植物寄生性担子菌類の生活環の進化と多様性に関する分子的解析とその制御

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はしがき

本研究では、生活様式や形態的特徴がユニークであり、また農林業における重要な病原菌となっているさび菌、くろぼ菌、もち病菌などの植物寄生性担子菌類について、生活環などの生態的特徴と分散や感染に関わる胞子などの形態的特徴の進化学的な実体を分子系統学的な解析を通じて明らかにするとともに、これらの生活環を制御している分子的要因を解明することを目的とした。そのため、国内および国外から標本や試料などの材料が収集し易く、多様な生活様式が存在し、且つ農林業上の重要な病原菌が含まれるマメ科、イネ科、キク科、バラ科、ツツジ科、ヒノキ科などに寄生する菌類について、それぞれの研究者が分担し、生活環と形態を再検討するとともに、リボゾームＤＮＡなどを用いて分子系統学的解析を行い、進化の実体や生活環の制御要因を解明した。この研究により解明された成果の主な概要は以下のとおりである。なお、研究内容の詳細については、それぞれの研究論文を参照していただきたい。

（１）イネ科のサトウキビ寄生する *Puccinia* 属さび菌について、多くの標本を収集し検討を行った結果、これらは、形態的にも分子系統学的にも２種とすることが妥当であると結論された。（２）マメ科の栽培豆類などに寄生する *Uromyces* 属さび菌について、これまで生活環の不明であった種について、その生活環を明らかにした。また、多くの植物への接種試験を行い、それぞれの種または変種の寄生性を明らかにした。さらに、多くの標本を収集し、形態学的および分子的系統学的解析を行い、これらの系統関係を明らかにするとともに、その進化を類推し、分類システムの再構築の必要性を明らかにした。（３）キク科のフキ属植物に寄生する *Puccinia* 属さび菌３種について検討を行った結果、これらは形態的、分子的また生態的にも１種とすることが妥当であると結論された。また、キク科のヨモギ属植物に寄生する *Puccinia* 属さび菌について、形態学的および分子系統学的解析を行い、これらの系統関係を明らかにし、生活環の進化とその多様性の要因を解明した。（４）バラ科に寄生する約３０種の *Phragmidium* 属菌について、世界各地から多数の標本を収集し、形態的特徴を明らかにするとともに、分類学的検討を行った結果、新種１種を含む１６種に整理するのが妥当であると結論された。（５）ツツジ科およびハイノキ科に寄生する *Exobasidium* 属菌について、形態学的および培養学的性状を明らかにし、その分類学的再検討を行うとともに、分子系統学的解析により、その進化を明らかにした。（６）ヒノキ科に寄生する *Blastospora* 属菌の生活環、核相交代、分子系統学的位置を明らかにした。（７）ツガ属に寄生する *Crysomyxa* 属菌について、形態学的および分子系統学的解析を行い、その生活環の進化を明らかにした。（８）ポプラ類に寄生する *Melampsora* 属菌について、形態学的および分子的系統学的解析を行い、これらの系統関係を明らかにするとともに、その進化を類推し、分類システムの再構築の必要性を明らかにした。（９）さび菌類の生活環における核相交代のタイプとその多様性を明らかにし、その進化的意義を明らかにした。

以上のように、本研究では、研究分担者を始め、多くの研究者のご協力を得て多くの成果を上げることができた。ここに厚く御礼申し上げる。

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研究発表

（１）学会誌等

1. Ono, Y., Ishimiya, K. and Kakishima, M.
Taxonomic identity of caricicolous puccinias host-alternating on *Petasites* in Japan.
Mycoscience 42: 101-106, 2001.
2. Ono, Y., Kakishima, M. and Ishimiya, K.
Aecidium dispori is the aecial anamorph of *Puccinia albispora*, sp. nov. (Uredinales).
Mycoscience 42: 149-153, 2001.
3. Virtudazo, E. V., Nojima, H. and Kakishima, M.
Taxonomy of *Puccinia* species causing rust diseases on sugarcane.
Mycoscience 42: 167-175, 2001
4. Virtudazo, E. V., Nakamura, H. and Kakishima, M.
Ribosomal DNA-ITS sequence polymorphism in the sugarcane rust, *Puccinia kuehnii*.
Mycoscience 42: 447-453, 2001.
5. Dono Wahyuno, Kakishima, M. and Ono, Y.
Morphological analyses of urediniospores and teliospores in seven *Phragmidium* species parasitic on ornamental roses.
Mycoscience 42: 519-533, 2001.
6. Nagao, H., Ezuka, A., Ohkubo, H. and Kakishima, M.
A new species of *Exobasidium* causing witches'broom on *Rhododendron wadanum*.
Mycoscience 42: 549-554, 2001.
7. Virtudazo, E. V., Nakamura, H. and Kakishima, M.
Phylogenetic analysis of sugarcane rusts based on sequences of ITS, 5.8S rDNA and D1/D2 region of LSU rDNA.
Journal of General Plant Pathology 67: 28-36, 2001.

8. Ono, Y. and Ishimiya, K.
Puccinia speciosa, sp. nov., host-alternates between *Tricyrtis* and *Carex*.
Mycologia 93: 796-801, 2001.
9. Ono, Y.
Life cycle and nuclear behavior in three rust fungi (Uredinales).
Mycoscience 43: 37-45, 2002.
10. Wahyuno, D., Kakishima, M. and Ono, Y.
Aeciospore-surface structures of *Phragmidium* species parasitic on roses.
Mycoscience 43: 159-167, 2002.
11. Ono, Y.
The diversity of nuclear cycle in microcyclic rust fungi (Uredinales) and its ecological and evolutionary implications.
Mycoscience 43: 421-439, 2002.
12. Yamaoka, Y., Fujiwara, Y., Kakishima, M., Katsuya, K., Yamada, K. and Hagiwara, H.
Pathogenic races of *Phakopsora pachyrhizi* on soybean and wild host plants collected in Japan.
Journal of General Plant Pathology 68: 52-56, 2002
13. Nagao, H., Akimoto, M., Kishi, K. Ezuka, A. and Kakishima, M.
Exobasidium dubium and *E. miyabei* sp. nov. causing Exobasidium leaf blisters on *Rhododendron* spp. in Japan.
Mycoscience 44: 1-9, 2003
14. Nakamura, H., Kaneko, S., Yamaoka, Y. and Kakishima, M.
Correlation between pathogenicity and molecular characteristics in the willow leaf rusts *Melampsora epitea* and *M. humilis* in Japan.
Mycoscience 44: 253-256, 2003.
15. Nagao, H., Ogawa, S., Sato, T. and Kakishima, M.
Exobasidium symploci-japonicae var. *carpogenum* var. nov. causing Exobasidium fruit deformation on *Symplocos lucida* in Japan.
Mycoscience 44: 369-375, 2003.
16. Chung, W. H., Ono, Y. and Kakishima, M.
Life cycle of *Uromyces appendiculatus* var. *azukicola* on *Vigna angularis*.
Mycoscience 44: 425-430, 2003.
17. Ono, Y.
Does *Puccinia hemerocallidis* regularly host-alternate between *Hemerocallis* and *Patrinia* plants in Japan?
Journal of General Plant Pathology 69: 240-243, 2003.
18. Chung, W. H., Tsukiboshi, T., Ono, Y. and Kakishima, M.
Phylogenetic analyses of *Uromyces viciae-fabae* and its varieties on *Vicia, Lathyrus* and *Pisum* in Japan.
Mycoscience 45:1-8, 2004.
19. Tian, C. H., Shang, Y. Z., Zhuang, J. Y., Wang, Q. and Kakishima, M.
Morphological and molecular phylogenetic analysis of *Melampsora* species on poplars in China.
Mycoscience 45: 56-66, 2004.
20. Nagao, H., Sato, T. and Kakishima, M.
Three species of *Exobasidium* causing Exobasidium leaf blight on subgenus *Hymenanthes*, *Rhododendron* spp. in Japan.
Mycoscience 45: 85-95, 2004
21. Chung, W. H., Tsukiboshi, T., Ono, Y. and Kakishima, M.
Morphological and phylogenetic analyses of *Uromyces appendiculatus* and *Uromyces vignae* on legumes in Japan.
Mycoscience 45 (in press)

（2）口頭発表

1. 長尾英幸，江塚昭典，柿嶌　眞 (2001)
ツツジ属に寄生するもち病菌８種の分子系統解析
平成１３年度日本植物病理学会大会，東北大学
2. Virtudazo, E. V., 中村　仁，柿嶌　眞 (2001)
サトウキビ黄さび病菌 *Puccinia kuehnii* の ITS 領域における多型
平成１３年度日本植物病理学会大会，東北大学
3. Wahyuno, D., 小野義隆，柿嶌　眞 (2001)
生活環とさび胞子・冬胞子の形態によるバラ属植物に寄生する *Phragmidium* 属菌の識別
日本菌学会第４５回大会，日本獣医畜産大学，東京
4. Virtudazo, E. V., 野島秀伸，柿嶌　眞 (2001)
サトウキビ黄さび病菌 *Puccinia kuehnii* 冬胞子の発芽過程
日本菌学会第４５回大会，日本獣医畜産大学，東京
5. Chung, W. H., 月星隆雄，柿嶌　眞 (2001)
栽培マメ類に寄生する *Uromyces* 属菌の分類学的再検討
日本菌学会第４５回大会，日本獣医畜産大学，東京
6. 長尾英幸，江塚昭典，指田玲子，柿嶌　眞 (2001)
日本産もち病菌１４種の分子系統解析
日本菌学会第４５回大会，日本獣医畜産大学，東京
7. 今津道夫，伊藤知子，原田幸雄 (2001)
ヒノキアスナロ・クロベ類天狗巣病菌の生活環と核相交代について
日本菌学会第４５回大会，日本獣医畜産大学，東京
8. 長尾英幸，秋本正信，江塚昭典，柿嶌　眞 (2001)
ヨドガワツツジ平もち病菌の再検討
日本植物病理学会北海道部会，北海道大学
9. 長尾英，原田幸雄，江塚昭典，柿嶌　眞 (2001)
青森県でヒメウスノキとウスノキに発生した裏白もち病について
日本植物病理学会東北部会，秋田市
10. 小口　悠，柿嶌　眞 (2002)
タデ科植物に寄生するさび菌 *Puccinia polygoni-amphibii* の変種についての再検討
日本菌学会第４６回大会，信州大学農学部，伊那
11. 星川圭一，今津道夫，福島和貴，滝澤香代子 (2002)
ツガ属植物に寄生する短世代型 *Chrysomyxa* 属菌２種について
日本菌学会第４６回大会，信州大学農学部，伊那
12. Chung, W. H., Tsukiboshi, T. and Kakishima, M. (2002)
Taxonomic revision of *Uromyces* species on cultivated legumes in Japan.
7th International Mycological Congress (IMC7), Oslo, Norway, August
13. Imazu, M., Kakishima, M., and Harada, Y. (2002)
Nuclear behavior and life cycle of witches' broom rust of Japanese hiba and thuja, *Blastospora betulae*
IUFRO Working Party Symposium, Rust of Forest Trees, Yangling, China, August
14. Tian, C. M., Shang, Y. Z., Zhuang, J. Y., Wang, Q. and Kakishima, M. (2002)
Morphological and phylogenetic analyses of poplar rusts in China
IUFRO Working Party Symposium, Rust of Forest Trees, Yangling, China, August
15. Hoshikawa, K., Imazu, M. and Fukushima, K. (2002)
Phylogenetic analysis of two *Chyrsomyxa* rusts on *Tsuga* spp in Japan.
IUFRO Working Party Symposium, Rust of Forest Trees, Yangling, China, August

16. Kaneko, S. (2002)
A taxonomic overview of some important tree rusts in Japan.
IUFRO Working Party Symposium, Rust of Forest Trees, Yangling, China, August
17. Chung, W. H., Tsukiboshi, T. and Kakishima, M. (2002)
Genetic variation of *Uromyces* species on cultivated legumes in Japan.
3rd Asia-Pacific Mycological Congress on Biodiversity and Biotechnology, Kunming, China, November
18. Chung, W. H., 小野義隆, 柿嶌 眞 (2003)
栽培マメ類に寄生する *Uromyces* 属菌３種の寄生性の検討
平成１５年度日本植物病理学会大会, 明治大学, 東京
19. 田 呈明, 尚 衍重, 庄 剣雲, 王 琦, 柿嶌 眞 (2003)
ポプラ類に寄生する中国産 *Melampsora* 属さび菌の分類学的検討
平成１５年度日本植物病理学会大会, 明治大学, 東京
20. 小口 悠, 柿嶌 眞 (2003)
タデ科植物に寄生する日本産さび菌 *Puccinia polygoni-amphibii* の分類学的再検討
日本菌学会第４７回大会, 北海道大学, 札幌
21. Engkhaninun Jintana, To-anun Chaiwat, 小野義隆, 柿嶌 眞 (2003)
タイ北部で新たに記録されたサビキン２種について
日本菌学会第４７回大会, 北海道大学, 札幌
22. Chung, W. H., 小野義隆, 柿嶌 眞 (2003)
アズキ類に寄生する *Uromyces appendiculatus* var. *azukicola* の生活環
日本菌学会第４７回大会, 北海道大学, 札幌
23. 田 呈明, 今津道夫, 柿嶌 眞 (2003)
形態的および分子系統的解析による *Pucciniastrum tiliae* と *P. styracinum* の関係
日本菌学会第４７回大会, 北海道大学, 札幌
24. 今津道夫, 大西麻友紀, 原田幸雄 (2003)
Blastospora betulae によるヒノキアスナロ天狗巣病罹病部における菌糸の分布と進展
日本菌学会第４７回大会, 北海道大学, 札幌
25. Denchev, C. M., Kakishima, M and Kadota, Y. (2003)
Current status of *Sphacelotheca hydropiperis* gr. (smut fungi).
XIV Congress of Europian Mycologists, Yalta, Crimea, Ukraine, September
26. 梁 英梅, 柿嶌 眞 (2004)
ブナおよびリョウブに寄生する日本産 Pucciniastrum 属さび菌について
日本菌学会第４８回大会, 長崎シーボルト大学, 長崎
27. Engkhaninum, J., Ono, Y., Kakishima, M. (2004)
Possible phylogenetic relationship of four *Puccinia* species parasitic on *Altemisia* and *Carex* in Japan
日本菌学会第４８回大会, 長崎シーボルト大学, 長崎
28. Chung, W. H., 月星隆雄, 小野義隆, 柿嶌 眞 (2004)
マメ類に寄生する日本産 *Uromyces appendiculatus* および *U. vignae* の形態学的および系統学的解析
日本菌学会第４８回大会, 長崎シーボルト大学, 長崎
29. 田 呈明, 梁 英梅, 柿嶌 眞 (2004)
Populus yunnanensis に寄生する中国産 *Melampsora* 属さび菌の１新種について
日本菌学会第４８回大会, 長崎シーボルト大学, 長崎
30. 君和田香織, 今津道夫, 小野義隆 (2004)
トウヒーシャクナゲ類さび病菌 *Chrysomyxa succinea* の発生生態と生活環
日本菌学会第４８回大会, 長崎シーボルト大学, 長崎

31. 今津道夫，西方恵理，大澤正嗣 (2004)
ハリモミとミツバツツジに発生した *Chrysomyxa* 属さび菌について
日本菌学会第４８回大会，長崎シーボルト大学，長崎

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Taxonomic identity of caricicolous *Puccinia* host-alternating on *Petasites* in Japan

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Uredinial-telial *Puccinia* fungi on *Carex shimizuensis* in Nagano Pref. and on *C. dimorpholepis* in Ibaraki Pref. were proven to host-alternate on *Petasites japonicus* by field observations and inoculation experiments. These fungi from the two localities were morphologically similar and were compared with three described *Puccinia* species that host-alternate between *Carex* species and *P. japonicus* in Japan, i.e., *P. caricis-petasitidis*, *P. caricis-flabellatae* and *P. caricis-podogynae*. The three previously described species and the newly found *Puccinia* fungi were morphologically indistinguishable at all stages of the life cycle; therefore, it was concluded that three species and the two newly found fungi are taxonomically identical, in which *P. caricis-petasitidis* has nomenclatural priority.

Key Words——*Carex*; Compositae; Cyperaceae; life cycle; Uredinales.

Uredinial-telial *Puccinia* fungi were found on *Carex shimizuensis* Franch. at Sugadaira, Nagano Pref. and on *C. dimorpholepis* Steud. at Okami, Ibaraki Pref. The *Puccinia*-infected *Carex* plants were associated with *Aecidium*-infected plants of *Petasites japonicus* (Sieb. & Zucc.) Maxim. at each location. The close association of the *Puccinia*-infected *Carex* and the *Aecidium*-infected *Petasites* plants indicated their life-cycle relationship. This paper reports experimental proof of the predicted anamorph-teleomorph relationship between the *Puccinia* on *Carex* species and the *Aecidium* on *Petasites japonicus*.

Three *Puccinia* species have been described as having a heteroecious life cycle on *Carex* species and *Pet. japonicus* in Japan, i.e., *P. caricis-petasitidis* Y. Harada (Harada, 1977), *P. caricis-flabellatae* Y. Harada and *P. caricis-podogynae* Y. Harada (Harada, 1986). All the three *Puccinia* species were described from Aomori Pref. and were stated to differ in the teliospore morphology and the number and distribution of urediniospore germ pores and to be specific on *C. sadoensis* Franch., *C. presicottiana* Boott subsp. *flabellata* (Lév. & Vant.) T. Koyama and *C. podogyna* Franch. & Savat., respectively (Harada, 1977, 1986). Determination of the taxonomic identity of the two *Puccinia* fungi found in Nagano and Ibaraki necessitated their comparison with the three previously described *Puccinia* species host-alternating on *P. japonicus* and the taxonomic reevaluation of the three described species themselves. This paper discusses the taxonomic identity of the two newly found *Puccinia* fungi and the three previously described species.

Materials and Methods

Specimens examined On *Carex dimorpholepis*, Ibaraki, Kuji-gun, Satomi-mura, Okami, 31 March 1998. Y. Ono (Y. O.) & K. Ishimiya (K. I.) 4092 (T: IBA-8034); 21 Aug. 1998. Y. O. & K. I. 4220 (U, T: IBA-8163); 2 March 1999. Y. O. & K. I. 4268 (T: IBA-8219); 2 March 1999. Y. O. & K. I. 4269 (T: IBA-8220). On *C. presicottiana* subsp. *flabellata*, Aomori, Kitatsugaru-gun, Shiura-mura, Katsuragawa, 22 Oct. 1978. Y. Harada (Y. H.) 11500 (U, T; Holotype of *P. caricis-flabellatae*). On *C. podogyna*, Aomori, Nakatsugaru-gun, Nishimeya-mura, Anmon-ohashi, 13 Nov. 1979. Y. H. 11201 (U, T; Holotype of *P. caricis-podogynae*). On *C. sadoensis*, Aomori, Hirosaki, Koguriyama, 1 Dec. 1974. Y. H. 4129 (U, T: HU-741201; Holotype of *P. caricis-petasitidis*); 20 May 1975. Y. H. 11201 (U, T: HU-750501). On *C. shimizuensis*, Nagano, Chiisagata-gun, Sanada-machi, Sugadaira, 24 Aug. 1997. J. Abe (U, T: TSH-R1702); 26 April 1997, M. Kakishima (T: TSH-R1703). On *Petasites japonicus*, Aomori, Hirosaki, 14 March 1975. Y. H. (result of basidiospore inoculation of *P. caricis-petasitidis*, S. A: HU750301); 12 March 1979. Y. H. 10020 (result of basidiospore inoculation of *P. caricis-flabellatae*, S. A); 12 March 1980. Y. H. 10668 (result of basidiospore inoculation of *P. caricis-podogynae*, S, A); Ibaraki, Kuji-gun, Satomi-mura, Okami, 8 June 1990. Y. O. 2114 (S, A: IBA-4806); 7 June 1998. Y. O. & K. I. 4150 (S, A: IBA-8093); Mito, 10 May 1991. Y. O. 2336 (result of basidiospore inoculation, S, A: IBA-5692); 6 May 1998. Y. O. 4096 (result of basidiospore inoculation, S, A: IBA-8038); 6 May 1998. Y. O. 4097 (result of basidiospore inoculation, S, A: IBA-8039); Mito, 8 June 1999. Y. O. 4342 (result of basidiospore inoculation, S, A: IBA-8292); Nagano, Chiisagata-gun, Sanada-machi, Sugadaira, Aug. 1996. S. Iwamoto (S, A: TSH-R1704).

Capital letters S, A, U and T preceding the herbarium accession number denote spermogonial, aecial, uredinial

Table 1. Teliospore characteristics of *Puccinia* species host-alternating between *Carex* and *Petasites* in Japan.

Species/fungus	Specimen	Length (Mean) (μm)	Width (Mean) (μm)	Apical wall-thickening (Mean) (μm)	Pedicel length (Mean) (μm)
P. caricis-flabellatae Harada	YH-11500 (Holotype)	42.5–(52.5)–67.5	13.8–(16.9)–20.0	6.3–(8.3)–11.3	25.0–(36.2)–46.3
P. caricis-petasitidis Harada	YH-4129 (Holotype)	41.3–(49.8)–65.0	15.0–(19.1)–22.5	7.5–(11.4)–13.8	25.0–(40.0)–51.3
P. caricis-podogynae Harada	YH-11201 (Holotype)	41.3–(55.6)–67.5	15.0–(19.1)–22.5	10.0–(12.3)–15.0	18.8–(31.4)–47.5
The Okami fungus	IBA-8163	37.5–(49.7)–61.3	13.8–(18.1)–22.5	7.5–(10.7)–12.5	28.8–(37.9)–50.0
The Sugadaira fungus	TSH-R1702	40.0–(50.0)–62.5	13.8–(16.6)–20.0	6.3–(7.9)–10.0	20.0–(30.5)–47.5

and telial stage, respectively. All the above-cited specimens have been deposited in the mycological herbaria of the Faculty of Education, Ibaraki University (IBA), the Institute of Agriculture and Forestry, University of Tsukuba (TSH) and the Faculty of Agriculture and Life Science, Hirosaki University (HU).

Basidiospore inoculation Basidiospore inoculations were undertaken by the method described by Ono (1994) and Ono and Azbukina (1997). Telium-bearing leaves of *C. shimizuensis* were collected at Sugadaira, Nagano in April, 1997 and of *C. dimorpholepis* at Okami, Ibaraki in March, 1991, February, 1998 and March, 1999, respectively. The telium-bearing leaves were preserved in a refrigerator at ca. 5°C until the time when they were soaked in running tap water for 7–14 d to induce germination. Then, the leaves were cut into small pieces (ca. 2×5 mm), placed on water-saturated filter paper in a petri dish and incubated in the dark at ca. 18°C. The leaf pieces with germinated teliospores were placed on adaxial surfaces of apparently healthy leaves of the *Pet. japonicus* plants, which had been planted with loam soil in clay pots (18 cm diam). The inoculated plants were sprayed with distilled water and placed in a moist chamber at room temperature (18–22°C) for 48 h. The plants were subsequently transferred to a glasshouse for further observations. The inoculation experiments were repeated four times for the Sugadaira fungus population in 1997 and five times for the Okami fungus population, twice in 1991 and 1998 and once in 1999. In each basidiospore inoculation, two to five leaves of one or two plants were inoculated.

Microscopic observation To examine morphology and structure of spermogonium and aecium, fresh infected materials and dried herbarium specimens were sectioned freehand under a binocular dissecting microscope. Thin sections were mounted in a drop of lactophenol solution without stain. To examine morphology and to measure size, spores were scraped from sori on herbarium specimens and mounted as described above. Fifty or 100 randomly selected spores were measured in each specimen.

For scanning-electron microscopy (SEM), rust-infected leaves from dried herbarium specimens were cut into ca. 3×3 mm pieces containing a few sori, and each piece was placed on double-adhesive tape on a specimen holder. The preparations were subsequently coated with platinum-paladium using a Hitachi E-1030 Ion Sputter and examined with a Hitachi S-4200 SEM at 15 kV

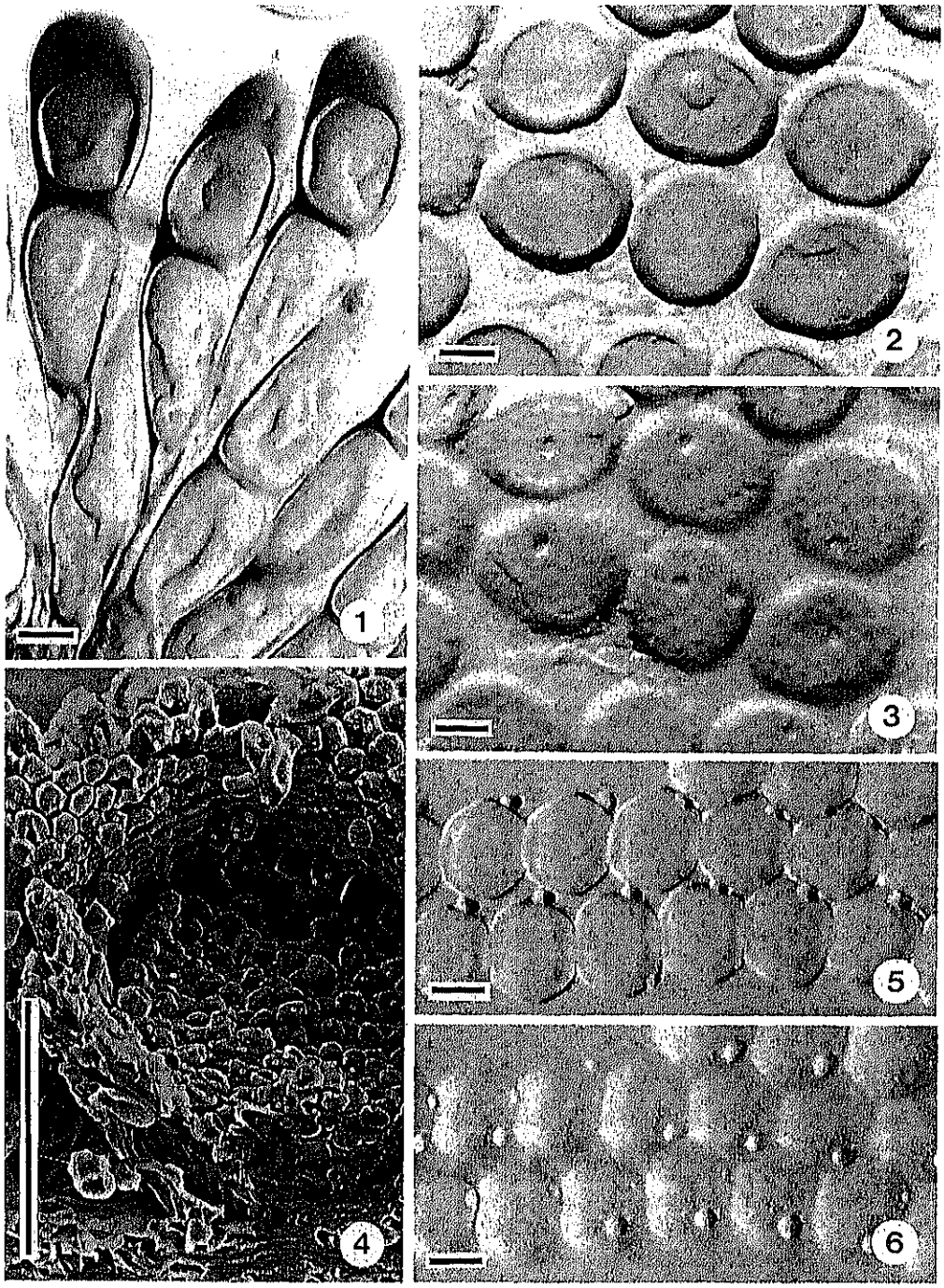
Results

Life cycle In the Sugadaira fungus, the basidiospores were successfully inoculated twice on the apparently healthy leaves of *Pet. japonicus*. Spermogonia were formed 12–15 d after the basidiospore inoculation, and aecia followed in the subsequent 10–15 d. In the Okami fungus, the basidiospores always successfully infected the *Petasites* plants, on which spermogonia appeared 5–7 d after the basidiospore inoculation and aecia were formed in the subsequent 6–7 d.

Aeciospores formed on the *Petasites* plants by the basidiospore inoculation were inoculated on either *C. shimizuensis* or *C. dimorpholepis*, from which the inoculum of the basidiospore inoculation was derived. Repeated inoculations were unsuccessful, however. Although aeciospore infection resulting in uredinio- and teliospore production on the *Carex* plants failed, the life-cycle connection between the spermogonial-aecial fungus on the *Petasites* plants and uredinial-telial fungi on the *Carex* plants both at Sugadaira and at Okami was confirmed. Because of the failure of the aeciospore inoculation, cross-inoculation to determine host range of both Sugadaira and Okami fungi was not undertaken.

Table 2. Urediniospore characteristics of *Puccinia* species host-alternating between *Carex* and *Petasites* in Japan.

Species/fungus	Specimen	Length (Mean) (μm)	Width (Mean) (μm)	Wall thickness (μm)
P. caricis-flabellatae Harada	YH-11500 (Holotype)	16.3–(19.2)–25.0	13.8–(17.0)–18.8	2.5
P. caricis-petasitidis Harada	YH-24000 (Holotype)	16.3–(20.4)–23.8	15.0–(17.8)–18.8	2.5
P. caricis-podogynae Harada	YH-11201 (Holotype)	16.3–(19.0)–23.8	13.8–(16.2)–18.8	2.5–3.8
The Okami fungus	IBA-8163	17.5–(20.8)–25.0	13.8–(17.8)–20.0	2.5–3.8
The Sugadaira fungus	TSH-R1702	18.8–(19.9)–23.8	16.3–(18.3)–20.0	2.5–3.8



Figs. 1–6. *Puccinia caricis-petasitidis sensu lato*. 1. Teliospores (IBA-8034). 2. Urediniospores fo-cused on a median plane (IBA-8163). 3. Urediniospores focused on a tangential plane (IBA-8163). 4. Aecium (TSH-R1704; SEM). 5. Aeciospores focused on a median plane (IBA-8038). 6. Aeciospores focused on a tangential plane (IBA-8038). 7. Aeciospore surface structure (TSH-R1704; SEM). Scale bar=10 μm in Figs. 1–3, 5–7; 100 μm in Fig. 4.

Morphology In the Okami fungus, teliospores were variable in shape, mostly clavate to oblong-ellipsoid, rounded, truncate or conical at the apex; slightly to moderately constricted at the septum, attenuate toward the base, and 37.5–61.3×13.8–22.5 μm in size (Fig. 1; Table 1). The wall was light chestnut-brown and prominently thickened at the apex (7.5–12.5 μm). The pedicel was persistent and 28.8–50.0 μm long. Teliospores of the Sugadaira population were similar to and did not seem to be morphologically distinct from those of the Okami population (Table 1).

Urediniospores of the two geographically separated populations were also similar; mostly obovoid-ellipsoid or broadly ellipsoid and 17.5–25.0×13.8–20.0 μm in size

Table 3. Urediniospore germ pores of *Puccinia* species host-alternating between *Carex* and *Petasites* in Japan.

Species/fungus	Specimen	2, infraequatorial	2, equatorial	3, equatorial	4, equatorial
P. caricis-flabellatae Harada	YH-11600 (Holotype)	2*	22	65	11
P. caricis-petasitidis Harada	YH-24000 (Holotype)	2	15	76	7
P. caricis-podogynae Harada	YH-11201 (Holotype)	2	11	68	19
The Okami fungus	IBA-8163	0	17	79	4
The Sugadaira fungus	TSH-R1702	1	20	73	6

* Frequency in 100 observed urediniospores.

(Fig. 2; Table 2). The wall was evenly 2.5–3.8 μm thick, cinnamon-brown and completely echinulate (Fig. 3). Number and distribution of urediniospore germ pores were variable. When the urediniospore was divided longitudinally into three zones, i.e., apical, equatorial and basal zones, most urediniospores in the two geographically separated populations possessed three germ pores at the equatorial zone (Fig. 3; Table 3).

As with the uredinio- and teliospores, no significant difference was observed in aecium and aeciospore morphology of the two fungus populations. Spermogonia were subepidermal and globose, flask-shaped or depressed ovoid, 110–185 μm high and 115–135 μm wide. Aecia were cupulate surrounded by a well-developed peridium that ruptures and becomes reflected upon maturity (Fig. 4). Aeciospores were subglobose or depressed ellipsoid, often angular and 12.5–22.5 × 10.5–17.5 μm in size (Fig. 5; Table 4). The wall was ca. 1 μm thick, colorless and verrucose with refractive granules on the upper side (type 5 of Savile, 1973; Fig. 6, Table 4).

Discussion

Identity of the two geographically separate fungi The *Puccinia* fungi on *C. shimizuensis* at Sugadaira, Nagano and on *C. dimorpholepis* at Okami, Ibaraki have the same heteroecious life cycle with *Pet. japonicus* as the common spermogonial-aecial host, as proven by field observations and artificial inoculations. Beside having the same life cycle, as shown in Tables 1–4, the two fungi are morphologically indistinguishable at all stages of the life cycle. The same heteroecious life cycle and morphological similarity indicate that the two geographically separated fungi constitute a single species under the species concept of interbreeding populations reproductively isolated from others, which is adopted from Mayr and Ashlock (1991).

As stated previously for caricicolous rust fungi (Ono, 1983), two morphologically similar fungal populations may be recognized as distinct and placed in different taxa if they exhibit distinct host specificity, taxa being species, subspecies, varieties or formae speciales depending on how well their biology is understood. Thus, morphologically indistinguishable rust populations may be recognized as distinct species if they have different life cycles with different spermogonial-aecial host(s) and distinct ranges of uredinial-telial host(s).

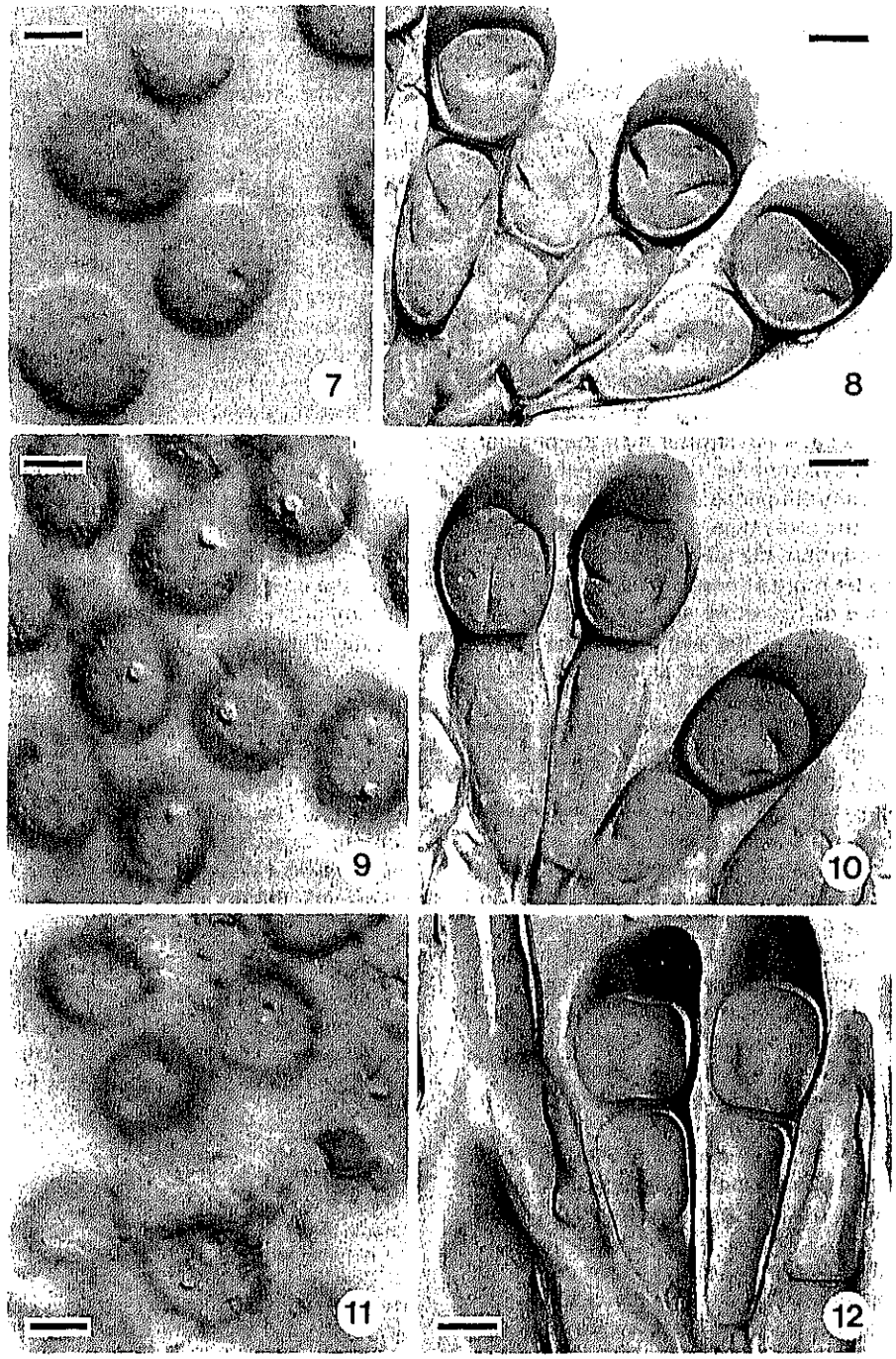
For rust populations that are morphologically indistinguishable, have similar uredinial-telial hosts and share common spermogonial-aecial host(s), it would be practical to treat them as a single species. Thus circumscribed species might be found to be composed of two or more reproductively isolated populations, i.e., two or more species, by additional properties to be found in future study. Accordingly, it is concluded that the geographically separated *Puccinia* fungi under discussion constitute a single species.

Taxonomic identity of *Puccinia caricis-flabellatae* and *P. caricis-podogynae* with *P. caricis-petasitidis* Three *Puccinia* species host-alternating between *Carex* species and *Pet. japonicus* have been described as distinct in Japan. *Puccinia caricis-petasitidis* was the first to be described and was characterized by globose or subglobose urediniospores (Fig. 7) of 18–21 × 16–20 μm in size with 2–4 subequatorial or scattered germ pores and broadly clavate teliospores of 40–55 × 15–20 μm in size with moderate constriction at the septum (Fig. 8; Harada, 1977). This fungus was assumed to be specific on *C. sadoensis*, giving negative results upon inoculation onto *C. thunbergii* Steud., *C. heterolepis* Bunge and *C. forficula* Franch. & Savat. (Harada, 1977).

Table 4. Aeciospore characteristics of *Puccinia* species host-alternating between *Carex* and *Petasites* in Japan.

Species/fungus	Specimen	Length (Mean) (μm)	Width (Mean) (μm)	Wall thickness (μm)	Surface structure type**
P. caricis-flabellatae Harada	YH-10021*	15.0–(16.7)–20.0	12.5–(14.7)–16.3	ca.1.0	type 5
P. caricis-petasitidis Harada	YH-4143*	13.8–(17.5)–20.0	12.5–(15.3)–17.5	ca.1.0	type 5
P. caricis-podogynae Harada	YH-10668*	15.0–(16.8)–20.0	12.5–(14.5)–17.5	ca.1.0	type 5
The Okami fungus	IBA-8095	15.0–(16.7)–20.0	12.5–(14.6)–17.5	ca.1.0	type 5
The Okami fungus	IBA-8038*	12.5–(17.7)–22.5	12.5–(15.2)–17.5	ca.1.0	type 5
The Sugadaira fungus	TSH-R1704	14.5–(16.8)–19.5	10.5–(14.0)–17.5	ca.1.0	type 5

* Aeciospores formed by basidiospore inoculation.
** The type is according to Savile's (1973) classification.



Figs. 7–12. *Puccinia caricis-petasitidis sensu lato*. 7. Urediniospores focused on a tangential plane (YH-4129: Holotype of *P. caricis-petasitidis*). 8. Teliospores (YH-4129: Holotype of *P. caricis-petasitidis*). 9. Urediniospores focused on a tangential plane (YH-11500: Holotype of *P. caricis-flabellatae*). 10. Teliospores (YH-11500: Holotype of *P. caricis-flabellatae*). 11. Urediniospores focused on a tangential plane (YH-11201: Holotype of *P. caricis-podogynae*). 12. Teliospores (YH-4129: Holotype of *P. caricis-podogynae*). Scale bar=10 μm.

The second species, *P. caricis-flabellatae*, was characterized by obovate or broadly ellipsoid urediniospores (Fig. 9) of 18–21×15–19 μm in size with 2–3 primarily equatorial germ pores and broadly clavate or ellipsoid teliospores of 45–75×15–20 μm in size with strong constriction at the septum (Fig. 10; Harada, 1986). This species seems to be restricted to *C. presicottiana* subsp. *flabellata*, giving negative results upon inoculation onto *C. heterolepis*, *C. podogyna*, *C. sadoensis*, and *C. stipata* Muhl. (Harada, 1986).

The third species, *P. caricis-podogynae*, was stated to possess globose or subglobose urediniospores (Fig.

11) of 18–21 × 15–19 μm in size with 3–4 (rarely 2 or 5) equatorial gem pores and broadly clavate or ellipsoid teliospores of 50–70 × 13–19 μm in size with weak constriction at the septum (Fig. 12; Harada, 1986). This species was assumed to be specific on *C. podogyna*, giving negative results upon inoculations onto *C. sadoensis*, *C. heterolepis*, *C. forficula*, *C. olivacea* Boott var. *angustior* and *C. shimizuensis* (Harada, 1986).

Our measurements of the type specimens of the three species are slightly different from the original description (Tables 1–4). The longest teliospore of *P. caricis-flabellatae* was ca. 8 μm shorter and that of *P. caricis-petasitidis* was 10 μm longer than the original description (Table 1). The shortest teliospore of *P. caricis-flabellatae* was 2.5 μm shorter and that of *P. caricis-podogynae* was ca. 9 μm shorter than the original description (Table 1). Except for the difference in the size range, no significant difference in the measurements was detected among the three type specimens. As stated in the original description, the teliospores of *P. caricis-flabellatae* appear to be more strongly constricted at the septum than are those of two other species. However, the degree of constriction at the septum of the teliospores is variable and the difference among the specimens is not prominent (Figs. 8, 10, 12).

No significant differences were observed among the urediniospores from the three type specimens (Table 2). The most significant difference in the urediniospores of the three species was stated to be in the number and distribution of germ pores, i.e., 2–4 subequatorial or scattered in *P. caricis-petasitidis*, 2–3 equatorial in *P. caricis-flabellatae* and 3–4 (rarely 2 or 5) equatorial or scattered in *P. caricis-podogynae* (Harada, 1977, 1986). Distribution of the germ pores is highly variable, primarily because spores are distorted rather than ellipse oriented to the longitudinal axis. When the spores are longitudinally divided into three zones, the number and distribution pattern are found to be similar among the three species: most of the spores examined possess 3 equatorial germ pores (Figs. 7, 9, 11; Table 3).

In the morphology of aeciospores formed on the *Petasites* plants by the basidiospore inoculation, no significant difference was observed among the three species (Table 4).

These observed similarities noted upon the reexamination of the type specimens lead to the conclusion that the three described species are actually a single species, and that *P. caricis-petasitidis* has nomenclatural priority. Putative host specificity of the species is not conclusive and needs further investigation to see if the host specificity actually causes reproductive isolation of the rust populations or if the degree of host specificity is at a stage at which the different rust populations are recognized as formae speciales.

Following the conclusion that *P. caricis-petasitidis* embraces the rust populations formerly named *P. caricis-flabellatae* and *P. caricis-podogynae*, it is also concluded that the *Puccinia* populations on *C. shimizuensis* at Sugadaira, Nagano and on *C. dimorpholepis* at Okami, Ibaraki belong to *P. caricis-petasitidis*. The description of *Puccinia caricis-petasitidis* is revised as follows:

Puccinia caricis-petasitidis Y. Harada, Trans. mycol. Soc. Japan 18: 173. 1977.

Synomyms: *Puccinia caricis-flabellatae* Y. Harada, Trans. mycol. Soc. Japan 27: 359. 1986.

Puccinia caricis-podogynae Y. Harada, Trans. mycol. Soc. Japan 27: 362. 1986.

Spermogonia mostly epiphyllous or petiolicolous, subepidermal, subglobose or depressed obovoid, 100–185 μm high and 100–170 μm wide, Aecia mostly hypophyllous or petiolicolous, peridiate, cupulate: aeciospores subglobose, often angular, 12–23 × 10–20 μm, the wall ca. 1 μm thick, colorless, verrucose with refractive granules at upper side. Uredinia mostly hypophyllous, brown, powdery; urediniospores subglobose, obovoid or ellipsoid, 16–25 × 13–20 μm, the wall evenly 2.5–3.8 μm thick, cinnamon-brown, completely echinulate, germ pores (2–)3(–5) mostly equatorial. Telia hypophyllous, black, pulvinate; teliospores clavate, obovoid ellipsoid, oblong ellipsoid, rounded, truncate or conical at the apex, weakly to moderately constricted at the septum, attenuate toward the base, 37–70(–75) × 12–23 μm, the wall chestnut-brown, 5–15 μm thick at the apex, the pedicel colorless, persistent, 18–55(–70) μm long.

Holotype: On *Carex sadoensis* Franch., Japan, Aomori Pref., Hirosaki, Oguriyama, 1 Dec. 1974, Y. Harada 4129 (HU741201).

Host and geographic distribution: *C. dimorpholepis*-Ibaraki and Fukushima; *C. presicottiana* subsp. *flabellata*-Aomori; *C. podogyna*-Aomori; *C. sadoensis*-Aomori; *C. shimizuensis*-Nagano; *Pet. japonicus*-Hokkaido, Aomori, Fukushima, Ibaraki and Nagano.

Acknowledgements——We thank Prof. Y. Harada, Faculty of Agriculture and Life Science, Hirosaki University, for the loan of the holotype specimens for *Puccinia caricis-petasitidis*, *P. caricis-flabellatae* and *P. caricis-podogynae*.

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Literature cited

Harada, Y. 1977. *Puccinia caricis-petasitidis*, sp. nov., a heteroecious *Carex* rust fungus from Japan. Trans. Mycol. Soc. Japan 18: 170–176.

Harada, Y. 1986. Four new species of heteroecious *Puccinia* (Uredinales) on *Carex* from Japan. Trans. Mycol. Soc. Japan 27: 357-370.

Mayr, E. and Ashlock, P. D. 1991. Principles of systematic zoology. 2nd. edn. McGraw-Hill, Inc., New York.

Ono, Y. 1983. Aecial stage of *Puccinia dioicae* var. *micropuncta*, var. nov. occurs on *Artemisia* in Japan. Trans. Mycol. Soc. Japan 24: 1–6.

Ono, Y. 1994. *Tranzschelia asiatica* sp. nov. and its taxonomic relationship to *Tranzschelia arthuri*. Can. J. Bot. 72: 1178–1186.

Ono, Y. and Azubukina, Z. M. 1997. Heteroecious life cycle of two graminicolous *Puccinia* (Uredinales). Mycoscience 38: 281–286.

Savile, D. B. O. 1973. Aeciospore types in *Puccinia* and *Uromyces* attacking Cyperaceae, Juncaceae and Poaceae. Rep. Tottori Mycol. Inst. (Japan) 10: 225–241.

Mycoscience 42: 149–153, 2001

Aecidium dispori is the aecial anamorph of *Puccinia albispora*, sp. nov. (Uredinales)

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Aecidium dispori **forms spermogonium and aecium on** ***Disporum sessile*** **and** ***D. smilacinum*****, which are distributed in East Asia. The** ***Aecidium*** **species is found to be an aecial anamorph of a** ***Puccinia*** **fungus, with its urediniał-telial stage being formed on** ***Carex conica, C. dolichostachya*** **subsp.** ***multifolia, C. pisiformis*** **subsp.** ***alterniflora*** **and** ***C. rugata*****. Urediniospores of this fungus are large, colorless, thick-walled with 4–5 equatorial germ pores. The morphological characteristics of urediniospores and the spermogonial-aecial host do not fit to any set of circumscribing characters of previously described species. We consider the fungus to be a new species and propose a new name,** ***Puccinia albispora*****, for the fungus.**

Key Words——*Carex*; Cyperaceae; *Disporum*; Liliaceae; rust fungus.

Aecidium dispori Dietel was first described for a fungus found on *Disporum sessile* Don at Togakushi, Nagano Pref., Japan (Dietel, 1899). It has since been found to occur also on *D. smilacinum* A. Gray and to be widely distributed in Japan, China and Taiwan (Hiratsuka et al., 1992).

It is empirically known that *A. dispori* does not repeat the spermogonial-aecial stage on the same host species. No urediniał-telial fungus has been discovered on *Disporum* species in Japan, although *Puccinia dispori* Sydow is know to occur on *Disporum* species in the Philippines (Sydow and Petrak, 1931), China (Zhuang et al., 1998) and Taiwan (Hiratsuka and Hashioka, 1937). Thus, *A. dispori* is believed to be an aecial anamorph of a fungus that may belong either to *Puccinia* or to *Uromyces*.

This paper reports the life-cycle connection between *A. dispori* on *Disporum* and a urediniał-telial *Puccinia* on *Carex*, which was proven by field observations and repeated inoculations, and proposes a name for the new holomorphic fungus based on morphological examinations of closely related species.

Materials and Methods

Specimens examined Specimens deposited in the mycological herbaria of the Faculty of Education, Ibaraki University (IBA), the Institute of Agriculture and Forestry, University of Tsukuba (TSH), the Faculty of Agriculture, Hokkaido University (SAPA) and the Swedish Museum of Natural History, Sweden (S) were examined and are listed under each species in the description section below.

Basidiospore inoculation Basidiospore inoculations were undertaken by the method described by Ono (1995) and Ono and Azubukina (1997). Telium-bearing leaves of *Carex* species were collected at various locations in different years as follows: *C. pisiformis* subsp. *alterniflora* at Asakawa, Tokyo, in April, 1976; *C. conica* at Mt. Tsukubasan, Ibaraki, in March, 1979; *C. dolichostachya* subsp. *multifolia* at Takefu, Ibaraki, in 1979, at Mt. Gozenyama, Ibaraki, in March 1980 and at Tokuda, Ibaraki, in March 1999; and *C. rugata* at Okami, Ibaraki, in March, 1999 and February 2000. In each year, the collected telium-bearing leaves were preserved in a refrigerator at ca. 5°C until the time when they were soaked in running tap water at room temperature for 7–14 d to induce germination. Then, the leaves were cut into small pieces (ca. 3–5 mm long), placed on water-saturated filter paper in a Petri dish and incubated in the dark at ca. 18°C. The leaf pieces with germinated teliospores were placed on the adaxial surface of apparently healthy leaves of the following plants, which had been planted with loam soil in clay pots (18 cm diam): *Disporum smilacinum, Cardiocrinum cordatum* Makino, *Aster scaber* Thunb. and *A. ageratoides* Turzc. subsp. *ovatus* (Fr. & Sav.) Nakai were inoculated with the fungus on *C. pisiformis* subsp. *alterniflora* in 1976; *D. sessile, D. smilacinum, C. cordatum* and *Polygonatum lasiantum* Maxim. with the fungus on *C. dolichostachya* subsp. *multifolia* from Takefu in 1979; *D. sessile* and *D. smilacinum* with the fungus on *C. dolichostachya* subsp. *multifolia* from Mt. Gozenyama in 1980; *D. sessile, D. smilacinum* and *C. cordatum* with the fungus on *C. conica* in 1979; *D. smilacinum, C. cordatum, Majanthemum dilatatum* Nels. & Macbr., *Tricyrtis macropoda* Miq., *A. ageratoides* subsp. *ovatus* and *Circaea mollis* Sieb. & Zucc. with the fungus on *C. dolichostachya* subsp. *multifolia* in 1999; and *D. sessile* with the fungus on *C. rugata* in 2000. The inoculated plants were chosen because

they were often found infected with an *Aecidium* and located near the *Puccinia*-infected *Carex* species, except for *M. dilatatum* and *T. macropoda*, which were not aecidium-bearing or located near the rusted sedges, but which were shown by previous records to be possible aecial hosts of the *Puccinia* fungus. The inoculated plants were sprayed with distilled water and placed in a moist chamber at room temperature (18–22°C) for 48 h, then transferred to a glasshouse for further observation.

Aeciospore inoculation Aeciospores formed a plant inoculated with basidiospores of the fungus on *C. conica* or *C. dolichostachya* subsp. *multifolia* were inoculated onto apparently healthy leaves of *C. conica*, *C. dolichostachya* subsp. *multifolia* and *C. pisiformis* subsp. *alterniflora*. Aeciospores were scraped from the sori and dusted on small pieces (ca. 3×3 mm) of water-saturated filter paper, which were then placed on the abaxial surfaces of leaves. The inoculated plants were sprayed with distilled water and placed in a moist chamber at room temperature (18–22°C) for 48 h, then transferred to a glasshouse for further observation.

Microscopic observation To examine morphology and structure of spermogonia and aecia, fresh infected materials and dried herbarium specimens were freehand-sectioned under a binocular dissecting microscope. Thin sections were mounted in a drop of lactophenol solution without staining. To examine morphology and measure size, the spores were scraped from sori on herbarium specimens and mounted by the same method as described above.

To observe germ pores in urediniospores, the spores were placed in a drop of lactic acid on a microscopic slide, heated to boiling for a few seconds and mounted with an additional drop of lactophenol solution with aniline blue. The spores on the slide were smashed by applying gentle pressure over a cover slip on the preparation.

For scanning electron microscopy (SEM), rust-infected leaves from dried herbarium specimens were cut into ca. 3×3 mm pieces containing a few sori, and each piece was placed on double-adhesive tape on a specimen holder. The preparations were coated with platinum-palladium using a Hitachi E-1030 Ion Sputter and examined with a Hitachi S-4200 SEM at 15kV.

Results and Discussion

Life cycle Basidiospores were abundantly formed under the conditions described above; however, the time lag before initiation of basidiospore production varied from a few hours to a few days depending on the conditions of teliospores used for inoculation experiments and the year of collection. The basidiospores of the fungus on *C. pisiformis* subsp. *alterniflora* infected *D. smilacinum*, resulting in spermogonium production 7–10 d after the inoculation and aecium production 5–7 d later. No sign of infection was detected on *C. cordatum*, *A. scaber* and *A. ageratoides* subsp. *ovatus*. Similarly, basidiospores of the fungus on *C. conica* were successfully inoculated only on *D. smilacinum*, with spermogonium and aecium production. No sign of infection was observed on *D. sessile* and *C. cordatum*. Basidiospores of the fungus on *C. rugata* also infected only *D. smilacinum*, resulting in spermogonium and aecium production. *Cardiocrinum cordatum*, *M. dilatatum*, *T. macropoda*, *A. ageratoides* subsp. *ovatus* and *C. mollis* were not infected.

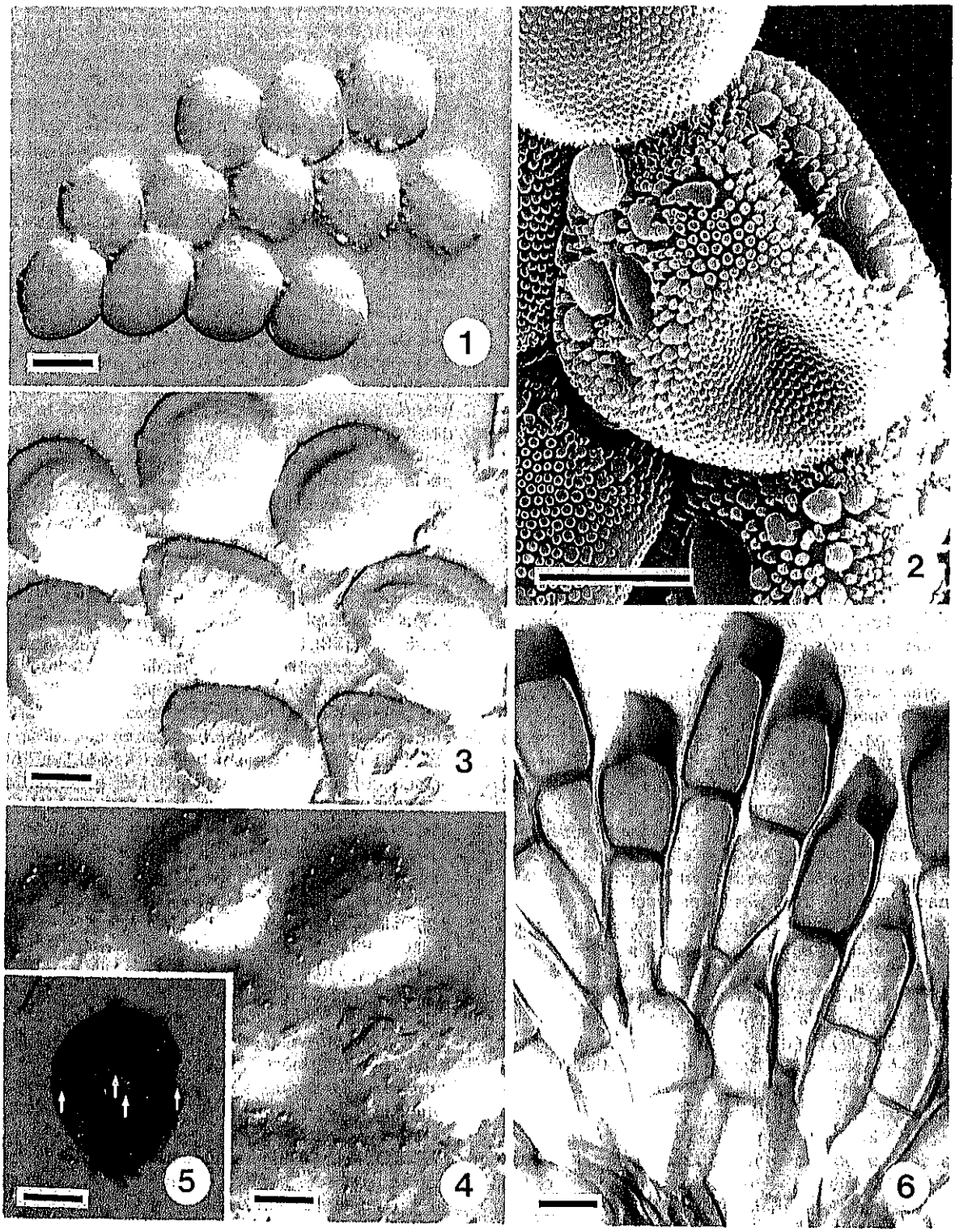
On the other hand, basidiospores of the fungus on *C. dolichostachya* subsp. *multifolia* from Takefu were successfully inoculated both on *D. smilacinum* and on *D. sessile* resulting in spermogonium and aecium production. No infection took place on *C. cordatum* and *P. laslanthum*. Basidiospores of the fungus on *C. dolichostachya* subsp. *multifolia* from Mt. Gozenyama infected only *D. sessile*, resulting in spermogonium and aecium production. No sign of infection was detected on *D. smilacinum*.

Aeciospores formed by the basidiospore inoculation of the fungus on *C. dolichostachya* subsp. *multifolia* from Takefu were successfully inoculated only on the same host species, resulting in uredinium production 10–14 d after the inoculation. No infection occurred on *C. conica* and *C. pisiformis* subsp. *alterniflora*. Similarly, aeciospores formed by the basidiospore inoculation of the fungus on *C. conica* successfully infected only the same host species, resulting in uredinium production. Naturally formed aeciospores on *D. smilacinum* collected at Okami was also successfully inoculated on *C. rugata*, resulting in uredinium and telium production.

The successful basidiospore inoculations proved the life-cycle connection between the *Aecidium* fungus on *D. smilacinum* and the *Puccinia* fungus on *C. conica*, *C. dolichostachya* subsp. *multifolia*, *C. pisiformis* subsp. *alterniflora* and *C. rugata* and between the *Aecidium* fungus on *D. sessile* and the *Puccinia* fungus on *C. dolichostachya* subsp. *multifolia*.

Both basidiospore and aeciospore inoculations indicated some degree of host specificity among the fungal populations studied. Because the inoculation experiment was not comprehensive, however, the degree of host specificity of both the spermogonial-aecial hosts and the uredinial-telial hosts and its taxonomic implications were not determined. Nevertheless, the fact that the fungus on *C. dolichostachya* subsp. *multifolia* from Takefu infected both *D. smilacinum* and *D. sessile* indicates that the *Aecidium* on both *D. smilacinum* and *D. sessile* is an aecial anamorph of the *Puccinia* on the four *Carex* species.

Morphology No significant difference was observed in aecia and aeciospores formed, either naturally or by the basidiospore inoculation, on *D. smilacinum* and *D. sessile*. Spermogonia occurred on the adaxial leaf surface or on petiole and were subepidermal, subglobose or ovoid, 110–150 μm high and 90–140 μm wide. Aecia were formed mostly on abaxial leaf surfaces or on petioles and were subepidermal, cupulate with a well-developed peridium reflexing upon maturity. Aeciospores were catenulate, subglobose or broadly ellipsoid, often angular, 16–21×15–18 μm (Fig. 1). The aeciospore wall was ca. 1 μm thick, colorless and verrucose with refractive granules at the upper side (type 5 of Savile



Figs. 1–6. *Puccinia albispora*. 1. Aeciospores (IBA-8521). Bar=10 μm. 2. Aeciospores surface structure, SEM (IBA-8521). Bar=5 μm. 3. Urediniospores focused on a median plane (Holotype, IBA-8492). Bar=10 μm. 4. Urediniospores focused on a tangential plane (Holotype, IBA-8492). Bar=10 μm. 5. Urediniospore germ pores (arrows) (Holotype, IBA-8492). Bar=10 μm. 6. Tellospores (Holotype, IBA-8492). Bar=10 μm.

1973; Fig. 2).

The morphology of the *Aecidium* fungus on the *Disporum* plants agrees with that of *A. dispori* Dietel originally described for a fungus on *D. sessile* from Nagano

Pref. (Dietel, 1899). Although Dietel (1899) did not mention refractive granules on the wall, other similar morphological characteristics and the host relationship are sufficient to conclude the taxonomic identity of the *Aecidium* fungus with *A. dispori*.

Uredinial and telial characteristics of the fungi on the four *Carex* species were also identical. Uredinia occurred on the abaxial leaf surface and were small, subepidermal, long-covered by the epidermis, rupturing at maturity and pale brownish or whitish. Urediniospores were subglobose, broadly obovoid or ellipsoid and 21–34 × 20–31 μm (Fig. 3). The urediniospore wall was evenly (2.5–) 3.0–5.0(–6.0) μm thick, pale yellowish or almost colorless and completely echinulate (Fig. 4). Four or five germ pores were distributed in the equatorial zone (Fig. 5). Telia occurred also on abaxial leaf surface and were subepidermal, soon rupturing at maturity, pulvinate and black. Teliospores were clavate or oblong-ellipsoid, rounded, truncate or conical at the apex, not to weakly constricted at the septum and 32–55 × 12–23 μm (Fig. 6). The teliospore wall was brown, smooth and 7–15 μm thick at the apex. The pedicel was persistent and 25–50 μm long.

Uredinial characteristics of the *Disporum-Carex* alternating fungus under discussion are similar to *P. breviculmis* Dietel (Dietel, 1907), *Uredo breviculmis* Hennings (Hennings, 1901), *P. iriensis* Y. Morimoto (Morimoto, 1962) and *P. caricis-conicae* Homma (Ito and Homma, 1938). The number and distribution of urediniospore germ pores were not mentioned in the original description of the three species (Morimoto, 1962; Hennings, 1901; Ito and Homma, 1938). Therefore, holo- and/or isotype specimens of *U. breviculmis* (Herb. SAPA), *P. iriensis* (Herb. TSH) and *P. caricis-conicae* (Herb. S & SAPA) were examined for the comparison.

Although the urediniospores of *U. breviculmis* were described as subglobose, ovoid or ellipsoid and 22–32 × 20–25 μm, with a thick, colorless wall (Hennings, 1901), our measurement of the spores was much larger (25–36 × 21–34 μm) than that of the original description and significantly different from that of the fungus under discussion. The wall was colorless and (2.5–)3.0–6.0 μm thick. Four or five (infrequently 3) germ pores were distributed on an equatorial zone. In the original description, Dietel (1907) cited *U. breviculmis* as the uredinial state of *P. breviculmis* without mentioning the uredinial morphology.

In contrast, the urediniospore size of *P. iriensis* (24.2–29.6 × 20.1–25.5 μm; 20–34 × 17–19 μm in the original description, Morimoto 1962) was similar to those of *P. breviculmis* and the fungus under discussion, but the wall thickness was thinner [2.5–5 μm; 2–3.5(–5) μm in the original description]. The germ pore seemed not to be differentiated in *P. iriensis*.

In addition to the difference in the urediniospore morphology, *P. iriensis* was proven to host-alternate on *Smilax china* L. (Morimoto, 1962). No cross-inoculation was undertaken between *P. iriensis* and the fungus under discussion. However, a distant taxonomic relationship between *Smilax* (Smilacaceae in Dioscoreales) and *Disporum* (Uvulariaceae in Liliales) (Dahlgren et al., 1985) suggests that the two fungi do not share both plant species as common spermogonial-aecial hosts and, thus, the two fungi are presumed to be reproductively isolated.

According to Ito and Homma (1938), *P. caricis-conicae* forms globose or ovate urediniospores of 31–39 × 23–37 μm with thick (4–5 μm), colorless and echinulate urediniospores and clavate teliospores of 52–84 × 17–28 μm (Ito and Homma, 1938). The number and distribution of urediniospore germ pores was stated to be obscure in the original description. Examination of the holotype (Herb. S) and isotype (Herb. SAPA) revealed that the urediniospores were subglobose, broadly obovoid or broadly ellipsoid and 38–45 × 31–39 μm in size. The wall was 3.4–4.8(–5.7) μm thick, colorless and echinulate. Five or six germ pores were distributed in the equatorial zone. Thus, the fungus under discussion is apparently different from *P. caricis-conicae* in the uredinial morphology.

Because the fungus under discussion possesses morphological features and life cycle distinct from those of any previously described species, the fungus is concluded to be a new species and the following name is proposed:

Puccinia albispora Ono & Kakishima, sp. nov. Figs. 1–6
Aecial anamorph: *Aecidium dispori* Dietel, Bot. Jahrb. 27: 671. 1899.
Uredinia hypophylla, subepidermalia, diu epidermide tecta, pulverulenta, pallide brunnea vel alba. Urediniosporae subglobosae, late obovoideae vel late ellipsoideae, 21–34 × 20–31 μm, episporio aequaliter (2.5–) 3.0–5.0(–6.0) μm crasso pallide flavido vel hyalino echinulato, poris germinationis 4–5 aequaliterialibus. Telia subepidermalia, mox nuda, pulvinata, atra. Teliosporae clavatae, ad apicem roundatae, truncatae vel conicae, basin versus attenuatae, non vel leniter constrictae ad septum, 32–55 × 12–23 μm, episporio brunneo laevi ad apicem 7–15 μm crasso, pedicello persistenti 25–50 μm longo.
HOLOTYPE: On *Carex rugata* Ohwi, JAPAN: Ibaraki, Mito (obtained from aeciospore inoculation), 22 Nov. 1999, *Y. Ono 4533* (IBA-8492).
Spermogonia epiphyllous or petiolicolous, subepidermal, subglobose or ovoid, 110–150 μm high and 90–140 μm wide. Aecia mostly hypophyllous or petiolicolous, subepidermal, cupulate with a well-developed peridium reflexing upon maturity. Aeciospores catenulate, subglobose or broadly ellipsoid, often angular, 16–25 × 15–21 μm in size. The aeciospore wall ca. 1 μm thick, colorless and verrucose with refractive granules at the upper side. Uredinia hypophyllous, small, subepidermal, long-covered by the epidermis, rupturing at maturity and pale brownish or whitish. Urediniospores subglobose, broadly obovoid or ellipsoid, 21–34 × 20–31 μm in size. The wall evenly (2.5–)3.0–5.0(–6.0) μm thick, pale yellowish or colorless and completely echinulate. Germ pores 4–5, equatorial. Telia hypophyllous, subepidermal, soon rupturing at maturity, pulvinate and black. Teliospores clavate or oblong-ellipsoid, rounded, truncate or conical

at the apex, attenuate toward the base, not to weakly constricted at the septum and 32–55 × 12–23 μm in size. The wall brown, smooth and 7–15 μm thick at the apex. The pedicel persistent and 25–50 μm long.

Host and distribution: On *Disporum sessile, D. smilacinum, Carex conica, C. dolichostachya* subsp. *multifolia, Carex pisiformis* subsp. *alterniflora* and *C. rugata*—widely distributed in Japan and perhaps also in China and Taiwan

Specimens examined: On *Disporum sessile*, **JAPAN: Gumma**, Tone-gun, Niiharu-mura, Mikuni-toge, 18 Jul. 1973, *Y. Ono* (*Y. O.*) *67* (IBA-1608); **Ibaraki**, Kuji-gun, Sulfu-mura, Takefu, 16 Aug. 1978, *Makoto Kakishima* (*M. K.*) (TSH-R1733); Mito, Watari, 10 May 1980, *Y. O. 363* (IBA-1899); Tsukuba (obtained from basidiospore inoculation), 14 May 1979, *M. K.* (TSH-R1723); 7 May 1979, *M. K.* (TSH-R1724); 23 May 1979, *M. K.* (TSH-R1726); 14 May 1980, *M. K.* (TSH-R1727); 28 May 1980, *M. K.* (TSH-R1729); 1 Jun. 1979, *M. K.* (TSH-R1730). On *D. smilacinum*, **JAPAN: Hokkaido**, Nemuro, Mt. Rausudake, 6 Jul. 1998, *Y. O. 4181* (IBA-8124); **Ibaraki**, Higashiibaraki-gun, Katsura-mura, Mt. Gozenyama, 10 Jul. 1980. *Y. O. 423* (IBA-1960); 3 Jul. 1999, *Y. O. & Kaori Ishimiya* (*K. I.*) *4366* (IBA-8316); Kuji-gun, Daigo-machi, Mt. Nantaisan, 14 Jun. 1999, *Y. O. & K. I. 4353* (IBA-8306); 9 May 1998, *Y. O. & K. I. 4110* (IBA-8053); 27 May 1990, *Y. O. 2085* (IBA-4777); 27 May 1990, *Y. O. 2088* (IBA-4780); 31 May 1994, *Y. O. 3001* (IBA-7168); 27 May 1990, *Y. O. 2096* (IBA-4788); 31 May 1989, *Y. O. 1695* (IBA-3242); 31 May 1989, *Y. O. 1697* (IBA-3244); 31 May 1989, *Y. O. 1702* (IBA-3249); Kuji-gun, Satomi-mura, Nanatan, 8 June 1990, *Y. O. 2112* (IBA-4804); Okami, 14 Jun. 1999, *Y. O. 4349* (IBA-8299); Kuji-gun, Sulfu-mura, between Okubo and Kamegafuchi, 9 Jun. 1991, *Y. O. et al. 2367* (IBA-5723); Mito, Watari, 4 Jun. 1980, *Y. O. 380* (IBA-1918); 9 Apr. 1999, *Y. O. & K. I. 4298b* (IBA-8248); Mito (obtained from basidiospore inoculation), 18 Jun. 1999, *Y. O. & K. I. 4357* (IBA-8307); 18 Jun. 1999, *Y. O. & K. I. 4358* (IBA-8308); 2 May 1999, *Y. O. & K. I. 4350* (IBA-8300); 25 May 2000, *Y. O. & K. I. 4572* (IBA-8520); 31 May 2000. *Y. O. & K. I. 4573a* (IBA-8521); 31 May 2000. *Y. O. & K. I. 4573b* (IBA-8522); Mito, Narusawa, Mito Forest Park, 28 Jun. 1980, *Y. O. 407* (IBA-1945); Tsukuba (obtained from basidiospore inoculation), 25 May 1977, *M. K.* (TSH-R1712); 2 June 1977, *M. K.* (TSH-R1713); 17 Jun. 1977, *M. K.* (TSH-R1714); 14 Jun. 1977, *M. K.* (TSH-R1715); 27 May 1979, *M. K.* (TSH-R1717); 31 May 1979, *M. K.* (TSH-R1718); 17 May 1979, *M. K.* (TSH-R1719); 5 Jun. 1979. *M. K.* (TSH-R1721); Tsukuba-gun, Tsukuba-machi, Mt. Tsukubasan, 17 Jun. 1978, *M. K.* (TSH-R1732); **Nagano**, Minamisaku-gun, Minamimaki-mura, Nobeyama, 25 Jul. 1978, *M. K.* (TSH-R1731); **Tochigi**, Nikko, between Kotoku and Karenuma, alt. 1650 m, 18 Jun. 1990. *Y. O. 2126* (IBA-4818). On *Carex conica*, **Ibaraki**, Tsukuba-gun, Tsukuba-machi, Mt. Tsukubasan, 6 Mar. 1979, *M. K.* (TSH-R1716); 12 Mar. 1980, *M. K.* (TSH-R1720). On *C. dolichostachya* subsp. *multifolia*, **Japan: Ibaraki**, Kuji-gun, Sulfu-mura, Takefu, 23 Mar. 1979, *M. K.* (TSH-R1732); Satomi-mura, Tokuda, 2 Mar. 1999, *Y. O. & K. I. 4266* (IBA-8217); Tsukuba (obtained from aeciospore inoculation), 17 Aug. 1979, *M. K.* (TSH-R1728); Kuji-gun, Katsura-mura, Mt. Gozenyama, 14 Mar. 1979, *M. K.* (TSH-R1725). On *C. pisiformis* subsp. *alterniflora*, **JAPAN: Tokyo**, Hachioji, Asakawa, 24 April 1977, *M. K.* (TSH-R1711). On *C. rugata* Ohwi, **JAPAN: Ibaraki**, Kuji-gun, Satomi-mura, Okami, 11 Sep. 1999, *Y. O. & K. I. 4381* (IBA-8331; 15 Feb. 2000, *Y. O. & K. I. 4535* (IBA-8538); 15 Feb. 2000, *Y. O. & K. I. 4536* (IBA-8539); 2 Mar. 1999, *Y. O. & K. I. 4270* (IBA-8221); 2 March 1999, *Y. O. & K. I. 4271* (IBA-8222); 9 Aug. 1999, *Y. O. 4369* (IBA-8319); Mito (obtained from aeciospore inoculation), 22 Nov. 1999, *Y. O. 4533* (IBA-8492); 30 Nov. 1999, *Y. O. 4534* (IBA-8493).

Additional specimens examined: *Puccinia breviculmis* Dietel on *Carex breviculmis* R. Br., **JAPAN: Kochi**, Sakawa, Jun. 1901, *T. Yoshinaga* (Isotype of *Uredo breviculmis* Hennings in Herb. SAPA). *Puccinia caricis-conicae* Homma on *C. conica*, **JAPAN: Hokkaido**, Makomanai, 16 Oct. 1910, *M. Miura* (Holotype in Herb. S and Isotype in Herb. SAPA). *Puccinia iriensis* Y. Morimoto on *C. maximowzii* Miq., **JAPAN: Hiroshima**, Kaiji, 23 Sept. 1960, *Y. Morimoto* (Isotype in Herb. TSH).

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Literature cited

Dietel, P. 1899. Uredineae japonicae I. Bot. Jahrb. **27**: 564–576 & 2 pl.

Dietel, P. 1907. Uredineen aus Japan. Ann. Mycol. **5**: 70–77.

Hennings, P. 1901. Einige neue japanische Uredineen II. Hedwigia Beibl. **40**: 124–125.

Hiratsuka, N. and Hashioka, Y. 1937. Uredinales collected from Formosa. VI. Bot. Mag. (Tokyo) **51**: 41–47.

Hiratsuka, N., Sato, S., Katsuya, K., Kakishima, M., Hiratsuka, Y., Kaneko, S., Ono, Y., Sato, T., Harada, Y., Hiratsuka, T. and Nakayama, K. 1992. The rust flora of Japan. Tsukuba-shuppankai, Tsukuba, Japan.

Ito, S. and Homma, Y. 1938. Notae mycologicae Asiae orientalis III. Trans. Sapporo Nat. Hist. Soc. **15**: 113–128.

Morimoto, Y. 1962. Notes of rust fungi of Japan (4). J. Japanese Bot. **37**: 1–2.

Ono, Y. 1995. *Aecidium alangii* is the aecial state of *Puccinia miyakei* (Uredinales). Nova Hedwig. **60**: 157–164.

Ono, Y. and Azubukina, Z. M. 1997. Heteroecious life cycle of two graminicolous *Puccinia* (Uredinales). Mycoscience **38**: 281–286.

Savile, D. B. O. 1973. Aeciospore types in *Puccinia* and *Uromyces* attacking Cyperaceae, Juncaceae and Poaceae. Rep. Tottori Mycol. Inst. (Japan) **10**: 225–241.

Sydow, H. and Petrak, F. (1931) Micromycetes philippinenses. Series secunda. Ann. Mycol. **29**: 145–279.

Zhuang, J.-y., Wei, S.-x. and Wang, Y.-c. (1998) Flora fungorum sinicorum. Vol. 10. Uredinales (I). Science Press, Beijing, China. (In Chinese.)

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Taxonomy of *Puccinia* species causing rust diseases on sugarcane*

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A taxonomic revision of *Puccinia* species causing rust diseases on sugarcane was conducted to clarify their morphological characteristics. Specimens including previously reported species, *Puccinia melanocephala*, *P. kuehnii* and *Puccinia* sp. *sensu* Muta, 1987, were collected in Japan and the Philippines and borrowed from various herbaria worldwide. Morphological characteristics of these specimens were examined under light and scanning electron microscopes. Comparative morphological studies of the specimens showed that rust fungi infecting sugarcane could be classified into two species, *Puccinia melanocephala* and *P. kuehnii*. *Puccinia* sp. *sensu* Muta was morphologically identical with *P. kuehnii*. Results of this study corroborate previous phylogenetic analysis results of D1/D2 regions of LSU rDNA gene.

Key Words——*Puccinia kuehnii*; *Puccinia melanocephala*; sugarcane rusts; taxonomy; Uredinales.

Puccinia melanocephala Syd. et P. Syd. and *Puccinia kuehnii* Butler are reported to cause rust diseases on sugarcane (Cummins and Hiratsuka, 1983; Hiratsuka and Kaneko, 1983; Sivanesan and Waller, 1986; Ryan and Egan, 1989). These two species have been reported in various parts of the world where sugarcane is cultivated. In addition, Muta (1987) reported *Puccinia* sp. as an unidentified rust pathogen on sugarcane in the Nansei Islands, Kagoshima Pref., Japan.

Puccinia kuehnii was first described as *Uromyces kuehnii* Krueger because of the presence of apically thick-walled urediniospores that were apparently mistaken as teliospores. It was later renamed as *Uredo kuehnii* (Kruegar) Wakker et Went, since the telial stage was not found and the apically-thick walled spores were proven to be urediniospores (Sydow et al., 1906a; Ito, 1909; Butler, 1914; Ryan and Egan, 1989). Butler (1914) found teliospores of this fungus on *Saccharum spontaneum* L. and named the species as *Puccinia kuehnii*. Most of subsequent descriptions of the telial stage of *P. kuehnii* were cited from his description because no teliospore was found in either sugarcane or the other grass hosts (Laundon and Waterson, 1964; Cummins, 1953, 1971; Sivanesan and Waller, 1986; Ryan and Egan, 1989). Although the telial stage of *P. kuehnii* was reported by Hiratsuka (1958), Teng and Ou (1937, cited by Tai, 1947), Tai (1947), Patel et al. (1950) and Chona and Munjal (1950), these descriptions were inconsistent with those of Butler (1914) and similar to descriptions of *P. miscanthi* Miura or *P. melanocephala*. In 1986, Hennen found teliospores on specimens collected in Taiwan. They were different from those described by Butler (1914) in color of telia and size of teliospores. However, he reported them as mature teliospores of *P. kuehnii* and suggested that Butler (1914) observed immature teliospores (Hennen, 1986). In 1987, Muta also found teliospores similar to those described by Hennen (1986) on sugarcane collected in the Nansei Islands and reported them as *Puccinia* sp., because these specimens were different from *P. kuehnii* in the absence of paraphyses in uredinia and telia.

There was also confusion in the naming of *P. melanocephala*, due to an apparent misidentification of the host from which the original description was made. Sydow et al. (1906b) were the first to name it as *P. melanocephala* on *Bambusa* sp., which was later found to be *Erianthus* sp. (Cummins, 1971; Sathe, 1971). When Padwick and Khan (1944) found a rust on *Erianthus rufipilis* (Steud.) Griseb. (=*E. fulvus* Nees ex Stend.), they gave it a different name, *P. erianthi* Padwick et Khan, which became the widely used name for the rust later found causing epidemics in commercial sugarcane. When Cummins (1971) and Sathe (1971) found that the rust described by Sydow et al. (1906b) actually occurs on an *Erianthus* sp., they proposed that it should be named *P. melanocephala*, since this name antedates the name *P. erianthi*, which becomes a nomenclatural duplication. However, misidentification of *P. melanocephala* still occurs in more recent literature such as that of Presley et al. (1978) and other reports cited by Egan (1980). Egan (1980) showed that reports of *P. kuehnii* in Africa and the Americas were in fact *P. melanocephala* based on previous records of sugarcane rusts and the non-suscep-

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tibility of the sugarcane varieties cultivated in these areas to *P. kuehnii*.

Although recent reviews of rust diseases including sugarcane rusts described the history, distribution, and characteristics of these rusts (Ryan and Egan, 1989; Purdy, 1985), there are still inconsistencies in descriptions of certain morphological characteristics and variations among authors. In view of the lack of comprehensive taxonomic treatment of rust pathogens on sugarcane and the recent report of an unidentified rust from Japan, a taxonomic revision of these sugarcane rust pathogens was considered necessary.

A previous study on the phylogenetic relationships of these sugarcane rusts revealed that sugarcane rusts can be clearly separated into two main phylogenetic groups based on D1/D2 regions of LSU rDNA, although there was considerable divergence in the ITS regions (Virtudazo et al., 2001).

This study was conducted to clarify the taxonomy of the *Puccinia* species causing rust diseases on sugarcane and the taxonomic status of *Puccinia* sp. *sensu* Muta (1987) reported in the Nansei Islands, Kagoshima Pref., Japan.

Materials and Methods

Collections were conducted in sugarcane fields and agricultural experiment stations in Amamioshima Is., Kagoshima Pref. in June, 1996, in Okinawa Is., Miyako Is., Ishigaki Is., and Iriomote Is., Okinawa Pref. in December, 1996, and in the provinces of Negros Occidental and Davao del Sur in the Philippines in August, 1996. These collections were dried and kept at the Mycological Herbarium, Institute of Agriculture and Forestry, University of Tsukuba (TSH). Dried herbarium specimens identified as *Puccinia melanocephala* and *P. kuehnii* were borrowed from the National Fungus Collections, United States Department of Agriculture, Beltsville, USA (BPI), the Arthur Herbarium, Purdue University, West Lafayette, USA (PUR), the Herbarium of the Plant Disease Division, Landcare Research, Auckland, New Zealand (PDD), the Rijksherbarium, Leiden, Netherlands (L), Institute of Botany, Jagiellonian University, Krakow, Poland (KRAM), the Mycological Herbarium, Swedish Museum of Natural History, Stockholm, Sweden (S), and from the collections of the Shikoku Agricultural Experiment Station, Japan. Specimens examined are listed in the description of the species.

Morphological characteristics were examined under light and scanning electron microscopes (SEM). Spore dimensions were measured using an Olympus Color Image Analyzer CIA 102. Urediniospores, teliospores, and cross-sections of uredinia and telia were mounted in SEM specimen holders using double adhesive tape and coated with platinum-palladium using a Hitachi Ion Sputter E-1030. Surface structures of uredinia, telia and spores were examined with a Hitachi Scanning Electron Microscope S4200 operating at 15.0 kV.

Results

Uredinia The lesions formed around the uredinia on the specimens examined could be classified into two major types. The first type is generally brown to dark brown, with dark necrotic areas around uredinia, sometimes coalescing to form large necrotic areas with many uredinia. The second type is generally lighter brown, sometimes yellowish to yellow-orange, and with some brown necrotic areas around uredinia.

However, the sugarcane rust specimens cannot be readily distinguished based only on observations of the symptoms in old herbarium specimens, in specimens collected from the field, and in those produced from inoculation experiments (data not shown). Most of the herbarium specimens labeled as *P. melanocephala* were found to have the first type of lesion, while most of the herbarium specimens labeled as *P. kuehnii* had the second type of lesion. The lesions in most of the *P. kuehnii* specimens, which were mostly from very old collections, ranged from pale yellow to yellow-orange. In certain specimens, reddish brown lesions were found and could not be distinguished from those found in most of the *P. melanocephala* specimens. Most of the specimens collected from sugarcane fields in Japan and the Philippines had the second type of lesion, similar to those of specimens labeled as *P. kuehnii*: they were yellow or reddish brown, and sometimes coalesced into large necrotic areas. The remaining specimens had the same type of lesions as herbarium specimens of *P. melanocephala*.

Uredinia observed in the specimens examined could also be grouped into two types based mainly on color and paraphyses. The first type generally were cinnamon-brown to dark brown, mainly hypophyllous and linear, and with abundant paraphyses, which were sometimes more numerous than urediniospores in the uredinia (Fig. 1B). The paraphyses were usually capitate, sometimes spathulate, colorless to golden brown, with walls in the head thicker than in the stipe (Fig. 1C). Uredinia found in most of the herbarium specimens of *P. melanocephala* and some of the specimens collected in Japan and the Philippines were of this type.

The second type of uredinia ranged from orange to yellowish brown, sometimes cinnamon-brown, and distinct paraphyses like those in the first type were absent. Extremely thin-walled, sometimes obovoid or small, but more often irregularly shaped and hyaline paraphyses-like structures were observed in the second type (Fig. 2C, D). Under the SEM, they seemed to occur underneath the urediniospores and could usually be observed only when the urediniospores were removed from the uredinia (Fig. 2B). When urediniospores were still present, they could be seen in the periphery of the uredinia in some specimens. *Puccinia kuehnii* specimens and most of the specimens collected in Japan and the Philippines had this type of uredinia. Specifically, specimens collected from the Nansei Islands, Kagoshima Pref., Japan, where *Puccinia* sp. *sensu* Muta (1987) was reported, could not be distinguished from other specimens having this type of uredinia in characteristics of

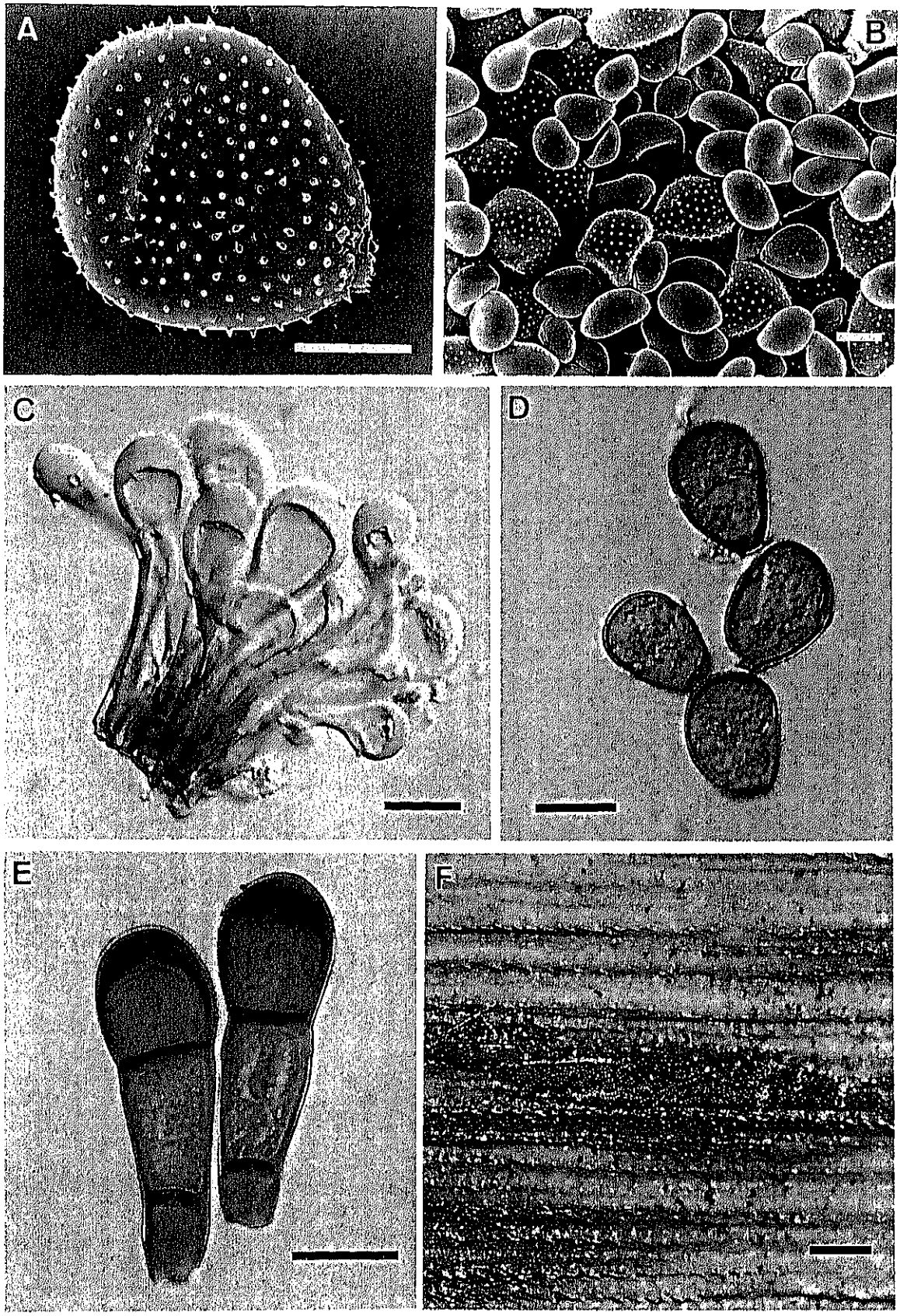

Fig. 1. *Puccinia melanocephala*. A. Urediniospore (SEM), B. Uredinium (SEM) showing abundant paraphyses among urediniospores, C. Paraphyses, D. Urediniospores, E. Teliospores, F. Telium. Scale bars: A, B=10 μm; C–E=20 μm; F=0.5 mm.

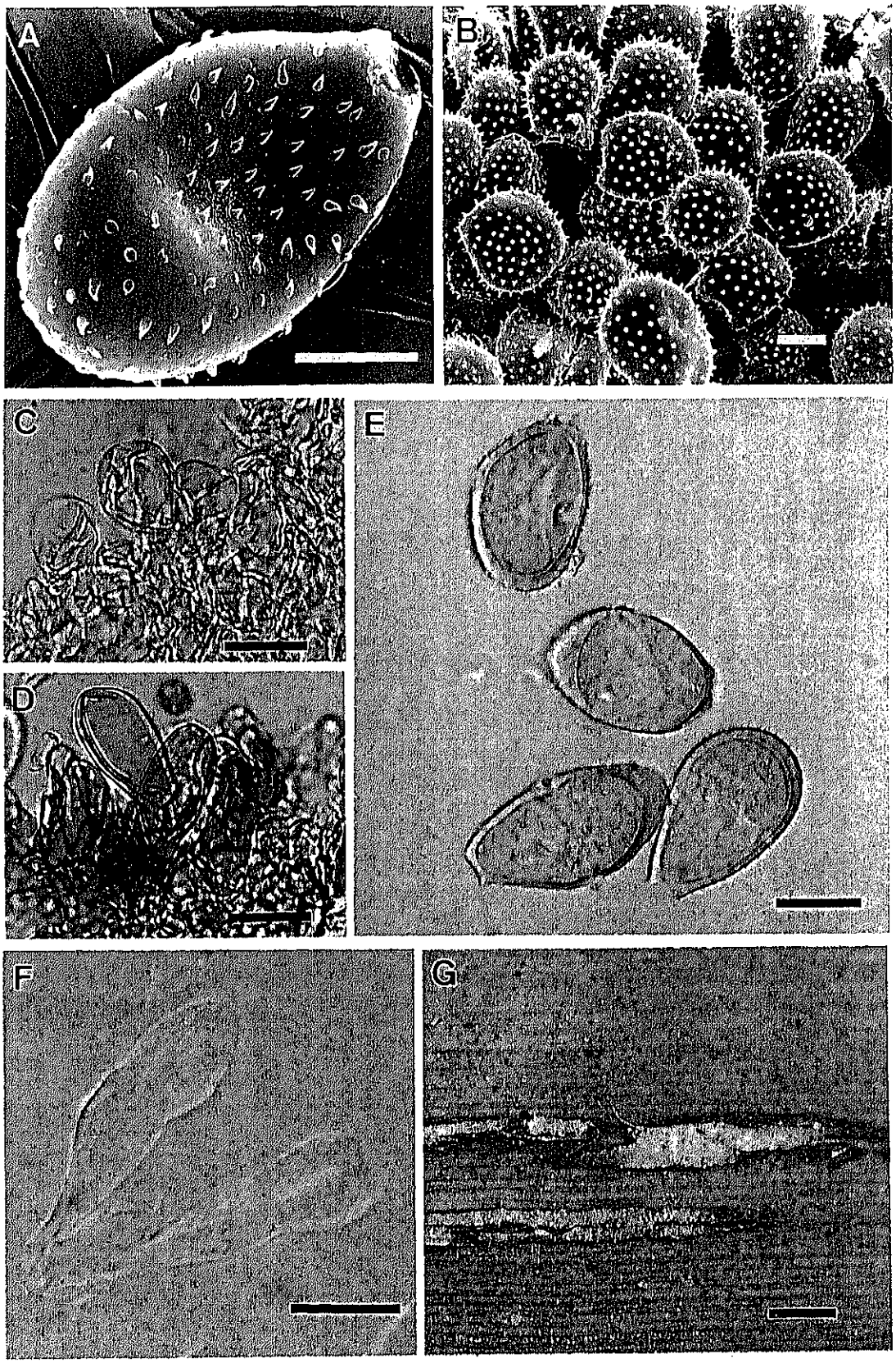

Fig. 2. *Puccinia kuehnii*. A. Urediniospore (SEM), B. Uredinium (SEM), paraphyses not seen, C–D. Paraphyses, E. Urediniospores, F. Teliospores, G. Telia. Scale bars: A, B=10 μm; C–F=20 μm; G=0.5 mm.

paraphyses. Furthermore, since the paraphyses were irregular in size and shape, their presence sometimes could not be clearly ascertained.

Urediniospores Based on color and wall thickness of urediniospores, the specimens could be separated into two types. Most of the herbarium specimens of *P. melanocephala* and some specimens collected in Japan and the Philippines had urediniospores that were mostly obovoid, sometimes ellipsoidal, with uniformly thick walls. The spores of this type were cinnamon-brown to dark brown (Fig. 1D). On the other hand, urediniospores of most herbarium specimens of *P. kuehnii* were mostly obovoid or pyriform, sometimes ellipsoidal. Some of their spores had slight to pronounced apical thickening around 5 μm or more, while others had uniformly thick walls. Urediniospores of this type ranged from golden yellow to orange, sometimes cinnamon-brown (Fig. 2E). Urediniospores in most of the specimens collected in Japan and the Philippines were similar to this type in shape, color and wall thickness.

SEM examinations showed that the urediniospore surface ornamentation in the specimens could be distinguished into two types. The first type was observed in urediniospores of most herbarium specimens of *P. melanocephala* and some specimens from Japan and the Philippines. In this type, the spore surface was densely echinulate and the spines were regularly spaced (Fig. 1A) except near the pores, where they tended to be more closely spaced. Spines at base of the spores in this type also tended to be bigger and more developed than spines in the other parts of the spore. The second type of urediniospore surface ornamentation was observed in most *P. kuehnii* specimens and most of the specimens collected in Japan and the Philippines. In this type, the echinulations were less dense than those found in urediniospores of specimens of the first type and were more or less evenly distributed over the spore surface (Fig. 2A). The spines of this type were also bigger, longer, more pointed and had a wider base than spines of the first type.

In urediniospore size, herbarium specimens of *P. melanocephala* tend to be in the smaller range, while those of *P. kuehnii* in the larger range. Among the specimens collected in Japan and the Philippines, those having characteristics similar to those of herbarium specimens of *P. melanocephala* were in the smaller range, while those having characteristics similar to herbarium specimens of *P. kuehnii* were distributed in the larger range (Fig. 3). However, these size ranges overlap and the specimens could not be separated based on urediniospore size.

Telia and teliospores In specimens producing the telial stage, two types of telia were observed. The first type was found in herbarium specimens of *P. melanocephala* and some specimens collected in Japan and the Philippines, whose uredinial stage was morphologically similar

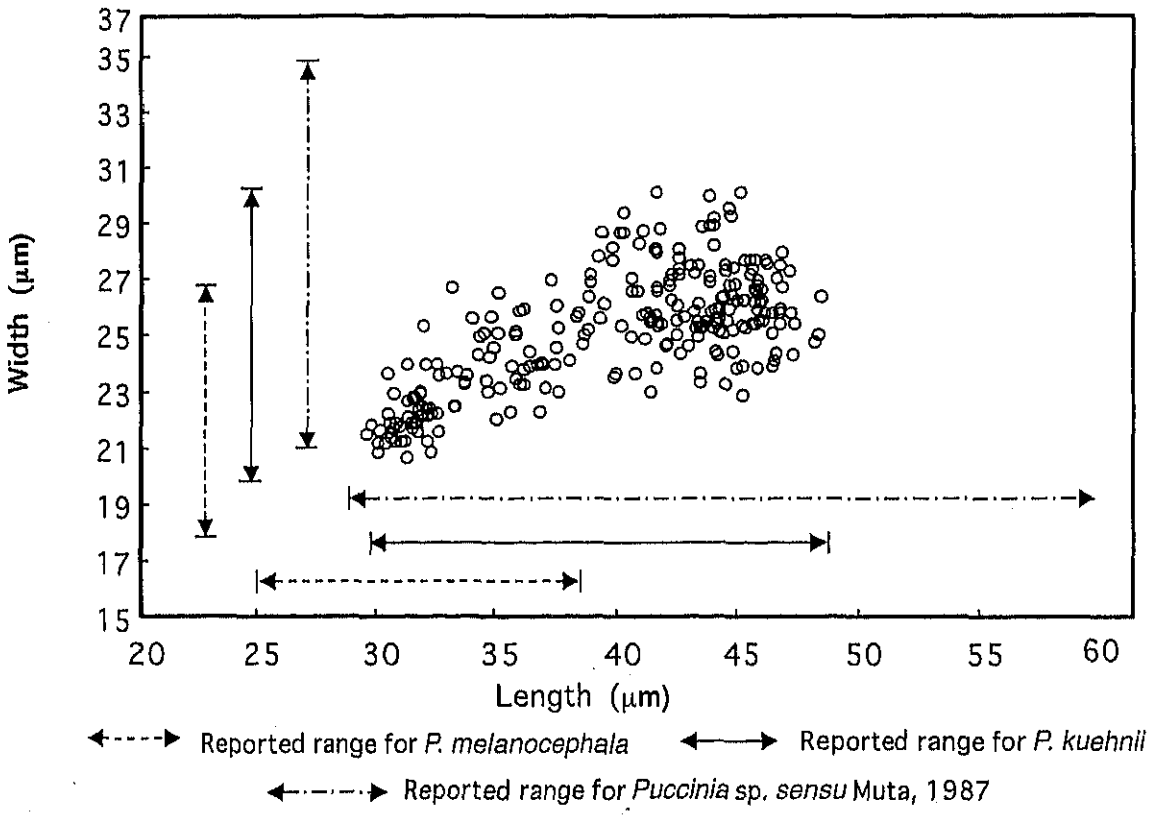

Fig. 3. Distribution plot of specimens of sugarcane rusts based on urediniospore size.

to *P. melanocephala* specimens. Telia in this type were blackish brown and occurred together with uredinia (Fig. 1F). The teliospores were brown to dark brown, mostly clavate, with apically thickened walls, and usually their upper cells were darker than the lower cells (Fig. 1E). Capitate and colorless to golden brown paraphyses were observed among teliospores of this type.

The second type of telia was found in a few herbarium specimens of *P. kuehnii* and some specimens collected in Japan and the Philippines (Fig. 2G). The telia of this type were whitish and fuzzy due to metabasidia formed *in situ*. The teliospores were hyaline, obclavate, with slight or no constriction at the septum, and walls uniformly thin (Fig. 2F). Three-celled teliospores were sometimes observed. Basidiospores were found in the metabasidial layer above the teliospores. These teliospores were also observed in the specimens used by Hennen (1986) to describe teliospores of *P. kuehnii* (PUR-89541 and PUR-89542). Except for these specimens, teliospores of this type were found in only one other herbarium specimen of *P. kuehnii* collected on *S. officinarum* L. in the Philippines in 1918 (BPI-79621).

Telia and teliospores of this type were also found in some specimens collected from Amamioshima Is. and the Okinawa Islands, Japan and the Philippines, whose uredinial stages were morphologically similar to those of herbarium specimens of *P. kuehnii*. These telial characteristics were also similar to those of *Puccinia* sp. *sensu* Muta (1987) reported in the Nansei Islands, Kagoshima Pref., Japan. Their teliospores are smaller than those of the first type (Fig. 2F).

Discussion

Comparative morphological examinations of uredinial and telial stage characteristics showed that the specimens could be classified into two morphologically distinct groups. The specimens examined could not be clearly distinguished based on color of lesions and uredinia, and the size of urediniospores. However, they were distinguishable based on the presence or absence of abundant capitate paraphyses in uredinia, echinulation, color and wall thickness of urediniospores, color of the telia and color and wall thickness of teliospores. One group of specimens, including most herbarium specimens of *P. melanocephala*, had abundant capitate paraphyses in uredinia and urediniospores with dense echinulation, darker brown and uniformly thick walls. They also had dark brown to blackish telia with brown to dark brown teliospores with apically thickened walls. The other group of specimens, including most herbarium specimens of *P. kuehnii* had morphologically indistinct paraphyses in uredinia and urediniospores with moderate echinulation, lighter brown and sometimes apically or uniformly thickened walls. They also had whitish telia and hyaline thin-walled teliospores exhibiting *in situ* germination. Specimens collected in Japan and the Philippines could also be separated into these two groups.

The presence or absence of conspicuous paraphyses in uredinia and telia was found to be an important characteristic for distinction of the two groups. In previous reports, paraphyses of *P. kuehnii* are variously and sometimes ambiguously described by different authors. Butler (1914) mentioned the occurrence of paraphyses which were described as "club-shaped or cylindrical and brownish found at the margin of the sori." On the other hand, Cummins (1953), Laundon and Waterson (1964), Sivanesan and Waller (1986), and Ryan and Egan (1989) mentioned "inconspicuous, peripheral, cylindrical or obovoid to capitate, hyaline or pale brownish." However, Ito (1909) and Hennen (1986) did not describe paraphyses in *P. kuehnii*. In this study, paraphysis-like structures observed in herbarium specimens of *P. kuehnii* and specimens collected in Japan and the Philippines were inconspicuous and of various irregular shapes and sizes. Furthermore, their occurrence was not consistent within a specimen. Therefore, we considered that paraphyses are unstable characteristics of this group. As such, we concluded that *Puccinia* sp. reported by Muta (1987) as a species producing no paraphyses is included in this group.

These two groups could also be clearly distinguished based on urediniospore surface characteristics. The first group had dense echinulations, while the second group had bigger, more widely spaced and evenly distributed spines. These results more or less coincide with previous reports of urediniospore surface differences between *P. melanocephala* and *P. kuehnii*. Mordue (1985) reported that *P. melanocephala* and *P. kuehnii* could be differentiated based on spine density and distribution, spines in the former being denser and more regularly spaced except near the pores, where they tend to cluster together. Hiratsuka and Kaneko (1983) reported that *P. melanocephala* spines at base of the spores are bigger and more developed than spines in the other parts of the spore. We also observed these spines in the first group of specimens.

Apical thickening of urediniospore walls was characteristic of the morphology in the second group of specimens. Urediniospores with uniformly thick walls and urediniospores with conspicuous apical thickening were often found together in the same specimen. However, urediniospores with apical thickening were not observed in specimens of the first group.

Telial characteristics also clearly support the separation of sugarcane rusts into the two groups. Previously reported characteristics of *P. melanocephala* coincide with those of the first group. On the other hand, telial stage characteristics of *P. kuehnii* reported by Hennen (1986) and of *Puccinia* sp. reported by Muta (1987) were found to be similar to those of specimens of the second group. It is believed that the teliospores observed by Butler (1914) were indeed immature, as he reported and as suggested by Hennen (1986). This accounts for their smaller size compared to those observed in this study and those reported by Hennen (1986) and Muta (1987). Furthermore, Butler (1914), who described teliospores from *S. spontaneum*, not from sugarcane, apparently did not observe germination of teliospores. His description of the telia as "blackish" is inconsistent with his description

of the teliospores as hyaline or pale yellow, and it is probable that he mistook black hyperparasitic infections as mature telia.

Separation of the sugarcane rusts into these two groups by morphological characteristics corroborates the results of molecular phylogenetic analysis of the D1/D2 regions of the LSU rDNA (Virtudazo et al., 2001). However, the analysis of ITS regions showed that specimens of one group could be further separated into two groups. Although morphological variation was observed among these specimens, it did not correlate with the divergence observed in the ITS regions. Hence, variation in the ITS regions, though considerable, is believed to reflect intraspecific polymorphism rather than inter-species variation.

Taxonomy

Results of comparative morphology showed that sugarcane rust specimens could be clearly distinguished into two morphologically and phylogenetically distinct groups. The characteristics of the uredinial and telial stages of these two groups correspond to previously reported taxonomic characteristics of *P. melanocephala* and *P. kuehnii*. Therefore, sugarcane rust fungi are classified into two species: *Puccinia melanocephala* and *P. kuehnii*.

Puccinia kuehnii Butler, Ann. Mycol. 12: 82, 1914.

Synonyms: *Uromyces kuehnii* Krueger, Ber. Versuchs Stat. f Zuckerrohr West-Java, Kogot-Tegal 1: 120, 1890.

Uredo kuehnii (Krueger) Wakker et Went, De Ziekten van het suekerviet Java, Lieden, p. 144, 1898.

(*Puccinia* sp. *sensu* Muta, Proc. Assoc. Pl. Prot. Kyushu 33: 36, 1987.)

Spermogonia and aecia unknown. Uredinia mainly hypophyllous, sometimes amphigenous, linear up to 3–4 mm, yellow-orange to reddish brown, develop subepidermally, erumpent; paraphyses inconspicuous, not always observed, if present basal and/or peripheral, irregular in shape, usually pyriform to clavate, extremely thin walled (>1 μm) and delicate, hyaline to pale brown. Urediniospores mostly obovoid or pyriform, sometimes broadly ellipsoidal, size highly variable, (26.4–) 33.3–52.2 (–67.7) × (16.0–)21.3–30.5(–39.2) μm, walls orange- to cinnamon-brown, 1–2.3 μm thick at the sides, sometimes uniformly thick but usually with pronounced apical wall thickenings up to 10 μm or more, with 4 or 5 equatorial germ pores. The wall moderately echinulate with evenly distributed spines. Telia hypophyllous, erumpent, arising from uredinia, translucent at first, turning whitish upon formation of metabasidia, paraphyses probably present but indistinguishable from immature teliospores. Teliospores sometimes sessile, or with hyaline pedicel, mostly ca. 12 μm long, sometimes more than one spore borne in one pedicel; fusiform to clavate, two- rarely three-celled, with slight or no constriction at the septa, (25.8–)31.4–54.8(–65.9) × (8.3–)10.7–16.6(–19.4) μm. The wall hyaline, smooth, and uniformly thin (0.5–1.2 μm). Teliospores germinate without dormancy, germ pores undifferentiated, but germination apical in both cells, basidiospores 7–10 × 5–7 μm.

Holotype: on *Saccharum spontaneum* L., E. J. Butler, Bassein, Myanmar (HCIO). (not seen)

Specimens examined: On *S. officinarum* L.: Malaysia –(PDD-60536); Philippines–Luzon Is. (BPI-79608, BPI-0079615, BPI-79617~22), Negros Is. (BP I-79616, 79623, 79625~7, 79629; TSH-R11201~16, 11229~35), Mindanao Is. (TSH-R11236~7, 11239~50, 11252~5, 11258~61); Indonesia–Java Is. (BPI-79614); Taiwan-(BPI-79610~1, 79630, 79634; PUR-89541~2); Hawaii-(BPI-79624); Australia-(BPI-79612); Micronesia-(PDD-50993); Western Samoa-(PDD-34296, 34297, 36600, 36403~5, 34298, 34173, 34031); Vanuatu-(PDD-43982, 49233, 46817); Cook Islands-(PDD-39571, 32989); Fiji-(PDD-36402); French Polynesia-(PDD-44462); Japan–Amamioshima Is. (TSH-R11001~2, 11004, 11010~3, 11015, 11024, 11026~9, 11032, 11034, 11301~4, 11306~8, 11335~7), Okinawa Is. (TSH-R11061~5, 11067, 11070, 11072~9, 11081, 11085, 11087~9, 11092, 11095, 11097, 11099, 11102-3, 113414), Iriomote Is. (TSH-R11105~6, 11110), Ishigaki Is. (TSH-R11113~4, 11121~2, 11125~6, 11131, 11133~4, 11323~5, 11327), Miyako Is. (TSH-R11137~9, 11144, 11146~7, 11149, 11152~3, 11157~8), Tanegashima, Is. (TSH-R11309, 11311~3, 11315~6, 11318~9, 11321, 11322). On *S. arundinaceum* Retz.: India-(PDD-14040, 9362; HCIO-75, 573, 1592; BPI-79606, PUR-F15855, F11422; L-955052-317; S-01, 573, 1592; Japan-Okinawa Is. (BPI-79607). On *S. spontaneum*: Indonesia–Java Is. (BPI-79613); India-(BPI-79635, S-2146, KRAM-2146); Hongkong-(PDD-57590); Solomon Islands-(PDD-38201). On *S. edule*: Fiji-(PDD-34271, 36401, 36599, 36406~7); Solomon Islands-(PDD-38367, 42120); Vanuatu–(PDD-45002). On *Saccharum* sp.: China-(BPI-79605, 199088), Taiwan-(PUR). On *Sclerostachya fusca* (Roxb.) A. Camus: India-(PUR-F8803).

Hosts and distribution: On *S. officinarum* L., Japan (Ito, 1909; Muta, 1987), Australia (Cobb, 1893, cited by Butler, 1914), Indonesia (Krueger, 1890, cited by Butler, 1914), Philippines (Lee, 1922; Ocfemia, 1939), Taiwan (Hsieh et al., 1977); Pacific Islands, Sri Lanka, Malaysia, Thailand, New Caledonia, China (Egan, 1980; Sivanesan and Waller, 1986), India (Mukerji and Bhasin, 1986); On *S. spontaneum* L., India (Sydow et al., 1906a), Burma (Butler, 1914); On *S. arundinaceum*, India (Sydow et al., 1906a; Butler, 1918); On *S. robustum* Brandes and Jesw. ex Grassl, *S. edule* Hassk., New Guinea (Koike et al., 1979); On *S. narenga* Wall., (Laundon and Waterson, 1964; Sivanesan and Waller, 1986; Mukerji and Bhasin, 1986); On *S. barberi* Jeswelt and *S. sinense* Roxb. (Ryan and Egan, 1989). On *Sclerostachya fusca* (Roxb.) A. Camus (=*Saccharum fuscum* Roxb.), India (Sydow et al., 1906a; Butler, 1918, Laundon and Waterson, 1964, Mukerji and Bhasin, 1986).

Puccinia melanocephala Sydow et P. Sydow, in (H.)Sydow, (P.)Sydow et Butler, Ann. Mycol. 5: 500,

1906.

Synonyms: *Puccinia eulaliae* Barclay, J. Asiatic Soc. Bengal 60: 216, 1891, *nomen dubium.*

Puccinia erianthi Padwick et Khan, Imp. Mycol. Inst. Kew, Mycol. Papers 10: 32–33, 1944.

Puccinia sacchari Patel, Kamat et Padhye, Curr. Sci. 19: 122, 1950, *nomen nudum.*

Spermogonia and aecia unknown. Uredinia mainly hypophyllous, sometimes amphigenous, linear up to 4 mm, cinnamon-brown to dark brown to blackish in some varieties, develop subepidermally, erumpent; paraphyses abundant, capitate or spathulate, colorless to golden brown, 32–98 μm long, with the head 12–25 μm in diam, the wall 1.0–2.8 μm thick in the stipe and 4–15 μm in the head. Urediniospores obovoid or ellipsoidal, cinnamon-brown to dark brown, (20.6–)25.8–38.7(–44.3) × (14.8–)17.8–27.5(–32.1) μm. The wall uniformly thick (0.8–2.3 μm), with usually 4, sometimes 5 equatorial germ pores, finely echinulate with regularly spaced spines, sometimes clustered at the pores and more developed at the spore base. Telia hypophyllous, black, erumpent, arising from uredinia, long capitate paraphyses present. Teliospores mostly clavate, two-celled with slight constriction at the septum, (31.3–)34.5–55.2(–61.0) × (14.8–)16.4–23.2(–25.0) μm. The wall smooth, 2–3.5 μm thick at sides, 2.5–8 μm apically, upper cells chestnut brown to dark brown with lower cells paler, the pedicels dark brown, 4.7–16.5 μm long.

Holotype: on *Erianthus* sp. (probably *E. ravennae* (L.) P. Beauv.) (originally identified as *Arundinaria* sp.), 5 May 1905, E. J. Butler, Nahjan, Khasi Hills, India (S).

Specimens examined: On *S. officinarum* L.: Jamaica-(BPI-33035, 188671, 188688); Dominican Republic-(BPI-188669, 188670); Mexico-(BPI-188643~51, 188653~6, 188658~62, 188664~5, 193824; DAOM-181745; L-983071-886); Costa Rica-(BPI-37734); Puerto Rico-(BPI-188685~6, 188688); Nicaragua-(BPI-188666~7); USA-Texas (BPI-188642, 188652, 188661, 188663, 188690~2, 188694; PUR-F11084); Ecuador-(BPI-189697); Australia-(BPI-113635~6, 113641); Japan-Amamioshima Is. (TSH-R11411~4, TSH-R11416~8); Philippines-Negros Is. (TSH-R11401, 11403, 11419). On *E. rufipilis* (Steud.) Griseb: India-(PUR-F14544, USNH-1607370), China-(PUR-F11750, 11753; ENH-1505263, 1722949). On *E. fulvus* Nees ex Stend: India-(PUR-F17947, F16074-type for *P. erianthi*). On *E. ravennae* (L.) Beauv. (originally identified as *Arundinaria* sp).: India-E. J. Butler No. 512 (S-holotype, designated by G. B. Cummins).

Hosts and distribution: On *S. officinarum* L., India (Patel, et al., 1950; Mukerji and Bhasin, 1986), Japan (Ohtsu, 1975 (cited by Muta, 1987)), Philippines (Serra et al., 1983), Australia (Egan and Ryan, 1979), Taiwan (Hsieh et al., 1977), Dominican Republic (Presley et al., 1978), Jamaica (Burgess, 1979; Kolke et al., 1979), Puerto Rico (Liu, 1979), Cuba (Sandoval et al., 1983), Carribean and Central America (Purdy et al., 1983), Hawaii (Comstock et al., 1982), Angola, Kenya, Malagasy R., Tanzania, Uganda, Zambia, Zimbabwe, South Africa, Mozambique, Malawi (Egan, 1980; Sivanesan and Waller, 1986); On *S. barberi* Jesweit and *S. sinense* Roxb., India (Srinivasan, 1966); On *S. spontaneum* L., India (Singh and Tiwari, 1964); On *S. robustum* Brandes and Jesw. ex Grassl., Puerto Rico (Chu et al., 1982). On *E. ravennae* (L.) P. Beauv., India (Sydow et al., 1906b; Sathe, 1971; Cummins, 1971); On *E. rufipilis* (Stued.) Griseb. (=*E. fulvus* Nees ex Stend.), India (Padwick and Khan, 1944; Cummins, 1953), China (Cummins, 1953).

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Literature cited

Burgess, R. A. 1979. An outbreak of sugarcane rust in Jamaica. Sugarcane Pathol. Newsl. 22: 4–5.

Butler, E. J. 1914. Notes on some rusts in India. Ann. Mycol. 12: 76–82.

Butler, E. J. 1918. Fungi and diseass in plants. Thacker, Spink, and Co., Calcutta and Simla.

Chona, B. L. and Munjal, R. L. 1950. *Puccinia kuehnii* (Krueg.) Butler on sugarcane in India. Curr. Sci. 5: 151–152.

Chu, T. L., Serapion, J. L. and Rodriguez, J. L. 1982. Varietal *reaction and inheritance trends of susceptibility of sugarcane to rust (Puccinia melanocephala* Syd. & H. Syd.) J. Agric. Univ. P. R. 66: 99–108.

Comstock, J. C., Tew, T. L. and Ferreira, S. A. 1982. Sugarcane rust in Hawaii. Plant Dis. 66: 1193–1194.

Cummins, G. B. 1953. The species of *Puccinia* parasitic on Andropogoneae. Uredineana, Encyc. Mycol. 24: 1–89.

Cummins, G. B. 1971. The rust fungi of cereals, grasses and bamboos. Springer-Verlag, New York.

Cummins, G. B. and Hiratsuka, Y. 1983. Illustrated genera of rust fungi. Revised ed. Amer. Phytopath. Soc., Minnesota.

Egan, B. T. 1980. A review of the world distribution of *Puccinia* spp. attacking sugarcane. Proc. Intl. Sugarcane Tech. 17: 1373–1381.

Egan, B. T. and Ryan, C. C. 1979. *Sugarcane rust, caused by Puccimia melanocephala*, found in Australia. Plant Dis. Rep. 63: 822–823.

Hennen, J. F. 1986. Teliospores of the sugar cane rust *Puccinia kuehnii* Butler on *Saccharum officinarum* L. in Taiwan. Acta Mycol. Sinica Suppl. 1: 149–152.

Hiratsuka, N. and Kaneko, S. 1983. A provisional list of *Puccinia* species on the grasses in Japan. Rept. Tottori Mycol. Inst. 21: 61–75.

Hiratsuka, T. 1958. The species of rust fungi parasitic on the grasses collected in the Southern Kyushu and the Ryukyu Islands, Japan. Sci. Bull. Agric, Home Econ. & Engg. Div. Univ. Ryukyus No. 5.

Hsieh, W. H., Lee, C. S. and Chan, S. L. 1977. Rust disease of sugarcane in Taiwan. Taiwan Sugar Res. Inst. Ann. Rep., pp. 38–39.

Ito, S. 1909. On the Uredineae parasitic on the Japanese Gramineae. J. Sapporo Agric. Coll. Japan III., pp. 244–245.

Koike, H., Flora, G., Pollack, G., Lacy, S. and Dean, J. L. 1979. Rust of sugarcane in the Caribbean. Plant Dis. Rep. 63: 253–255.

Laundon, G. F. and Waterston, J. M. 1964. *Puccinia kuehnii*. CMI Descriptions of Fungi and Bacteria No. 10.

Lee, H. A. 1922. Observations on previously unreported or noteworthy diseases in the Philippines. Phil. Agric. Rev. 14: 422–434.

Liu, L-J. 1979. Rust of sugarcane in Puerto Rico. Plant Dis. Rep. 63: 256–258.

Mordue, J. E. M. 1985. Urediniospore ornamentation in the sugarcane pathogens *Puccinia kuehnii* and *P. melanocephala*. Trans. Br. Mycol. Soc. 84: 758–760.

Mukerji, K. G. and Bhasin, J. 1986. Plant Diseases of India: A Source Book, pp. 218–219. Tata McGraw-Hill Publishing Company Ltd. New Delhi.

Muta, T. 1987. Occurrence of sugarcane rust (*Puccinia melanocephala* and *Puccinia* sp.) in Kagoshima Prefecture. Proc. Assoc. Pl. Prot. Kyushu 33: 36–38.

Ocfemia, G. O. 1939. A review of sugarcane diseases in the Philippines. Proc. 6th Cong. I.S.S.C.T., Baton Rouge, pp. 183–189.

Padwick, G. W. and Khan, A. 1944. Notes on Indian Fungi II. Mycol. Pap. 10: 10–11.

Patel, M. K., Kamat, M. N. and Padhye, Y. A. 1950. A new record of *Puccinia* on sugarcane in Bombay. Curr. Sci. 19: 121–122.

Presley, J. T., Perdomo, R. and Ayats, J. D. 1978. Sugarcane rust found in the Dominican Republic. Plant Dis. Rep. 62: 843.

Purdy, L. H. 1985. Sugarcane rusts. Chap. VIII. In The Cereal Rusts. v. II., (ed. by Roelfs, A. P. and Bushnell, W. R.), pp. 237–256. Academic Press, Inc., Orlando.

Purdy, L. H., Liu, L. J. and Dean, J. L. 1983. Sugarcane rust, a newly important disease. Plant Dis. 67: 1292–1296.

Ryan, C. C. and Egan, B. T. 1989. Rust, Chap. XIII. In Diseases of Sugarcane, Major diseases. (ed. by Ricand, C., Egan, B. T. Gillaspie, Jr., A. G. and Hughes, C. G.), pp. 189–210. Elsevier, Amsterdam.

Sandoval, I., Picornell, V., Chavez, R. and Ramos, M. 1983. *Puccinia melanocephala*, H. & P. Syd.: Biological and ecological aspects. Proc. Int. Soc. Sugar Cane Techonol. 18: 845–853.

Sathe, A. V. 1971. Nomenclatural revision of the common rust fungus affecting sugarcane. Curr. Sci. 2: 42–43.

Serra, R. J. and Barredo, F. C. and Tiangco, A. P. 1983. Incidence of sugarcane rust (*Puccinia melanocephala* H. et. P. Sydow) in the Victorias Milling Company District Occidental Negros, Philippines in 1982. Philipp. Phytopath. 19: 50–53.

Singh, K. and Tiwari, M. M. 1964. Sugarcane rust-collateral hosts and physiologic specialization. Indian J. Sugarcane Res. Dev. 8: 275–276.

Sivanesan, A. and Waller, J. M. 1986. Sugarcane diseases. Commonwealth Mycological Institute Phytopath. Paper No. 29.

Srinivasan, K. V. 1966. Studies of resistance to rust of sugarcane. Sugarcane Breed. Newsl. 18: 80–81.

Sydow, H., Sydow, P. and Butler, E. J. 1906a. Fungi Indiae orientalis I. Ann. Mycol. 4: 422–445.

Sydow, H., Sydow, P. and Butler, E. J. 1906b. Fungi Indiae orientalis II. Ann. Mycol. 4: 485–501.

Tai, F. L. 1947. Uredinales of Western China. Farlowia 3: 95–139.

Virtudazo, E. V., Nakamura, H. and Kakishima, M. 2001. Phylogenetic analysis of sugarcane rusts based on sequences of ITS, 5.8S rDNA and D1/D2 region of LSU rDNA. J. Gen. Plt. Path. 67: 28–36.

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Ribosomal DNA-ITS sequence polymorphism in the sugarcane rust, *Puccinia kuehnii**

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The two highly divergent ITS types previously observed in isolates of *Puccinia kuehnii* were amplified from the same isolates through the use of ITS type-specific primers for PCR and are therefore considered as polymorphisms of ITS regions within the species. Although homology of sequences of one of the ITS types with other *Puccinia* species was of expected levels, significantly high homology of sequences of the other type with those of *Cronartium* members indicates that abnormal genetic events led to the occurrence of these polymorphic regions. These results indicate that ITS gene tree phylogeny may not reflect true species phylogeny in this group of rust fungi. Rather, D1/D2 region tree phylogeny, which was concordant with the differences in morphology with and among related rusts, more correctly reflects phylogenetic relationships of sugarcane and related grass rusts.

Key Words——*Puccinia kuehnii*; ribosomal DNA ITS polymorphism; sugarcane rust.

The analysis of nucleotide sequences of the ribosomal RNA gene to determine phylogenetic relationships and genetic variations in fungi is widely established. The rDNA unit is organized into three genes coding for the ribosomal units and two internal transcribed spacer (ITS) regions (Hibbet, 1992). One of the reasons for the usefulness of the nuclear rDNA in phylogenetic analysis lies in its occurrence as tandem repeat arrays with several hundred copies per genome, allowing for easy accessibility and amplification by PCR (White et al., 1990; Bruns et al., 1991). Furthermore, rDNA genes, like other multigene families, are subject to concerted evolution, resulting in homogeneity among the different copies of the gene in the cell, and making them ideal phylogenetic markers (Hillis and Dixon, 1991; Li, 1997; Page and Holmes, 1998). However, despite the homogenizing effect of concerted evolution and although the gene cluster may be derived from a single ancestral unit, the different copies of the rDNA array within a single nucleus are all replicated independently, allowing, although rarely, independent mutation and crossover that can result in various levels of polymorphism in the gene cluster within individual cells (Fatehi and Bridge, 1998; Hillis and Dixon, 1991). These intraspecific or intragenomic variations are considered to occur at low rates, mostly as length variations in both non-transcribed and transcribed regions of the gene (Hillis and Dixon, 1991; Hillis and Davis, 1988). Heterogeneity resulting from sequence differences that do not lead to size variation will only be detected through sequencing and may affect RFLP patterns (Harlton et al., 1995; Fatehi and Bridge, 1998). Occurrence of multiple forms of ITS regions in a single individual has been reported in other organisms (Zijstra, et al, 1995; Baldwin et al., 1995; Vogler and DeSalle, 1994), and in fungi (Sanders et al., 1995; Harlton et al., 1995; O' Donnell and Cigelnick, 1997; Hijri et al., 1999; Antoniolli et al., 2000).

The authors have previously detected two highly divergent ITS sequence types in morphologically indistinguishable isolates of *P. kuehnii* Butler (Virtudazo et al., 2001a). However, despite the divergence in ITS sequences, all isolates were found to have identical sequences in the D1/D2 region of the nuclear LSU rDNA and hence belonged to a single cluster in the phylogenetic tree constructed from D1/D2 region sequences. Despite having identical D1/D2 sequences and morphological characters, the high divergence and the occurrence of two nucleotide differences in the conserved 5.8S rDNA region between the two ITS types may support separation into different populations within *Puccinia kuehnii*. However, the unusual homology of ITS sequences of *P. kuehnii* with *Cronartium* members shows a homoplastic pattern of evolution and indicates that the D1/D2 region tree, which clearly separated the morphologically distinguishable units, rather than the ITS region tree, reflects true species phylogeny in this group of rust fungi (Virtudazo et al., 2001a, b). Therefore, rather than showing support for interspecific divergence, the possibility that the two highly divergent sequences may have resulted from unusual genetic events leading to non-orthologous, possibly intragenomic polymorphisms in the species, seemed to be more likely and is further

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examined in this study.

Materials and Methods

Isolates used in this study are listed in Table 1. ITS type-specific primers were designed from previously determined sequences and are shown in Table 2. They were tested for percentage homology and maximum matching with the target and non-target ITS types using Genetyx-Mac v. 10.1 (Software Development Co.) and were found highly specific to the respective target sequence type.

PCR was conducted with DNA extracted from urediniospores by the same method used in the original amplification with ITS 1F-ITS 4 primer pair (Virtudazo et al., 2001a). Amplifications were done in 100 μl PCR reaction mixtures containing different combinations of 2 μM type-specific primer pairs, 2.5 units of TaKaRa Ex Taq DNA polymerase (Takara, Japan), and the supplied dNTP mixture (containing 2.5 mM dNTPs) and Ex Taq reaction buffer (containing 2 mM Mg^{2+}). PCR was carried out using an ATTO Zymoreactor II (Atto Co., Japan) under the following conditions: 95°C for 3 min, then 35 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min, and a final step of 72°C for 10 min. The type-specific primers were also tested further on purified PCR products (ITS 1F-ITS 4 primer products previously used as templates in sequencing reactions) of isolates of both ITS types. Although the band sizes of the type-specific PCR products in agarose gel electrophoresis were consistent with predicted sizes computed from the location of the primers, showing that the target ITS type sequences were amplified, we further verified this by sequencing the type-specific PCR reaction products. Furthermore, we also conducted sequencing reactions using the original PCR products (ITS 1F-ITS4) as templates and the type-specific primers as sequencing primers. Sequencing was done according to previously reported procedures (Virtudazo et al., 2001a).

Results and Discussion

Amplification from DNA extracted from urediniospores

Table 2. Primers designed for specific amplification of ITS types I and II.

Primer[a]	Primer sequence	*Tm*[b] (°C)
ITS type I		
PK 1-1F	GTGTGCCTTTTTTGGTATAGCATC	68
PK 1-1B	AATGGGGGTTAGGAAGCTATT	60
PK 1-2F	CCCTTTATAAGTGACCCCCTTT	64
PK 1-2B	AATAAAGAATTGGAATGAGAGGG	62
ITS type II		
PK 2-1F	AATATGGGGGAAACCTCATTA	58
PK 2-1B	GGCAGGTAACACCTTCCTTGATG	70
PK 2-2F	CCACATATATGAAAATGAATGTA	58
PK 2-2B	GTTAAGGGGGAGGAGGAACTTGA	70

[a] F primers are forward primers, B primers are backward primers. Sequences are written 5'-3'.
[b] *Tm*'s were calculated by the formula: $Tm = 2(A+T)+4(G+C)$.

Initial PCR tests showed that ITS type I-specific primers did not yield amplification products, while ITS type II-specific primers yielded amplification products in isolates of both ITS types (Table 3). However, upon lowering the annealing temperature of the PCR reactions to 50°C, amplifications were detected using ITS type I-specific primers for isolates of both types (Fig. 1).

Amplification from both isolates using specific primers for ITS types I and II demonstrated that both ITS types are present in the same isolate. However, ITS type II is apparently preferentially amplified. This could be due to difference in copy number, with the ITS type II representing the major ITS form and occurring in higher copy number than the ITS type I. This agrees with the observation that most isolates of *P. kuehnii* exhibited the ITS type II and only a small percentage yielded ITS type I. It could also be due to amplification bias due to PCR selection, wherein primer efficiencies vary between the type-specific primers designed in this study (Wagner et al., 1994). Further studies, such as hybridization experiments, may provide insight into copy number differences between these two types.

Amplification from original PCR products Tests con-

Table 1. Isolates of ITS groups I and II used in the study.

Isolate number	Locality	Date collected	Voucher specimen[a]	ITS Type (Virtudazo et al., 2001a)
PSPFS2-2	Naha, Japan	1997. 6.18	TSH-R11164	I
PSPFS3-2	Miyako, Japan	1997. 6.19	TSH-R11165	I
PSPFS20-2	Naha, Japan	1997. 6.18	TSH-R11170	I
PSP1211	Naha, Japan	1997. 6.18	TSH-R11175	I
PKUDS50-1	Australia	1935. 9.15	BPI-79612	I
PKUDS51-1	Hawaii	1916. 6.26	BPI-79624	I
PSPFS32-1	Miyako, Japan	1997. 6.19	TSH-R11174	II
PSPFS16-1	Naha, Japan	1997. 6.18	TSH-R11169	II
PSPFS19-1	Amamioshima, Japan	1996.10.29	TSH-R11042	II
PSP1163	Amamioshima, Japan	1996.10.29	TSH-R11043	II

[a] TSH-, Mycological Herbarium, University of Tsukuba, Japan; BPI-, USDA National Fungus Collections, USA.

Table 3. Results of PCR using ITS type-specific primers[a].

Primer pair	*Predicted* Prod. size (bp)	ITS type I isolates						ITS type II isolates		
		P. sp 20	P. sp 1211	P. sp FS2	P. sp FS3	P. ku DS1	P. ku DS2	P. sp 19	P. sp 1163	P. sp FS32
ITS type 1 primers										
PK1-1F–PK1-1B	484	—	—	—	—	—	—	—	—	—
PK1-1F–PK1-2B	297	—		—	—	—	—			
PK1-2F–PK1-2B	223	—	—	—	—	—	—	—	—	—
PK1-2F–PK1-1B	410	—		—	—	—	—			
ITS1F–PK1-1B	636	—	—		—			—	—	
ITS1F–PK1-2B	449	—	—		—			—	—	
PK1-1F–ITS4	551	—	—		—			—	—	
PK1-2F–ITS4	477	—	—		—			—	—	
ITS type 2 primers										
PK2-1F–PK2-1B	417	+	+		+	—	—	+	+	+
PK2-1F–PK2-2B	345	+		—	+	—	—			
PK2-2F–PK2-2B	258	+	+		+	—	—	+	+	+
PK2-2F–PK2-1B	330	+		—	+	—	—			
ITS1F–ITS4	704/678	+	+	+	+	—	—	+	+	—

[a] Band size matched predicted sizes for different primer pairs. Predicted product size is the size of the region amplified minus the primers. Blank means not tested.

ducted with purified PCR products (ITS 1F-ITS 4 primer products previously used as templates in sequencing reactions) of isolates of both ITS types yielded amplification with both primers for products that previously yielded the different ITS types (Fig. 2A, 2B). Results from sequencing of type-specific PCR products showed that

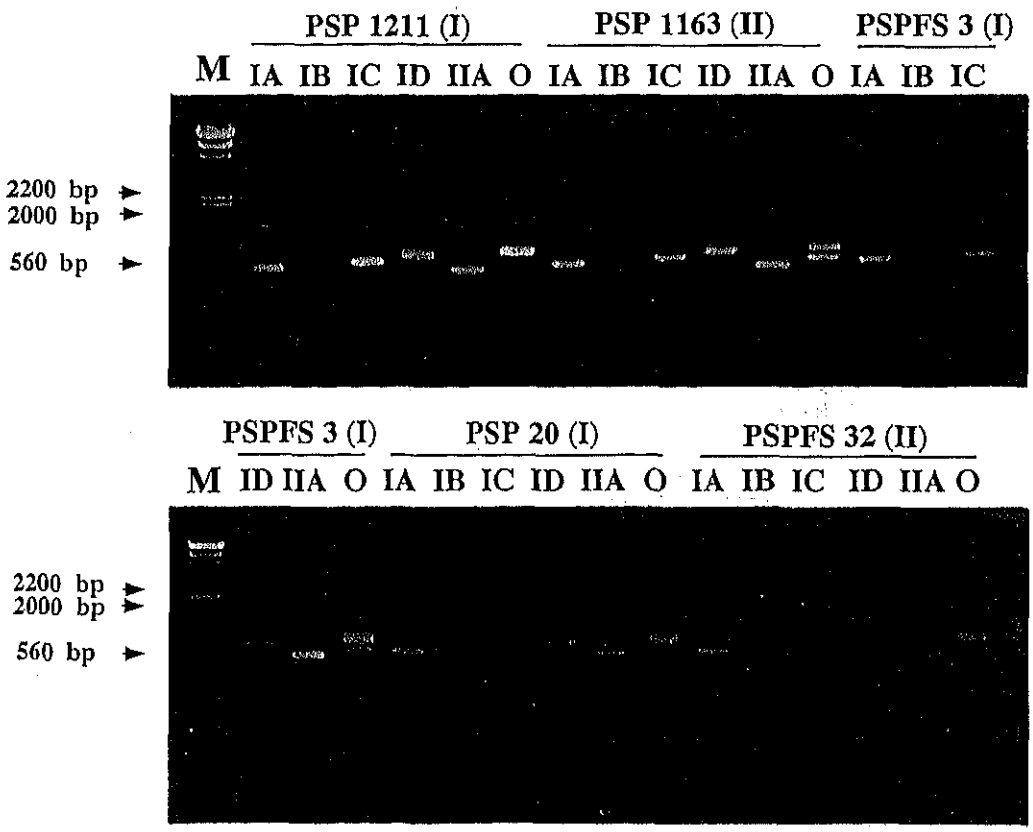

Fig. 1. *Results of PCR at lowered annealing temperatures* (50°C). Isolates PSP 1211, PSPFS3, PSP 20 are ITS type I isolates, while PSP 1163, PSPFS 32 are ITS type II isolates. Lanes IA to ID were amplified with ITS type I-specific primers (IA: PK1-1F, PK1-1B; IB: PK1-2F, PK1-2B; IC:PK1-1F, ITS 4; ID: ITS1F, PK1-1B), while lane IIA was amplified with ITS type II-specific primers (PK2-1F, PK2-1B). Lane O was amplified with the original primer pair, ITS1F, ITS 4.

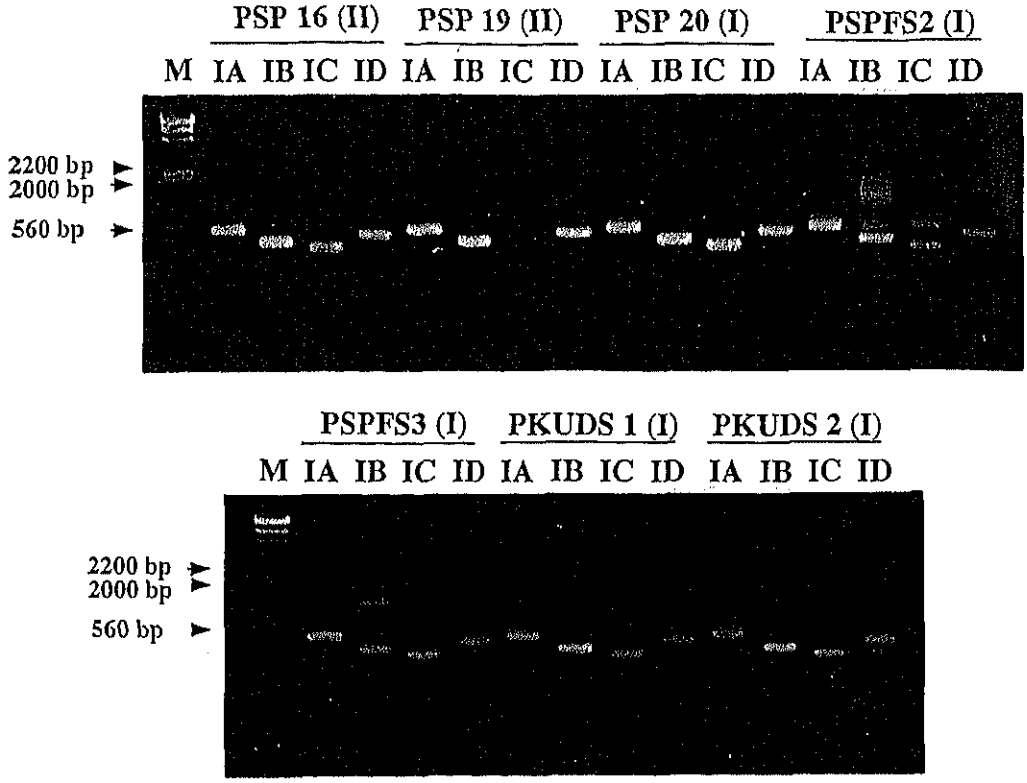

Fig. 2A. Results of PCR from original PCR products (amplified with primer pair ITS1F, ITS 4) using ITS type I-specific primers. Isolates PSP 20, PSPFS2, PSPFS3, PKUDS 1, PKUDS 2 are ITS type I isolates, while PSP 16 and PSP19 are ITS type II isolates. Lanes IA to ID were amplified with ITS type I-specific primers (IA: PK1-1F, PK1-1B; IB: PK1-1F, PK1-2B; IC:PK1-2F, PK1-2B; ID: PK1-2F, PK1-1B).

the type-specific PCR reaction products were true to the type. In cases where type I isolates yielded type II sequences, products amplified by the type II primers were indeed type II sequences, and vice versa.

Amplification of both types with templates obtained from the original PCR products of the ITS1F-ITS4 primer pair indicate that both types were amplified in the original PCR reaction but in unequal proportions. This is conceivable because the primer pair ITS1F-ITS4 amplifies both the ITS types. Thus, PCR products of isolates that yielded ITS type I in sequencing reactions also possessed small amounts of amplified ITS type II sequences, and vice versa. Although the two ITS sequence types differ in length by around 20 bp, they were not detected in agarose gel electrophoresis, possibly because one of the types was present in too small an amount to produce a detectable electrophoretic band. Thus, using these "mixed" amplification products as template for ITS type-specific PCR reactions resulted in amplification of sequences of both types from one supposedly "pure" PCR product. In many cases, however, PCR with the original primer pair ITS1F-ITS 4 resulted in distinctly double electrophoretic bands in agarose, from which subsequent sequencing analysis was not done.

Homology with ITS sequences of other rusts The phylogenetic tree (Fig. 3) showed that these two ITS types in *P. kuehnii* are highly divergent, and that divergence exceeds the level of variation normally found between and among related species. Homology search by BLAST of the DNA sequence database showed that ITS type II sequences were similar to ITS sequences of rusts of sugarcane and other hosts belonging to *Puccinia*. The ITS type I sequence, on the contrary, showed significantly high homology with ITS sequences of rust fungi belonging to the genus *Cronartium* rather than *Puccinia*. This type may have arisen through abnormal accidental insertions resulting from interspecies hybridization with rust from other lineages and thus represent non-orthologous, specifically xenologous sequences (O' Donnell and Cigelnik, 1997).

Members of *Puccinia*, belonging to Pucciniaceae, alternate on a wide range of host families, while members of *Cronartium*, belonging to Cronartiaceae, have a more limited host range. Although *Cronartium* aecial hosts are not hosts of *Puccinia*, certain telial host families such as Saxifragaceae, Scrophulariaceae and Gentanaceae are also hosts of some *Puccinia* members (Cummins and Hiratsuka, 1983; Hiratsuka et al., 1992). These common host families could have served as venues for the putative introgressive hybridization event between members of these two different rust lineages.

On the other hand, these polymorphisms could also be products of an ancient gene duplication event that occurred prior to the evolutionary radiation of the *Puccinia* lineage and may represent paralogous polymorphic sequences of rDNA (Sanderson and Doyle, 1992; O' Don-

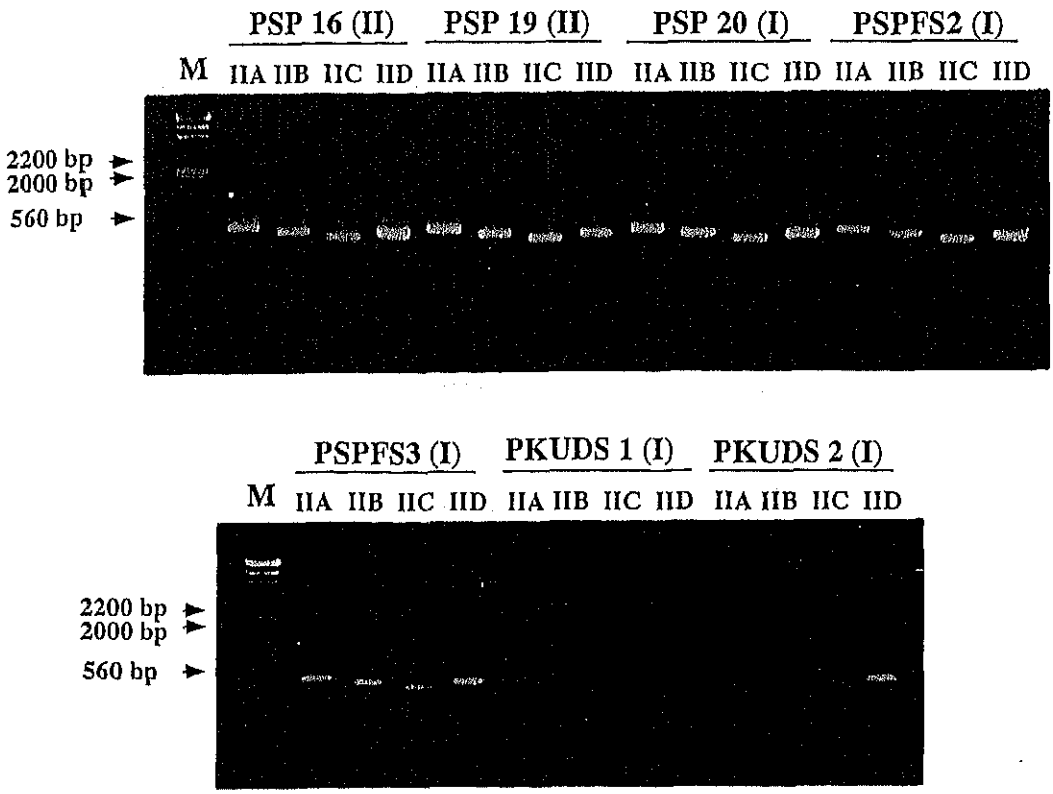

Fig. 2B. Results of PCR from original PCR products (amplified with primer pair ITS1F, ITS 4) using ITS type II-specific primers. Isolates PSP 20, PSPFS2, PSPFS3, PKUDS 1, PKUDS 2 are ITS type I isolates, while PSP 16 and PSP19 are ITS type II isolates. Lanes IIA to IID were amplified with ITS type II-specific primers (IA: PK2-1F, PK2-1B; IB: PK2-1F, PK2-2B; IC:PK2-2F, PK2-2B; ID: PK2-2F, PK2-1B).

nell and Cigelnick, 1997). Previously reported rDNA polymorphisms (Zijstra, et al., 1995; Suh et al., 1993; Vogler and DeSalle, 1994; Sanders et al., 1995; Harlton et al., 1995; O' Donnell and Cigelnick, 1997; Hijri et al., 1999; Antonlolli et al., 2000) are composed of a small number of nucleotide differences that either span the entire ITS region or occur in either of the two spacer regions. Divergence of the two nonorthologous sequences observed in this study is extremely high and even involves two nucleotide differences in the conserved 5.8S rDNA region. One of the nucleotide differences in the 5.8S rDNA region between the two types is shared with the other *Puccinia* species, while the other is shared with members of *Cronartium*. Unlike the D1/D2 region sequences, which were conserved among the isolates that showed different ITS types, the 5.8S rDNA region is apparently implicated in the putative hybridization or gene duplication events that brought about this heterogeneity.

Since they appear to be more ancient than the species in which they occur, they can also be considered as transspecies polymorphisms that were sorted among *Puccinia* and *Cronartium* lineages in such a way that coalescence of these polymorphic ITS types did not occur within the *Puccinia* lineage but prior to separation of *Puccinia* and *Cronartium* lineages (Doyle, 1992; Li, 1997; Page and Holmes, 1998). Since the 5.8S rDNA region is also heterogeneous and carries nucleotide substitutions shared by both lineages, its gene tree phylogeny may provide an insight into the coalescence of these two ITS types and phylogenetic relationships between these two rust lineages.

Analysis of members of Urediniomycetes placed *Puccinia* and *Cronartium* in distant clades in the phylogenetic trees (Sjamsuridzal et al., 1999). Furthermore, ontogeny and morphology of spermogonia and telia, which are considered to reflect evolutionary and phylogenetic relationships in rust fungi, are clearly differentiated in *Puccinia* and *Cronartium* members, which respectively form two distinct families of rust fungi, Pucciniaceae and Cronartiaceae, (Cummins and Hiratsuka, 1983). Pucciniaceae belong to Group V and Cronartiaceae to Group II based on spermogonial morphology and have telial Type IV and Type II, respectively (Hiratsuka and Cummins, 1963; Hiratsuka, 1988). These molecular and morphological evidences indicate that they are phylogenetically distant lineages. Thus, the extremely high homology of ITS type I to sequences of a quite unrelated lineage indicates a homoplastic pattern of evolution (Hillis and Dixon, 1991). If ITS type I sequences are products of an ancient gene duplication event synchronous to the divergence of the lineages that led to *Puccinia* and *Cronartium*, then these paralogous polymorphisms complicate the hierarchical relationships among rDNA genes within and between species of these two lineages (Sanderson and Doyle, 1992) and mask true species phylo-

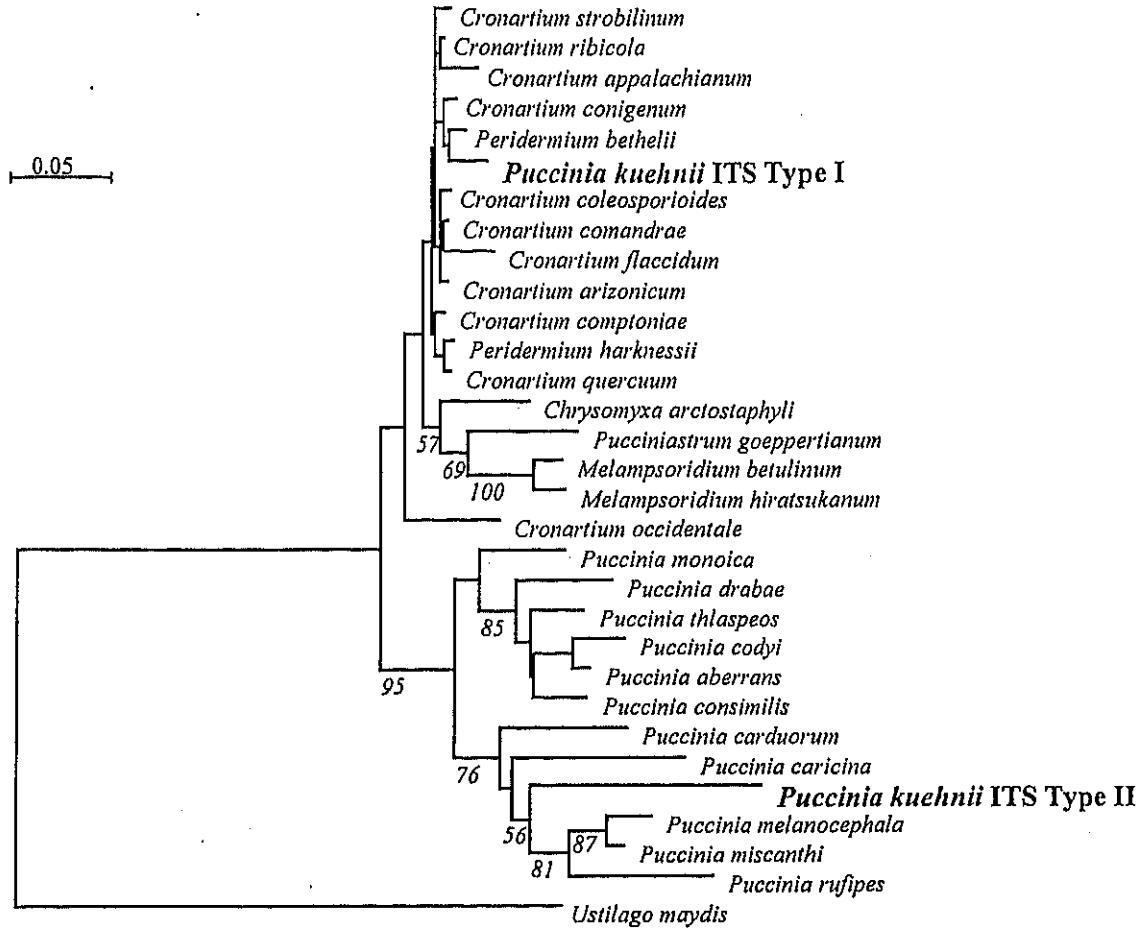

Fig. 3. Neighbor-joining tree inferred from sequences of ITS and 5.8S rDNA regions. Bootstrap support for 1000 resamplings is shown for branches with more than 50% support. Length of branches is proportional to number of base changes indicated by the scale above.

genetic relationships (Doyle, 1992).

Further evidence must be obtained to determine the exact origin of these highly divergent polymorphic sequences. In addition, the occurrence of similar homologous regions in rDNA or in other loci should be examined between other members of *Cronartium* and *Puccinia*.

ITS type I sequences apparently persisted over a long time, spanning several divergence and speciation events. O' Donnell and Cigelnik (1997) discussed the mechanisms by which intragenomic heterogeneity could have escaped the homogenizing effects of concerted evolution and become established across many speciation events in *Fusarium*. Among the factors that affect the rate of concerted evolution is the arrangement of the repeats in the genome (Graur and Li, 2000). Concerted evolution is least favorable when the repeats of an array are highly dispersed throughout the genome (Vogler and DeSalle, 1994; Li, 1997). The polymorphisms could be located in the chromosomes in such a way that they were highly diverged and inaccessible to inter-chromosomal conversions (Li, 1997; O' Donnell and Cigelnik, 1997).

Since the DNA was extracted from urediniospores, which are binucleate (n+n), it is also possible that these two types occur in different nuclei of the spore (Harlton et al., 1995) and thus represent intra-sporal heterogeneity similar to that observed in multinucleate spores of Glomales (Sanders et al., 1995; Hijri et al., 1999; Antoniolli et al., 2000).

This heterogeneity in ITS regions may have implications for the interpretation of phylogenetic relationships of these rusts based on rDNA ITS sequence data alone (O' Donnell and Cigelnik, 1997; Hillis and Davis, 1988). Non-orthologous rDNA loci are reported to possibly replace major orthologous loci during speciation and lead to disruptions in an evolutionary lineage (Dubcovsky and Dvorak, 1995). In addition, these rDNA polymorphisms may also sometimes go undetected by direct sequencing of PCR products (Wendel et al., 1995; Baldwin et al., 1995). The occurrence of rDNA polymorphisms shows that thorough sampling to detect possible polymorphisms and comparisons with other regions or gene loci that more appropriately reflect the species phylogeny must be done (Hillis and Dixon, 1991; Sanderson and Doyle, 1992; Doyle, 1992).

Our results showed that these two highly divergent ITS types occur in the same individual as polymorphic ITS forms and confirm previous speculations that ribosomal DNA polymorphism occurs in ITS regions of the sugar-

cane rust *Puccinia kuehnii*. They also coincide with the grouping together of these isolates into a single cluster based on sequences of the more conserved D1/D2 region of the LSU rDNA, and with results from comparative morphological studies showing that *Puccinia* sp. and *P. kuehnii* isolates could not be separated morphologically (Virtudazo et al., 2001b). They therefore support the grouping of these isolates into one species, *P. kuehnii* (Virtudazo et al., 2001a). In addition, this shows that the D1/D2 regions of the LSU rDNA of *P. kuehnii* is more appropriate for resolving phylogenetic relationships in sugarcane and other related rusts.

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Literature cited

Antoniolli, Z. I., Schachtman, D. P., Ophel-Keller, K. and Smith, S. E. 2000. Variation in rDNA ITS sequences in *Glomus mossaea* and *Gigaspora margarita* spores from a permanent pasture. Mycol. Res. 104: 708–715.

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S. and Donoghue, M. J. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on anglosperm phylogeny. Ann. Missouri Bot. Gard. 82: 247–277.

Bruns, T. D., White, T. J. and Taylor, J. W. 1991. Fungal Molecular Systematics. Annu. Rev. Ecol. Syst. 22: 525–564.

Cummins, G. B. and Hiratsuka, Y. 1983. Illustrated genera of rust fungi, revised ed. The American Phytopathological Society, Minnesota.

Doyle, J. J. 1992. Gene trees and species trees: molecular systematics as one-character state taxonomy. Syst. Bot. 17: 144–163.

Dubcovsky, J. and Dvorak, J. 1995. Ribosomal RNA multigene loci: nomads of the Triticeae genomes. Genetics 140: 1367–1377.

Fatehi, J. and Bridge, P. 1998. Detection of multiple rRNA-ITS regions in isolates of *Ascochyta*. Mycol. Res. 102: 762–766.

Graur, D. and Li, W-H. 2000. Fundamentals of Molecular Evolution. 2nd ed. Sinauer Associates, Inc. Massachusetts.

Harlton, C. E., Levesque, C. A. and Punja, Z. K. 1995. Genetic diversity in *Sclerotium (Athelia) rolfsii* and related species. Phytopathology 85: 1269–1281.

Hibbet, D. S. 1992. Ribosomal RNA and fungal systematics. Trans. Mycol. Soc. Japan 33: 533–556.

Hijri, M., Hosnen, M., van Tuinen, D. and Dulieu, H. 1999. Intraspecific ITS polymorphism in *Scutellospora castanea* (Glomales, Zygomycota) is structured within multinucleate spores. Fung. Gen. Biol. 26: 141–151.

Hillis, D. M. and Davis, S. K. 1988. Ribosomal DNA: Intraspecific polymorphism, concerted evolution, and phylogeny reconstruction. Syst. Zool. 37: 63–66.

Hillis, D. M. and Dixon, M. T. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quart. Rev. Biol. 64: 411–453.

Hiratsuka, N., Sato, S., Kakishima, M., Kaneko, S., Sato, T., Hiratsuka, T., Katsuya, K., Hiratsuka, Y., Ono, Y., Harada, Y. and Nakayama, K. 1992. The rust flora of Japan. Tsukuba Shuppankai, Ibaraki.

Hiratsuka, Y. 1988. Ontogeny and morphology of teliospores (probasidia) in Uredinales and their significance in taxonomy and phylogeny. Mycotaxon 31: 517–531.

Hiratsuka, Y. and Cummins, G. B. 1963. Morphology of spermogonia of the rust fungi. Mycologia 55: 487–507.

Li, W-H. 1997. Molecular Evolution. Sinauer Associates, Inc. Masssachusetts.

O' Donnell, K. and Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phylogen. Evol. 7: 103–116.

Page, R. D. and Holmes, E. C. 1998. Molecular evolution: a phylogenetic approach. Blackwell Science Ltd., Oxford.

Sanders, I. R., Alt, M., Groppe, K., Boller, T. and Wiemken, A. 1995. Identification of ribosomal DNA polymorphisms among and within spores of the Glomales: application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. New Phytol. 130: 419–427.

Sanderson, M. J. and Doyle, J. J. 1992. Reconstruction of organismal and gene phylogenies from data on multigene families: concerted evolution, homoplasy, and confidence. Syst. Biol. 41: 4–17.

Sjamsuridzal, W., Nishida, H., Ogawa, H., Kakishima, M. and Sugiyama, J. 1999. Phylogenetic relationships of rust fungi parasitic on ferns: Evidence from 18S rDNA sequence analysis. Mycoscience 40: 21–27.

Suh, Y., Thien, L. B., Reeve, H. E. and Zimmer, E. A. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. Am. J. Bot. 80: 1042–1055.

Virtudazo, E. V., Nakamura, H. and Kakishima, M. 2001a. Phylogenetic analysis of sugarcane rusts based on sequences of ITS, 5.8S rDNA and D1/D2 region of LSU rDNA. J. Gen. Plant Pathol. 67: 28–36.

Virtudazo, E. V., Nojima, H. and Kakishima, M. 2001b. Taxonomy of *Puccinia* species causing rust diseases on sugarcane. Mycoscience 42: 167–175.

Vogler, A. P. and DeSalle, R. 1994. Evolution and phylogenetic information content of the ITS-1 region in the tiger beetle *Cicindela dorsalis*. Mol. Biol. Evol. 11: 393–405.

Wagner, A., Blackstone, N., Cartwright, P., Dick, M., Misof, B., Snow, P., Wagner, G. P., Bartels, J., Murtha, M. and Pendleton, J. 1994. Surveys of gene families using polymerase chain reaction: PCR selection and PCR drift. Syst. Biol. 43: 250–261.

Wendel, J. F., Schnabel, A. and Seelanan, T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc. Natl. Acad. Sci. USA 92: 280–284.

White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols (Innis, M. A., Gelfrand, D. H., Sninsky, J. J. and White, J., eds.). pp. 315–322. Academic Press, San Diego, California.

Zijstra, C., Lever, A. E. M., Uenk, B. J. and Van Silfhuot, C. H. 1995. Differences between ITS regions of isolates of root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi*. Phytopathology 85: 1231–1237.

Mycoscience 42: 519–533, 2001

Morphological analyses of urediniospores and teliospores in seven *Phragmidium* species parasitic on ornamental roses

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Ornamental roses worldwide harbor 10 *Phragmidium* species. Among these, *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum*, *P. rosae-pimpinellifoliae*, *P. rosae-rugosae* and *P. tuberculatum* are frequently reported; however, these species are morphologically similar and difficult to distinguish. For better circumscription and correct identification of the species, this study examined morphological features in urediniospores and teliospores of the seven *Phragmidium* species collected on ornamental rose cultivars and wild species. The results indicated that some host-specific groups could be well circumscribed by the morphological properties of urediniospores and teliospores. However, without the precise identification of hosts, these morphological properties were not effective key characters for the identification of the species.

Key Words——identification; *Phragmidium*; *Rosa*; Rosaceae; taxonomy; Uredinales.

The rust genus *Phragmidium* Link is characterized by linearly arranged multicellular teliospores with a persistent pedicel, the lower half of which is usually thickened and hygroscopic (Cummins and Hiratsuka, 1983). Most species produce subcuticular spermogonium, caeomatoid aecium, uredinium and telium in the autoecious macrocyclic life cycle. Species of the genus are restricted to plants of the family Rosaceae, especially the genera *Rosa*, *Rubus* and *Potentilla*. Among 60 or more species of *Phragmidium*, some 30 species have been reported to infect wild *Rosa* species and ornamental cultivars.

Ornamental rose cultivars are selections from more than 70 wild species and their hybrids (Beales et al., 1998). Species of the genus *Rosa* are classified in subgenera *Hulthemia*, *Hesperhodes* and *Eurosa*; and the subgenus *Eurosa* is further divided into 10 sections. Domesticated species and hybrid cultivars are distributed in these subgenera and sections, except for *Hulthemia*.

Because of the heterogeneity of rose species and cultivars in their origin and geographic distribution, several *Phragmidium* species are involved in the rust diseases of ornamental roses. Two or more species are reported to occur in any particular area of the world and, overall, 10 species are considered to be the pathogens: *P. americanum* (Peck) Dietel, *P. fusiforme* Schröter, *P. montivagum* Arthur, *P. mucronatum* (Persoon: Persoon) Schlectendal, *P. rosae-californicae* Dietel, *P. rosae-pimpinellifoliae* Dietel, *P. rosae-rugosae* Kasai, *P. rosicola* (Ellis & Everhart) Arthur, *P. speciosum* (Fries) M. C. Cooke and *P. tuberculatum* J. M. Müller (Pirone et al., 1960; Howden and Jacobs, 1973; Horst, 1983).

The causal species of the rose rusts have been variously circumscribed and classified by combinations of the morphological characteristics of a maximum of four spore stages produced in the life cycle (Arthur, 1934; Hiratsuka, 1935; Gäumann, 1959; Wilson and Henderson, 1966; Azbukina, 1984; Wei, 1988; Hiratsuka et al., 1992). Although these *Phragmidium* species are autoecious and macrocyclic in the life cycle, spermogonial-aecial stage is short-lived and quickly replaced by uredinia in the early growing season. Thus, the rose rust species persist in the uredinial or uredinial-telial stage for almost the entire growing season, and either uredinia or a mixture of uredinia and telia can be found at any part of the growing season or on any particular specimen. Because of the prevalence of the uredinial-telial stage and because of the morphological diversity of teliospores, circumscription and identification of the rose rust species have relied heavily on morphological characteristics of the teliospores with supplementary urediniospore characteristics.

Because the morphological circumscription of species does not seem clear and also because a variation range of each morphological feature is broad and continuous, the genus *Phragmidium* has been among the most difficult taxa in the Uredinales to classify and to identify its species, particularly those on *Rosa*. Among the 10 species parasitic on ornamental roses, *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum*, *P. rosae-pimpinellifoliae*, *P. rosae-rugosae* and *P. tuberculatum* are morphologically similar and widespread in the world. Consequently, the identification of the species causing

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the rust diseases of ornamental roses has often been problematic.

It is of mycological and horticultural importance to know exactly how many *Phragmidium* species are involved in the rose rusts, what their biological nature is and how they can be correctly identified. This study aims at examining morphological properties in the uredinial and telial stages to find which properties can be reliable key characters for the identification.

Materials and Methods

Specimens examined Herbarium specimens examined were loaned from the Arthur Herbarium, Purdue University (PUR); the Faculty of Agriculture, Hokkaido University (SAPA); the Botanical Garden and Museum, Berlin-Dahlem (B); the National Fungus Collections, the United States Department of Agriculture (BPI); the Herbarium of Systematic Mycology, the Faculty of Education, Ibaraki University (IBA); the Museum of Natural History, Wroclaw University (WRSL); and the Herbarium of the University of Tsukuba (TSH). The specimens had been identified as *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum*, *P. rosae-pimpinellifoliae*, *P. rosae-rugosae* and *P. tuberculatum*. To examine both uredinia and telia in the same life-cycle, 198 specimens with both spore stages were selected from more than 600 specimens (Table 1). Among them, the host species had been identified in 119 specimens. The specimens included the type specimens of *P. montivagum* (holotype on *Rosa acicularis* collected by A. Nelson in Wyoming in 26 July 1895, in Herb. PUR), *P. rosae-pimpinellifoliae* (holotype on *R. pimpinellifolia* collected by Kemmler at Donnstetten no. 1671 in Herb. B) and *P. rosae-rugosae* (one of the syntypes on *R. rugosa* collected by M. Kasai in Sapporo, Japan in 30 October 1908, in Herb. SAPA).

The ontogenic terminology system refined by Hiratsuka (1973) and Cummins and Hiratsuka (1983) was employed in identifying and describing life-cycle stages of the *Phragmidium* specimens throughout the study.

Microscopy Urediniospores and tellospores were scraped from the specimens and mounted in a drop of lactophenol solution on a microscopic slide. For each specimen, 50 spores were randomly chosen and observed under an Olympus BH 100 microscope for the selected morphological features listed. Measurements were made with a Leica Q-Win Image Analyzer.

Spore-wall color was determined under an Olympus BH 100 microscope with a tungsten lamp without filter. The light intensity was adjusted by controlling the voltage of the lamp. The color was described according to Rayner (1970).

Number and distribution of germ-pores in urediniospores were determined by the aniline-blue squash method (Jennings et al., 1989). The distribution pattern of the pores was categorized according to Cummins and Hiratsuka (1983).

For scanning-electron-microscopy, urediniospores and teliospores were dusted on double-adhesive tape on a specimen holder and coated with platinum-palladium at 25 nm thickness under a Hitachi E-1030 ion sputter. The coated specimens were observed under a Hitachi S-4200 operating at 15 kV. The urediniospore-surface type was determined according to Cummins and Hiratsuka (1983).

Statistical analyses Statistics including multivariate analyses of measured continuous numerical variables were performed using the software package Systat™ version 5.2 (Wilkinson, 1989) run on a Macintosh Power Mac G4. Discrete numerical or qualitative attributes or host species were superimposed on two-or three-dimensional scatter diagrams generated from the analyses to detect possible groups.

Results

Morphological analyses of 119 specimens with identified host plants were essentially the same as those of 198 specimens, which included those on unidentified host plants. Therefore, only results from the analyses of host-identified specimens are presented here to facilitate discussion of morphological variations in relation to putative host specificity.

Teliospore morphology Shape and size of teliospores varied both among specimens and within a specimen (Fig. 1A, Table 2). Mean teliospore length in individual specimens ranged from 61.6 to 106.7 μm and mean width from 26.3 to 37.3 μm. Among these specimens, BPI0126320 on *R. macdaugall*, PUR-56692 on *R. manca* (Fig. 4A), PUR7836 on *R. engelmannii*, PUR 8164 on *R. gallica* and PUR7761 on *R. suffulta* bore small spores. The spore length ranged from 50.1 to 83.0 μm and the width from 21.8 to 34.4 μm in the five specimens. PUR44755 on *R. nutkana*, PUR48167 on *R. eastwoodii*, PUR7847 on *R. fendleri* (Fig. 4B), PUR7887 on *R. macounii*, PURF1514 on *R. tomentosa* were among medium-spore bearing specimens. The spore length ranged from 61.0 to 106.4 μm and the width from 26.2 to 37.1 μm in the five specimens. On the other hand, PUR7725 on *R. setigera*, PURF11650 on *R. rugosa*, SAPA (Tokachi) on *R. rugosa*, BPI0126648 on *R. centifolia* and BPI0126642 on *R. alba* (Fig. 4C) were among large-spore bearing specimens. The spore length ranged from 65.0 to 135.6 μm and the width from 29.2 to 45.0 μm in the five specimens. As shown in Fig. 1A, there were many specimens with the spores of various sizes covering all three groups, and no disjunction was detected in the variation range to circumscribe spore-size groups among the specimens.

No correlation was detected between teliospore length and width (Fig. 1A, not significant in Spearman's rank correlation analysis). Thus, the teliospore length/width ratio also varied both among specimens and within a specimen, mean value ranging from 2.0 in BPI0126306 on *R. arkansana*, PURF11649 on *R. canina*, PURF1507 and PURF9504 on *R. gallica* (Fig. 4D) and PURF11645 on *R. rugosa* to 3.3 in PUR 48581 on *R. acicularis*; 3.4 in BPI0126299 on *R. acicularis* and TSH on *R. rugosa*; 3.5 in IBA0311 on *R. acicularis* and 4.0 in

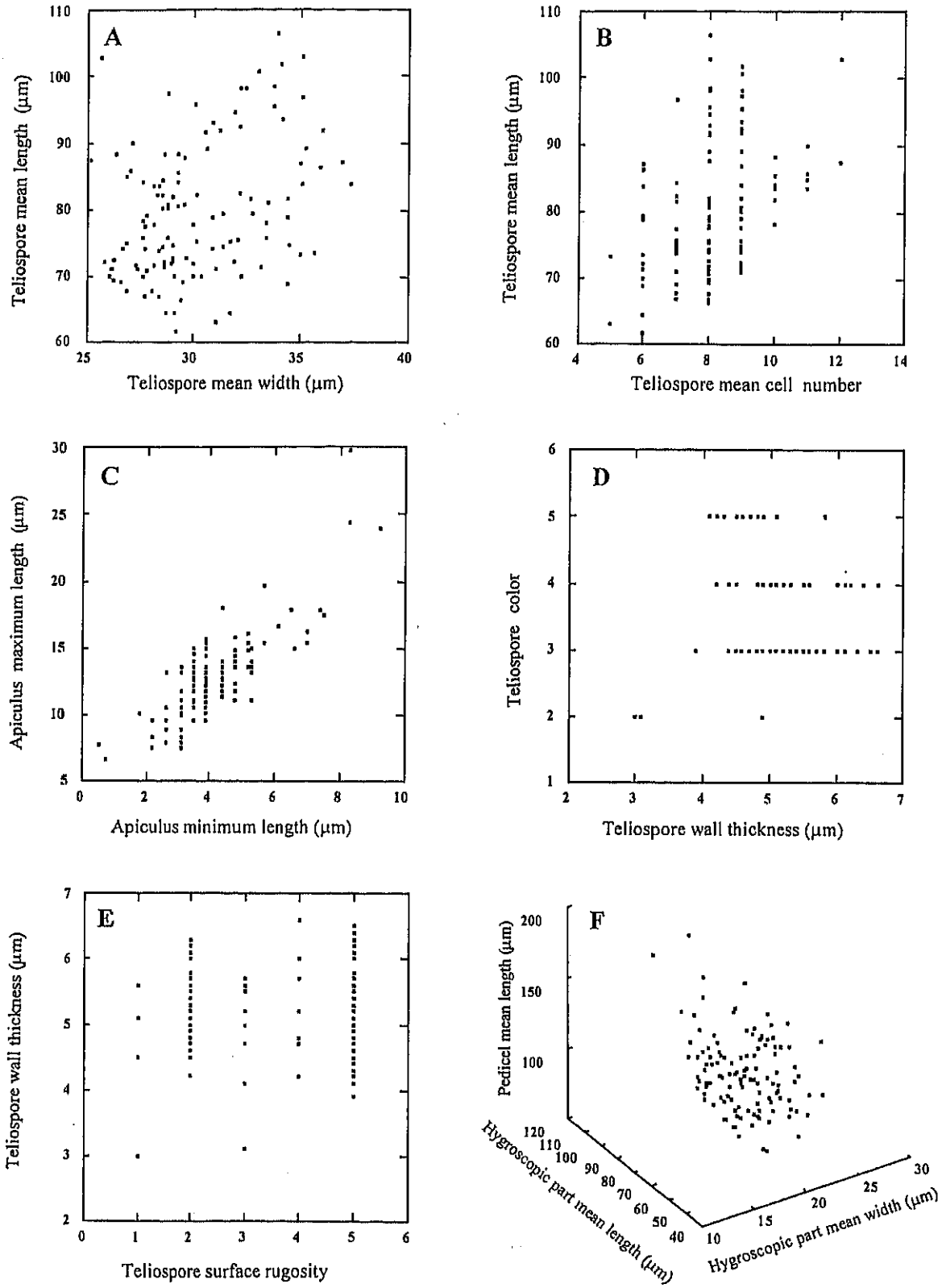

Fig. 1. Variations in the teliospore characteristics observed in the *Phragmidium* specimens. A. Mean lengths against mean widths. B. Mean lengths against mean cell numbers. C. Apiculus maximum lengths against apiculus minimum lengths. D. Darkness of wall color (light brown, 1, to blackish brown, 5) against wall thickness. E. Wall thickness against degrees of wall-surface rugosity (faint, 1, to conspicuous, 5). F. Relationship among the pedicel mean length and mean length and mean width of the hygroscopic part of pedicels.

Table 1. *Phragmidium* specimens examined in this study.

Host	Locality	Accession number[1)]
Rosa acicularis Lindl.	Alaska, U.S.A.	BPI 0126284, PUR F48581
	Colorado, U.S.A.	PUR 7925, 7935
	Michigan, U.S.A.	IBA 0311 and 1387, PUR 7797
	Montana, U.S.A.	BPI 0126338 and 0126270, PUR 55312, 7746, 7927 and 7930
	Russia	BPI 0126299
	Russia	SAPA
	Wyoming, U.S.A.	PUR 61189, 61191, 7934, 7933, 7923 and 7926
	New Mexico, U.S.A.	PUR 61979
R. aciculata Cockerell	Colorado, U.S.A.	PUR 7811
R. alba L.	Alaska, U.S.A.	PUR 8161
	New York, U.S.A.	PUR 8162
	No locality given	BPI 0126642
R. aleutensis Crép.	Alaska U.S.A.	PUR 48583
R. blanda Ait.	Wisconsin, U.S.A.	PUR 65617 and 7660
	Canada	PUR 7657
R. bourgeaniana Crép.	U.S.A.	PUR 44750
	Indiana, U.S.A.	PUR 44752
R. canina L.	Germany	PUR F1567 and F1561
	Belgium	PUR F11649
	Turkey	PUR F16657
R. carolina L.	Indiana, U.S.A.	PUR 88322
	New York, U.S.A.	PUR 7708
	Wisconsin, U.S.A.	PUR 44733
R. centifolia L.	No locality given	BPI 0126648
R. cinnamomea L.	Germany	PUR F1552
R. damascena Mill.	California, U.S.A.	PUR 59483
	Iowa, U.S.A.	PUR 7705
R. davurica Pall.	China	SAPA
	Japan	SAPA
R. eastwoodii Rydb.	California, U.S.A.	PUR 48167
R. engelmannii S. Wats	U.S.A.	PUR 7836 and 7845
	Wyoming, U.S.A.	PUR 7826
R. fendleri Crép	Colorado, U.S.A.	PUR 7847, 7853, and 7860
	New Mexico, U.S.A.	PUR 7861
	Wyoming, U.S.A.	PUR 7868
	Montana, U.S.A.	PUR 7910
	Nevada, U.S.A.	PUR 48561
	New Mexico, U.S.A.	PUR 48092 and 7908
	Utah, U.S.A.	PUR 51058 and 7914
	Wyoming, U.S.A.	PUR 63194, 7904 and 7105
R. gallica L.	Argentina	PUR F9504
	Ecuador	PUR F1507
	Alaska, U.S.A.	PUR 8164
R. gymnocarpa Nutt.	Wyoming, U.S.A.	BPI 0126317
	Oregon, U.S.A.	PUR 7877 and 7878
R. macdaugalii Holz	Idaho, U.S.A.	PUR 49821
	Montana, U.S.A.	BPI 0126320
R. macounii Greene	Columbia, U.S.A.	PUR 7887
	Idaho, U.S.A.	PUR 7875
	Nevada, U.S.A.	PUR 7892
	Utah, U.S.A.	PUR 7872
R. manca Greene	Arizona, U.S.A.	PUR 56689 and 56692

Table 1. (Continued)

R. marretii Lev.	Kushiro, Japan	SAPA and SAPA
R. mirifica Cockerell	New Mexico, U.S.A.	PUR 51945 and 51814
R. nutkana Presl.	Alaska, U.S.A.	PUR 48582, 7770 and 7782
	Montana, U.S.A.	PUR 44755
	Oregon, U.S.A.	BPI 0126326
	Washington, U.S.A.	PUR 53448
R. pendulina L.	Germany	PUR F1548
	Switzerland	WRSL
R. pimpinellifolia L.	Wyoming, U.S.A.	BPI 0126625 and B
R. pisocarpa G.Gray.	Idaho, U.S.A.	PUR 7787
R. pulverulenta Rydb.	Nevada, U.S.A.	PUR 7916
R. rubiginosa L.	Nebraska, U.S.A.	PUR 8226
	Switzerland	PUR F1157
R. rugosa Thunb.	Hokkaido, Japan	TSH and SAPA
	Ishikari, Japan	SAPA and SAPA
	Japan	BPI 0126669
	Shiribeshi, Japan	BPI 0126671
	Tokachi, Japan	SAPA
	Tsukuba, Japan	TSH
R. setigera Michx.	Indiana, U.S.A.	PUR 44734, 7731 and 7725
	Ohio, U.S.A.	PUR 44740
	Tennessee, U.S.A.	PUR 44735 and 44737
	Wisconsin, U.S.A.	PUR 53303
R. stellata Wooton	U.S.A.	PUR 59705
R. suffulta Greene	Canada	PUR 44758
	Montana, U.S.A.	BPI 0126643 and 0126306
	North Dakota, U.S.A.	PUR 7761 and BPI 0126318
R. tomentosa Sm.	Germany	PUR F1514
R. virginiana Mill.	Maine, U.S.A.	PUR 7669
	New Hampshire, U.S.A.	PUR 44741
Rosa sp.	Alaska, U.S.A.	PUR 48491, 48497, 7789 and 53449
	California, U.S.A.	PUR 44893, 7975, 8182, and 50718, BPI 0125302
	Colorado, U.S.A.	PUR 52482
	District of Columbia, U.S.A.	PUR 7703
	Hawaii, U.S.A.	BPI 0126634
	Kansas, U.S.A.	PUR 44796
	Massachusetts, U.S.A.	PUR 49172 and 7696
	Michigan, U.S.A.	PUR 8190, 89295, 51672 and 7796
	Montana, U.S.A.	BPI 0126293, IBA 1320 and 1363, PUR 7808
	New York, U.S.A.	PUR 7697 and 8194
	New Jersey, U.S.A.	PUR 8214, 7963 and 7691, BPI 0126291
	New Mexico, U.S.A.	PUR 7963
	North Dakota, U.S.A.	PUR 7683 and 8173
	Oregon, U.S.A.	PUR 8216
	Pennsylvania, U.S.A.	PUR 61459
	South Dakota, U.S.A.	PUR 44744
	Utah, U.S.A.	PUR 60983
	Vermont, U.S.A.	PUR 8177
	Washington, U.S.A.	PUR 8198, 50845
	West Virginia, U.S.A.	PUR 8208 and 44746
	Wisconsin, U.S.A.	PUR 7798 and 7684
	Wyoming, U.S.A.	PUR 62432
	Argentina	PUR F9501

Table 1. (Continued)

	Bolivia	PUR F1526
	Brazil	PUR F1524
Rosa sp.	Canada	PUR 48594, 8215, 59839, 65693, 49165, 7932, and 7702, BPI 0126627
	Canary Island	PUR F1516
	China	PUR F14181
	Chile	PUR F1523
	Denmark	BPI 0125304
	Ethiopia	PUR F16304
	Ecuador	PUR F1528 and BPI 0125309
	Germany	BPI 0126632, 0126633 and 0125308; PUR F1521 and F 1484
	Guatemala	BPI 0126290, 0126292 and 0126288
	Himalaya	PUR F1527
	Italy	PUR F9485
	Japan	JSH
	Mexico	BPI 0125310 and 0125301
	Netherlands	PUR F11834 and 11650
	New Zealand	BPI 0125306
	Peru	PUR F1530 and F1529
	Sweden	BPI 0126631

1) B: The Botanical Garden and Museum Berlin-Dahlem, Germany.
BPI: The U.S. National Fungus Collection, USA.
IBA: The Herbarium of Systematic Mycology, Faculty Education, Ibaraki University, Japan.
WRSL: The Museum of Natural History, Wroclaw University.
PUR: The Arthur Herbarium, Purdue University, West Lafayette, USA.
SAPA: The Herbarium Botanical Institute, Faculty of Agriculture, Hokkaido University, Japan.
TSH: The Mycological Herbarium of the Institute of Agriculture and Forestry, University of Tsukuba, Japan.

PURF1548 on *R. pendulina* (Fig. 4E). Between the extremes, a continuum of ratios was found, with a median value of 3.0 in BPI0126284 on *R. acicularis*, PUR7877 on *R. gymnocarpa*, PUR7925 on *R. acicularis* (Fig. 4F) and PUR44735 on *R. setigera* among others.

The teliospore length/width ratio was believed to be a numerical indicator of the teliospore shape, i.e., small values indicate broadly ellipsoidal shape while large

Table 2. Size variation in teliospores from selected *Phragmidium* specimens on *Rosa*.

Specimen	Host	Length (μm)		Width (μm)	
		Mean	Range	Mean	Range
BPI0126320	*R. macdaugall*	70.8	51.6–83.0	26.3	23.6–28.8
PUR56692	*R. manca*	67.6	55.2–79.3	26.9	23.7–29.8
PUR7836	*R. engelmannii*	66.8	50.8–80.6	27.6	24.5–27.7
PUR8164	*R. gallica*	64.3	50.1–79.4	28.7	24.9–31.8
PUR7761	*R. suffulta*	61.6	50.1–75.4	29.2	21.8–34.4
PUR44755	*R. nutkana*	80.9	63.7–103.3	33.5	29.7–36.6
PUR48167	*R. eastwoodii*	82.4	64.0–99.0	32.2	28.9–35.5
PUR7847	*R. fendleri*	79.3	64.4–94.6	32.7	25.8–32.7
PUR7887	*R. macounii*	75.3	61.8–92.9	31.8	28.9–34.6
PURF1514	*R. tomentosa*	79.2	61.0–106.4	31.4	26.2–37.1
PUR7725	*R. setigera*	106.4	65.0–135.6	33.9	29.2–37.9
PURF11650	*R. rugosa*	87.2	65.8–103.8	36.9	33.1–44.0
SAPA (Tokachi)	*R. rugosa*	101.8	79.1–121.5	35.2	29.7–38.9
BPI0126648	*R. centifolia*	91.8	70.8–116.2	36.0	32.3–39.3
BPI0126642	*R. alba*	83.9	60.7–105.3	37.3	32.8–45.0

values indicate oblong or oblong-ellipsoidal shape. However, the mean ratio value of a specimen did not represent the general shape of its teliospores. Within individual specimens, the length/width ratio, and thus shapes of the spores varied. Among the specimens with mean ratios of less than 2.0, the ratio range was 1.5–2.4 in PURF9504 on *R. gallica* and in PURF11645 on *R. canina*. Among those with mean ratios of more than 3.4, the ratio range was 2.4–4.4 in BPI0126299 on *R. acicularis*, 3.0–4.1 in IBA0311 on *R. acicularis* and 3.1–5.5 in PURF1548 on *R. pendulina*. Among those with mean ratios with a median value of 3.0, the ratios ranged 2.4–3.9 in BPI0126284 on *R. acicularis*, 2.1–3.9 in PUR7877 on *R. gymnocarpa*, 2.6–3.6 in PUR7925 on *R. acicularis* and 2.5–3.8 in PUR44735 on *R. setigera*.

Teliospore length and cell numbers were not correlated (Fig. 1B, not significant in Spearman's rank correlation analysis): teliospores with larger numbers of cells were not necessary longer than those with fewer numbers of cells and vice versa (Table 3, Fig. 1B). IBA0311 on *R. acicularis* (mean cell number of 12 and range of 10–14 cells; mean spore length of 87.4 μm) (Fig. 4G), PURF1548 on *R. pendulina* (12, 8–14 cells; 103 μm), PUR48581 on *R. acicularis* (11, 8–13 cells; 90 μm) and WRSL on *R. pendulina* (11, 8–12 cells; 84.8 μm) were among the specimens bearing teliospores with the largest number of cells. In contrast, PURF1557 on *R. rugosa* (5, 4–7 cells; 73.2 μm), PURF1507 on *R. gallica* (5, 4–6 cells; 62.9 μm) (Fig. 4H) and PUR7761 on *R. suffulta* (5, 3–5 cells; 61.6 μm) were the specimens bearing teliospores with fewest cells.

Significant disequilibrium in the relationship between the cell number and the length of teliospores was observed in those specimens that bore teliospores with 8 or 9 cells on average (Fig. 1B, Table 3). PUR7725 on *R. setigera* (9, 6–11 cells; 106.4 μm), SAPA (Tokachi) on *R. rugosa* (8, 6–10 cells; 101.8 μm), SAPA (Ishikari) on *R. rugosa* (8, 6–10 cells; 100.8 μm) (Fig. 5A) and BPI0126671 on *R. rugosa* (8, 7–9 cells; 98.5 μm) bore longer teliospores, being distributed in the middle top in Fig. 1B. In contrast, PUR48092 on *R. fendleri* (9, 8–10 cells; 70.9 μm) (Fig. 5B), PUR7878 on *R. gymnocarpa* (9, 6–10 cells, 72.0 μm), PUR7836 on *R. engelmannii* (8, 6–10 cells; 66.8 μm), PUR7853 on *R. fendleri* (8, 7–9 cells; 67.6 μm) and BPI0126625 on *R. pimpinellifolia* (8, 6–10 cells; 69.8 μm) bore shorter teliospores, being distributed in the middle bottom in Fig. 1B. Between these extremes, specimens bearing teliospores with different cell numbers and lengths were scattered across the entire range of variation.

In addition to the teliospore length/width ratios, degree of attenuation or roundness of the teliospore apex and base was considered as an important determinant of the general shape of teliospores. The teliospore shape was generally cylindrical. However, there were two extremes in the attenuation of the teliospore apex, i.e., abruptly rounded with the acute apiculus (Fig. 4B) vs. continuously attenuate toward the apiculus (Fig. 4G). In each specimen, the degree of attenuation in both teliospore ends seemed fairly constant, but it varied greatly and continuously among specimens.

The apical wall of teliospores elongated to form an apiculus. Teliospore apiculi were acute, acuminate or obtuse, their appearance being determined by the length and degree of gradation from the wall of teliospore apical cell. The mean length of apiculi varied from 3.9 μm (with minimum length of 0.8 μm and maximum length of 6.6 μm) in BPI0126669 on *R. rugosa* (Fig. 5C, Table 4) to

Table 3. Variation in the cell number and length in teliospores from selected *Phragmidium* specimens on *Rosa*.

Specimen	Host	Cell number		Length (μm)	
		Mean	Range	Mean	Range
IBA0311	*R. acicularis*	12	10–14	87.4	71.7–104.9
PURF1548	*R. pendulina*	12	8–14	103.0	76.7–133.0
PUR48581	*R. acicularis*	11	8–13	90.0	72.8–106.4
WRSL	*R. pendulina*	11	8–12	84.8	67.6–101.2
PUR7725	*R. setigera*	9	6–11	106.4	65.0–135.6
SAPA (Tokachi)	*R. rugosa*	8	6–10	101.8	79.1–121.5
SAPA (Ishikari)	*R. rugosa*	8	6–10	100.8	69.5–122.8
BPI0126671	*R. rugosa*	8	7–9	98.5	83.0–127.4
PUR48092	*R. fendleri*	9	8–10	70.9	54.8–83.2
PUR7878	*R. gymnocarpa*	9	6–10	72.0	52.1–85.9
PUR7836	*R. engelmannii*	8	6–10	66.8	50.8–80.6
PUR7853	*R. fendleri*	8	7–9	67.6	60.4–77.5
BPI0126625	*R. pimpinellifolia*	8	6–10	69.8	56.8–83.5
PURF1557	*R. rugosa*	5	4–7	73.2	56.2–95.5
PURF1507	*R. gallica*	5	4–6	62.9	48.4–77.2
PUR7761	*R. suffulta*	5	3–5	61.6	50.1–75.4

Table 4. Extreme values in the apiculus length in teliospores from selected *Phragmidium* specimens on *Rosa*.

Specimen	Host	Mean (μm)	Range (μm)
PURF1552	*R. cinnamomea*	15.3	9.2–24.0
PUR7892	*R. pendulina*	13.3	7.5–17.5
IBA0311	*R. acicularis*	12.8	7.4–17.9
PURF1557	*R. rugosa*	12.0	5.7–19.6
WRSL	*R. pendulina*	11.0	6.5–17.9
PUR7669	*R. lucida*	4.9	2.2–8.3
PUR7761	*R. suffulta*	4.7	2.6–7.9
SAPA (Tokachi)	*R. rugosa*	4.0	2.2–7.4
BPI0126671	*R. rugosa*	4.0	0.6–7.7
PUR44741	*R. virginiana*	3.9	2.2–7.5
BPI0126669	*R. rugosa*	3.9	0.8–6.6

15.3 μm (with minimum length of 8.3 μm and maximum length of 24.4 μm) in PURF11834 on *R. rugosa* (Fig. 5D, Table 4). Among specimens examined (Fig. 1C, Table 4), those bearing teliospores with short apiculi included PUR44741 on *R. virginiana* (mean 3.9 μm, range 2.2–7.5 μm), BPI0126671 on *R. rugosa* (4.0 μm, 0.6–7.7 μm), SAPA (Tokachi) on *R. rugosa* (4.0 μm, 1.8–7.4 μm), and PUR7761 on *R. suffulta* (4.7 μm, 2.6–7.9 μm) and PUR7669 on *R. lucida* (4.9 μm, 2.2–8.3 μm). In contrast, specimens bearing teliospores with long apiculi were PURF1552 on *R. cinnamomea* (15.3 μm, 9.2–24.0 μm), PUR7892 on *R. macounii* (13.3 μm, 7.5–17.5 μm), IBA0311 on *R. acicularis* (12.8 μm, 7.4–17.9 μm), PURF1557 on *R. rugosa* (12.0 μm, 5.7–19.6 μm) and WRSL on *R. pendulina* (11.0 μm, 6.5–17.9 μm).

Teliospore-wall color graded continuously from apricot (II9'b), bay (17km), rust (II7'k), sienna (II3b) to chestnut (II7'm) among specimens, although the color did not vary significantly within individual specimens. The wall color was arbitrarily divided into five grades (light brown, 1, to blackish brown, 5). However, the wall color was not correlated with the wall thickness (Fig. 1D, not significant in Spearman's rank correlation analysis) or other teliospore attributes (data not shown).

Similarly, the teliospore-wall thickness was not correlated with the wall-surface rugosity (faint, 1, to conspicuous, 5) (Fig. 1E, not significant in Spearman's rank correlation analysis). Degrees of variation in the wall-surface rugosity of teliospores varied continuously even within a single specimen, the rugosity ranging from conspicuous (as in PUR7892 on *R. macounii*, Fig. 5E) to faint (as in BPI0126284 on *R. acicularis*, Fig. 5F).

Teliospore-pedicel length variation was small within individual specimens. However, large and continuous variations were observed among specimens. The lower half of the pedicel in all specimens was enlarged in both wet and dry conditions as observed under LM and SEM, respectively. The pedicel enlargement was greater in a wet condition due to hygroscopicity than in a dry condition. The degree of pedicel hygroscopicity varied greatly and continuously among specimens, while it did not vary significantly within individual specimens. Length and width of the swollen part were not correlated, giving various shapes to the pedicel from subglobose, broadly ellipsoid to lanceolate among specimens (Fig. 1F, not significant in Spearman's rank correlation analysis), while the shape of the swollen part appeared fairly constant in individual specimens.

The pedicel length was not correlated with the teliospore length among specimens. Similarly, the shapes of teliospores and swollen parts of pedicels were not correlated (Fig. 2A, not significant in Spearman's rank correlation analysis), giving diverse gross morphology of the spore-pedicel complex.

Urediniospore morphology Urediniospores were subglobose to broadly ellipsoid; and the degree of variation in urediniospores, as shown by the length/width ratio, with mean values ranging from 1.0 to 1.4 (–1.5) (Fig. 2B), was smaller than that of teliospores or enlarged parts of pedicels. However, the size of urediniospores varied significantly and continuously among specimens. Specimens bearing large-sized urediniospores were PUR7746 on *R. acicularis* (mean length 24.8 μm, range 21.2–30.7 μm; mean width 21.9 μm, range 18.6–27.2 μm), BPI0126671 on *R. rugosa* (23.6 μm, 15.7–28.9 μm; 19.5 μm, 13.4–24.2 μm), SAPA (Tokachi) on *R. rugosa* (23.4 μm, 18.2–28.3 μm; 21.3 μm, 17.0–23.8 μm), PUR53303 on *R. setigera* (22.9 μm, 20.4–25.8 μm; 18.2 μm, 20.4–22.1 μm) and PUR7933 on *R. acicularis* (22.9 μm, 18.8–27.4 μm; 21.4 μm, 16.0–24.3 μm). In contrast, small urediniospores were observed in PUR51508 on *R. fendleri* (17.5 μm, 15.8–20.2 μm; 16.0 μm, 14.5–17.5 μm), SAPA (Kushiro) on *R. davurica* (18.0 μm, 15.0–20.4 μm; 15.8 μm, 12.8–18.7 μm), PUR61191 on *R. acicularis* (18.6 μm, 16.2–20.6 μm; 15.4 μm, 13.4–17.1 μm), PUR65617 on *R. blanda* (18.8 μm, 16.2–21.5 μm; 16.1 μm, 14.7–18.2 μm) and BPI0126320 on *R. macdougali* (18.9 μm, 14.4–23.0 μm; 15.6 μm, 12.7–18.8 μm). Between these extremes, medium-sized urediniospores were observed, among many others, in BPI0126648 on *R. centifolia* (21.0 μm, 17.5–24.5 μm; 17.4 μm, 15.4–19.5 μm), PUR7910 on *R. fendleri* (21.2 μm, 19.1–24.1 μm; 17.6 μm, 14.7–22.1 μm), PUR44750 on *R. bourgeaniana* (20.7 μm, 17.3–23.0 μm; 17.6 μm, 15.3–20.0 μm), PUR7657 on *R. blanda* (20.4 μm, 17.3–23.4 μm; 18.0 μm, 14.7–20.6 μm) and IBA1387 on *R. acicularis* (19.8 μm, 16.4–23.7 μm; 18.0 μm, 11.9–22.1 μm).

The urediniospore-wall was almost colorless or pale yellowish and did not show significant difference both within a specimen and among specimens. Similarly, the urediniospore-wall thickness was fairly uniform, mean values ranging from 1.1 to 2.2 μm (mostly 1.5–2.0 μm), and thus showed no correlation with the urediniospore size and other attributes.

The urediniospore-wall surface was echinulate in all specimens observed. Density of echinae was measured and found to be different among specimens, mean values ranging from 4.9 (as in PUR55312 on *R. acicularis*, Fig. 5G) to 9.8 (as in PUR59483 on *R. damascena*, Fig. 5H) echinae/10 μm². However, the density variation was

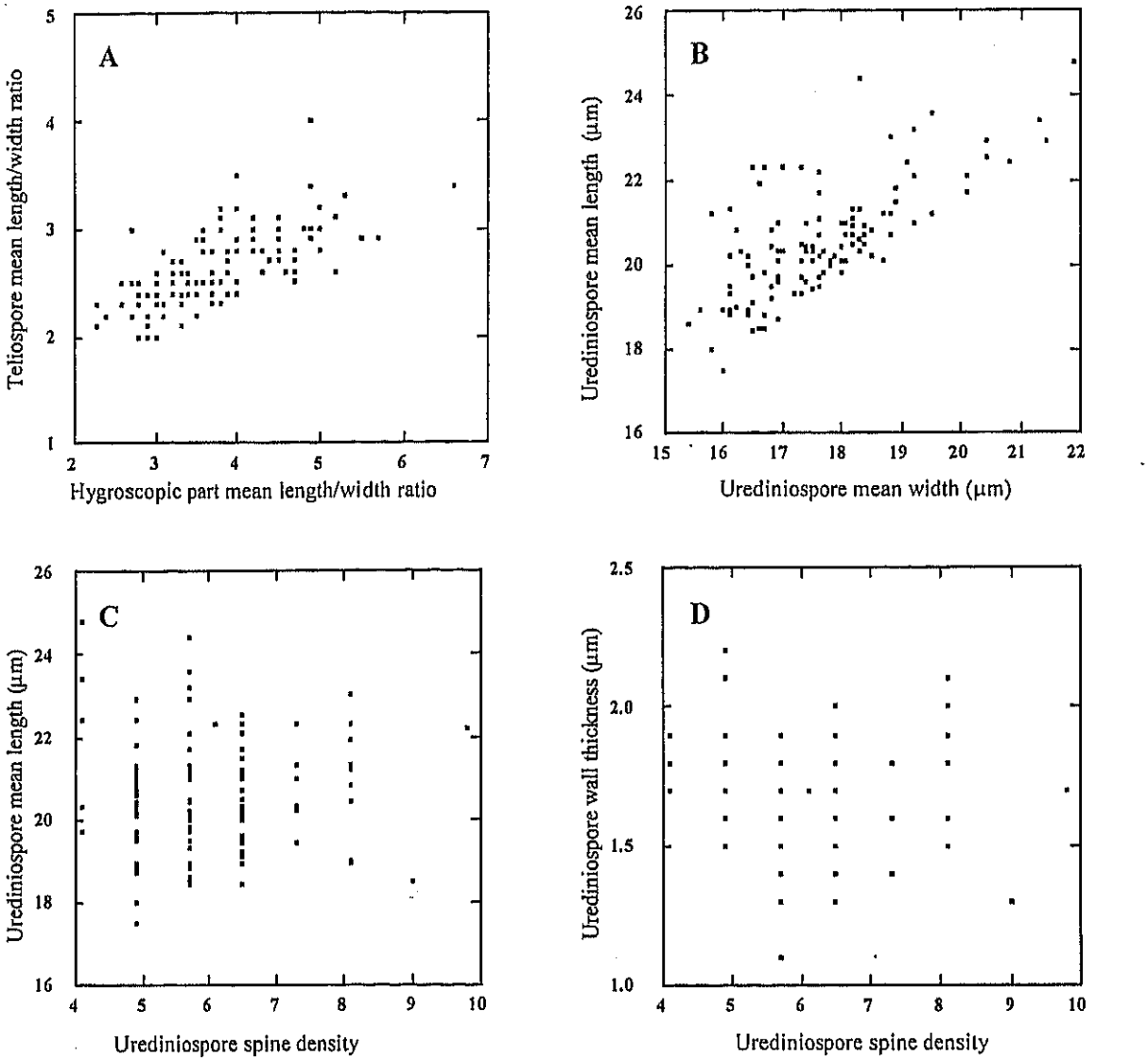

Fig. 2. Variations in the teliospore and urediniospore characteristics observed in the *Phragmidium* specimens. A. Relationship between the mean length/width ratio of teliospores and the mean length/width ratio of hygroscopic part of pedicels. B. Urediniospore mean lengths against urediniospore mean widths. C. Urediniospore mean lengths against mean spine density (echinulae/10 μm^2). D. Mean spine density (echinulae/10 μm^2) against mean urediniospore-wall thickness.

continuous and was not correlated with urediniospore size (Fig. 2C, not significant in Spearman's rank correlation analysis), wall thickness (Fig. 2D, not significant in Spearman's rank correlation analysis) or other urediniospore characteristics observed (data not shown).

Germ pores ranged from 5 (rarely 4) to 10 (rarely 11) with mean values of 6, 7 or 8 depending on specimens. Mean germ-pore numbers did not correlate with urediniospore size or wall thickness (data not shown). The pores were scattered over the wall. In certain specimens, the wall at the germ pores appeared to intrude into the urediniospore lumen. However, this attribute was not correlated with urediniospore size or wall thickness.

Uredinial-telial paraphyses were cylindrical to weakly clavate, weakly to strongly incurved. The wall was colorless, ventrally thin-walled (1.0–2.0 μm) and dorsally thick-walled (1.5–6.6 μm). The size of paraphyses varied from 34.8–74.9 × 12.8–26.4 μm in BPI0126671 on *R. rugosa* to 23.0–39.4 × 7.9–14.9 μm in PUR51814 on *R. mirifica*; mean length varied from 53.8 to 30.3 μm and mean width from 12.7 to 19.1 μm.

Principal component analyses Principal component analyses were undertaken with various combinations of numerical variables in urediniospores and teliospore morphology. Figure 3A is one of representative results. In this analysis, mean values of the following variables were used: paraphysis length and width, paraphysis-wall thickness, urediniospore length and width, urediniospore-wall thickness, teliospore-apiculus length, teliospore length and width, teliospore-wall thickness, pedicel length and width, and length and width of enlarged pedicel part. After the Varimax rotation, the calculated factors one

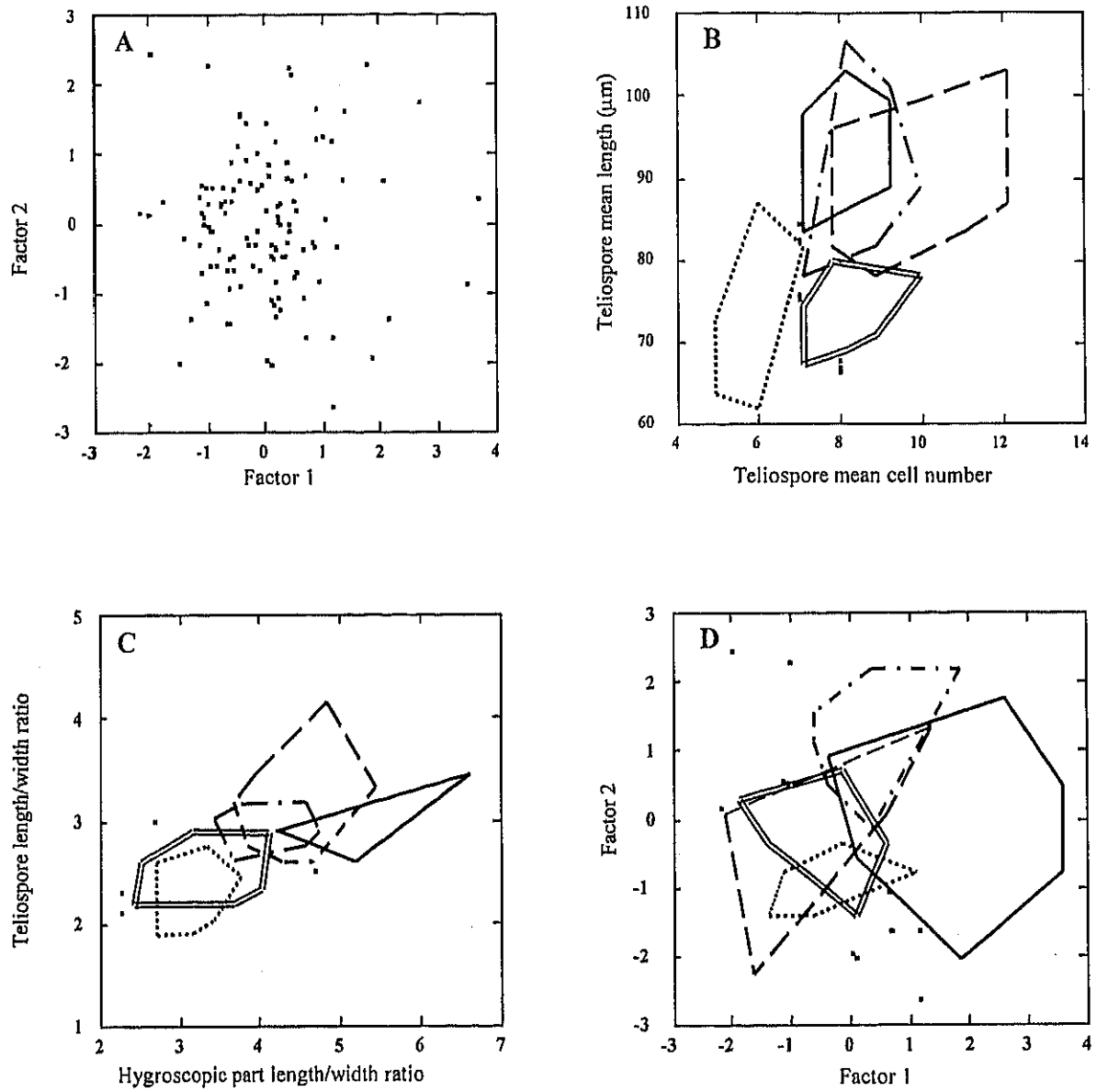

Fig. 3. Variations in the telial and uredinial characteristics observed in the *Phragmidium* specimens. A. A result of a principal component analysis. See the text for a detailed explanation. B. Teliospore dimensions observed among possible host-specific groups. C. Length/width ratios of teliospores and hygroscopic part of pedicels observed among possible host-specific groups. D. Distribution of possible host-specific groups in a scatter diagram generated by a principal component analysis. See text for a detailed explanation. –·–·: *Phragmidium americanum*; — —: *P. fusiforme*; ═══: *P. montivagum*; ·····: *P. mucronatum*; ——: *P. rosae-rugosae*.

and two explained 70.4% and 8.3% of the total variance, respectively.

The scatter diagram with factor one as the horizontal axis and the factor two as the vertical axis did not reveal discrete groups even after qualitative attributes, e.g., teliospore-wall color or teliospore-wall rugosity, were superimposed. The three-dimensional scatter diagram (not shown) with factors one, two and three also did not reveal any discrete groups.

Discussion

The causal species of the rose rusts under discussion have been circumscribed variously and classified by combinations of morphological characteristics of a maximum of four spore stages, which are produced in a sequence in the macrocyclic, autoecious life cycle. However, a uredinial or telial stage or both stages are frequently encountered in most herbarium specimens, and thus their features have been employed as taxonomic and key characters in the *Phragmidium* classification (Dietel, 1905, 1906; Arthur, 1934; Cummins, 1931), because a sper-

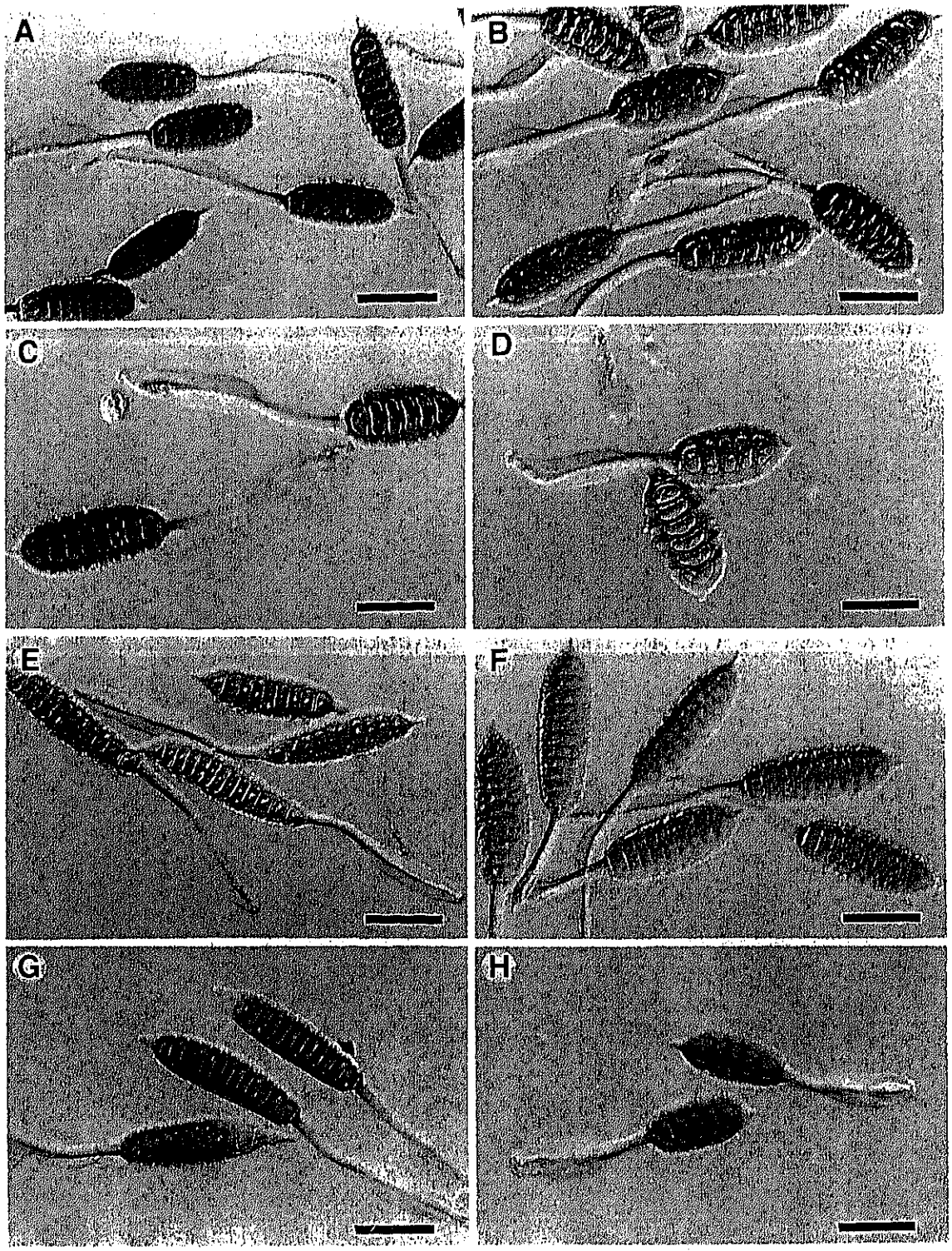

Fig. 4. Tellospore morphology observed in the *Phragmidium* specimens. A. PUR56692 on *Rosa manca*. B. PUR7847 on *R. fendleri*. C. BPI0126642 on *R. alba*. D. PURF9504 on *R. gallica*. E. PURF1548 on *R. pendulina*. F. PUR7925 on *R. acicularis*. G. IBA0311 on *R. acicularis*. H. PURF1507 on *R. gallica*. Scale bar=50 μm.

mogonial-aecial stage is produced in a short period during the early growing season.

Length, width, degrees of tapering toward both ends and apiculus length were believed to determine gross morphology of the tellospores; and these have been considered important taxonomic characters. The length/

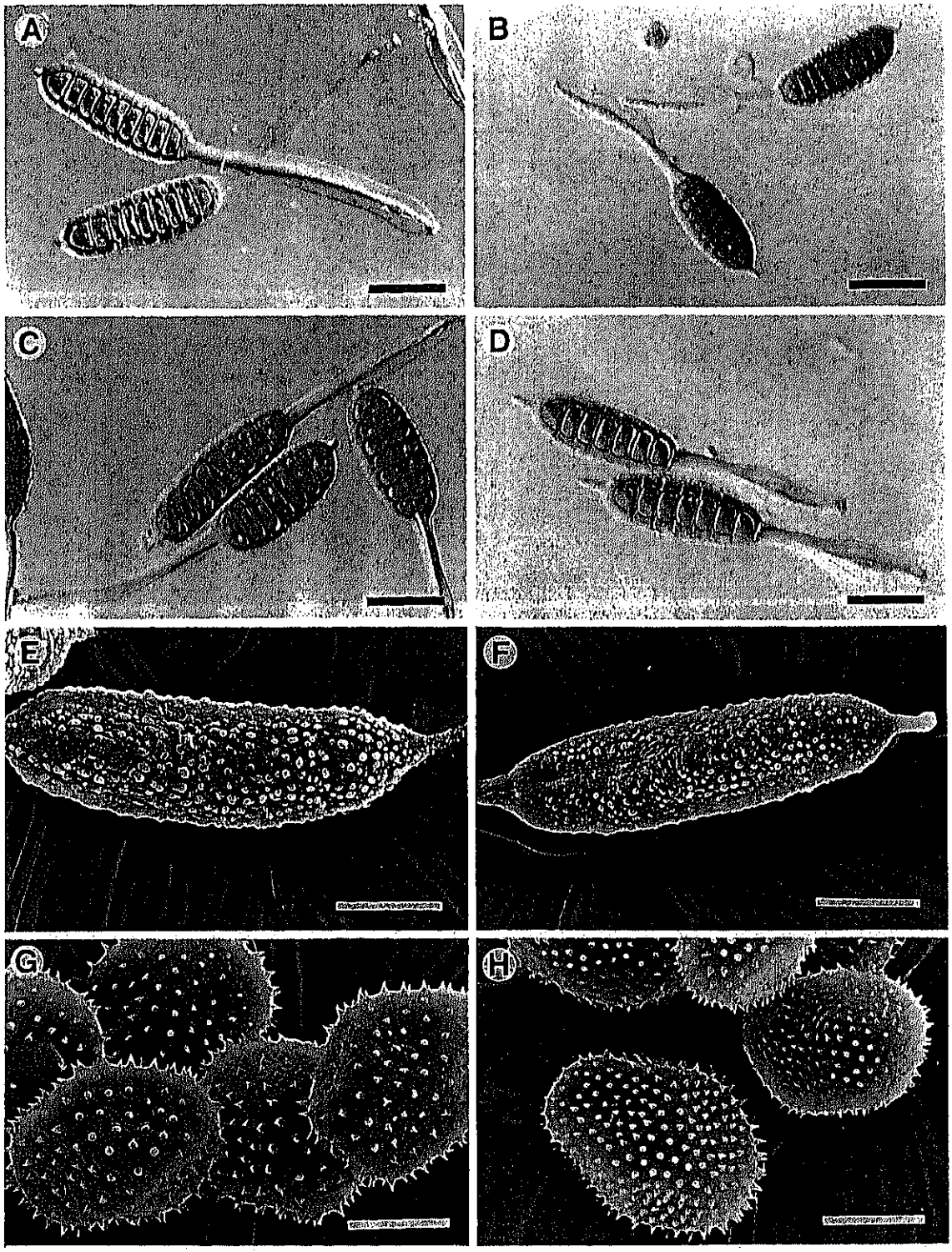

Fig. 5. Teliospore and urediniospore morphology observed in the *Phragmidium* specimens. A. Teliospores from SAPA (Ishikari) on *Rosa rugosa*. B. Teliospores from PUR48092 on *R. fendleri*. C. Teliospores from BPI0126669 on *R. rugosa*. D. Teliospores from PUR11834 on *R. rugosa*. E. Teliospore (SEM) from PUR7892 on *R. pendulina*. F. Teliospore (SEM) from BPI0126284 on *R. acicularis*. G. Urediniospores (SEM) from PUR55312 on *R. acicularis*. H. Urediniospores (SEM) from PUR59483 on *R. damascena*. Scale bars: A, B, C, D=50 μm; E, F=25 μm; G, H=10 μm.

width ratio was also considered as important. In addition to the gross morphology, cell number, wall color and surface rugosity have been used as taxonomic characters at the telial stage. Equally important was hygroscopicity of lower part of the pedicel. In contrast, only minuteness and density of echinae-verrucae on the wall surface, and sometimes wall thickness, have been stressed for urediniospores in distinguishing the species.

Thus, *P. mucronatum* was characterized by cylindrical teliospores composed of 5–9 cells and the size of 64–90 × 22–33 μm (Arthur, 1934; Cummins, 1931). Both ends of the teliospores were described as being rounded with a prominent apiculus of 7–13 μm long. The wall was dark chocolate-brown and coarsely verrucose. The pedicel length was about one and a half times the teliospore length, and the lower part of the pedicel becomes abruptly swollen to become broadly clavate or globose. Among related species, conspicuous hygroscopicity was reported only in this species (Cummins, 1931). The urediniospores were described as obovate or obovate-globoid and 20–26 × 16–19 μm (Arthur, 1934; Wilson and Henderson, 1966). The wall was closely echinulate with 8 or more indistinct, scattered pores.

Phragmidium mucronatum var. *americanum* was segregated from var. *mucronatum* because it possessed dark-walled teliospores with 8–10 cells (Peck, 1876). Var. *americanum* was raised to the species rank by Dietel (1905). This species has been characterized by long teliospores (80–100 μm) composed of 8–11 cells (Arthur, 1909) or by long telispores (64–125 μm) with dark chocolate-brown walls and thin-walled, moderately and finely verrucose aeciospres (Cummins, 1931).

Narrowly fusoid-cylindrical teliospores (7–13-celled, 90–110 × 19–30 μm) were said to be characteristic of *P. fusiforme* (Schröter, 1872). The wall was described as most finely verrucose among related species (Cummins, 1931).

Phragmidium tuberculatum was characterized by forming (3–)5(–6)-celled teliospores (54–81 × 27–35 μm) with an acute apiculus on a rounded apex (Müller, 1886). The wall was dark brown and verrucose. The lower half of the pedicel was swollen to clavate. Although not mentioned in the original description, the urediniospore wall at germ pores was stated to be conspicuously intruding in the spore lumen (Wilson and Henderson, 1966). This character of urediniospores was unique to this species among related species.

A fungus on *R. pimpinellifolia*, which was included in the "*P. subcorticium* group", was segregated and named as a new species, *P. rosae-pimpinellifoliae* (Dietel, 1906). This species was characterized by teliospores (6–8-celled, 65–87 × 28–30 μm) with a non-opaque, chestnut-brown wall. This is only the species having a translucent teliospore wall among related species (Cummins, 1931).

On the other hand, *P. montivagum* was described as distinct because it possessed 6–9-celled teliospores of 64–96 μm in length and 24–29 μm in width, with a conical subhyaline papilla (7–10 μm long) and a coarsely and moderately verrucose wall (Arthur, 1909). The urediniospores were stated to be obovate-globoid and 19–23 × 16–19 μm with a pale yellow and closely verrucose-echinulate wall (1–1.5 μm thick). Arthur (1909) stated that this species was distributed only in the Rocky Mountains and most variable morphologically among the species distributed in North America.

In comparison with *P. rosae-pimpinellifoliae*, Cummins (1931) stated that *P. montivagum* was similar to *P. rosae-pimpinellifoliae* in the teliospore size, measuring 64–95 × 24–32 μm. However, the two species were different in the teliospore-cell number (5–7 in *P. rosae-pimpinellifoliae* vs. 5–9 in *P. montivagum*) and the teliospore-wall color (dark chocolate-brown in the former and chestnut-brown and non-opaque in the latter).

Phragmidium rosae-rugosae was characterized by large cylindrical teliospores (7–11-celled, 72–128 × 28–32 μm) with lighter (yellowish brown) walls than those of related species (Kasai, 1910). The lower half of the pedicel was described as being only slightly swollen, while related species possessed pedicels with abruptly or gradually and moderately swollen lower parts.

As briefly described above, each of the seven species under consideration seems to be distinguishable by comparing the described characters. Thus, combinations of a few characters seem to be sufficient to separate these species, i.e., *P. americanum* by the large teliospores (up to 11-celled, up to 125 μm long), *P. fusiforme* by the fusoid-spindle-shaped teliospores, *P. mucronatum* by the abruptly swollen pedicels and the coarsely verrucose teliospore wall, *P. rosae-pimpinellifoliae* by the non-opaque chestnut-brown and finely verrucose teliospore wall, *P. rosae-rugosae* by the yellowish brown teliospore wall and the least hygroscopic pedicels and *P. tuberculatum* by the acute and long apiculi (up to 22 μm long) and the intruding wall at germ pores in the urediniospores.

However, it appears from the examinations of the uredinial and telial features in 119 specimens identified as one of the seven species, that no single morphological property or combination of properties is sufficient to distinguish morphological groups of the specimens that seem to correspond with the seven *Phragmidium* species under discussion. For example, mean size (Fig. 1A), cell number (Fig. 1B) and length/width ratio of the teliospores and pedicels (Fig. 2A) among others, continuously vary among the specimens and are not correlated with any other morphological features. Similarly, the mean apiculus length (Fig. 1C) continuously varies among the specimens. These results show that, using telial and uredinial features either singly or in combination, it is very difficult, if not impossible, to identify the seven causal species of the ornamental rose rusts.

Nevertheless, the non-discrete distribution patterns of uredinial-telial morphological features as described by continuous and/or discrete numerical variables do not necessarily mean that the specimens examined in the study comprise a single morphological taxon rather than two or more species. Each of the closely related but distinct rust taxa maintains characteristic biological properties. The properties of these taxa may or may not be

unique or distinct to each taxon. If a descriptor of the properties, particularly morphological properties is a continuous numerical variable, the properties of the taxa thus described are likely to overlap. When these morphological properties are employed as taxonomic characters, which is a common practice in the taxonomy of rust and other fungi, the distinction of taxa circumscribed in this way is likely to be difficult (see Stuessy (1990) for taxonomic characters and related terminology). Thus, it is not rare in the rust fungi that are reproductively isolated, host-restricted species are morphologically similar or even indistinguishable (Ono, 2001; Pfunder et al., 2001; Roy et al., 1998).

To overcome the current difficulties in distinguishing the *Phragmidium* species on ornamental roses, biological properties other than those described by a continuous numerical variable must be taken into account as taxonomic characters. One possible property that is currently available is the putative host specificity of the *Phragmidium* species. Assuming that the host identification and the putative host specificity are correct, certain *Rosa* species of the specimens examined are considered to harbor only one or two rust species. Accordingly, *R. blanda* and *R. setigera* harbor *P. americanum*; *R. acicularis*, *R. nutkana* and *R. pendulina* harbor *P. fusiforme*; *R. acicularis*, *R. engelmannii*, *R. fendleri*, *R. macounii*, *R. manca* and *R. mirifica* harbor *P. montivagum*; *R. alba*, *R. blanda* and *R. gallica* harbor *P. mucronatum*; *R. pimpinellifolia* harbors *P. rosae-pimpinellifoliae*; *R. canina* and *R. cinnamomea* harbor *P. tuberculatum*; and *R. rugosa* harbors *P. rosae-rugosae*. When these host species are plotted over the specimen scatter diagrams, host-specific morphological groups seem to be detected in the scatter diagrams of some teliospore-feature combinations, even though the ranges of groups overlap. In the scatter diagram with the tellospore cell number as a horizontal axis and the mean teliospore length as a vertical axis, the specimens that seem to correspond to *P. mucronatum*, *P. montivagum* and *P. fusiforme* are separated (Fig. 3B). However, those that seem to correspond to *P. americanum* and *P. rosae-rugosae* are not separated well and locate between those of *P. mucronatum* and *P. fusiforme*. The specimens that seem to correspond to *P. rosae-pimpinellifoliae* and *P. tuberculatum* are scattered over the range of *P. mucronatum*, *P. montivagum* and *P. fusiforme*.

In the scatter diagram with the length/width ratio of the enlargement in the pedicels as a horizontal axis and the teliospore length/width ratio as a vertical axis, five host-specific groups which seem to correspond to *P. fusiforme*, *P. montivagum* and *P. rosae-rugosae* are detected (Fig. 3C), while the distribution range of those specimens that seem to correspond to *P. americanum* and *P. mucronatum* overlap the specimens of *P. fusiforme*, *P. montivagum* and *P. rosae-rugosae*. The specimens that seem to correspond to *P. rosae-pimpinellifoliae* and *P. tuberculatum* are again enclosed in the range of both *P. montivagum* and *P. mucronatum*.

Similarly, in the scatter diagram generated from a principal component analysis, five host-specific groups, which are considered to correspond to *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum* and *P. rosae-rugosae*, appear to be morphologically circumscribed even though their distribution ranges overlap (Fig. 3D). Specimens that seem to correspond to *P. rosae-pimpinellifoliae* and *P. tuberculatum* are scattered over the whole range.

In contrast to the teliospore features, no host-specific groups are detected by urediniospore features, except for the wall-thickening over the urediniospore germ-pores, which seems to be characteristic of *P. tuberculatum*. Minuteness and density of verrucae-echinae in the urediniospores have been considered to be key characters in the taxonomy of the seven *Phragmidium* species. However, the urediniospore-surface ornamentation is exclusively echinulate and the density of echinae does not separate the host-specific groups.

Our results indicate that host-specific groups of the specimens that are considered to correspond to *P. americanum*, *P. fusiforme*, *P. montivagum*, *P. mucronatum* and *P. rosae-rugosae* can be circumscribed well by size, shape, cell number of teliospores, size and shape of pedicels and wall-thickening over urediniospore germpores. However, these morphological properties are considered not to be suitable as taxonomic characters for species identification. In order to find mutually exclusive morphological groups that correspond to the well-established species, further detailed analyses of the uredinial-telial properties together with the spermogonial-aecial properties of the *Phragmidium* species are now being undertaken.

Acknowledgments——A number of specimens examined in this study were loaned from the Arthur Herbarium, Purdue University (PUR); the Faculty of Agriculture, Hokkaido University (SAPA); the Botanical Garden and Museum, Berlin-Dahlem (B); the National Fungus Collections, the United States Department of Agriculture (BPI); and the Museum of Natural History, Wroclaw University (WRSL), for which we are grateful.

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Literature cited

Arthur, J. C. 1909. North America rose rusts. Torreya 9: 21–28.

Arthur, J. C. 1934. Manual of the rust in United States and Canada, Hafner Publ. Co., New York.

Azbukina, Z. M. 1984. Key to the rust fungi of the Soviet Far East. Nauka, Moscow. (In Russian.)

Beales, P., Cairns, T., Duncan, W., Fagan, G., Grant, W., Grapes, K., Harkness, P., Hughes, K., Mattock, J. and Ruston, D. 1998. Botanica's roses. Randomhouse Australia Pty. Ltd., Sydney.

Cummins, G. B. 1931. *Phragmidium* species of North America: Differential teliospore and aecial characters. Mycologia 23: 433–445.

Cummins, G. B. and Hiratsuka, Y. 1983. Illustrated genera of rust fungi. Revised edn. Amer. Phytopathol. Soc., St. Paul, Minnesota.

Dietel, P. 1905. Uber die arten der Gattung *Phragmidium*.

Hedwigia 44: 112–132.
Dietel, P. 1906. Uber die arten der Gattung *Phragmidium* II. Hedwigia 44: 330–346.
Gäumann, E. 1959. Die Rostpilze Metteleuropas. Beiträge zur Kryptogamenflora der Schweiz 12: 1–1407.
Hiratsuka, N. 1935. *Phragmidium* of Japan. Jpn. J. Bot. 7: 227–299.
Hiratsuka, N., Sato, S., Katsuya, K., Kakishima, M., Hiratsuka, Y., Kaneko, S., Ono, Y., Sato, T., Harada, Y., Hiratsuka, T. and Nakayama, K. 1992. The rust flora of Japan. Tsukuba Shuppankai, Tsukuba.
Hiratsuka, Y. 1973. The nuclear cycle and terminology of spore state in Uredinales. Mycologia 65: 432–443.
Horst, R. K. 1983. Compendium of rose disease. Amer. Phytopathol. Soc., St. Paul, Minnesota.
Howden, J. C. W. and Jacobs, L. 1973. Report on the rust work at Bath. Ann. Natl. Rose Soc. England 1973: 113–119.
Jennings, D. M., Ford-Ilyoid, B. V. and Butler, G. M. 1989. An aniline blue squash technique for observation of urediniospore germ pores. Mycol. Res. 92: 230–251.
Kasai, M. 1910. On the Japanese species of *Phragmidium*. Trans. Sapporo Nat. Hist. Soc. 3: 25–51.
Laundon, G. F. 1970. Additions to the rust fungi of New Zealand-5. New Zealand J. Bot. 8: 310–319.
Müller, J. 1886. Die Rostpilze der *Rosa*- und *Rubus*- Arten und die auf ihnen vorkomenden parasiten. Ber. Deutsch. Bot. Ges. 3: 391–395.
Ono, Y. 2000. Taxonomy of the *Phakopsora ampelopsidis* species complex on vitaceous hosts in Asia including a new species, *P. euvitis*. Mycologia 92: 154–173.
Phillips, R. and M. Rix. 1994. Roses. Pan Books Ltd., London.
Peck, C. H. 1876. Report of the botanist. Ann. Rep. New York State Mus. 28: 31–88.
Pirone, P. P., Dodge, B. O. and Ricket, H. W. 1960. Disease and pests of ornamental plants. 3rd edn. Ronald Press Co., New York.
Pfunder, M., Schürch, S. and Roy, B. A. 2001. Sequence variation and geographic distribution of pseudoflower-forming rust fungi (*Uromyces pisi* s. lat.) on *Euphorbia cyparissias*. Mycol. Res. 105: 57–66.
Rayner, R. W. 1970. A mycological colour chart. Commonwealth Mycological Institute, Kew.
Roy, B. A., Vogler, D. R., Bruns, T. D. and Szaro, T. M. 1998. Cryptic species in the *Puccinia monoica* complex. Mycologia 90: 846–853.
Schröter, J. 1872. Die Brand- und Rostpilze Schlesiens. Abh. Schles. Ges. Nat. Med. 1869–72: 1–31.
Stuessy, T. F. 1990. Plant taxonomy. The systematic evaluation of comparative data. Columbia Univ. Press, New York.
Wei, S. 1988. A taxonomic study of the genus *Phragmidium* of China. Mycosystema 1: 179–210.
Wilkinson, L. 1989. SYSTAT: the system for statistics. Version 5.2 edition. Systat, Inc., Evanston, Illinois.
Wilson, M. and Henderson, D. M. 1966. British rust fungi. Cambridge Univ. Press, London.

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A new species of *Exobasidium* causing witches' broom on *Rhododendron wadanum*

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Witches' broom on *Rhododendron wadanum* has been observed since 1994 in Nagano Pref. and recently, in 2000, it was found at Mt. Tsukuba, Ibaraki Pref., Japan. In comparison with the morphology, the mode of germination of basidiospores and the cultural characteristics of the 99 validly described taxa, this fungus was considered as a new species and named *Exobasidium nobeyamense*.

Key Words——Basidiomycetes; Japan; *Rhododendron wadanum*; taxonomy; yeast-like growth.

Witches' broom on *Rhododendron wadanum* Makino has been observed since 1994 in Nagano Pref. and was found in 2000 at Mt. Tsukuba in Ibaraki Pref., Japan. *Rhododendron wadanum*, endemic in Japan, belongs to Subgen. *Sciadorhodion*, Sect. *Brachycalyx*. Most plants in this section are distributed in China and Japan. Two *Exobasidium* species have been reported on Sect. *Brachycalyx* (Ezuka, 1991a). Hymenia of *E. yoshinagae* Henn. appear on *R. wadanum*, *R. dilatatum* Miq., *R. kiyosumense* Makino, *R. reticulatum* D. Don, *R. viscistylum* Nakai var. *viscistylum*, *R. viscistylum* var. *glaucum* Sugim. and *R. weyrichii* Maxim. This fungus causes small leaf spot (*Exobasidium* leaf blister) (Ezuka, 1991a). On the other hand, *E. otanianum* Ezuka var. *otanianum* on *R. reticulatum* causes systemic infection of leaves and produces white hymenia on the lower side of leaves (*Exobasidium* leaf blight) (Ezuka, 1991a). Eventually, the infected leaves dry up and fall. *Exobasidium otanianum* var. *satsumense* X. Y. Zhang et K. Arai on *R. dilatatum* var. *satsumense* T. Yamaz. causes systemic infection of leaves and produces white hymenia on the lower side of leaves (*Exobasidium* leaf blight) (Zhang et al., 1995). These fungi cause symptoms on newly developing leaves but do not affect branches and twigs; accordingly witches' broom does not occur. Furthermore, *E. pentasporium* Shirai, described in 1896, causes witches' broom of *R. obtusum* (Lindl.) Planch. var. *kaempferi* (Planch.) E. H. Wilson and *R. macrosepalum* Maxim., which belong to other Subgen. *Tsutsusi* (Ezuka, 1990). However, no species was reported to cause witches' broom in Sect. *Brachycalyx*.

Therefore, we carried out morphological comparisons of the specimens collected in Ibaraki and Nagano Pref. with other *Exobasidium* spp. on *Rhododendron* spp. We propose the fungus producing witches' broom on *R. wadanum* as a new *Exobasidium* species.

Materials and Methods

Morphological observations Fresh specimens on *R. wadanum* collected in the field were used for morphological observations. Specimens examined are listed in the description of the species. Morphological observations were conducted by light and scanning electron microscopy. The basidiospores and conidia or thin sections of hymenia were mounted in Shear's solution on glass slides for LM observations. For SEM, the infected leaves with hymenia stored in FAA were used. Samples for SEM were prepared by modifying a standard procedure (Tanaka, 1992), using a glutaraldehyde (2% v/v) fixation procedure, dehydration, then t-butyl alcohol freeze-drying and coating with platinum-palladium in a high vacuum with a Hitachi E-1030 ion sputter. They were examined with a Hitachi S-4200 SEM operating at 15 kV.

Taxonomy

Exobasidium nobeyamense Nagao et Ezuka, sp. nov.
Figs. 1–6

Ramuli caespitosi in ramis cum nodis agangulioneis formantes. Hymenuim hypophyllum, effusum, saepe

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totam infrasuperficiem folii tegens. Folia infecta supra flava vel viridiflava, infra viridescentia dein albofarinosa, leviter carnosa. Basidia hyalina, clavato-cylindracea, 35–40×7–10 μm, terminaliter cum 3–6 sterigmatibus longiconicis 3.5–5.5×2–2.5 μm praedita. Basidiosporae hyalinae, laeves, cylindricae vel falcatae, ad apicem semiorbiculares, ad basim curvatae et angustatae, 12–21×2–5.5 μm, primo continuae dein 2–4 septatae, per hyphas germinantes. Conidia hyalina, continua, laevia, linearia, 7–12×1–2 μm. Coloniae in PDA restricte crescentes, ad ambitum irregulariter rugosae, ex hyphis circa 1 μm latis et conidiis constantes, pallide persicinae vel pallide aurantiacae, in agaro non pigmentiferae; reversum coloniis concolor.

Holotypus in foliis *Rhododendri wadani* Makino, Nobeyama, Minami-maki-mura, Minami-saku-gun, Nagano Pref. in Japonia, 25 V 1994. A. Ezuka leg., in Herbario Instituti Nationalis Scientiae Agro-Environmentalis, Tsukuba, Japania conservatus (NIAES 10569).

Etymology: Nobeyama, referring to the type locality.

Specimens examined: TSH-B0001, TSH-B0002 (Nagao 99775, 99776, Minami-maki-mura, Nagano Pref., May 26, 1999), TSH-B0003 (Nagao 12935, Mt. Tsukuba, Ibaraki Pref., May 25, 2000). All materials were deposited in the Herbarium of Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba (TSH).

Hymenium was composed of basidia with 3 to 6 sterigmata and conidia (Fig. 2A). Hyphae did not develop directly on the surface of the epidermis. Basidia were clavate to cylindrical, 35–40×7–10 μm. Sterigmata were 2–2.5 μm in diam at the base and 3.5–5.5 μm in height, emerging outwardly and tapering toward the tip (Fig. 2B). Basidiospores were ellipsoid to ovoid, 12–21×2–5.5 μm, hyaline, smooth, one-celled when formed, becoming 2–4-septated (Fig. 1A, C). Septate basidiospores germinated after 6 h when dropped on the agar surface (Fig. 3). Germ tubes were 3–5×2–3 μm, emerging from each cell and producing conidia at the tip 12 h after of the dropping. Hyphae grew into pseudohyphae and branched. Conidia were long fusiform, 7–12×1–2 μm and budded polarly (Fig. 1B, D). Conidia budded to produce daughter cells polarly but did not develop germ tubes or hyphae. Colonies on PDA grew tightly, heaping up to form a mound, and wrinkled irregularly around the periphery. Surface of colonies was pale pink to pale orange and rather dry in appearance. Colonies composed of branching, intricate hyphae and conidia. The reverse of colonies was also pale pink to pale orange. Dark pigment was not produced on PDA (Fig. 4). Colonies from conidia showed the same morphological features as those from basidiospores.

The taxonomy of *Exobasidium* has been subjected to controversy because of the simple morphology and the highly variable symptoms (Burt, 1915; Ezuka, 1991b; Nannfeldt, 1981; McNabb, 1962; Savile, 1959; Sundström, 1964). Savile (1959) examined the morphology of basidia, basidiospores and sterigmata and synonymized several species described on *Rhododendron* from various parts of the world into *Exobasidium vaccinii*. In his judgment, the shape and size of basidiospores, the width of basidia, the size and number of sterigmata are more valuable taxonomic characters. Ninety-nine taxa of *Exobasidium* have been validly described at present. Of these, 16 taxa show similarities in some morphological measurements. However, *E. burtii* Zeller differs in the number of septa. Six taxa, *E. asebi* Hara et Ezuka, *E. canadense* Savile, *E. caucasicum* Woron., *E. japonicum* Shirai var. *japonicum*, *E. japonicum* Shirai var. *hypophyllum* Ezuka and *E. unedonis* Mre, differ in the sizes of basidia and numbers of septa of basidiospores. Three taxa, *E. cylindricum* Ezuka, *E. otanianum* Ezuka var. *otanianum* and *E. gracile* (Shirai) Syd. et P. Syd., differ in the sizes of basidia but agree in the number of septa of basidiospores. *Exobasidium karstenii* Sacc. et Trott. differ in the sizes of basidia and the mode of germination of basidiospores (budding). Five taxa, *E. affine* Racib., *E. dimorphosporum* Savile, *E. euryae* Syd. et P. Syd., *E. hachijoense* Y. Otani et al. and *E. sakishimense* Y. Otani, differ in both sizes of basidiospores and number of septa of basidiospores. The new species on *R. wadanum* differs morphologically from *E. yoshinagae*, causing leaf blister on *R. wadanum* and *E. pentasporium*, and witches' broom on *R. obtusum* var. *kaempferi*. Basidiospores of the new species are narrower than those of *E. yoshinagae* at their middle part. Basidiospores of the new species also differ from those of *E. pentasporium* in the number of septa. Sterigmata of the new species emerge outwardly, while those of *E. pentasporium*

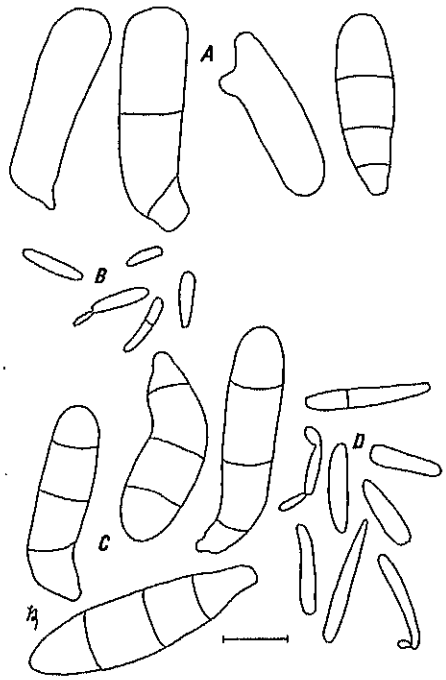


Fig. 1. Basidiospores and conidia of *Exobasidium nobeyamense* formed on the infected leaf of witches' broom on *Rhododendron wadanum*. Basidiospores (A) and conidia (B) collected in Nagano Pref. (TSH-B 0001). Basidiospores (C) and conidia (D) collected in Ibaraki Pref. (TSH-B 0003). Scale bar=10 μm.

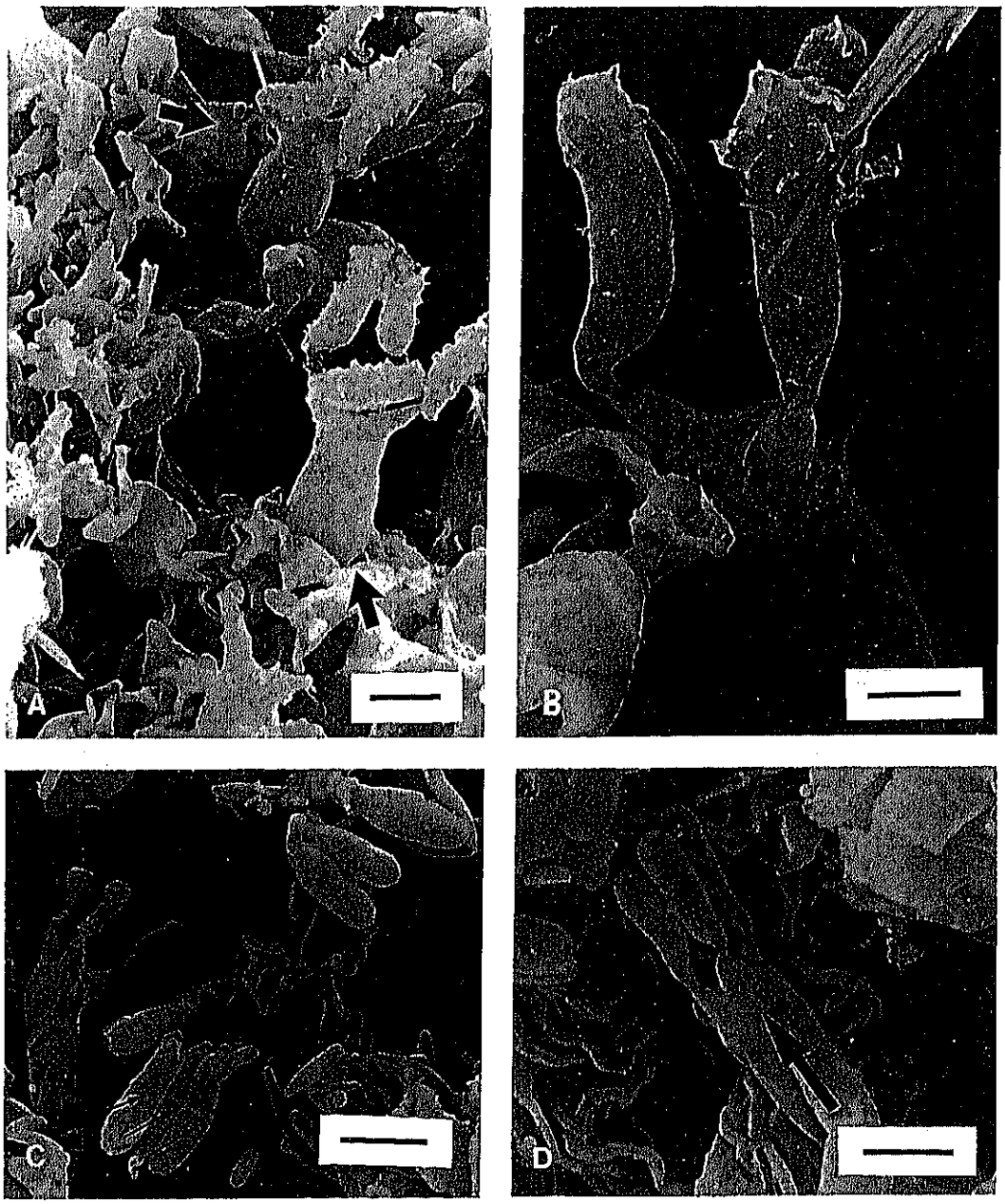
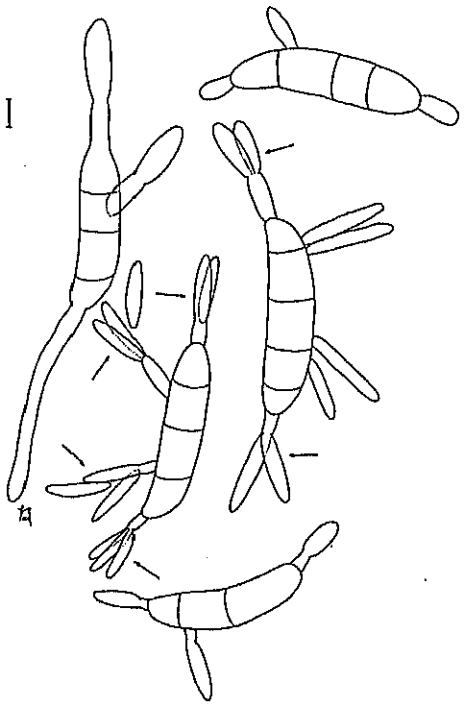


Fig. 2. Comparison of basidia of *E. nobeyamense* and *E. pentasporium*. *Exobasidium nobeyamense* observed by SEM. A. Hymenium of *E. nobeyamense* on *R. wadanum*. Arrows indicate two basidia bearing basidiospores. B. Basidium with immature basidiospores. Sterigmata were obviously emerging outwardly (arrowed). C. Hymenium of *E. pentasporium* on *R. obtusum* var. *kaempferi*. Septated basidiospores were observed (arrowed). D. Basidium with immature basidiospores. Sterigmata were emerging vertically (arrowed). Scale bar=5 μm.

emerge vertically (Fig. 2C, D). Basidiospores germinate into a germ tube in all these species, but the new species differs from *E. pentasporium* in the number of germ tubes. Colonies of *E. pentasporium* on PDA produce dark pigment, but cultures of *E. nobeyamense* and *E. yoshinagae* do not show dark pigmentation on PDA (Fig. 4).

Graafland (1960) performed cross-inoculations between *E. vaccinii* and *E. japonicum* var. *japonicum*, which were assigned to one species because of the morphological similarities by Savile (1959). These cross-inoculations demonstrated a difference in pathogenicity between these fungi. Graafland (1960) considered that the physiological specialization of the fungus and the difference in aspect of the pure cultures were sufficient to distinguish the species. Although not all descriptions of *Exobasidium* species indicate the aspect of the pure cultures, this aspect would be as useful as the mode of germination to distinguish the species. In our observations, the germination mode and absence of dark pigment



in culture of the new species were distinctive characteristics.

Numerous twigs characterize witches' broom on *R. wadanum* and smaller young leaves, the abaxial surface of which is covered by white hymenia. Leaves from infected branches were smaller and slightly thicker than healthy ones, and white powdery hymenia were formed entirely or partially on the lower side of these leaves (Fig. 6). Hymenia were not observed on twigs and branches. Infected leaves then dried up rapidly, turned dark and fell. In witches' broom on *R. wadanum*, the branch was slightly thickened at the beginning of the infection. The number of twigs subsequently increased and the basis of the infected branch thickened year by year. The witches' broom grew to around 40×50 cm (Figs. 5ABC). Twigs on the infected branches did not thicken. Witches' broom on *R. obtusum* var. *kaempferi* infected by *E. pentasporium* formed a gall at the basis of infected branches (Fig. 5D). However, gall formation on *R.*

Fig. 3. Germination of the basidiospore of *E. nobeyamense* of witches' broom on *R. wadanum* after 8 h of incubation. Some of the basidiospores produced conidia at the tip of germ tubes (arrowed). Scale bar=3 μm.

Fig. 4. Morphology and coloration of colonies formed by *E. nobeyamense* and *E. pentasporium* on PDA. Left, Surface of colonies of *E. nobeyamense* (upper) and *E. pentasporium* (lower). Unlike *E. pentasporium*, colonies of *E. nobeyamense* did not produce pigment on this medium. Right, Reverse of colonies of *E. nobeyamense* (upper) and *E. pentasporium* (lower). Submerged hypha in colonies of *E. nobeyamense* were not pigmented.

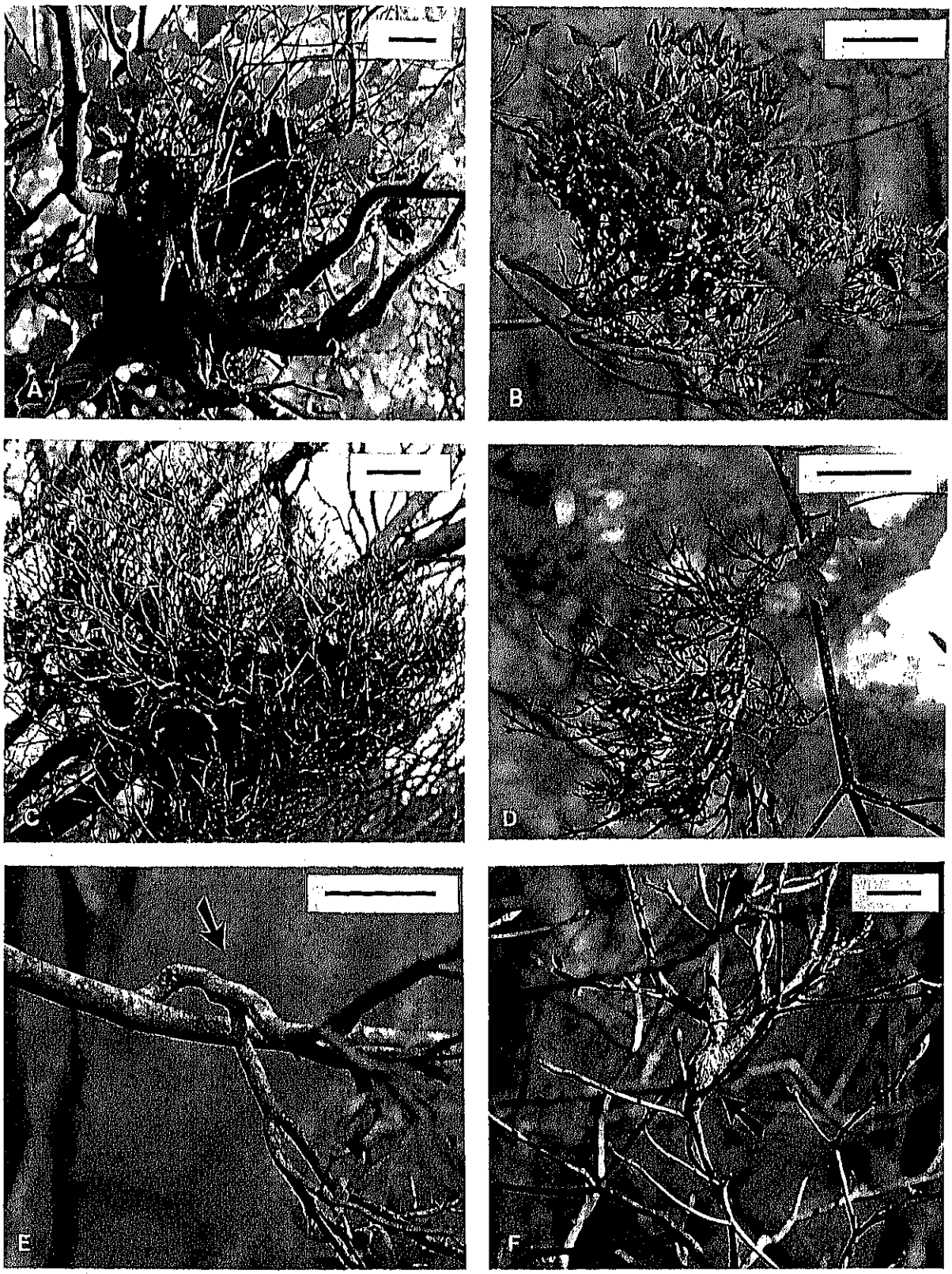


Fig. 5. Symptom of witches' broom on *Rhododendron* spp. by *Exobasidium* spp. A. Symptom of witches' broom observed on May, 2000 in Ibaraki Pref. B. Symptom of witches' broom observed on May, 1994 in Nagano Pref. C. Symptom of witches' broom before blooming observed on April, 2000 in Ibaraki Pref. D. Symptom of witches' broom on *R. obtusum* var. *kaempferi* by *E. pentasporium*. E. Typical symptom on branch of witches' broom on *R. wadanum* by *E. nobeyamense*. Infected branch was thickened but did not form gall (arrow). F. Symptom on branch of witches' broom on *R. wadanum* by *E. nobeyamense*. Infected branch formed round gall (arrow). Scale bars: A, B, C, D=50 mm; E=25 mm; F=20 mm.

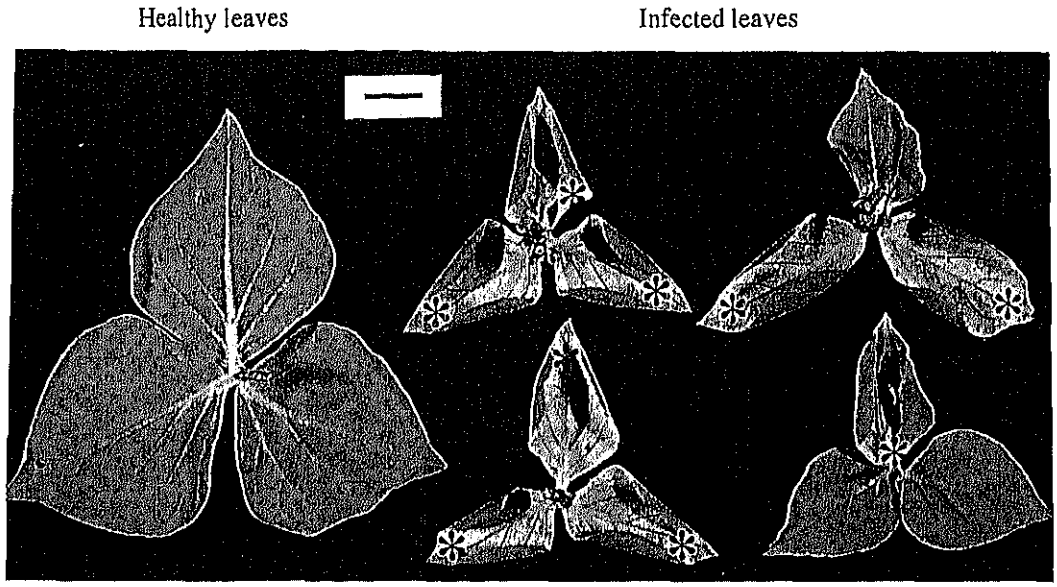


Fig. 6. Symptoms on leaf of witches' broom on *R. wadanum* by *E. nobeyamense*. White hymenia occurred on the lower surface of leaves (middle and right) and the infected leaves were smaller than healthy leaves (left). White hymenia formed partially on the lower surface of leaves (right). Asterisks indicate the regions of hymenia. Scale bar=10 mm.

wadanum by *E. nobeyamense* was rarely observed at the basis of infected branches of witches' broom (Fig. 5E). No gall was observed on *R. wadanum* in Nagano Pref., but galls were found on the specimens in Ibaraki Pref. (Fig. 5F). *Rhododendron wadanum* infected by *E. yoshinagae* exhibited *Exobasidium* leaf blister but not witches' broom (Ezuka, 1991a). In most cases, blooming decreased on infected branches.

In contrast, the symptoms on *R. obtusum* var. *kaempferi* caused by *E. pentasporium* were characterized by increased number of twigs, smaller infected leaves, no blooming and formation of a round gall at the base of infected branches. Thickening of infected branches is a typical symptom of witches' broom on *R. wadanum* except a case observed at Mt. Tsukuba, Ibaraki Pref. Most of witches' brooms at Mt. Tsukuba were larger than those in Minami-maki-mura, Nagano Pref. Thickening of branches and the increase in the number of twigs of *R. wadanum* are considered to be the results of physiological reactions to *Exobasidium* infection, and therefore the mechanism of gall formation, and the effect of the age of infected plants and growth conditions should be studied *in situ* and/or with inoculation tests.

The difference in host plant and morphological characters of the species producing witches' broom on *R. wadanum* suggest that it is a new species.

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Literature cited

Burt, E. A. 1915. The Thelephoraceae of North America. IV. *Exobasidium*. Ann. Mo. Bot. Gard. **2**: 627–659.

Ezuka, A. 1990. Notes on some species of *Exobasidium* in Japan (II). Trans. Mycol. Soc. Japan **31**: 439–455.

Ezuka, A. 1991a. Notes on some species of *Exobasidium* in Japan (III). Trans. Mycol. Soc. Japan **32**: 71–86.

Ezuka, A. 1991b. Notes on some species of *Exobasidium* in Japan (IV). Trans. Mycol. Soc. Japan **32**: 169–185.

Graafland, W. 1960. The parasitism of *Exobasidium japonicum* Shir. on Azalea. Acta Botanica Neerlandica **9**: 347–379.

McNabb, R. F. R. 1962. The genus *Exobasidium* in New Zealand. Trans. Roy. Soc. N. Z. **1**: 259–268.

Nannfeldt, J. A. 1981. *Exobasidium*, a taxonomic reassessment applied to the European species. Symb. Bot. Ups. **28**: 1–72.

Savile, D. B. O. 1959. Notes on *Exobasidium*. Can. J. Bot. **37**: 641–656.

Sundström, K. R. 1964. Physiological and morphological studies of some species belonging to the genus *Exobasidium*. Phytopathol. Z. **40**: 213–217.

Tanaka, K. 1992. *Sousa-densi-kenbikyo-gijutu*. Koudansya, Tokyo. (In Japanese.)

Zhang, X. Y., Arai, K., Sakoda, T. and Iwai, H. 1995. A new variety of *Exobasidium otanianum* isolated from *Rhododendron dilatatum* var. *satsumense*. Nippon Kingakukai Kaiho **36**: 97–102.

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Phylogenetic Analysis of Sugarcane Rusts Based on Sequences of ITS, 5.8 S rDNA and D1/D2 Regions of LSU rDNA†

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ABSTRACT

Phylogenetic analysis of sugarcane rusts based on sequences of ITS and the 5.8 S rDNA revealed two highly divergent ITS groups among isolates of *Puccinia* sp. sensu Muta, 1987 and *P. kuehnii* specimens. Although there is sufficient divergence (exceeding normal intraspecific variation) between the ITS regions of the two groups to support separation into different species, unusually high homology of the ITS group I sequences with those of members of *Cronartium* and identical sequences of the D1/D2 regions of the LSU rDNA for all the isolates of "*Puccinia* sp." and *P. kuehnii* that otherwise exhibited different ITS sequences, suggest that the two highly divergent sequences may have resulted from abnormal genetic events leading to non-orthologous, intraspecific polymorphisms. The other sugarcane rust, *P. melanocephala* and the grass rusts, *P. miscanthi* and *P. rufipes*, were separated from "*Puccinia* sp." and *P. kuehnii* and from each other in D1/D2 region analyses, indicating that D1/D2 region sequences may more correctly reflect phylogenetic relationships in these rusts than do the ITS regions. Further studies to examine differences in pathogenicity or finer morphological features within *P. kuehnii* that may be correlated with the high divergence in ITS sequences and experiments to determine if these two sequence types represent intraspecific polymorphism are necessary.

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Key words : sugarcane rusts, *Puccinia kuehnii*, *P. melanocephala*, phylogenetic analysis, ITS, D1/D2 region.

INTRODUCTION

Rust is considered one of the important diseases affecting sugarcane (*Saccharum officinarum* L.) and when occurring in epidemic proportions may cause heavy economic losses[26,27,40]. Presently, two species of rust fungi are known to cause rust in sugarcane ; namely, *Puccinia melanocephala* Syd. et P. Syd. and *Puccinia kuehnii* Butler[3,29,33]. *P. melanocephala* is known as the common rust and is reported in a wider area of distribution, including India, China, Asia-Pacific, Africa, Australia, South and North America. *P. kuehnii*, known as the orange rust, has a more limited distribution, including Asia-Pacific, Australia, India and China[5]. In addition to these two species, Muta[16] reported a "*Puccinia* sp." as a rust pathogen of sugarcane in Nansei Shoto, Kagoshima Pref., Japan. It was similar to *P. kuehnii* except for the absence of paraphyses in its uredinia and telia[16]. Likewise, the two previously reported sugarcane rusts are also quite commonly misidentified and confused for each other in some early reports[2,5,23,25,38]. To clarify the taxonomy of these sugarcane rusts, we are currently comparing their morphology.

Molecular phylogenetic analyses of rust fungi are not as widely done as are those with other fungi because rust fungi are obligate parasites that are impossible or difficult to obtain or maintain in pure culture. However, the advent of the polymerase chain reaction and related techniques has allowed analysis of DNA sequences from specimens other than pure cultures of obligate parasites such as members of the Uredinales[14,17,34,44,46] and Erysiphales[12,30,39]. Using our simplified method to extract DNA from a small amount of spores of rust fungi including samples from dried herbarium specimens[43], we used molecular phylogenetic analysis to determine phylogenetic relationships among these rusts to help clarify the taxonomic position of "*Puccinia* sp." and the taxonomy of the sugarcane rusts in general. In this study, in addition to sequences of the ITS regions, which are commonly used to resolve phylogenetic relationships between and within species in fungi, we also determined and analyzed

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sequences of the variable D1/D2 region at the 5′ end of the LSU rDNA gene.

MATERIALS AND METHODS

Materials Forty-five specimens and isolates of "*Puccinia* sp.", *P. kuehnii*, and *P. melanocephala* were used for phylogenetic analysis (Tables 1 and 2). Specimens used for DNA analysis were either fresh specimens stored at −80°C prior to extraction or dry herbarium specimens. For comparison and as outgroup taxa, a rust of *Miscanthus sinensis*, *P. miscanthi*, and a rust of *Imperata cylindrica*, *P. rufipes*, were also included in the analysis.

PCR amplification and sequencing of ITS and D1/D2 regions DNA was extracted from about 100-200 urediniospores obtained from a single uredinium. Spores were crushed between two sterile glass slides and suspended in 20 μl extraction buffer containing 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% Proteinase K, 0.01% SDS, then incubated at 37°C for 60 min, then at 95°C for 10 min[36,43]. From this crude extract, a 5 μl sample was used directly for each PCR amplification. Amplifications were done using 100 μl PCR reactions each containing 2 μM of each primer, 2.5 units of TaKaRa Ex Taq DNA polymerase (Takara, Japan), and the supplied dNTP mixture (containing 2.5 mM of each dNTP) and Ex Taq reaction buffer (containing 2 mM Mg^{2+}). PCR was carried out using an ATTO Zymoreactor II (Atto Co., Japan) under the following conditions : 95°C for 3 min, then 35 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min, and a final step of 72°C for 10 min. PCR of the ITS regions used the primer pair ITS 1F (5′-CTTGGTCATTTAGAGGAAGTAA)[8] and ITS 4 (5′-TCCTCCGCTTATTGATATGC)[45], while the D1/D2 region was amplified using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG)[19] and NL4 (5′-GGTCCGTGTTT

Table 1. Rust isolates and specimens used in phylogenetic analysis of ITS regions

Voucher specimen[a]	Host plant (Cultivar)	Locality	Date collected	DNA sequence accession No.
Puccinia sp.				
TSH-R11164	*Saccharum officinarum* (Ni6)	Naha, Japan	1997.6.18	AJ406050
TSH-R11165	*S. officinarum* (RK89-241)	Miyako, Japan	1997.6.19	AJ406051
TSH-R11169	*S. officinarum* (Nco310)	Naha, Japan	1997.6.18	AJ406053
TSH-R11142	*S. officinarum* (NiF5)	Amamioshima, Japan	1996.10.29	AJ406054
TSH-R11170	*S. officinarum* (Nco310)	Naha, Japan	1997.6.18	AJ406052
TSH-R11174	*S. officinarum* (RK89-241)	Miyako, Japan	1997.6.19	AJ406055
TSH-R11002	*S. officinarum* (F177)	Amamioshima, Japan	1996.6.14	AJ406056
TSH-R11319	*S. officinarum* (KF75-398)	Tanegashima, Japan	1991.2.1	AJ406057
TSH-R11108	*S. officinarum*	Iriomote, Japan	1996.11.30	AJ406058
TSH-R11129	*S. officinarum* (Nco310)	Ishigaki, Japan	1996.11.29	AJ406059
TSH-R11146	*S. officinarum* (Nco310)	Miyako, Japan	1996.12.1	AJ406060
TSH-R11221	*S. officinarum*	Negros, Philippines	1996.8.16	AJ406061
TSH-R11097	*S. officinarum*	Nago, Japan	1996.12.4	AJ406062
TSH-R11110	*S. officinarum*	Iriomote, Japan	1996.11.30	AJ406063
TSH-R11303	*S. officinarum*	Amamioshima, Japan	1996.3	AJ406065
TSH-R11113	*S. officinarum* (NiF4)	Ishigaki, Japan	1996.11.29	AJ406066
TSH-R11125	*S. officinarum* (RK89-241)	Ishigaki, Japan	1996.11.29	AJ406067
TSH-R11139	*S. officinarum* (RK89-241)	Miyako, Japan	1996.12.1	AJ406068
P. kuehnii				
BPI-79612	*S. officinarum*	Australia	1935.9.15	AJ 406048
BPI-79624	*S. officinarum*	Hawaii	1916.6.26	AJ 406049
P. melanocephala				
TSH-R11402	*S. officinarum* (RN82-311)	Amamioshima, Japan	1997.2.2	AJ406064
TSH-R11403	*S. officinarum* (F176)	Amamioshima, Japan	1996.7.8.	AJ406069
TSH-R11404	*S. officinarum* (RN82-311)	Amamioshima, Japan	1996.11.1	AJ406070
P. rufipes				
TSH-R11602	*Imperata cylindrica*	Tsukuba, Japan	1998.11.3	AJ406071
P. miscanthi				
TSH-R11503	*Miscanthus sinensis*	Tsukuba, Japan	1996.10.31	AJ406072

a) TSH-, Mycological Herbarium, University of Tsukuba, Japan ; BPI-, USDA National Fungus Collections, USA.

Table 2. Rust isolates and specimens used in phylogenetic analysis of D1/D2 regions

Voucher specimen[a]	Host plant (Cultivar)	Locality	Date collected	DNA sequence accession No.
Puccinia sp.				
TSH-R11163	*Saccharum officinarum* (Nco310)	Naha, Japan	1997.6.18	AJ296527
TSH-R11165[b]	*S. officinarum* (RK89-241)	Miyako, Japan	1997.6.19	AJ296528
TSH-R11166	*S. officinarum* (Nco310)	Miyako, Japan	1997.6.19	AJ296529
TSH-R11140	*S. officinarum* (KF75-398)	Amamioshima, Japan	1997.10.18	AJ296530
TSH-R11167	*S. officinarum* (NiN7)	Okinawa, Japan	1996.10.30	AJ296531
TSH-R11002	*S. officinarum* (F177)	Amamioshima, Japan	1996.6.14	AJ296532
TSH-R11030	*S. officinarum* (F176)	Naha, Japan	1996.12.3	AJ296533
TSH-R11108[b]	*S. officinarum*	Iriomote, Japan	1996.11.30	AJ296534
TSH-R11129[b]	*S. officinarum* (Nco310)	Ishigaki, Japan	1996.11.29	AJ296535
TSH-R11001	*S. officinarum*	Amamioshima, Japan	1996.6.14	AJ296536
TSH-R11036	*S. officinarum*	Tanegashima, Japan	1996.10.29	AJ296537
TSH-R11061	*S. officinarum*	Naha, Japan	1996.12.3	AJ296538
TSH-R11113[b]	*S. officinarum* (NiF4)	Ishigaki, Japan	1996.11.29	AJ296539
TSH-R11125[b]	*S. officinarum* (RK89-241)	Ishigaki, Japan	1996.11.29	AJ296540
TSH-R11139[b]	*S. officinarum* (RK89-241)	Miyako, Japan	1996.12.1	AJ296541
TSH-R11209	*S. officinarum*	Negros, Philippines	1996.8.16	AJ296542
P. kuehnii				
PUR	*S. officinarum*	Taiwan	1983	AJ296523
BPI-79612[b]	*S. officinarum*	Australia	1935.9.15	AJ296524
BPI-79624[b]	*S. officinarum*	Hawaii	1916.6.26	AJ296525
PDD-57590	*S. officinarum*	Hongkong	1990.7.7	AJ296526
P. melanocephala				
TSH-R11403[b]	*S. officinarum* (F176)	Amamioshima, Japan	1996.7.8	AJ296543
TSH-R11407	*S. officinarum* (RN82-311)	Amamioshima, Japan	1996.10.29	AJ296544
P. rufipes				
TSH-R11602[b]	*Imperata cylindrica*	Tsukuba, Japan	1998.11.3	AJ296545
P. miscanthi				
TSH-R11503[b]	*Miscanthus sinensis*	Tsukuba, Japan	1996.10.31	AJ296546

a) TSH-, Mycological Herbarium, University of Tsukuba, Japan ; BPI-, USDA National Fungus Collections, USA ; PUR-, Arthur Herbarium, Purdue University, USA ; PDD-, Landcare Research, Herbarium of Plant Disease Division, New Zealand.

b) Isolates used also in ITS region analysis.

CAAGACGG)[19]. After amplification, 8 μl of the reaction product was electrophoresed on 1% (w/v) agarose gels containing 0.5 μg/ml ethidium bromide in TAE buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.4). PCR products were purified using MicroSpin S-400 HR columns (Amersham Pharmacia Biotech, Buckinghamshire, England) and were sequenced directly using a Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Co., Foster, CA, USA) using the same primers used for PCR. Cycle sequencing reaction products were purified using Centri-Sep spin columns (Princeton Separations, Adelphia, NJ, USA). Data were collected using an ABI 377 Automated DNA Sequencer (Perkin Elmer Co., Foster, CA, USA).

Phylogenetic analysis Multiple alignment was performed using the programs Sequence Navigator v. 1.0.1 (Perkin Elmer Co., Foster, CA, USA) and Genetyx-Mac v. 10.1 (Software Development Co., Tokyo, Japan). Further visual alignment was done in portions of ITS1 and ITS2 and more variable portions of the D1/D2 region that were not aligned clearly by the programs. The aligned sequence data file is obtainable from the authors. Phylogenetic analysis of the data was done by distance and parsimony methods. The distance matrix for the aligned sequences was calculated using Kimura's two-parameter method[13] and was analyzed with the neighbor-joining (NJ) method[31] using the program Clustal W[41], excluding positions with gaps and correcting for multiple substitutions. Reliability of the inferred trees was estimated by 1000 bootstrap[7] resamplings using the same program. Parsimony analysis was done by PAUP v. 3.1.1[37] using the heuristic search option to search for the

most parsimonious tree(s). Insertions and deletions (single and multiple) in the aligned ITS sequences were not included as character-states because of ambiguous nucleotide positioning. However, for the more conservative D1/D2 region sequences, the few single isolated nucleotide indels were included as gaps as a fifth character in the analysis since they may be informative for inferring phylogenies. Bootstrap[7] values were generated with 100 replicate heuristic searches to estimate support for clade stability of the consensus tree using the same program.

In addition to sequences determined in this study, sequences of wheat leaf rust (*P. recondita* f. sp. *tritici*, accession number L08729), and flax rust (*Melampsora lini*, accession number L20283) were also obtained from GenBank Database and used for comparison and as outgroups in the analysis of D1/D2 regions.

RESULTS

The DNA sequences of the entire ITS regions (including the 5.8 S rDNA) of sugarcane rusts ranged from 605 to 654 bp. Of the 693 aligned bases, 272 varied among the sequences examined ; of the 509 bases selected for analysis, 190 bases were variable and 148 were informative sites for parsimony analysis. All sequences had the same length for the 5.8 S rDNA region.

ITS sequences of the sugarcane rust species examined in this study had a characteristic A nucleotide repeat site (12-13 bases) in the ITS2 region that sometimes resulted in unreadable nucleotide signals at the remaining regions after the repeat site in the electropherograms of both strands in certain isolates. Complete ITS sequences were determined for these isolates by assembling data from both strands at the location of the repeats. The incoherent electropherogram nucleotide signals could be attributed to variability in the number of repeating A nucleotides within an individual and could be better examined by cloning PCR products from the same isolate. However, since the signals immediately before the repeat sites were very clear, assembling single-strand data at these locations was considered sufficient to determine the entire ITS sequences for these isolates. Furthermore, comparison with double strand data from isolates that otherwise exhibited clear nucleotide signals throughout the entire region showed that single-strand assembly data are cor-

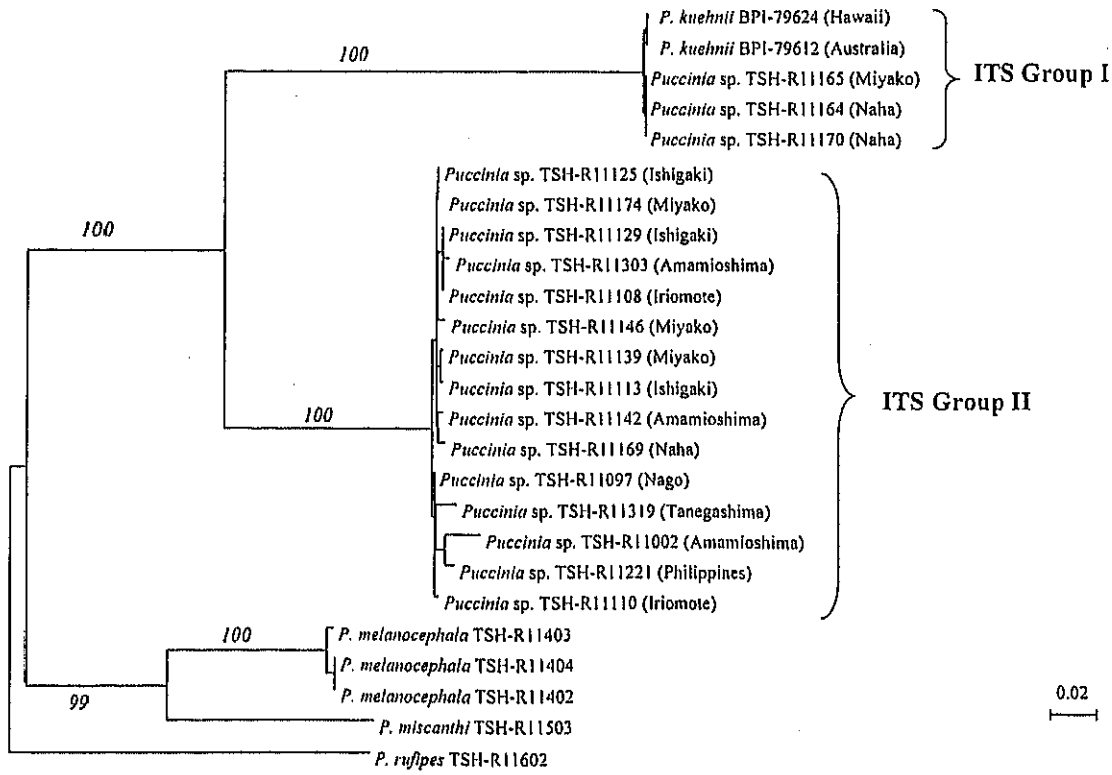

Fig. 1. Neighbor-joining tree inferred from sequences of ITS and 5.8 S rDNA regions using Clustal W. Values above the branches indicate percentage bootstrap support for 1000 resamplings. Length of branches is proportional to number of base changes indicated by the scale above. Isolates used in the analysis are listed in Table 1.

Table 3. Comparison of divergence within and between ITS groups I and II isolates

	Total		ITS1		5.8S		ITS2		D1/D2	
	Length[a]	% Ndiv.[b]	Length	% Ndiv.	Length	% Ndiv.	Length	% Ndiv.	Length	% Ndiv.
Within group I	654	0	244	0	155	0	255	0	624	0
Within group II	627-630	1.3-2.1	201-202	1.0-3.0	155	0	271-274	2.2-3.7	624	0
Between I and II		32.5-34.3		41.4-44.7		1.3		38.8-41.8		0

a) In basepairs (bp).

b) Shows percentage nucleotide divergence in all pairwise comparisons of different sequences.

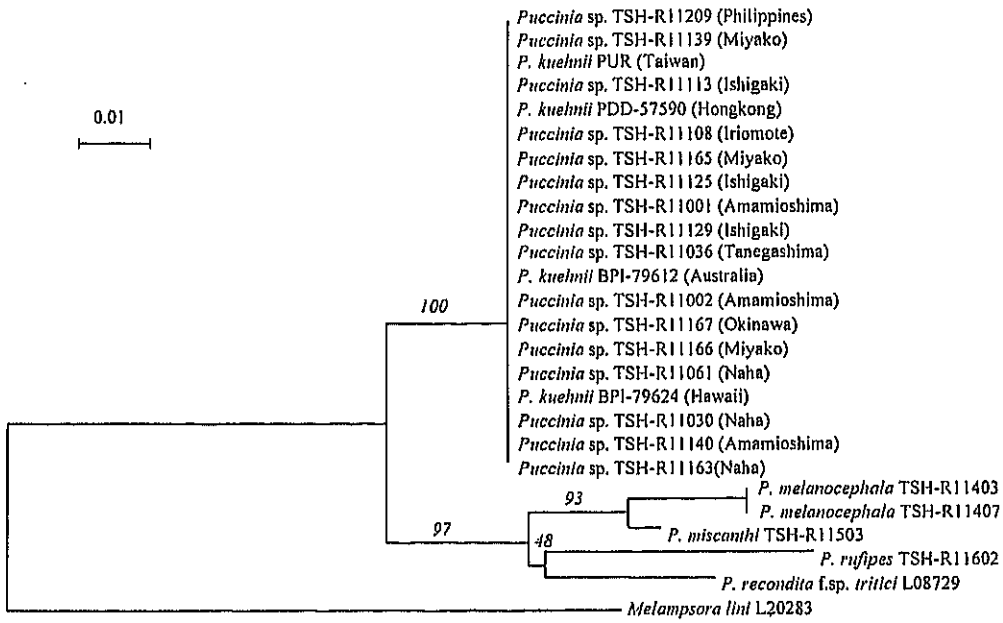

Fig. 2. Neighbor-joining tree inferred from sequences of D1/D2 region of the 28S rDNA using Clustal W. Values above the branches indicate percentage bootstrap support for 1000 resamplings. Length of branches is proportional to number of base changes indicated by the scale above. Isolates used in the analysis are listed in Table 2.

rect.

Neighbor-joining tree constructed from the ITS and 5.8 S rDNA regions showed that specimens of "*Puccinia* sp." collected from Nansei Shoto Islands, Japan and the Philippines, and dry herbarium specimens identified as *P. kuehnii* can be separated into two distinct clades, referred to here respectively as ITS group I and II, with each clade having high bootstrap support (Fig. 1). The strict consensus tree among 78 most parsimonious trees (not shown) obtained using PAUP had similar topology and clade support as the neighbor-joining tree. However, specimens of "*Puccinia* sp." and *P. kuehnii* do not separate respectively into the two clades, wherein some specimens of "*Puccinia* sp." belong together with *P. kuehnii* specimens in the ITS group I clade. "*Puccinia* sp." specimens that bear teliospores similar to those of *P. kuehnii*[42] were found in both groups. We also observed that specimens of "*Puccinia* sp." belonging to ITS group I clade were collected from the same localities as some specimens in ITS group II clade (Naha and Miyako specimens).

Within members of the ITS group I, none of the nucleotides differed; sequences in this group matched perfectly for the 5.8 S rDNA and the intervening ITS 1 and 2 regions. On the other hand, for members of the second group, ITS group II, 1.3 to 2.1% of the nucleotides differed among isolates. Among the three regions, most variation between group I and II occurs in ITS 1 with the highest at 44.7% nucleotide difference, while the highest variation at ITS 2 is 41.8%. Thus, there is much divergence between Group I and II ITS sequences with very little intra-group divergence and no intermediate forms (Table 3).

Sequences of the D1/D2 region of the LSU rDNA determined for the isolates used in this study ranged from 604 to 620 bp in length. "*Puccinia* sp." and *P. kuehnii* had the same length for all isolates at 620 bp, *P.*

melanocephala had 608 bp, *P. miscanthi* had 604 bp, and *P. rufipes* had 610 bp. The D1/D2 region sequence for *P. recondita* f.sp. *tritici* and *Melampsora lini* used as an outgroup in this analysis had 544 bp and 556 bp, respectively. During alignment of the bases, regions in excess and external to the *P. recondita* f. sp. *tritici* sequences, which were not variable, were omitted, such that only 570 bp were aligned for the analysis . Although, there was not much variation among the rusts used in the study, the sequences provided sufficient characters for phylogenetic analysis. Of the 570 aligned bases, 156 varied among the sequences examined ; of the 523 bases selected for analysis, 122 bases were variable and 35 were informative sites for parsimony analysis. There was 0% nucleotide difference for all isolates of "*Puccinia* sp." and *P. kuehnii*.

Analysis from the more conserved sequences of the D1/D2 regions of the LSU rDNA showed that, unlike results from ITS regions, specimens of both "*Puccinia* sp." and *P. kuehnii* were grouped together in a single cluster separate from specimens of the other sugarcane rust, *P. melanocephala* (Fig. 2). The single most parsimonious tree (not shown) obtained using PAUP had similar topology and clade support as the neighbor-joining tree. Thus, although there was divergence in the ITS regions, D1/D2 region analysis group all "*Puccinia* sp." and *P. kuehnii* specimens together in a single clade in both neighbor-joining and maximum parsimony analyses. Meanwhile, isolates of the other species, *P. melanocephala*, *P. miscanthi* and *P. rufipes* formed separate clades, although *P. melanocephala* and *P. miscanthi* were more closely related than with *P. rufipes*.

DISCUSSION

The level of divergence between the ITS sequences of Group I and II in specimens of "*Puccinia* sp." and *P. kuehnii* is extraordinarily high and exceeds the normal expected variation in ITS regions for the same and even closely related species (Table 3). In fact, they exceed the 30% maximum allowable divergence that would be expected to give unambiguous and informative alignment for phylogenetic analysis[11]. This is perplexing because comparative morphological studies so far have not revealed any morphological characters that correspond to and separate the members of the two ITS groups enough to morphologically distinguish isolates of "*Puccinia* sp." and *P. kuehnii*[12]. Thus, this divergence suggests the possible occurrence of a cryptic species within *P. kuehnii sensu lato* that is not morphologically evident[28]. Reproductive isolation events could be sensitively reflected in the evolution of the nonconserved ITS regions such that phylogenetic relationships revealed by the ITS regions also reflect biological species relationships[20]. Since isolates of both types were found in similar localities, the ITS types may represent morphologically indistinguishable but reproductively isolated sympatric species within *P. kuehnii s. l.* Furthermore, the occurrence of, at most, two nucleotide differences between the highly conserved 5.8 S rDNA regions of the two ITS sequence types (a transversion with T in ITS type I instead of A in type II in the 22nd base, and a transition with A in type I instead of G in Type II at the 133rd base of the 155-bp-length region) supports the possibility that these two ITS groups represent separate species.

However, it is very interesting and perplexing that BLAST searches for ITS type I sequences conducted against the DNA databases yielded significantly high homology with ITS regions of rust species belonging to the genus *Cronartium* (at most 96% homology) rather than to the same members of the genus *Puccinia* (only 66 to 72%). On the other hand, ITS type II sequences had 63 to 67% homology with *Cronartium* species and 69 to 72% homology with other *Puccinia* species, showing that ITS type I sequences are unusually similar to sequences of rusts belonging to an entirely different group of rust fungi.

Despite the homogenizing effects of concerted evolution on the rDNA cluster[10,11,22], the different copies of the rDNA array within a single nucleus are all replicated independently, allowing, although rarely, independent mutation and crossover that can result in various levels of polymorphism in the gene cluster within individual cells[6,11]. Aside from these intraspecific polymorphisms that normally occur at low rates and mostly as length variations in both nontranscribed and transcribed regions of the gene[11], highly divergent and even non-orthologous ITS polymorphisms can occur through gene duplications or ancient interspecies hybridization that may subsequently escape the homogenizing effects of concerted evolution due to physical constraints within the genome[1,21]. These polymorphisms can then become fixed in a species, and result in the occurrence of non-orthologous sequences that may persist across many speciation events[1,35].

The unusually high homology of ITS type I sequences with those of a morphologically very distinct and apparently phylogenetically distant rust lineage and its high divergence from ITS type II sequences suggest that this ITS sequence type may have resulted from rare genetic events such as interspecific hybridization or ancient gene duplication events that occurred prior to the evolutionary radiation of the *Puccinia* lineage. Thus, it may represent a non-orthologous ITS region that is coexistent with the ITS type II sequences as intraspecific ITS polymorphisms within *P. kuehnii*. Unlike the ITS types observed in *Fusarium*[21] however, divergence observed in this study

spans the entire ITS regions and is at a much higher rate. In plants, non-orthologous ITS polymorphisms occurred in both ITS 1 and 2 regions but not the 5.8 S rDNA gene, and sequence divergences are much lower compared to those found in this study[35]. The occurrence of two nucleotide differences between the two ITS sequence types in the 5.8 S rDNA region indicate that this coding region could also be heterogeneous like the flanking spacer regions and be implicated in the putative events that led to heterogeneity in ITS sequences.

In contrast to results with the ITS regions, analysis of sequences of the D1/D2 regions of the LSU rDNA showed that all "*Puccinia* sp." and *P. kuehnii* specimens are grouped together in a single clade in both neighbor-joining and maximum parsimony analyses. The LSU rDNA is therefore sufficiently more conserved than the ITS regions among isolates of "*Puccinia* sp." and *P. kuehnii*. If the ITS sequence divergence reflects the existence of a cryptic species within *P. kuehnii s. l.*, such that ITS groups I and II represent different species, then these results indicate that despite being contiguous, the ITS and D1/D2 regions must have been affected by sufficiently different evolutionary processes[22], such that very high divergence in ITS regions is not concordant with variation in this relatively variable region of the LSU rDNA. Likewise, if they represent intraspecific polymorphism, then this shows that, unlike the 5.8 S rDNA gene, the LSU rDNA region is apparently not implicated in the putative hybridization or gene duplication events that resulted in the occurrence of heterogeneous ITS sequences. The discordance between tree phylogenies of ITS and D1/D2 sequences shows that only one or the other, or possibly neither of them, reflects the true species phylogeny[32]. In such cases, concordance with other gene tree phylogenies must be determined and the use of other regions or loci that more appropriately reflect the relationships is desirable[4,22].

However, although intraspecific divergence is apparently non-existent in D1/D2 regions in the case of the rusts of sugarcane and the two related grasses used in the study, they clearly delineate the more or less morphologically distinguishable taxonomic units (*P. kuehnii s. l.*, *P. melanocephala*, *P. miscanthi* and *P. rufipes*). It may thus more appropriately define species boundaries in this group of rust fungi. In other fungi, Peterson and Kurtzman[24] and Kurtzman and Robnett[15] examined the nucleotide sequence divergence in the 5′ end of the LSU rRNA and demonstrated that the amount of nucleotide substitutions in the region is sufficient to allow reliable separation of all known clinically significant yeast species. Also, O'Donnell[19] and Guadet *et al.*[9] demonstrated the utility of this divergent domain in the resolution of phylogenetic relationships of species of *Fusarium* and some of its hypocrealean relatives.

Furthermore, if these two ITS groups indeed represent phylogenetically distinct species, extremely high homology of the ITS group I sequences with the phylogenetically and morphologically different *Cronartium* members indicates a homoplastic pattern of evolution, wherein ITS tree phylogeny obscures true species phylogenetic relationships even with rusts of lineages outside *Puccinia*. It is thus more likely that the phylogeny of the D1/D2 region sequences, rather than the ITS sequences, reflect the true species phylogeny of these rusts. As such, it is more probable, although not a common event for ITS regions, that the two highly divergent ITS sequences represent intraspecific polymorphisms rather than interspecific divergence.

To clarify this, we need further studies to determine if this ITS sequence divergence is reflected in or correlated with other phenotypic characters, such as finer morphological differences or differences in pathogenicity. In contrast to early observations that *P. kuehnii* is a less serious and less prevalent pathogen than *P. melanocephala*[27,29], our recent field observations and inoculation experiments (unpublished results) as well as disease reports in Japan[18] show that *P. kuehnii* tends to be more widespread and to infect more cultivars than *P. melanocephala*. This may reflect a change in pathogenicity or the possible development of races with altered pathogenicity to newly developed sugarcane cultivars that apparently do not carry resistance to *P. kuehnii*. The pathogenicity of "*Puccinia* sp." and *P. kuehnii* must be examined to determine possible correlation with the ITS divergence. Differences in pathogenicity with *P. kuehnii* may be correlated with the molecular divergence observed in the ITS regions and may support separation into distinct taxonomic entities, either at the species or subspecific level. Likewise, the possibility that these ITS sequence divergences occur as intraspecific polymorphisms must be studied further.

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LITERATURE CITED

1. Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S. and Donoghue, M.J. (1995). The ITS region of nuclear ribosomal DNA : a valuable source of evidence on angiosperm phylogeny. Ann. Missouri Bot. Gard. 82 : 247-277.

2. Chona, B.L. and Munjal, R.L. (1950). *Puccinia kuehnii* (Krueg.) Butler on sugarcane in India. Curr. Sci. 5 : 151-152.
3. Cummins, G.B. and Hiratsuka, Y. (1983). Illustrated Genera of Rust Fungi. Revised edition. p. 95, American Phytopathology Society, St. Paul, Minnesota.
4. Doyle, J.J. (1992). Gene trees and species trees : molecular systematics as one-character state taxonomy. Syst. Bot. 17 : 144-163.
5. Egan, B.T. (1980). A review of the world distribution of *Puccinia* spp. attacking sugarcane. Proc. Intl. Sugarcane Tech. 17 : 1373-1381.
6. Fatehi, J. and Bridge, P. (1998). Detection of multiple rRNA-ITS regions in isolates of *Ascochyta*. Mycol. Res. 102 : 762-766.
7. Felsenstein, J. (1985). Confidence limits on phylogenies : an approach using the bootstrap. Evolution 39 : 783-791.
8. Gardes, M. and Bruns, T.D. (1993). ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Molecular Ecology 2 : 113-118.
9. Guadet, J., Julien, J., Lafay, J.F. and Brygoo, Y. (1989). Phylogeny of some *Fusarium* species, as determined by large-subunit rRNA sequence comparison. Mol. Biol. Evol. 6 : 227-242.
10. Hibbett, D.S. (1992). Ribosomal RNA and fungal systematics. Trans. Mycol. Soc. Japan 33 : 533-556.
11. Hillis, D.M. and Dixon, M.T. (1991). Ribosomal DNA : molecular evolution and phylogenetic inference. Quart. Rev. Biol. 64 : 411-453.
12. Hirata, T. and Takamatsu, S. (1996). Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37 : 265-270.
13. Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16 : 111-120.
14. Kropp, B.R., Hansen, D.R., Wolf, P.G., Flint, K.M. and Thomson, S.V. (1997). A study on the phylogeny of the Dyer's woad rust fungus and other species of *Puccinia* from Crucifers. Phytopathology 87 : 565-571.
15. Kurtzman, C.P. and Robnett, C.J. (1997). Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. J.Clin. Microbiol. 35 : 1216-1223.
16. Muta, T. (1987). Occurrence of sugarcane rust (*Puccinia melanocephala* and *Puccinia* sp.) in Kagoshima Prefecture. Proc. Assoc. Pl. Prot. Kyushu 33 : 36-38 (in Japanese with English summary).
17. Nakamura, H., Kaneko, S., Yamaoka, Y. and Kakishima, M. (1998). Differentiation of *Melampsora* rust species on willows in Japan using PCR-RFLP analysis of ITS regions of ribosomal DNA. Mycoscience 39 : 105-113.
18. Nojima, H. and Muta, T. (1993). Varietal resistance of sugarcane cultivars or lines to sugarcane rusts caused by *Puccinia melanocephala* and *Puccinia* sp. *In* Amami Island. Proc. Assoc. Pl. Prot. Kyushu 39 : 11-12 (in Japanese with English summary).
19. O'Donnell, K. (1993). *Fusarium* and its near relatives. *In* The Fungal Holomorph : Mitotic, Meiotic, and Pleomorphic Speciation in Fungal Systematics (Reynolds, D.R. and Taylor, J.W., eds.). pp. 225-236, CAB International, Wallingford, UK.
20. O'Donnell, K. and Gray, L.E. (1995). Phylogenetic relationships of the soybean sudden death syndrome pathogen *Fusarium solani* f. sp. *phaseoli* inferred from rDNA sequence data and PCR primers for its identification. Mol. Plant Microbe Interact. 8 : 709-716.
21. O'Donnell, K. and Cigelnik, E. (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Mol. Phylogen. Evol. 7 : 103-116.
22. Page, R.D. and Holmes, E.C. (1998). Molecular Evolution : A Phylogenetic Approach. Blackwell Science Ltd., Oxford, UK.
23. Patel, M.K., Kamat, M.N. and Padhye, Y.A. (1950). A new record of *Puccinia* on sugarcane in Bombay. Curr. Sci. 19 : 121-122.
24. Peterson, S.W. and Kurtzman, C.P. (1991). Ribosomal RNA sequence divergence among sibling species of yeasts. Syst. Appl. Microbiol. 14 : 124-129.
25. Presley, J.T., Perdomo, R. and Ayats, J.D. (1978). Sugarcane rust found in the Dominican Republic. Plant Dis. Reptr. 62 : 843.
26. Purdy, L.H., Liu, L.J. and Dean, J.L. (1983). Sugarcane rust, a newly important disease. Plant Dis. 67 : 1292-1296.
27. Purdy, L.H. (1985). Sugarcane Rusts. Chap. VIII. *In* The Cereal Rusts. Vol. II (Roelfs, A.P. and Bushnell, W. R., eds.). pp. 237-256, Academic Press, Inc., Orlando, Florida.
28. Roy, B.A., Vogler, D.R., Bruns, T.D. and Szaro, T.M. (1998). Cryptic species in the *Puccinia monoica* complex. Mycologia 90 : 846-853.
29. Ryan, C.C. and Egan, B.T. (1989). Rust, Chap. XIII. *In* Diseases of Sugarcane, Major Diseases (Ricaud, C., Egan, B.T., Gillaspie, Jr., A.G. and Hughes, C.G., eds.). pp. 189-210, Elsevier, Amsterdam.
30. Saenz, G.S., Taylor, J.W. and Gargas, A. (1994). 18 S rRNA gene sequences and supraordinal classification of the Erysiphales. Mycologia 86 : 212-216.
31. Saitou, N. and Nei, M. (1987). The neighbor-joining method : a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4 : 406-425.
32. Sanderson, M.J. and Doyle, J.J. (1992). Reconstruction of organismal and gene phylogenies from data on multigene families : concerted evolution, homoplasy, and confidence. Syst. Biol. 41 : 4-17.
33. Sivanesan, A. and Waller, J.M. (1986). Sugarcane

diseases. CMI Phytopath. Paper No. 29. CAB International, Oxon, UK.
34. Sjamsuridzal, W., Nishida, H., Ogawa, H., Kakishima, M. and Sugiyama, J. (1999). Phylogenetic relationships of rust fungi parasitic on ferns : evidence from 18 S rDNA sequence analysis. Mycoscience 40 : 21-27.
35. Suh, Y., Thien, L.B., Reeve, H.E. and Zimmer, E.A. (1993). Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. Am. J. Bot. 80 : 1042-1055.
36. Suyama, Y., Kawamuro, K., Kinoshita, I., Yoshimura, K., Tsumura, Y. and Takahara, H. (1996). DNA sequence from a fossil pollen of *Abies* spp. from Pleistocene peat. Genes Genet. Syst. 71 : 145-149.
37. Swofford, D.L. (1993). PAUP : Phylogenetic Analysis Using Parsimony. v. 3.1.1. Illinois Natural History Survey, Champaign, Illinois.
38. Tai, F.L. (1947). Uredinales of Western China. Farlowia 3 : 95-139.
39. Takamatsu, S., Hirata, T. and Sato, Y. (1998). Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (Erysiphaceae). Mycoscience 39 : 441-453.
40. Taylor, P.J.W., Croft, B.J. and Ryan, C.C. (1986). Studies into the effect of sugarcane rust (*Puccinia melanocephala*) on yield. Proc. Int. Soc. Sugar Cane Technol. 19 : 411-419.
41. Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W : improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22 : 4673-4680.
42. Virtudazo, E.V., Muta, T., Nojima, H. and Kakishima, M. (1998). Taxonomic study of sugarcane rusts (Abstr). Ann. Phytopathol. Soc. Jpn. 64 : 437.
43. Virtudazo, E.V., Nakamura, H. and Kakishima, M. (1998). A simple method of nucleic acid extraction from spores of rust fungi for PCR amplification. p. 61. *In* Abstr., Progress in Mycological Sciences in Japan and the United Kingdom : 6th International Symposium of the Mycological Society of Japan, Chiba University, Japan.
44. Vogler, D.R. and Bruns, T.D. (1998). Phylogenetic relationships among the pine stem rust fungi (*Cronartium and Peridermium* spp.). Mycologia 90 : 244-257.
45. White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR Protocols (Innis, M.A., Gelfrand, D.H., Sninsky, J.J. and White, T.J., eds.). pp. 315-322, Academic Press, San Diego, California.
46. Zambino, P.J. and Szabo, L.J. (1993). Phylogenetic relationships of selected cereal and grass rusts based on rDNA sequence analysis. Mycologia 85 : 401-414.

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Puccinia speciosa, sp. nov., host-alternates between *Tricyrtis* and *Carex* in Japan

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Abstract: A spermogonial-aecial fungus was found on *Tricyrtis affinis* (Liliaceae) in Hiroshima Pref., Japan. The fungus was experimentally proven to be the spermogonial-aecial stage of a heteroecious macrocyclic *Puccinia* species on *Carex blepharicarpa* (Cyperaceae). Because morphological characteristics of the uredinial and telial stage were different from any of the described caricicolous *Puccinia* species and because no rust fungus had been known on *Tricyrtis*, *P. speciosa* sp. nov. was described. In addition to *T. affinis*, *T. flava*, *T. hirta*, and *T. macropoda* were proven to be spermogonial-aecial hosts.

Key Words: Cyperaceae, heteroecism, life cycle, Liliaceae, rust fungus, taxonomy, Uredinales, Uvulariaceae

INTRODUCTION

Species of the plant genus *Tricyrtis* (Liliaceae s. l. or Uvulariaceae, Dahlgren et al 1985) are distributed from the Himalayas to East Asia where its species are most abundant. In Japan, 11 species and 5 varieties have been recorded (Satake et al 1982). *Tricyrtis* is commonly found in deciduous hardwood forests. No rust fungi were known on *Tricyrtis* spp. before a spermogonial-aecial stage was found on *T. affinis* Makino at Sandankyo, Hiroshima Pref., in 1997.

In Japan, the spermogonial-aecial stage of various rust fungi had been recorded on a variety of liliaceous genera (Hiratsuka et al 1992): *Allium* (*Uromyces japonicus* Berkeley & Curtis), *Cardiocrinum* (*Puccinia hibayamensis* Y. Morimoto), *Disporum* (*Aecidium dispori* Dietel), *Erythronium* (*U. erythronii* Passerini), *Hosta* (*P. sessilis* Schröter), *Lilium* (*U. holwayi* Lagerheim), *Maianthemum* (*P. sessilis*), *Ornithogalum* (*P. hordei* Otth) and *Polygonatum* (*P. sessilis*). *Aecidium dispori* was recently found to be the spermogonial-aecial stage of a new *Puccinia* species (Ono et al unpubl).

When found, the spermogonial-aecial stage of a fungus on the *Tricyrtis* species did not seem to repeat or to form a uredinial-telial stage on the same plant species. Furthermore, it was found that the plants with the spermogonial-aecial stage grew near *Puccinia*-infected plants of *Carex blepharicarpa* Franch. *Carex blepharicarpa* was known to harbor the uredinial-telial stage of *P. caricis-blepharicarpae* S. Ito & Murayama (Ito and Murayama 1949). The field observations suggested a life-cycle relationship between the *Aecidium* and the *Puccinia* and its possible taxonomic identity with *P. caricis-blepharicarpae*, *P. breviculmis* Dietel or *P. hibayamensis* Y. Morimoto.

This paper deals with the life-cycle connection of the *Aecidium* on *T. affinis* with the *Puccinia* species on *C. blepharicarpa* and discusses its taxonomic difference from morphologically related caricicolous *Puccinia* species.

MATERIALS AND METHODS

Inoculation experiment.—Artificial inoculations were undertaken by the method described by Ono (1995) and Ono and Azbukina (1997). Naturally infected, telium-bearing leaves of *C. blepharicarpa* were collected at Sandankyo, Hiroshima Pref. in Dec 1997. At the same time, leaves of the same host species, on which telia were formed by aeciospore inoculation in Mito, Ibaraki Pref., were also collected. The telium-bearing leaves were preserved in a refrigerator at ca 5 C until Apr 1998, when they were soaked in running tap water at room temperature for 7–14 d to induce germination. Then, the leaves were cut into small pieces (ca 3–5 mm long) and placed on water-saturated filter paper in a petri dish and incubated in the dark at ca 18 C. The leaf pieces with metabasidia and basidiospores formed by the teliospore germination were placed on the adaxial surfaces of apparently healthy leaves of *T. affinis*, *T. flava* Maxim., *T. hirta* (Thunb.) Hook., *T. macropoda* Miq. and an unidentified *Tricyrtis* cultivar, which had been planted with loam soil in clay pots (18 cm diam). The inoculated plants were sprayed with distilled water and placed in a moist chamber at room temperature (18–22 C) for 48 h. The plants were subsequently transferred to a glasshouse for further observations.

Aeciospores naturally formed on *T. affinis* at Sandankyo were collected in May 1997 and inoculated onto leaves of *C. blepharicarpa* in June. Aeciospores formed on the same host species after basidiospore inoculation were also trans-

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ferred to various *Carex* species, which had been grown as described above, in May 1998. The aeciospores were scraped from fresh sori and dusted on a small piece (ca 3 × 3 mm) of water-saturated filterpaper. The spore-dusted pieces of filterpaper were then placed on the abaxial surface of the leaves of *C. blepharicarpa*, *C. breviculmis* R. Br., *C. conica* Boott, *C. dimorpholepis* Steud., *C. dolichostachya* Hayata subsp. *multifolia* (Ohwi) T. Koyama. The *Carex* species to be inoculated included the host of *P. breviculmis*, *P. caricis-conicae* Dietel and *P. hibayamensis*. The inoculated plants were treated in the same manner as in the basidiospore inoculation.

Microscopic observation.—To examine morphology and structure of spermogonium and aecium, fresh infected materials and dried herbarium specimens were freehand-sectioned under a binocular dissecting microscope. Thin sections were mounted in a drop of lactophenol solution without staining on a microscopic slide. To examine morphology and to measure size, the spores were scraped from sori on herbarium specimens and mounted by the same method as described above.

To observe germ pores in urediniospores, the spores were placed in a drop of lactic acid on a microscopic slide, heated to boil for a few seconds and mounted with an additional drop of lactophenol solution with aniline blue. The spores on the slide were smashed by applying gentle pressure over a cover slip on the preparation.

For scanning-electron microscopy (SEM), rust-infected leaves from dried herbarium specimens were cut into ca 3 × 3 mm pieces containing a few sori, and each piece was placed on double-adhesive tape on a specimen holder. The preparations were subsequently coated with platinum-palladium using a Hitachi E-1030 Ion Sputter and examined with a Hitachi S-4200 SEM at 15 kV.

TAXONOMY

Puccinia speciosa Y. Ono et K. Ishimiya, sp. nov.

FIGS. 1–9

Spermogonia plerumque epiphylla, subepidermalia, globosa, subglobosa vel ovoidea, 140–220 μm alta et 130–190 μm lata. Aecia hypophylla, cupulata, peridio bene evoluto. Aeciosporae subglobosae vel late ellipsoideae, 16–25 × 14–22 μm; episporio hyalino, verrucoso, granulis grandis in dimidio superiore. Uredinia hypophylla, subepidermalia, diu epidermide tecta, pulverulenta, alba. Urediniosporae subglobosae, late obovoideae vel late ellipsoideae, 27–37 × 22–32 μm; episporio aequaliter 2.5–5.0 μm crasso, hyalino, echinulato; poris germinationis 6–10, plerumque 8, aequatorialibus. Telia subepidermalia, mox nuda, pulvinata, atra. Teliosporae clavatae, ad apicem rotundatae vel truncatae, basin versus attenuatae, leniter vel modice constrictae ad septum, 42–80 × 12–23 μm; episporio brunneo, laevi, ad apicem 9–21 μm crasso; pedicello persistenti, usque ad 40 μm longo.

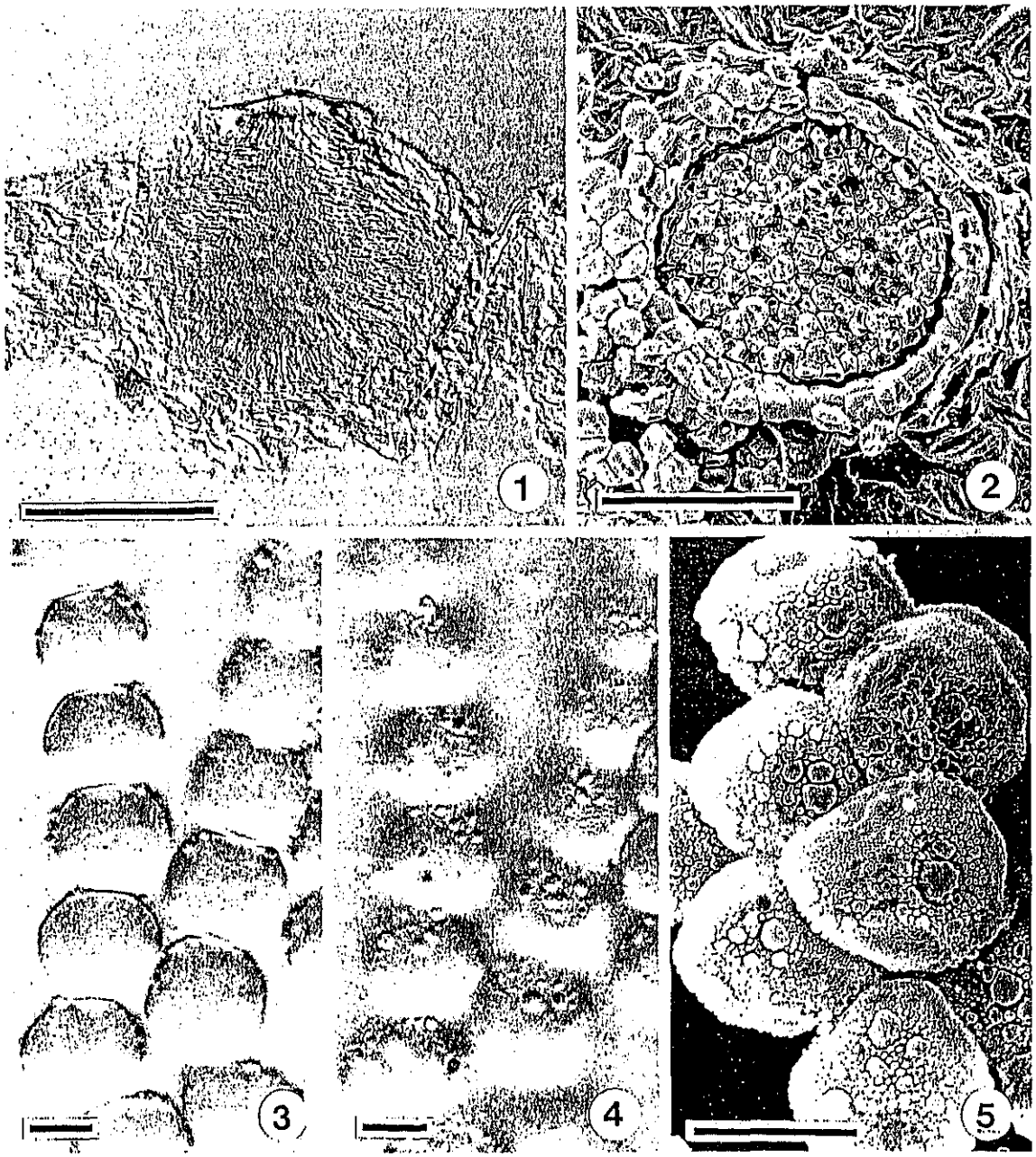
Spermogonia amphigenous, mostly epiphyllous, subepidermal, globose, subglobose or ovoid, 140–220 μm high and 130–190 μm wide (FIG. 1). Aecia hypophyllous, cupulate with a peridium. (FIG. 2). The peridial cells becoming disjointed as the sori mature. Aeciospores subglobose or broadly ellipsoid, 16–25 × 14–22 μm (FIG. 3). The wall thin, hyaline, verrucose with refractive granules on the upper side (FIGS. 4 & 5: type 5 of Savile 1973). Uredinia hypophyllous, long covered by the epidermis, becoming erumpent, pulverulent, and whitish. Urediniospores subglobose, broadly obovoid or broadly ellipsoid, 27–37 × 22–32 μm (FIG. 6). The wall evenly 2.5–5.0 μm thick, hyaline, completely echinulate (FIG. 7). Germ pores (6–) 8(–10), equatorial (FIG. 8). Telia hypophyllous, soon naked, pulvinate, black. Teliospores clavate, rounded or truncate at the apex, attenuate toward the base, weakly or moderately constricted at the septum, 42–80 × 12–23 μm (FIG. 9). The wall brown, smooth, 9–21 μm thick at the apex. The germ pore of the upper cell apical and of the lower septal. The pedicel persistent, up to 40 μm long.

Specimens examined. JAPAN. HIROSHIMA: Yamagata-gun, Sandankyo, on *Carex blepharicarpa* Franch., 2 Dec 1997, *Y. Ono & K. Ishimiya 4073* (*HOLOTYPE* IBA-8008); 25 May 1997, *Y.O. 3291* (IBA-7813); on *Tricyrtis affinis* Makino, 25 May 1997, *Y.O. 3290* (IBA-7812); IBARAKI: Mito, on *C. blepharicarpa*, 3 Jul 1997, *Y.O. 7860* (IBA-7860); 10 Dec 1997, *Y.O. 4075* (IBA-8010); 12 Dec 1998, *Y.O. & K.I. 4265* (IBA-8186); on *T. affinis*, 30 May 1998, *Y.O. & K.I. 4153* (IBA-8096); *Y.O. & K.I. 4154* (IBA-8097); *Y.O. & K.I. 4157* (IBA-8100); on *T. flava* Maxim., 30 May 1998, *Y.O. & K.I. 4156* (IBA-8099); on *T. hirta* (Thunb.) Hook., 30 May 1998, *Y.O. & K.I. 4159* (IBA-8102); on *T. macropoda* Miq., 30 May 1998, *Y.O. & K.I. 4155* (IBA-8098); *Y.O. & K.I. 4158* (IBA-8101); *Y.O. & K.I. 4160* (IBA-8103).

Note. All Ibaraki specimens are vouchers in inoculation experiments.

DISCUSSION

The *Aecidium* on *T. affinis* was successfully connected with the *Puccinia* on *C. blepharicarpa*, which occurred at Sandankyo, Hiroshima Pref. In addition to *T. affinis*, *T. flava*, *T. hirta* and *T. macropoda* were proven to serve as the spermogonial-aecial host of the fungus under consideration. In all basidiospore inoculations, spermogonia appeared on the inoculated *Tricyrtis* plants 7–17 d after the basidiospore inoculation and aecia were formed in subsequent 7–14 d, except for *T. flava* on which spermogonium-bearing lesions became necrotic before aecia were formed. In contrast to the wild species, no infection took place on an unidentified *Tricyrtis* cultivar. Aeciospores formed after the basidiospore inoculation of *T. affinis* were successfully transferred to *C. blepharicarpa* resulting in uredinia 10–20 d and in telia 2 mo after the aeciospore inoculation. No infection was detected on the

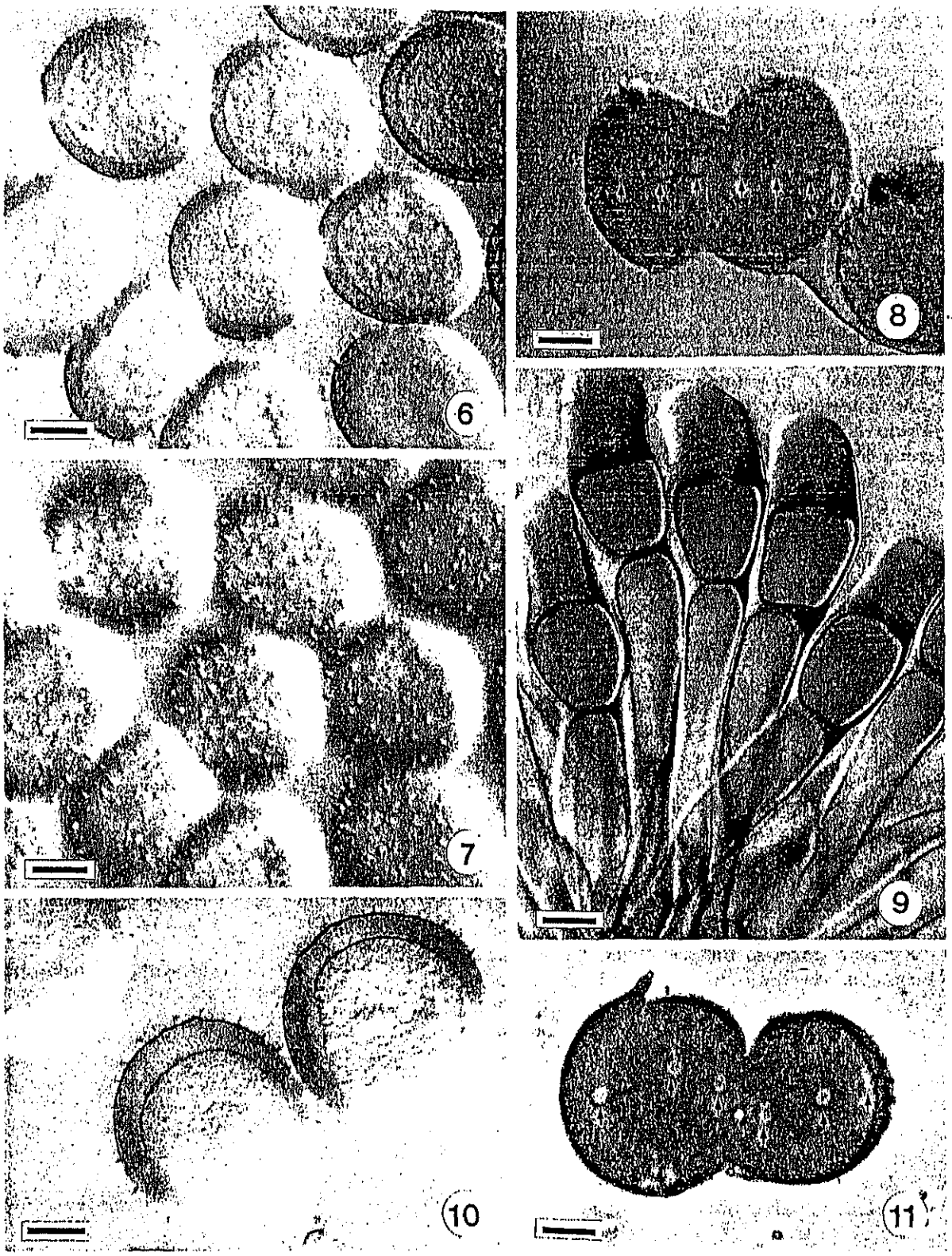


FIGS. 1–5. *Puccinia speciosa* on *Tricyrtis affinis* (IBA-7812). 1. Spermogonium. 2. Aecium (SEM). 3. Aeciospores focused on a median plane. 4. Aeciospores focused on a tangential plane. 5. Surface structure of aeciospores (SEM). Bars: 1, 3–5 = 10 μm; 2 = 100 μm.

inoculated leaves of *C. breviculmis, C. conica, C. dimorpholepis* and *C. dolichostachya* subsp. *multifolia*.

The fungus under consideration forms colorless, thick-walled urediniospores with 6–10 germ pores being distributed on an equatorial zone. These urediniospore characteristics and putative spermogonial-aecial host specificity to *Tricyrtis* species (Uvulariaceae-Tricyrtideae, Dahlgren et al 1985), which is phylogenetically distantly related to the other liliaceous-uvulariaceous plants (Shinwari et al 1994, Chase et al 1995), distinguish the fungus from any of morphologically similar species, particularly *P. caricis-conicae*. Thus, a new name, *P. speciosa*, is proposed for the fungus.

For *C. blepharicarpa*, only one species, *P. caricis-blepharicarpae* S. Ito & Murayama has been recorded (Ito and Murayama 1949). Neither the type specimen nor authentic materials were available for the study.



FIGS. 6–11. *Puccinia speciosa* and *P. caricis-conicae*. 6–9. *Puccinia speciosa* on *Carex blepharicapa*. 6. Urediniospores focused on a median plane (IBA-7860). 7. Urediniospores focused on a tangential plane (IBA-7860). 8. Eight equatorially arranged germ pores (arrows) on the urediniospore wall. 9. Teliospores (HOLOTYPE, IBA-8008). Scale bars: 6–9 = 10 μm. 10, 11. *Puccinia caricis-conicae* (HOLOTYPE in Herb. S). 10. Urediniospores. 11. Six equatorially arranged germ pores (arrows) on the urediniospore wall. Bars: 6–11 = 10 μm.

According to the description by Ito and Murayama (1949), this species possesses larger urediniospores (31–48 × 29–40 μm) with 3–5 μm thick, colorless wall and smaller teliospores (36–70 × 13–23 μm). In addition to these morphological differences, this species forms the spermogonial-aecial stage on *Smilax china* L. (Smilacaceae, Dahlgren et al 1985), which is distantly related to *Tricyrtis* (Uvulariaceae) (Chase et al 1995, Judd et al 1999).

Puccinia hibayamensis forms its spermogonia and aecia on *C. cordatum* (Liliaceae, Dahlgren et al 1985; Hiratsuka and Kaneko 1977). Unlike the other caricicolous *Puccinia* spp. under discussion, the fungus forms large (27–55 × 17–27 μm) amphispores with yellowish brown to chestnut-brown wall of 2–4 μm thick and pale-colored teliospores, 38–77 × 11–22 μm (Morimoto 1959).

Puccinia breviculmis forms subglobose, broadly obovoid or ellipsoid urediniospores, 20–34 × 20–30 μm, with thick (2.5–5.0 μm), colorless and echinulate walls. *Puccinia speciosa* and *P. breviculmis* are indistinguishable by the urediniospore morphology. In the original description of *Uredo breviculmis* Hennings (uredinial anamorph of *P. breviculmis* Dietel, 1907), Hennings (1901) did not mention the number and distribution of urediniospore germ pores. The isotype specimen (Herb. S) of *U. breviculmis* (on *C. breviculmis* collected at Sakawa, Kochi Pref., in Jul 1901 by T. Yoshinaga) was examined. *Puccinia breviculmis* was found to have 4 or 5 germ pores which were distributed equatorially. In addition to the difference in the number of urediniospore germ pores, teliospores of *P. breviculmis* are significantly shorter (32–55 μm long) than those of *P. speciosa*.

Puccinia caricis-conicae is the species morphologically closer to *P. speciosa* (Ishimiya and Ono 1999). This fungus forms globose or ovate urediniospores of 31–39 × 23–37 μm with thick (4–5 μm), colorless and echinulate urediniospores and clavate teliospores of 52–84 × 17–28 μm (Ito and Homma 1938). The urediniospore germ pore was stated to be obscure in the original description. The holotype specimen (Herb. S) and isotype specimen (Herb. SAPA) (on *C. conica* collected at Makomanai, Hokkaido on 16 Oct 1910 by M. Miura) were examined in this study. The urediniospores observed were subglobose, broadly obovoid or broadly ellipsoid and 38–45 × 31–39 μm (FIG. 10). The urediniospore size was significantly larger than that described by Ito and Homma (1938). The wall was 3.4–4.8(–5.7) μm thick, colorless and echinulate. Five or 6 germ pores were distributed along the equatorial zone (FIG. 11). The prominent differences in the urediniospore dimension and the germ pore number distinguish *P. speciosa* from *P. caricis-conicae*.

A spermogonial-aecial stage of *P. caricis-conicae* is not known so far. Because morphologically similar species that form large, colorless, thick-walled urediniospores on *Carex* form their spermogonial-aecial stage on Liliaceae s.l., it is likely that *P. caricis-conicae* also forms its spermogonial-aecial stage on Liliaceae s.l.

ACKNOWLEDGMENTS

The holotype of *Uredo breviculmis* Hennings and *Puccinia caricis-conicae* Homma were loaned from the Swedish Museum of Natural History (S) and the isotype of *P. caricis-conicae* from the Mycological Herbarium of the Faculty of Agriculture, Hokkaido University (SAPA). Scanning-electron-microscopy was done at the Institute of Agriculture and Forestry, University of Tsukuba. The *Carex* species were identified by Mr. M. Michikawa, the Institute of Biological Science, University of Tsukuba.

LITERATURE CITED

Chase MW, Duvall MR, Hills HG, Conran JG, Cox AV, Eguiarte LE, Hartwell J, Fay MF, Caddick LR, Cameron KM, Hoot S. 1995. Molecular systematics of Lilianae. In: Rudall PJ, Cribb PJ, Cutter DF, Humphies CJ, eds. Monocotyledons: systematics and evolution. Kew: The Royal Botanic Gardens. p 109–137.

Dahlgren RM, Clifford HT, Yeo PF. 1985. The families of the monocotyledons. Structure, evolution and taxonomy. Berlin: Springer-Verlag. 520 p.

Dietel P. 1907. Uredineen aus Japan. Ann Mycol 5:70–77.

Hennings P. 1901. Einige neue japanische Uredineen II. Hedwigia Bibl 40:124–125.

Hiratsuka N, Kaneko S. 1977. Life-cycles of three rust species. Rep Tottori Mycol Inst 15:6–12.

———, Sato S, Katsuya K, Kakishima M, Hiratsuka Y, Kaneko S, Ono Y, Sato T, Harada Y, Hiratsuka T, Nakayama K. 1992. The rust flora of Japan. Tsukuba: Tsukuba-shuppankai. 1205 p.

Ishimiya K, Ono Y. 1999. Host range and taxonomic identity of the *Tricyrtis* rust fungus. Ann Phytopathol Soc Japan 65:411. (abstract, in Japanese)

Ito S, Homma Y. 1938. Notae mycologicae Asiae orientalis III. Trans Sapporo Natl Hist Soc 15:113–128.

———, Murayama D. 1949. Notae mycologicae Asiae orientalis V. Trans Sapporo Natl Hist Soc 18:84–88.

Judd WS, Campbell CS, Kellog EA, Stevens PF. 1999. Plant systematics. A phylogenetic approach. Massachusetts: Sinauer Associates, Inc. 464 p.

Morimoto Y. 1959. Notes of rust fungi of Japan. J Jap Bot 34:185–189.

Ono Y. 1995. *Aecidium alangii* is the aecial state of *Puccinia miyakei* (Uredinales). Nova Hedwigia 60:157–164.

———, Azbukina ZM. 1997. Heteroecious life cycle of two graminicolous *Puccinia* (Uredinales). Mycoscience 38: 281–286.

Satake Y, Ohwi J, Kitamura S, Watari S, Tominari T, eds. 1982. Wild flowers of Japan. Vol. 1. Herbaceous plants–

Monocotyledoneae. Tokyo: Heibonsha Ltd. 305 p. (in Japanese)

Savile DBO. 1973. Aeciospore types in *Puccinia* and *Uromyces* attacking Cyperaceae, Juncaceae and Poaceae. Rep Tottori Mycol Inst 10:225–241.

Shinwari ZK, Terauchi R, Htech FH, Kawano S. 1994. Recognition of New World *Disporum* section *Prosartes* (Liliaceae) based on the sequence data of the *rbcL* gene. Taxon 43:353–366.

Mycoscience (2002) 43:37–45

FULL PAPER

Yoshitaka Ono

Life cycle and nuclear behavior in three rust fungi (Uredinales)

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Abstract *Kuehneola japonica* has a microcyclic life cycle with a regular alternation of generations. Single basidiospore inoculations onto *Rosa wichuraiana* resulted in teliospore production, indicating its homothallic nature. Dikaryotization in a vegetative mycelium in the host seemed to occur through nuclear division that was not followed by septum formation. Karyogamy and meiosis took place through teliospore and metabasidium development; this fungus was considered to reproduce genetically homogeneous progenies. *Puccinia lantanae* and *P. patriniae* were also microcyclic in their life cycle; however, these fungi differed from *K. japonica* in the mode of nuclear behavior. In the former two fungi, both vegetative and reproductive cells were uninucleate. No karyogamy was observed, and nuclear division in the metabasidium development was thought to be mitotic. In *P. lantanae*, a basidiospore was formed on a sterigma, whereas a whiplike hypha emerged from each metabasidium cell in *P. patriniae*. Inoculations of *Justicia procumbens* with a single basidiospore of *P. lantanae* resulted in teliospore production. The fungus seemed to remain uninucleate, either haploid or diploid, throughout the life cycle. Thus, reproduction was considered to be apomictic.

Key words Apomixis · Homothallism · *Kuehneola japonica* · *Puccinia lantanae* · *Puccinia patriniae*

Introduction

Rust fungi exhibit a diverse array of life cycles and accompanying nuclear behaviors through teliospore (probasidium) and metabasidium development. Most rust species producing only a telial stage associated or not associated with a spermogonial stage are believed to have been derived from macrocyclic parental species, producing spermogonial, aecial, uredinial, and telial stages through various pathways (Jackson 1931; Hennen and Buriticá 1980). These microcyclic species constitute a larger proportion of the rust biota in arcto-alpine regions or oceanic islands, whereas macrocyclic species predominate in temperate regions. The life cycle producing only the telial stage is said to be reduced in these arcto-alpine or island species. In contrast, many tropical species producing only a telial stage are considered to be unexpanded in their life cycle (Hennen and Buriticá 1980). The microcyclic life cycle in the rust fungi includes those with two different evolutionary backgrounds.

Rust species with a reduced life cycle are most diverse in nuclear behaviors and development of metabasidia and basidiospores (Jackson 1931; Petersen 1974; Walker 1928). Some of the best examples have been depicted by Gardner (1981, 1987, 1988, 1994, 1996) and Hodges and Gardner (1984). Possible pathways of life cycle reduction and interpretations of various patterns in nuclear behavior and associated morphological changes in metabasidium and basidiospore development are still controversial. For better understanding of the evolution of the microcyclic life cycle and nuclear behavior, detailed studies on a broad range of rust species are indispensable.

This article reports the life cycle, nuclear behavior, and morphology of the metabasidia and basidiospores of three rust fungi with a reduced life cycle that are distributed in Japan.

Materials and methods

Fungal species and isolates examined

Kuehneola japonica (Dietel) Dietel on *Rosa wichuraiana* Crép., Ibaraki, Kasama, Mt. Sashirosan, 18 October 1993, Y. Ono 2942; Ibaraki, Higashiibaraki-gun, Oarai-machi, 12

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August 1998, Y. Ono 4231. *Puccinia lantanae* W. G. Farlow on *Justicia procumbens* L., Ibaraki, Kuji-gun, Diago-machi, Shimonomiya, 1998 and Mito, 1999 (no voucher specimen). *Puccinia patriniae* P. Hennings on *Patrinia villosa* (Thunb.) Juss., Gumma, Tone-gun, Katashina-mura, Oshimizu, 16 September 1999, Y. Ono 4390.

The fungi were collected together with the host plants, and the infected plants were transplanted in a clay pot and maintained at Ibaraki University campus in Mito. The voucher specimens have been deposited in the Herbarium of Systematic Mycology, Ibaraki University (IBA).

Fixation and staining

Fresh fungal spores were scraped from the sori on the host plants and floated on a drop of distilled water or 0.1% water agar on a microscopic slide. The slide preparation was incubated in the dark at either 18°C or 20°C for 12–24 h, depending on the fungal species. The incubated slide preparation was then air-dried and fixed with Carnoy's fluid (absolute ethanol:chloroform:glacial acetic acid = 6:3:1) at about 20°C for 30 min and stained with propidium iodide solution according to a standard protocol (Fujita and Minamikawa 1990; Nagle 1996; Mukai 1996): the fixed materials in a vial were hydrated through an ethanol series (75% for 2 min, 70% for 2 min, 50% for 2 min, and distilled water for 2 min), soaked in phosphate-buffered saline solution (Sigma, St. Louis, MO, USA) for 2 min, incubated in RNase A (type I-AS; Sigma, 100 mg/ml) solution at 37°C for 1 h, rinsed with Tris-ethylenediaminetetraacetic acid (EDTA) buffer (pH 7.4), and stained with a solution composed of 5 mg/ml propidium iodide in 10% Tris-EDTA buffer and antifade [1.25% 1,4-diazabicyclo (2.2.2) octane; Sigma) in the dark at 20°C for 1 h. The stained materials were mounted with the propidium iodide solution as described above and the cover slip was sealed with fingernail polish. The preparations were examined immediately or stored at 4°C in the dark until examined (maximum, 1 month).

The fungus-infected lesions of the host plants were free-hand sectioned under a binocular dissecting microscope, fixed, and stained within a screw vial with the same protocol as above.

Microscopy and photomicrography

The slide preparations were examined with an Olympus (Tokyo, Japan) BX50 epifluorescent microscope equipped with U-MWIG cube (a 520 to 550-nm excitation filter BP520–550, a 565-nm dichroic mirror DM565, and a 590-nm barrier filter BA580IF) and UPlanFl 40/0.75 and UPlanFl100/1.30 objective lenses. The microscope was also equipped with a differential interference contrast optic (DIC) unit so that both fluorescent and DIC images were simultaneously observed and photographed. Photomicrographs were taken at a magnification of 670× or 270× on 35-mm Fuji Presto film (ISO 400) with an Olympus PM-20 automatic photomicrograph unit.

Single basidiospore inoculation

Single basidiospores spread on a microscopic slide were isolated with an Olympus ON-M1 micromanupilator under an Olympus IX50 inverted microscope. The isolated basidiospores were individually transferred onto young shoots or leaves of the uninfected host plants. The inoculated plants were kept under saturated humidity in the dark at 20°C for 48 h, and then transferred to a growth chamber at about 20°C with artificial illumination of 12-h-light and 12-h-dark intervals.

Successfully infected parts of the host plants as manifested by production of yellow flecks or by production of sorus primordia were cut into small pieces, free-hand sectioned, and stained as already described.

Results

Kuehneola japonica

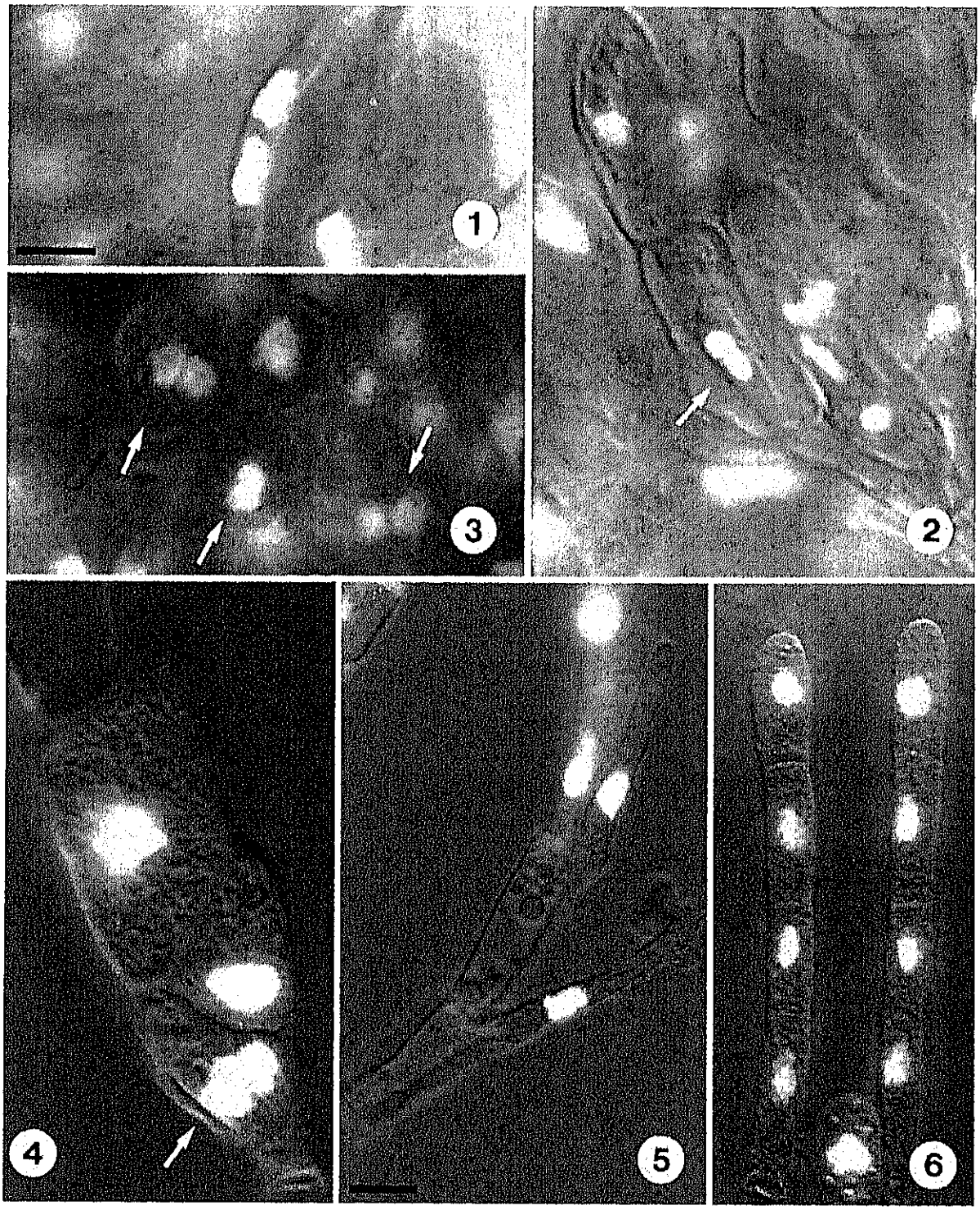
A vegetative mycelium in the tissue of both successfully inoculated and field-collected host plants was binucleate (Fig. 1). The binucleate hyphae ramified and grew intercellularly in the host tissue. The intercellular hyphae gave rise to a cylindrical or club-shaped haustorium in the host mesophyll cells. The haustorium was one-celled, unbranched, and also binucleate (Fig. 2).

The hyphae became aggregated to form a telial primordium beneath the host epidermis. From binucleate teliospore initials (sporogenous cells) produced in the telial primordium (Fig. 3), binucleate teliospore mother cells arose. The teliospore mother cell successively divided 3 to 7 times so as to form a linear 3- to 7-celled teliospore and a subtending pedicel cell. The immature teliospore cells and the subtending pedicel cell were binucleate. As the teliospores mature, the two nuclei in each teliospore cell fused, while the two nuclei in the pedicel cell remained separated (Fig. 4).

The teliospores germinated to become a metabasidium, into which the fused nucleus migrated (Fig. 5). The nucleus divided twice, presumably by meiosis, resulting in four daughter nuclei in the metabasidium (Fig. 6). No clearly condensed chromosomes were observed in the nuclear division (Fig. 7). A septum was laid down between the daughter nuclei on completion of each nuclear division.

From each of four metabasidium cells, a sterigma arose and a basidiospore was formed, into which the nucleus migrated (Fig. 8). After being forcibly discharged from the sterigma, the nucleus in the basidiospore divided once (Fig. 9).

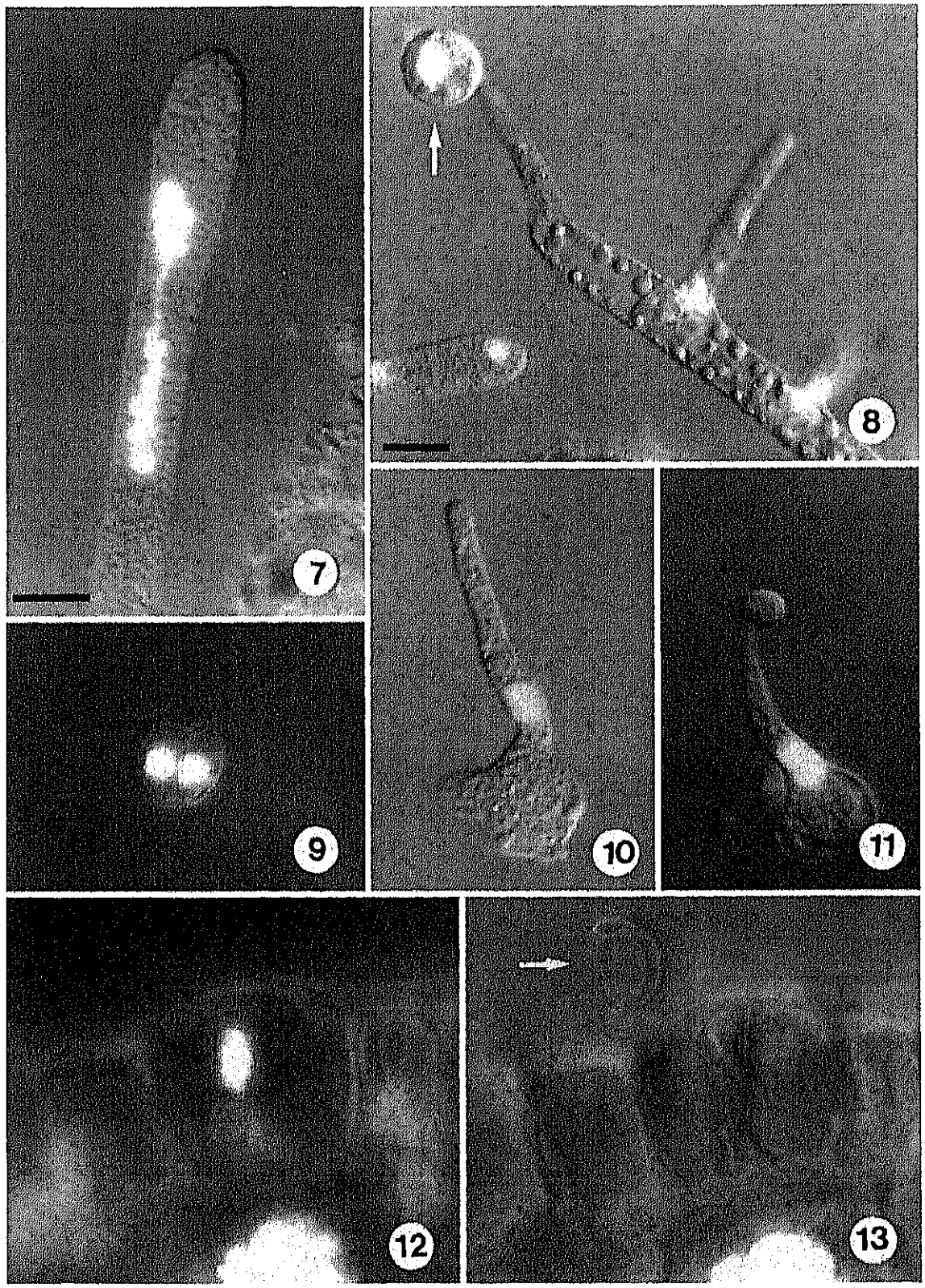
The basidiospore germinated either to a germ tube or to produce a secondary spore. Before the germination, most of the basidiospores became uninucleate by disintegration of one of the two nuclei and the remaining nucleus migrated either into a short germ tube (Fig. 10) or to a secondary spore (Fig. 11). Some basidiospores remained binucleate during germination; however, only one of the two nuclei



Figs. 1–6. *Kuehneola japonica.* **1** Binucleate vegetative mycelium. **2** Binucleate haustorium (*arrow*) in a host mesophyl cell. **3** Binucleate telial primordium cells (*arrows*). **4** Diploid, uninucleate telial cells and a subtending haploid, binucleate pedicel cell (*arrow*). **5** Diploid nucleus moving into a developing metabasidium. **6** Haploid, uninucleate metabasidium cells. *Bars* **1–4** 5 μm; **5, 6** 10 μm

migrated either into a germ tube or to a secondary spore. No tertiary or quaternary spores were observed.

Single-basidiospore inoculations resulted in infection and teliospore production, although the rate of successful infection was very low (7 successful infections with the telium production of 100 single-basidiospore inoculations). The inoculation was successful only when the spores were placed on young etiolated shoot tips, and no infection was



Figs. 7–13. *Kuehneola japonica.* **7** Presumed anaphase of meiosis I in a developing metabasidium. **8** Uninucleate basidiospore (*arrow*) formed on a sterigma arising from a metabasidium cell. **9** Binucleate basidiospore. **10** Germinating basidiospore with a single nucleus. **11** Secondary spore production from a uninucleate basidiospore. **12** Uninucleate infection hypha in a host epidermal cell. **13** Collapsed basidiospore (*arrow*) on host epidermis and infection hypha in host epidermal cell (same as in Fig. 12 but not fluorescent). *Bars* **7, 9–13** 5 μm; **8** 10 μm

obtained when the spores were inoculated on fully expanded leaflets of a compound leaf.

Upon inoculation on the host, a short germ tube from the basidiospore penetrated the host cuticle and cell wall. The penetration hypha entered into the epidermal cell and did not become enlarged. The nucleus migrated into the penetration hypha (Figs. 12, 13). From the penetration hypha, intercellular hyphae arose. The intercellular hyphae became binucleate at an early stage of the infection; however, the mode of dikaryotization was not determined.

Puccinia lantanae

Intercellular hyphae in the host mesophyll were mostly uninucleate (Fig. 14). Binucleate hyphae were seldom observed. Haustorial cells were also uninucleate. Cells of hyphal aggregates that would give rise to a telial primordium were still uninucleate (Fig. 15). Teliospore initials arose from a cell layer beneath the host epidermis. The teliospore initial was uninucleate (Fig. 16) and seemed to originate directly from the telial primordium cell without dikaryotization and immediate karyogamy in the teliospore initial. The teliospore initial divided to form a distal teliospore mother cell and a proximal cell, both of which remained uninucleate (Fig. 16). The teliospore mother cell divided to become a one-celled teliospore and a subtending pedicel cell. Two-celled teliospores were infrequently observed. The mature teliospores, either one-celled or two-celled, were uninucleate with a few exceptions.

On germination, the teliospores gave rise to a metabasidium, into which the nucleus migrated. The nucleus divided twice, and the four daughter nuclei were separated subsequently by septa to form a four-celled metabasidium (Fig. 17). Each of four metabasidium cells gave rise to a sterigma, on which a uninucleate basidiospore was formed (Figs. 17, 18). The nucleus in the metabasidium cell migrated into a basidiospore where the nucleus further divided so that the basidiospore became binucleate. Either one or two nuclei migrated into a germ tube arisen from the basidiospore (Fig. 19).

Single basidiospore inoculations were successful at a very low rate (less than 5%), resulting in the telium production. The infection process was not observed. The teliospores formed by the single basidiospore inoculations were unicellular.

Puccinia patriniae

Intercellular hyphae were uninucleate throughout the host mesophyll (Fig. 20). A telial primordium that was composed of an intercellular hyphal aggregate was uninucleate (Fig. 21). A layer of cells beneath the host epidermis gave rise to teliospore initials, which were also uninucleate (Fig. 22). The teliospore initial divided into a distal teliospore mother cell and a proximal cell. The teliospore mother cell further divided to become a two-celled teliospore and a subtending pedicel cell. Both immature and mature teliospores were uninucleate (Fig. 23). Very rarely, a few telial primordium cells appeared to be binucleate (Fig. 24).

The teliospore germinated into either a short or elongated metabasidium (Figs. 25, 26). The nucleus migrated into the metabasidium from the teliospore cell. The nucleus divided twice to become four daughter nuclei. The four nuclei were separated by septa concomitantly laid down with the nuclear division, the metabasidium becoming four celled with a single nucleus in each cell.

Each of four cells of the metabasidium gave rise to a peg- or a whiplike germ tube instead of producing a normal basidiospore. The nucleus in the metabasidium cell migrated into the peg- or whiplike projection (Fig. 27). No additional nuclear division took place in the germ tube. No further observation was made as to the infection process of the basidial germ tube.

Discussion

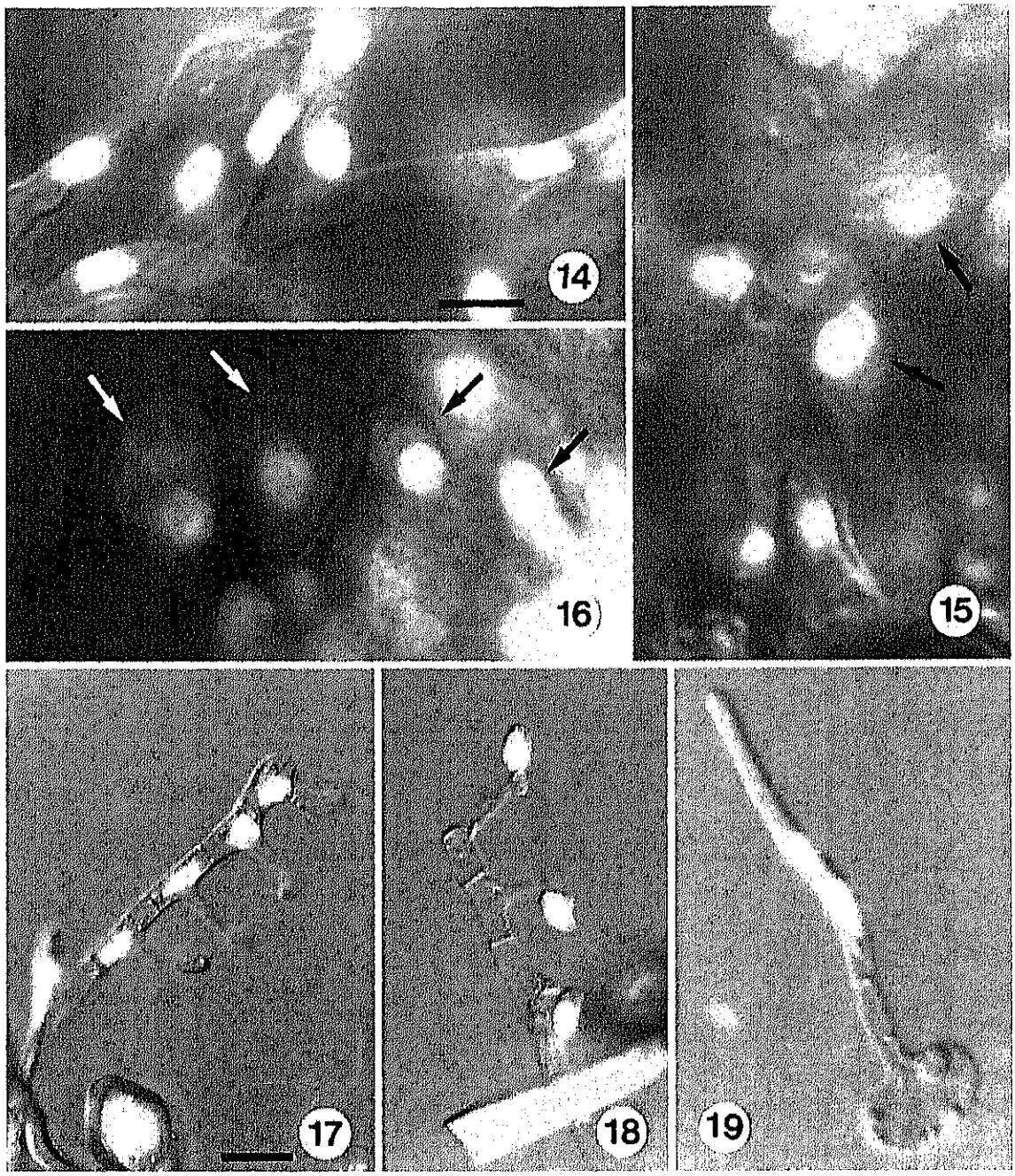
In the rust species with a macrocyclic life cycle, either autoecious or heteroecious, a regular alternation of generation is commonly observed, i.e., karyogamy and meiosis take place at the end of a sporophytic generation (teliospores), and dikaryotization at the initiation of sporophytic generation (aeciospores and urediniospores), and the gametophytic generation (spermogonium and aecial primordium) intercalates between the two phases of the sporophytic generation. Examples of this nuclear behavior observed in the microcyclic life cycle rusts include *Puccinia malvacearum* Bertero (Blackman and Fraser 1906; Olive 1911; Werth and Ludwig 1912; Moreau 1914; Lindfors 1924; Allen 1933), *Endophyllum sempervivi* (Alb. & Schw.) de Bary (Maire 1900; Hoffman 1912; Moreau and Moreau 1918b; Ashworth 1935), *P. grindeliae* Peck (Brown 1940), *P. xanthii* Schw. (Brown 1940), *P. ruelliae* (Berk. & Br.) Lagerh. (Singh 1979), *Cystopsora oleae* Butler, pro parte (Thirumalachar 1945), *Uromyces rayssiae* Anikst. & Wahl (Anikister et al. 1980), and *Puccinia pampeana* Speg. (Hennen et al. 1984). *Puccinia pampeana* possesses the *Endophyllum*-type teliospore in addition to the *Puccinia*-type teliospore, both of which are formed only after the cross-spermatization on the basidiospore-infected plant.

On the other hand, in the microcyclic rust species, i.e., those with secondarily reduced life cycle, a variety of nuclear behaviors have been observed (Jackson 1931; Petersen 1974).

Kuehneola japonica

In this study, *K. japonica* was shown, as has previously been reported by Kohno et al. (1975, 1977), to exhibit the regular nuclear cycle commonly observed in macrocyclic rust species.

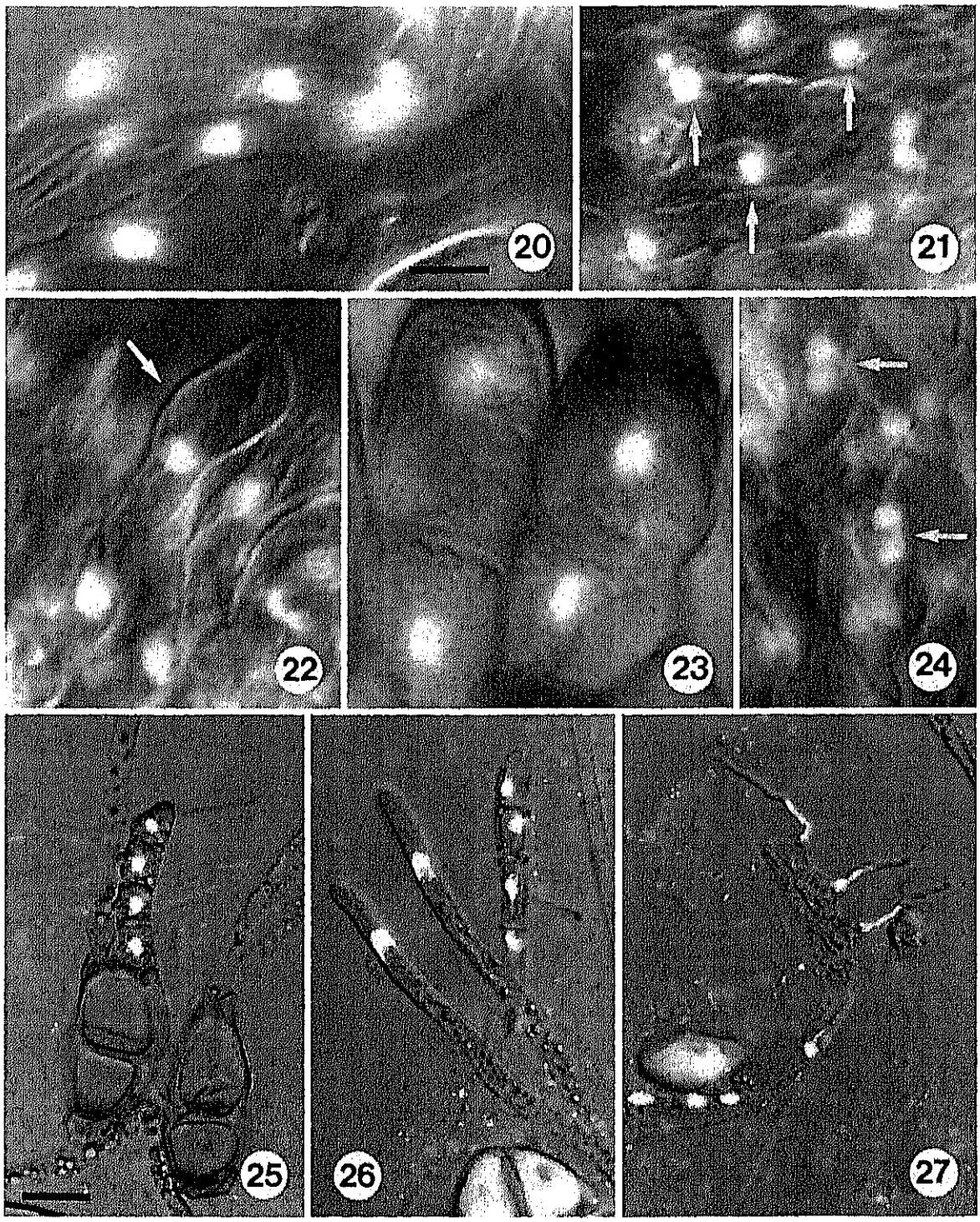
Successful single basidiospore inoculation proved that this fungus is homothallic and that the uninucleate infection



Figs. 14–19. *Puccinia lantanae.* **14** Uninucleate vegetative mycelium. **15** Uninucleate telial primordium cells (*arrows*). **16** Uninucleate teliospore mother cells (*thin black arrows*) and young teliospores (*thick white arrows*). **17** Uninucleate metabasidium cells, on which a basidiospore is being produced. **18** Uninucleate basidiospores on a sterigma. **19** Binucleate germ tube from a basidiospore. *Bars* **14–16, 19** 5 μm; **17, 18** 10 μm

hypha gives rise to the binucleate mycelium. The dikaryotization seems to take place by mitotic nuclear division that is not followed by septum formation (Maire 1899; Walker 1928; Dodge 1929) at an early point of the infection. The results, that well-isolated single basidiospore inoculation resulted in the teliospore production and that dikaryotization took place at an early stage of infection, may rule out the two possiblities of dikaryotization by nuclear migration from an adjacent mycelial cell (Blackman 1904) or by anastomosis of uninucleate mycelial cells (Christman 1905; Kursanov 1917).

In the course of teliospore differentiation, two nuclei in a teliospore mother cell fused while those in a pedicel cell remained separated. Therefore, nuclear division observed in the metabasidium developing upon germination of the teliospore is believed to be meiosis.



Figs. 20–27. *Puccinia patriniae.* **20** Uninucleate vegetative mycelium. **21** Uninucleate telial primordium cells (*arrows*). **22** Uninucleate teliospore mother cell (*arrow*). **23** Two-celled, uninucleate teliospores. **24** Binucleate vegetative mycelium cells (*arrows*) were rarely observed. **25** Short, four-celled metabasidium with a single nucleus in each cell. **26** Long, four-celled metabasidium with a single nucleus in each cell (*right*). Two developing metabasia with a single nucleus (*left*). **27** Whip-like hyphae from metabasidium cells. A single nucleus is moving into each hypha. *Bars* **20–24** 5 μm; **25–27** 10 μm

The uninucleate basidiospores often became binucleate by an additional mitotic division as commonly observed in other rust species (Anikister 1983, 1984). Either one of the two or both nuclei migrated into the germ tube. However, only one of them seems to take part in the infection, another being degenerated. Consequently, upon infection of a uninucleate infection hypha, a uninucleate intracellular hypha resulted from the basidiospore infection.

Nuclei involved in the dikaryotization, karyogamy, and meiosis in the alternation of generation possess the same genetic constituent. Thus, this fungus is considered to reproduce genetically homogeneous progenies in the homothallic sexual process, which is highly contrasted to the macrocyclic species with regular alternation of generation such as *P. helianthi* Schw., *P. graminis* Pers., *P. recondita* Desm., *P. coronata* Corda, *P. sorghi* Schw., *P. phragmitis* (Schm.) Koern., *Melampsora lini* (Ehrenb.) Lév., *Uromyces appendiculatus* (Pers.) Unger, *U. vignae Barcl.*, *Gymnosporangium asiaticum* Yamada, *G. juniperi-virginianae* Schw., *G. globosum* Farl., and *Cronartium ribicola* J. C. Fischer. These species are proven or assumed to be heterothallic (Cragie 1927a,b; Allen 1933; Brown 1940), and sexual recombination is an essential part of the life cycle in these macrocyclic species.

Puccinia lantanae

The results, showing that the fungus was uninucleate throughout the life cycle and single basidiospore inoculations resulted in telium production, suggest that the fungus reproduces apomictically. Although four uninucleate basidiospores are formed on the four-celled metabasidium as normally observed in sexually reproducing species, the nuclear division during the development of the metabasidium and the basidiospore is considered to be mitosis.

This type of nuclear behavior and metabasidium formation have been reported for a large uninucleate form of *E. euphorbiae-sylvaticae* (DC.) Wint. (=*E. euphorbiae* Plowr., *E. uninucleatum* Moreau) (Mme. Moreau 1911, 1914, 1915; Moreau and Moreau 1918a, 1919; Dodge 1929; Olive 1953), *E. centranthi-rubri* Poir. (Poirault 1915), and a uninucleate form of *Gymnoconia nitens* (Schw.) Kern & Thurst. (=*Caeoma nitens* Burrill) (Dodge 1929). This type has also been reported for a form of a demicyclic species *Puccinia podophylli* (Brumfield, Ined; cited from Dodge 1929).

Dodge (1929) considered that the nucleus observed in the vegetative mycelium and the teliospore of *E. euphorbiae-sylvaticae* is diploid because the spores of the uninucleate form are as large as those of the binucleate forms and that the nucleus is as large as two nuclei of the binucleate spore. He assumed that the uninucleate form of *E. euphorbiae-sylvaticae* arose from the parental form by fusion of two haploid nuclei in the vegetative mycelium, which does not normally occur in the parental form.

In contrast, the nucleus of a uninucleate form of *G. nitens* was considered as haploid because the spore is smaller in this form than the binucleate spore and because the nucleus is the same as that of the binucleate spore (Dodge 1929). The nucleus of *E. centranthi-rubri* was also considered as haploid without a stated reason (Dodge 1929). These two forms and species are different from the uninucleate form of *E. euphorbiae-sylvaticae* in producing a two-celled metabasidium.

It is unknown whether the nucleus in the *P. lantanae* life cycle is haploid or diploid. If haploid, *P. lantanae* is unique in being haploid, uninucleate throughout the life cycle, and reproducing apomictically with the production of the four-celled metabasidium.

Puccinia patriniae

The uninucleate life cycle and the apomictic reproduction observed in this species are the same as those in *P. lantanae*. Binucleate cells were rarely observed in the telial primordium. If this is not aberrant nuclear behavior, this may indicate that the haploid uninucleate vegetative mycelium becomes dikaryotized by an unknown method, immediately followed by karyogamy. However, no binucleate teliospores have been observed, and thus this fungus is better considered to be uninucleate, either diploid or haploid, throughout the life cycle. The nuclear division took place in the development of the four-celled metabasidium is considered to be mitosis.

Although the nuclear behavior in *P. patriniae* is the same as that of *P. lantanae*, the four metabasidium cells give rise to a peg- or a whiplike germ tube in the former species, instead of producing ordinary basidiospores as in the latter. The nucleus in the metabasidium cell migrated into the peg- or a whiplike projection. No additional nuclear division took place in the germ tube.

It is empirically known that, in water or under condition of oxygen deprivation, teliospores germinate into elongated germ tubes or metabasidia, which when formed give rise to narrow, whiplike germ tubes. However, in this study, teliospores were germinated on an agar film on a microscopic slide. Because ordinary metabasidia and basidiospores were formed in *K. japonica* and *P. lantanae* under the same condition and because whiplike germ tubes were exclusively observed in *P. patriniae*, the latter mode of germination is considered to be normal in this fungus, as has been reported in *Uromyce aloes* (Ck.) Magn. (Sato et al. 1980), *U. hobsoni* Vize (Payak 1953), *Endoraecium acaciae* Hodges & Gardner, and *E. hawaiiense* Hodges & Gardner (Hodges and Gardner 1984).

Finally, it is important to note that any type of nuclear behavior observed for one or a few populations from a microcyclic species may not represent the entire population of that species. Microcyclic derivatives might have arisen repeatedly from a parental macrocyclic species (Jackson 1931). Thus, different populations, even though originated from the same parental species, may have different types of nuclear behavior. Microcyclic derivatives may remain morphologically similar and thus are taxonomically conspecific; however, a reproductive isolation mechanism might have

developed among those populations because of their different nuclear behaviors.

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References

Allen RF (1933) A cytological study of the teliospores, promycelia, and sporidia in *Puccinia malvacearum*. Phytopathology 23:572–586

Anikister Y (1983) Binucleate basidiospores – a general rule in rust fungi. Trans Br Mycol Soc 81:624–626

Anikister Y (1984) Contribution to the knowledge of nuclear history in some Pucciniaceae. Rep Tottori Mycol Inst 22:120–123

Anikister Y, Moseman JG, Wahl I (1980) Development of basidia and basidiospores in *Uromyces* species on wild barley and Liliaceae in Israel. Trans Br Mycol Soc 75:377–382

Ashworth D (1935) An experimental and cytological study of the life history of *Endophyllum sempervivi*. Trans Br Mycol Soc 19:240–258

Blackman VH (1904) On the fertilization, alternation of generations, and general cytology of the Uredineae. Ann Bot 18:323–373

Blackman VH, Fraser HCI (1906) Further studies on the sexuality of the Uredineae. Ann Bot 20:35–48

Brown AM (1940) The sexual behaviour of several plant rusts. Can J Res 18:18–25

Christman AH (1905) Sexual reproduction in rusts. Bot Gaz 39:267–275

Cragie JH (1927a) Experiments on sex in rust fungi. Nature 120:116–117

Cragie JH (1927b) Discovery of the function of the pycnia of the rust fungi. Nature 120:765–767

Dodge BO (1929) Cytological evidence bearing on the sexuality and origin of life cycles in the Uredinales. Proc Int Cong Plant Sci 1926 (Ithaca) 2:1751–1766

Fujita T, Minamikawa T (1990) Confocal laser microscopy (in Japanese). Jikken-igaku 5:459

Gardner DE (1981) Nuclear behavior and clarification of the spore stages of *Uromyces koae*. Can J Bot 59:939–946

Gardner DE (1987) Teliospore germination of *Uromyces alyxiae*, and endemic Hawaiian rust. Mycologia 79:914–917

Gardner DE (1988) Teliospore germination of *Puccinia vitata*, an endemic Hawaiian rust. Mycologia 80:590–593

Gardner DE (1994) Teliospore germination and nuclear behavior of *Puccinia rutainsulara*, a microcyclic Hawaiian rust. Mycologia 86: 486–493

Gardner D.E. (1996) *Puccinia rugispora*: an unusual microcyclic rust endemic to Hawaii. Mycologia 88:671–676

Hennen JF, Buriticá P (1980) A brief summary of modern rust taxonomic and evolutionary theory. Rep Tottori Mycol Inst 18:243–256

Hennen JF, Figueiredo MB, Pimental CP, Russomanno OMR (1984) The life cycle and taxonomy of *Puccinia pampeana* Speg. and *Endophyllum pampeanum* (Speg.) Lindq. on *Capsicum* spp. and other Solanaceae. Rep Tottori Mycol Inst 22:209–220

Hodges CS Jr, Gardner DE (1984) Hawaiian forest fungi. IV. Rusts on endemic *Acacia* species. Mycologia 76:332–349

Hoffman AWH (1912) Zur Entwicklungsgeschichte von *Endophyllum Sempervivi*. Centralbl Bakt 2 Abt 32:137–158

Jackson HS (1931) Present evolutionary tendencies and the origin of life cycles in the Uredinales. Bull Torrey Bot Club 18:1–108

Kohno M, Nishimura T, Ishizaki H, Kunoh H (1975) Cytological studies on rust fungi (III). Nuclear behaviors during the process from teliospore stage through sporidial stage in two short-cycled rusts, *Kuehneola japonica* and *Puccinia horiana*. Bull Fac Agric Mie Univ 49:21–29

Kohno M, Ishizaki H, Kunoh H (1977) Cytological studies on rust fungi. (VI.) Fine structure of infection process of *Kuehneola japonica* (Diet.) Diet. Mycopathologia 61:35–41

Kursanov L (1917) Recherches morphologiques et cytologiques sur les Urédinées (a French version published in 1922). Bull Soc Nat Moscou N S 31:1–129, 4 pl

Lindfors T (1924) Studien über den Entwicklungsverlauf bei einigen Rostpilzen aus zytologischen und anatomischen Gesichtpunkten. Sven Bot Tidskr 18:1–84

Maire R (1899) Sur les phénomenes cytologiques précédant et appagnant la formation de la téleutospore ches la *Puccinia liliacearum* Duby. C R Acad Paris 129:839–841

Maire R (1900) L'evolution nucléaire chez les *Endophyllum*. J Bot 14:80–97

Moreau Mme F (1911) Sur l'existence d'une forme écidienne uninucléée. Bull Soc Mycol Fr 27:489–493

Moreau, Mme F (1914) Les phénomenones de la sexualité chez les Urédinées. Botaniste 3:145–283

Moreau Mme F (1915) Note sur la variété uninucléée de l'*Endophyllum Euphorbiae* (DC.) Winter. Bull Soc Mycol Fr 31:68–70

Moreau F, Moreau Mme F (1918a) L'écidiospore de l'*Endophyllum Euphorbiae-silvaticae* (DC.) Winter est-elle le siege d'une karyogamie? Bull Soc Mycol Fr 33:97–99

Moreau F, Moreau Mme F (1918b) L'evolution nucléaire chez l'*Endophyllum Sempervivi* Lév. Bull Soc Mycol Fr 33:70–72

Moreau F, Moreau Mme F (1919) Les Urédinées du groupe *Endophyllum*. Bull Soc Bot France 66:14–44

Mukai Y (1996) In situ hybridization. In: Fukuik, Nakayama S (eds) Plant chromosomes: laboratory methods. CRC Press, Boca Raton, pp 155–170.

Nagle W (1996) Polyten chromosome. In: Fukui F, Nakayama S (eds) Plant chromosomes: laboratory methods. CRC Press, Boca Raton, pp 51–83

Olive EW (1911) Nuclear divisions in certain short-cycled rusts. Science 33:194

Olive LS (1953) The structure and behavior of fungal nuclei. Bot Rev 19:439–586

Payak MM (1953) On the existence of endo-condition in *Uromyces hobsoni* Vize. Cellule 56:73–84, 2 pl.

Petersen RH (1974) The rust fungus life cycle. Bot Rev 40:453–513

Poirault G (1915) Sur quelques champignons parasites rares ou noueaux obsérves dans les Alpes-Maritimes. Bull Assoc Nat Alpes-Maritimes 20:105–108

Sato T, Katsuya K, Sato S (1980) Host range, tellospore germination and infection process of *Uromyces aloes*. Trans Mycol Soc Jpn 21:273–282

Singh UP (1979) Cytology of *Puccinia ruelliae*. Mycologia 71:1197–1205

Thirumalachar MJ (1945) Development of spore-forms and the nuclear cycle in the autoecious opsis rust, *Cystopsora oleae*. Bot Gaz 107:74–86

Walker RI (1928) Cytological studies of some of the short-cycled rusts. Trans Wis Acad Sci Art Lett 23:567–582, 3 pl

Werth E, Ludwig K (1912) Zur Sporenbildung bei Rost- und Brandpilzen. Ber Dtsch Bot Ges 30:522–528

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FULL PAPER

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Aeciospore-surface structures of *Phragmidium* species parasitic on roses

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Abstract Aeciospore-surface structures of *Phragmidium* species parasitic on roses were investigated by scanning electron microscopy. Seven kinds of structures were distinguished according to gross shape of ornamentations and their distribution on the aeciospore wall. The seven different structures were categorized into three major types: echinulate, annulate, and verrucose. The echinulate type was further classified into five types and designated in a numerical series, echinulate types 1–5. Application of the aeciospore-surface structures in the classification of *Phragmidium* species is discussed.

Key words Aeciospore · *Rosa* · Scanning electron microscopy · Taxonomy · Uredinales

Introduction

Morphological features of aeciospores have been suggested as important characters in the taxonomy of *Cronartium* (Hiratsuka 1971), *Puccinia* and *Uromyces* on Gramineae, Cyperaceae, and Juncaceae (Savile 1973), on Liliaceae, Gramineae, and Juncaceae (Holm 1984), and *Gymnosporangium* and *Roestelia* (Holm 1984). However, no systematic account on aeciospore-surface structures was published until Sato and Sato (1982) studied aeciospore-surface structures of 70 species in 39 genera of the Uredinales. They recognized eight major types, i.e., aciculate, annulate, coronate, echinulate, nailheaded, reticulate, tubulate, and verrucose, within which several subtypes were included. In the study of aeciospores of *Gymnosporangium* and *Roestelia*, Lee and Kakishima (1999) classified aeciospore-surface structures into ten groups, i.e., large coronate, small coronate, minutely coronate, mountain shaped, echinulate, small annulate, large annulate, tubulate, nailheaded, large verrucose, small verrucose, and verrucose with refractive granules. These studies indicated that the aeciospore-surface structures could be a good taxonomic character when employed concomitantly with other selected characters.

In the taxonomy of the genus *Phragmidium*, less attention was paid to morphological features of aeciospores than those of teliospores or urediniospores. However, Cummins (1931) showed that aeciospore wall thickness and wall sculptures as revealed by light microscopy could be employed to segregate some *Phragmidium* species on roses. Aeciospore-surface ornamentations and wall thickness at germ pores were considered important in distinguishing *P. tuberculatum* J. Müller from *P. mucronatum* Schlectendal by Wilson and Henderson (1966) and Laundon (1970).

Aeciospore-surface structures of a few *Phragmidium* species have been studied by scanning electron microscopy (Sato and Sato 1982; Bedland 1984; Preece and Hick 1990). However, no systematic study of aeciospore-surface structures of *Phragmidium* species was undertaken and, therefore, no thorough consideration of the aeciospore-surface structures as a taxonomic character has been carried out in the taxonomy of *Phragmidium*.

This study reports aeciospore-surface structures of all *Phragmidium* species recorded on the genus *Rosa* and discusses a possible application of the structural features in the classification of *Phragmidium* species parasitic on roses.

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Materials and methods

Specimens examined

A total of 290 aecial specimens deposited in the Herbarium of Systematic Mycology, the Faculty of Education, Ibaraki University (IBA) and the Mycological Herbarium, the Institute of Agriculture and Forestry, the University of Tsukuba (TSH) and loaned from the following herbaria were studied: the Arthur Herbarium, Purdue University (PUR); the Mycological Herbarium, Faculty of Agriculture, Hokkaido University, Japan (SAPA); the Swedish Museum of Natural History, Stockholm, Sweden (S); the Herbarium of Mycology and Lichenology, the Institute of Microbiology, Academica Sinica (HMAS); and the Museum of Natural History, Wroclaw University, Poland (WRSL) (Table 1). The specimens represented *P. americanum* Dietel, *P. butleri* Sydow, *P. cinnamomeum* Durrieu, *P. fusiforme* Schröter, *P. montivagum* Arthur, *P. mucronatum* (Pers.) Schlectendal, *P. rosae-alpinae* (DC.) Winter, *P. rosae-arkansanae* Dietel, *P. rosae-californicae* Dietel, *P. rosae-lacerantis* Dietel, *P. rosae-moschatae* Dietel, *P. rosae-multiflorae* Dietel, *P. rosae-pimpinellifoliae* Dietel, *P. rosae-rugosae* Kasai, *P. rosae-setigerae* Dietel, *P. speciosum* (Fr.) Cooke, and *P. tuberculatum* J. Müller.

Table 1. Aeciospore-surface structure types of *Phragmidium* species on roses with their host range and geographic distributions

Rosa species	Geographic locality	Accession number[a] (number of specimens)	Surface structure type[b]
R. acicularis Lindl.	Alaska, U.S.A.	PUR7741, 7747, and 7749	E-1
	Canada	PUR44747 and 44749	E-1
	Canada	PUR53193	E-1
	Colorado, U.S.A.	PUR7925	E-1
	Japan	PURF11991, SAPA (3)	E-1
	Kansas, U.S.A.	PUR8113, 8132, and 8125	E-1
	Montana, U.S.A.	PUR7792, 55311, 7746, and 7836	E-1
	Russia	IBA7267, 6734, and 6750, SAPA	E-1
	U.S.A.	PUR7836	E-1
	Wyoming, U.S.A.	PUR61190 and 63222	E-1
	Unknown	PURF1587	E-1
R. alba L.	Maryland, U.S.A.	PUR8158	E-1
	Sweden	TSH-R1973	E-1
R. blanda Ait.	Canada	PUR65694 and 8484	E-1
	Iowa, U.S.A.	PUR8110 and 8481	E-1
	New York, U.S.A.	PUR8485	E-1
	Wisconsin, U.S.A.	PUR7660 and 44785	E-1
R. bourgeaniana Crép.	Canada	PUR44752	E-1
	Colorado, U.S.A.	PUR8571	E-1
	Montana, U.S.A.	PUR7799, 7800, and 7802	E-1
R. brunonii Lindl.	India	PURF18144	A
R. californica Cham. & Schlect.	California, U.S.A.	PUR8027, 53849, 8004, 8018, 62463, 8025, 8026, 8006, and 8010, S	E-1
	Nevada, U.S.A.	PUR8028	E-1
R. canina L.	Germany	PURF1560	E-3
	Sweden	PURF1563	E-3
R. carolina L.	Alabama, U.S.A.	PUR8530	E-1
	Delaware, U.S.A.	PUR7662	E-1
	Illinois, U.S.A.	PUR7718	E-1
	Indiana, U.S.A.	PUR8533, 8534, 8546, 8549, 8489, and 8539, S	E-1
	Massachusetts, U.S.A.	PUR44693 and 8491	E-1
	Missouri, U.S.A.	PUR47977	E-1
	Mississippi, U.S.A.	PUR44695 and 8493	E-1
	New York, U.S.A.	PUR7663, 7713, 7710, 8540, 8550, and 8536	E-1
	North Carolina, U.S.A.	PUR8552	E-1
	Vermont, U.S.A.	PUR8542	E-1
	West Virginia, U.S.A.	PUR44694	E-1
R. carolina L.	Wisconsin, U.S.A.	PUR7719	E-1
	Unknown	PUR7715	E-1
R. centifolia L.	Germany	PURF1503	E-1
R. cinnamomea L.	Germany	PURF1492	E-1
	Germany	PURF1552 and F1553	E-3
	Switzerland	PURF17039	E-3
R. damascena Miller	Alaska, U.S.A.	PUR8163	E-1
R. davurica Pall.	Russia	SAPA (4)	E-1
R. engelmannii Wats	Nebraska, U.S.A.	PUR8096	E-1

Table 1. *Continued*

Rosa species	Geographic locality	Accession number[a] (number of specimens)	Surface structure type[b]
R. fendleri Crép.	Alaska, U.S.A.	PUR50575	E-1
	Colorado, U.S.A.	PUR7886 and 8559	E-1
	Montana, U.S.A.	PUR8098, 8556, and 8586	E-1
	Nebraska, U.S.A.	PUR8101 and 8102	E-1
	Utah, U.S.A.	PUR7866	E-1
	Wyoming, U.S.A.	PUR7904, SAPA	E-1
R. filifera Rydb.	California, U.S.A	PUR8070	E-1
R. foetida J. Herrmann	Wyoming, U.S.A.	PUR62725	E-1
R. foliosa Nutt.	Tennessee, U.S.A	PUR8497	E-1
R. gallica L.	Scandinavia	PURF1518	E-1
R. gymnocarpa Nutt.	California, U.S.A.	SAPA, PUR8045, 8048, 53854, 8043, 8047, 53852, and 8053	E-1
	Washington, U.S.A.	PUR8035, 8038, 8039, and 8044	E-1
	Oregon, U.S.A	PUR44776	E-1
R. lacerans Boiss	Iran	*S (3)*	V
R. macdaugalii Holz.	Idaho, U.S.A.	PUR7766 and 49821	E-1
	Montana, U.S.A.	PUR7773	E-1
R. macounii Greene	Colorado, U.S.A.	PUR8553 and 8554	E-1
	North Dakota, U.S.A.	PUR8528	E-1
	Utah, U.S.A.	PUR7872	E-1
R. macrophylla L.	Unknown	PURF10545	E-3
R. marretii Lév.	Russia	PURF12006, SAPA (3)	E-1
R. minutifolia Englm.	California, U.S.A	PUR8054	E-1
R. moschata Miller	India	PURF10548	E-4
	India	IBA4929	E-3
	India	PURF1584 and F10547 PURF1573, F1574, and F17982	V
R. multiflora Thunb.	Japan	IBA1897, 5696, 6592, 2148, 1786, and 4736, PURF1579, SAPA (6), TSH-R3759	E-2
R. nutkana Presl.	Alaska, U.S.A.	PUR7784, 48583, and 8065	E-1
	California, U.S.A.	PUR53855	E-1
	Canada	PUR44757 and 8057	E-1
	Montana, U.S.A.	PUR44755, 44756, and 7768	E-1
	Oregon, U.S.A.	PUR8068 and 65059	E-1
	Washington, U.S.A.	PUR8069, 8055, 8060, and 8062	E-1
	Wyoming, U.S.A.	PUR48847	E-1
	Unknown	PUR1473, 53856, and 53857	E-1
R. palustris Marsh.	Tennessee, U.S.A.	PUR44696	E-1
R. pecosensis Cockerell	New Mexico, U.S.A.	PUR8563	E-1
R. pendulina L.	Switzerland	PURF1558	E-1
	Austria	PURF1568	E-1
	Poland	WRSL	E-1
R. pimpinellifoliae L.	Austria	PURF1535	E-1
	Germany	PURF1532	E-1
	Wisconsin, U.S.A.	PUR8233	E-1
	Unknown	PURF1535	E-1
R. pisocarpa Gray	Canada	PUR58015	E-1
	California	PUR8076, 48209, and 53850	E-1
	Oregon, U.S.A.	PUR8603	E-1
	Washington, U.S.A.	PUR8074	E-1
R. prattincola Greene	Kansas, U.S.A.	PUR8113	E-1
R. rubiginosa L.	Massachusetts, U.S.A.	PUR 8227	E-1
	Nebraska, U.S.A.	PUR8228 and 8225	E-1
	New York, U.S.A.	PUR 8231	E-1
	Switzerland	PURF1558	E-3
R. rugosa Thunb.	Japan	IBA8132 and 1890, PURF12022, SAPA (11)	E-1
	Russia	IBA6182 and 7238, SAPA	E-1
	Wisconsin, U.S.A.	PUR8568	E-1
R. saundersiae Rolfe	India	PURF1575	E-3
R. sericea Lindl.	China	TSH-R1975, 1976, and 1977	A
R. sepia Koch	Switzerland	PURF1556	E-3
R. setigera Michx.	Indiana, U.S.A.	PUR7726	E-1
	Unknown	PUR7716	E-1
R. sikangensis Yu & Ku	China	HMAS 45217	E-5
R. spithamea Wats.	California, U.S.A.	PUR49614	E-1
R. subnuda J. Lunnel	North Dakota, U.S.A.	PUR8146	E-1

Table 1. *Continued*

Rosa species	Geographic locality	Accession number[a] (number of specimens)	Surface structure type[b]
R. suffulta Greene	Alaska, U.S.A.	PUR8131 and 8525	E-1
	Canada	PUR7750 and 8106	E-1
	Indiana, U.S.A.	PUR8510	E-1
	Iowa, U.S.A.	PUR8502, 8504, and PUR N37	E-1
	Kansas, U.S.A.	PUR8506, S	E-1
	Missouri, U.S.A.	PUR8137	E-1
	North Dakota, U.S.A.	PUR7760 and 8499	E-1
	South Dakota, U.S.A.	PUR8123 and 8513	E-1
R. virginiana Miller	Canada	PUR8522 and 8576	E-1
	Connecticut, U.S.A.	PUR8581	E-1
	Indiana, U.S.A.	PUR8582	E-1
	Maine, U.S.A.	PUR7680 and 7673	E-1
R. virginiana Miller	Massachusetts, U.S.A.	PUR7678	E-1
	Missouri, U.S.A.	PUR8585	E-1
	New York, U.S.A.	PUR7681 and 8584	E-1
R. webbiana Wallich ex Royle	India	PURF10549 and	E-4
		F15706	E-3
	Pakistan	IBA4937 and 4897	E-3
	Tibet, China	HMAS67833	A
R. wilmottiae Hemsl.	Tibet, China	HMAS67838	A
Rosa sp.	Alaska, U.S.A.	PUR7809 and 7790	E-1
	Arizona, U.S.A	PUR8084	E-1
	California, U.S.A	PUR8090, 8091, 8082, 8086, 8087, and 8597	E-1
	Canada	PUR8085	E-1
	Colorado, U.S.A.	PUR8601 and 49702	E-1
	Indiana, U.S.A.	PUR44699	E-1
	Michigan, U.S.A.	PUR8592	E-1
	Mississippi, U.S.A.	PUR8600 and 8602	E-1
	Missouri, U.S.A.	PUR51235	E-1
	Montana, U.S.A.	PUR8153	E-1
	Nebraska, U.S.A.	PUR7736 and 7689	E-1
	New York, U.S.A.	PUR7698	E-1
	Nevada, U.S.A.	PUR8030	E-1
	North Dakota, U.S.A.	PUR8148	E-1
	Pakistan	PURF1586	E-3
	India	PURF15878	E-1
	Oregon, U.S.A	PUR8088	E-1
	Russia	IBA7282	E-1
	South Dakota, U.S.A.	PUR7810	E-1
	Sweden	TSH-R1982 and 1984	E-1
	Washington, U.S.A.	PUR7969 and 8083	E-1
	Wisconsin, U.S.A.	PUR8591 and 8594	E-1
	Wyoming, U.S.A.	SAPA	E-1

[a] HMAS, The Herbarium of Mycology and Lichenology, Institute of Microbiology, Academia Sinica, China; IBA, The Herbarium of Systematic Mycology, Faculty Education, Ibaraki University, Japan; PUR, The Arthur Herbarium, Purdue University, U.S.A.; SAPA, The Mycological Herbarium, Faculty of Agriculture, Hokkaido University, Japan; S, The Swedish Museum of Natural History, Sweden; TSH, The Mycological Herbarium, Institute of Agriculture and Forestry, University of Tsukuba, Japan; WRSL, The Museum of Natural History, Wroclaw University, Poland

[b] A, annulate; E-1, echinulate type 1; E-2, echinulate type 2; E-3, echinulate type 3; E-4, echinulate type 4; E-5, echinulate type 5; V, verrucose

Scanning electron microscopy

Sori were determined as aecia when they were associated with spermogonia on the herbarium specimens examined. Aeciospores were scraped from the aecia by a fine surgical knife and dusted on a small piece of double-adhesive tape on a specimen holder. The preparation was coated with platinum-palladium with an E-1030 Ion Sputter and examined under an S-4200 SEM (Hitachi, Ibaraki, Japan) operating at 15kV. Observed surface structures were categorized according to Sato and Sato (1982) and Cummins and Hiratsuka (1983).

Results and discussion

Seven kinds of surface structures were revealed by scanning electron microscopy (SEM) and categorized into echinulate, annulate, or verrucose types (Figs. 1–3). The echinulate type was further classified into five types (Fig. 1). Each type is described as follows.

Echinulate type 1 (E-1)

Echinulate type 1 is characterized by even distribution of stout, pointed conical echinae (Figs. 1E-1, 2A). The apex

may be weakly curved. The structure is essentially the same as the echinulate structure most commonly observed in urediniospores, and the process of echina development in the aeciospores is assumed to be the same as that in the urediniospores (Littlefield and Heath 1979). Length and density of echinae on the spores were fairly uniform within a specimen but varied among the specimens. The length ranged from 0.9 to 1.2μm, and density ranged from 2 to 4 echinae per 10μm². The spore surface structures of a majority of the specimens fell into this category (see Table 1). Holm et al. (1970) observed this type of echinulation in aeciospores from a specimen that they identified as *P. mucronatum*.

Echinulate type 2 (E-2)

Echinulate type 2 is essentially the same as type 1 except for the lack of echinae on the apical area of the spore wall (Figs. 1E-2, 2B). The echina size is decreased gradually toward the smooth area. The apical smooth area ranged from 10% to 30% of the total spore wall surface. As in type 1, the size and density of echinae were fairly uniform in the spores from a specimen but varied among the spores from different specimens. Echina length was ~1μm on average, ranging from 0.3 to 1.5μm; density ranged from 2 to 4 echinae/10μm².

Type 2 was observed only in the spores produced on *R. multiflora* Thunb. in Japan (Table 1). Spores from 14 specimens on *R. multiflora* showed only this type of surface structure. The SEM photomicrograph published by Sato and Sato (1982) showed echinulate aeciospores from 1 of 2 specimens on *R. multiflora* they examined. They did not mention the presence of a smooth area on the spore wall (Table 2). Echinulate aeciospores with an apical smooth area have been reported in *Naohidemyces fujisanensis* S. Sato et al. (Sato et al. 1993). This type of surface ornamentation is not uncommon in urediniospores and has been reported in *Melampsoridium asiaticum* S. Kaneko & Hiratsuka, f. (Kaneko and Hiratsuka 1983), *Melampsora* spp. (Preece and Hick 1990), *Blastospora smilacis* Dietel (Ono et al. 1986), *Tranzschelia* species (López-Franco and Hennen 1990; Ono 1994), and *N. fujisanensis* (Sato et al. 1993).

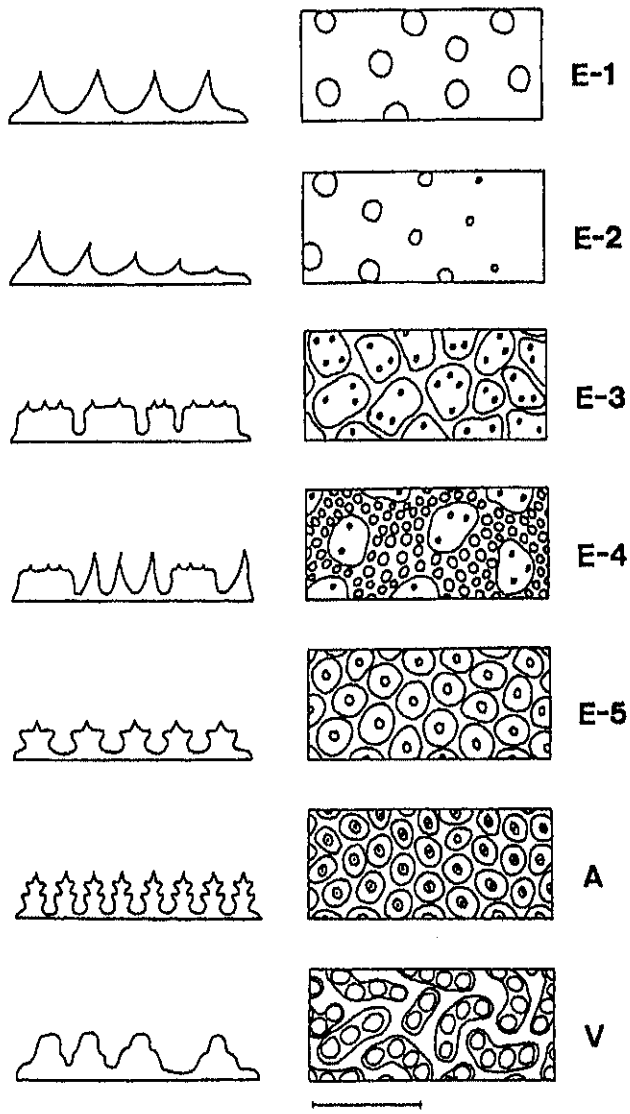

Fig. 1. Schematic presentation of aeciospore-surface structures of *Phragmidium* species on roses. *E-1*, echinulate type 1; *E-2*, echinulate type 2; *E-3*, echinulate type 3; *E-4*, echinulate type 4; *E-5*, echinulate type 5; *A*, annulate; *V*, verrucose. *Left*, side view; *right*, top view. *Bar* 2μm

Echinulate type 3 (E-3)

This surface-structure type is characterized by one or more echinae arising on a plateau-shaped base (Figs. 1E-3, 2C,D). Number of echinae on each plateau-shaped base was mostly two or three. The echinae of this type were shorter (0.4–0.8μm) than those in echinulate types 1 and 2 (0.9–1.2μm). The plateau-shaped bases were various in shapes, mostly irregularly angular, and their length also varied from 1.4 to 5.0μm. This type of surface structure was observed in the specimens on *R. canina* L., *R. cinnamomea* L., *R. macrophylla* Lind., *R. moschata* L., *R. rubiginosa* L., *R. saundersiae* Rolfe, *R. sepia* Koch., *R. webbiana* Wallich ex Royle, and *Rosa* sp. from Europe and the Himalayas (Table 1).

An SEM photomicrograph of aeciospores from a specimen identified as *P. tuberculatum* was published by Preece and Hick (1990). The surface structure shown in the photomicrograph is considered to be echinulate type 3, although the photomicrograph is out of focus and the authors did not describe the structure in detail. The nailheaded type observed in aeciospores of *Phakopsora meliosmae* Kusano (Sato and Sato 1982) resembles this type of surface structure. However, in the nailheaded type, one echina occurs on each peltate base, which is connected to adjacent peltate bases by buttresses (see Table 2).

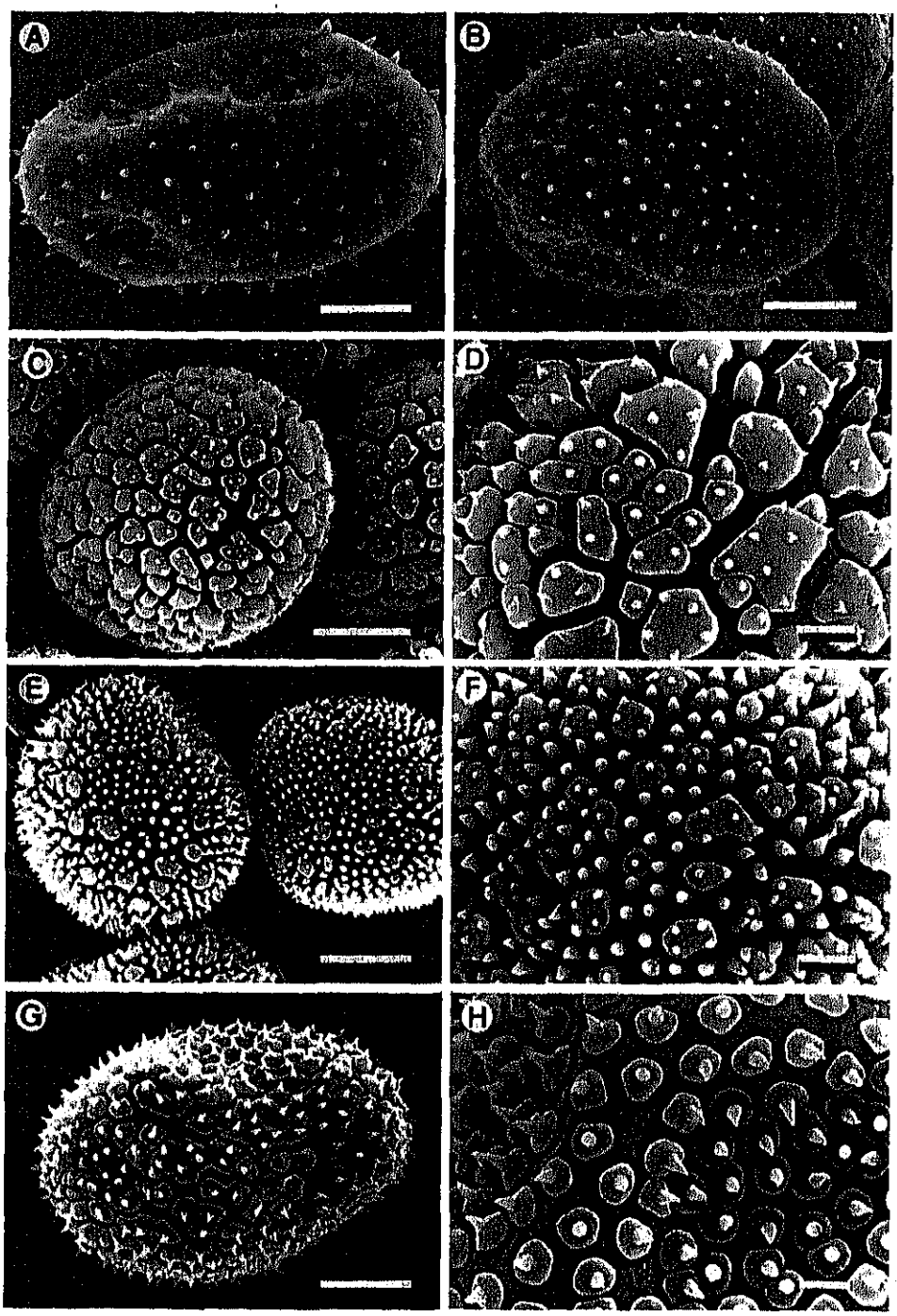

Fig. 2. Aeciospore-surface structures of *Phragmidium* species on roses. **A** Echinulate type 1 (E-1) on *Rosa* species (PUR44699). **B** Echinulate type 2 (E-2) on *R. multiflora* (IBA5696). **C, D** Echinulate type 3 (E-3) on *Rosa* sp. (PURF1586). **E, F** Echinulate type 4 (E-4) on *R. moschata* (PURF10548). **G, H** Echinulate type 5 (E-5) on *R. sikangensis* (HMAS45217). *Bars* **A–C, E, G** 5 μm; **D, F, H** 1.5 μm

Echinulate type 4 (E-4)

Type 4 is characterized by mixed distribution of echinae with or without a plateau-shaped base (Figs. 1E-4, 2E,F). The echinae without a plateau-shaped base are long and aciculate or subulate, whereas those arising on a plateau-shaped base are short and conical. The number of echinae formed on each plateau-shaped base varied from one to five. Length was 0.8–1.2 μm in the echinae without a plateau-shaped base and 0.4–0.7 μm in those arising on a plateau-shaped base.

This type of surface structure was observed in the specimens on *R. moschata* and *R. webbiana* from the Himalayas (Table 1) and has not been reported previously in the Uredinales (Table 2).

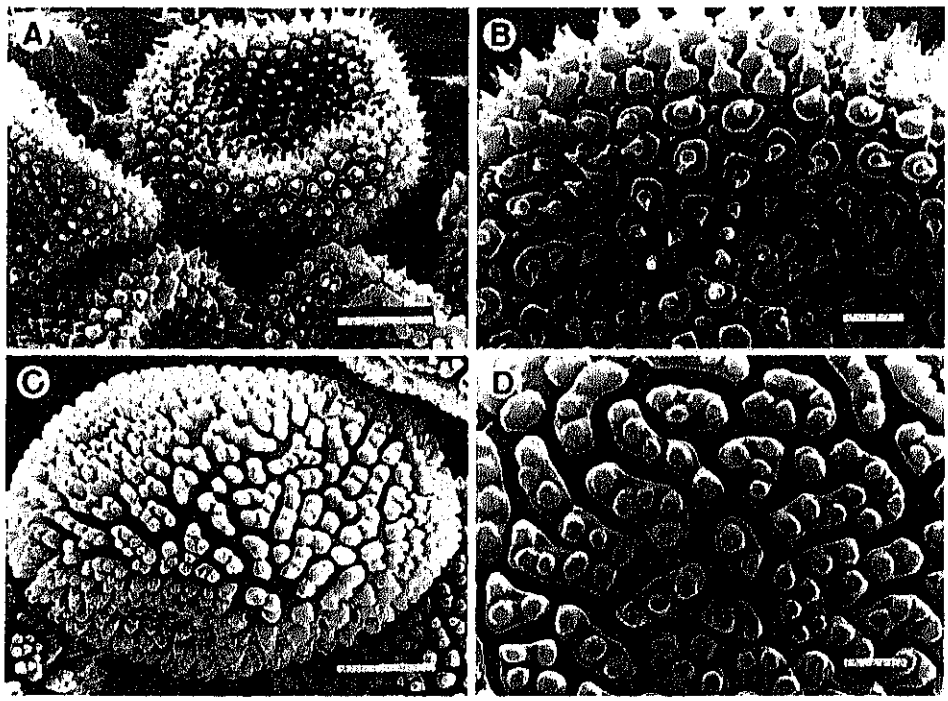

Fig. 3. Aeciospore-surface structures of *Phragmidium* species on roses. **A, B** Annulate type (A) on *R. sericea* (TSH-R1976). **C, D** Verrucose type (V) on *R. moschata* (PURF17982). *Bars* **A, C** 5 μm; **B, D** 1.5 μm

Table 2. Correspondence among different classifications of aeciospore-surface structures

Present study[a]	Sato and Sato (1982)	Lee and Kakishima (1999)[b]
–	Aciculate	–
Annulate	Annulate	Large and small annulate
–	Coronate	Coronate (large coronate, small coronate, minutely coronate) and mountain shape
Echinulate (type 1–5)	Echinulate	Echinulate
–	Nailheaded	Nailheaded
–	Reticulate	–
–	Tubulate	Tubulate
Verrucose	Verrucose	Verrucose (large verrucose, small verrucose, verrucose with refractive granules)

–, not observed
[a] Aeciospore-surface structures of *Phragmidium*
[b] Aeciospore-surface structures of *Gymnosporangium* and *Roestelia*

Echinulate type 5 (E-5)

Type 5 is characterized by an echina arising from a mushroom-shaped base (Figs. 1E-5, 2G,H). The echinae were straight or weakly curved, sharp pointed, and ~0.9 μm long. The mushroom-shaped bases appeared more or less rounded on a spore surface area where they were separated, whereas they were variously shaped on a spore area where they were densely packed. This type of surface structure was observed in one specimen on *R. sikangensis* Yu & Ku from China (Table 1).

Sato and Sato (1982) described an echina produced on a broad moundlike base on the aeciospore surface as a variant of the echinulate type. In contrast, the echina in echinulate type 5 is unique in having the constriction at the lower half of the base, thus giving the base a mushroom-like appearance (Table 2). This type of aeciospore-surface structure was unknown in species of *Phragmidium*.

Annulate type (A)

The annulate type of surface structure is characterized by a two-layered torulose base on which an echina is produced (Figs. 1A, 3A,B). The echinae were conical, straight, or slightly curved at the apex and ~1 μm long. The height of the two-layered bases was 1.5–2.7 μm. The density of the ornamentations varied from 7 to 11 per 10 μm^2 depending on the specimens examined. This type of surface structure was observed in specimens on *R. brunonii* Lindl., *R. sericea* Lindl., *R. webbiana*, and *R. wilmottiae* Hemsl. (Table 1).

This type of surface structure resembles echinulate type 5 in that an echina is produced on a mushroom-like base. However, in this type of surface structure, the echina is produced on an additional small base, the lower half of which is also constricted, formed on a large mushroom-like base (Table 2).

The annulate type as defined by Sato and Sato (1982) was characterized by two or more annulations (or stacked layers) in the processes. The surface structure of aeciospores of *Aecidium balanse* Cornu ex Pat. (Punithalingam and Jones 1971) and *Coleosporium*

phellonderi Komarov (Hiratsuka and Kaneko 1975) represents the annulate type. Annulate-type ornamentation was also characterized by a peltate or flat apex (Sato and Sato 1982). In contrast, the annulate type we described here has a pointed apex, as already described.

Verrucose type (V)

The verrucose type is characterized by one or more verrucae produced on a distinct base on the spore wall (Figs. 1V, 3C,D). The verrucae are short and cylindrical with an obtuse apex and ~1 μm long. The base was rounded, triangular, broadly ellipsoidal, or curved beltlike in shape depending on the number and arrangement of verrucae produced on the base. SEM photomicrographs of aeciospores and urediniospores of *P. fragariae* (DC.) Rabh. published by Preece and Hick (1990) appear to be the same as the verrucose type we describe here. However, details of the structures were not determined because the photomicrographs were out of focus. This type of surface structure was observed in the specimens on *R. lacerans* Boiss and on *R. moschata* (Table 1).

Application of aeciospore-surface structures as a taxonomic character

Classification and identification of *Phragmidium* species have relied heavily on selected morphological characteristics in the telial-uredinial stage and putative host specificity (Wahyuno et al. 2001). The reasons for this taxonomic practice are (1) the telial-uredinial stage is often observed both in the field and in herbarium specimens; (2) morphological features of teliospores and their pedicels are diverse, characteristic, and easy to observe; (3) a few unique features are observed in urediniospores; and (4) different sets of morphological characteristics are often associated with different hosts or host groups.

Nevertheless, ambiguities have not been excluded with the species circumscriptions and identifications by the selected telial-uredinial characters. To overcome this taxonomic difficulty, search for new taxonomic characters has been urgently needed; the aeciospore-surface structures have became the most important candidate for taxonomic characters in the classification of *Phragmidium* as indicated by the successful application of the aeciospore-surface structure in the classification of *Coleosporium* (Kaneko 1981) or *Gymnosporangium* (Lee and Kakishima 1999), among others.

In the SEM study of aeciospores of all the currently accepted species of *Phragmidium* species on *Rosa*, seven different surface structures have been revealed. Although aeciospores from a majority of specimens on a broad spectrum of host species collected across the world exhibited the echinulate type 1 of surface structure, the other six types of surface structure seemed to be restricted to specimens on certain host species and with a narrow geographic distribution. Thus, echinulate type 2 is specific to the specimens on *R. multiflora* in Japan; echinulate type 3 is restricted to the specimens on *R. canina*, *R. cinnamomea*, *R. macrophylla*, *R. moschata*, *R. rubiginosa*, *R. saundersiae*, *R. sepia*, and *R. webbiana* in Europe and the Himalayas; echinulate type 4 is restricted to *R. moschata* and *R. webbiana* in the Himalayas; and echinulate type 5 is specific to specimens on *R. sikangensis* in southwestern China. The annulate type, which has not been reported previously, was observed on *R. brunonii*, *R. sericea*, *R. webbiana*, and *R. wilmottiae* from China and India. The verrucose type is observed from specimens on *R. lacerans* and *R. moschata* in Iran and India.

Among the seven specimen groups circumscribed by the aeciospore-surface structures, the first group with the echinulate type 1 aeciospores is highly likely to contain two or more species. However, from the host relationship and the geographic distribution, the second group with echinulate type 2 aeciospores is highly likely to be *P. rosae-multiflorae*. No rust species other than *P. rosae-multiflorae* is known on *R. multiflora*. Similarly, the fourth group with the echinulate type 4 is predicted to be *P. rosae-moschatae*. Only *P. rosae-moschatae* has been known to occur on both *R. moschata* and *R. webbiana* within the Himalayan region. *Rosa lacerans* is the only host for *P. rosae-lacerantis* in the Himalayas and, thus, the verrucose-type aeciospores observed in this study are likely to be those of *P. rosae-lacerantis*. These predictions of the three species by the aeciospore-surface structures can be justified only after the structure differences are proven to be correlated or associated with other morphological differences or host specificities. Covariations among different morphological features in different spore stages in the species of *Phragmidium* under discussion will prove the aeciospore-surface structures to be a good taxonomic character.

Acknowledgments A number of specimens examined in this study were loaned from the Arthur Herbarium, Purdue University, USA (PUR); the Mycological Herbarium, Faculty of Agriculture, Hokkaido University, Japan (SAPA); the Swedish Museum of Natural History, Sweden (S); the Herbarium of Mycology and Lichenology, Institute of Microbiology, Academia Sinica, China (HMAS); and the Museum of Natural History, Wroclaw University, Poland (WRSL), for which we are grateful.

References

Bedland G (1984) Die Gattung *Phragmidium* Link. mit besonder Berucksichtigung des Formenkreises um *Phragmidium mucronatum* und *Phragmidium potentillae* in Mitteleuropa. Pflanzenschutzberichte 46:33–60

Cummins GB (1931) *Phragmidium* species of North America: differential teliospores and aecial characters. Mycologia 23:433–445

Cummins GB, Hiratsuka Y (1983) Illustrated genera of rust fungi, 2nd edn. American Phytopathological Society, St. Paul, MN

Hiratsuka Y (1971) Spore surface morphology of pine stem rust of Canada as observed under scanning electron microscope. Can J Bot 49:371–372

Hiratsuka N, Kaneko S (1975) Surface structure of *Coleosporium* spores. Rep Tottori Mycol Inst 12:1–13

Holm L (1984) The importance of aecial characters in the taxonomy of rusts. In: Subramanian CV (ed) Taxonomy of fungi. University of Madras, India, pp 548–552

Holm L, Dunbar A, Hofsten A (1970) Studies on the fine structure of aeciospores. II. Sven Bot Tidskr 64:380–382

Kaneko S (1981) The species of *Coleosporium*, the causes of pine needle rusts, in the Japanese Archipelago. Rep Tottori Mycol Inst 19:1–159

Kaneko S, Hiratsuka N (1983) A new species of *Melampsoridium* on *Carpinus* and *Ostrya*. Mycotaxon 18:1–4

Laundon GF (1970) Additions to the rust fungi of New Zealand. 5. NZ J Bot 8:310–319

Lee SK, Kakishima M (1999) Aeciospore surface structure of *Gymnosporangium* and *Roestelia* (Uredinales). Mycoscience 40: 109–120

Littlefield H, Heath M (1979) Ultrastructure of rust fungi. Academic Press, New York

López-Franco RM, Hennen JF (1990) The genus *Tranzschelia* (Uredinales) in the Americas. Syst Bot 15:560–591

Ono Y (1994) *Tranzschelia asiatica* sp. nov. and its taxonomic relationship to *Tranzschelia arthurii*. Can J Bot 72:1179–1186

Ono Y, Kakishima M, Kudo A, Sato S (1986) *Blastospora smilacis*, a teleomorph of *Caeoma makinoi*, and its sorus development. Mycologia 78:253-262

Preece TF, Hick AI (1990) An introductory scanning electron microscope atlas of rust fungi. Farrand Press, London

Punithalingam E, Jones D (1971) *Aecidium* species on *Agathis*. Trans Br Mycol Soc 57:325–331

Sato T, Sato S (1982) Aeciospore surface structure of the Uredinales. Trans Mycol Soc Jpn 23:51–63

Sato S, Katsuya K, Hiratsuka Y (1993) Morphology, taxonomy and nomenclature of *Tsuga*-Ericaceae rusts. Trans Mycol Soc Jpn 34:47–62

Savile DBO (1973) Aeciospore types in *Puccinia* and *Uromyces* attacking Cyperaceae, Juncaceae and Poaceae. Rep Tottori Mycol Inst 10:225–241

Wahyuno D, Kakishima M, Ono Y (2001) Morphological analyses of urediniospores and teliospores in seven *Phragmidium* species parasitic on ornamental roses. Mycoscience 42:519–533

Wilson M, Henderson DM (1966) British rust fungi. Cambridge University Press, Cambridge

Mycoscience (2002) 43:421–439

REVIEW

Yoshitaka Ono

The diversity of nuclear cycle in microcyclic rust fungi (Uredinales) and its ecological and evolutionary implications

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Abstract Nine types with 11 variations of nuclear cycle and associated metabasidium development were distinguished in microcyclic rust fungi. An additional type was recognized in rust fungi with an expanded life cycle. A significant proportion of rust fungi with a reduced life cycle is assumed to have lost a sexual genetic recombination process, being either apomictic or asexual in reproduction. Most species that retain a sexual process in the microcyclic life cycle seem to have become homothallic. During life cycle evolution by the omission of spore stages, these traits might have had a selective advantage for those species that had less opportunity to encounter a genetically different but sexually compatible mate because of isolated patchy distribution or a short growing season. The findings that different populations of a morphologically identifiable species exhibit two or more distinct patterns of nuclear cycle and different metabasidium development indicate that microcyclic lineages might have evolved independently and repeatedly from a macrocyclic parental species. Those lineages are morphologically the same but would differ from each other in their genetics and biology.

Key words Basidiospore · Ecology · Evolution · Life cycle · Metabasidium

Introduction

The rust fungi (Uredinales, Basidiomycota) consist of 6929 species in 163 genera in 14 families and comprise approximately 10% of all described and named species in the kingdom Fungi (Kirk et al. 2001). The Uredinales has been believed to be a monophyletic taxon, and recent molecular phylogenetic analyses (Swann et al. 1998; Sjamsuridzal et al. 1999) support this perspective. A phylogenetic placement of families has not been critically evaluated, however (Swann et al. 2001). All the rust fungi are ecologically obligate parasites on ferns, gymnosperms, and angiosperms (perhaps on mosses as well). The parasitism of rust fungi to the host plant is highly specific. However, the degree of host specialization varies with species. Thus, some species are limited to a single species or a few closely related species in a genus or a few allied genera of a plant family. Others, e.g., *Phakopsora pachyrhizi* Syd. & P. Syd. and *P. meibomiae* Arthur (Ono et al. 1992), occur on a large number of species in several genera of the plant family Leguminosae. Only a limited number of species have been axenically cultured on semidefined media (MaClean 1982).

Besides the characteristic nature of obligate parasitism on vascular plants, rust fungi are unique in having complex life cycle patterns with elaborate spore forms in the life cycle stages. Rust fungi produce up to five distinct spore forms in respective stages of the life cycle. Accordingly, the life cycle may be composed of spermogonial, aecial, uredinial, telial, and basidial stages. This life cycle is said to be macrocyclic. When all the stages are produced on plants of a single species or closely related species, the life cycle is called autoecious. On the other hand, when spermogonial and aecial stages occur on plants of a group of species and uredinial and telial stages on a different group of host plants, the life cycle is called heteroecious. The spermogonial aecial host(s) and the uredinial telial host(s) are eco-geographically closely associated. They are phylogenetically distantly related, however. The heteroecious life cycle is widespread in the Uredinales, whereas the mode of life cycle similar to heteroecism has been reported only in a few species of *Ceolomomyces* (Blastocladiales, Chytridiomycota) (Whisler et al. 1974, 1975) and in *Pyxidiophora* (Laboulbeniales, Ascomycota) (Blackwell and Malloch 1989).

A life cycle is said to be macrocyclic when all spore forms are produced in a unidirectional order in the life cycle. In the demicyclic life cycle, the uredinial stage is omitted. When only a telial stage with or without a spermogonial stage is formed on a plant throughout the season, the life

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cycle is said to be microcyclic. In some species whose aeciospores are morphologically indistinguishable from urediniospores, their life cycle is called either macrocyclic or brachycyclic depending on the terminology and definition of spore stages. Terminology of spore forms and life cycle has been discussed by Arthur (1925), Hiratsuka (1973a,b, 1975, 1991), Hiratsuka and Sato (1982), Holm (1973, 1984, 1987), Laundon (1967, 1972), and Savile (1968, 1988). The ontogenic system of terminology (Hiratsuka 1973a, 1975) is employed throughout this article.

The complex life cycle with elaborated spore forms produced in each stage and the obligate parasitism of rust fungi have long drawn the attention of mycologists. The origin and evolution of obligate parasitism and the complex life cycle in rust fungi have been the subject of considerable debate, and a wide variety of hypotheses have been submitted for verification [Fitzpatrick 1918a,b; Orton 1927; Dodge 1929; Jackson 1931, 1944; Couch 1937, 1938; Linder 1940; Stanley 1940; Leppik 1953, 1955, 1956, 1959, 1961, 1965, 1967, 1972, 1973; Savile 1955, 1976; see Hennen and Buriticá (1980) and Petersen (1974) for a brief review]. Currently, there is no universally accepted theory to reasonably explain this important subject, however.

A widespread assumption is that parasitism and host specialization were acquired at an early stage of the rust–fungus evolution. Speciation, ecophysiological diversification, and geographic distribution changes of host plants are believed to have played a vital role in the evolution and ecological diversification of rust fungi.

Thus, no matter which fungus, i.e., a *Herpobasidium*-related fungus parasitic on ferns (Jackson 1935), an auriculariaceous parasitic fungus related to *Jola*, *Eocronartium*, *Herpobasidium*, and *Platycarpa* (Leppik 1955, 1965), an ascomycetous proto-*Taphrina* parasitic on ferns (Savile 1955, 1976), or a *Pachnocybe*- or *Helicobasidium*-related fungus (Swann et al. 1998), was an immediate ancestor of rust fungi, the "first rust fungus" certainly possessed only a basidial stage with morphologically undifferentiated probasidia (teliospores). The life cycle of the ancestral rust fungus only with the basidium stage is said to be "unexpanded" (Hennen and Buriticá 1980). From this ancestor, progenies have evolved through jumping to other plants and entering different environments, particularly with seasonally changing climates. The life cycle might have expanded with the addition of vegetative spore forms (aeciospores and urediniospores) and sex-related structures (spermogonia), as depicted by Jackson (1931) and Hennen and Buriticá (1980).

Through the process of speciation and concomitant ecophysiological changes, which followed host speciation and their changes in ecogeographic distribution, some rust fungi might have reduced their life cycle by eliminating one or more spore stages. This evolutionary process of life cycle reduction ultimately resulted in rust fungi that produce only a telial/basidial stage in their life cycle. This life cycle is said to be reduced (Hennen and Buriticá 1980). During the reduction of life cycle in some, particularly heteroecious, species, the function of the telial stage was transferred to the aecial stage. This phenomenon has been explained under "Tranzschel's law." The life cycle of those species that produce morphological aeciospores with the function of teliospores is called endocyclic, a special case of the microcyclic life cycle.

Consequently, those rust fungi that possess a microcyclic life cycle are of two groups with different evolutionary backgrounds, one retaining the most primitive trait and another the most advanced. Microcyclic species constitute a larger proportion of the rust biota in arcto-alpine regions (Arthur 1928; Hiratsuka 1935; Savile 1953) than in temperate regions. Endemic microcyclic rust fungi have also been found in oceanic islands (Hennen and Hodges 1981; Hodges and Gardner 1984; Gardner 1990, 1996). The life cycle of those species is considered to be reduced (Ono 2002). These arcto-alpine species are often isolated and patchy in distribution. In contrast, many tropical species producing only a telial stage are considered to be unexpanded in their life cycle (Hennen and Buriticá 1980; Ono and Hennen 1983; Ono 2002). Hereafter, the term microcyclic refers to a life cycle that has been reduced to form only the telial stage.

Rust fungi with a reduced microcyclic life cycle are most diverse in nuclear behavior and accompanying development of metabasidia and basidiospores (Walker 1928; Jackson 1931; Petersen 1974). A general tendency that microcyclic rust fungi with ecogeographically isolated distribution exhibit a wide variation in their nuclear cycle implies the evolutionary and ecological significance of life cycle reduction because the variation is likely to be associated with the loss of the sexual genetic recombination process.

Cytological studies of the rust life cycle have been reviewed by Moreau and Moreau (1919), Dodge and Gaiser (1926), Dodge (1929), Jackson (1931, 1935), and Olive (1953) and summarized with some revisions by Petersen (1974) and Hiratsuka and Sato (1982). Hiratsuka (1973a) discussed the nuclear cycle in relation to the terminology of the spore stages.

This article reexamines previously reviewed studies on the nuclear cycle and associated metabasidium development in microcyclic rust fungi and incorporates those works that were published in the past 20 years and are pertinent to the discussion.

Types of nuclear cycle and accompanying morphological changes in metabasidium and basidiospore production

Type I: Sexual cycle with haploid binucleate mycelium and four haploid uninucleate basidiospores produced on the four-celled metabasidium (Fig. 1)

This type is equivalent to the "variation 1" of Jackson (1935), the "type 1" of Petersen (1974), and the "type I" of Hiratsuka and Sato (1982).

In this nuclear cycle, the vegetative mycelium is at first uninucleate, becoming binucleate during the initiation of a telium (basidiosorus) by various methods described elsewhere. Nuclear fusion takes place in young teliospores

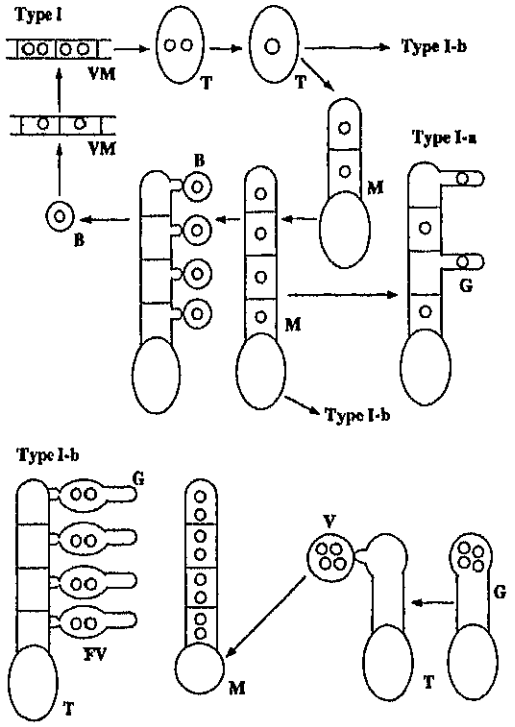

Fig. 1. Types of nuclear cycle in microcyclic rust fungi. *Type I.* A uninucleate vegetative mycelium (*VM*) becomes binucleate during telium (basidiosorus) production. Nuclear fusion takes place in a teliospore (*T*). A fusion (diploid) nucleus divides twice meiotically in a metabasidium (*M*). Each of four daughter (haploid) nuclei is delimited by septa. Each metabasidium cell produces a uninucleate basidiospore (*B*). *Type I-a.* A germ tube (*G*), instead of an ordinary basidiospore, arises from a metabasidium cell. Two (less frequently one or three) germ tubes are produced from a metabasidium. *Type I-b.* Each metabasidium cell gives rise to a fusiform vesicle (*FV*), into which a nucleus (presumably haploid) migrates. The nucleus divides mitotically to form two daughter nuclei, which then move to a germ tube (*G*). In another variant, a nucleus (presumably diploid) in a germ tube (*G*) from a teliospore divides (presumably meiotically) to form four daughter nuclei. Four daughter nuclei are not delimited by septa; instead, they migrate into a vesicle-like structure (*V*) formed on an elongated sterigma-like process derived from a germ tube. From the vesicle, another germ tube arises where the four nuclei are delimited by septa and each of four nuclei further divides once mitotically. The germ tube that becomes four celled, each cell containing either one or two nuclei, is referred to as the metabasidium (*M*). Each binucleate cell may give rise to a germ tube that functions as an infection hypha

(probasidia). During metabasidium production by the germination of teliospores, the fusion (diploid) nucleus divides twice meiotically and each of the four daughter (haploid) nuclei is delimited by septa laid down concomitantly with the nuclear division, the mature metabasidium thus becoming four celled. Each metabasidium cell gives rise to a basidiospore into which the nucleus migrates from the metabasidium cell. In endocyclic species, teliospores may morphologically resemble aeciospores of related macrocyclic species.

This type of nuclear cycle commonly occurs in macrocyclic and demicyclic species but less frequently in microcyclic species. Microcyclic species that exhibit this nuclear cycle type include *Puccinia malvacearum* Bertero (Blackman and Fraser 1906; Olive 1911; Werth and Ludwigs 1912; Moreau 1914; Lindfors 1924; Allen 1933), *Gymnoconia nitens* (Schwein.) F. Kern & Thurst., pro parte (Kunkel 1914), *Endophyllum sempervivi* (Alb. & Schwein.) de Bary (Maire 1900; Hoffman 1912; Moreau and Moreau 1918b; Ashworth 1935), *E. paederiae* F. Stevens & Mendiola (Ono 2003), *P. prostii* Moug. (Lamb 1934; Olive 1953), *P. grindeliae* Peck (Brown 1940), *P. xanthii* Schwein. (Brown 1940), *P. ruelliae* (Berk. & Broome) Lagerh. (Singh 1979), *P. tiarellaecola* Hiratsuka, f. (Ono 2003), *Cystopsora oleae* Butler, pro parte (Thirumalachar 1945), *Kuehneola japonica* (Dietel) Dietel (Kohno et al. 1975b, 1977; Ono 2002), and *Uromyces rayssiae* Y. Anikster & I. Wahl (Anikster et al. 1980).

Puccinia pampeana Speg. was considered to have a demicyclic life cycle with spermogonia, aecia, and telia on *Capsicum* spp. and other solanaceous plants until Hennen et al. (1984) determined its true biology. They found that *Endophyllum*-type and *Puccinia*-type teliospores are produced from the same mycelium after cross-fertilization in spermogonia and that both types of spores germinate to form a four-celled metabasidium with a basidiospore on each metabasidium cell (Hennen et al. 1984). This life cycle is better considered as microcyclic with dimorphic telial states (*Puccinia* and *Endophyllum*) because either one of the telial stages can be deleted for persistence (see also *Puccinia japonica* Dietel; Kakishima et al. 1984).

Haploid uninucleate basidiospores commonly become binucleate by an additional mitotic division of the nucleus (Anikster 1983, 1984, 1986). Either one of the two or both nuclei migrate into a germ tube. Only one of them seems to take part in the infection, another becoming degenerated. Consequently, a uninucleate intercellular mycelium results by the infection of an uninucleate infection hypha. When the basidiospore gives rise to a second basidiospore by "germination by repetition," one of the two nuclei migrates into the secondary basidiospore.

Type I-a: A variation of type I with the production of a uninucleate germ tube, instead of an ordinary basidiospore, from each uninucleate metabasidium cell (Fig. 1)

Meiosis takes place in a metabasidium developed from aeciospore-like teliospores, where karyogamy preceded the meiosis. The metabasidium becomes four celled. However, a germ tube, instead of an ordinary basidiospore, arises from the metabasidium cell. Two (less frequently one or three) germ tubes are produced from the metabasidium. This variation has been reported for *Endocronartium harknesii* (J.P. Moore) Y. Hirats. and *E. pini* (Pers.) Y. Hirats. (Hiratsuka 1968, 1969, 1973a, b, 1986; Hiratsuka and Maruyama 1968; Hiratsuka et al. 1966). Contrary to the repeated reports of the above-described nuclear cycle in *Endocronartium* species by Hiratsuka and coworkers, Epstein and Buurlage (1988) and Vogler et al. (1997) have shown both cytologically and by an isozyme study that the populations of the fungus named *E. harknesii* reproduce vegetatively. The two nuclei in the aeciospores do not fuse,

and no meiosis takes place in the rust populations they studied. Therefore, they believe that what is named *E. harknesii* must be asexually reproducing and that the fungus name should be *Peridermium harknessii* J.P. Moore.

The same disagreement exists for *E. pini* (Hiratsuka 1969, 1973b) and *Peridermium pini* (Pers.) Lév. (Gibbs et al. 1988). Kasanen (1998) concluded from a molecular analysis that *P. pini* is vegetatively propagated. It has not been determined whether these disagreements are the result of the difference in rust populations they studied or a matter of interpretation of nuclear behavior.

Type I-b: A variation of type I with the production of a binucleate vesicle or a binucleate germ tube, instead of an ordinary basidiospore, from each uninucleate metabasidium cell (Fig. 1)

Gardner (1988) described this variation of nuclear cycle in *Puccinia vittata* J.F. Hennen & Hodges which occurs on *Euphorbia olowaluana* Sherff var. *gracilis* (Rock) Sherff in Hawaii (Hennen and Hodges 1981). A four-celled metabasidium is formed after division (presumably meiosis) of a nucleus (presumably diploid) that has migrated from a teliospore. Each of the four metabasidium cells gives rise to a fusiform vesicle-like structure, into which the nucleus migrates. The nucleus then divides mitotically to form two daughter nuclei. The binucleate vesicle can be referred to as a sedentary basidiospore, and a slender germ tube arising from the vesicle may function as an infection hypha.

A slightly complex, but still basically the same, process of nuclear behavior and associated morphological change in a metabasidium has been reported in *Uromyces alyxiae* Arthur parasitic on *Alyxia olivaeformis* Guad. in Hawaii (Gardner 1987). In a germ tube developing from a teliospore, a nucleus (presumably diploid) divides twice (presumably meiotically) to form four daughter nuclei. The nuclei are not delimited by septa; instead, they migrate into a vesicle-like structure formed on an elongated sterigma-like process derived from the germ tube. From the vesicle, another germ tube arises where the four nuclei are delimited by septa and each of the four nuclei further divides once mitotically. Perhaps, each of the binucleate cells gives rise to a germ tube that functions as an infection hypha although no further observation was made by Gardner (1987). The germ tube that becomes four celled, each cell containing either one or two nuclei, is referable to as a metabasidium and a germ tube possibly developed from each cell is a sedentary basidiospore. This interpretation is contrary to Gardner's view, which considers the tetranucleate vesicle as a basidiospore (Gardner 1987).

Type I-c: A variation of type I with the production of a septate trinucleate or a nonseptate tetranucleate metabasidium (Fig. 2)

This nuclear cycle type has been reported in *Uromyces aloes* (Cook) Magnus on *Aloe* spp. (Thirumalachar 1946; Sato et al. 1980). In this fungus, the vegetative mycelium is at first

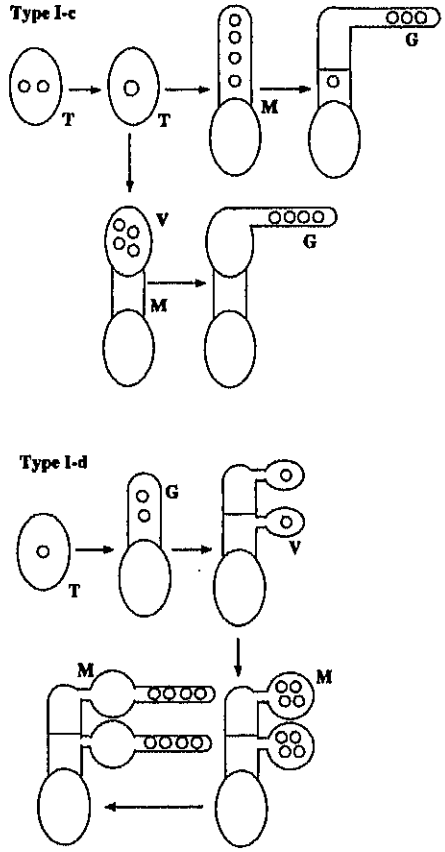

Fig. 2. Types of nuclear cycle in microcyclic rust fungi. *Type I-c.* Distal three haploid nuclei are delimited by a septum from a proximal nucleus in a metabasidium (*M*) or four haploid nuclei locate in an apical vesicle-like structure (*V*) in a metabasidium. The metabasidium or the vesicle gives rise to a thin germ tube (*G*), into which three or four nuclei migrate. *Type I-d.* A uninucleate (presumably diploid) teliospore (*T*) germinates into a germ tube (*G*), where a nucleus divides (presumably mitotically) once to form two daughter nuclei. Two daughter nuclei are delimited by a septum and the germ tube becomes two celled. The apical portion of the germ tube becomes vesicular (*V*). The nucleus migrates into the vesicle. The vesicle becomes a metabasidium (*M*), where the nucleus divides (presumably meiotically) to form four (presumably haploid) daughter nuclei. The four nuclei migrate into a thin germ tube that arises from the vesicle

uninucleate (presumably haploid), and cell fusion is stated to occur between the uninucleate hyphae in a telium. The binucleate mycelium gives rise to binucleate teliospores. The two nuclei then fuse to form a single large (presumably diploid) nucleus. The fusion nucleus undergoes two consecutive divisions (presumably meiosis) to form four daughter nuclei in a metabasidium produced upon the germination of teliospores. A single septum usually divides the metabasidium into a lower uninucleate cell and an upper trinucleate cell. Instead of producing a basidiospore, a whiplike germ tube develops from the upper cell and the three nuclei migrate into it. The germ tube is referable to a nondetachable basidiospore and functions as an infection hypha. Thirumalachar (1946) assumed that uninucleate hyphae are the result of the septation of the trinucleate infec-

tion hypha. In contrast, Sato et al. (1980) speculated that, when intercellular hyphae arise from a trinucleate vesicle formed by the infection of the trinucleate infection hypha, only one nucleus is delimited by a septum. A single teliospore inoculation resulted in telium production with or without spermogonia (Thirumalachar 1946). The spermogonia are apparently nonfunctional. Because of the assumed haploid uninucleate condition in the vegetative mycelium and because of telium production by a single basidiospore inoculation, the homothallic nature of this fungus is proven (Thirumalachar 1946). Olive (1953) stated that this species is basically heterothallic and that the infection hypha is "miktopalontic" homothallic, i.e., secondary homothallic.

This type of nuclear behavior and the associated morphological change of the metabasidium has also been observed in *Endocronartium sahoanum* Imazu & Kakish. var. *sahoanum* (Imazu et al. 1989), *E. sahoanum* var. *hokkaidoense* Imazu & Kakish. (Imazu and Kakishima 1992), and *E. yamabense* (Saho & I. Takah.) Paclt (Imazu et al. 1991a,b). In these fungi, spermogonia are believed to play an important role in the initiation of a binucleate vegetative mycelium. The binucleate mycelium gives rise to binucleate teliospores, which morphologically resemble aeciospores of related *Cronartium ribicola* J.C. Fischer. The two nuclei (presumably haploid) in the teliospores migrate into a metabasidium where the two nuclei fuse to become a single nucleus (presumably diploid). The fusion nucleus then divides (presumably meiotically) twice to form four daughter nuclei in a terminal vesicle-like structure, which is separated from the rest of the metabasidium by a septum. The tetranucleate vesicle gives rise to a thin germ tube, into which the four nuclei migrate. The germ tube is comparable to a sedentary basidiospore and may function as an infection hypha.

In *E. yamabense*, another type (type IX-a) of nuclear cycle was occasionally observed (Imazu et al. 1991a). Hiratsuka (1986) believed that the fungus is uninucleate throughout the life cycle, where no karyogamy and meiosis take place, and that it should be referred to as anamorphic *Peridermium yamabense* Saho & I. Takah. because of the "asexual" nature of reproduction. The population studied by Hiratsuka (1986) might be a haploid variant derived from the population of *E. yamabense* studied by Imazu et al. (1991a).

Endoraecium acaciae Hodges & D.E. Gardner and *E. hawaiiense* Hodges & D.E. Gardner produce teliospores, which morphologically resemble aeciospores and urediniospores of the related species *Atelocauda koae* (Arthur) Cummins & Y. Hirats. on *Acacia koa* Gray var. *koa* in Hawaii (Hodges and Gardner 1984). The nuclear behavior is essentially the same as in *Endocronartium* species. However, in these fungi, one or more short branches arise from the distal vesicular cell. Hodges and Gardner (1984) speculated that the haploid mycelium would result from the infection of one or more of the branches derived from the distal cell and that the binucleate mycelium would be produced by fusion of the uninucleate hyphal cells.

Type I-d: A variation of type I with the production of one or two tetranucleate vesicular metabasidia from a two-celled germ tube derived from teliospores (Fig. 2)

Atelocauda koae (Arthur) Cummins & Y. Hirats. parasitic on *A. koa* var. *koa* in Hawaii exhibits this kind of nuclear behavior and metabasidium production (Gardner 1981; Chen et al. 1996). Uninucleate (presumably diploid) teliospores germinate into a germ tube, into which a nucleus migrates. The nucleus divides (presumably mitotically) once to form two daughter nuclei, and less frequently one of the two nuclei further divides (presumably mitotically). The daughter nuclei are delimited by a septum laid down concomitantly with the nuclear division. Thus, the germ tube is now two celled, or less frequently three celled, and becomes branched. The distal end of the branched germ tube becomes vesicular. The nucleus migrates into the terminal vesicle of the germ tube branch and divides (presumably meiotically) to form four daughter nuclei. The four nuclei then migrate into a thin germ tube that arises from the terminal vesicle.

Gardner (1981) and Chen et al. (1996) interpreted the tetranucleate vesicle as representing a vestigial basidiospore or a nondetachable basidiospore, suggesting the two- or three-celled germ tube from the teliospores to be a metabasidium. Their interpretation of the tetranucleate vesicle as the basidiospore might be derived from a general observation that mature basidiospores of various rust fungi have been often reported to become binucleate or even regularly tetranucleate (Anikster 1983, 1984). The tetranucleate vesicle observed in *A. koae* resembles the tetranucleate basidiospores of a number of *Uromyces* and *Puccinia* species (Anikster 1984). However, the four nuclei in basidiospores of various rust fungi reported by Anikster (1984) are the product of a mitotic division of the two nuclei originally contained in the basidiospores. On the other hand, the four nuclei in the vesicle of *A. koae* are a meiotic tetrad. Therefore, the terminal vesicle in which meiosis takes place is better referred to as a metabasidium and the thin germ tube that arises from the vesicle as a basidiospore without dispersal ability.

Atelocauda koae produces spermogonia and urediniod aecia in addition to telia. However, aeciospores are found to be nonfunctional, i.e., they do not infect the host even though germinating on the host surface (Chen et al. 1996). Chen et al. (1996) speculated that the tetranucleate infection hypha could create a uninucleate hypha by septation of the infection hypha, whereas Gardner (1981) assumed that single infection of the tetranucleate hypha would result in spermogonia of both mating types.

Puccinia rutainsulara D.E. Gardner occurs on *Melicope anisata* (H. Mann) T. Hartley & B. Stone in Hawaii (Gardner 1994). In this fungus, upon germination, a teliospore nucleus (presumably diploid) migrates into a germ tube where it divides (presumably mitotically) to form two daughter nuclei. The germ tube becomes two celled by a septum laid down between the two nuclei. Infrequently, the germ tube becomes tri- or tetranucleate, but only the distal nucleus in a row of three or four becomes delimited

by a septum. From the distal cell, a vesicle-like structure arises and the nuclei that migrate from the cell divide (presumably meiotically) to form four daughter nuclei. The proximal cell(s) remains unchanged. Gardner (1994) interpreted this to indicate that the tetranuclear vesicle is a nondetachable basidiospore as in *A. koae*. However, as stated earlier, the tetranucleate structure derived from the proximal germ tube cell is a metabasidium.

The nuclear behavior and associated morphological changes taking place during the germination of teliospores in *Puccinia rugispora* D.E. Gardner, which occurs on *Zanthoxylum dipetalum* Mann in Hawaii, is somewhat complicated (Gardner 1996), yet it is basically the same as that which is observed in *A. koae* and *P. rutainsulara*. In this fungus, a germ tube that arises from teliospores is a large bubble-like vesicle, instead of a cylindrical tube as in the latter two species. In the vesicular germ tube, a nucleus (presumably diploid) that has migrated from the teliospores divides (presumably mitotically) to form two daughter nuclei. The two nuclei are separated by a septum, the vesicular germ tube thus becoming two celled. Either only the upper cell or both cells give rise to a small vesicle on a sterigma. The nucleus migrates from the germ tube into the vesicle, where it divides twice (presumably meiotically) to form four daughter nuclei. Again, Gardner (1996) interpreted the tetranucleate vesicle as a basidiospore. However, it is an infection hypha that is comparable to a basidiospore and the vesicle is a metabasidium.

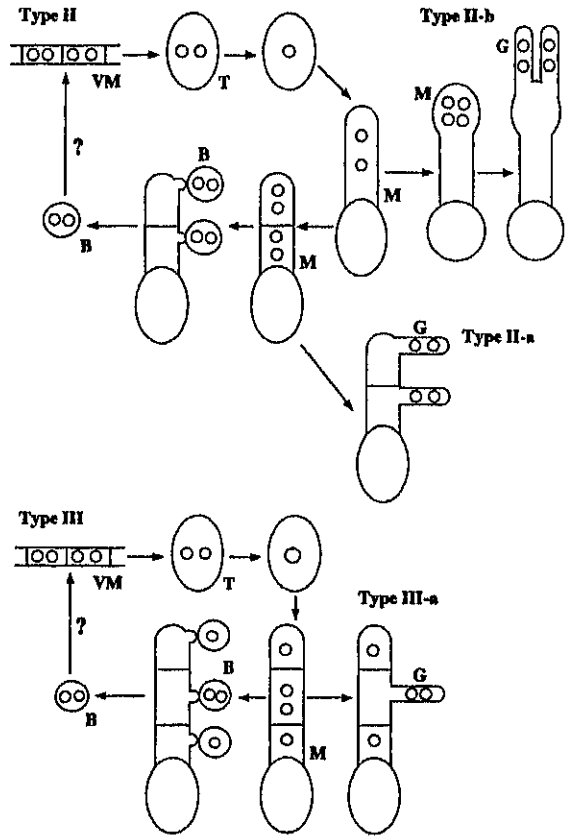

Fig. 3. Types of nuclear cycle in microcyclic rust fungi. *Type II.* A binucleate vegetative mycelium (*VM*) gives rise to a teliospore (*T*) where two nuclei fuse. After the first meiotic division of a fusion nucleus, two daughter nuclei are delimited by a septum. In each metabasidium cell (*M*), the second meiotic division takes place without laying down a septum. Each binucleate metabasidium cell gives rise to a binucleate basidiospore (*B*). *Type II-a.* A metabasidium cell gives rise to a binucleate germ tube (*G*), instead of an ordinary basidiospore. *Type II-b.* A nucleus divides once (presumably meiotically) in a metabasidium (*M*), and two daughter nuclei migrate into a terminal vesicle where the two nuclei undergo second division (presumably meiosis). A pair of the daughter nuclei migrates into bifurcate germ tubes (or infection pegs) (*G*) arising from the terminal vesicle. *Type III.* A teliospore (*T*) arises from a binucleate vegetative mycelium (*VM*). Karyogamy occurs normally in the teliospore, and a fusion (diploid) nucleus migrates into a metabasidium (*M*). Two successive meiotic divisions and concomitant production of septa usually result in proximal and distal uninucleate cells and one middle binucleate cell. Subsequently, two uninucleate basidiospores (*B*) and a binucleate basidiospore (*B*) are produced. *Type III-a.* A middle cell of the metabasidium (*M*) gives rise to a binucleate germ tube (*G*) instead of an ordinary basidiospore. A lower uninucleate cell does not germinate, and an upper uninucleate cell may or may not germinate

Type II: Sexual cycle with the haploid binucleate mycelium and two haploid binucleate basidiospores produced on the two-celled metabasidium (Fig. 3)

This type is equivalent to the "variation 3" of Jackson (1935), the "type 3" of Petersen (1974), and the "type III" of Hiratsuka and Sato (1982).

The vegetative mycelium is binucleate. Two nuclei fuse in young teliospores. After the first meiotic division of the fusion (diploid) nucleus in a metabasidium developing from the teliospores, a septum is laid down between the two daughter (haploid) nuclei. The mature metabasidium is two celled. In each of the two metabasidium cells, the second meiotic division takes place but no septum is formed following the second division. Each metabasidium cell gives rise to a basidiospore, which receives the two nuclei from the metabasidium cell. The two nuclei in the basidiospore are meiotic diads and, thus, the binucleate condition exhibited in this type must not be confused with the binucleate basidiospores commonly observed in many species (Anikster 1983, 1984), whose nuclei are the result of mitosis. Infection of the binucleate basidiospore is believed to initiate a binucleate vegetative mycelium. The two nuclei in any of the basidiospores may be heterokaryotic or homokaryotic, i.e., heterozygous or homozygous in any given gene locus.

This type has been reported in *Puccinia arenariae* (Schumach.) G. Winter (Lindfors 1924), *P. horiana* Hennings (Kohno et al. 1974, 1975a), *P. anemones-virginianae* Schwein., *P. heucherae* (Schwein.) Dietel (Lehmann, cited from Jackson 1935), *Cystopsora oleae* Butler, pro parte (Thirumalachar 1945), *Uromyces oliveirae* Y. Anikster & I. Wahl, nine formae speciales of *U. schillarum* (Grev.) G. Winter (Anikster et al. 1980), and *P. mesnieriana* Thüm. (Anikster and Wahl 1985). Three suspected macrocyclic rusts, *U. christensenii* Y. Anikster & I. Wahl, *U. viennot-bourginii* Y. Anikster & I. Wahl, and *U. hordeastri* Guyot f. sp. *marini* Y. Anikster and f. sp. *bulbosi-bellevaliae-flexuosae* Y. Anikster, also show this type of

nuclear cycle (Anikster et al. 1980). *Uromyces oliveirae*, nine formae speciales of *U. schillarum*, *P. mesnieriana*, *U. christensenii*, *U. viennot-bourginii*, and *U. hordeastri* f. sp. *bulbosi-bellevaliae-flexuosae* regularly produce tetranucleate basidiospores by additional mitosis of two nuclei in basidiospores (Anikster 1984).

Teliospores of *Chrysomyxa weirii* H.S. Jacks. were found to be unique in being a diaspore and germinating on free water, often after water dispersal (Crane et al. 2000). In this fungus, the vegetative mycelium is mostly uninucleate. At the base of a telium, dikaryotization and subsequent karyogamy occur in the mycelium, from which teliospores are produced. During the development of a metabasidium, nuclear division and concomitant septum formation occur, resulting in the production of a two-celled metabasidium. The nucleus in each metabasidium cell further divides and the two daughter nuclei move into a basidiospore subsequently produced on each metabasidium cell. The two nuclei in the basidiospore were stated to divide once or more, the basidiospore thus becoming tetranucleate or multinucleate. They stated that tetranucleate basidiospore could result either if the first and second divisions are meiosis and the third division is mitosis or if the first division is mitosis and the second and third division are meiosis.

Type II-a: A variation of type II with the production of a germ tube, instead of an ordinary basidiospore, from the metabasidium (Fig. 3)

Teliospores of *Monosporidium machili* (Hennings) T. Sato resemble aeciospores of many *Puccinia* species and germinate into a two-celled metabasidium (Stevens 1932; Hiratsuka and Kaneko 1977). The nucleus (presumably diploid) in the teliospores migrates into a metabasidium, where the nucleus divides once (presumably the first meiotic division). The two daughter nuclei are delimited by a septum and then the second division (presumably the second meiotic division) takes place, resulting in two daughter nuclei in each of the two metabasidium cells. The metabasidium cell gives rise to a germ tube, instead of an ordinary basidiospore, into which the two nuclei migrate (Hiratsuka and Kaneko 1977).

In a *M. machili* population examined by Ono (2003), the vegetative mycelium was exclusively uninucleate. Teliospore initials seemed to arise from the mycelial aggregate, whose cells had already become binucleate. The mode by which the binucleate cell arose was not determined. The teliospores were binucleate and the two nuclei did not seem to fuse, even in the mature teliospores. This observation is contrary to that reported by Hiratsuka and Kaneko (1977), who believed that the two nuclei fuse in immature teliospores because uninucleate spores were frequently observed. The teliospores germinated into the two-celled metabasidium after a nuclear division. The two metabasidium cells became binucleate and gave rise to a binucleate whiplike germ tube, instead of producing ordinary basidiospores. Rarely, no septum was laid down between the two nuclei in the metabasidium.

If karyogamy takes place in the teliospores, then the nuclear divisions in the teliospores and metabasidium are meiosis; thus, the nuclear cycle is type II-a. However, if karyogamy does not occur in the teliospores, the nuclear division in the metabasidium is mitosis; thus, the nuclear cycle is type VII-b. The two types of nuclear cycle may represent the existence of two distinct populations in this morphologically circumscribed species, although a possibility that two variations of nuclear cycle may occur in a single population is not ruled out.

Puccinia japonica Dietel was believed to be demicyclic with aecia and telia in the life cycle. According to Kakishima et al. (1984), however, the spores previously referred to as aeciospores are functionally teliospores (endophylloid teliospores). The endophylloid teliospores are initially binucleate but the two nuclei (presumably haploid) fuse in the spores. Nuclear division (presumably meiosis) takes place in a metabasidium produced upon the germination of teliospores. The two-celled, binucleate metabasidium results and a binucleate hypha emerges from each metabasidium cell. Ordinary puccinioid teliospores also germinate into a metabasidium. The nuclear behavior in the metabasidium and basidiospores developing from the puccinioid teliospores follows the pattern of type III-a. Similar to *P. pompeana*, the life cycle of this fungus is considered to be microcyclic with dimorphic telial states, i.e., *Puccinia* and *Endophyllum*.

Type II-b: A variation of type II with the development of bifurcate infection hyphae, instead of an ordinary basidiospore, from the metabasidium (Fig. 3)

Although the nuclear cycle observed in most populations of *Hemileia vastatrix* Berk. & Broome is type I (Chinnappa and Sreenivasan 1965, 1968; Countinho et al. 1995), a population studied by Rajendren (1967a–c) exhibits a unique nuclear behavior in teliospores that resemble urediniospores, i.e., the uredinioid teliospores. The nuclear cycle is called the "Kamat phenomenon" (Rajendren 1967a–c).

In the population studied by Rajendren (1967a,b), the binucleate vegetative mycelium became uninucleate, presumably by karyogamy, then produced sporogenous (basidiogenous) cells. Uredinioid spores were produced from the uninucleate (presumably diploid) mycelium. Upon the germination of spores, the nucleus divided once (presumably first meiotic division) and the two daughter nuclei migrated into a terminal vesicle of a germ tube. The two nuclei then underwent a second division (presumably second meiotic division). A pair of the four daughter nuclei migrated into each of the bifurcate infection pegs arising from the terminal vesicle. This process often repeated once or twice so that secondary or tertiary vesicles were produced. The binucleate uredinioid spores were considered as functional teliospores because the two nuclei in the spores were interpreted as transient meiotic diads. The terminal vesicle was comparable to a metabasidium.

In addition to the aforementioned "atypical, nonsporidial" germination in the uredinioid teliospore, "the typi-

cal, sporidial" germination was reported in the same population (Rajendren 1967c). The spores gave rise to a two- to four-celled metabasidium, from which a maximum of two basidiospores were produced. No nuclear condition for this process was reported, however. Hennen and Figueiredo (1984) believed that the spores functioning as teliospores in *H. vastatrix* are derived from aeciospores, rather than urediniospores as Rajendren (1967a–c) stated, because the life cycle reduction normally occurs either in the "telioid" pathway or in the "endophylloid pathway" (Hennen and Buriticá 1980) and because the *Hemileia* population that exhibits the Kamat phenomenon is most likely to be derived through the endophylloid pathway. In the so-called endophylloid pathway, the aeciospores of the parental macrocyclic species become the functional teliospores whereas the morphology remains unchanged. However, a spermogonial aecial stage has not been found in any *Hemileia* species.

Type III: Sexual cycle with the haploid binucleate mycelium and one haploid binucleate and two haploid uninucleate basidiospores produced on the three-celled metabasidium (Fig. 3)

This type is equivalent to the "type 7" of Petersen (1974) and the "type IV" of Hiratsuka and Sato (1982). No equivalent "variation" is found in Jackson (1935).

The vegetative mycelium is binucleate. Teliospores arise from the binucleate mycelium. Karyogamy occurs normally in the teliospores, and the fusion (diploid) nucleus migrates into a metabasidium developing by the germination of teliospores. Two successive meiotic divisions take place, resulting in four haploid nuclei. Instead of laying down three septa to delimit the four daughter nuclei, however, only two septa are laid down, usually forming two proximal and distal uninucleate cells and one middle binucleate cell. Subsequently, three basidiospores are produced, i.e., two uninucleate basidiospores from the proximal and distal metabasidium cells and a binucleate basidiospore from the middle metabasidium cell. Again, the two nuclei in the basidiospore are meiotic diads, and thus the binucleate condition exhibited in the type must not be confused with binucleate basidiospores commonly observed in many other species (Anikster 1983, 1984), in which mitosis results in the binucleate condition. Infection of the binucleate basidiospore is believed to initiate the binucleate mycelium.

This type has not been known in microcyclic rust species but is reported for a macrocyclic (or brachycyclic) species, *Sphenospora kevorkianii* Linder (Olive 1947), and two demicyclic species, *Gymnosporangium clavipes* (Cooke & Peck) Cooke & Peck (Olive 1949) and *Uromyces erythronii* (DC.) Pass. (Fukuda and Nakamura 1990). In *S. kevorkianii*, the middle metabasidium cell is binucleate and the apical and basal cells are uninucleate. Occasionally, two- and four-celled metabasidia are produced (Olive 1947). This type of nuclear behavior was rarely observed in *G. clavipes* (Olive 1949).

Fukuda and Nakamura (1990) reported that populations of *U. erythronii* on *Erythronium japonicum* Decne. and *Amana edulis* (Miq.) Honda produced three-celled metabasidia whereas a population on *A. latifolia* (Makino) Honda formed four-celled metabasidia. In the former populations, the middle cell of the metabasidium was binucleate whereas the apical and basal cells were uninucleate. They attributed the frequent lack of spermogonia in the aecial stage in the former populations to the consistent production of binucleate basidiospores, indicating the origin of a binucleate vegetative mycelium by the infection of binucleate basidiospores. In the latter population, which consistently produced four uninucleate basidiospores, however, aecia were always associated with spermogonia in the natural infection.

Whether the two nuclei of the binucleate basidiospores are heterozygous for any given gene would depend on two factors: (1) which two of the four daughter nuclei are included in the binucleate metabasidium cell and (2) the segregation pattern of the genes involved (Petersen 1974). If *S. kevorkianii* and *U. erythronii* should prove to be heterothallic, then it would appear that the binucleate basidial cells and the basidiospores produced by them are "miktohaplontic" homothallic (Buller 1941; Olive 1953) or secondary homothallic.

Type III-a: A variation of type III with the production of a germ tube, instead of an ordinary basidiospore, from the metabasidium (Fig. 3)

This mode of nuclear cycle has been known only in *P. japonica*. As described in nuclear cycle type II-a, this fungus produces two (puccinioid and endophylloid) types of teliospores in the life cycle. The puccinioid teliospores form a three-celled metabasidium upon germination, with the middle cell being binucleate. The middle cell gives rise to a germ tube, into which two nuclei (presumably haploid) migrate. The lower uninucleate cell does not germinate, and the upper uninucleate cell may or may not germinate. This species possesses the endophylloid teliospores whose nuclear cycle is of type II-a, as described earlier. It is interesting to note that, when the puccinioid teliospores are inoculated, the production of the endophylloid telium precedes the puccinioid telium, whereas both endophylloid and puccinioid telium arise simultaneously when the endophylloid teliospores are inoculated (Kakishima et al. 1984).

Type IV: Sexual cycle with the haploid binucleate or uninucleate mycelium and two haploid uninucleate basidiospores produced on the two-celled metabasidium (Fig. 4)

This type of nuclear behavior and metabasidium formation was found for the first time in *Herpobasidium filicinum* (Rostr.) Linder (Platygloeaceae, Platygloeales) (Jackson 1935). In *H. filicinum*, the vegetative mycelium both in and on the surface of the host plant is binucleate. The two nuclei

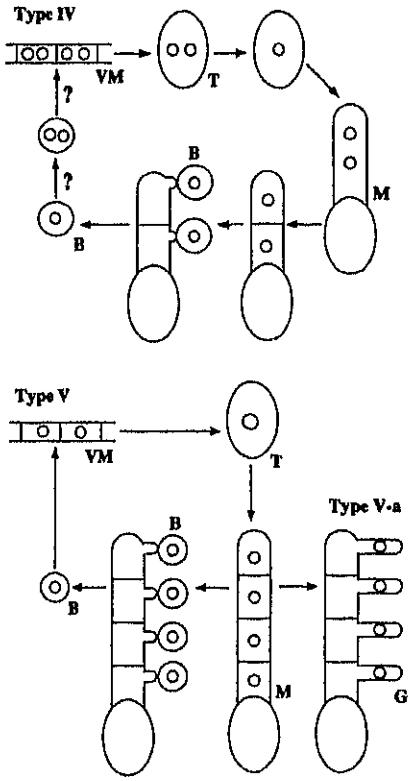

Fig. 4. Types of nuclear cycle in microcyclic rust fungi. *Type IV*. A binucleate teliospore (*T*) arises from a binucleate vegetative mycelium (*VM*). Karyogamy takes place in the teliospore, and a fusion (diploid) nucleus divides once (presumably first meiosis) and septum formation follows in the metabasidium (*M*). Each metabasidium cell gives rise to a uninucleate basidiospore. *Type V*. A teliospore (*T*) arises from a uninucleate (presumably diploid) vegetative mycelium (*VM*). A nucleus divides twice (presumably mitotically) in a metabasidium (*M*). The four (presumably diploid) nuclei are delimited by septa laid down between the nuclei. Each metabasidium cell gives rise to a uninucleate basidiospore. *Type V-a*. A whiplike germ tube (*G*), instead of an ordinary basidiospore, arises from each metabasidium cell (*M*)

fuse in a distal cell of the branched mycelium. The distal cell containing a fusion nucleus (presumably diploid) then becomes a metabasidium. The fusion nucleus divides once (presumably first meiosis) and the daughter nuclei (presumably haploid) are separated by a septum laid down between them. Each of the two metabasidium cells gives rise to a sterigma on which a basidiospore is formed. The nucleus in the metabasidium cell migrates into the basidiospore. A mode of dikaryotization was unknown to Jackson (1935), but he assumed that a second meiosis took place in the basidiospore, suggesting the origin of the binucleate vegetative mycelium by infection of the binucleate basidiospore. If this is true, then *H. filicinum* is considered to be homothallic.

In the rust fungi, this nuclear cycle type was found to occur in *Puccinia circaeae* Pers. (Ono 2003). The vegetative mycelium was mostly uninucleate, and the binucleate condition was rarely observed. Dikaryotization seemed to occur in a telium primordium, although the mode of dikaryotization was not determined. Teliospores were initially binucleate but became uninucleate through karyogamy in the course of the maturation. The teliospores germinated into a two-celled metabasidium. Each of the two metabasidium cells contained one nucleus and formed a uninucleate basidiospore. The nuclear division that takes place in the metabasidium development is believed to be the first meiotic division. The nucleus in the basidiospore then divided once more to form two daughter nuclei. This additional division can be interpreted as the second meiotic division. Very often, two nuclei migrated into a germ tube upon the germination of basidiospores. Although this has not been observed, the infection of the binucleate basidiospore is believed to initiate the uninucleate vegetative mycelium with an unidentified mode. Thus, this fungus is homothallic in the sexual reproduction.

Type V: Apomictic cycle with the entirely diploid uninucleate mycelium and four diploid uninucleate basidiospores produced on the four-celled metabasidium (Fig. 4)

This type is equivalent to the "variation 6" of Jackson (1935), the "type 6" of Petersen (1974), and the "type VIII" of Hiratsuka and Sato (1982).

In this type of nuclear cycle, the vegetative mycelium is believed to be diploid uninucleate. Teliospores arise from the uninucleate mycelium. The nucleus of teliospores passes into a metabasidium developing through the germination of teliospores and divides twice (presumably mitotically). The four nuclei (presumably diploid) are delimited by septa laid down between the nuclei. Thus, the mature metabasidium becomes four celled. Each of the four metabasidium cells gives rise to a sterigma on which a basidiospore is formed. The basidiospore receives the single nucleus from the metabasidium cell. Thus, the nuclear cycle is wholly diploid.

This type of nuclear behavior and metabasidium formation have been reported for a large uninucleate form of *Endophyllum euphorbiae-sylvaticae* (DC.) G. Winter (=*E. euphorbiae* Plowr., *E. uninucleatum* Moreau) (Moreau 1911, 1914, 1915; Moreau and Moreau 1918a, 1919; Dodge 1929; Olive 1953). This type has also been observed in a form of demicyclic *Puccinia podophylli* Schwein. (Brumfield, cited from Dodge 1929).

Dodge (1929) considered that the nucleus observed in the vegetative mycelium and the teliospores of the large uninucleate form of *E. euphorbiae-sylvaticae* is diploid, because the spores of the uninucleate form are as large as those of the binucleate form, and that the four-celled metabasidium is produced by nuclear divisions without actual reduction. In contrast to Dodge (1929), Petersen (1974) interpreted the nuclear division during metabasidium development in *E. euphorbiae-sylvaticae* as meiosis, and Hiratsuka and Sato (1982) apparently followed him without referring to Dodge's (1929) interpretation. Dodge (1929) assumed that *E. uninucleatum* and the uninucleate form of *E. euphorbiae-sylvaticae* have evolved from the parental form by fusion of two haploid nuclei in the vegetative mycelium, which does not normally occur in the parental form.

A uninucleate form of *Aecidium punctatum* Pers. and of *A. leucospermum* DC. reported by Kursanov (1917) may be interpreted as an endocyclic derivative of *Tranzschelia pruni-spinosae* (Pers.) Dietel and *Ochropsora ariae* (Fuckel) Ramsb., respectively. The nucleus is assumed to be diploid; thus, the nuclear cycle of these fungi is expected to follow this type. However, Dodge (1929) predicted that the uninucleate aeciospores would result in uninucleate teliospores upon infection on the alternate host.

The nuclear cycle of *Puccinia lantanae* Farl. (Ono 2002) is temporarily classified as this type, although the ploidy of the nucleus observed is not determined. If the nucleus is proven to be haploid, the nuclear cycle would be a new variation of type VI.

Type V-a: A variation of type V with the production of a whiplike germ tube, instead of an ordinary basidiospore, from the metabasidium (Fig. 4)

This variation of nuclear cycle is reported only in *Puccinia patriniae* Hennings (Ono 2002). The nuclear cycle of this fungus is the same as that of *P. lantanae*; however, a whiplike germ tube, instead of an ordinary basidiospore, arises from each metabasidium cell (Ono 2002). As in *P. lantanae*, the ploidy of the nucleus observed throughout the nuclear cycle is not determined; thus, the placement is arbitrary.

Type VI: Apomictic cycle with the haploid binucleate mycelium and four haploid uninucleate basidiospores produced on the four-celled metabasidium (Fig. 5)

This type is equivalent to the "variation 2" of Jackson (1935), the "type 2" of Petersen (1974), and the "type II" of Hiratsuka and Sato (1982).

The vegetative mycelium is binucleate. Teliospores arise from the binucleate mycelium. No nuclear fusion takes place, and the two haploid nuclei migrate into a metabasidium produced by the germination of teliospores. The two nuclei subsequently divide (presumably mitotically) to form four daughter nuclei, which are concomitantly separated by septa. Each of the four metabasidium cells gives rise to a basidiospore that receives a single nucleus. Although the pattern is similar to type I, karyogamy and subsequent meiosis do not occur. Thus, the nuclear cycle is entirely haploid.

This mode of nuclear cycle has been observed so far only in a binucleate form of *Gymnoconia nitens* (Kunkel 1914; Dodge and Gaiser 1926; Dodge 1929; Olive 1953) and a binucleate form of *E. euphorbiae-sylvaticae* (Sappin-Trouffy 1896; Moreau 1911, 1914, 1915; Moreau and Moreau 1918a, 1919; Dodge 1929; Olive 1953).

Type VII: Apomictic cycle with the production of two haploid binucleate basidiospores produced on the two-celled metabasidium (Fig. 5)

This type was first recognized as distinct and designated as type V by Hiratsuka and Sato (1982).

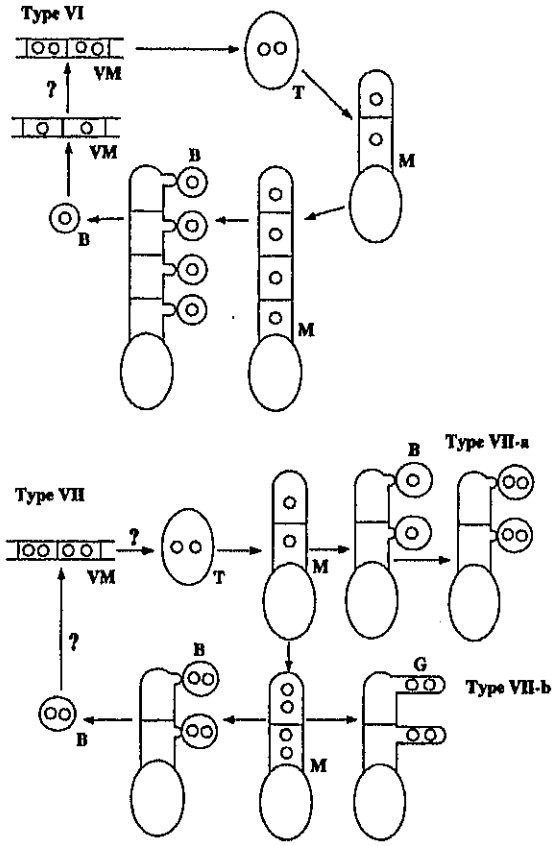

Fig. 5. Types of nuclear cycle in microcyclic rust fungi. *Type VI.* A teliospore (*T*) arises from a binucleate vegetative mycelium (*VM*). No nuclear fusion takes place, and two haploid nuclei migrate into a metabasidium, where they divide (presumably mitotically) to form four daughter nuclei. Each metabasidium cell gives rise to a uninucleate basidiospore. *Type VII.* A teliospore is binucleate. No nuclear fusion takes place in the binucleate teliospore, and two nuclei divide (presumably mitotically) once in a metabasidium (*M*). Each binucleate metabasidium cell gives rise to a binucleate basidiospore. *Type VII-a.* A binucleate teliospore (*T*) arises from a binucleate vegetative mycelium (*VM*). Two nuclei (presumably haploid) migrate into a metabasidium (*M*). The nuclei are separated by a septum, and each nucleus moves into a basidiospores (*B*). The nucleus in each basidiospore further divides (presumably mitotically). *Type VII-b.* Instead of producing an ordinary basidiospore, a binucleate germ tube (*G*) arises from each metabasidial cell (*M*)

Only *Endophyllum spilanthes* Thirum. & Govindu exhibits this type of the nuclear behavior associated with the metabasidium and basidiospore production (Thirumalachar and Govindu 1954). Teliospores are binucleate, perhaps being derived from a binucleate mycelium. No nuclear fusion takes place in the binucleate teliospores, and the two nuclei divide (presumably mitotically) once in a metabasidium. Each of the two metabasidium cells, which are binucleate, gives rise to a binucleate basidiospore. Upon infection, the binucleate basidiospore is assumed to initiate the binucleate condition of the vegetative mycelium.

Type VII-a: A variation of type VII with the delay of nuclear division in the basidiospores (Fig. 5)

This method has been known only in *Endophyllum heliotropii* Thirum. & Naras. (Thirumalachar and Narasimhan 1950). Teliospores are initially binucleate, and each of the two nuclei migrates into a developing metabasidium upon the germination of teliospores. The nuclei are separated by a septum, and each nucleus moves into a basidiospore subsequently developed on the metabasidium. The nucleus (presumably haploid) in the basidiospore then divides (presumably mitotically). Infection of the binucleate basidiospore is assumed to initiate the binucleate vegetative mycelium.

Type VII-b: A variation of type VII with the production of a binucleate germ tube, instead of an ordinary basidiospore, from the metabasidium cell (Fig. 5)

This nuclear cycle has been known only in one of three endocyclic forms of *Uromyces hobsoni* Vize on *Jasminum grandiflorum* L. (Payak 1953). Teliospores arise from a binucleate vegetative mycelium. A two-celled uninucleate metabasidium is produced upon the germination of teliospores, where karyogamy and subsequent meiosis do not occur. The nucleus (presumably haploid) in each of the two-celled metabasidium divides (presumably mitotically). Each metabasidium cell gives rise to a binucleate germ tube instead of producing an ordinary basidiospore.

Type VIII: Apomictic cycle with the haploid binucleate mycelium and one haploid uninucleate basidiospore produced on the two-celled metabasidium (Fig. 6)

This type is equivalent to the "variation 4" of Jackson (1935), the "type 4" of Petersen (1974), and the "type VI" of Hiratsuka and Sato (1982).

This nuclear cycle has been observed only in *Endophyllum valerianae-tuberosae* R. Maire (Maire 1900; Poirault 1915; Jackson 1931, 1935). The vegetative mycelium is at first uninucleate and becomes binucleate by an unknown mode of dikaryotization. Teliospores are binucleate, and no nuclear fusion takes place in the teliospores. One of the two nuclei degenerates, and the remaining nucleus (presumably haploid) migrates into a metabasidium developing upon the germination of teliospores. The nucleus undergoes another division (presumably mitosis), and a septum divides the metabasidium into two cells. The nucleus in the lower cell degenerates. The upper cell gives rise to a sterigma on which a basidiospore is formed. The basidiospore receives a nucleus from the metabasidium cell.

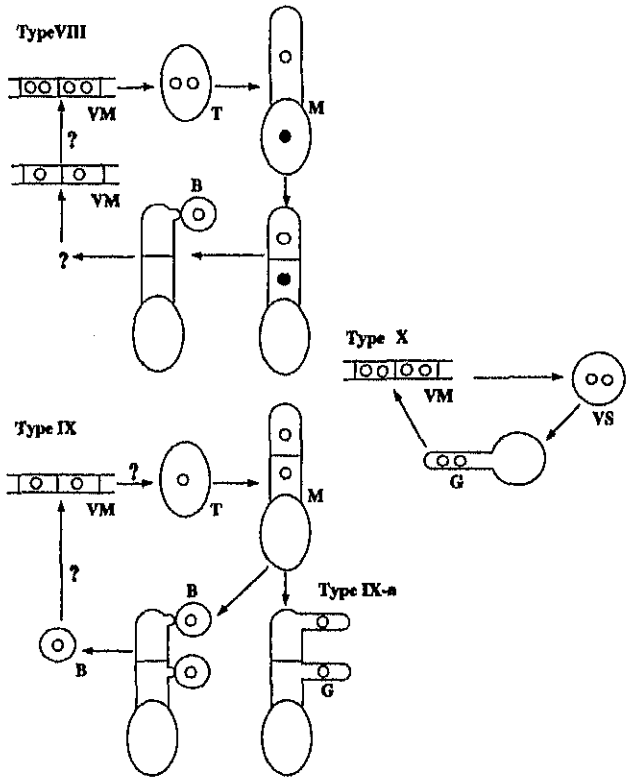

Fig. 6. Types of nuclear cycle in microcyclic rust fungi. *Type VIII.* A vegetative mycelium (*VM*) is uninucleate. A binucleate teliospore (*T*) arises from a vegetative mycelium that dikaryotizes through an unidentified method. One of the two nuclei (*solid circle*) degenerates, and the remaining nucleus (presumably haploid) migrates into a metabasidium. The nucleus undergoes a single division (presumably mitotic), and a septum divides the metabasidium into two cells. The nucleus (*solid circle*) in the lower cell degenerates. The upper cell gives rise to a uninucleate basidiospore. *Type IX.* A vegetative mycelium (*VM*) and a teliospore (*T*) are haploid uninucleate. In a metabasidium (*M*), a single nucleus (presumably haploid) divides once (presumably mitotically), and a septum is laid down between the daughter nuclei. The mature metabasidium is two celled and each cell gives rise to a uninucleate basidiospore (*B*). *Type IX-a.* Either an upper cell or both cells of a metabasidium produce a germ tube (infection hypha) (*G*), instead of an ordinary basidiospore. *Type X.* A vegetative mycelium (*VM*) is binucleate. No karyogamy and meiosis take place in the production of vegetative spores (*VS*). The binucleate spore germinates into a binucleate germ tube (*G*). The process is wholly asexual

Type IX: Apomictic cycle with the haploid uninucleate mycelium and two haploid uninucleate basidiospores produced on the two-celled metabasidium (Fig. 6)

This type is equivalent to the "variation 5" of Jackson (1935), the "type 5" of Petersen (1974), and the "type VII" of Hiratsuka and Sato (1982).

The vegetative mycelium and young teliospores are haploid uninucleate. In a metabasidium developed by the germination of teliospores, the single nucleus (presumably haploid) divides (presumably mitotically) once and a septum is laid down between the daughter nuclei. The mature metabasidium is two celled, and each of the two cells gives rise to a sterigma on which a basidiospore is formed. The

basidiospore receives a single nucleus from the metabasidium cell. Thus, the nuclear cycle is entirely haploid uninucleate.

This type of nuclear behavior has been reported for a small uninucleate form of *G. nitens* (Dodge 1924, 1929; Jackson 1935; Olive 1953), *Uromyces rudbeckiae* Dietel & Holw. (Olive 1911; Jackson 1931), *Endophyllum centranthi-rubri* G. Poirault (Poirault 1913, 1915; Jackson 1935), and one of three endocyclic forms of *U. hobsoni* (Payak 1953).

Dodge (1929) considers that the single nucleus in the teliospores of a small uninucleate form of *G. nitens* is haploid by contrasting the small-sized teliospores of this form to the large-spored form of *G. nitens*. The nucleus size of the small-spored form is stated to be the same as each of the two nuclei in the large-spored form.

In an endocyclic form of *U. hobsoni* (Payak 1953), the vegetative mycelium, teliospores, and two-celled metabasidium are uninucleate. A binucleate basidiospore seems to arise from each of the two uninucleate (presumably haploid) metabasidium cells after division (presumably mitosis) of the nucleus. Because the vegetative mycelium is uninucleate, one of the two nuclei in the basidiospore is assumed to degenerate during the infection process.

Type IX-a: A variation of type IX with the production of a germ tube, instead of an ordinary basidiospore, from the two-celled metabasidium (see Fig. 6)

A two-celled uninucleate (presumably haploid) metabasidium is produced from uninucleate teliospores. Either an upper cell or both cells of the metabasidium produce a germ tube or an infection hypha, instead of producing an ordinary basidiospore. Populations of the *P. yamabense–E. yamabense* complex exhibit this type of nuclear cycle. Hiratsuka (1986) considered that the life cycle of *P. yamabense* he examined is wholly vegetative, however. Imazu et al. (1991a,b) only occasionally observed this nuclear behavior in their material of *E. yamabense*. As mentioned elsewhere, the population referred to as *P. yamabense* (Hiratsuka 1986) may well be a haploid variant derived from the *E. yamabense* population that is truly endocyclic in the life cycle (Imazu et al. 1991a,b).

One of three endocyclic forms of *U. hobsoni* on *Jasminum malabaricum* has also been known to form two uninucleate whiplike germ tubes from the two-celled metabasidium, and the nucleus has been assumed to be haploid (Payak 1953).

Type X: Asexual life cycle with the haploid binucleate vegetative mycelium and spores without the production of metabasidium (see Fig. 6)

The vegetative mycelium is binucleate. Karyogamy and subsequent meiosis do not take place in the production of spores and their germination. Regular conjugate division of the two nuclei and following septation result in spores that are morphologically aeciospores. The process is wholly asexual. *Aecidium mori* Barclay (Kaneko 1973), *A. rhaphiolepidis* Syd., and *A. pourthiaeae* P. Syd. & Syd. (Sato and Sato 1981) exhibit this type of life cycle.

From long-term field observations, inoculation experiments, and cytological observations, it is apparent that these anamorphic fungi only produce morphological aeciospores in the peridiate *Aecidium*-type sori. The spores and sori of three *Aecidium* species are referred to as the aecidioid urediniospores and aecidioid uredinia, respectively (Kaneko 1973; Sato and Sato 1981). These observations suggest that many unconnected *Aecidium, Peridermium, Roestelia*, and *Caeoma* species reproduce entirely by the haploid binucleate vegetative spore without a sexual stage in the life cycle. Similarly, many unconnected *Uredo* species are believed to have lost the sexual recombination process.

Loss of sexual recombination process is associated with reduction of life cycle in the rust fungi

The mode of dikaryotization

Sexuality in rust fungi has been a vexing question ever since the suggestion made by Meyen (1841, cited from Blackman 1904) that spermogonia and aecia represent the male and female organ, respectively. This view was first supported by the observations of Tulasne (1854, cited from Blackman 1904) and De Bary (1853, 1884, cited from Blackman 1904), who showed that spermogonia and aecia were closely associated in a large number of species and that spermatia produced in spermogonia apparently lacked ability of germination (cited from Blackman 1904). Since Blackman (1904) first discovered the dikaryotization (fertilization) of the haploid mycelium by nuclear migration, a variety of methods of dikaryotization have been documented with the monumental work on the function of the spermogonium by Craigie (1927a,b). Although the sexual process seems to occur uniformly in macrocyclic rust fungi with the spermogonial–aecial stage, diverse modes of sexual reproduction have been documented or suggested for microcyclic fungi (Walker 1928; Jackson 1931; Petersen 1974).

Jackson (1931) classified microcyclic rust fungi into two categories according to the nuclear status of the vegetative mycelium and suggested two modes of dikaryotization: (1) species with a uninucleate vegetative mycelium, with the binucleate mycelium being initiated by cell fusion or other undetermined methods in the sorus primordium (22 species in *Puccinia, Uromyces, Tranzschelia, Kunkelia* (=*Gymnoconia*), *Gallowaya* (=*Coleosporium*), *Chrysomyxa*, and *Endophyllum*); and (2) species predominantly with a binucleate vegetative mycelium that is initiated by unidentified mode(s) at an early stage of infection (21 species in *Puccinia, Uromyces*, and *Botryorhiza*). *Uromyces ficariae* and *P. xanthii* were included in both categories.

The methods of dikaryotization so far reported in microcyclic rust fungi are summarized as follows.

1. Nuclear migration in the telium primordium

Blackman (1904) documented the origin of the binucleate condition by nuclear migration in *Phragmidium violaceum* (Schultz) G. Winter. He first confirmed the nuclear cycle illustrated by Sappin-Trouffy (1896) for the Uredinales. In *P. violaceum*, mature uninucleate teliospores gave rise to four uninucleate basidiospores. A mycelium derived from the basidiospore infection was uninucleate and formed spermatia in spermogonia. Young aecia became binucleate, and the binucleate condition persisted throughout the life cycle until the nuclei fused in mature teliospores. Blackman (1904) interpreted the spermatium as a male cell and the aecium as a female reproductive organ. However, he considered the spermatium that formerly took part in fertilization no longer retained its sexual ability in *P. violaceum* and confirmed that a vegetative mycelial cell was fertilized by a nucleus from an adjacent cell. This process was called a "reduced form of fertilization" by Blackman (1904).

Werth and Ludwigs (1912) found in *Puccinia malvacearum* that cell fusion occurred between uninucleate cells of unequal size and that the nucleus of the smaller migrated to the larger cell (Walker 1928). Lindfors (1924) showed that cell fusion occurred in *Tranzschelia fusca* (Pers.) Diet., although nuclear migration occurred through a small pore if the cells were in contact with each other (Walker 1928). Walker (1928) assumed that the binucleate condition in *U. ficariae* originated, as in *P. malvacearum*, by nuclear migration between cells at the base of a telial primordium, although a possibility of dikaryotization by cell fusion was not ruled out. A similar process was observed in *U. hobsoni* (Payak 1952). In *P. prostii*, the dikaryotic condition was stated to arise at the base of a telial primordium, either by nuclear migration or by cell fusion (Lamb 1934; Olive 1953).

2. Cell fusion in the telium primordium

This second mode of dikaryotization was documented by Christman (1905) for *Gymnoconia nitens* and *Phragmidium speciosum* (Fr.) Cooke. In these rust fungi, uninucleate cells in a telium primordium became aligned in a pair. Each pair of cells came in contact where the wall of the two cells dissolved and the protoplasts were brought into contact. By gradual enlargement of the dissolved area of the walls, the upper halves of the protoplasts of the gametes united to form a continuous cell mass, which still showed the two distinct bases. In all cases, "the two distinct bases remain throughout as an evidence of the double origin of each row of aecidiospores" (Christman 1905). Christman (1905) considered the fused cells observed in *P. speciosum* and *G. nitens* are equal gametes. Christman (1905) also stated that the important problem relating this discovery was that the function of the spermogonium and the spermatium remained unexplained in the life cycle of rust fungi. Craigie (1927a,b) eventually answered this question by sophisticated experiments.

Following Christman's discovery, Olive (1908a) found that the binucleate condition in *Puccinia transformans* Ellis & Everh. [=*Prospodium transformans* (Ellis & Everh.) Cummins] was brought about by the absorption of the walls of two adjacent cells, which came in contact in a telium (Walker 1928). However, Olive (1908b) pointed out that the seeming disagreement between the "Blackman-type" and "Christman-type" of dikaryotization methods may be a matter of degree by which the wall of two adjacent gametic cells are dissolved; in the cytological study of *Triphragmium ulmariae* (Schlect.) Lagerh. and three other rust fungi, he observed Blackman-type dikaryotization through a narrow pore and Christman-type through a broad pore side by side in the same fungal mycelium.

Consequently, intermediate conditions have been observed between the migration of the nucleus from a smaller to a larger cell and the fusion of equal-sized cells. *Puccinia prostii* Moug. was reported to dikaryotize at the base of a telial primordium either by means of cell fusion or by nuclear migration (Lamb 1934; Olive 1953). Lindfors (1924) showed that cell fusion occurred in *T. fusca*, while Walker (1928) stated that dikaryotization took place by nuclear division not followed by septum formation, although cell fusion was occasionally observed.

Dodge (1929) stated that cell fusion is known to occur in more than 40 species. Characteristic cell fusion has been reported in *Gallowaya pinicola* Arth. (=*Coleosporium pinicola* (Arthur) Arthur) (Dodge 1925; Walker 1928), *Puccinia morthieri* Körn. (Lindfors 1924), *P. eatoniae* Arthur (Fromme 1914), *P. thwaitesii* Berk. (=*P. lantanae*) (Payak 1952), *P. xanthii*, *U. ficariae*, and *Chrysomyxa abietis* (Wallr.) G. Winter (Walker 1928), *Endophyllum sempervivi* (Hoffman 1912), and *Uromycladium tepperianum* (Sacc.) McAlpine (Olive 1953).

3. Anastomosis of vegetative mycelia at an early stage of basidiospore infection

Kursanov (1917) noted the migration of a nucleus into a fertile cell from a purely vegetative mycelium in *Uromyces alchemillae* (Pers.) Fuckel (=*Trachyspora alchemillae* Fuckel). Lindfors (1924) showed that cell fusion occurred, at an early stage of infection, between cells of young telia in *P. malvacearum* and that the binucleate cells were often separated by sterile cells. Similarly, dikaryotization in *P. adoxae* and *U. scillarum* seemed to take place by cell fusion of a vegetative mycelium at an early stage of infection (Walker 1928).

Dodge (1929) distinguished the "anastomosis" from the "migration" and the "fusion" as the mode of dikaryotization in rust fungi, whereas Lindfors (1924) stated that the three methods were observed with practically all intergrades in dikaryotization in *T. alchemillae*. In the observed cell fusions, fusing (gametic) cells are stated to be more or less differentiated (Dodge 1929); however, the distinction of the fusing cells from the vegetative cells in a telium primordium is arbitrary. Furthermore, as variously stated here, the difference between migration and fusion is a matter of degree by which the wall of adjacent two cells is dissolved.

4. Mitotic division of a nucleus not accompanied by cell division

The origin of binucleate condition by mitosis not followed by septum formation in a uninucleate mycelial cell was first suggested by Sappin-Trouffy (1896). Walker (1928) interpreted for *Puccinia asteris* Duby, *P. cryptotaeniae* Peck, and *T. fusca* that a binucleate mycelium originated from a uninucleate mycelium by nuclear division not associated with septum formation. Ono (2002) believed that this phenomenon occurred in *K. japonica*. The origin of binucleate mycelium at an early stage of infection by a uninucleate basidiospore, as suggested by Jackson (1931), can be attributed to this mode.

5. Fusion of compatible spermatia with receptive hyphae in the spermogonium

The function of spermatia in spermogonia in the sexual reproduction of rust fungi was first proven by Craigie (1927a,b), who undertook intensive crossing experiments with *P. helianthi* and *P. graminis*. From the results he obtained, Craigie (1927a,b) deduced that hyphae and spermatia in the spermogonium were female and male gametes, respectively, which were produced by two sexually different types of basidiospores, and concluded that the vegetative mycelium and subsequent aeciospores were produced only when the fusion of spermatia of one sexual type with hyphae of another sexual type occurred in the spermogonium. In controlled experiments, he also deduced that insects would play an important role in cross-spermatization in nature.

Thereafter, sexual reproduction by cross-spermatization in the spermogonia has been reported across macrocyclic rust species, e.g., *Puccinia triticina* Eriks. (=*P. recondita* Desm) (Allen 1932), *P. sorghi* Schwein. (Allen 1933), *P. phragmitis* (Schum.) Körn (Lamb 1935), *P. anomala* Rostr. (=*P. hordei* Otth), *P. coronata* Corda, *Uromyces appendiculatus* (Pers.) Unger, *U. vignae* Barclay, *U. graminis* (Niessl) Dietel, *U. trifolii-hybridi* H.G.K. Pau (=*U. trifolii-repentis* Liro), *U. fabae* de Bary (=*U. viciae-fabae* J. Schröt.), *Gymnosporangium haraeanum* Syd. & P. Syd. (=*G. asiaticum* G. Yamada), *G. juniperi-virginianae* Schwein., *G. globosum* Farl., *Cronartium ribicola* J. C. Fischer, *Melampsora lini* (Eremb.) Lév. (last 13 species cited from Brown 1940), and many other macrocyclic or demicyclic species.

6. Fusion of spermatia with stomatal hyphae (Andrus 1931; Allen 1934)

Andrus (1931) reported, in *U. appendiculatus* and *U. vignae*, that a uninucleate mycelium in an aecial primordium was dikaryotized by fusion of spermatia with receptive hyphae that emerged through the host epidermis. However, conclusive evidence to support this perspective is needed (Olive 1953).

7. Germination of spermatia and entrance of the germ tube through a stoma "to become effective" (Allen 1934)

Conclusive evidence to support this method is lacking (Olive 1953). Budding of spermatia in *Coleosporium tussilaginis* (Pers.) Lév. was once reported (Deml et al. 1982); however, it was found that the reported observation was based on nonrust yeasts (Bauer 1986).

8. Fusion of spermatia in pairs (Allen 1934)

No conclusive evidence to support of this method is available (Olive 1953).

Heterothallism

As listed in the mode of dikaryotization 4 (fusion of compatible spermatia with receptive hyphae in the spermogonium), many, probably most, macrocyclic and demicyclic species with spermogonia in the life cycle would prove to be heterothallic. Contrary to macrocyclic species, few cases have been documented in which dikaryotization, either between compatible spermatia and receptive hyphae in the spermogonium or between compatible vegetative mycelia, is prerequisite for the development of teliospores.

In *P. prostii* (Lamb 1934; Olive 1953), the dikaryotic condition is reported to arise at the base of a telium primordium, either by means of cell fusion or by nuclear migration, and this species is suspected to be heterothallic. *Puccinia pampeana* produces both *Endophyllum*-type and *Puccinia*-type teliospores, only after cross-spermatization in the spermogonium (Hennen et al. 1984). Without cross-spermatization, the systemic infection forming the spermogonium remains so for a considerable period of time. The result indicates the heterothallic nature of sexual reproduction in this species. *Endocronartium sahoanum* var. *sahoanum* (Imazu et al. 1989), *E. sahoanum* var. *hokkaidoense* (Imazu and Kakishima 1992), and *E. yamabense* (Saho & I. Takah.) Paclt (Imazu et al. 1991a,b) are suggested be heterothallic.

Homothallism

The diverse modes of nuclear behavior and accompanying morphological change in metabasidium and basidiospore production, as described here, clearly indicate that a large number of microcyclic rust fungi are either homothallic, when reproducing sexually, or apomictic or asexual in their reproduction. Nevertheless, homothallism has been proven by a single basidiospore inoculation only in the following species: *P. malvacearum* (Ashworth 1931), *P. mesnieriana* (Anikster and Wahl 1985), *K. japonica* (Ono 2002), *U. aloes* (a single teliospore inoculation; Thirumalachar 1946), *U. viennot-bourginii* (Anikster et al. 1980), and *U. scillarum* f. sp. *leopoldiae-maritimae* (Anikster et al. 1980).

Variants in the type I nuclear cycle indicate that many microcyclic rust fungi have become homothallic and have

simplified the nuclear cycle and associated sexual structures during their evolution. During the course of life cycle reduction and the simplification of the sexual process, most microcyclic rust fungi seem either to have lost spermogonia entirely or their function where the spermogonia are still formed. For this reason, Buller (1950) and others believed that the absence or imperfect development of spermogonia indicates the homothallic nature of the sexual reproduction in microcyclic rust fungi.

Buller (1941) distinguished two kinds of homothallism in fungi: "haplomonoecious" homothallic, where the life cycle starts with a single uninucleate basidiospore, and "miktohaplontic" homothallic (sexually heterokaryotic; secondarily homothallic), where a basidiospore is bisexual, containing the nuclei of both sexes. In those species whose basidiospores are produced on a two-celled metabasidium and contain two nuclei (meiotic diads), the two nuclei may be homokaryotic or heterokaryotic at any given gene locus, depending on the segregation of the genes over the two divisions (Petersen 1974), and may be homothallic or secondarily homothallic. Both kinds of homothallism commonly exist among microcyclic rust fungi, which include *K. japonica* (Ono 2002), *P. anemones-virginianae* (Jackson 1935), *P. arenariae* (Lindfors 1924), *P. circaeae* (Ono 2003), *P. grindeliae* (Brown 1940), *P. heucherae* (Jackson 1935), *P. horiana* (Kohno et al. 1974, 1975a), *P. malvacearum* (Blackman and Fraser 1906; Ashworth 1935), *P. mesnieriana* (Anikster and Wahl 1985), *P. rutainsulara* (Gardner 1994), *P. xanthii* (Brown 1940), *U. aloes* (Thirumalachar 1946), *U. alyxiae* (Gardner 1987), *U. oliveirae* (Anikster et al. 1980), nine formae speciales of *U. schillarum* (Anikster et al. 1980), *U. viennot-bourginii* (Anikster et al. 1980), *E. heliotropii* (Thirumalachar and Narasimhan 1950), and *C. weirii* (Crane et al. 2000).

Homothallism generally implies the genetic homogeneity of the fungal population; however, it does not prevent but reduces the frequency of outcrossing in the population (Carlile 1987). When two homothallic populations of different genotypes are brought together, heterokaryosis and the heterozygous condition at any gene locus are expected by infection of the basidiospores of two genetically different populations. Karyogamy and subsequent meiosis result in nuclei of different genetic constitution through a random assortment of homologous chromosomes.

Even in a single population, two haploid nuclei in the mycelium become genetically different if gene or chromosomal mutations occur in either one or both nuclei. Uninucleate basidiospores originated from heterokaryotic mycelium thus formed are different genetically and may infect a plant in sufficiently close proximity. The infection hyphae derived from the two basidiospores may result in heterokaryosis and heterozygosity in a new generation of the population that had been homozygous. Through these processes, genetic diversity might have been and would be maintained even in the homothallic microcyclic rust fungi. Olive (1953) predicted that heterokaryosis, heterozygosity, and segregation would be found to occur quite commonly among homothallic rust fungi.

Apomixis

Sexual recombination is a widespread phenomenon in eukaryotic organisms. It is often believed that sex promotes genetic variability and that sexually reproducing organisms evolve faster than asexually reproducing ones (Barton and Charlesworth 1998). The common occurrence of sex among eukaryotic organisms suggests the existence of strong driving forces that favor the evolution and persistence of sex (Maynard Smith 1978; Wuerthrich 1998). However, sex still maintains uncertainty or inherent disadvantages in evolution (Maynard Smith 1978; Barton and Charlesworth 1998): it is not certain a priori that heritable variance in fitness is significantly increased by sex; sexual recombination would break up adaptive gene complexes that have been established through natural selection; and sexually reproducing organisms must allocate much resource to reproductive organs. Consequently, in environments where sex does not significantly increase fitness or genetic recombination breaks up adaptive gene complexes, apomictic or vegetative reproduction would have been favored.

This scenario can be applied to rust fungi. The apomictic reproduction has been proven in *P. lantanae* (Ono 2002) by cytology and artificial inoculations and assumed in the following rust fungi: *P. patriniae* (Ono 2002), three endocyclic forms of *U. hobsoni* (Payak 1953), *U. rudbeckiae* (Olive 1911; Jackson 1931), a binucleate form of *G. nitens* (Kunkel 1914; Dodge and Gaiser 1926; Dodge 1929; Olive 1953), a small uninucleate form of *G. nitens* (Dodge 1924, 1929; Jackson 1935; Olive 1953), *E. centranthi-rubri* (Poirault 1913, 1915; Jackson 1935), a uninucleate form of *E. euphorbiae-sylvaticae* (Moreau 1911, 1914, 1915; Moreau and Moreau 1918a, 1919; Dodge 1929; Olive 1953), a binucleate form of *E. euphorbiae-sylvaticae* (Sappin-Trouffy 1896; Moreau 1911, 1914, 1915; Moreau and Moreau 1918a, 1919; Dodge 1929; Olive 1953), *E. heliotropii* (Thirumalachar and Narasimhan 1950), *E. spilanthes* (Thirumalachar and Govindu 1954), *E. valerianae-tuberosae* (Maire 1900; Poirault 1915; Jackson 1931, 1935), and *P. yamabense* (Hiratsuka 1986).

In rust fungi, through the evolution of apomictic reproduction, metabasidia and basidiospores might have been so simplified that they can hardly be distinguished from a germ tube or infection hypha arisen from vegetative spores.

Secondarily asexual reproduction

It is highly likely that many unconnected *Aecidium*, *Peridermium*, *Roestelia*, and *Caeoma* species have the entirely haploid binucleate condition without a sexual stage in the life cycle. Wholly asexual reproduction with an aeciospore-type of vegetative spores has been confirmed by cytology and artificial inoculations in such anamorphic fungi as *A. mori* (Kaneko 1973), *A. raphidolepidis*, and *A. portheriae* (Sato and Sato 1981), *P. harknesii* (Epstein and Buurlage 1988; Vogler et al. 1997), and *P. pini* (Gibbs et al. 1988). Hantula et al. (2002) suggested by molecular genetic analyses that *P. pini* had originated from sexually reproduc-

ing *Cronartium flaccidum* (Alb. & Schwein.) G. Winter. These examples suggest reduction of life cycle to the ultimate vegetative reproduction through the endophylloid pathway, i.e., conferring the function of a telial stage to an aecial stage of macrocyclic parental species, as advocated by Hennen and Figueiredo (1984).

The foregoing discussion does not exclude the possibility that truly vegetative reproduction by urediniospores could have been evolved by omission of spermogonial, aecial, and telial stages in the life cycle. Many unconnected *Uredo* species would be proven as truly asexual "species."

Ecological and evolutionary significance of homothallism, apomixis, and asexuality in microcyclic rust fungi

A number of data have indicated that microcyclic rust fungi have become homothallic, apomictic, or asexual in reproduction during the course of life cycle reduction. The development of homothallism during the evolution of microcyclic rust fungi, mostly from macrocyclic heteroecious parental species (Jackson 1931), may bear special importance in their biology.

In examining rust-infected plant materials from northwestern Greenland, Arthur (1928) noticed that most common rust fungi observed there are microcyclic in the life cycle and that those rust fungi that possess a macrocyclic heteroecious life cycle persist by a uredinial stage, whose mycelium survives in the host tissue for a considerable period of time. In the study of rust flora in the alpine region of Japan, Hiratsuka (1935) showed that the number of microcyclic rust fungi increase with the increase of latitude of localities and altitude of mountains and that 35 species (~42%) of 83 species found in the alpine regions were microcyclic.

Savile (1953) also noticed that microcyclic rust fungi, which seem self-fertile, are prevalent in the arctic region in northern Canada and that many heteroecious species persist by a uredinial stage or an aecial stage, whose mycelium overwinters in evergreen host tissues. Savile (1953, 1976) believed that facultative or permanent reduction of life cycle and self-fertility are an effective adaptation to a short growing season.

After having become macrocyclic, either autoecious or heteroecious, teliospores in many rust fungi have become deciduous, acquiring a function of dissemination with increased wall thickness and melanization. For those rust fungi, vegetative reproduction by aeciospores and urediniospores is not economical under severe ecological conditions where their hosts are scattered and the suitable growing period is short. Under such conditions, aeciospores and urediniospores are not functioning, i.e., no new infection by those spores will result in effective reproduction in places where potential hosts are not easily available and the favorable climate persists for only a short period. Production of highly tolerant and migratory teliospores is the most economical and safest way of life and, to become so, the acquisition of self-fertility becomes prerequisite. On the other hand, a totally clonal life cycle with repeating aeciospores or urediniospores would also be highly adaptive as well.

Similar interpretation has been presented for the prevalence of short-cycled, self-fertile *Uromyces* species (Anikster et al. 1980) and *Puccinia mesnieriana* (Anikster and Wahl 1985) in Israel, where the rust fungi must survive a short growing season under the semiarid climatic condition.

These observations and interpretations suggest that homothallic, apomictic, or vegetative reproduction is highly adaptive for rust fungi inhabiting severe ecological conditions and that homothallic, apomictic, or asexually reproducing variants could have been selected from heterothallic macrocyclic parental species under such adverse conditions. Some of the incipient populations of reduced life cycle derived from parental populations might have succeeded to become distinct species whereas most might have become extinct under still harsh conditions.

Brasier (1987) presented, with some convincing examples of plant pathogenic fungi, a possible mode of the speciation of clonal or self-fertile populations in response to severe ecological disturbance ("episodic selection"). Under a severe ecological condition, a genetically uniform population may arise from an originally variable population by reduction or loss of outcrossing ability or acquisition of self-fertility. If episodic selection continues and if a reproductive isolating mechanism develops, the self-fertile or clonal populations would rapidly speciate. If the speciating populations enter the narrow and recurrently distributed niche where the populations are partitioned in a small size, the population would become a self-fertile or vegetatively reproducing species.

As in vascular plants (Barton and Charlesworth 1998), secondary asexuality can evolve in fungi independently many times at different localities. The evidence seems to support the possibility of multiple origins of microcyclic or clonal rust lineages from the same ancestral rust species.

It is readily accepted that asexually reproducing rust fungi persist for much shorter periods in evolutionary time than their sexually reproducing relatives (Maynard Smith 1978; Barton and Charlesworth 1998). Buller (1950) pointed out that the advantage of life cycle reduction with a short generation time was gained at the cost of reduced genetic variability, which eventually leads to evolutionary stagnancy. That apomictically or vegetatively reproducing species are widespread, particularly in cool temperate and arctic regions, however, indicates that a dynamic equilibrium between extinction and reestablishment of microcyclic or asexual species exists as a common evolutionary phenomenon among the rust fungi. Repeated derivation of microcyclic lineages from macrocyclic parental species might have compensated the extinction in the past evolutionary history.

The *Gymnoconia nitens* species complex, the *E. euphoribiae-silvaticae* species complex, the *U. hobsoni* species complex, the *C. flaccidum–E. pini–P. pini* complex, the *C. coleosporioides–E. harknesii–P. harknesii* complex, and

the *C. ribicola–E. yamabense–P. yamabense* complex may represent examples of repeated derivation of microcyclic or clonal populations in different times and places. Because of the possibility of independent evolution, these morphologically delimited microcyclic or clonal species may consist of populations that are heterogeneous in terms of nuclear behavior and genetic constitution.

In addition to the mode of adaptation under cold environmental conditions, through which apomictically or asexually reproducing rust fungi evolved, there is another possibility that microcyclic species with similar reproductive traits have evolved under warm and humid climatic conditions or "mild and stable" ecological conditions. After having become macrocyclic, either autoecious or heteroecious, certain rust fungi might have entered mild and stable environments where conditions are favorable for continuous growth and reproduction and the hosts are almost always available throughout the year. Under those stable and favorable environmental conditions, the production of pleomorphic spores might be redundant. Allocation of available resources to the vegetative mycelium and one kind of spores might have been favored. The spores produced might be sexual, apomictic, or clonal in their genetic nature. Those fungi that have deleted aecial and uredinial stages from the life cycle seem to have the highest selective advantage. Those rust species that produce only teliospores that germinate without dormancy to initiate new generations repeatedly in a single growing season would be highly adaptive. *Puccinia pampeana*, *U. alyxiae*, *P. rutainsulara*, *P. rugispora*, *P. vittata*, *E. acaciae*, *E. hawaiiense*, and *Monosporidium machili* and many other tropical *Endophyllum* species might represent these microcyclic species.

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References

Allen RF (1932) A cytological study of heterothallism in *Puccinia triticina*. J Agric Res 44:733–754

Allen RF (1933) A cytological study of the teliospores, promycelia, and sporidia in *Puccinia malvacearum*. Phytopathology 23:572–586

Allen RF (1934) A cytological study of heterothallism in flax rust. J Agric Res 49:765–791

Andrus CF (1931) The mechanism of sex in *Uromyces appendiculatus* and *U. vignae*. J Agric Res 42:539–587

Anikster Y (1983) Binucleate basidiospores – a general rule in rust fungi. Trans Br Mycol Soc 81:624–626

Anikster Y (1984) Contribution to the knowledge of nuclear history in some Pucciniaceae. Rep Tottori Mycol Inst 22:120–123

Anikster Y (1986) Teliospore germination in some rust fungi. Phytopathology 76:1026–1030

Anikster Y, Wahl I (1985) Basidiospore formation and self-fertility in *Puccinia mesnieriana*. Trans Br Mycol Soc 84:164–167

Anikster Y, Moseman JG, Wahl I (1980) Development of basidia and basidiospores in *Uromyces* species on wild barley and Liliaceae in Israel. Trans Br Mycol Soc 75:377–382

Arthur JC (1925) Terminology of the Uredinales. Bot Gaz 80:219–233

Arthur JC (1928) Notes on arctic Uredinales. Mycologia 20:41–43

Ashworth D (1931) *Puccinia malvacearum* in monosporidial culture. Trans Br Mycol Soc 16:177–202

Ashworth D (1935) An experimental and cytological study of the life history of *Endophyllum sempervivi*. Trans Br Mycol Soc 19:240–258

Barton NH, Charlesworth B (1998) Why sex and recombination? Science 281:1986–1989

Bauer R (1986) Uredinales – germination of basidiospores and pycnospores. Stud Mycol 30:111–125

Blackman VH (1904) On the fertilization, alternation of generations, and general cytology of the Uredineae. Ann Bot 18:323–373

Blackman VH, Fraser HCI (1906) Further studies on the sexuality of the Uredineae. Ann Bot 20:35–48

Blackwell M, Malloch D (1989) *Pyxidiophora*: life histories and arthropode associations of two species. Can J Bot 67:2552–2562

Brasier CM (1987) The dynamics of fungal speciation. In: Rayner ADM, Brasier CM, Moore D (eds) Evolutionary biology of the fungi. Cambridge University Press, Cambridge, pp 231–260

Brown AM (1940) The sexual behaviour of several plant rusts. Can J Res 18:18–25

Buller AHR (1941) The diploid cell and the diplodisation process in plants and animals, with special reference to the higher fungi. Bot Rev 16:355–431

Buller AHR (1950) Research on fungi, vol 7. University of Toronto Press, Toronto

Carlile MJ (1987) Genetic exchange and gene flow: their promotion and prevention. In: Rayner ADM, Brasier CM, Moore D (eds) Evolutionary biology of the fungi. Cambridge University Press, Cambridge, pp 203–214

Chen WQ, Gardner DE, Webb DT (1996) Biology and life cycle of *Atelocauda koae*, an unusual demicyclic rust. Mycoscience 37:91–98

Chinnappa CC, Sreenivasan MS (1965) Cytological studies on germinating teliospores of *Hemileia vastatrix*. Caryologia 18:625–631

Chinnappa CC, Sreenivasan MS (1968) Cytology of *Hemileia vastatrix*. Caryologia 21:75–82

Christman AH (1905) Sexual reproduction in rusts. Bot Gaz 39:267–275

Couch JN (1937) A new fungus intermediate between the rusts and *Septobasidium*. Mycologia 29:665–673

Couch JN (1938) The genus *Septobasidium*. University of North Carolina Press, Chapel Hill

Countinho TA, Rijkenberg FHJ, Van Ash MAJ (1995) Teliospores of *Hemileia vastatrix*. Mycol Res 99:932–934

Craigie JH (1927a) Experiments on sex in rust fungi. Nature (Lond) 120:116–117

Craigie JH (1927b) Discovery of the function of the pycnia of the rust fungi. Nature (Lond) 120:765–767

Crane PE, Hiratsuka Y, Currah RS (2000) Reproductive biology and evidence for water dispersal of teliospores in *Chrysomyxa weirii*, a microcyclic spruce needle rust. Can J Bot 92:754–763

De Bary A (1853) Untersuchungen über die Brandpilze. Berlin (cited from Blackman, 1904)

De Bary A (1884) Vergleichende Morphologie u. Biologie der Pilz u.s.w. Leipzig. (cited from Blackman 1904)

Deml G, Bauer R, Oberwinkler F (1982) Studies in Heterobasidiomycetes. Part 9. Axenic cultures of *Coleosporium tussilaginis* (Uredinales). I. Isolation, identification, and characterization of the cultures. Phytopathol Z 104:39–45

Dodge BO (1924) Uninucleated aecidiospores in *Caeoma nitens* and associated phenomena. J Agric Res 28:1045–1058

Dodge BO (1925) Organization of the telial sorus in the pine rust, *Gallowaya pinicola* Arth. J Agric Res 31:641–651

Dodge BO (1929) Cytological evidence bearing on the sexuality and origin of life cycles in the Uredinales. Proc Int Congr Plant Sci 1926 (Ithaca) 2:1751–1766

Dodge BO, Gaiser LO (1926) The question of nuclear fusions in the blackberry rust. J Agric Res 32:1008–1012, 1022–1023

Epstein L, Buurlage MB (1988) Nuclear division in germinating aeciospores and its taxonomic significance for the western gall rust fungus, *Peridermium harknessii*. Mycologia 80:235–240

Fitzpatrick HM (1918a) The cytology of *Eocronartium muscicola*. Am J Bot 5:397–419

Fitzpatrick HM (1918b) The life history and parasitism of *Eocronartium muscicola*. Phytopathology 8:197–218

Fromme FD (1914) The morphology and cytology of the aecidium cup. Bot Gaz 58:1–35

Fukuda T, Nakamura S (1990) Karyology and nuclear condition of the basidia and basidiospores in *Uromyces erythronii* on *Erythronium* and *Amana*. Trans Mycol Soc Jpn 31:431–438
Gardner DE (1981) Nuclear behavior and clarification of the spore stages of *Uromyces koae*. Can J Bot 59:939–946
Gardner DE (1987) Teliospore germination of *Uromyces alyxiae*, an endemic Hawaiian rust. Mycologia 79:914–917
Gardner DE (1988) Teliospore germination of *Puccinia vitata*, an endemic Hawaiian rust. Mycologia 80:590–593
Gardner DE (1990) New rusts on endemic Hawaiian Rutaceae. Mycologia 82:141–144
Gardner DE (1994) Teliospore germination and nuclear behavior of *Puccinia rutainsulara*, a microcyclic Hawaiian rust. Mycologia 86:486–493
Gardner DE (1996) *Puccinia rugispora*: an unusual microcyclic rust endemic to Hawaii. Mycologia 88:671–676
Gibbs JN, England N, Wolstenholme R (1988) Variation in the pine stem rust fungus *Peridermium pini* in the United Kingdom. Plant Pathol 37:45–53
Hantula J, Kasanen R, Kaitera J, Moricca S (2002) Analyses of genetic variation suggest that pine rusts *Cronartium flaccidum* and *Peridermium pini* belong to the same species. Mycol Res 106:203–209.
Hennen JF, Buriticá P (1980) A brief summary of modern rust taxonomic and evolutionary theory. Rep Tottori Mycol Inst 18:243–256
Hennen JF, Figueiredo MB (1984) The life cycle *of Hemileia vastatrix*? Comunicaçoes, Simpósio sobre Ferrugens do Cafeeiro, Centro de Investigaçno das Ferrrugens do Cafeeiro, Oeiras, Portugal, pp 47–56
Hennen JF, Hodges CS Jr (1981) Hawaiian forest fungi. II. Species of *Puccinia* and *Uredo* on *Euphorbia*. Mycologia 72:1116–1122
Hennen JF, Figueiredo MB, Pimental CP, Russomanno OMR (1984) The life cycle and taxonomy of *Puccinia pampeana* Speg. and *Endophyllum pampeanum* (Speg.) Lindq. on *Capsicum* spp. and other Solanaceae. Rep Tottori Mycol Inst 22:209–220
Hiratsuka N (1935) A contribution to the knowledge of the rust-flora in the alpine regions of high mountains in Japan. Mem Tottori Agric Coll 3:125–247
Hiratsuka N, Kaneko S (1977) Life cycles of three rust species. Rep Tottori Mycol Inst 15:6–12
Hiratsuka Y (1968) Morphology and cytology of aeciospores and aeciospore germ tubes of host-alternating and pine-to-pine races of *Cronartium flaccidum* in northern Europe. Can J Bot 46:1119–1122, 4 pl
Hiratsuka Y (1969) *Endocronartium*, a new genus for autoecious pine stem rusts. Can J Bot 47:1493–1495
Hiratsuka Y (1973a) The nuclear cycle and the terminology of spore states in the Uredinales. Mycologia 65:432–443
Hiratsuka Y (1973b) Nuclear cycle, taxonomy and nomenclature of autoecious pine stem rusts in North America and Europe (in Japanese). Rep Tottori Mycol Inst 10:243–248
Hiratsuka Y (1975) Recent controversies on the terminology of rust fungi. Rep Tottori Mycol Inst 12:99–104
Hiratsuka Y (1986) Cytology of an autoecious soft pine blister rust (*Peridermium yamabense*) in Japan. Mycologia 78:637–640
Hiratsuka Y (1991) Nuclear cycle, taxonomy, and nomenclature of western gall rust. In: Hiratsuka Y, Samoil JK, Blenis PV, Crane PE, Laishley BL (eds) Rust of pines. Information rep NOR-X-317. Forestry Canada, Northwest Region, Northern Forest Center, Edmonton, ALB, pp 92–101
Hiratsuka Y, Maruyama PJ (1968) Identification of *Peridermium harknesii* in eastern Canada on the basis of nuclear condition of aeciospore germ tubes. Plant Dis Rep 52:650–651
Hiratsuka Y, Sato S (1982) Morphology and taxonomy of rust fungi. In: Scott KJ, Chakravorty AK (eds) The rust fungi. Academic Press, London, pp 1–36
Hiratsuka Y, Morf W, Powell JM (1966) Cytology of the aeciospores and aeciospore germ tubes of *Peridermium harknessii* and *P. stalactiforme* of the *Cronartium coleosporioides* complex. Can J Bot 44:1639–1643, 3 pl
Hodges CS Jr, Gardner DE (1984) Hawaiian forest fungi. IV. Rusts on endemic *Acacia* species. Mycologia 76:332–349
Hoffman WHA (1912) Zur Entwicklungsgeschichte von *Endophyllum Sempervivi*. Centralbl Bakt 2 Abt 32:137–158
Holm L (1973) Some notes on rust terminology. Rep Tottori Mycol Inst 10:183–187
Holm L (1984) Terminology of life cycle in rust fungi. Rep Tottori Mycol Inst 22:221–225
Holm L (1987) The terminology of rusts. Suggestions for a compromise. Notes R Bot Gard Edinb 44:433–435
Imazu M, Kakishima M (1992) A new variety of *Endocronartium sahoanum* found on *Pinus pumila* in Hokkaido, Japan. Trans Mycol Soc Jpn.33:167–176
Imazu M, Kakishima M, Kaneko S (1989) *Endocronartium sahoanum*, a new stem rust fungus on *Pinus pumila* in Japan. Trans Mycol Soc Jpn 30:301–310
Imazu M, Kakishima M, Katsuya K (1991a) Morphology and nuclear cycle of *Endocronartium yamabense*. Trans Mycol Soc Jpn 32:371–379
Imazu M, Kakishima M, Katsuya K (1991b) Morphology, cytology, and taxonomy of stem rusts on five-needle pines in Japan. In: Hiratsuka Y, Samoil JK, Blenis PV, Crane PE, Laishley BL (eds) Rust of pines. Information rep NOR-X-317. Forestry Canada, Northwest Region, Northern Forest Center, Edmonton, ALB, pp 76–91
Jackson HS (1931) Present evolutionary tendencies and the origin of life cycles in the Uredinales. Bull Torrey Bot Club 18:1–108
Jackson HS (1935) The nuclear cycle in *Herpobasidium filicinum* with a discussion of the significance of homothallism in Basidiomycetes. Mycologia 27:553–572
Jackson HS (1944) Life cycles and phylogeny in the higher fungi. Trans R Soc Can Sect V Ser III 38:1–30
Kakishima M, Sato T, Sato S (1984) Life-cycle, morphology and cytology of *Puccinia japonica* on *Anemone flaccida*. Trans Br Mycol Soc 82:469–475
Kaneko S (1973) Life cycle and behavior of nuclei of *Aecidium mori* Barclay, the causal fungus of mulberry rust. Trans Mycol Soc Jpn 14:294–301
Kasanen R (1998) The genetic variation within cankers of *Peridermium pini*. In: Jarkanen R, Crane PE, Walla JA, Aalto T (eds) Proceedings, 1st IUFRO Rusts of Forest Trees Working Party Conference, 2–7 Aug 1998, Sariselka, Finland, pp 163–165
Kirk PM, Cannon PF, David JC, Stalper JA (eds) (2001) Dictionary of fungi, 9th ed. CAB International, Wallingford, UK
Kohno M, Nishimura T, Ishizaki H, Kunoh H (1974) Cytological studies on rust fungi. I. Teliospore germination and nuclear behaviors in *Chrysanthemum* white rust (*Puccinia horiana* P. Hennings) (in Japanese). Bull Fac Agric Mie Univ 47:1–9
Kohno M, Nishimura T, Ishizaki H, Kunoh H (1975a) Cytological studies on rust fungi. II. Ultrastructure of sporidia of *Puccinia horiana* P. Hennings. Bull Fac Agric Mie Univ 49:9–15
Kohno M, Nishimura T, Ishizaki H, Kunoh H (1975b) Cytological studies on rust fungi. III. Nuclear behaviors during the process from teliospore stage through sporidial stage in two short-cycled rusts, *Kuehneola japonica* and *Puccinia horiana*. Bull Fac Agric Mie Univ 49:21–29
Kohno M, Ishizaki H, Kunoh H (1977) Cytological studies on rust fungi. VI. Fine structure of infection process of *Kuehneola japonica* (Diet.) Diet. Mycopathologia 61:35–41
Kunkel LO (1914) Nuclear behavior in the promycelia of *Caeoma nitens* Burrill and *Puccinia peckiana* Howe. Am J Bot 1:37–46
Kursanov L (1917) Recherches morphologiques et cytologiques sur les Urédinées (French version published in 1922). Bull Soc Nat Moscou NS 31:1–129, 4 pl
Lamb IM (1934) On the morphology and cytology of *Puccinia prostii* Moug., a micro-form with pycnidia. Trans R Soc Edinb 58:143–162
Lamb IM (1935) The initiation of the dikaryophase in *Puccinia Phragmitis* (Schum.) Kühn. Ann Bot 49:403–438
Laundon GF (1967) Terminology in the rust fungi. Trans Br Mycol Soc 50:189–194
Laundon GF (1972) Delimitation of aecial from uredinial states. Trans Br Mycol Soc 58:344–346
Leppik EE (1953) Some viewpoints on the phylogeny of rust fungi. I. Coniferous rusts. Mycologia 45:46–74
Leppik EE (1955) Evolution of angiosperms as mirrored in the phylogeny of rust fungi. Arch Soc Zool-Bot Fenn 'Vanamo' 9:149–160
Leppik EE (1956) Some viewpoints on the phylogeny of rust fungi. II. *Gymnosporangium*. Mycologia 48:637–654
Leppik EE (1959) Some viewpoints on the phylogeny of rust fungi. III. Origin of grass rusts. Mycologia 51:512–528
Leppik EE (1961) Some viewpoints on the phylogeny of rust fungi. IV. Stem rust genealogy. Mycologia 53:378–405

Leppik EE (1965) Some viewpoints on the phylogeny of rust fungi. V. Evolution of biological specialization. Mycologia 57:6–22
Leppik EE (1967) Some viewpoints on the phylogeny of rust fungi. VI. Biogenic radiation. Mycologia 59:568–579
Leppik EE (1972) Evolutionary specialization of rust fungi (Uredinales) on the Leguminosae. Ann Bot Fenn 9:135–148
Leppik EE (1973) Origin and evolution of conifer rusts in the light of continental drift. Mycopathol Mycol Appl 49:21–136
Linder DH (1940) Evolution of the Basidiomycetes and its relation to the terminology of the basidium. Mycologia 32:419–447
Lindfors T (1924) Studien iter den Entwicklungsverlauf bei einigen Rostpilzen aus zytologischen und anatomischen Gesichtpunkten. Svensk Bot Tidskr 18:1–84
MaClean DJ (1982) Axenic culture and metabolism of rust fungi. In: Scott KJ, Chakravorty AK (eds) The rust fungi. Academic Press, London, pp 37–84
Maire R (1900) L'evolution nucléaire chez les *Endophyllum*. J Bot 14:80–97
Maynard Smith J (1978) The evolution of sex. Cambridge University Press, Cambridge
Meyen FJF (1841) Pflanzen-Pathologie. Berlin (cited from Blackman 1904)
Moreau Mme F (1911) Sur l'existence d'une forme écidienne uninucléée. Bull Soc Mycol Fr 27:489–493
Moreau Mme F (1914) Les phénomenones de la sexualité chez les Urédinées. Botaniste 3:145–283
Moreau Mme F (1915) Note sur la variété uninuclée de l'*Endophyllum Euphorbiae* (DC.) Winter. Bull Soc Mycol Fr 31:68–70
Moreau F, Moreau Mme F (1918a) L'écidiospore de l'*Endophyllum Euphorbiae-silvaticae* (DC.) Winter est-elle le siege d'une karyogamie? Bull Soc Mycol Fr 33:97–99
Moreau F, Moreau Mme F (1918b) L'evolution nucléaire chez l'*Endophyllum Sempervivi* Lév. Bull Soc Mycol Fr 33:70–72
Moreau F, Moreau Mme F (1919) Les Urédinées du groupe *Endophyllum*. Bull Soc Mycol Fr 66:14–44
Olive EW (1908a) Sexual cell fusions and vegetative nuclear divisions in the rusts. Ann Bot 22:331–361
Olive EW (1908b) The relation of "conjugation" and "nuclear migration" in the rusts. Science 27:213–214
Olive EW (1911) Nuclear divisions in certain short-cycled rusts. Science 33:194
Olive LS (1947) Cytology of the teliospores, basidia, and basidiospores of *Sphenospora kevorkianii* Linder. Mycologia 39:409–425
Olive LS (1949) Cytological study of typical and atypical basidial development in *Gymnosporangium clavipes*. Mycologia 41:420–426
Olive LS (1953) The structure and behavior of fungal nuclei. Bot Rev 19:439–586
Ono Y (2002) Life cycle and nuclear behavior in three rust fungi (Uredinales). Mycoscience 43:37–45
Ono Y (2003) Nuclear behaviors during teliospore germination in four microcyclic rust fungi (Uredinales). Bull Fac Educ Ibaraki Univ 52 (in press)
Ono Y, Hennen JF (1983) Taxonomy of the chaconiaceous genera (Uredinales). Trans Mycol Soc Jpn 24:369–402
Ono Y, Butiticá P, Hennen JF (1992) Delimitation of *Phakopsora*, *Physopella* and *Cerotelium* and their species on Leguminosae. Mycol Res 96:825–850
Orton CR (1927) A working hypothesis on the origin of rusts, with special reference to the phenomenon of heteroecism. Bot Gaz 84:113–138
Payak MM (1952) Diploidisation in *Uromyces hobsoni* Vize and *Puccinia thwaithesii* Berk. Indian Phytopathol 5:104–109
Payak MM (1953) On the existence of endo-condition in *Uromyces hobsoni* Vize. Cellule 56:73–84, 2 pl
Petersen RH (1974) The rust fungus life cycle. Bot Rev 40:453–513
Poirault G (1913) Sur quelques Urédinées nouvelles. Bull Mens Nat Nice Alpes-Maritimes 1:105–108
Poirault G (1915) Sur quelques champignons parasites rares ou noueaux obsérves dans les Alpes-Maritimes. Bull Assoc Nat Alpes-Maritimes 2:7–19
Rajendren RB (1967a) New type of nuclear life cycle in *Hemileia vastatrix*. Nature (Lond) 213:105–106
Rajendren RB (1967b) A new type of nuclear life cycle in *Hemileia vastatrix*. Mycologia 59:279–285
Rajendren RB (1967c) Atypical and typical germination of uredinoid teliospores of *Hemileia vastatrix*. Mycologia 59:918–921
Sappin-Trouffy M (1896) Recherches histologique sur la famille des Uredinées. Botaniste 5:59–244, 69 fig
Sato T, Sato S (1981) *Aecidium rhaphiolepidis* and *A. pourthiaeae*: two imperfect rust fungi with aecidioid uredinia. Trans Mycol Soc Jpn 22:173–179
Sato T, Katsuya K, Sato S (1980) Host range, teliospore germination and infection process of *Uromyces aloes*. Trans Mycol Soc Jpn 21:273–282
Savile DBO (1953) Short-season adaptations in the rust fungi. Mycologia 45:75–87
Savile DBO (1955) A phylogeny of the Basidiomycetes. Can J Bot 33:60–104
Savile DBO (1968) The case against "Uredinium." Mycologia 60:459–464
Savile DBO (1976) Evolution of the rust fungi (Uredinales) as reflected by their ecological problems. Evol Biol 9:137–207
Savile DBO (1988) Terminology of spore states in Uredinales. Mycotaxon 33:387–389
Singh UP (1979) Cytology of *Puccinia ruelliae*. Mycologia 71:1197–1205
Sjamsuridzal W, Nishida H, Ogawa H, Kakishima M, Sugiyama J (1999) Phylogenetic positions of rust fungi parasitic on ferns: evidence from 18S rDNA sequence analysis. Mycoscience 40:21–27
Stanley IN (1940) Development of the basidium of *Eocronartium muscicola*. Trans Am Microsc Soc 59:407–413
Stevens FL (1932) Additional Philippine Urednineae. Univ Philipp Nat Appl Sci Bull 2:441–447
Swann EC, McLaughlin DJ, Frieder EM, Szabo LJ (1998) The origin of the rust fungi: the Uredinales are not primitive! Inoculum 49: 51
Swann EC, Frieder EM, McLaughlin DJ (2001) Urediniomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) The Mycota. VII. Systematics and evolution, part B. Springer, Berlin, pp 37–56
Thirumalachar MJ (1945) Development of spore-forms and the nuclear cycle in the autoecious opsis rust, *Cystopsora oleae*. Bot Gaz 107:74–86
Thirumalachar MJ (1946) A cytological study of *Uromyces aloës*. Bot Gaz 108:245–254
Thirumalachar MJ, Govindu HC (1954) Morphological and cytological studies of a bisporidial species of *Endophyllum*. Bot Gaz 115:391
Thirumalachar MJ, Narasimhan MJ (1950) Cytology and life history of a bisporidal *Endophyllum*. New Phytol 49:117–120
Tulasne LR (1854) Second mémoire sur les Urédinées et les Ustilaginées. Ann Sci Nat 2e Ser. 4:77.
Vogler DR, Epstein L, Cobb FW Jr (1997) Nuclear behaviour and evolution of two populations of the western gall rust fungus. Mycol Res 101:791–797
Walker RI (1928) Cytological studies of some of the short-cycled rusts. Trans Wis Acad Sci Art Lett 23:567–582, 3 pl
Werth E, Ludwigs K (1912) Zur Sporenbildung bei Rostund Brandpilzen. Ber Dtsch Bot Ges 30:522–528
Whisler HC, Zebold SL, Shemanchuk JA (1974) Alternate host for mosquito parasite *Coelomomyces*. Nature (Lond) 251:715–716
Whisler HC, Zebold SL, Shemanchuk JA (1975) Life history of *Coelomomyces psorophorae*. Proc Natl Acad Sci USA 72:693–696
Wuerthrich B (1998) Why sex? Putting theory to the test. Science 281:1980–1982

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Short Communication

Pathogenic Races of *Phakopsora pachyrhizi* on Soybean and Wild Host Plants Collected in Japan

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ABSTRACT

A total of 45 single uredinial isolates of *Phakopsora pachyrhizi* were collected from rust-infected soybean and wild host plants (*Pueraria lobata* and *G. soja*) at different localities in central and southwestern Japan. Eighteen pathogenic races were identified using a set of differential varieties composed of nine cultivars of soybean and two accession lines of *G. soja*. Nine and 11 races were found on soybean and wild host plants, respectively. Two races were common to soybean and wild host plants.

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Key words : *Phakopsora pachyrhizi*, pathogenic race, soybean rust, *Glycine max*, *Glycine soja*, *Pueraria lobata*.

Phakopsora pachyrhizi H. et P. Sydow is a causal organism of soybean rust in Australia, Asia and Hawaii and may cause serious yield losses[14]. Pathogenic races in populations of the fungus have been detected in several studies using various sets of differentials[1-4,10,11]. The Asian Vegetable Research and Development Center (AVRDC)[1] identified nine races in Taiwan using a set of differentials composed of nine varieties of soybean (*Glycine max* (L.) Merrill) and two accessions of *G. soja* Sieb. et Zucc. This set of differentials includes important varieties used in studies on race detection by McLean and Byth[11], Bromfield *et al.*[2] and Bromfield[3] and was recommended for identifying soybean rust races[15]. Yamada *et al.*[16] first demonstrated the presence of two races of soybean rust in Japan using this set of differentials. Fujiwara *et al.*[6] then identified six more races among eight isolates of the fungus collected on soybean and on a wild host plant, *Pueraria lobata* (Willd.) Ohwi.

Phakopsora pachyrhizi is able to infect not only soybean but also various leguminous plants in the field[12]. In Japan, *G. soja* and *P. lobata* as well as soybean are common host plants of *P. pachyrhizi*[9]. Populations of *P. pachyrhizi* from soybeans are able to infect *P. lobata*[13]. The same race was found on both soybean and *P. lobata*[6]. To accumulate more information on the pathogenic races of *P. pachyrhizi* in Japan, we conducted a study to identify races of the rust fungus collected from cultivated soybean and wild host plants from central to southwestern Japan between 1993 and 1997. We report here the presence of 18 races of the rust fungus in Japan, including eight races identified by Yamada *et al.*[16] and Fujiwara *et al.*[6].

Uredinia of *Phakopsora pachyrhizi* on leaves of soybean or on wild host plants, *P. lobata* and *G. soja*, were collected from soybean fields or roadside areas (Table 1). They were stored in a deep freezer (−80°C) until use. Frozen samples were thawed under warm running water (37-40°C) for 1 min. Leaves of soybean cultivar or *P. lobata*, grown for 2 weeks in a growth cabinet controlled at 25°C for 12 hr light : 12 hr dark, were inoculated with urediniospores. Six to seven days after inoculation, when flecking appeared, leaves were cut into segments with a

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Table 1. Origin of single uredinium isolates of *Phakopsora pachyrhizi*

Isolates	Number of isolates	Original Collection		
		Host plant	Locality	Date
GE1 to GE4	4	*Glycine max* cv. Enrei	Soybean field at NARC[a], Tsukuba, Ibaraki	27.Oct.93
GN1	1	*G. max* cv. Nattoushyouryu	Soybean field, Shimodate, Ibaraki	19.Oct.93
GT1, GT2	2	*G. max* cv. Tachinagaha	Soybean field, Shimodate, Ibaraki	19.Oct.93
GW1 to GW12	12	*G. max* cv. Wayne	Soybean field at UofT[b], Tsukuba, Ibaraki	7.Oct.97
GA1, GA2	2	*G. max*	Soybean field, Kumamoto, Kumamoto	Oct.93
GB1	1	*G. max*	Soybean field, Ekawasaki, Kochi	30.Oct.96
Gs1, Gs2	2	*G. soja*	Roadside, Shimotsuma, Ibaraki	19.Oct.93
PA1	1	*Pueraria lobata*	Roadside at UofT, Tsukuba, Ibaraki	27.Oct.93
PB1	1	*P. lobata*	Roadside, Shimotsuma, Ibaraki	19.Oct.93
PC1	1	*P. lobata*	Roadside, Atami, Shizuoka	25.Nov.93
PD1	1	*P. lobata*	Roadside, Azusagawa, Nagano	12.Nov.93
PE1	1	*P. lobata*	Roadside, Katsuura, Chiba	25.Oct.95
PF1	1	*P. lobata*	Roadside, Matsuyama, Ehime	29.Oct.96
PG1 to PG9	9	*P. lobata*	Roadside at UofT, Tsukuba, Ibaraki	7.Oct.97
PH1 to PH3	3	*P. lobata*	Roadside at UofT, Tsukuba, Ibaraki	27.Oct.97
PJ1 to PJ3	3	*P. lobata*	Botanic Garden, Tsukuba, Ibaraki	21.Oct.97

a) NARC, National Agriculture Research Center.
b) UofT, University of Tsukuba.

single fleck, and each leaf segment was placed on a paper towel moistened with 50 ppm benzimidazole (about 5 ml) in plastic Petri dishes. The plates were incubated in a growth cabinet until the uredinium ruptured on the leaf segment, thus establishing a single uredinium isolate. Urediniospores of the single uredinial isolates were propagated by inoculating leaves of intact soybean plants grown in another growth cabinet. Soybean cultivars used for isolating and propagating single uredinial isolates were the same cultivars from which the original uredinial samples were collected except for four isolates—cultivar Tachinagaha for isolate GE1 and cultivar Wayne for isolates GA1, GA2 and GB1. For the isolates from wild host plants, *P. lobata* collected in Tsukuba, Ibaraki was used. One to 12 single uredinial isolates were established from each field collection (Table 1). Isolates GE1 and GE2 used by Yamada *et al.*[16] and isolates GA1, GA2, GB1, PB1, PC1, PD1, PE1 and PF1 used by Fujiwara *et al.*[6] were also included in the present study because precise data on infection types of the differential varieties of the races that they identified have not been published.

Sets of differentials recommended by Tschanz[15] were used in the present study. They included nine cultivars of soybean and two accession lines of *G. soja* (PI239871A, PI239871B). Three seeds of each variety were sown in plastic pots with soil and incubated in a growth cabinet controlled as described earlier for 3 weeks. The first trifoliate leaves were inoculated with urediniospores of each isolate using a paintbrush. Two weeks after inoculation, the color of lesions and number of uredinia per lesion on the leaves were recorded and infection types were estimated following Bromfield[3] : type 0, no macroscopically visible signs or symptoms ; type 1, dark reddish necrotic lesions lacking uredinia ; type 2, dark reddish necrotic lesions with one or two sparsely sporulating uredinia per lesion ; type 3, dark reddish necrotic lesions with three or more profusely sporulating uredinia per lesion ; type 4, tan lesions with two to five uredinia per lesion ; and type 5, tan lesions with more than five uredinia per lesion. Infection type 0 indicates host immunity or near immunity ; types 1, 2 and 3 with dark reddish necrotic lesions show specific resistance of the host ; types 4 and 5 with tan lesions indicates host susceptibility[3].

A total of 45 isolates (22 isolates on soybean and 23 isolates on wild host plants) were used in the present study. Infection types on the differential varieties for each isolate are shown in Table 2. Pathogenic races of the isolates were determined by regarding infection types 0 to 3 as resistant reactions and infection types 4 and 5 as susceptible reactions. Among the 45 isolates, 18 races were recognized (Table 3). Races Ppj1 to 18 were temporarily assigned. Nine races were present on soybean and 11 on wild host plants. Two races, Ppj2 and Ppj18, were common to soybean and wild host plants. Races Ppj5 and Ppj7 were recognized by Yamada *et al.*[16]. Races Ppj2, 3, 10, 13, 16, and 18 were recognized by Fujiwara *et al.*[6].

Race Ppj1 was the most pathogenic race among the 18 races. Only PI459025 had resistance. This race was isolated only from the soybean cultivar Wayne, which is not popular in Japan. It was cultivated in the experimental

Table 2. Infection types of 45 isolates of *Phakopsora pachyrhizi* on differential varieties[a)]

Isolate No.	Differential varieties										
	PI230970	PI230971	Ankur	Wayne	PI459024	PI459025	TK#5	Tainung#4	PI200492	PI239871A	PI239871B
GW1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12	4	4	4	4	4	1,2	4	4	4	4	4
GA2, PE1	2	4	4	4	4	1,2	4	4	4	4	4
GA1	2	2,3	4	4	4	2,3	4	4	4	4	4
GT1	4	4	4	4	4	2	4	2	4	4	4
GE2	2	2	1	4	4	4	4	1	4	4	4
GE3	3	2	1	4	4	1	4	3	4	4	4
GN1, GT2	2	2	1	4	4	2	4	1	4	4	4
GE1	2	2	1	4	2	2	4	1	4	4	4
GE4	3	4	2	4	4	2	4	3	4	4	4
Gs1	4	4	—	4	2	2	4	2	4	4	4
PB1	1,2	4	4	4	1,2	1,2	4	4	1,2	4	4
PG7	1	1	4	4	4	2	1	0	4	4	4
PJ3	1	1	4	4	4	1,2	1,2	0	4	4	4
PG1, 2, 6, PJ1	1	1	1	4	4	1,2	1	0	4	4	4
PG4, 5, PH1	1	1	1	4	4	1	1	0	4	4	4
PG8, 9, PJ2	1	1	1	4	4	2	2	0	4	4	4
PC1	1	1	2	4	2	1	1,2	1	4	4	4
PH2	1	1	1	4	4	1	1	0	4	4	2
PH3	1	1	1	4	4	1	1	0	2	4	2
PD1	1	1	1,2	4	1,2	1,2	4	1,2	1,2	1,2	1,2
PG3	1	1	2	1,2	4	1	1	0	4	1,2	1,2
GB1	1,2	2	1	2	1,2	1	2	0	2,3	2,3	2,3
Gs2	2	2	2	3	3	2	2	2	3	3	3
PA1	1	1	1	1	1	1	1	1	1	1	1
PF1	1,2	1,2	1	1,2	1,2	1,2	1,2	0	1,2	1,2	1,2

a) Infection type 0, no macroscopically visible signs or symptoms ; type 1, dark reddish necrotic lesions lacking uredinia ; type 2, dark reddish necrotic lesions with one or two sparsely sporulating uredinia per lesion ; type 3, dark reddish necrotic lesions with three or more profusely sporulating uredinia per lesion ; type 4, tan lesions with two to five uredinia per lesion ; and type 5, tan lesions with more than five uredinia per lesion.

field at the University of Tsukuba to propagate the differential varieties. The same race was recorded as a dominant race in the study conducted by AVRDC[1)] in Taiwan, where cultivar Wayne was used for isolation and propagation of urediniospores of each culture.

Race Ppj6 was isolated from three Japanese soybean cultivars, Enrei, Tachinagaha and Nattousyouryu, cultivated in Ibaraki Prefecture. These three are popular recommended cultivars in Ibaraki Prefecture[5)]. Yamada *et al.*[16)] reported that two races, Ppj5 and Ppj7, were isolated from soybean in Tsukuba, with Ppj7 more frequently isolated. Race Ppj7 differed from Ppj6 only in the reaction on cultivar PI459024. Because Yamada *et al.*[16)] did not use cultivar PI459024 as one of the differential varieties, they could not detect the difference between race Ppj7 and Ppj6. Thus, the isolates identified as the predominant race by Yamada *et al.*[16)] might also include race Ppj6 in addition to Ppj7.

Four single uredinial isolates (GE1 to GE4) were established from a uredinial culture on soybean cultivar Enrei collected from the same field at the National Agriculture Research Center, Tsukuba. Each of the isolates were a different race. That is, isolates GE1, GE2, GE3 and GE4 were races Ppj7, Ppj5, Ppj6 and Ppj8, respectively. The results indicate that several races might exist on one cultivar of soybean in one small field.

Isolate GA1 from Kumamoto, Kyushu Island was identified as race Ppj3. It caused the same reaction pattern on the differential varieties as tentative race No. 11 identified by AVRDC in Taiwan[1)]. Race Ppj3 as well as Ppj1 were recognized both in Japan and Taiwan. Isolate GB1, collected in Kochi, Shikoku Island, was race Ppj18, an

Table 3. Eighteen races of *Phakopsora pachyrhizi* and their reactions on differential varieties

Race No.	Isolate No.	Reaction on differential varieties[a]										
		PI230970	PI230971	Ankur	Wayne	PI459024	PI459025	TK#5	Tainung#4	PI200492	PI239871A	PI239871B
Ppj1	GW1-GW12	S	S	S	S	S	R	S	S	S	S	S
Ppj2	GA2, PE1	R	S	S	S	S	R	S	S	S	S	S
Ppj3	GA1	R	R	S	S	S	R	S	S	S	S	S
Ppj4	GT1	S	S	S	S	S	R	S	R	S	S	S
Ppj5	GE2	R	R	R	S	S	S	S	R	S	S	S
Ppj6	GE3, GN1, GT2	R	R	R	S	S	R	S	R	S	S	S
Ppj7	GE1	R	R	R	S	R	R	S	R	S	S	S
Ppj8	GE4	R	S	R	S	S	R	S	R	S	S	S
Ppj9	Gs1	S	S	—	S	R	R	S	R	S	S	S
Ppj10	PB1	R	S	S	S	R	R	S	S	R	S	S
Ppj11	PG7, PJ3	R	R	S	S	S	R	R	R	S	S	S
Ppj12	PG1, 2, 4, 5, 6, 8, 9, PH1, PJ1, 2	R	R	R	S	S	R	R	R	S	S	S
Ppj13	PC1	R	R	R	S	R	R	R	R	S	S	S
Ppj14	PH2	R	R	R	S	S	R	R	R	S	S	R
Ppj15	PH3	R	R	R	S	S	R	R	R	R	S	R
Ppj16	PD1	R	R	R	S	R	R	S	R	R	R	R
Ppj17	PG3	R	R	R	R	S	R	R	R	S	R	R
Ppj18	GB1, Gs2, PA1, PF1	R	R	R	R	R	R	R	R	R	R	R

a) Infection types 0 to 3 were regarded as a resistant reaction (R) and infection types 4 and 5 as a susceptible reaction (S).

unusual race among those obtained from soybean and was avirulent on all 11 differential varieties.

Eight of nine races on soybean were compatible with Wayne, TK#5, PI200492, PI239871A, and PI239871B. Of these, PI200492 is known to possess the resistant gene *Rpp1*[7]. Most of the races collected from soybean seemed to possess a virulent gene for *Rpp1*. All but race Ppj5 were avirulent on PI459025, which has resistant gene *Rpp4*[8]. All nine races identified by AVRDC in Taiwan were also avirulent on this cultivar[1]. Many of the resistant reactions on the differential varieties against the races collected from soybean were infection type 2 or 3 (Table 2), in which uredinia were produced on lesions. Highly resistant infection types, type 0 and 1 (no uredinia produced) were observed in a few cases.

Eleven races were detected on wild host plants, *G. soja* and *P. lobata*. Compared with the interactions of races on soybean, those from wild host plants tended to be less pathogenic on soybean. Most of the races were avirulent on soybean cultivars, PI230970, PI230971, Ankur, PI459025, TK#5, and Tainung#4. Cultivars PI230970 and Ankur are known to possess resistant genes *Rpp2* and *Rpp3*, respectively[7].

Isolates Gs2, PA1 and PF1, as well as isolate GB1, were identified as race Ppj18, which was avirulent on all the differentials. However, isolate PA1 produced infection type 1 on all the differentials, while isolate Gs2 gave type 2 or 3, isolate PF1 type 0 to 2 and isolate GB1 type 0 to 3. In the present study, reactions with reddish brown lesions, *i.e.*, type 0, 1, 2, and 3, were regarded as resistant. Thus, these three isolates were identified as the same race, although they might have different virulent genes that were not detected clearly on the differential varieties used in this study. Additional differentials may be required for more precise identification of the races on soybean and on wild host plants.

Phakopsora pachyrhizi spends the uredinial-telial stage on soybean and other leguminous plants, but the spermogonial-aecial stage is unknown. Thus, it is uncertain if sexual reproduction and thus new races occur every year. Our results showed that many races were present in a specific area. The presence of wild hosts over a wide area of Japan might help to maintain the diversity of races of the fungus.

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LITERATURE CITED

1. Asian Vegetable Research and Development Center (1985). Progress Report 1983. AVRDC Publication, Shanhua, Taiwan.
2. Bromfield, K.R., Melching, J.S. and Kingsolver, C.H. (1980). Virulence and aggressiveness of *Phakopsora pachyrhizi* isolates causing soybean rust. Phytopathology 70 : 17-21.
3. Bromfield, K.R. (1984). Soybean Rust. Monograph No. 11. p. 65, The American Phytopathological Society, St. Paul, MN.
4. Burdon, J.J. and Speer, S.S. (1984). A set of differential *Glycine* hosts for the identification of races of *Phakopsora pachyrhizi* Syd. Euphytica 33 : 891-896.
5. Characteristics of recommended varieties of major crops (1994). Ibaraki Prefecture, Mito, Ibaraki, Japan (in Japanese).
6. Fujiwara, Y., Yamaoka, Y., Kakishima, M. and Katsuya, K. (1998). Identification of pathogenic races of *Phakopsora pachyrhizi* on soybean and Kudzu. Ann. Phytopathol. Soc. Jpn. 64 : 362-363 (Abstr. in Japanese).
7. Hartwig, E.E. and Bromfield, K.R. (1983). Relationships among three genes conferring specific resistance to rust in soybean. Crop Sci. 23 : 237-239.
8. Hartwig, E.E. (1986). Identification of a fourth major gene conferring resistance to soybean rust. Crop Sci. 26 : 1135-1136.
9. Hiratsuka, N., Sato, S., Katsuya, K., Kakishima, M., Hiratsuka, Y., Kaneko, S., Ono, Y., Sato, T., Harada, Y., Hiratsuka, T. and Nakayama, K. (1992). The Rust Flora of Japan. p. 1205, Tsukuba Shuppankai, Tsukuba.
10. Lin, S.Y. (1966). Studies on the physiologic races of soybean rust fungus, *Phakopsora pachyrhizi* Syd. J. Taiwan Agric. Res. 15 : 24-28 (Chinese with English summary).
11. McLean, R.J. and Byth, D.E. (1976). Resistance of soybean to rust in Australia. Aust. Plant. Pathol. Soc. Newsl. 5 : 34-36.
12. Ono, Y., Buritica, P. and Hennen, J.F. (1992). Delimitation of *Phakopsora*, *Physopella* and *Cerotelium* and their species on Leguminosae. Mycol. Res. 96 : 825-850.
13. Sato, T. and Sato, S. (1982). Infective ability of soybean rust to several leguminous plants. Soybean Rust Newsl. 5 : 22-26.
14. Sinclair, J.B. and Hartman, G.L. (1999). Soybean rust. *In* Compendium of Soybean Diseases. 4th ed. (Hartman, G.L., Sinclair, J.B. and Rupe, J.C., eds.). pp. 25-26, The American Phytopathological Society Press, St. Paul, MN.
15. Tschanz, A.T. (1989). Rust. *In* Compendium of Soybean Diseases. 3rd ed. (Sinclair, J. B. and Backman, P. A., eds.). pp. 24-27, The American Phytopathological Society, St. Paul, MN.
16. Yamada, K., Yamaoka, Y., Kakishima, M., Katsuya, K. and Hagiwara, H. (1996). Identification of races of soybean rust, *Phakopsora pachyrhizi*, in Japan. Ann. Phytopathol. Soc. Jpn. 62 : 271 (Abstr. in Japanese).

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FULL PAPER

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Exobasidium dubium and *E. miyabei* sp. nov. causing Exobasidium leaf blisters on *Rhododendron* spp. in Japan

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Abstract Two *Exobasidium* species causing Exobasidium leaf blister on *Rhododendron* spp. are described. An Exobasidium leaf blister on *Rhododendron yedoense* var. *yedoense* f. *yedoense* has been recognized in Hokkaido Prefecture, Japan, since the first report was issued in 1950. The causal fungus is identified with *Exobasidium dubium* from the morphology of its hymenial structure and mode of germination of the basidiospores. Another Exobasidium leaf blister on *Rhododendron dauricum* has been observed in Hokkaido Prefecture, Japan. In comparison with morphology based on hymenial structure and mode of germination of the basidiospores of the 100 validly described taxa, this fungus differs from those known taxa in the size of basidia and basidiospores, the numbers of sterigmata and septa of basidiospores, and the mode of germination of basidiospores. Thus, a new species, *Exobasidium miyabei*, is established and illustrated.

Key words Basidiomycetes · Culture · *Exobasidium* · Japan · Taxonomy

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Introduction

In Japan, three Exobasidium leaf blisters causing small leaf spots (Ezuka 1991a) have been recorded on ericaceous plants: viz., on six species with a variety of *Rhododendron* L. belonging to subgenus *Sciadorhodion* section *Brachycalyx* (Yamazaki 1989) caused by *Exobasidium yoshinagae* Henn, on *Pieris japonica* D. Don ex G. Don by *E. asebi* Hara et Ezuka, and on *Lyonia ovalifolia* (Wall.) Drude var. *elliptica* (Siebold et Zucc.) Hand.-Mazz. by *E. pieridis-ovalifoliae* Sawada. Another Exobasidium leaf blister on *R. yedoense* Maxim. var. *yedoense* f. *yedoense* was reported by Sawada (1950) in Iwate Prefecture, assigning the causal agent to *E. magnusii* Woron. Ito (1955) and Ezuka (1992) excluded this fungus from their monograph or list of *Exobasidium* in Japan and placed it among the doubtful species. However, Nannfeldt (1981) examined Sawada's specimens in Iwate University and treated *E. magnusii* as a synonym of *E. dubium* Racib. Taxonomic reassessment of *E. magnusii* using different herbarium materials was preceded by Nannfeldt, and no additional collection in Japan has been reported since Sawada's publication. In June 2001, Akimoto, one of the authors here, found an Exobasidium leaf blister on *R. yedoense* var. *yedoense* f. *yedoense* at Hakodate-shi, Hokkaido Prefecture. Identification of this *Exobasidium* species leads to revealing whether the causal agent is *E. dubium*.

Since 1996, an Exobasidium leaf blister on *Rhododendron dauricum* L. has been reported at Bibai (Akimoto 1999) and at Hidaka (Nagao et al. 2000) in Hokkaido Prefecture. *Rhododendron dauricum* belongs to subgenus *Rhodorastrum* (Yamazaki 1989). No species has been reported to cause *Exobasidium* diseases on *R. dauricum* in Japan, whereas two species, *E. caucasicum* Woron. and *E. rhododendri* (Fuckel) C.E. Cramer apud Geyler, were described in the USSR to infect leaves and branches (Murashkinsky and Sieling 1928; cited in Farr et al. 1996). In the herbarium of University of Hokkaido (SAPA), similar spotted leaves of *R. dauricum* are deposited as a specimen infected by *Exobasidium* sp., but the taxonomic examina-

tion has not appeared in the literature. There is no comment and no annotation slip in and on the specimen envelopes. In 2001, we could obtain several specimens of diseased leaves in new localities at Hakodate and Teshikaga, Hokkaido Prefecture. These fresh materials gave us an opportunity to identify the causal agent on *R. dauricum* and to determine how the fungus differed from that on *R. yedoense* var. *yedoense* f. *yedoense*, which also causes an Exobasidium leaf blister in the same season.

Therefore, we examined the morphology of these specimens collected in Hokkaido Prefecture compared with the described *Exobasidium* spp. We propose the fungus that caused an Exobasidium leaf blister on *R. dauricum* is a new *Exobasidium* species.

Materials and methods

Morphological observations

Fresh specimens on *R. dauricum* and *R. yedoense* var. *yedoense* f. *yedoense* (simplified as *R. yedoense* hereafter) collected in the field were used for morphological observations. Specimens examined are listed in the description of the species. Morphological observations were conducted by light (LM) and scanning electron microscopy (SEM). The basidia, basidiospores, and conidia were scratched from hymenia, mounted in Shear's mounting fluid on glass slides, and were occasionally stained with 1% (w/v) Phloxine B dye solution or 0.01% (w/v) lacto-phenol Cotton blue solution for LM observations. Germination of basidiospores was also examined according to Graafland (1960) and Sundström (1964). The germination method was followed as described next. Germinated basidiospores on Difco potato dextrose agar (PDA) were fixed with formalin-acetic acid solution (10ml formalin, 5ml acetic acid, 85ml distilled water). After microscopic examination through the bottom of the Petri dish, the areas of fallen basidiospores and germinated basidiospores were marked with a marker pencil on the bottom surface of the Petri dish. The agar was cut with a small knife along the marking on the bottom of the dish and transferred to the glass slide. Shear's mounting fluid was poured first to prevent overstaining of agar. Then, the lacto-phenol Cotton blue solution was added. Excess staining solution was absorbed with filter paper. Finally, the agar surface was again covered with Shear's mounting fluid and then covered with a coverslip. Samples for SEM were prepared as described previously (Nagao et al. 2001). All specimens were deposited in the Mycological Herbarium of Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba (TSH).

Culture of basidiospore isolate

Fresh materials were kept in a plastic bag for vegetables until newly sporulating lesions were observed. Leaves with lesions were cut into small pieces about 5mm square and fixed with a water agar block about 10mm square to the inside of the lid of a sterile Petri dish, poured with PDA acidified with 10% (v/v) lactic acid. The dish was kept at 22°C in the dark. Basidiospores then fell down from the hymenium onto the agar surface. After microscopic examination through the bottom of the Petri dish, a single basidiospore was isolated from the dish, and 18 isolates were transferred to a new PDA dish to grow. Then, three to five colonies that were growing well were selected among these colonies and stored on PDA slants as the representative strains. Cultures were kept in the Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba and also deposited in National Institute of Agrobiological Resources (MAFF).

Taxonomy

Exobasidium dubium Racib. Figs. 1,2

=*Exobasidium* sp. Magnus, Acta Hortic. Petropol. 16:540, 1900

=*Exobasidium vaccinii* (Fuckel) Woron. f. *rhododendrifavi* Bubák, Ann. Naturh. Hofmus. Wien 23:101, 1909

=*E. magnusii* Woron., Monit. Jard. Bot. Tiflis 28:18, 1913

Specimens examined: TSH-B0076, TSH-B0077 (Nagaoh 13119, 13120, Donan Branch, Hokkaido Forestry Research Institute, Hakodate, Hokkaido Prefecture, June 14, 2001).

The hymenium was composed of basidia with 3–5 sterigmata and conidia (Fig. 3A). Hyphae did not develop directly on the surface of the epidermis. Basidia were clavate to cylindrical, 20–30 × 6.5–10μm. Apices of basidia were obtuse. Basidia emerged directly from the host surface or through stomata. Basidia were not fasciculate. Sterigmata were 1.5–2μm in diameter at the base and 3–7μm in height, emerging outwardly and tapering toward the tip (Fig. 3B). Basidiospores were ellipsoid to ovoid, 15–25 × 3.8–5μm, hyaline, smooth, one-celled when formed, becoming septate with 1–4 (6) septa (see Fig. 1A,B). Septate basidiospores germinated after 6h when dropped on the agar surface (Fig. 2). Germ tubes of the basidiospores emerged from the cells of both ends at first, then from other cells, and produced conidia at the tip of germ tubes or laterally 22h after dropping (Fig. 2). Hyphae grew into pseudohyphae and branched. Conidia were bacilliform, lacrimiform, subfusiform, and clavulate (Fig. 1C), 6–14 × 1–2μm, and budded polarly. Conidia budded to produce daughter cells polarly and also developed hyphae. Colonies on PDA grew gradually, to a maximum 11mm diameter in 21-day incubations, and were wrinkled irregularly around the periphery. The surface of the colonies was pale yellow to pale pink and corrugate. Colonies were gelatinous and fixed on the agar surface. Conidial formation did not produce a powdery appearance. Colonies were composed of branching, intricate hyphae and pseudohyphae and conidia. The reverse of colonies was also pale yellow to pale pink. Dark pigmentation was not observed on PDA (see Fig. 6A). Colonies from conidia showed the same morphological features as those from basidiospores.

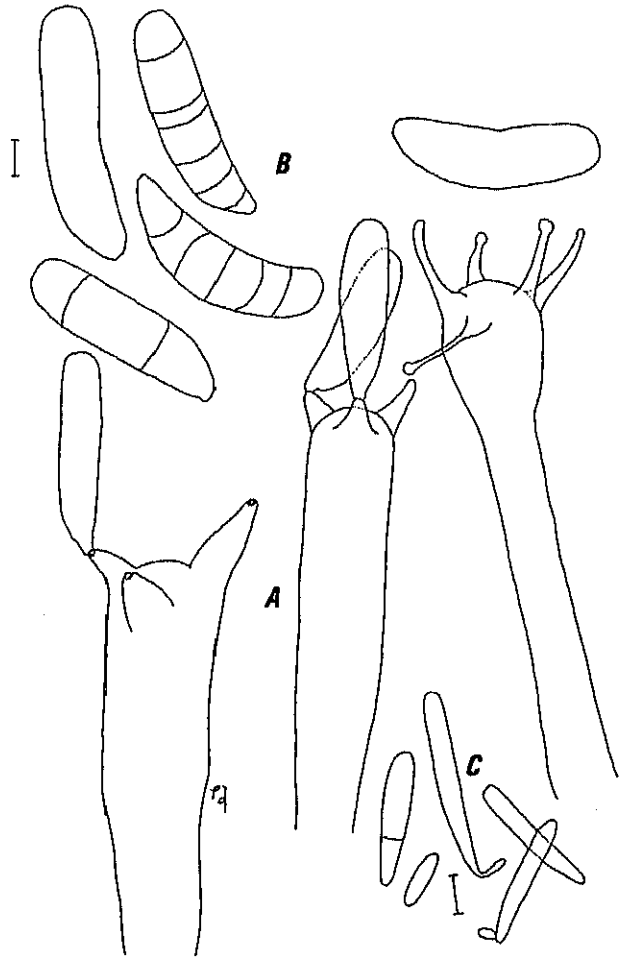


Fig. 1. Basidia and basidiospores of *Exobasidium dubium* formed on the infected leaf on *Rhododendron yedoense*. Basidia (**A**) and basidiospores (**B**) collected in Hokkaido Prefecture (TSH-B 0076). Conidia (**C**) produced on potato dextroseagar (PDA) in 21-day incubations at 22°C. *Bars* **A**, **B** 3 μm; **C** 2 μm

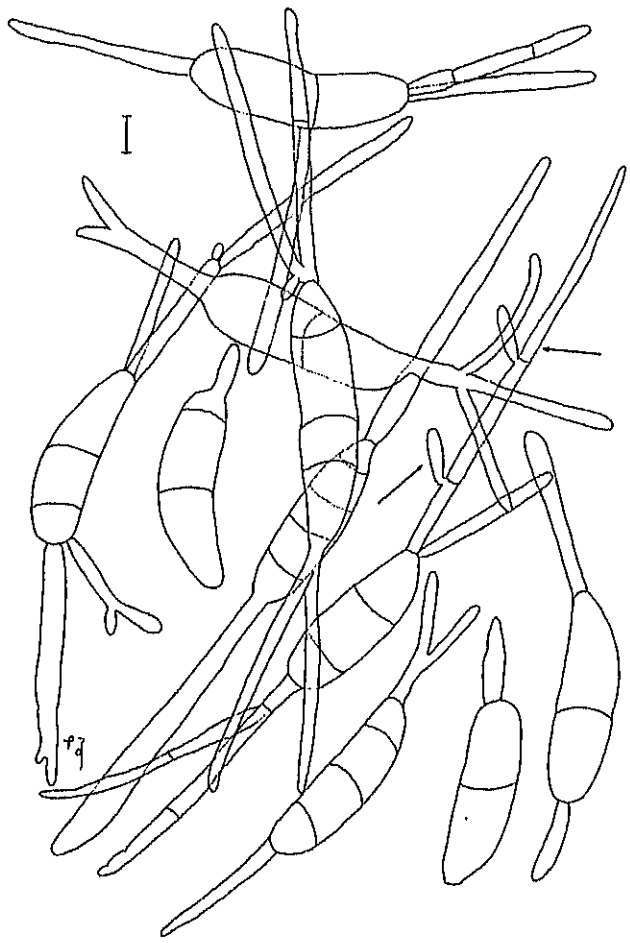


Fig. 2. Germination of the basidiospore of *E. dubium* on PDA after 8-h incubation. Some of the basidiospores produced conidia on the germ tube (*arrows*). *Bar* 3 μm

Sawada (1950) identified the causal agent of the *Exobasidium* leaf blister of *R. yedoense* with *E. magnusii*. As his note simply described the sizes of basidiospores and did not show an illustration, his description was not enough to identify his material with *E. magnusii*. Unfortunately, we could not obtain Sawada's specimens in Iwate University. In the original description, Woronichin (1913) described the morphology of basidia, basidiospores, and sterigmata. These numerical data matched very well with those of the Hakodate specimens. His illustrations pointed out a germinated basidiospore. After Sundström (1964), the modern criterion for germination of basidiospores in *Exobasidium* is determined as the conidial form, in which the conidia bud directly from basidiospores without a germ tube or hyphae, and the mycelial form, in which basidiospores germinate with a hyphal tube. Thus, the mode of germination of *E. magnusii* was interpreted as the mycelial form from Woronichin's illustration. This mode of germination of basidiospores also agrees with the specimens obtained from Hokkaido (Fig. 2).

Woronichin (1913) established *E. magnusii* based on the Caucasian specimens. He commented that he noticed a very similar *Exobasidium* spp. mentioned in Bubák (1909) and Raciborski (1909a) and wondered if these fungi were the same. Although he borrowed and checked Raciborski's specimens, he could not find basidiospores and basidia (Woronichin 1913). However, Raciborski (1909b) described *E. dubium* with a type mentioned in a previous paper (Raciborski 1909a). Nannfeldt (1981) proposed to synonymize *E. magnusii* into *E. dubium*. *Exobasidium dubium* has basidia 6–11 μm wide with (2) 4 (5) sterigmata, basidiospores (15) 18–24 × 4–5 μm with 0–1 (–3) septation, and conidia 5–9 (12) × 0.7–1.5 μm, and forms small spots (0.5 mm diameter) on *R. luteum* Sweet (= *A. pontica* L. = *R. flonum* G. Don).

Among 100 taxa of *Exobasidium* hitherto validly described, 3 taxa show similarities in some morphological measurements. *Exobasidium dubium* is distinguishable from *E. burtii* Zeller by the number of its sterigmata and by having a vertical septum in basidiospores, from *E. asebi*

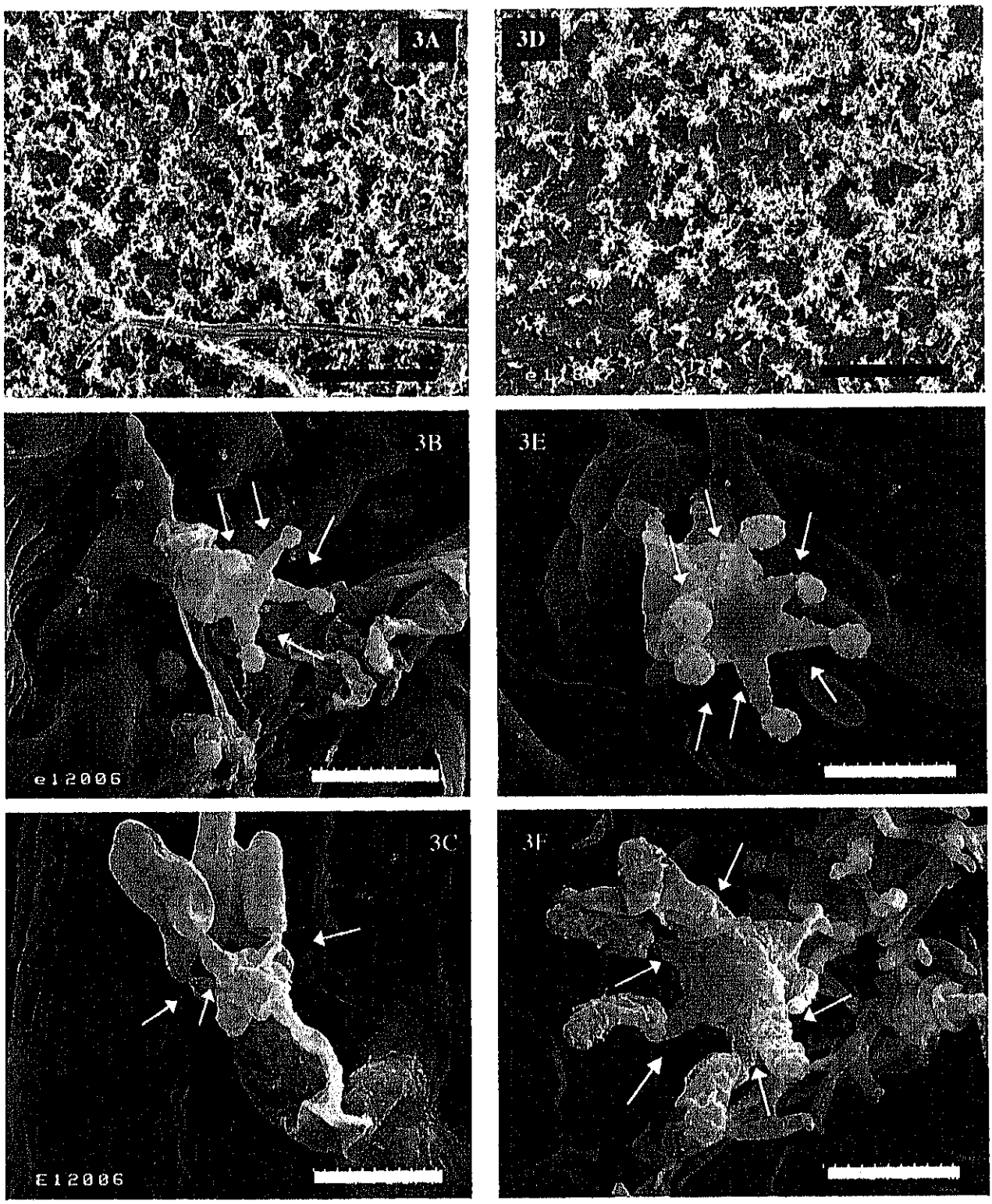


Fig. 3. Hymenium and basidia of *E. dubium* and *E. miyabei* observed by scanning election microscopy (SEM). **A** Hymenium of *E. dubium* on *R. yedoense*. **B, C** Basidium with immature basidiospores. *Arrows* indicate sterigmata. **D** Hymenium of *E. miyabei* on *R. dauricum*. **E, F** Basidium with immature basidiospores. *Arrows* indicate sterigmata. *Bars* **A, D** 150 μm; **B** 10 μm; **C** 7.5 μm; **E** 6.7 μm

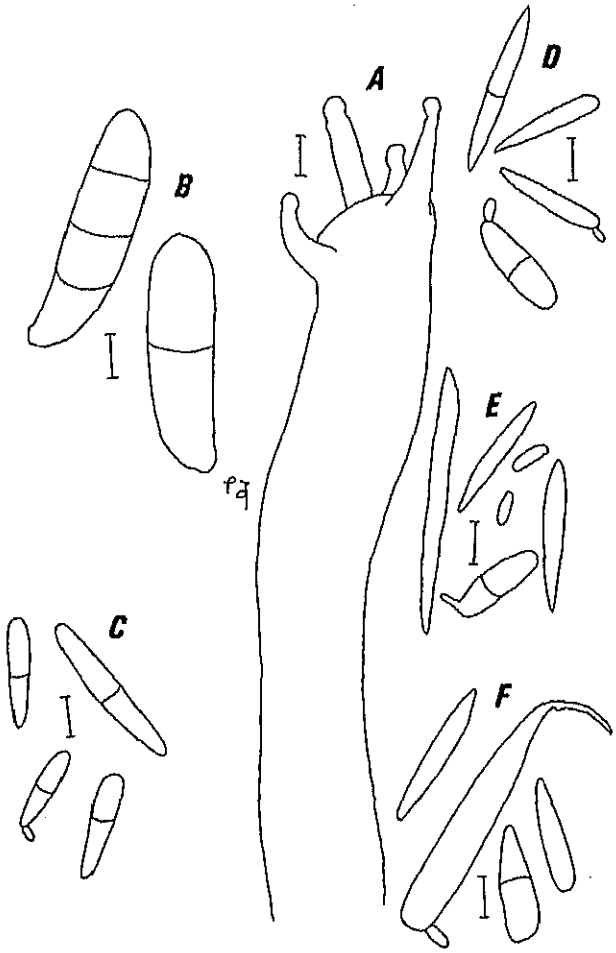


Fig. 4. Basidia and basidiospores of *E. miyabei* formed on the infected leaf of *R. dauricum*. Basidia (**A**) and basidiospores (**B**) collected in Hokkaido Prefecture (TSH-B 0073). Conidia produced on PDA in 21-day incubations at 22°C in nagaoh-13116 (**C**), nagaoh-13130 (**D**), nagaoh-99796 (**E**), or nagaoh-13118 (**F**). *Bars* **A**, **C–F** 2μm; **B** 3μm

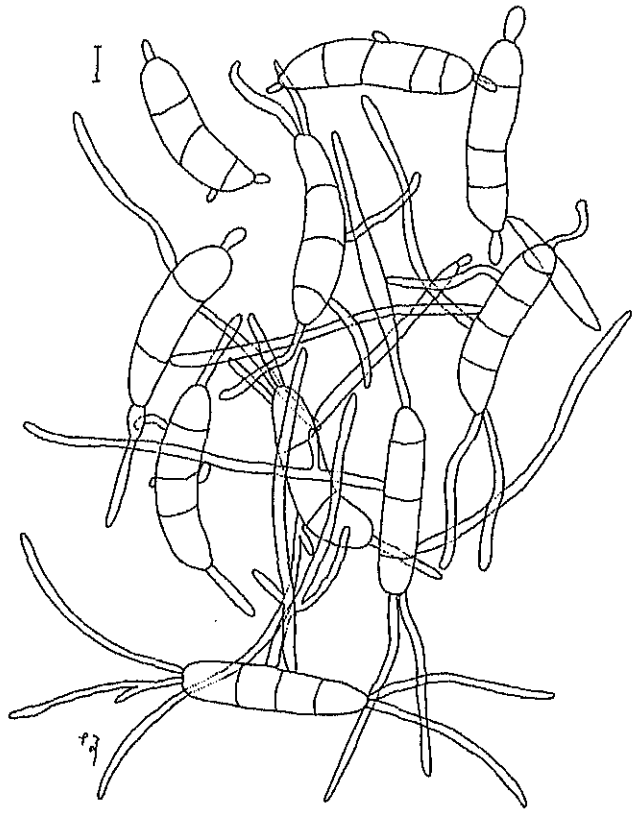


Fig. 5. Germination of the basidiospore of *E. miyabei* on PDA after 8-h incubation. *Bar* 3μm

Hara et Ezuka by the size of its basidia and number of sterigmata, and from *E. fraserii* McNabb by the size of its basidia, size and number of sterigmata, and number of septa of basidiospores. Therefore, *E. dubium* is a unique species that is completely identical in the morphological characters. The mode of germination of the Japanese specimens was the same mycelial form as that of *E. dubium*. Hokkaido was thus recorded as a new locality for the distribution of this fungus.

Leaf lesions are a chlorotic spot circumscribed with a dark-colored rind (Fig. 7A), whereas the chlorotic spot sometimes lacks the dark rind when infected trees grow in the shade (Fig. 7B). Irrespective of the dark rind, the diameter of lesions ranges from 3 to 12mm. Lesions are sometimes confluent to develop larger and ambiguous ones, 5–24 × 3–10mm, when several infections occur coincidentally in timing and location. A hymenium is formed on the underside of leaves (Fig. 7C). Chlorotic spots are uniquely observed, as reported by Woronichin (1913) and Nannfeldt (1981).

Exobasidium miyabei Nagao, Akimoto et Kishi, sp. nov.
Figs. 4,5

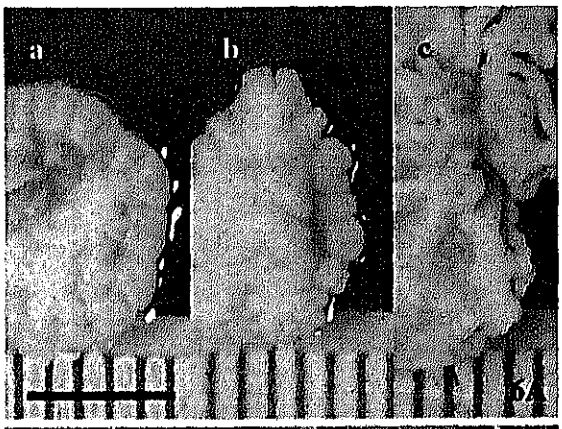
Maculae in foliis rotundae, 3–9mm diameter, planae et haud incrassatae, supra flavae vel viridiflavae et infra albofarinosae. Hymenium hypophyllum, determinatum infra maculas. Basidia hyalina, clavato-cylindracea, 25–41 × 7–9μm, ad apicem obtusata vel deplanata, terminaliter cum 3–5 sterigmatibus longiconicis 3–5 × 1–2μm praedita. Basidiosporae hyalinae, laeves, cylindricae vel falcatae, apice rotundatae, ad basim curvatae et angustatae, 14–23 × 4–5μm, primo continuae dein 1–6-septatae, per hyphas germinantes. Conidia hyalina, continua, laevia, linearia, 3–12 × 1–1.5μm. Coloniae in PDA restricte crescentes, ad ambitum irregulariter rugosae, ex hyphis circa 1μm latis et conidiis constantes, cremeae vel pallide aurantiacae, in agaro non pigmentiferae; reversum coloniis concolor.

Holotypus in foliis *Rhododendri dauricí* Makino, Kikyo, Hakodate, Hokkaido Prefecture in Japonia, 14 VI 2001, H. Nagao et M. Akimoto leg., in Herbario Instituti Agriculturae et Silviculturae, University of Tsukuba, Tsukuba, Japonia conservatus (TSH-B 0075).

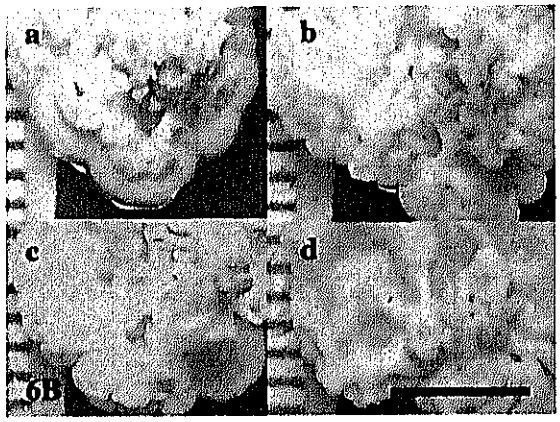
Etymology: Referring to a Japanese mycologist, Prof. K. Miyabe, who collected materials of this species for the first time.

Specimens examined: TSH-B0017 (Nagaoh 99796, Miyamae, Kawasaki, Kanagawa Prefecture, June 8, 1999); TSH-B0073, TSH-B0075 (Nagaoh 13116, 13118, Donan

6

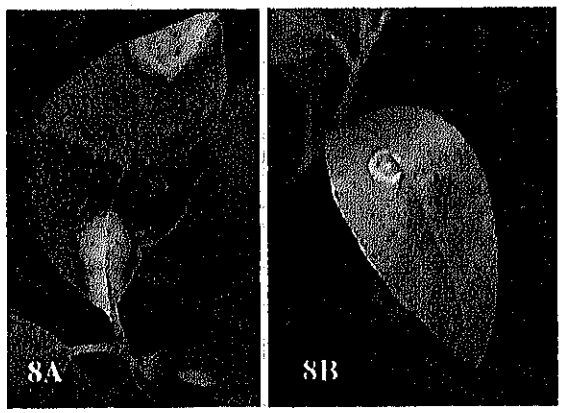


6A



6B

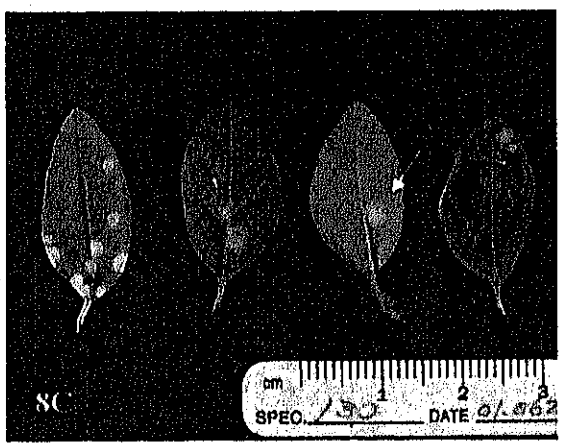
7A



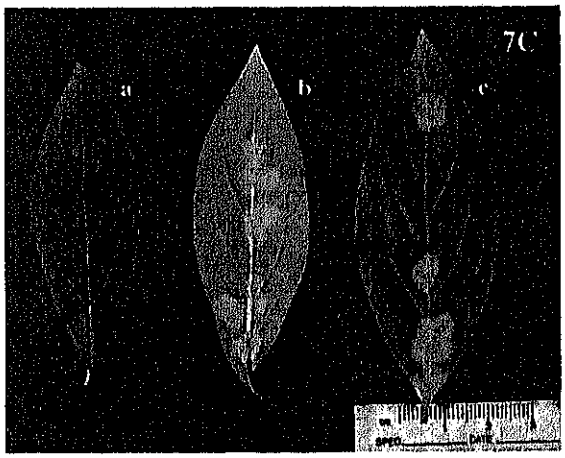
8A

8B

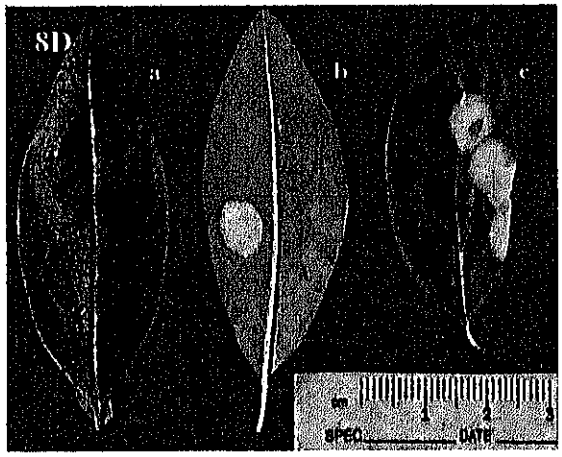
7B



8C



7C



8D

Branch, Hokkaido Forestry Research Institute, Hakodate, Hokkaido Prefecture, June 14, 2001); TSH-B0087 (Nagaoh 13130, on the roadside, Teshikaga, Hokkaido Prefecture, June 21, 2001).

The hymenium was composed of basidia with 3–5 sterigmata and conidia (Fig. 3C). Hyphae did not develop directly on the surface of the epidermis. Basidia were clavate to cylindrical, 25–41 × 7–9 μm (Fig. 4A). The apex of basidia was blunt or flattened (Fig. 3E,F). Basidia emerged directly from the host surface or through stomata. Basidia were not fasciculate. Sterigmata were 1–2 μm in diameter at the base and 3–5 μm in height, emerging outwardly and tapering toward the tip (Fig. 3D). Basidiospores were ellipsoid to ovoid, 14–23 × 4–5 μm, hyaline, smooth, one-celled when formed, becoming septate with 1–6 septa (Fig. 4B). Septate basidiospores germinated after 6 h when dropped on the agar surface. Germ tubes of the basidiospores emerged from each cell and produced conidia at the tip 12 h after dropping (Fig. 5). Hyphae grew into pseudohyphae and branched. Conidia were bacilliform, lacrimiform, subfusiform, or clavulate, 3–12 × 1–1.5 μm, and budded polarly (Fig. 4C–F) to produce daughter cells polarly and also developed a germ tube or hyphae. Colonies on PDA grew gradually, to a maximum 15 mm diameter in 21-day incubations, and were wrinkled irregularly around the periphery. The surface of the colonies was pale yellow to pale orange and corrugate. Colonies were thin and fixed on the agar surface. Formed conidia did not produce a powdery appearance. Colonies were composed of branching, intricate hyphae and pseudohyphae, and conidia. The reverse of colonies was also pale yellow to pale orange. Dark pigment was not produced on PDA (Fig. 6B). Colonies from conidia showed the same morphological features as those from basidiospores.

The first report of an Exobasidium leaf blister on *R. dauricum* was in 1999 by Akimoto, whereas earlier samplings of an Exobasidium leaf blister on *R. dauricum* were in 1909 and 1919; these were deposited in the herbarium of University of Hokkaido (SAPA). The first recognition of this disease was at Sapporo, Hokkaido Prefecture, in August 1909 by Prof. K. Miyabe. The second collection was made at Sapporo, Hokkaido Prefecture on August 1, 1919, by Prof. S. Ito. These two specimens showed circular lesioms on the leaves of 5–8 mm and 3–6 mm in diameter, respectively. Two *Exobasidium* species, *E. caucasicum* and *E. rhododendri*, were recorded as infecting leaves and branches of *R. dauricum* in Russia (Murashkinsky and Sieling 1928; cited in Farr et al. 1996). Woronichin (1920) described *E. caucasicum* on *R. caucasicum* Pall. and indicated that its hymenia were formed all over the lower leaves. *Exobasidium caucasicum* showed systemic infection (Nannfeldt 1981). The symptom caused by these two species is clearly different from this Exobasidium leaf blister on *R. dauricum* in Japan. However, these Japanese specimens were just treated as *Exobasidium* sp. and did not appear in the later literature (Ito 1955). We examined these materials, and sufficient basidia and basidiospores to identify the taxon were not obtained because of the secondary infection on these lesions or because it was not the correct season for the collection of these materials. It is supposed that taxonomic investigations have not treated the causal fungus of this Exobasidium leaf blister.

The taxonomy of *Exobasidium* has been debated because of the simple morphology of taxonomic characters and the highly variable symptoms and wide host range (Burt 1915; Ezuka 1991b; Nannfeldt 1981; McNabb 1962; Savile 1959; Sundström 1964). We compared the morphology of basidia, basidiospores, and sterigmata and the mode of germination of basidiospores. One hundred taxa of *Exobasidium* have been validly described to date. Among these, 19 taxa show similarities in some morphological measurements (Table 1). The new species is distinguishable from *E. dracophylli* McNabb and *E. vaccinii-uliginosi* Boud. apud Boud. et E. Flesch. by width of basidia, length and number of sterigmata, and width and numbers of septa of basidiospores. It is also distinguishable from *E. otanianum* Ezuka var. *otanianum* and *E. yoshinagae* by length of basidia, size of sterigmata, and number of septa of basidiospores; from *E. cylindricum* Ezuka by size of basidia, length of sterigmata, and number of septa of basidiospores; from *E. bisporum* Sawada ex Ezuka in width of basidia and basidiospores, number of sterigmata, and mode of germination of basidiospores (conidial form); from *E. pieridis* Henn. by length of basidia, size and number of sterigmata, number of septa of basidiospores, and mode of germination of basidiospores (conidial form); from *E. burtii* Zeller and *E. uvae-ursi* (Maire) Juel by number of sterigmata and septa of basidiospores; from *E. aequale* Sacc. by number of sterigmata, width of basidiospores, and number of septa of basidiospores; from *E. dimorphosporum* Savile and *E. empetri* S. Ito et Y. Otani by size of basidiospores and number of septa of basidiospores; from *E. asebi* by size of basidia and number of septa of basidiospores; and from 6 other taxa, *E. canadense* Savile, *E. caucasicum*, *E.*

Fig. 6. Morphology and coloration of colonies formed by *E. dubium* and *E. miyabei* on PDA. **A** Surface of colonies of *E. dubium* MAFF 238581 (nagaoh-13119) collected in Hakodate (*a*), *E. dubium* MAFF 238582 (nagaoh-13120) collected in Hakodate (*b*), and *E. yoshinagae* MAFF 238607 (nagaoh-99807) (*c*). **B** Surface of colonies of *E. miyabei* MAFF 238583 (nagaoh-99796) collected in Kawasaki (*a*), *E. miyabei* MAFF 238593 (nagaoh-13116) collected in Hakodate (*b*), *E. miyabei* MAFF 238594 (nagaoh-13118) collected in Hakodate (*c*), and *E. miyabei* MAFF 238595 (nagaoh-13130) collected in Teshikaga (*d*). Submerged hyphae in colonies of *E. dubium* and *E. miyabei* were not pigmented

Fig. 7. Symptoms of Exobasidium leaf blister on *R. yedoense* by *E. dubium*. **A** Chlorotic spots observed on June 2001 in Hokkaido Prefecture. **B** Chlorotic spots without black rinds. **C** *a*, Healthy leaf; *b*, white hymenia occurred on the lower surface of leaf; *c*, symptom on the upper surface

Fig. 8. Symptoms on Exobasidium leaf blister on *R. dauricum* by *E. miyabei*. **A** Chlorotic spots observed on June 2001 in Hokkaido Prefecture. **B** Red pigmentation on the chlorotic spot. **C** Small chlorotic spots collected in Teshikaga. White hymenia occurred on the lower surface of leaf (*arrow*). **D** *a*, Healthy leaf; *b*, white hymenia occurred on the lower surface of leaf; *c*, symptom on the upper surface

Table 1. Morphological measurements of *Exobasidium* spp.

Species	Size of basidia (μm)	Size of sterigmata (μm)	Number of sterigmata	Size of basidiospores (μm)	Number of septa basidiospores	References
miyabei	25–41 × 7–9	3–5 × 1–2	3–5	14–23 × 4–5	1–6	In this article
aequale	6–8 wide	4–6 long	2	(14) 15–22 × (6) 7–9 (10)	0	Symb. Bot. Ups. 28 (1981):1–72
asebi	60–80 × 4–7	4–6 × 1.5–2.5	3–4	16–23 × 3–5.5	1–3	Nougyo Oyobi Engei 34 (1959):1353–1354
bisporum	40 × 6–7	4–6 long	2	15–24 × 5–7	0–7	Tohoku Biol. Res. 1 (1950):97–98
burtii	36–50 × 8–10	5–6.5 × 1.5–1.7	4 (5)	(14) 16–24 × 3.2–5.5	0–1 (3)	Mycologia 26 (1934):291–304
canadense	24–40 × 5.5–8	2.5–4.5 × 1.5–2	2–4 (5)	14–20 × 3–4.7	1–3	Can. J. Bot. 37 (1959):641–656
caucasicum	7–9 wide	nd	(2) 4	13–18 × 5–5.5	1	Symb. Bot. Ups. 28 (1981):1–72
cylindricum	50–60 × 5–7	5–6 × 2	(4) 5 (6)	12–22 × 2.8–4.4	(1) 3	Trans. Mycol. Soc. Jap. 31 (1990):439–455
decolorans	18–35 × 6.5–8.5 (10)	3.5–5 (8) × 1.8–2.5	(2) 3–5 (6)	14.5–22 × 4.2–6.5	1–3	Can. J. Bot. 37 (1959):641–656
dimorphosporum	26–40 × 5–9.5	5–7 × 1.5–2.5	2–3	13–18.5 × 6.5–8.5; 18.5–28.5 (32) × 5–7 (8)	0 (1)	Can. J. Bot. 37 (1959):641–656
dracophylli	25–45 × 5–6.5	6–8.5 long	2 (3)	20–27 × 5–8	0 (1)	Trans. N.Z. Bot.1 (1962):259–268
dubium	6–11 wide	nd	(2) 4 (5)	(15) 18–24 × 4–5	0–1 (3)	Symb. Bot. Ups. 28 (1981):1–72
	20–30 × 6.5–10	3–7 × 1.5–2	3–5	15–25 × 3.8–5	1–4 (6)	In this article
empetri	20–28 × 8.5	nd	2–4	8.5–14 × 5.5–9.5	0–1	Trans. Mycol. Soc. Jpn. 1 (1958):3
nobeyamense	35–40 × 7–10	3.5–5.5 × 2–2.5	3–6	12–21 × 2–5.5	2–4	Mycoscience 42 (2001):549–554
otanianum var. otanianum	50–70 × 6–8	4.5–5.5 × 2	(2) 4–5 (8)	13–21 (23) × 3.5–6	0–1 (3)	Trans. Mycol. Soc. Jpn. 32 (1991):71–86
pierids	50–60 × 7–9	5–9 × 2.5–3	2–3 (4)	15–22 × 4–5.5	1–3	Trans. Mycol. Soc. Jap. 32 (1991):71–86
unedonis	35–45 × 7	nd	4	13–21 × 4–6	1	Symb. Bot. Ups. 28 (1981):1–72
uvae-ursi	nd	nd	2–3 (4)	15–22 × 5–6	1–3	Symb. Bot. Ups. 28 (1981):1–72
vaccinii-uliginosi	9–10 wide	7 long	2	16–23 (28) × 6.5–9 (12)	0	Symb. Bot. Ups. 28 (1981):1–72
yoshinagae	50–70 × 7.5–10 (12)	5–6 × 2–2.5	(3) 4–5 (6)	13–23 × 3.5–6	0–1 (3)	Trans. Mycol. Soc. Jpn. 32 (1991):71–86

nd, Datum was not indicated in its original description

decolorans Harkn. *E. dubium, E. nobeyamense* Nagao et Ezuka, and *E. unedonis* Maire, in numbers of septa of basidiospores. In addition, *E. caucasicum* germinates by conidia (conidial form), whereas the new species germinates by hypha (mycelial form). There are several morphological differences in *E. dubium* and *E. yoshinagae*, which cause Exobasidium leaf blisters on *Rhododendron* spp. in Japan. This new species is distinguished from *E. dubium* by number of septa of basidiospores and from *E. yoshinagae* by length of basidia, shape of the apex of basidia (see Fig. 3B) (Ezuka 1991a), size of sterigmata, and number of septa of basidiospores. The new species on *R. dauricum* morphologically differs from the known species in size of basidia and basidiospores, number of sterigmata and septa of basidiospores, and mode of germination of basidiospores.

Compared with the conidia of *E. yoshinagae* MAFF 238607 (nagaoh-99807), isolated from an Exobasidium leaf blister of *R. wadanum* Makino, 2–7 × 1 μm, those of *E. dubium* MAFF 238581 (nagaoh-13119), isolated from an Exobasidium leaf blister of *R. dauricum*, were 6–14 × 1–2 μm and those of *E. miyabei* 3–12 × 1–1.5 μm. The conidial size of *E. miyabei* overlaps that of the former two species. The shapes of the conidia of these three species are bacilliform, lacrimiform, subfusiform, and clavulate. From the anamorphic examination, there is not any common morphological character to distinguish among these three leaf blister species. Colonies of *E. miyabei* on PDA do not show dark pigmentation (Fig. 6B).

The typical symptom of an *Exobasidium* leaf blister on *R. dauricum* is a chlorotic spot on the upper side of the leaves (Fig. 8A,C), corresponding to the hymenium that appears on the lower side (Fig. 8C,D). At the beginning of the infection, a minute yellow spot occurs on the leaves. This spot then gradually develops up to 3–9 mm in diameter. One of the symptoms is a red pigmentation on the upper side of leaves (Fig. 8B); these lesions actually match with hymenial parts. On the specimens collected in Misato, Teshikaga, Hokkaido Prefectue, chlorotic spots were limited as lesions at the beginning, but hymenial areas developed up to 2 mm in diameter (Fig. 8C). This observation suggests that hymenial development does not necessarily correspond with symptom development. As the occurrence of disease and the day of our samplings in Hakodate and Teshikaga, Hokkaido Prefecture, were within 8 days, environmental conditions, i.e., sunshine, temperature, and humidity, may have affected symptom development. The host range of *E. dubium* and *E. miyabei* will be studied with inoculation tests to determine their pathogenicity.

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References

Akimoto M (1999) Tsutsuji: Syakunage rui-no byoki-no miwakekata to boujo (in Japanese). Kojunai-kiho 115:1–6

Bubák F (1909) Fungi. In: Handel-Mazzetti HV (ed) Botanische Reise in das Pontische Randgebrge. Ann Naturhist Hofmus Wien 23:101–108

Burt EA (1915) The Thelephoraceae of North America. IV. *Exobasidium*. Ann Mo Bot Gard 2:627–659

Ezuka A (1991a) Notes on some species of *Exobasidium* in Japan (III). Trans Mycol Soc Jpn 32:71–86

Ezuka A (1991b) Notes on some species of *Exobasidium* in Japan (IV). Trans Mycol Soc Jpn 32:169–185

Ezuka A (1992) *Exobasidium* in Japan (3) (in Japanese). For Pests 41:41–48

Farr D, Esteban HB, Palm ME (1996) Fungi on *Rhododendron*: a world reference. Parkway, Boone, p 193

Graafland W (1960) The parasitism of *Exobasidium japonicum* Shir. on Azalea. Acta Bot Neerl 9:347–379

Ito S (1955) Basidiomycetes no. 4. In: Mycological flora of Japan, vol II. Yokendo, Tokyo, pp 46–55

McNabb RFR (1962) The genus *Exobasidium* in New Zealand. Trans R Soc NZ 1:259–268

Nagao H, Kishi K, Ezuka A, Kakishima M (2000) The occurrence of *Exobasidium* leaf blister on *Rhododendron dauricum* L. (abstract in Japanese). Jpn J Phytopathol 66:307

Nagao H, Ezuka A, Ohkubo H, Kakishima M (2001) A new species of *Exobasidium* causing witches' broom on *Rhododendron wadanum*. Mycoscience 42:549–554

Nannfeldt JA (1981) *Exobasidium*, a taxonomic reassessment applied to the European species. Symb Bot Ups 23:1–72

Raciborski M (1909a) *Azalea pontica* in Sandomierer Wald und ihre Parasiten. Bull Int Acad Sci Cracovie Cl Sci Math Nat B 3:385–391

Raciborski M (1909b) Mycotheca polonica. Kosmos 34:1166–1172

Sawada K (1950) Fungi from the North-Eastern region of Honshu, Japan (2): Basidiomycetes. Tohoku Biol Res 1:95–100

Savile DBO (1959) Notes on *Exobasidium*. Can J Bot 37:641–656

Sundström KR (1964) Physiological and morphological studies of some species belonging to the genus *Exobasidium*. Phytopathol Z 40:213–217

Woronichin NN (1913) Mycoflorae Caucasicae novitates (in Russian). Monit Jard Bot Tiflis 28:3–12

Woronichin NN 1921 (1920) Notes Mycologiques 2–4 (in Russian). Monit Jard Bot Tiflis 51:1–6

Yamazaki T (1989) Ericaceae. In: Satake Y, Hara H, Watari S, Tominari T (eds) Wild flowers of Japan. Woody plants, vol II. Heibonsha, Tokyo, pp 122–156

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SHORT COMMUNICATION

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Correlation between pathogenicity and molecular characteristics in the willow leaf rusts *Melampsora epitea* and *M. humilis* in Japan

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Abstract We studied the correlation between pathogenicity and restriction fragment length polymorphism (RFLP) type, which was determined by polymerase chain reaction-based RFLP analysis of the internal transcribed spacer regions of ribosomal DNA, in the willow leaf rust fungi *Melampsora epitea* and *M. humilis*. Eighteen clones of eight *Salix* species were inoculated with urediniospores from seven collections of the two rust species. *M. epitea* and *M. humilis* (RFLP type-5 collections) were pathogenic to six to eight *Salix* species. RFLP type-7 collections of *M. epitea* were pathogenic to only two *Salix* species. The taxonomic relationships of the two rust species are discussed.

Key words Inoculation · *Melampsora epitea* · *Melampsora humilis* · Pathogenicity · Willow leaf rust

Willows, which belong to the Salicaceae, are a valuable source of biomass because of their fast growth and easy propagation (Newsholme 1992). Willow leaf rust diseases, caused by *Melampsora* species, often occur in willow plantations and occasionally result in serious damage owing to premature defoliation. *Melampsora epitea* (Kunze et Schmidt) Thümen is one of the most destructive rust species to cultivated willow and is distributed in Europe, North America, New Zealand, and central and eastern Asia (Wilson and Henderson 1966; Ziller 1974; Hiratsuka et al. 1992; Spiers and Hopcraft 1996). This fungus has a heteroecious life cycle and produces uredinia and telia on a large number of *Salix* species and spermogonia and aecia on various woody and herbaceous plants.

European *M. epitea* were separated into six races based on the aecial hosts (Wilson and Henderson 1966). In addition, one of the six races, *M. larici-epitea* Klebahn., whose alternative hosts are larches, was separated into seven formae speciales based on its uredinial and telial hosts (Gäumann 1959). Consequently, *M. epitea* has been treated as a species complex in Europe and North America (Gäumann 1959; Wilson and Henderson 1966; Ziller 1974).

In *M. epitea* in Japan, the spermogonial and aecial hosts are species of *Larix*, and the uredinial and telial hosts are seven species of *Salix* (Hiratsuka et al. 1992; Kondo et al. 1994). However, races or formae speciales within the species were unrecognized because of few inoculation experiments with basidiospores and urediniospores. Recently, Nakamura et al. (1998) separated *M. epitea* collected from three *Salix* species into three groups based on their restriction fragment length polymorphism (RFLP) types (types 5, 6, and 7), which were determined by polymerase chain reaction (PCR)-based RFLP analysis of the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA). They suggested a positive correlation between the RFLP type of *M. epitea* and its uredinial and telial hosts because each of the three groups of *M. epitea* was collected from the different uredinial host plants. They also found that the RFLP type 5 of *M. epitea* was identical to that of *M. humilis* Dietel in RFLP band patterns. *M. humilis* is distributed in a limited area of eastern Asia, including Japan (Hiratsuka and Kaneko 1982). The pathogenicity of the uredinial state of *M. humilis* to the host plants of *M. epitea* was not reported precisely.

We investigated the pathogenicity of the uredinial state of RFLP types 5 and 7 of *M. epitea*, as well as that of *M. humilis* (RFLP type 5), to clarify the correlation between pathogenicity and the RFLP types, and discussed the taxonomic considerations on these species.

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Table 1. Collections and isolates of *Melampsora epitea* and *M. humilis*

Species and collection no.	Host	Locality	RFLP type[a]	Isolates used in inoculation experiments[b]
M. epitea				
ETf-1	*Salix futura*	Niigata	Type 7	ETf-1-m
ETg-1	*S. gilgiana*	Nagano	Type 5	ETg-1-S, ETg-1-S2, ETg-1-S3
ETg-3	*S. gilgiana*	Fukushima	Type 5	ETg-3-S, ETg-3-S2, ETg-3-S3
ETj-1	*S. japonica*	Yamanashi	Type 7	ETj-1-S, ETj-1-S2, ETj-1-S3
M. humilis				
HUi-2	*S. integra*	Nagano	Type 5	HUi-2-S, HUi-2-S2, HUi-2-S3
HUi-3	*S. integra*	Iwate	Type 5	HUi-3-S, HUi-3-S2, HUi-3-S3
RS1	*S. integra*	Gunma	Type 5	HU1[c]

RFLP, restriction fragment length polymorphism
[a] Determined according to Nakamura et al. (1998)
[b] Isolate ETf-1-m is a mass-uredinial isolate; the other isolates are single-uredinial isolates
[c] Provided by R. Suzuki, University of Tsukuba

The uredinial and telial states of four rust materials (collections ETg-1, ETg-3, ETj-1, and ETf-1) were collected along with host-plant leaves from three *Salix* species (*S. futura* Seemen, *S. gilgiana* Seemen, and *S. japonica* Thunb.), and of two rust materials (collections HUi-2 and HUi-3) from *S. integra* Thunb., from June 1995 to November 1996 in Japan (Table 1). The four "ET" collections were identified as *M. epitea*, and the two "HU" collections as *M. humilis*, on the basis of the morphology of the uredinial and telial states, according to the criteria of Hiratsuka and Kaneko (1982). Voucher specimens of our collections were deposited in the Mycological Herbarium, Institute of Agriculture and Forestry, University of Tsukuba (Nakamura et al. 1998).

Isolates that were derived from several uredinia, which we call a mass-uredinial isolate, from each collection were maintained separately on detached host leaves on 1% (w/v) agar plates containing 40 µg/ml benzimidazole in Petri dishes (9 cm diameter) at 23°C under fluorescent lighting (16 h light/day). Inoculations with the isolate to new leaves were repeated at intervals of 2–3 weeks. Three single-uredinial isolates were also obtained from each mass-uredinial isolate from three collections of *M. epitea* (ETg-1, ETg-3, and ETj-1) and from two collections of *M. humilis* (HUi-2 and HUi-3) (Table 1). These isolates were derived from a single uredinium produced after inoculation with a mass-uredinial isolate to a new host plant leaf and were maintained described as above. A single-uredinial isolate of another *M. humilis* collection (RS1) was also used. RFLP types in the collections were determined as in our previous report (Nakamura et al. 1998) (see Table 1).

Eighteen clones from eight *Salix* species, which were reported to be uredinial host plants for *M. epitea* or *M. humilis*, were obtained from various sites in Japan and maintained in growth chambers (Table 2). Two *Salix* species (*S. integra* and *S. koriyanagi* Kimura) are known to be host plants for *M. humilis* and seven *Salix* species (all except *S. koriyanagi*) for *M. epitea* (Hiratsuka et al. 1992; Kondo et al. 1994). The scientific names of the *Salix* species used in this study follow the nomenclature of A. Kimura (Iizumi et al. 1980).

Table 2. *Salix* species used for inoculation experiments with urediniospores of *Melampsora epitea* and *M. humilis*

Salix species	Clone no.	Origin in Japan
S. futura[a]	SF-1	Nagano
	SF-A1	Niigata
S. gilgiana[a]	HB-101	BG1
	HB-500	BG1
	SGL-1	Nagano
S. gracilistyla[a]	SGR-B1	BG2
var. *pendula* f. *pendula*	HB-60	BG2
var. *adscendens* f. *latifolia*	HB-528	BG1
S. integra[a,b]	SI-1	Nagano
	SI-B1	BG2
f. *pendula*	HB-602	BG1
S. japonica[a]	HB-606	BG1
	SJ-1	Chiba
S. koriyanagi[b]	1H	BG1
	HB508	BG1
S. miyabeana[a]	SM-S1	Hokkaido
S. reinii[a]	SR-1	Nagano
	SR-2	Iwate

BG1, Botanical Garden, Tohoku University, Sendai, Miyagi; BG2, Botanical Garden, Tsukuba University, Ibaraki
[a] Uredinial hosts of *M. epitea* reported by Hiratsuka et al. (1992) and Kondo et al. (1994)
[b] Uredinial hosts of *M. humilis* reported by Hiratsuka et al. (1992)

Inoculation experiments were conducted with detached leaves from these clones, as follows. Fully expanded young leaves detached from potted plants were placed topside downward onto a 1% agar plate containing 40 µg/ml benzimidazole in a glass Petri dish. Urediniospores were put onto small pieces (3 × 3 mm) of filter paper wet with sterilized water, and the pieces were placed upside down on the lower surface of the detached leaves. The Petri dishes containing the inoculants and leaves were kept in a dark moist chamber at 20°C for 2 days and then were transferred to a growth chamber at 23°C under fluorescent lights (16 h/day). The inoculated leaves were kept under these conditions for a month to observe uredinia produced on them.

Inoculation experiments with urediniospores from each isolate were done one to four times, mostly twice, per plant clone. The 18 *Salix* clones were inoculated with

Table 3. Results of inoculation experiments with urediniospores in *Melampsora epitea* and *M. humilis* collections

Plants inoculated		Collection no. (RFLP type[a]) and isolate no.[b]																	
Salix species	Clone no.	*M. epitea*										*M. humilis*							
		ETg-1 (type 5)			ETg-3 (type 5)			ETf-1 (type 7)	ETj-1 (type 7)			HUi-2 (type 5)			HUi-3 (type 5)			RS1 (type 5)	
		ETg-1-S	ETg-1-S2	ETg-1-S3	ETg-3-S	ETg-3-S2	ETg-3-S3	ETf-1-m	ETj-1-S	ETj-1-S2	ETj-1-S3	HUi-2-S	HUi-2-S2	HUi-2-S3	HUi-3-S	HUi-3-S2	HUi-3-S3	HU1	
S. futura	SF-1	−	nt	−[c]	+	+	+	+	−	+	−	−	(+)	−	−	−	−*[d]	−	
	SF-A1	−	−	−	−	−	−	+	−	−	−	−	−	−	nt	−	−	−	
S. gilgiana	HB-101	+	−	+	+	+	+	−	−	−	−	−	−	+	−	−	−*	+	
	HB-500	+	−	−	+	+	+	−	−	−	−	−	+	+	−	−	−*	+	
	SGL-1	+	+	+	+	+	+	−	−	−	−	+	+	+	−	−	−	+	
S. gracilistyla	SGR-B1	−	−	−	−	−	−	nt	−	−	−	−	+	−	−	−	−	−	
	HB-60	−	−	−	+	−	−	nt	−	−	−	−	+	−	nt	−	−	+	
	HB-528	−	nt	−	+	+	−	−	−	−	−	+	+	+	−	nt	−	+	
S. integra	SI-1	+	+	+	+	+	+	nt	−	−	nt	+	+	+	−	+	−	+	
	SI-B1	+	+	+	+	+	+	−	−	−	−	−	−	−*	+	+	+	+	
	HB-602	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+	
S. japonica	HB-606	−	+	−	−	−	+	+	+	+	+	−	+	+	−	−	−*	+	
	SJ-1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	
S. koriyanagi	1H	−	−	+	+	+	+	−	−	−	−	+	−	+	−	−	−*	+	
	HB508	−	+	−	−	+	+	nt	−	−	−	+	+	+	−	−	−*	−	
S. miyabeana	SM-S1	−	+	−	−	−	−*	nt	−	−	nt	−	−	−	−	−	−	−	
S. reinii	SR-1	+	+	+	+	+	+	−	−	−	−	+	+	+	nt	−	−	−	
	SR-2	−	nt	−	+	+	+	nt	−	nt	−	+	−	+	−	−	−*	+	

nt, not tested
[a] Determined according to Nakamura et al. (1998)
[b] Isolate ETf-1-m is a mass-uredinial isolate; the other isolates are single-uredinial isolates
[c] −, No uredinia appeared; +, uredinia appeared; (+), immature uredinia without urediniospores appeared
[d] A mass-uredinial isolate produced uredinia in preliminary experiments

urediniospores from each of 3 single-uredinial isolates from our five collections (ETg-1, ETg-3, and ETj-1 from *M. epitea*; HUi-2 and HUi-3 from *M. humilis*), a single-uredinial isolate from the RS1 collection of *M. humilis*, and a mass-uredinial isolate from the ETf-1 collection of *M. epitea*. Pathogenicity of the single-uredinial isolates in the five collections of the two rust species did not necessarily correspond within a species and a collection. Also, differences in the pathogenicity of a rust isolate to the clones of each *Salix* species were recognized in most. These differences were conspicuous in the inoculations with ETg-1 and HUi-2 to *S. gilgiana* clones and collection ETg-3 to *S. gracilistyla* Miquel clones (Table 3). The results of inoculations can be summarized as follows (Table 3). Two type-5 collections of *M. epitea* (ETg-1 and ETg-3, which were collected on *S. gilgiana*) produced uredinia on six and seven *Salix* species, respectively. A mass-uredinial isolate from collection ETg-3 was pathogenic to *S. miyabeana* Seemen in preliminary experiments, although its single-uredinial isolates did not show pathogenicity. Two type-7 collections of *M. epitea* (ETj-1 from *S. japonica* and ETf-1 from *S. futura*) were each pathogenic to both these *Salix* species. Two type-5 collections of *M. humilis* (HUi-2 and RS1, from *S. integra*) produced uredinia on six *Salix* species. Single-uredinial isolates of collection HUi-3 were pathogenic to only two *Salix* species (*S. integra* and *S. japonica*), although in preliminary experiments a mass-uredinial isolate from the same collection was pathogenic on six *Salix* species, including these two species.

Table 4. Summary of results of inoculation experiments with urediniospores of *Melampsora epitea* and *M. humilis*[a]

Salix species inoculated	*M. epitea*		*M. humilis*
	RFLP type 5[b]	RFLP type 7[b]	RFLP type 5[b]
S. futura	(+)	+	(+)
S. gilgiana	+	−	+
S. gracilistyla	(+)	−	(+)
S. integra	+	−	+
S. japonica	+	+	+
S. koriyanagi	+	−	+
S. miyabeana	+	−	−
S. reinii	+	−	+

[a] +, uredinia were produced by all collections; (+), uredinia were produced by one of two collections in *M. epitea* and one or two of three collections in *M. humilis*; −, no uredinia was produced in any collection
[b] According to Nakamura et al. (1998)

To facilitate a comparison of the host ranges of *M. humilis* and the two types of *M. epitea*, the results of the inoculation experiments are summarized in Table 4. Type-5 *M. epitea* could infect all eight host *Salix* species tested. The host range of *M. humilis* (all collections were type 5) was the same except that it did not infect *S. miyabeana*. Type-7 *M. epitea* infected only two *Salix* species. These results show

a strong correlation between pathogenicity and RFLP type in these fungi.

Gäumann (1959) separated *M. larici-epitea* into seven formae speciales based on their uredinial and telial hosts. Pei et al. (1999) reported, on the basis of crossing experiments, that three formae speciales of *M. epitea* infecting larches (i.e., *M. larici-epitea*) were genetically different populations. The ITS regions of rDNA, which we used to determine the RFLP types of our fungal collections, are considered very suitable for identification and phylogenetic studies of fungal species because they show high genetic variation (Bruns et al. 1991; Hibbett 1992). Therefore, each of the two RFLP types, types 5 and 7, in *M. epitea* could be considered to be an independent species. In addition, RFLP type 5 of *M. epitea* probably has an extremely close genetic relationship to *M. humilis*. However, precise morphological comparisons among these fungal groups were not made in this study.

Hiratsuka and Kaneko (1982) provisionally treated *M. humilis* as an independent species because *M. humilis* was distinguished from *M. epitea* by only a slight difference in teliospore length. More precise morphological examinations are required for taxonomic reevaluation of *M. humilis* and the two groups of *M. epitea*. A group of *M. epitea* showing RFLP type 6 (Nakamura et al. 1998), although not used in this study, should also be reevaluated. In addition, the alternative hosts of *M. humilis* and three groups of *M. epitea* need to be confirmed. It will be especially important to examine the teliospore size produced on different host species by inoculation with a single rust collection. Comparative investigations of type specimens or specimens collected from type localities of *M. epitea sensu stricto* (Europe) and *M. humilis* (Asia) will be necessary to clarify the taxonomic situation of this group of rust.

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References

Bruns TD, White J, Taylor JW (1991) Fungal molecular systematics. Annu Rev Ecol Syst 22:525–564

Gäumann E (1959) Die Rostpilze Mitteleuropas mit besonderer Berucksichtigung der Schweiz. Beitrage zur Kryptogamenflora der Schweiz, vol 12. Buckdruckerei Buchler, Bern

Hibbett DS (1992) Ribosomal RNA and fungal systematics. Trans Mycol Soc Jpn 33:533–556

Hiratsuka N, Kaneko S (1982) A taxonomic revision of *Melampsora* on willows in Japan. Rep Tottori Mycol Inst 20:1–32

Hiratsuka N, Sato S, Katsuya K, Kakishima M, Hiratsuka Y, Kaneko S, Ono Y, Sato T, Harada Y, Hiratsuka T, Nakayama K (1992) Rust flora of Japan. Tsukuba Shuppankai, Tsukuba

Iizumi S, Toda H, Naito T, Saito K, Okazaki H (1980) Index Saliceti Sendaiensis sive enumeratio Salicacearum Japonicarum atque exoticarum in horto botanico, Tohoku Universitatis cultarum (in Latin and Japanese). Botanical Garden, Tohoku University, Sendai

Kondo S, Yamaoka Y, Kakishima M (1994) Life cycle and morphology of *Melampsora* species on *Salix integra* (abstract in Japanese). Proc 38th Annu Meet Mycol Soc Jpn 38:27

Nakamura H, Kaneko S, Yamaoka Y, Kakishima M (1998) Differentiation of *Melampsora* rust species on willows in Japan by using PCR-RFLP analysis of ITS regions of ribosomal DNA. Mycoscience 39:105–113

Newsholme C (1992) Willows: the genus *Salix*. Timber Press, Oregon

Pei MH, Royle DJ, Hunter T (1999) Hybridization in larch-alternating *Melampsora epitea* (*M. larici-epitea*). Mycol Res 103: 1440–1446

Spiers AG, Hopcraft DH (1996) Morphological and host range studies of *Melampsora* rusts attacking *Salix* species in New Zealand. Mycol Res 100:1163–1175

Wilson M, Henderson DM (1966) British rust fungi. Cambridge University Press, Cambridge

Ziller WG (1974) The tree rusts of western Canada. Canadian Forestry Service, Ottawa

Mycoscience (2003) 44:369–375
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FULL PAPER

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Exobasidium symploci-japonicae var. *carpogenum* var. nov. causing Exobasidium fruit deformation on *Symplocos lucida* in Japan

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Abstract *Exobasidium symploci-japonicae* var. *carpogenum*, causing Exobasidium fruit deformation on *Symplocos lucida* collected in Fukuoka Prefecture, Japan, is newly described based on morphological observations of hymenial structure and mode of basidiospore germination. This new variety differs morphologically from the type variety, particularly in the septal number of basidiospores and in the shapes and sizes of conidia formed on the medium. Colonies of this new variety are also distinguishable from those of the type variety by yeast-like growth, morphology, and color of colonies.

Key words Basidiomycetes · Culture · *Exobasidium* · Fruit deformation · Japan

Introduction

Recently, fruit deformation of *Symplocos lucida* Siebold et Zucc. and *S. myrtacea* Siebold et Zucc. by *Exobasidium* species was reported in Fukuoka Prefecture, but the species of these causal fungi have not been determined (Ogawa 1996, 2000). Fruit deformation has not been reported on *Symplocos* spp. in Japan. Two species of *Exobasidium* have been recorded on *Symplocos* spp. in Japan (Ezuka 1991; Hirata 1981; Kusano 1907). *Exobasidium symploci-japonicae* Kusano et Tokubuchi invades shoots and undeveloped young leaves of *S. lucida* and causes Exobasidium leaf blight (Ezuka 1991; Kusano 1907). *Exobasidium sakataniense* S. Hirata invades shoots and undeveloped young leaves of *S. theophrastaefolia* Siebold et Zucc. and causes typical Exobasidium leaf blight and also small leaf spot on developing young leaves. This species is distinguished from *E. symploci-japonicae* in the number of sterigmata and size of basidiospores (Hirata 1981).

In 2001, we were able to obtain fresh specimens of fruit deformation of *S. lucida* in Fukuoka Prefecture and observe their morphology. We propose a new variety of *E. symploci-japonicae* based on comparative morphology with *Exobasidium* species on *Symplocos* spp.

Materials and methods

Morphological observations

For light microscopy, materials were prepared as described previously (Nagao et al. 2003). Observations and measurements of conidia were conducted from the pure culture isolates on Difco potato dextrose agar (PDA). For scanning electron microscopy (SEM), samples were prepared and observed as described previously (Nagao et al. 2001). Fresh materials of Exobasidium fruit deformation and leaf blight on *S. lucida* collected in the field were used for morphological observations. Materials were deposited in the Mycological Herbarium of Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba (TSH), and the Herbarium of the National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan (NIAES). Material of Exobasidium fruit deformation (TSH-B0090) was collected in Kuroyama, Okagaki-machi, Onga-gun, Fukuoka Prefecture, June 22, 2001, by S. Ogawa, where the culture was obtained and deposited

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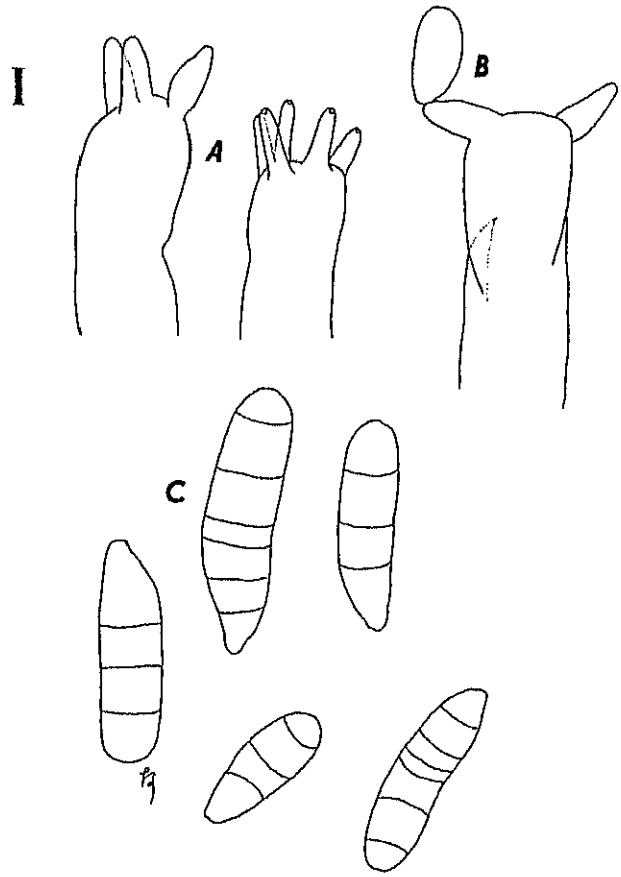

Fig. 1. Basidia and basidiospores of *Exobasidium symploci-japonicae* var. *carpogenum* formed on the infected fruit on *Symplocos lucida*. Basidia (**A**), basidium with immature basidiospore (**B**), and basidiospores (**C**) were collected in Fukuoka Prefecture (TSH-B 0090). *Bar* 3 μm

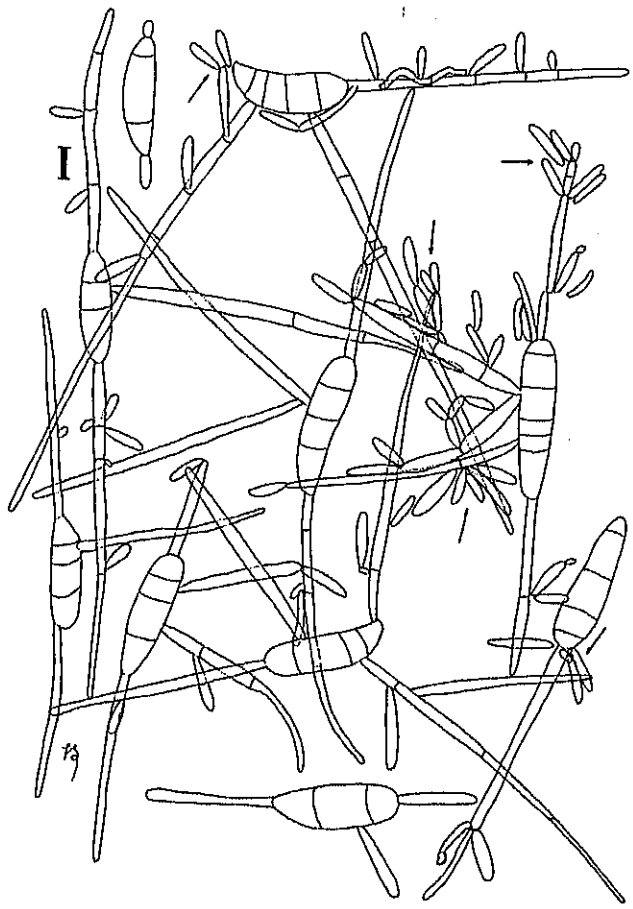

Fig. 2. Basidiospore germination of *E. symploci-japonicae* var. *carpogenum* (TSH-B 0090) on potato dextrose agar (PDA) after 12 h incubation. Some of the basidiospores produced conidia on the germ tube (*arrows*). *Bar* 5 μm

as MAFF238620. For comparison, three samples of *E. symploci-japonicae* var. *symploci-japonicae* were collected: TSH-B0040 in Shinji-machi, Ohara-gun, Shimane Prefecture, June 22, 2000, by T. Kobayashi; NIAES 20520 in Kuroiwa, Okagaki-machi, Onga-gun, Fukuoka Prefecture, June 2002, by J. Onagamitsu; and NIAES 20521 in Katuki-Shimin-no-mori, Chiyo, Yahata-Nishi-ku, Kitakyushu-shi, Fukuoka Prefecture, June 16, 2002, by Y. Usami. Cultures were obtained and deposited as MAFF238605, 238810, and 238811, respectively. Several herbarial materials were examined for comparative morphology; six specimens on *S. lucida* in the Herbarium of the Hokkaido University Museum (SAPA), two specimens on *S. lucida*, and four specimens on *S. theophrastaefolia* collected by Prof. Hirata in the Herbarium of Agricultural Museum, Faculty of Agriculture, Miyazaki University.

Culture of basidiospore isolate

Fresh infected materials were kept in a plastic bag for vegetables until newly sporulating lesions were observed. Colonies from a single basidiospore were obtained from the fresh materials as described previously (Nagao et al. 2003). Cultures were kept in the Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba, and also deposited in National Institute of Agrobiological Sciences (MAFF). An additional culture of *E. symploci-japonicae* var. *symploci-japonicae* IFO7790 was also examined.

Taxonomy

Exobasidium symploci-japonicae Kusano et Tokubuchi var. ***carpogenum*** Nagao et S. Ogawa, var. nov.

Figs. 1, 2

Fructus infecti superficie viridiflavi vel viridescentes, hypertrophici. A typo differt basidiosporis 3–6-septatis. Conidia hyalina, continua, laevia, linearia, globosa, ovata, lacrimiformia, subfusiformia vel claviformia, 2–9 × 1–2 μm. Coloniae in PDA restricte crescentes, post 21 dies maxime 16 mm diam. attingens, ad ambitum irregulares, corrugatae, glutinosae, ex hyphis circa 1 μm latis et conidiis constantes,

persicinae, in agaro non pigmentiferae; reversum pallide persicinum.

Holotypus in fructibus *Symploci lucidae* Siebold et Zucc., Kuroyama, Okagaki-machi, Onga-gun, Fukuoka Prefecture in Japonia, 22 VI 2001, S. Ogawa leg., in Herbario Instituti Agriculturae et silviculturae Universitatis Tsukubensis, Tsukuba, Japonia (TSH-B 0090) conservatus.

Isotypus in Herbario Instituti Nationalis Scientiae Agro-Environmentalis, Tsukuba, Japonia conservatus (NIAES 20530).

Etymology: The Latin word *carpogenus* refers to the infecting part of *S. lucida*.

The hymenium was composed of basidia with 2–5 sterigmata and conidia. Hyphae did not develop directly on the surface of the epidermis. Basidia were clavate to cylindrical, 8–68 × 6–9 μm (Fig. 1A,B). Basidia emerged directly from the host surface or through stomata. Basidia were not fasciculate. Sterigmata were 1.5–2.5 μm in diameter at the base and 4–6 μm in height, emerging outwardly and tapering toward the tip (Fig. 1A,B). Basidiospores were ellipsoid to ovoid, 13–23 × 4–6 μm, hyaline, smooth, one-celled when formed, becoming septate with 3–6 septa (Fig. 1C). Septate basidiospores germinated after 6 h when dropped on the agar surface. Germ tubes of the basidiospores emerged from each cell and produced conidia at the tip of germ tubes 12 h after dropping (Fig. 2). Hyphae grew into pseudohyphae and branched. Conidia were spherical, oval, lacrimiform, subfusiform, and clavulate, 2–9 × 1–2 μm, and budded or germinated polarly (Fig. 4A). Conidia budded to produce daughter cells polarly and also developed germ tube or hyphae. Colonies on PDA grew gradually, to a maximum 16-mm diameter in 21-day incubation, and were wrinkled irregularly around the periphery. The surface of the colonies was pink to pale pink and corrugate. Colonies were glutinous and did not fix on the agar surface. Colonies were composed of partially elongated pseudohyphae and conidia. Conidial formation did not produce a powdery appearance. The reverse of colonies was also pale pink. Dark pigment was not produced on PDA (Fig. 6A). Colonies from conidia showed the same morphological features as those from basidiospores.

Genus *Exobasidium* is characterized by the clavate to cylindrical basidia with erumpent sterigmata and ellipsoidal basidiospores, and there is no remarkable ornamentation on the surface of basidium and basidiospore. Its host range is wide, and the symptoms are galls on leaves, buds, flowers, fruit, and even on trunk, leaf blister, leaf blast, shoestring leaf, and witches' bloom. The taxonomy of *Exobasidium* has been argued (Burt 1915; Ezuka 1991; McNabb 1962; Nannfeldt 1981; Savile 1959; Sundström 1964). These arguments are attributed to the simple morphology of taxonomic characters and the variable symptoms and wide host range of this genus. We followed Nannfeldt's species concept and compared the morphology of basidia, basidiospores, and sterigmata and the mode of basidiospore germination.

Among 101 taxa of *Exobasidium* having been validly described so far, the following 2 species show some morphological similarities to the present taxon. *Exobasidium sakataniense* differed from *E. symploci-japonicae* var. *carpogenum* in number of sterigmata and size of basidiospores. Numbers of sterigmata of *E. symploci-japonicae* var. *carpogenum* were two to five, whereas *E. sakataniense* had two. Length of basidiospores of var. *carpogenum* ranged smaller than those of *E. sakataniense*. *Exobasidium symploci-japonicae* var. *symploci-japonicae* differed from *E. symploci-japonicae* var. *carpogenum* in the septal number of basidiospores. Basidiospores of *E. symploci-japonicae* var. *symploci-japonicae* have dominantly 0–4 septa at maturity (Table 1) (Ezuka 1991). Basidiospores of this new variety have 3–6 septa. The septal number of basidiospores overlapped, at about 3–4. Modes of septal number of the basidiospores varied with the two varieties. Variety *symploci-japonicae* deviated to less than 3-septated basidiospores, whereas the new variety had more than 3-septated ones (Fig. 9). Width of basidia, sizes of sterigmata, and sizes of basidiospores of this new variety were smaller than those of *E. symploci-japonicae* var. *symploci-japonicae*, but overlapped in the lower ranges of those of *E. symploci-japonicae* var. *symploci-japonicae* (Table 1). Numbers of sterigmata were the same in both varieties. Basidiospore germination of both varieties was of mycelial form.

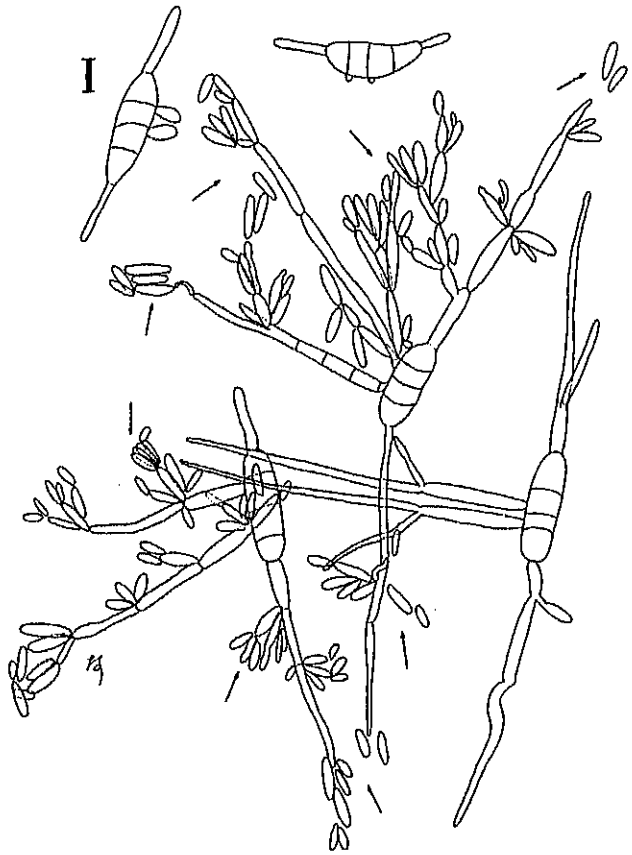

Fig. 3. Basidiospore germination of *E. symploci-japonicae* var. *symploci-japonicae* (TSH-B 0040) on PDA after 12 h incubation. Some of the basidiospores produced conidia on the germ tube (*arrows*). *Bar* 5 μm

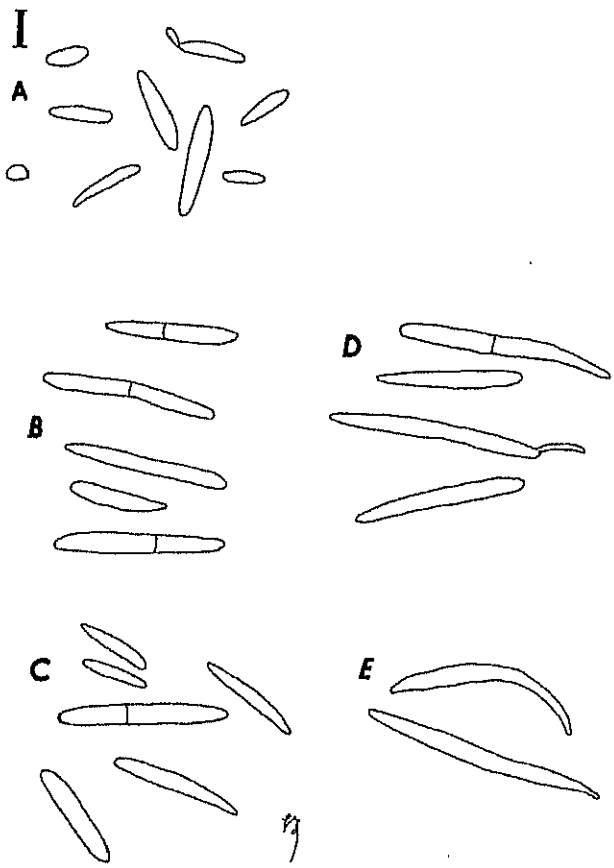

Fig. 4. Conidia of *E. symploci-japonicae* var. *carpogenum* MAFF 238620 (**A**) and var. *symploci-japonicae* MAFF 238605 (**B**), IFO7790 (**C**), MAFF 238810 (**D**), and MAFF 238811 (**E**) produced on PDA in 21-day incubations at 22°C. *Bar* 3 μm

The morphology of *Exobasidium* sp. on *S. myrtacea* has been briefly reported (Ogawa 2000). Size of basidiospores on *S. myrtacea* was in the same range as *E. symploci-japonicae* var. *carpogenum*, but septal number of basidiospores on *S. myrtacea* was two. Number of sterigmata was not determined. Although we could not precisely compare the morphology of *E. symploci-japonicae* var. *carpogenum* with *Exobasidium* sp. on *S. myrtacea*, the septal numbers of basidiospores of these two fungi were different. We considered that these morphological features rank this fungus as a new variety of *E. symploci-japonicae*.

In examining the herbarium materials, no fruit deformation was seen on *S. lucida* and on *S. theophrastaefolia*. All specimens showed the symptoms on shoots and undeveloped young leaves. Basidiospores and basidia could scarcely be detected on these materials.

Conidial shapes and sizes of the new variety markedly differed from those of *E. symploci-japonicae* var. *symploci-japonicae*. Conidia of *E. symploci-japonicae* var. *symploci-japonicae* (IFO7790, MAFF 238605, MAFF 238810, and MAFF 238811) were 7–23 × 1–2 μm, but those of the new variety (MAFF 238620) were 2–9 × 1–2 μm (Table 1). Ezuka (1991) reported conidial sizes of var. *symploci-*

Table 1. Morphological characteristics of *Exobasidium* species on *Symplocos* spp.

Taxon	Size of basidia (μm)	Size of sterigmata (μm)	Number of sterigmata	Size of basidiospores (μm)	Number of septa of basidiospores	Size of conidia (μm)	Number of septa of conidia	References
E. sakataniense	33–55 × 5–8	5–9 × 3–4	2	19–28 × 4–6	(2)3	7–18 × 1.5–2	0	Trans Mycol Soc Jpn 22 (1981):61–63
E. symploci-japonicae var. *carpogenum* TSH-B 0090	8–68 × 6–9	4–6 × 1.5–2.5	2–5	13–23 × 4–6	3–6	2–9 × 1–2	0	
E. symploci-japonicae var. *symploci-japonicae*	120–140 long	nd	(2)4(5)	17–22 × 6–7	nd	nd	nd	Bot Mag Tokyo 21 (1907):138–139
NIAES 10525	100–150 × 8–13	5–8 × 2–3	(2–)3–4(–5)	18–25(–27) × 5–6.5	(1–)3	10–27 × 2–3	0	Trans Mycol Soc Jpn 32 (1991):169–185
IFO7790	nd	nd	nd	nd	nd	(6)7–23 × 1–1.5	0–1	
TSH-B 0040	nd	nd	nd	nd	nd	11–21 × 1–1.5	0–1	
NIAES 20520	33–66 × 5–8	4–7 × 1.5–3	2–4	15–22 × 4.5–7	0–4(6)	(14)16–22(32) × 1.5–2	0–1	
NIAES 20521	22–46 × 7–9	4–7 × 1.5–2.5	2–3	13–23 × 4–7	0–4	13–22 × 1–2	0–1	

nd, not determined

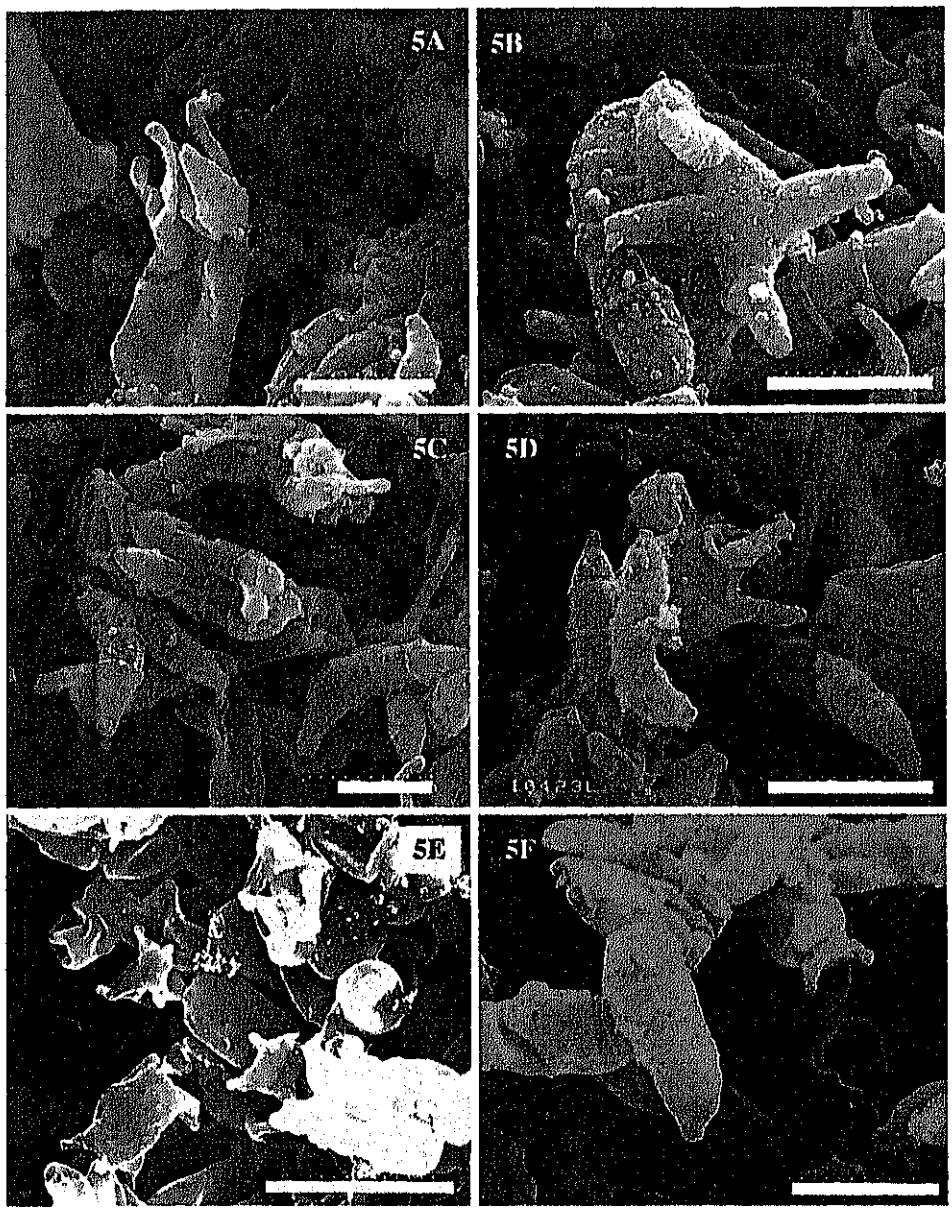

Fig. 5. Hymenium of *E. symploci-japonicae* var. *carpogenum* and var. *symploci-japonicae* observed by scanning election microscopy (SEM). **A** Basidium of *E. symploci-japonicae* var. *carpogenum* on the infected fruit of *S. lucida*. **B, D** Basidium and basidiospores of *E. symploci-japonicae* var. *carpogenum* on the infected fruit of *S. lucida*. **C** Basidiospores of *E. symploci-japonicae* var. *carpogenum* on the infected fruit of *S. lucida*. **E** Basidia of *E. symploci-japonicae* var. *symploci-japonicae* on infected leaf of *S. lucida*. Two to four sterigmata were seen. **F** Basidium and basidiospores of *E. symploci-japonicae* var. *symploci-japonicae*. *Bars* 6.5 μm

japonicae (NIAES 10525) as 10–27 × 2–3 μm without septum. These spore sizes were larger and wider than those of our observations. Conidia of the new variety were spherical, oval, lacrimiform, subfusiform, and clavulate (Fig. 4A), whereas those of *E. symploci-japonicae* var. *symploci-japonicae* were long fusiform to linear, sometimes being bent (Fig. 4B–E; Ezuka 1991), and its hyphae inclined to elongate rather than branching. Both germinations started from the tips of conidia. Although germination of basidiospores of both varieties was achieved with the mycelial form, the way of conidial germination was specific to the varieties. Conidial germinations of the new variety were both conidial and mycelial in form, but those of *E. symploci-japonicae* var. *symploci-japonicae* were mycelial in form.

Characteristics of colonies of both varieties were also remarkable. Colonies of *E. symploci-japonicae* var. *symploci-japonicae* composed of intricate pseudohyphae grew radially on PDA, to a maximum 33 mm diameter in 21 days incubation, and fixed on the agar surface (Fig. 6B–E), whereas colonies of the new variety composed of partially elongated pseudohyphae and conidia showed yeast-like growth and were wrinkled irregularly around the periphery (Fig. 6A). Anamorphic characteristics were remarkably different between the two varieties. Sundström (1964) demonstrated that the cultural characteristics (color, colony margination, texture, and mode of growth) and physiological activities led to grouping the strains into host races. Later, Nannfeldt (1981) proposed nine species based on the nine host races named by Sundström, which were classified into two species, *myrtilli* and *vaccinii*. In the case of var. *carpogenum*, the morphology of the teleomorph was not different enough to establish the new species.

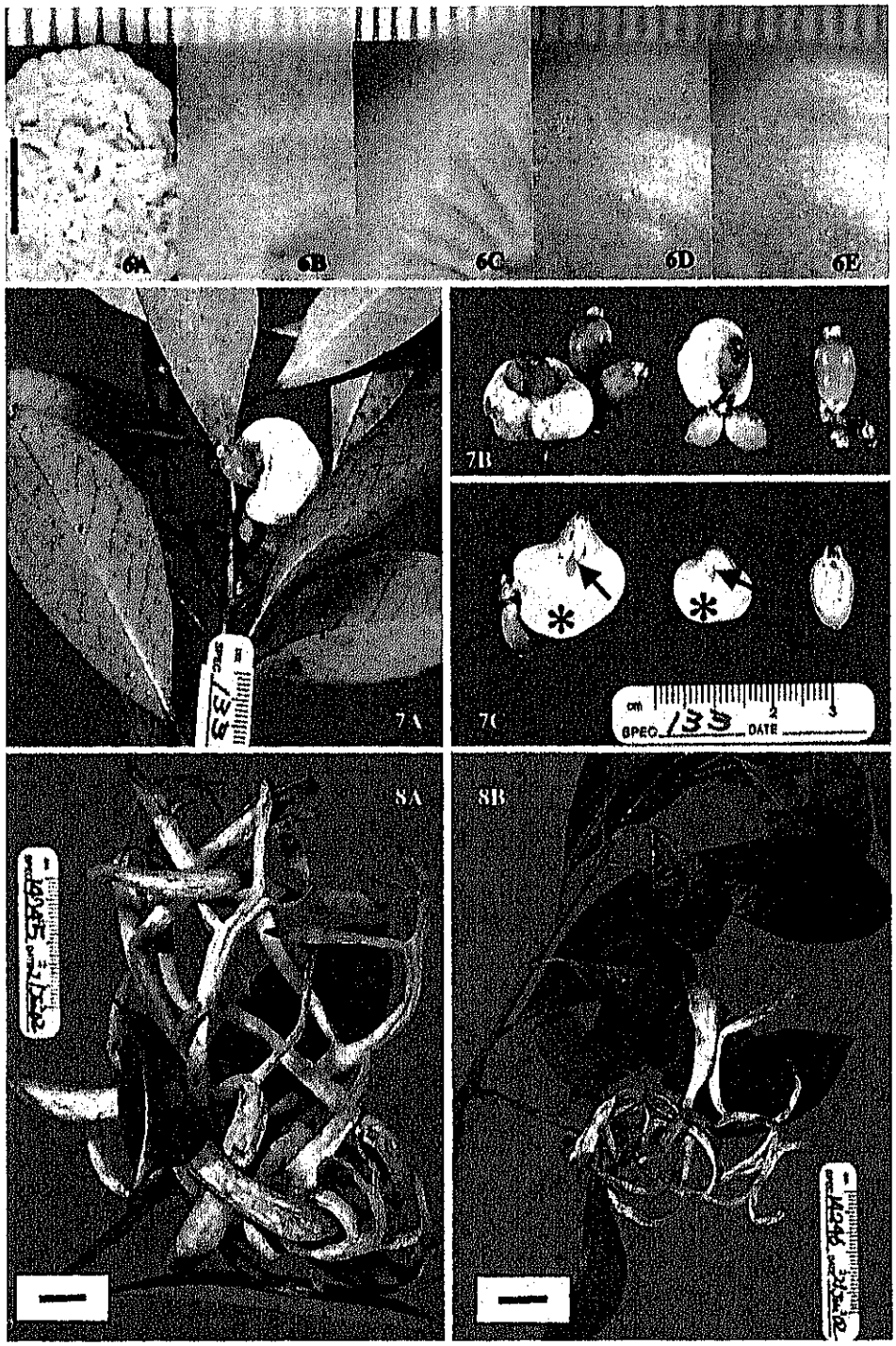

Fig. 6. Morphology and coloration on PDA. Surface of colonies of *E. symploci-japonicae* var. *carpogenum* MAFF 238620 (**A**). Surface of colonies of *E. symploci-japonicae* var. *symploci-japonicae* MAFF 238605 (**B**), IFO7790 (**C**), MAFF 238810 (**D**), and MAFF 238811 (**E**), respectively. *Bar* 5 mm

Fig. 7. Symptoms on Exobasidium fruit deformation on *S. lucida* by *E. symploci-japonicae* var. *carpogenum*. **A** Fruit deformation covered with white hymenia observed on June 2001 in Fukuoka Prefecture. **B** Appearance of fruit deformation (*left* and *center*) and healthy fruit. **C** Vertical section of infected and uninfected fruit. *Asterisk*, hyperplasia of tissue; *arrows*, abortive seed

Fig. 8. Symptoms on Exobasidium leaf blight on *S. lucida* by *E. symploci-japonicae* var. *symploci-japonicae*. **A** Infection occurred on the main branch of NIAES 20520. **B** Infection occurred on the lateral branch of NIAES 20521. *Bars* 10 mm

On infected fruit of *S. lucida*, white powdery hymenia were formed entirely on the swollen part of the fruit (Fig. 7A). Half the fruit were apparently bigger than the healthy ones and pale green or whitish-green (Fig. 7B). Sections of the infected fruit show hypertrophy and hyperplasia. Hyphae spread intercellularly and were branched. Haustorium was not determined in these hand sections. All seed were sterile (Fig. 7C). Hymenium formation was not observed on leaf, twig, and branch as appeared in the symptoms caused by var. *symploci-japonicae* (Fig. 8A,B). An infected fruit was overmatured and colonized by the secondary invaders. These fruits were decayed.

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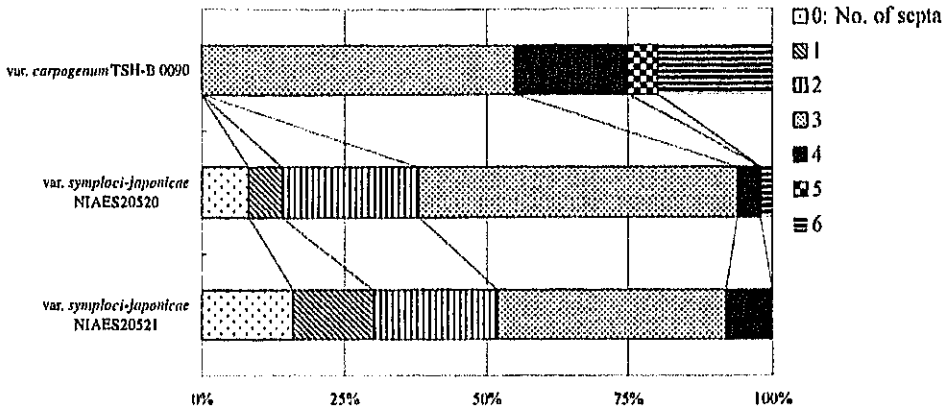

Fig. 9. Ratio of septal number in the basidiospores of *E. symploci-japonicae* var. *carpogenum* and var. *symploci-japonicae* NIAES 20520 and NIAES 20521

Dr. T. Utatsu at the Herbarium of Agricultural Museum, Faculty of Agriculture, Miyazaki University, for the loan of herbarium materials. This study was supported in part by a Grant-in-Aid for Scientific Research (B) (No.13460019), Japan Society for Promotion of Science (JSPS).

References

Burt EA (1915) The Thelephoraceae of North America. IV. *Exobasidium*. Ann Mo Bot Gard 2:627–659

Ezuka A (1991) Notes on some species of *Exobasidium* in Japan (IV). Trans Mycol Soc Jpn 32:169–185

Hirata S (1981) A new species of *Exobasidium* parasitic on *Symplocos theophrastaefolia*. Trans Mycol Soc Jpn 22:61–63

Kusano S (1907) *Exobasidium* – disease of *Symplocos japonica* Dc (in Japanese). Bot Mag Tokyo 21:138–139

McNabb RFR (1962) The genus *Exobasidium* in New Zealand. Trans R Soc N Z 1:259–268

Nagao H, Ezuka A, Ohkubo H, Kakishima M (2001) A new species of *Exobasidium* causing witches' broom on *Rhododendron wadanum*. Mycoscience 42:549–554

Nagao H, Akimoto M, Kishi K, Ezuka A, Kakishima M (2003) *Exobasidium dubium* and *E miyabei* sp. nov. causing Exobasidium leaf blisters on *Rhododendron* spp. in Japan. Mycoscience 44:1–9

Nannfeldt JA (1981) *Exobasidium*: a taxonomic reassessment applied to the European species. Symb Bot Ups 23:1–72

Ogawa S (1996) New diseases of trees recorded in Fukuoka Pref (I) (in Japanese). Forest Pests 45:31–35

Ogawa S (2000) New diseases of trees recorded in Fukuoka Pref. (II) (in Japanese). Forest Pests 49:28–33

Savile DBO (1959) Notes on *Exobasidium*. Can J Bot 37:641–656

Sundström KR (1964) Physiological and morphological studies of some species belonging to the genus *Exobasidium*. Phytopathol Z 40:213–217

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FULL PAPER

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Life cycle of *Uromyces appendiculatus* var. *azukicola* on *Vigna angularis*

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Abstract Field observations and inoculation experiments revealed that *Uromyces appendiculatus* var. *azukicola* has an autoecious and macrocyclic life cycle and produces spermogonia, aecia, uredinia, and telia on *Vigna angularis* var. *angularis* and *V. angularis* var. *nipponensis*. From inoculation experiments, it was suggested that this rust fungus has different host relationships from other varieties. Morphological examinations revealed that the characteristics of urediniospores and teliospores are different among varieties, although aeciospores are morphologically similar to each other.

Key words Life cycle · *Uromyces appendiculatus* var. *azukicola* · *Vigna angularis*

Introduction

A rust fungus on *Vigna angularis* (Willd.) Ohwi & Ohashi var. *angularis* (=*Phaseolus radiatus* L. var. *aurea* Prain.) was reported as *Uromyces appendiculatus* (Pers.) Link. because of morphological similarity with a rust fungus on *Phaseolus vulgaris* L. (Ito 1922, 1950). Hirata (1952) examined the rust fungus on *V. angularis* var. *angularis*, *V. angularis* var. *nipponensis* (Ohwi) Ohwi & Ohashi, and *V. umbellata* (Thunb.) Ohwi & Ohashi and found morphological differences from *U. appendiculatus*. Therefore, he newly described this fungus as *U. azukicola* S. Hirata (Hirata 1952). However, Hiratsuka et al. (1992) treated this rust as a variety of *U. appendiculatus* (Pers.) Unger. *Uromyces appendiculatus* var. *azukicola* (Hirata) Hiratsuka, f. has been reported to be morphologically different from *U. appendiculatus* var. *appendiculatus* in size of teliospores (Hiratsuka et al. 1992), although the difference is not clear because of overlap in spore size. This fungus is distributed throughout Japan (Hiratsuka 1973; Hiratsuka et al. 1992). However, the spermogonial and aecial stages of *U. appendiculatus* var. *azukicola* have not been reported, whereas other varieties of *U. appendiculatus* have been reported to have an autoecious and macrocyclic life cycle (Hiratsuka 1973; Hiratsuka et al. 1992).

In May 2002, we found the spermogonia and aecia of a rust fungus on leaves of *V. angularis* var. *nipponensis* on Mt. Nantai, Ibaraki Prefecture, Japan. The fungus was suspected to be *U. appendiculatus* var. *azukicola* from its morphology and host plants. We carried out inoculation experiments to clarify the life cycle and host plants of the rust fungus. We also report the morphology of the fungus and discuss its taxonomy.

Materials and methods

Basidiospore inoculation

Leaves of *V. angularis* var. *nipponensis* with abundant telia of *Uromyces* sp. were collected from Mt. Nantai, Ibaraki Pref., on September 1, 2002. The leaves were kept in a refrigerator at 4–5°C until use. The leaves were immersed in running tap water (about 20°C) for about 2–3 weeks to induce germination of teliospores. The teliospores germinated to produce numerous basidiospores within several days. For inoculation, small pieces of the leaves with germinating teliospores were placed on young, healthy leaves of *V. angularis* var. *nipponensis*. The inoculated plants were sprayed with distilled water and placed in a dark, moist chamber at about 20°C for 3 or 4 days, then transferred to a growth cabinet at about 20°C with controlled illumination

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(Kakishima et al. 1999; Kondo et al. 1997; Ono and Kakishima 1981).

Furthermore, teliospores formed on *V. angularis* var. *nipponensis* by aeciospore inoculation were also used as inocula to confirm the life cycle.

Aeciospore inoculation

Aeciospores formed on *V. angularis* var. *nipponensis* by the basidiospores inoculation, and those on the same host collected from Mt. Nantai, Ibaraki Pref. on May 27, 2002, were used as inocula. Aeciospores collected from aecia on the leaves were dusted with a scalpel onto pieces of wet filter paper (about 5 × 5 mm), which were then placed on the lower surface of young, healthy leaves of *V. angularis* var. *nipponensis* planted in clay pots. The inoculated plants were placed in a dark, moist chamber at about 20°C for 2 days, and then transferred to a growth cabinet at about 20°C with controlled illumination (Sato et al. 1983).

Urediniospore inoculation

Urediniospores from *V. angularis* var. *nipponensis* were dusted with a scalpel onto pieces of wet filter paper (~5 × 5 mm), which were then placed on the lower surface of healthy leaves of *V. angularis* var. *angularis*, *V. angularis* var. *nipponensis*, *V. unguiculata* ssp. *unguiculata*, and *Phaseolus vulgaris*. The inoculated plants were placed in a dark, moist chamber at about 20°C for 2 days, and then transferred to a growth cabinet at about 20°C with controlled illumination (Sato et al. 1983).

Furthermore, urediniospores on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref., from June to August of 2002 were inoculated onto *Vigna radiata* (L.) Wilczek, *V. mungo* (L.) Hepper, *Phaseolus vulgaris*, *Vicia cracca* L., *V. faba* L., *V. amoena* Fisch., *V. unijuga* Al. Br., *Lathyrus maritimus* Bigel., *L. palustris* L., and *Pisum sativum* L.

Morphological observations

Specimens on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref., and obtained from inoculation experiments were used for morphological observations. These specimens were deposited as dry herbarium specimens in the Mycological Herbarium, Institute of Agriculture and Forestry, University of Tsukuba (TSH).

For light microscopy, hand sections of spermogonia, aecia, uredinia or telia, and aeciospores, urediniospores, or teliospores were mounted in a drop of lactophenol solution on glass slides. Fifty spores of each spore state were measured with an Image Analyzer (Leica Qwin).

For scanning electron microscopy (SEM), spores were dusted onto double-sided adhesive tape on specimen holders, and then coated with platinum-palladium with a Hitachi E-1030 Ion Sputterer. The spores were examined with a Hitachi S-4200 SEM operating at 15 kV.

Results and discussion

Life cycle

About 12–13 days after leaves of *V. angularis* var. *nipponensis* were inoculated with basidiospores (Fig. 1), yellow and yellow-brown spermogonia appeared on the surface of the leaves, and 8–9 days later, aecia were mostly produced on the lower surface of the leaves (Table 1).

About 4–5 days after leaves of *V. angularis* var. *nipponensis* were inoculated with aeciospores, yellow spots appeared on the leaves. Five to 6 days later, the spots turned brown and brownish and uredinia were produced. After 12–13 days, telia started to appear on the inoculated leaves of *V. angularis* var. *nipponensis* (Table 2).

From the inoculation experiments it was proved that the rust fungus on *V. angularis* var. *nipponensis* has an autoecious and macrocyclic life cycle. Hiratsuka et al. (1992) reported that *U. appendiculatus* var. *appendiculatus* and *U. appendiculatus* var. *dispersus* are autoecious and macrocyclic rust fungi. Therefore, the three varieties have the same life cycle. We also found in the field that uredinia and telia occurred on *V. angularis* var. *nipponensis* on which spermogonia and aecia had been produced in spring (April to May).

Urediniospores formed on *V. angularis* var. *nipponensis* with aeciospore inoculation and those collected from Mt. Nantai, Ibaraki Pref., on the same host were used in

Table 1. Results of inoculation experiments with basidiospores taken from teliospores on *Vigna angularis* var. *nipponensis*

Inoculum[a]	Plant inoculated	Date of inoculation	Days required for the first appearance of	
			Spermogonia	Aecia
I	*V. angularis* var. *nipponensis*	Oct. 11, 2002	12	20
		Oct. 22, 2002	12	21
II	*V. angularis* var. *nipponensis*	Sept. 3, 2002	13	21

[a] I, Basidiospores from teliospores on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref.; II, basidiospores from teliospores formed on *V. angularis* var. *nipponensis* by aeciospore inoculation in June 2002

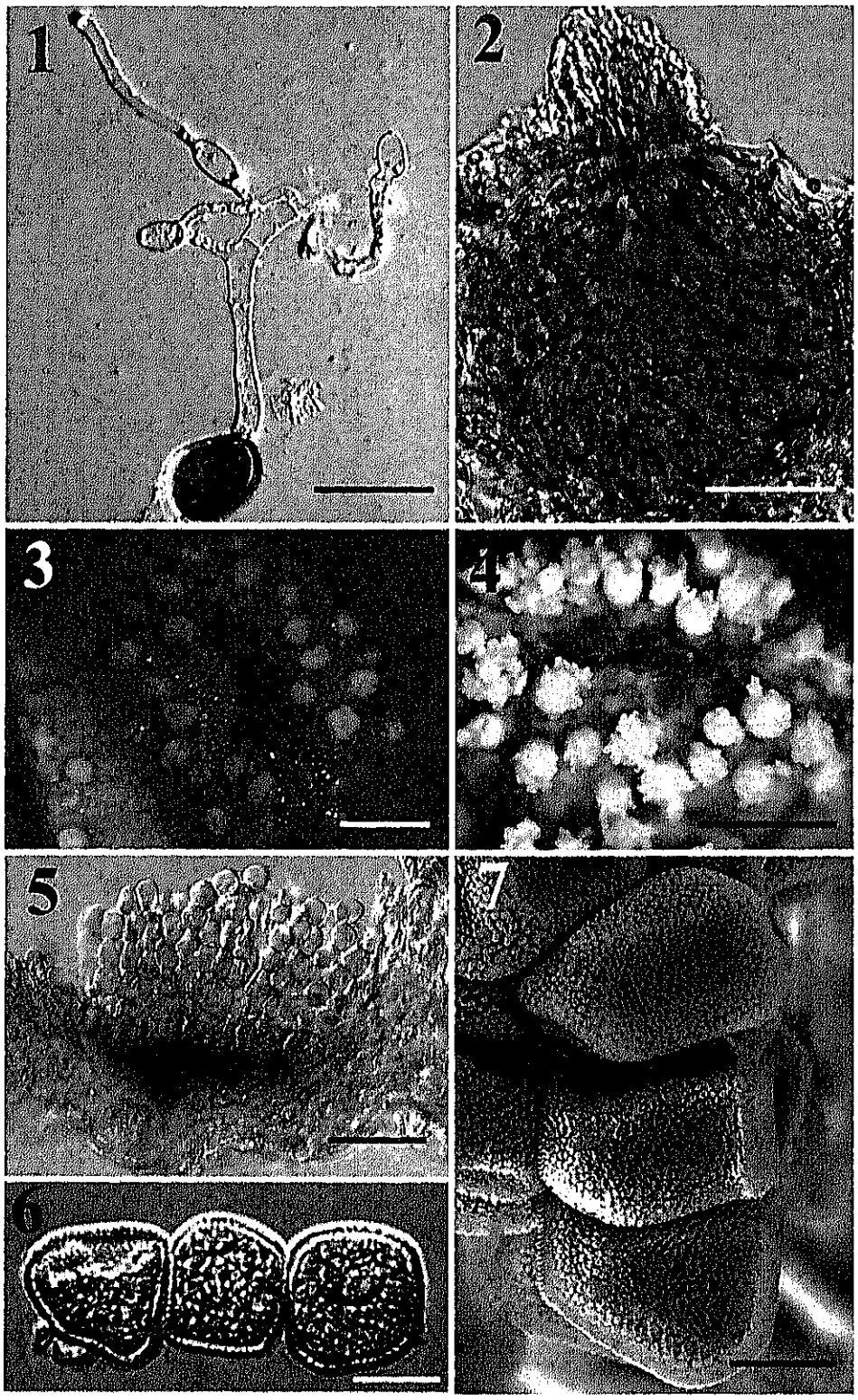

Fig. 1-7. *Uromyces appendiculatus* var. *azukicola* on *Vigna angularis* var. *nipponensis*. **1** A basidium and basidiospores from a teliospore. **2** A cross section of a spermogonium produced by inoculation experiment. **3, 4** Aecia resulting from basidiospore inoculation. **5** A cross section of an aecium. **6, 7** Aeciospores under light (**6**) and scanning electron (**7**) microscopes. *Bars* **1** 30μm; **2** 50μm; **3, 4** 1mm; **5** 50μm; **6** 10μm; **7** 5μm

inoculation for possible host plants of the fungus. Uredinia and telia were produced on *V. angularis* var. *nipponensis* and *V. angularis* var. *angularis* only. *Vicia amoena*, *V. cracca*, *V. faba*, *V. unijuga*, *Lathyrus maritimus*, *L. palustris*, *Pisum sativum*, *Vigna radiata*, *V. mungo*, *V. unguiculata* ssp. *unguiculata*, and *Phaseolus vulgaris* are not infected with this rust fungus (Table 3). Ito (1922, 1950) reported that the rust fungus on *V. angularis* was morphologically similar to

Table 2. Results of inoculation experiments with aeciospores on *Vigna angularis* var. *nipponensis*

Inoculum[a]	Plant inoculated	Date of inoculation	Days required for the first appearance of	
			Uredinia	Telia
I	*V. angularis* var. *nipponensis*	June 8, 2002	10	22
		June 10, 2002	10	23
II	*V. angularis* var. *nipponensis*	Oct. 10, 2002	10	22

[a] I, Aeciospores on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref.; II, aeciospores formed on *V. angularis* var. *nipponensis* by basidiospore inoculation in September 2002

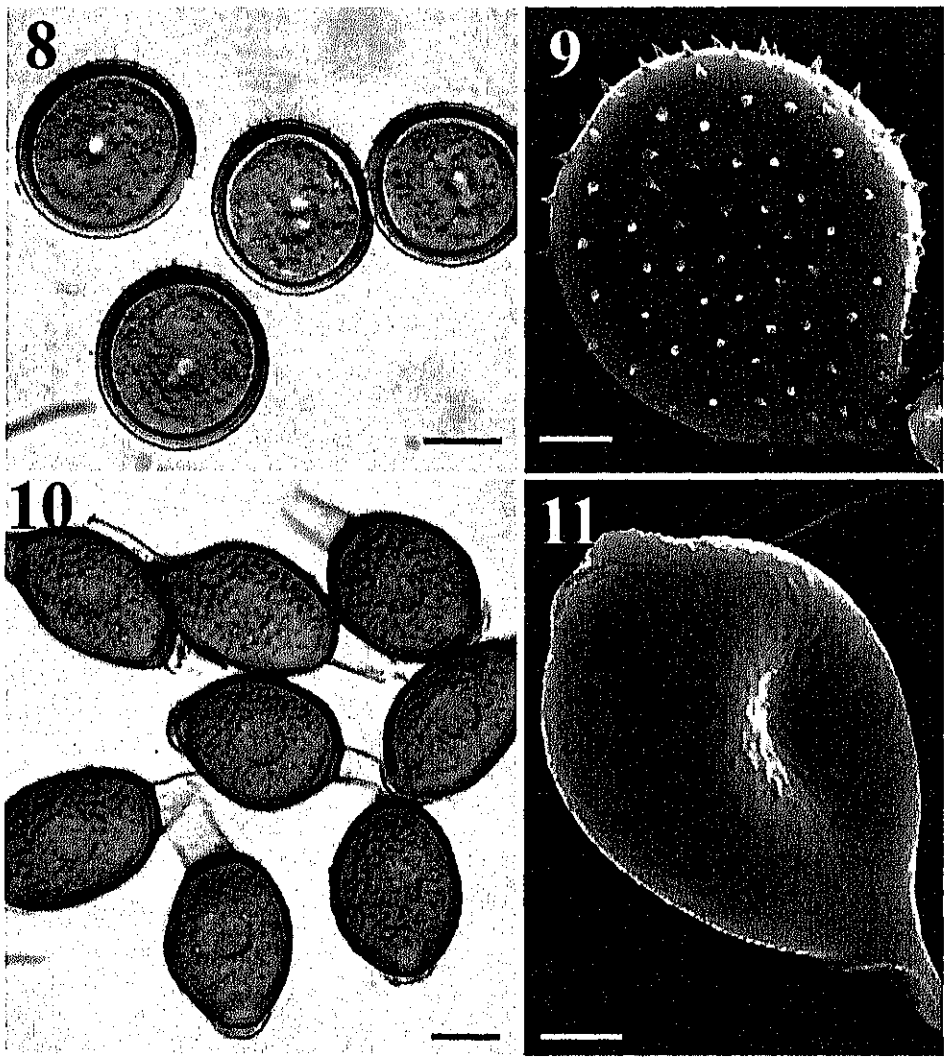

Fig. 8–11. *Uromyces appendiculatus* var. *azukicola* on *Vigna angularis* var. *nipponensis*. Urediniospores under light (**8**) and scanning electron (**9**) microscopes. Teliospores under light (**10**) and scanning electron (**11**) microscopes. *Bars* **8,10** 10 μm; **9,11** 4 μm

U. appendiculatus on *P. vulgaris*, and Duke (1981) also stated that *V. unguiculata* ssp. *unguiculata*, *V. mungo*, and *V. radiata* were host plants of *U. appendiculatus*. In our inoculation, however, the rust fungus from *V. angularis* var. *nipponensis* was not able to infect them. This result confirms that the rust fungus is specific to *V. angularis*, as reported by Hirata (1952). On the other hand, *Vicia*, *Lathyrus*, and *Pisum* are known as host plants of *U. viciae-fabae*. From these results, it is suggested that the rust fungus on *V. angularis* is different from these rust fungi in host relations.

Morphology

The spermogonia on *V. angularis* var. *nipponensis* were amphigenous or epiphyllous, surrounded by yellow lesions,

Table 3. Results of inoculation experiments with urediniospores on *Vigna angularis* var. *nipponensis*

Inoculum*	Plant inoculated	Appearance of spore stages	
		Uredinia	Telia
I	*Vigna angularis* var. *angularis*	+	+
	V. angularis var. *nipponensis*	+	+
	V. unguiculata ssp. *unguiculata*	–	–
	V. radiata	–	–
	V. mungo	–	–
	Phaseolus vulgaris	–	–
	Vicia cracca	–	–
	V. faba	–	–
	V. amoena	–	–
	V. unijuga	–	–
	Lathyrus maritimus	–	–
	L. palustris	–	–
	Pisum sativum	–	–
II	*Vigna angularis* var. *angularis*	+	+
	V. angularis var. *nipponensis*	+	+
	V. unguiculata ssp. *unguiculata*	–	–
	V. radiata	–	–
	V. mungo	–	–
	Phaseolus vulgaris	–	–
	Vicia cracca	–	–
	V. faba	–	–
	V. amoena	–	–
	V. unijuga	–	–
	Lathyrus maritimus	–	–
	L. palustris	–	–
	Pisum sativum	–	–
III	*Vigna angularis* var. *angularis*	+	+
	V. angularis var. *nipponensis*	+	+
	V. unguiculata ssp. *unguiculata*	–	–
	V. radiata	–	–
	V. mungo	–	–
	Phaseolus vulgaris	–	–
	Vicia cracca	–	–
	V. faba	–	–
	V. amoena	–	–
	V. unijuga	–	–
	Lathyrus maritimus	–	–
	L. palustris	–	–
	Pisum sativum	–	–
IV	*Vigna angularis* var. *angularis*	+	+
	V. angularis var. *nipponensis*	+	+
	V. unguiculata ssp. *unguiculata*	–	–
	Phaseolus vulgaris	–	–

*I–III, Urediniospores on *V. angularis* var. *nipponensis* collected from Mt. Nantai, Ibaraki Pref. in June (I), July (II), and August (III) 2002; IV, urediniospores formed on *V. angulris* var. *nipponensis* by aeciospore inoculations

scattered or aggregated, yellow to yellow-brown, subepidermal, and flask-shaped (type 4 of Cummins and Hiratsuka 2003) (Fig. 2). Spermogonia and aecia also appeared on the petioles of *V. angularis* var. *nipponensis*.

Aecia were amphigenous or hypophyllous, aggregated (rarely scattered), cupulate with peridia, and pale yellow (Figs. 3–5). Aeciospores were globose, subglobose, or angular, and measured 16.4–26.6 × 14.2–21.2 μm. Their walls were 0.9–1.3 μm thick, hyaline, and verrucose (Figs. 6, 7).

The uredinia on *V. angularis* var. *nipponensis* were amphigenous, scattered or aggregated, erumpent, and brown. Urediniospores were subglobose and obovoid, and measured 20.5–27.3 × 16.8–23.1 μm. Their walls were 0.9–1.5 μm thick, brown or light brown, and echinulate. The two germ pores were equatorial (superequatorial) (Figs. 8, 9). Telia were similar to uredinia. Teliospores were ellipsoid, apical papilla pale, mostly cuspidate, and 24.2–33.6 × 17.2–21.8 μm. Walls were brown or brownish, 1.3–2.0 μm thick, and smooth (Figs. 10, 11).

The morphology of the uredinial and telial stages of the specimens on *V. angularis* var. *nipponensis* is identical with the description of *U. appendiculatus* var. *azukicola* [=*U. azukicola*] described by Hirata (1952) and Hiratsuka et al. (1992). Therefore, we identified these specimens as *U. appendiculatus* var. *azukicola*. The morphology of the spermogonia and aecia on *V. angularis* var. *nipponensis* is described for the first time in the present study, and is similar to that of *U. appendiculatus* var. *appendiculatus* and *U. appendiculatus* var. *dispersus* (Hiratsuka et al. 1992). Size, shape, and wall structure of the aeciospores on *V. angularis* var. *nipponensis* are not distinguishable in these two varieties. Urediniospores, spore size, and wall structure are indistinguishable from other varieties of *U. appendiculatus*, but germ pore position and number are different from *U. appendiculatus* var. *appendiculatus* and *U. appendiculatus* var. *dispersus*. Although the rust fungus on *V. angularis* var. *nipponensis* has two equatorial or superequatorial pores, *Uromyces appendiculatus* var. *appendiculatus* and var. *dispersus* have two at the equatorial or subequatorial positions and two or three equatorial (Hiratsuka et al. 1992), respectively. Teliospore size is indistinguishable from other varieties of *U. appendiculatus*. However, teliospore wall thickness of the rust fungus on *V. angularis* var. *nipponensis* (1.3–2.0 μm) is different from *U. appendiculatus* var. *appendiculatus* (3.5 μm) and *U. appendiculatus* var. *dispersus* (1.5–3.0 μm). Therefore, we consider the morphological characteristics of the urediniospores and teliospores to be important in separating these varieties.

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References

Cummins GB, Hiratsuka Y (2003) Illustrated genera of rust fungi, 3rd edn. American Phytopathological Society, St. Paul, MN

Duke JA (1981) Handbook of legumes of world economic importance. Plenum Press, New York

Hirata Y (1952) On the rust fungi of cowpea, kidney bean and small red bean (in Japanese). Ann Phytopathol Soc Jpn 16:13–18

Hiratsuka N (1973) Revision of taxonomy of the genus *Uromyces* in the Japanese Archipelago. Rep Tottori Mycol Inst 10:1–98

Hiratsuka N, Sato S, Katsuya K, Kakishima M, Hiratsuka Y, Kaneko S, Ono Y, Sato T, Harada Y, Hiratsuka T, Nakayama K (1992) The rust flora of Japan. Tsukuba Shuppankai, Tsukuba

Ito S (1922) *Uromyces* of Japan. J Coll Agric Hokkaido Imp Univ, Sapporo 11:228–231

Ito S (1950) Mycological flora of Japan, vol 2. Basidiomyces no. 3: Urediniales-Pucciniaceae, Urediniales Imperfecti (in Japanese). Yokendo, Tokyo

Kakishima M, Yokoi M, Harada Y (1999) *Puccinia carici-adenocauli*, a new rust fungus on *Carex*, and its anamorph, *Aecidium adenocauli*. Mycoscience 40:503–507

Kondo S, Nishimoto F, Yamaoka Y, Kakishima M (1997) Life cycle and morphology of *Melampsora yezoensis* on *Salix serissaefolia*. Mycoscience 38:163–169

Ono Y, Kakishima M (1981) *Puccinia pulchella*: a new *Viola-Carex* rust from Japan. Can J Bot 59:1543–1546

Sato S, Goto M, Doi Y (1983) Laboratory guide for plant pathology (in Japanese). Kodansha, Tokyo, pp 32–38

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Short communication

Yoshitaka Ono

Does *Puccinia hemerocallidis* regularly host-alternate between *Hemerocallis* and *Patrinia* plants in Japan?

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Abstract Daylily rust fungus, *Puccinia hemerocallidis*, was proven to host-alternate between a wild daylily, *Hemerocallis fulva* var. *longituba*, and a patrinia, *Patrinia villosa*. No proof was obtained for the early belief that the fungus is pathogenic to plantainlilies, *Hosta* species, in addition to daylilies, *Hemerocallis* species. The fungus seems to alternate regularly between daylilies and patrinias in Japan because most daylily species are deciduous, and a vegetatively reproducing stage of the pathogen does not seem capable of successfully overwintering free of the living host tissue.

Key words Daylily · Host specificity · Life cycle · Plantainlily · Rust

Rust has been one of the few serious foliar diseases of daylilies (*Hemerocallis* spp., Liliaceae). The rust, which is widespread and common in Asia (Azbukina 1984; Hiratsuka et al. 1992; Zhuang et al. 1998), invaded the Americas in 2000 (Hernandez et al. 2002; Williams-Woodward et al. 2001) and Australia in 2001 (unreported specimens deposited in the Herbarium of Systematic Mycology, Ibaraki University: IBA). The causal fungus, *Puccinia hemerocallidis* Thümen, was described from telial material collected on *Hemerocallis flava* L. in Siberia (von Thümen 1880). Its uredinial stage was unknown until Dietel (1899) described uredinia and urediniospores produced on the leaves of *H. fulva* L. var. *longituba* Maxim. collected in Tokyo. The fungus has been proven to host-alternate on *Patrinia scabiosaefolia* Link and *P. rupestris* (Pallas) Dufresne. (Valerianaceae; spermogonial-aecial stage) and *H. minor* Mill. (uredinial-telial stage) in Russia (Tranzschel 1914), and between *P. scabiosaefolia* and *H. fulva* L. var. *disticha* (Donn) M. Hotta (= *H. fulva* var. *longituba*) (Hiratsuka 1938) and *H. thunbergii* Baker [= *H. citrina* Baroni var. *vespertiana* (Hara) M. Hotta] (Hiratsuka and Sato 1951) in Japan. In addition to *P. scabiosaefolia* and *P. rupestris*, *P. gibbosa* Maxim., *P. triloba* Miq., and *P. villosa* Juss. have been assumed to be the spermogonial-aecial hosts of this fungus (Hiratsuka et al. 1992), although there is no proof for this assumption.

Puccinia funkiae Dietel (Dietel 1898), described for a uredinial-telial fungus parasitic on a plantainlily, *Funkia ovata* Spreng. [= *Hosta albomarginata* (Hook.) Ohwi], has become synonynous with *P. hemerocallidis* since Hiratsuka and Hasebe's (1978) taxonomic treatment. Hence, *P. hemerocallidis* is now believed to parasitize four species and two varieties of *Hemerocallis* and six *Hosta* species (Hiratsuka et al. 1992). However, this taxonomic decision seems to have been based solely on the morphological similarity of the uredinial-telial stage, as there have been no critical evaluations of its life cycle or host specificity.

This study confirms the heteroecism of the fungus to *P. scabiosaefolia* and *P. villosa* using populations on *H. fulva* var. *longituba* as a representative of *P. hemerocallidis* and examined whether regular host alternation is needed for the continued disease cycle of daylily rust. The study also examined whether plantainlilies can harbor the fungus.

Heteroecism and host range. Fungal isolates used for basidiospore inoculations are as follows: teliospores produced on leaves of *H. fulva* var. *longituba* in Japan: Gumma Pref., Tano-gun, Ueno, Sanki, October 20, 2001, Y. Ono 4772, IBA-8745; Ibaraki Pref., Mito, Hirakue, December 1, 2001, Y. Ono 4778, IBA-8760; Ibaraki Pref., Kuji-gun, Daigo, Saigane, February 2002, Y. Ono, no voucher specimen; Ibaraki Pref., Higashiibaraki-gun, Gozenyama December 1, 2001, Y. Ono 4777, IBA-8759). Teliospores produced in the host tissue (Fig. 1A) were processed, and teliospore germination and basidiospore production were induced with the usual methods (Ono 1995; Ono and Azbukina 1997).

Leaves of *P. villosa* (Fig. 1C) and *P. scabiosaefolia* (Fig. 1D) were successfully inoculated with basidiospores (Fig. 1B) from each of the four fungal isolates during March and April 2002 at Ibaraki University campus in Mito. Spermogonia (Fig. 1E) appeared on the adaxial leaf surface

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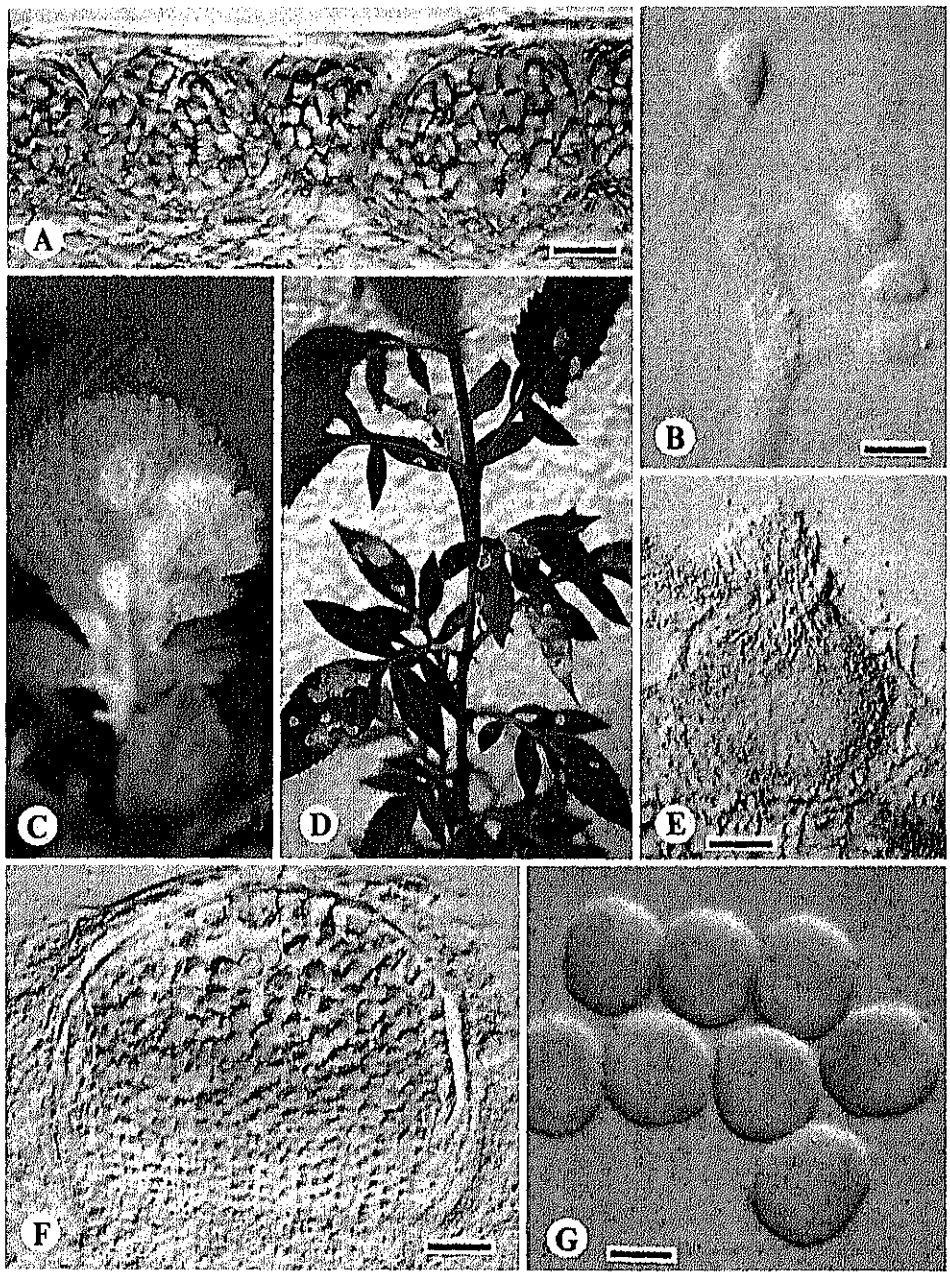

Fig. 1. *Puccinia hemerocallidis*. **A** Teliospores produced in subepidermal locules in leaf tissue of *Hemerocallis fulva* var. *longituba*. **B** Basidium with four basidiospores. **C** Chlorotic spots with orange-yellow spermogonia produced on *Patrinia villosa* after inoculation with basidiospore. **D** Coalescing chlorotic spots with spermogonia and aecia produced on *Patrinia scabiosaefolia* by basidiospore inoculation. **E** Spermogonium produced on *P. villosa*. **F** Aecium produced on *P. villosa*. The aecium is surrounded by a layer of fungal cells (peridium), within which aeciospores are produced in basipetal succession. **G** Aeciospores produced on *P. villosa*. *Bars* **A**, **E**, **F**: 40 μm; **B**, **G**: 10 μm

5–7 days after inoculation, and aecia (Fig. 1F) were produced on the abaxial surface 12–15 days after inoculation.

Aeciospores produced on *P. scabiosaefolia* and *P. villosa* after basidiospore inoculations were used to inoculate the abaxial leaf surface of *Hemerocallis fulva* var. *longituba*, *Hosta albo-marginata* (Hook.) Ohwi, *H. longipes* (Franch. et Savat.) Matsum., *H. montana* F. Maekawa, *H. rectifolia* Nakai, and *H. sieboldiana* (Lodd.) Engl. Successful infection was followed by abundant urediniospore production only on the inoculated leaves of *Hemerocallis fulva* var. *longituba*; no sign of infection appeared on any of the inoculated leaves of *Hosta* species.

Urediniospores produced after aeciospore inoculations were subsequently used to inoculate leaves of the same plant species described earlier. The urediniospore inoculation was successful only on leaves of *H. fulva* var. *longituba*; leaves of *Hosta* species were not infected.

The inoculation experiments proved that *P. villosa* is the spermogonial-aecial host of the daylily rust fungus, and that plantainlilies would not support uredinial-telial production of the fungus in nature. These differences in host specialization and life cycle necessitate taxonomic reevaluation of the *P. hemerocallidis–P. funkiae* species complex.

Overwintering of fungal vegetative mycelium in the host. Most daylily species in Japan are deciduous in winter. The exceptions are two perennial species, *H. littorea* Makino and *H. aurantiaca* Baker, which are distributed in southern coastal areas and the Danjo-gunto, respectively. It seems a natural consequence to believe that the rust fungus might invade a dormant bud or rhizome of diseased daylilies, and that the mycelium overwintering in the host tissue would produce a new crop of urediniospores (primary inoculum) in the spring.

A total of 100 individuals composed of a dormant bud and rhizome, which were apparently propagated clonally, were removed from a heavily rusted daylily population at Hirakue, Mito in December 2001. Half of the plants were thoroughly washed to remove soil from rhizomes and roots, but dead leaves bearing abundant uredinia and telia remained attached to the rhizome. The other 50 plants were similarly washed, but the dead, rusted leaves were completely removed. These plants, with or without dead, rusted leaves, were planted in separate groups of four in a seedling box of vermiculite. Seedling boxes with the thoroughly cleaned plants were placed in an insect-free growth chamber (20°C–25°C, 12-h light/dark intervals) for observation. The boxes with the washed plants with dead, rusted leaves were left outside the greenhouse. All plants were watered periodically, but no fertilizer was applied. They were then inspected periodically for rust pustules after new leaves emerged during late February.

From late February to October 2002, none of the plants, with or without dead, rusted leaves, developed uredinia on newly emerged leaves. These observations indicate that the fungal mycelium does not invade the dormant bud or rhizome of the rust-infected plant. Consequently, overwintering of the fungus by way of vegetative mycelium, which is often assumed, cannot be substantiated.

Overwintering of urediniospores on the host surface. Three natural daylily populations in Mito, Gozenyama, and Daigo, Ibaraki Prefecture, which were severely infected by *P. hemerocallidis* with abundant production of uredinia and telia in 2001, were chosen for continuous observation for disease occurrence in 2002. The sites of the daylily populations correspond with those where the inocula for basidiospore inoculations were collected.

At all three sites, new leaves started to expand during late February through early March. No rusted leaves were detected in the Gozenyama or the Mito populations until mid-October. A few *P. villosa* plants were observed near the two sites; however, no spermogonial-aecial infection was confirmed on the plants from early spring through early summer. These observations suggest that the urediniospores produced during a previous season and remaining on the host surface through the winter are not viable to infect newly expanding host leaves in a new growing season. When overwintered leaves were examined microscopically, only a few urediniospores were detected, and all observed spores looked collapsed.

On the other hand, in the Daigo population, uredinial pustules started to appear in mid-May, and infection become apparent in early June. From the foregoing observations, the source of infection was thought to be aeciospores produced by basidiospore infection on *Patrinia* growing near the rusted daylily.

In the field survey started in early March for *Aecidium*-infected *Patrinia* plants, a *P. villosa* plant at one site (of more than 50 *Patrinia*-growing sites in Ibaraki, Tochigi, and Fukushima Prefecture) was *Aecidium*-infected. The site was about 6 km from the site of the rusted Daigo population. Daylilies growing adjacent to the rusted *Patrinia* plant were producing uredinia. Hence, either aeciospores that were produced on the *Patrinia* plant or urediniospores produced on the daylilies were thought to be the source of the inoculum of the Daigo population that resulted in uredinial production. *Patrinia scabiosaefolia* and *P. villosa* were not uncommon in the Daigo area surveyed; therefore, some rusted *Patrinia* plants might have been overlooked in the survey.

Patrinia scabiosaefolia and *P. villosa* are common plants that are widely distributed in Japan, as are *Hemerocallis* species. However, *Aecidium*-infected *Patrinia* plants have rarely been collected [unpublished data in the Mycological Herbarium, University of Tsukuba (TSH); the Herbarium of Systematic Mycology, Ibaraki University (IBA); the Mycological Herbarium, the National Fungus Collection, USDA (BPI)], despite ubiquitous records of daylily rusts. Field surveys for *Aecidium*-infected *Patrinia* plants and specimen records have also been limited. Nevertheless, aeciospores that are produced on isolated *Patrinia* plants after a basidiospore infection could be the sole source of the primary infection of most, if not all, natural daylily populations. Daylilies growing near the aeciospore-producing patrinias would become infected, and the urediniospores thus produced would spread over widely distributed but discontinuous daylily populations. If the rust spreads stepwise by repeated uredinial infections on discontinuously distributed daylilies, it would take a long time for the rust to become prevalent or epidemic. This inference is in good agreement with empirical knowledge that daylily rust becomes prevalent during late summer or early autumn in Japan.

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References

Azbukina Z (1984) Key to the rust fungi of the Soviet Far East. Nauka, Moscow (in Russian)

Dietel P (1898) Einige Uredineen aus Ostasien. Hedwigia 37:212–218

Dietel P (1899) Uredinae japonicae I. Bot Jahrb 27:564–576

Hernandez JR, Palm ME, Castlebury LA (2002) *Puccinia hemerocallidis*, cause of daylily rust, a newly introduced disease in the Americas. Plant Dis 86:1194–1198

Hiratsuka N (1938) Miscellaneous notes on the East Asiatic Uredinales with special reference to the Japanese species (V). J Jpn Bot 15:433–439

Hiratsuka N, Sato S (1951) Inoculation experiments with heteroecious species of the Japanese rust fungi. IV. Bot Mag (Tokyo) 64:219–222

Hiratsuka N, Hasebe S (1978) A taxonomic revision of the species of *Puccinia* parasitic on the Liliales (Liliaceae, Amaryllidaceae, Dioscoreaceae, and Iridaceae) in the Japanese Archipelago: contributions to the rust-flora of eastern Asia. XII. Rep Tottori Mycol Inst 16:1–36

Hiratsuka N, Sato S, Katsuya K, Kakishima M, Hiratsuka Y, Kaneko S, Ono Y, Sato T, Harada Y, Hiratsuka T, Nakayama K (1992) The rust flora of Japan. Tsukuba, Tsukuba-shuppankai

Ono Y (1995) *Aecidium alangii* is the aecial state of *Puccinia miyakei* (Uredinales). Nova Hedwigia 60:157–164

Ono Y, Azbukina ZM (1997) Heteroecious life cycle of two graminicolous *Puccinia* (Uredinales). Mycoscience 38:281–286

Tranzschel W (1914) Kulturversuche mit Uredineen den Jahren 1911–1913. Mycol Centrbl 4:70–71

Von Thümen F (1880) Beiträge zur Pilzflora Sibiriens. III. Bull Soc Nat Moscou 55:72–104

Williams-Woodward JL, Hennen JF, Parda KW, Fowler JM (2001) First report of daylily rust in the United States. Plant Dis 85:1121

Zhuang Z-J, Wei S, Wang, Y-C (1998) Flora fungorum sinicorum, vol 10: Uredinales (1). Science Press, Beijing

Mycoscience (2004) 45:1–8
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FULL PAPER

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Phylogenetic analyses of *Uromyces viciae-fabae* and its varieties on *Vicia*, *Lathyrus*, and *Pisum* in Japan

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Abstract A pea rust fungus, *Uromyces viciae-fabae*, has been classified into two varieties, var. *viciae-fabae* and var. *orobi*, based on differences in urediniospore wall thickness and putative host specificity in Japan. In principal component analyses, morphological features of urediniospores and teliospores of 94 rust specimens from *Vicia*, *Lathyrus*, and *Pisum* did not show definite host-specific morphological groups. In molecular analyses, 23 *Uromyces* specimens from *Vicia*, *Lathyrus*, and *Pisum* formed a single genetic clade based on D1/D2 and ITS regions. Four isolates of *U. viciae-fabae* from *V. cracca* and *V. unijuga* could infect and sporulate on *P. sativum*. These results suggest that *U. viciae-fabae* populations on different host plants are not biologically differentiated into groups that can be recognized as varieties.

Key words *Lathyrus* · Phylogeny · *Pisum* · Rust fungus · Taxonomy · Uredinales · *Uromyces viciae-fabae* · *Vicia*

Introduction

Uromyces viciae-fabae (Pers.) J. Schroet., causing pea rust disease, is an autoecious and macrocyclic rust fungus occurring on wild and cultivated *Vicia*, *Lathyrus*, *Pisum*, and *Lens* throughout the world (Wilson and Henderson 1966; Cummins 1978; Duke 1981; Hiratsuka et al. 1992). Currently, *U. viciae-fabae* is separated into two varieties, var. *viciae-fabae* (= *U. fabae* de Bary) and var. *orobi* (Schumach.) Jørst. (= *U. orobi* Lév.), based on wall thickness difference of urediniospores and putative host specificity (Wilson and Henderson 1966; Hiratsuka et al. 1992).

The species name, *U. orobi* Lév. (= *U. viciae-fabae* var. *orobi*), was originally applied to a rust occurring on *Lathyrus montanus* Bernh. by Léveillé (1847, cited from Wilson and Henderson 1966), and it was noted that the urediniospore wall was thicker than that of *U. fabae* (= *U. viciae-fabae* var. *viciae-fabae*). Later, Jørstad (1936, cited from Wilson and Henderson 1966) merged *U. orobi* with *U. viciae-fabae*, designating the former fungus as a variety of the latter. On the other hand, *U. fabae* was originally applied to a rust on *Vicia faba* L. (de Bary 1863, cited from Wilson and Henderson 1966), having been reported on *Vicia*, *Lathyrus*, *Pisum*, and *Lens* (Wilson and Henderson 1966; Duke 1981). However, Gäumann (1934) stated that urediniospores of *U. fabae* on *Vicia sepium* showed different wall thickness and that those on stems were thicker than those on leaves. He listed six formae speciales for *U. fabae* and three formae speciales for *U. orobi*.

In Japan, Ito (1922) classified a rust fungus on *V. unijuga* Al. Br. as *U. orobi* because it had a thicker urediniospore wall than *U. fabae* on *Vicia*, *Lathyrus*, and *Pisum*. Later, Hiratsuka (1933) classified rust fungi on *V. unijuga*, *V. nipponica* var. *capitata* Nakai, and *L. davidii* Hance as *U. orobi*. Furthermore, *U. orobi* was stated not to infect the host plants of *U. fabae* (Hiratsuka 1933). Recently, the fungi on these three host species were transferred into *U. viciae-fabae* var. *orobi* (Hiratsuka 1973; Hiratsuka et al. 1992). Although the difference in urediniospore wall thickness of *U. viciae-fabae* was considered an important taxonomic characteristic, it is not necessarily distinct among the species compared. Therefore, there has been confusion about the identity of, and relationship between, the two varieties causing the rust disease in different geographic areas and occur-

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ring on closely related host plants (Gäumann 1934; Wilson and Henderson 1966; Hiratsuka et al. 1992).

Recently, molecular methods have been applied to filamentous fungi for the study of genetic variation and the phylogeny of species that are morphologically indistinct (Foster et al. 1993). Molecular phylogenetic analyses of rust fungi were not widely carried out because they are obligate parasites that are impossible or difficult to obtain or maintain in pure culture. However, it is now possible to extract DNA from a single spore of dry herbarium specimens and to amplify target DNA by polymerase chain reaction (PCR) (Bruns et al. 1990; Lee and Taylor 1990). Virtudazo et al. (2001) modified DNA extraction methods from Suyama et al. (1996) and extracted genomic DNA from spores from a single uredinium, then amplified the template DNA by PCR. Ribosomal repeat units are generally informative for species and generic differentiation (Bruns et al. 1991; O'Donnell 1993; Piepenbring et al. 1999; Tehler et al. 2000; de Jong et al. 2001). Accordingly, ribosomal DNA sequences of rust fungi have been analyzed and registered in genetic databases (Zambino and Szabo 1993; Vogler and Bruns 1998; Ayliffe et al. 2001; Pfunder et al. 2001; Virtudazo et al. 2001).

The current study reevaluates morphological variations of urediniospores and teliospores of *Uromyces viciae-fabae* populations on *Vicia*, *Lathyrus*, and *Pisum* in Japan and analyzes their molecular phylogeny in relation to their taxonomy. The scientific names of cultivated and wild legumes follow Ohwi and Kitagawa (1992).

Materials and methods

Morphological observations and statistical analyses

Fresh material or dry herbarium specimens were used for light microscopic (LM) and scanning electron microscopic (SEM) observation. Most specimens examined were loaned from the Hiratsuka Herbarium, Tokyo, Japan (HH); the National Fungus Collections, the United States Department of Agriculture, Beltsville, MD, USA (BPI); the Mycological Herbarium of the Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Japan (TSH); the Herbarium of Systematic Mycology, the College of Education, Ibaraki University, Mito, Japan (IBA); and the Herbarium of the National Institute of Agro-Environmental Science, Tsukuba, Japan (NIAES).

Ninety-four specimens were gathered from seven species of *Vicia*, three species of *Lathyrus*, and *P. sativum* in Japan (Table 1). These host plants are reported to be susceptible to *U. viciae-fabae* var. *viciae-fabae* and *U. viciae-fabae* var. *orobi* (Hiratsuka et al. 1992). Specimens were examined for morphological characteristics of urediniospores and teliospores. Statistical analyses, including multivariate analyses of measured continuous numerical variables, were performed using the software package SPSS (SPSS Japan, Tokyo, Japan). Discrete numerical or qualitative attributes, or host species, were superimposed on two- or three-dimensional scatter diagrams generated from the analyses to detect possible groups.

For SEM, spores obtained from dry specimens were dusted onto double-sided adhesive tape on specimen holders, and then coated with platinum-palladium with a Hitachi E-1030 Ion Sputter Coater. The spores were examined by SEM with a Hitachi S-4200 instrument operating at 15kV.

Fungal taxa for rDNA sequencing

Twenty-three specimens were selected for large subunit (LSU) rDNA (D1/D2) and internal transcribed spacer (ITS) sequence analyses (Table 2). For comparison, and as outgroup taxa, the fabaceous rusts *U. appendiculatus* (Pers.) Unger, *U. vignae* Barclay, *U. fabae* de Bary, *U. pisi* (DC.) Otth, and *U. minor* Schroet., a rust of *Gagea lutea* (L.) Ker Gawl., *U. gageae* Beck, and a rust of *Miscanthus sinensis*, *Puccinia miscanthi*, were also included in the analyses. These GenBank sequences of fungi used for phylogenetic comparison are listed in Table 3.

DNA extraction, PCR amplification, and purification and sequencing of LSU rDNA (D1/D2) and ITS regions

Genomic DNA was extracted from about 150–200 urediniospores or teliospores from a single uredinium or

Table 1. Specimens of *Uromyces viciae-fabae* used for morphological observations

Host plants	Locality (no. of specimens)	Stage*
Vicia amoena Fisch.	Honshu (3)	II, III
V. cracca L.	Honshu (22), Hokkaido (3), Kyushu (1)	0, I, II, III
V. faba L.	Honshu (7), Hokkaido (4), Kyushu (4), Okinawa (1)	II, III
V. japonica A. Gray	Honshu (7), Hokkaido (6)	II, III
V. nipponica var. *capitata* Nakai	Honshu (1)	II, III
V. pseudo-orobus Fish. & C.A. Mey.	Honshu (2)	II, III
V. unijuga Al. Br.	Honshu (5), Kyushu (1)	II, III
Lathyrus davidii Hance	Honshu (2)	II, III
L. maritimus Bigel.	Honshu (5), Hokkaido (4), Kyushu (3)	II, III
L. palustris L.	Honshu (6), Hokkaido (1)	II, III
Pisum sativum L.	Honshu (3), Hokkaido (1), Kyushu (2)	I, II, III
Total	94	

*0, spermogonia; I, aecia; II, uredinia; III, telia

Table 2. Specimens of *Uromyces viciae-fabae* sequences used for phylogenetic analysis

Host plants	Locality in Japan	Voucher specimens[a]	GenBank accession no.	
			D1/D2	ITS
Vicia amoena	Yamanashi	TSH-R13227	AB115592	AB115650
V. cracca	Nagano	TSH-R16998	AB115597	AB115654
	Ibaraki	TSH-R16999	AB115598	AB115655
	Hokkaido	TSH-R16269	AB115595	AB115652
	Nagano	TSH-R18187	NA[b]	AB115659
	Nagano	TSH-R2986	NA	AB115660
V. faba	Chiba	BPI-0005425	AB115607	AB115663
	Fukuoka	BPI-0005454	AB115608	AB225664
V. pseudo-orobus	Yamanashi	TSH-R1743 (IBA-2652)	AB115601	AB115656
V. japonica	Hokkaido	TSH-R1738 (IBA-5836)	AB115600	AB085194
	Yamanashi	TSH-R13306	AB115592	NA
V. unijuga	Yamanashi	TSH-R1747 (IBA-3068)	AB115603	AB115657
	Kanagawa	H40928	AB115605	AB115661
	Yamaguchi	H67023	AB115611	AB115666
	Nagano	TSH-R18185	AB115604	AB115658
V. nipponica var. *capitata*	Tottori	BPI-0005541	AB115609	NA
Lathyrus maritimus	Ibaraki	TSH-R6320	AB115610	AB115665
	Hokkaido	TSH-R1736 (IBA-5842)	AB115599	AB085193
	Hokkaido	TSH-R1739 (IBA-3004)	NA	AB085195
	Ibaraki	TSH-R1744 (IBA-2894)	AB115602	AB085192
L. palustris	Iwate	BPI-0005266	AB115606	AB115662
	Hokkaido	TSH-R16270	AB115596	AB115653
Pisum sativum	Hokkaido	TSH-R16268	AB115594	AB115651

ITS, internal transcribed spacer region
[a]TSH, Mycological Herbarium, University of Tsukuba, Japan; BPI, USDA National Fungus Collections, USA; HH, Hiratsuka Herbarium, Tokyo, Japan; IBA, Herbarium of Systematic Mycology, Ibaraki University, Japan
[b]No analyses

Table 3. Additional taxa selected for D1/D2 and ITS analysis

Species	Host plants	GenBank accession no.	
		D1/D2	ITS
Uromyces minor Schroet.	*Trifolium lupinaster* L.	NA[a]	AB115737
U. vignae Barclay	*Vigna unguiculata* (L.) Walp. ssp. *unguiculata*	AB115629	AB115720
U. appendiculatus (Pers.) Unger var. *appendiculatus*	*Phaseolus vulgaris* L.	AB115644	AB115741
U. appendiculatus var. *azukicola* (Hirata) Hiratsuka, f.	*Vigna angularis* (Willd.) Ohwi & Ohashi var. *angularis*	AB115619	AB115710
U. fabae de Bary	*Vicia pannonica* Crantz	AF426199	NA
U. pisi (DC.) Otth	*Euphorbia cyparissias* L.	AF426201	NA
U. pisi	*Euphorbia cyparissias*	NA	AF180165
U. gageae Beck	*Gagea lutea* (L.) Ker Gawl.	AF426208	NA
Puccinia miscanthi Miura	*Miscanthus sinensis* Anderss.	AJ296546	NA

[a]No analyses

telium. DNA extraction methods followed Virtudazo et al. (2001). From this crude extract, 2–3 µl was used directly for each PCR amplification. Amplifications were done using 40-µl PCR reactions, each containing 0.2 µM of each primer, 1 unit TaKaRa Ex Taq DNA polymerase (Takara, Tokyo, Japan), and a commercial dNTP mixture (containing 2.5 mM of each dNTP) and Ex Taq reaction buffer (containing 2 mM Mg^{2+}). PCR was carried out using a Gene Amp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) under the conditions used by Virtudazo et al. (2001). The D1/D2 region was amplified using primers NL1 and NL4 (O'Donnell 1993). For ITS and 5.8S regions, the primers used at the beginning of this study were ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993). However, because amplification using these primers was successful for only a few specimens, we chose primers that worked better for *Uromyces* spp., ITS5-u (5'-AAGGTTTCTGTAGGTG-3') and ITS4-u (5'-GGCTTTTCCCTCTTCAT-3') (Pfunder et al. 2001). PCR products were run on 1% agarose gels containing 0.5 µg/ml ethidium bromide in TAE [Tris-acetate, ethylene diaminetetraacetic acid (EDTA)] or TBE (Tris-borate, EDTA) buffers. PCR products were first purified by spin columns (MicroSpin S-400 HR Columns). Purified PCR products were reacted with BigDie Terminator v3.0 Cycle Sequencing (Applied Biosystems) under the following conditions: 25 cycles of 96°C for 10 s, 50°C for 5 s,

and 60°C for 4 min. Cycle sequencing reaction products were purified by ethanol precipitation, and then analyzed by ABI PRISM 3100 automated sequencers (Applied Biosystems).

Sequence alignment and analyses

DNA sequences were aligned using Clustal X v1.8 (Thompson et al. 1997). Further visual alignments were done in Sequence Alignment (Se-Al) Editor v.2.0 (Rambaut 2000). Specimen sequences were analyzed together with sequences from GenBank.

The aligned sequences data file can be obtained from the authors. Phylogenetic analyses of the data were done by distance and parsimony methods. The distance matrix for the aligned sequences was calculated using Kimura's two-parameter method (Kimura 1980) and was analyzed with the neighbor-joining (NJ) method (Saitou and Nei 1987) using the program PAUP v 4.0 b (Swofford 1999), excluding positions with gaps and correcting for multiple substitutions. Reliability of the inferred trees was estimated by 1000 bootstrap resamplings using the same program. Parsimony analysis was also done by PAUP v 4.0 b using the heuristic search option with 100 random stepwise-addition sequences to search for the most parsimonious tree. Bootstrap (Felsenstein 1985) values were generated with 1000 replicate heuristic searches to estimate support for clade stability of the consensus tree using the same program.

Inoculation experiments

Urediniospores from *Vicia cracca* L. and *V. unijuga* were dusted with a scalpel onto pieces of wet filter paper (about 5 × 5 mm), which were then placed on the lower surface of healthy leaves of *V. amoena* Fisch., *V. cracca*, *V. faba*, *V. unijuga*, *Lathyrus maritimus* Bigel., *L. palustris* L., and *Pisum sativum* L. The inoculated plants were placed in a dark, moist chamber at about 20°C for 2 days, and then transferred to a growth cabinet at about 20°C with controlled illumination (Sato et al. 1983).

Results

Morphological features and principal component analyses

Morphology of urediniospores and teliospores on *Vicia*, *Lathyrus*, and *Pisum* was not significantly different. The urediniospores were globose, subglobose, or ellipsoid; the spore wall was pale yellow or yellow, and echinulate (Fig. 1A,B). Mean urediniospore length in individual specimens ranged from 20.7 to 30.5 µm and mean width from 16.5 to 26.7 µm. However, no discrete groups were detected for urediniospore length and width (Fig. 2A). Urediniospores on *V. unijuga*, *V. nipponica* var. *capitata*, and *L. davidii* did not show significant difference in wall thickness, ranging from 1.1 to 2.8 µm.

The teliospores were subglobose, ovate, or ellipsoid (Fig. 1C,D). Mean length of teliospores in individual specimens ranged from 29.1 to 37.1 µm and mean width from 18.4 to 28.3 µm. Mean teliospore apical thickness in individual specimens ranged from 4.1 to 8.4 µm and mean wall thickness from 1.7 to 3.2 µm. However, no discrete groups were detected for teliospore size and apical thickness (Fig. 2B). The wall surface was smooth in all specimens observed, and color was pale brown, brown, or dark brown. The principal component analyses were undertaken with various combinations of urediniospore size, wall thickness and teliospore size, apical thickness, and side-wall thickness. After the Varimax rotation, the calculated factors 1 and 2 explained 30.4% and 26.5% of the total variance, respectively. The scatter diagram with factors 1 and 2 did not form discrete groups (Fig. 2C).

Molecular phylogenetic analysis inferred from rDNA D1/D2 and ITS

The DNA sequences of the entire LSU rDNA (D1/D2) region of pea rust fungi, ranging from 604 to 607 bases, were used for phylogenetic analysis. Of the 617 aligned bases, 19 sites were variable and 24 sites were parsimony-informative characters. The NJ tree constructed from the LSU (D1/D2) rDNA regions showed that *Uromyces* on *Vicia*, *Lathyrus*, and *Pisum* formed a single genetic clade with high bootstrap support (Fig. 3). The LSU (D1/D2) bootstrap phylogram has a consistency index (CI) of 0.900, a retention index (RI) of 0.906, retention consistency (RC) of 0.815, and a tree length of 50.

The DNA sequences of the entire ITS regions of cultivated and wild legume rust, ranging from 615 to 623 bases, were used for phylogenetic analyses. Of the 660 aligned bases, 107 sites were variable and 70 sites were parsimony-informative characters. In the NJ tree constructed from the ITS and 5.8S rDNA regions, the rust fungi on *Vicia*, *Lathyrus*, and *Pisum* also formed a single genetic clade, with high bootstrap support (Fig. 4). The ITS bootstrap phylogram has a CI of 0.809, an RI of 0.667, an RC of 0.539, and a tree length of 251. Therefore, the sequence analyses of *Uromyces* on *Vicia*, *Lathyrus*, and *Pisum* revealed virtually no genetic variation based on the D1/D2 and ITS regions.

Host specificity of *Uromyces viciae-fabae* on *Vicia*, *Lathyrus*, and *Pisum*

Three fungal isolates, TSH-R16998, TSH-R16999, and TSH-R18187, from *V. cracca* could infect and sporulate on *V. cracca* and *P. sativum*; TSH-R16998 and TSH-R18187 could also infect and sporulate on *V. faba*; and TSH-R18187 could also infect and sporulate on *V. amoena*. One fungal isolate, TSH-R18185, from *V. unijuga* could infect and sporulate on *V. unijuga* and *P. sativum*. However, urediniospores of four fungal isolates from wild *Vicia* were also pathogenic to cultivated *P. sativum* (Table 4).

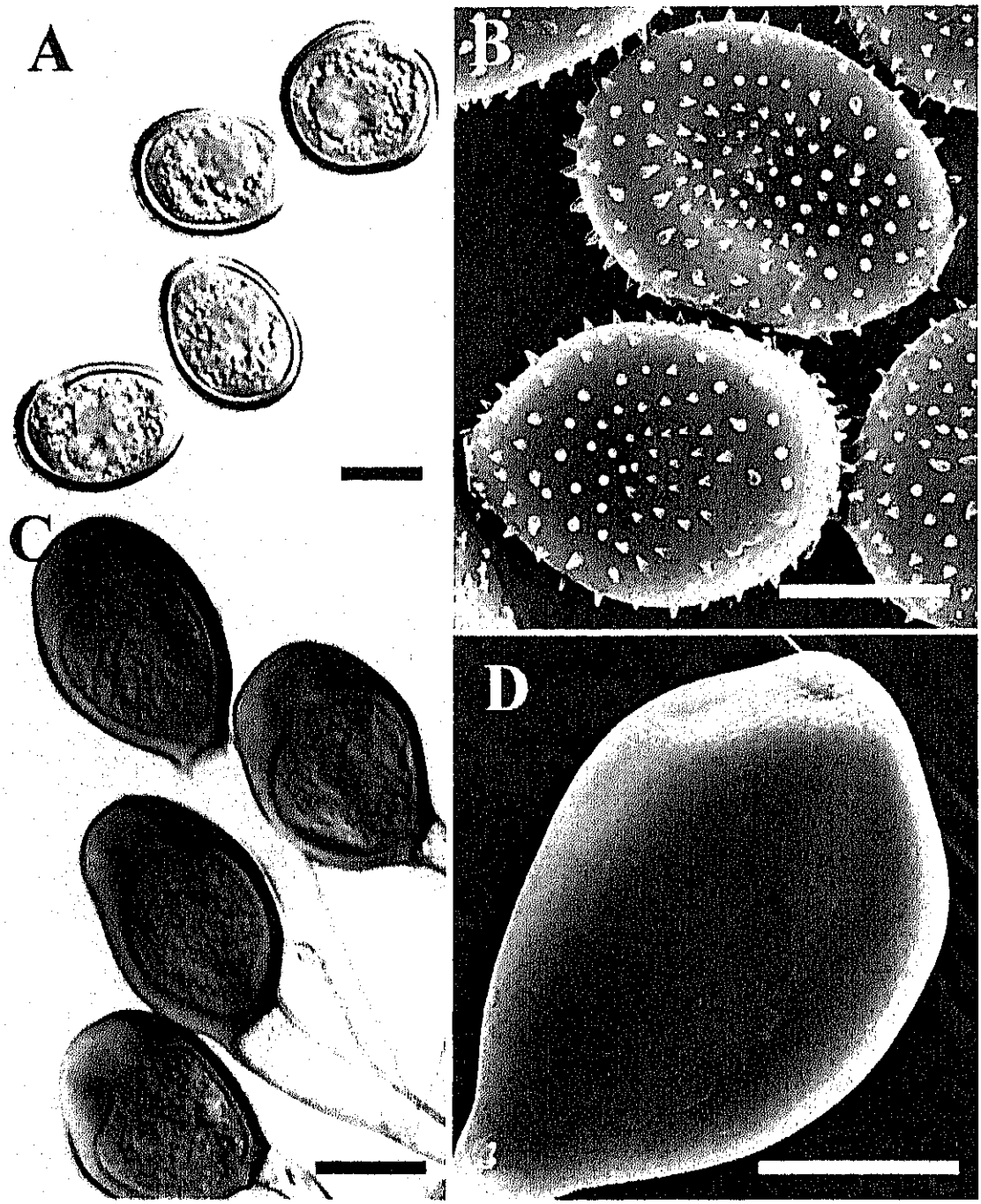

Fig. 1. *Uromyces viciae-fabae* (TSH-R18185) on *Vicia unijuga*. **A,B** Urediniospores. **C,D** Teliospores. *Bars* **A–D** 10 μm

Discussion

Uromyces viciae-fabae populations in Japan on *Vicia*, *Lathyrus*, and *Pisum* had morphologically similar urediniospores and teliospores. Likewise, molecular phylogenetic analyses revealed that specimens used for LSU rDNA D1/D2 and ITS regions analyses formed a single clade. Also, *Uromyces* isolates on wild *Vicia* showed the same pathogenicity on cultivated *P. sativum*, and the variations in virulence on *Vicia*, *Lathyrus*, and *Pisum* are recognized in our inoculation results.

In Japan, a rust on *V. unijuga* was first stated to have urediniospores with the wall 2–3 μm thick, thicker than those rusts on most other *Vicia*, *Lathyrus*, *and Pisum* (1.5–2.5 μm thick), and the fungus was classified into *U. orobi* (= *U. viciae-fabae* var. *orobi*) (Ito 1922). Hiratsuka (1933) stated that the rust on *V. nipponica* var. *capitata* and *L.*

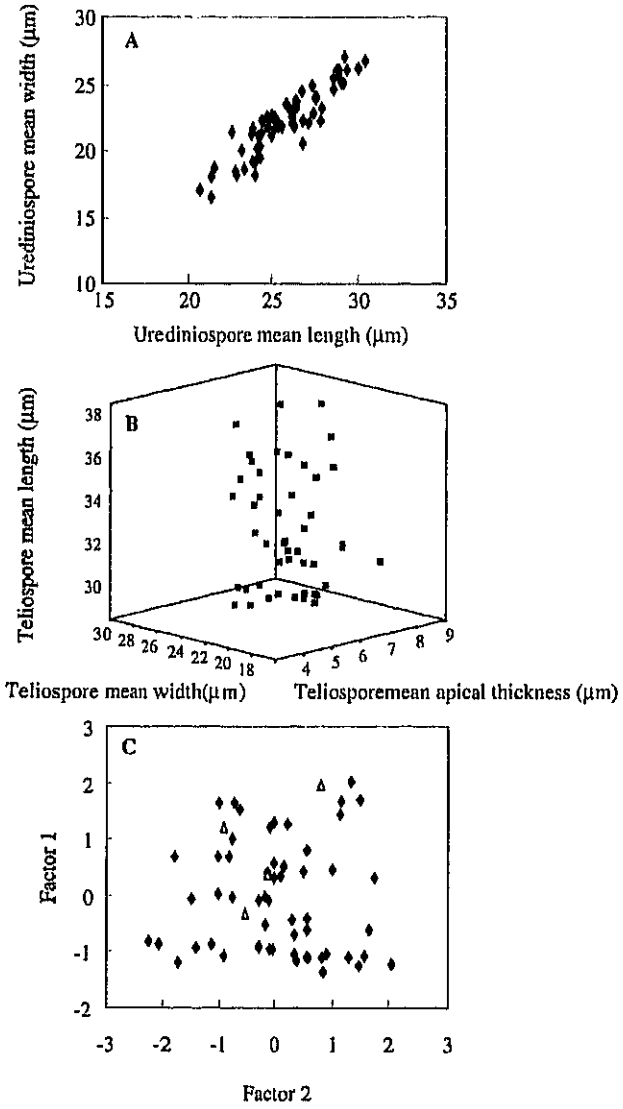

Fig. 2. Variations in characteristics of urediniospores and teliospores on *Vicia*, *Lathyrus*, and *Pisum*. **A** Urediniospore mean lengths against urediniospore mean widths. **B** Correlation of teliospore mean lengths, widths, and apical thickness. **C** Results of a principal component analysis of urediniospores and teliospores (△, specimens on *Vicia unijuga*, *V. nipponica* var. *capitata*, and *Lathyrus davidii*)

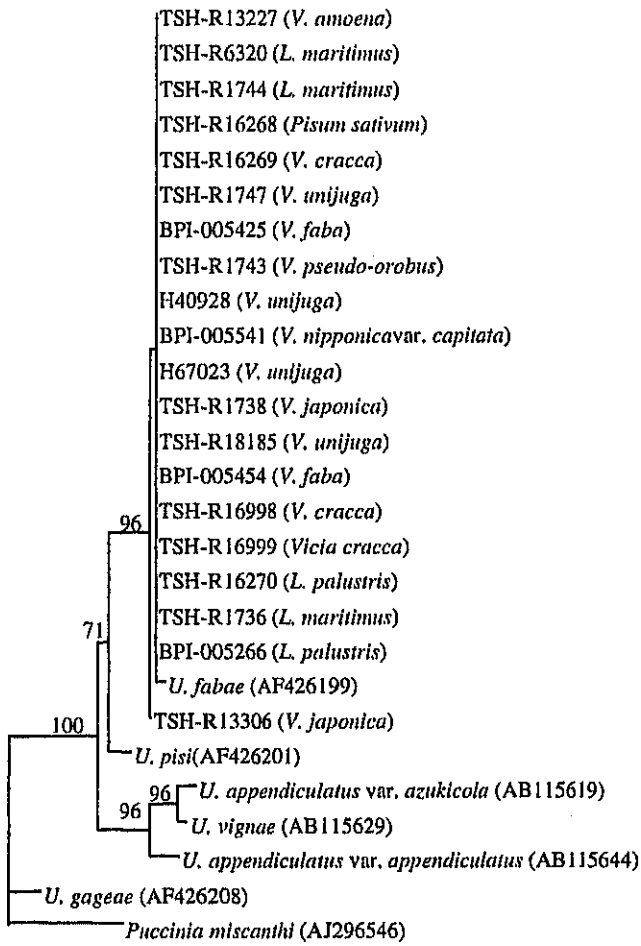

Fig. 3. A neighbor-joining tree inferred from sequences of large subunit (LSU) rDNA (D1/D2) regions using Clustal X. *Values above the branches* indicate percentage bootstrap support for 1000 resamplings. *Length of branches* is proportional to number of base changes, indicated by the *scale at bottom*

davidii also had thicker urediniospore walls and could be distinguished from *U. fabae*. Hiratsuka (1973) and Hiratsuka et al. (1992) agreed with Jørstad's taxonomic system and transferred *U. fabae* and *U. orobi* into *U. viciae-fabae* var. *viciae-fabae* and *U. viciae-fabae* var. *orobi*, respectively, because these fungi resemble each other except for the urediniospore wall thickness and putative host specificity (Hiratsuka 1973; Hiratsuka et al. 1992). Our morphological observations revealed that urediniospores and teliospores on *Vicia*, *Lathyrus*, and *Pisum* are similar. Similarly, principal component analyses did not show significant variation among these specimens. Although only six specimens of rust on *V. unijuga*, one on *V. nipponica* var. *capitata*, and two on *L. davidii* were measured in this study, statistical analyses showed that these specimens could not be grouped separately from other rust specimens on *Vicia*, *Lathyrus*, and *Pisum*. We do not consider variation in urediniospore wall thickness to be a useful and stable character to separate var. *viciae-fabae* and var. *orobi* in Japan.

Twenty-three rust specimens on *Vicia*, *Lathyrus*, and *Pisum* were used for molecular phylogenetic analyses. The analyses revealed that the 23 specimens have high homology values based on the D1/D2 and ITS regions. For D1/D2 analysis, homology of these specimens ranged from 95 to 100, and they formed a single genetic clade. Likewise, homology of ITS regions ranged from 97 to 100, and also formed a single genetic clade. Parsimony analysis of the two regions showed the same genetic tree as the NJ analysis (tree not showed). Moreover, *Uromyces* on *V. unijuga* and *V. nipponica* var. *capitata* did not have a significant genetic difference from other analyzed specimens based on D1/D2 and ITS regions. Furthermore, we also analyzed *U. fabae*

Table 4. Results of inoculation experiments on *Vicia*, *Lathyrus*, and *Pisum*

Plants inoculated	Voucher specimen number			
	TSH-R16999[b]	TSH-R16998[b]	TSH-R18187[b]	TSH-R18185[c]
Vicia faba	−[a]	+	+	−
V. cracca	+[a]	+	+	−
V. amoena	−	−	+	−
V. unijuga	−	−	−	+
Lathyrus maritimus	−	−	−	−
L. palustris	−	−	−	−
Pisum sativum	+	+	+	+

[a] −, no infection; +, uredinium production
[b] *Uromyces* isolates from *V. cracca*
[c] *Uromyces* isolate from *V. unijuga*

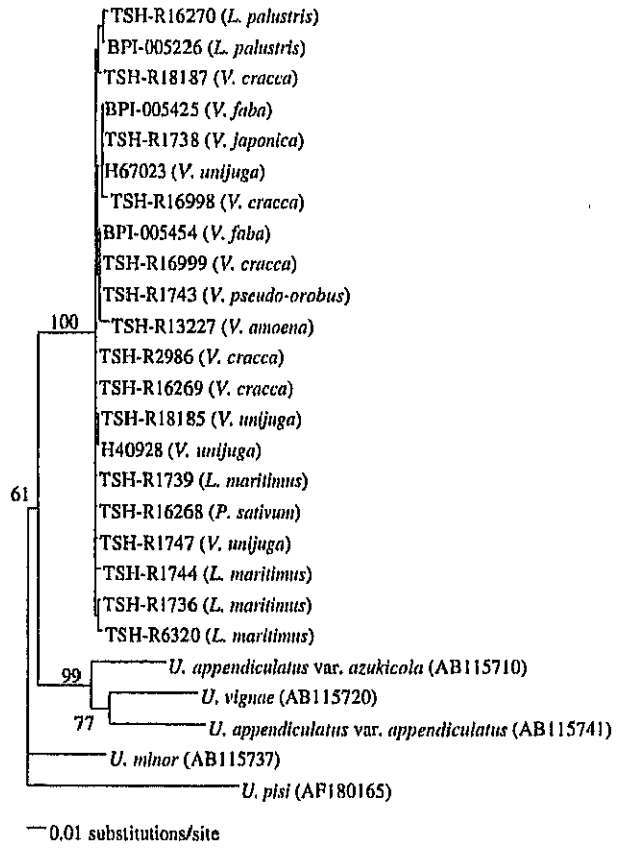

Fig. 4. A neighbor-joining tree inferred from sequences of internal transcribed spacer (ITS) and 5.8S rDNA regions using Clustal X. *Values above the branches* indicate percentage bootstrap support for 1000 resamplings. *Length of branches* is proportional to number of base changes, indicated by the *scale at bottom*

(GenBank: AF426199), *U. appendiculatus* var. *appendiculatus* (GenBank: AB115644, AB115741), *U. appendiculatus* var. *azukicola* (GenBank: AB115619, AB115710), *U. vignae* (GenBank: AB115629, AB115720), *U. pisi* (GenBank: AF426201, AF180165), *U. gageae* (GenBank: AF426208), and *P. miscanthi* (GenBank: AJ296546). Results revealed that our specimens formed a single genetic clade with *U. fabae* (GenBank). Our specimens are closely related to other *Uromyces* and distant from *Puccinia*, inferred from D1/D2 regions. Moreover, *Uromyces* on *Vicia*, *Lathyrus*, and *Pisum* are closely related genetically with *U. appendiculatus* and *U. vignae*, based on molecular phylogenetic analysis (see Figs. 3, 4).

For host specificity, Hiratsuka (1973) and Hiratsuka et al. (1992) reported var. *orobi* only on *V. unijuga*, *V. nipponica* var. *capitata*, and *L. davidii*. Historically, Gäumann (1934) stated that *Uromyces* on the three host plants were formae speciales of *U. fabae*. However, the host ranges of *U. fabae* and *U. orobi* are obscure (El-Gazzar 1981). Our inoculation results revealed that three *Uromyces* isolates from *V. cracca* showed variation in virulence on different *Vicia* species and *P. sativum*. We repeated the inoculation of urediniospores from *V. unijuga* onto *P. sativum*, then inoculated urediniospores from *P. sativum* onto *V. unijuga*. The cross-inoculations were successful (data not showed). However, the inoculation results suggest that host specificity is also not an appropriate characteristic for classification of our specimens.

Morphological observations, inoculation experiments, and molecular phylogenetic analyses revealed that *U. viciae-fabae* did not show significant variation among our specimens on *Vicia*, *Lathyrus*, and *Pisum* in Japan. According to these results, we suggest that *U. viciae-fabae* var. *viciae-fabae* and var. *orobi* should be included in *U. viciae-fabae* (= *U. fabae*).

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References

Ayliffe MA, Dodds PN, Lawrence GJ (2001) Characterisation of a β-tubulin gene from *Melampsora lini* and comparison of fungal β-tubulin genes. Mycol Res 105:818–826

Bruns TD, Fogel R, Taylor JW (1990) Amplification and sequencing of DNA from fungal herbarium specimens. Mycologica 82:175–184

Bruns TD, White TJ, Taylor JW (1991) Fungal molecular systematics. Annu Rev Ecol Syst 22:525–564

Cummins GB (1978) Rust fungi on legumes and composites in North America. University of Arizona Press, Tucson

de Jong SN, Levesque A, Verkley GJM, Abeln ECA, Rahe JE, Braun PG (2001) Phylogenetic relationships among *Neofabraea* species causing tree cankers and bull's-eye rot of apple based on DNA sequencing of ITS nuclear rDNA, mitochondrial rDNA, and the β-tubulin gene. Mycol Res 105:658–559

Duke JA (1981) Handbook of legumes of world economic importance. Plenum Press, New York

El-Gazzar A (1981) Systematic implications of susceptibility to *Uromyces* rusts in Leguminosae. In: Polhill RM, Raven PH (eds) Advances in legume systematics. HMSO, London, pp 979–994

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Foster LM, Kozak K, Loftus MG, Stevens J, Ross IK (1993) The polymerase chain reaction and its application to filamentous fungi. Mycol Res 97:779–781

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Gäumann VE (1934) Zur Kenntnis des *Uromyces fabae* (Pers.) de By. Ann Mycol 32:464–470

Hiratsuka N (1933) Studies on *Uromyces fabae* and its related species. Jpn J Bot 6:331–379

Hiratsuka N (1973) Revision of taxonomy of the genus *Uromyces* in the Japanese Archipelago. Rep Tottori Mycol Inst 10:1–98

Hiratsuka T, Sato S, Kakishima M, Kaneko M, Sato S, Hiratsuka T, Katsuya K, Hiratsuka Y, Ono Y, Harada Y, Nakayama K (1992) The rust flora of Japan. Tsukuba Shuppankai, Ibaraki

Ito S (1922) A preliminary report on the Japanese species of Uromyces. Ann Mycol 20:81–85

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Lee SB, Taylor JW (1990) Isolation of DNA from fungal mycelia and single spore. In: Gelfrand MA, Sninsky DH, White J (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 282–287

O'Donnell K (1993) *Fusarium* and its near relatives. In: Reynolds DR, Taylor JW (eds) The fungal holomorph: mitotic, meiotic, and pleomorphic speciation in fungal systematics. CAB International, Wallingford, pp 225–236

Ohwi J, Kitagawa M (1992) New flora of Japan revised (in Japanese). Shibundo, Tokyo

Pfunder M, Schürch S, Roy BA (2001) Sequence variation and geographic distribution of pseudoflower-forming rust fungi (*Uromyces pisi* s. lat.) on *Euphorbia cyparissias*. Mycol Res 105:57–66

Piepenbring M, Begerow D, Oberwinkler F (1999) Molecular sequence data assess the value of morphological characteristics for a phylogenetic classification of species of *Cintractia*. Mycologia 9:485–489

Rambaut A (2000) Se-Al: sequence alignment editor. Department of Zoology, University of Oxford, Oxford

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sato S, Koto M, Doi Y (1983) Laboratory guide for plant pathology (in Japanese). Kodansha, Tokyo

Suyama Y, Kawamuro K, Kinoshita I, Yoshimura K, Tsumura Y, Takahara H (1996) DNA sequence from a fossil pollen of *Abies* spp. from Pleistocene peat. Genes Genet Syst 71:145–149

Swofford DL (1999) PAUP*: phylogenetic analysis using pasimony (* and other methods). Version 4. Sinauer, Sunderland, MA

Tehler A, Farris JS, Lipscomb DL, Kallersjo M (2000) Phylogenetic analyses of the fungi based on large rDNA data sets. Mycologia 92:459–474

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Virtudazo EV, Nakamura H, Kakishima M (2001) Phylogenetic analysis of sugarcane rusts based on sequences of ITS, 5.8S rDNA and D1/D2 regions of LSU rDNA. J Gen Plant Pathol 67:28–36

Vogler DR, Bruns TD (1998) Phylogenetic relationships among the pine stem rust fungi (*Cronatium* and *Peridermium* spp.). Mycologia 90:224–257

White TJ, Bruns T, Lee SB, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand M, Sninsky D, White T (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

Wilson M, Henderson DM (1966) British rust fungi. Cambridge University Press, London

Zambino PJ, Szabo LJ (1993) Phylogenetic relationships of selected cereal and grass rusts based on rDNA sequence analysis. Mycologia 85:401–414

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FULL PAPER

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Morphological and molecular phylogenetic analysis of *Melampsora* species on poplars in China

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Abstract Many species of *Melampsora* on *Populus* have been reported in China, based on morphological characteristics of both uredial and telial states, and on host species, but their morphology and taxonomy are still poorly defined. In this study, 196 specimens representing *Melampsora* species on poplars and collected from various areas of China were used for morphological observations. The morphological characteristics of urediniospores and teliospores were examined with light and scanning electron microscopy. The specimens could be classified into five groups based on their morphology. For the sequencing of the nuclear large subunit rDNA (D1/D2), 5.8S rDNA and their internal transcribed spacers, ITS1 and ITS2 region, 54 specimens were selected from the specimens used in morphological observations. These specimens were separated into six clades by phylogenetic analyses of the D1/D2 and ITS regions. Correlations among morphological groups and phylogenetic clades based on these results suggest a revision of these species. In particular, no evidence to discriminate specimens of *M. acedioides*, *M. magnusiana*, and *M. rostrupii* was found from either morphological characteristics or sequence analysis.

Key words *Melampsora* · Phylogeney · *Populus* · Rust fungus · Taxonomy · Uredinales

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Introduction

Since the genus *Melampsora* was established by Castagne in 1843 based upon *M. euphorbiae* (Schub.) Cast., about 90 species, showing either an autoecious or a heteroecious life cycle pattern, have been described worldwide (Kirk et al. 2001). Most of these occur on poplars and willows. Shang et al. (1986b) examined 34 species of poplar rusts reported in the world by using the host and characteristics of uredinia and telia and recognized 12 species. Dai (1989) studied species of *Melampsora* on poplars using 24 characters from urediniospores and teliospores and the aecial and telial hosts by the numerical taxonomic method. He reported 14 species. Bagyanarayana (1998) studied the morphology of *Melampsora* species on *Populus* species and recognized 9 species and 5 f. sp. under *M. populnea* (Pers. ex Pers.) Karst. After this, Cellerino (1999) listed 14 species of *Melampsora* on poplars. However, they are not definitive studies, and the taxonomy of *Melampsora* on various poplar species is not clear at the present time.

Poplar rusts caused by *Melampsora* spp. is one of the most important tree diseases in China. Five species, *M. larici-populina* Kleb., *M. laricis* Hart., *M. magnusiana* Wagn., *M. rostrupii* Wagn., and *M. pruinosae* Tranz., have been reported by Tai (1979). Yuan (1984) reported another three species, *M. abietis-canadensis* (Farl.) Ludw., *M. allii-populina* Kleb., and *M. occidentalis* Jacks. Shang et al. (1986a) described a new species, *M. multa* Shang, Pei & Yuan on *P.* × *euramericana* Moench., and they recognized *M. magnusiana* and *M. rostrupii* as a synonym of *M. aecidioides* Plowr. (Shang et al. 1990). The species *M. abietis-populi* Imai occurring on *Populus wilsonii* Schneid. in Shaanxi and Hubei Provinces was reported recently (Guo

1989; Zhang et al. 1997; Cao and Li 1999). Previously, it was reported only from Japan (Hiratsuka et al. 1992; Bagyanarayana 1998). Zhuang and Wei (1994) reported *M. populnea* on *Populus pseudoglauca* Wang & Fu. and *P. szechuanica* Schneid. var. *tibetica* Schneid. for the first time in China. Although these species are classified mainly by morphology of urediniospores and teliospores and by host range, identification is very difficult for several reasons. Almost all morphological characteristics of teliospores are very similar, and teliospores of some species do not appear during the growing season. Most species infecting poplars are heteroecious, with species of *Abies*, *Allium*, *Arum*, *Chelidonium*, *Corydalis*, *Fumaria*, *Larix*, *Mercurialis*, *Papaver*, *Picea*, *Pinus*, and *Tsuga* as secondary hosts, but characteristics on these secondary hosts are not useful for identification; also, some species overwinter as mycelia in the dormant buds of *Populus* without going to the alternate hosts, and some rust species may occur on the same aecial hosts. Although telial host range often is used to distinguish the *Melampsora* species, the same species of *Populus* can be infected by different *Melampsora* species. Nonhost poplars can be infected when artificial inoculations by urediniospores. Therefore, the morphology and taxonomy of *Melampsora* species on poplars are still confused.

Recently, molecular methods have been developed to clarify genetic variation and phylogenetic relationships of rust fungi (Nakamura et al. 1998; Vogler and Bruns 1998; Edwards et al. 1999; Newcombe et al. 2000; Ayliffe et al. 2001; Virtudazo et al. 2001; Hantula et al. 2002; Maier et al. 2003; Weber et al. 2003). The purpose of this study is to analyze morphological characteristics and phylogenetic relationships of *Melampsora* species on poplars in China, including *M. larici-populina*, *M. laricis*, *M. magnusiana* (=*M. aecidioides*), *M. rostrupii*, *M. pruinosae*, *M. allii-populina*, *M. multa*, *M. abietis-populi*, and *M. populnea*.

Materials and methods

Morphological observations

One-hundred ninety-six *Melampsora* specimens from 14 provinces of China were used for morphological analyses (Table 1). These specimens have been kept in the following herbaria: the Mycological Herbarium of College of Forestry, Northwest Sci-Tech University of Agriculture and Forestry, China (HMNWFC); the Mycological Herbarium of Institute of Microbiology, Chinese Academy of Sciences (HMAS); the Herbarium of College of Forestry, Inner Mongolia Agricultural University, China (HIM); and the Mycological Herbarium of Institute of Agriculture and Forestry, University of Tsukuba, Japan (TSH).

Table 1. *Melampsora* specimens on *Populus* species used for morphological observations

Section of *Populus*	Species of host plants	Localities[a] (no. of specimens)
Leuce	*P. adenopoda*	Yunnan (3)
	P. alba	Xinjiang (10), Gansu (1)
	P. alba var. *pyramidalis*	Xinjiang (4), Gansu (2), Inner Mongolia (4), Shaanxi (2)
	P. davidiana	Shaanxi (2), Beijing (2), Heilongjiang (5), Tibet (1), Inner Mongolia (3), Jilin (2)
	P. hopeiensis	Inner Mongolia (1), Shaanxi (1)
	P. rotundifolia	Yunnan (1)
	Populus sp.	Shaanxi (1)
	P. tomentosa	Beijing (2), Jilin (4), Shaanxi (12), Henan (2)
	P. tremula	Xinjiang (4)
Leucoides	*P. pseudoglauca*	Tibet (4)
	P. wilsonii	Shaanxi (3)
Aigeiros	*P. berolinensis*	Inner Mongolia (2)
	P. × *beijingensis*	Liaoning (1), Jilin (2)
	P. × *canadensis*	Jilin (13), Liaoning (2), Hebei (1), Shaanxi (3)
	P. deltoides × *P. lasiocarp*	Shaanxi (1)
	P. nigra	Shaanxi (1), Heilongjiang (2)
	P. nigra var. *italica*	Jilin (13), Shaanxi (2), Qinghai (1)
	P. nigra var. *thevestina*	Shaanxi (1)
	P. nigra × *P. laurifolia*	Xinjiang (1)
Tacamahaca	*P. cathayana*	Gansu (2), Shaanxi (4), Inner Mongolia (2), Qinghai (1)
	P. laurifolia	Xinjiang (8)
	P. maximoniicizii	Inner Mongolia (1)
	P. opera	Inner Mongolia (2)
	P. purdomii	Shaanxi (4)
	P. pseudo-simonii × *P. deltoids*	Shaanxi (1)
	P. popularis	Shaanxi (1)
	P. simonii	Inner Mongolia (5), Jilin (9), Shaanxi (4)
	P. simonii var. *shomliftia*	Inner Mongolia (1)
	Populus sp.	Jilin (4), Xinjiang (1), Inner Mongolia (2), Shaanxi (4)
	P. szechuanica	Shaanxi (1)
	P. talassica	Xinjiang (1)
	P. yunnanensis	Yunnan (8)
Turanga	*P. euphratica*	Inner Mongolia (5), Xinjiang (2), Ningxia (1)

[a]Provinces of China

Urediniospores and teliospores from specimens were mounted in a drop of lactophenol solution. About 30–50 spores from each specimen were randomly chosen and observed under a BH 100 microscope (Olympus, Tokyo, Japan). Length, width, wall thickness of both apex and lateral, and distance between spines of urediniospores were measured with a Q-Win Image Analyzer (Leica, Tokyo, Japan). Statistics, including multivariate analyses of measured continuous numerical variables, were performed using the software package SPSS (SPSS Japan, Tokyo, Japan) run on Windows 2000 Professional. Discrete numerical or qualitative attributes or host species were superimposed on two- or three-dimensional scatter diagrams generated from the analyses to detect possible groups.

The surface features of urediniospores and teliospores were observed by scanning electron microscopy (SEM). For SEM, samples were coated with platinum-palladium and were observed with a S-4200 scanning electron microscope (Hitachi, Tokyo, Japan) operated at 15 kV.

Polymerase chain reaction amplification and sequencing of D1/D2 and internal transcribed spacer regions

Fifty-four specimens of *Melampsora* on poplars were selected from the specimens used in morphological observations and for molecular phylogenetic analysis (Table 2). DNA was extracted from about 100–200 urediniospores obtained from a single uredinium and teliospores obtained from a single telium. Spores were crushed between two sterile glass slides and suspended in 20 µl extraction buffer containing 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% Proteinase K, and 0.01% sodium dodecyl sulfate (SDS), then incubated first at 37°C for 60 min and then at 95°C for 10 min (Suyama et al. 1996; Virtudazo et al. 1998). From these crude extracts, a 5 µl aliquot was used directly for PCR amplification.

Amplifications were done using 40 µl polymerase chain reactions (PCR) each containing 0.2 µM primer, 1 unit of TaKaRa Taq DNA polymerase, a commercial deoxynucleoside triphosphate (dNTP) mixture (containing 2.5 mM of each dNTP), and Taq reaction buffer (containing 2 mM Mg^{2+}). PCR was carried out using a GeneAmp PCR System 9700 (Applied Biosystems, CA, USA) under the following conditions: 95°C for 3 min, then 35 cycles of 95°C for 30 s, 55°C for 1 min, 72°C for 1 min, and a final step of 72°C for 10 min. PCR of the D1/D2 region of nuclear large subunit rDNA was done using the primer pair NL1 (5'-GCATATC AATAAGCGGAGGAAAAG) and NL4 (5'-GGTCCGT GTTTCAAGACGG) (O'Donnell 1993). The internal transcribed spacer (ITS) and 5.8S region of rDNA was amplified with primers ITS1F (5'-CTTGGTCATTTAGA GGAAGTAA) (Gardes and Bruns 1993) and ITS4 (5'-TCCTCCGCTTATTGATATGC) (White et al. 1990). After amplification, 3 µl aliquots of the reaction products were electrophoresed on 1% (w/v) agarose gel containing 0.5 µg/ml ethidium bromide in TAE buffer [40 mM Tris-HCl, 20 mM sodium acetate, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4].

PCR products were purified by using MicroSpin S-400 HR columns (Amersham Pharmacia Biotech, NJ, USA). The purified PCR products were sequenced directly using a Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) with the same primers used for PCR. The reaction was set up as 25 cycles of 96°C for 10 s, 50°C for 5 s, 60°C for 4 min. The resulting fragments were finally purified using Centri-Sep spin columns CS-901 (Princeton Separations, Adelphia, NJ, USA) and loaded onto the sequencing gel. Data were collected using an ABI 377 Automated DNA Sequencer (PE Applied Biosystems).

Phylogenetic analysis

DNA sequences alignment was performed using the CLUSTAL X multiple alignment program, version 1.8 (Thompson et al. 1997). Further manual alignment was done in Se-Al v2.07a (Rambaut 2001). Alignment gaps were treated as a "fifth characters" base in the analysis. Phylogenetic trees were constructed using PAUP* v4.0b10 (Swofford 2002) with the neighbor-joining method (Saitou and Nei 1987) from LSU rDNA and ITS1–5.8S–ITS2 (ITS region) sequences. Neighbor-joining (NJ) analysis of a distance matrix produced by the Kimura two-parameter model (Kimura 1980) with a transition:transversion rate of 2.0 was applied with the default parameters of the program. Bootstrap analysis was based on 1000 bootstrap replicates using the NJ option for NJ trees (Felsenstein 1985).

Results

Morphology

All specimens observed could be divided into five groups based on position of sori in leaves, shape, size, and wall thickness of urediniospores, and distance between spines on the surface of urediniospores (Table 3). Group I and II differ from other in the smooth apex and length of urediniospores (>25 µm; Fig. 1A). Group I has the greatest difference in the laterally thickened urediniospore walls (=5.1 µm) with group II (=2.8 µm) and other groups, and all specimens of group I were clearly separated from other groups, as seen in the scatter plot (Fig. 1B). There was greater divergence between group V (= 1.1 µm) and other groups (>1.5 µm) in distance between spines of urediniospores (Fig. 1C). In addition, group V differs from group III and IV in the amphigenous telia and uredinia, and this group only occurred on sections of *Turanga* (Table 3). Group IV is similar to group III in shape, size, and distance between spines of urediniospores, but differs in having thin urediniospore wall (up to 2.7 µm) and the host plants of group III in section *Leuce* of *Populus*. Morphological characteristics of these groups were as follows.

Group I. Telia mainly epiphyllous (Fig. 2-1), small, single or coalescing in groups, light brown. Uredinia hypophyllous or rarely epiphyllous, especially in heavy infections.

Table 2. Specimens of *Melampsora* species on *Populus* species and their GenBank accession numbers used for phylogenetic analysis

Host plants	Localities[a]	Year and collector	Voucher specimens[b]	GenBank accession no.	
				D1/D2	ITS
P. adenopoda	Yunnan	1985, J.Y. Zhuang	TSH-R04133 (HMAS50159)	AB116807	AB116865
P. adenopoda	Yunnan	1985, J.Y. Zhuang	TSH-R04134 (HMAS50160)	AB116808	AB116866
P. adenopoda	Yunnan	1998, M. Kakishima	TSH-R20045	AB116819	AB116825
P. alba	Xinjiang	1966, Z.K. Liu	TSH-R04125 (HMAS37769)	AB116811	AB116854
P. alba	Xinjiang	1984, C.L. Wang	TSH-R04126 (HMAS58560)	AB116810	AB116855
P. alba	Xinjiang	1982, Z.K. Liu	HMNWFC-T041	AB116814	AB116850
P. alba var. *pyramidalis*	Xinjiang	1986, J.Y. Zhuang	TSH-R04136 (HMAS52886)	AB116813	AB116857
P. alba var. *pyramidalis*	Gansu	2001, C.M. Tian and Y.M. Liang	HMNWFC-T021 (TSH-R16945)	AB116815	AB116843
P. alba var. *pyramidalis*	Inner Mongolia	2001, C.M. Tian and Y.Z. Shang	HMNWFC-T022 (TSH-R16946)	AB116818	AB116849
P. alba var. *pyramidalis*	Xinjiang	1974, Z.Y. Zhao	TSH-R04129 (HMAS58565)	AB116817	AB116844
P. alba var. *pyramidalis*	Xinjiang	1983, C.L. Wang	TSH-R04130 (HMAS58578)	AB116812	AB116856
P. berolinensis	Inner Mongolia	1993, Z.S. Hou	HMNWFC-T008	AB116786	AB116830
P. cathayana	Gansu	2000, C.M. Tian	HMNWFC-T003 (TSH-R16927)	AB116769	AB116828
P. × *canadensis*	Jilin	2001, Q. Wang	TSH-R16983	AB116778	AB116840
P. davidiana	Tibet	1983, J.Y. Zhuang	TSH-R4149 (HMAS46905)	AB116809	AB116867
P. davidiana	Inner Mongolia	1991, Y.Z. Shang	HMNWFC-T033	AB116804	—
P. davidiana	Inner Mongolia	1994, Y.Z. Shang	HMNWFC-T038	AB116805	AB116863
P. euphratica	Ningxia	1980, N.X. Tian	HMAS49649	AB116793	AB116862
P. euphratica	Inner Mongolia	1992, Y.Z. Shang	HMNWFC-T036	AB116795	AB116858
P. euphratica	Inner Mongolia	1992, Y.Z. Shang	HMNWFC-T073	AB116794	AB116861
P. euphratica	Inner Mongolia	1992, Y.Z. Shang	HMNWFC-T045	AB116792	AB116859
P. euphratica	Inner Mongolia	1993, Y.Z. Shang	HMNWFC-T046	AB116796	AB116860
P. hopeiensis	Inner Mongolia	1992, Y.Z. Shang	HMNWFC-T031	AB116816	AB116846
P. laurifolia	Xinjiang	1984, Z.K. Liu	HMNWFC-T040	AB116788	AB116835
P. laurifolia	Xinjiang	1986, J.Y. Zhuang	TSH-R04141 (HMAS52890)	AB116801	AB116875
P. laurifolia	Xinjiang	1986, J.Y. Zhuang	TSH-R04139 (HMAS52888)	—	AB116873
P. laurifolia	Xinjiang	1986, J.Y. Zhuang	TSH-R04140 (HMAS52889)	AB116800	AB116874
P. laurifolia	Xinjiang	1986, J.Y. Zhuang	TSH-R04138 (HMAS52892)	AB116803	AB116872
P. maximowiczii	Inner Mongolia	1993, Z.S. Hou	HMNWFC-T013	AB116776	AB116832
P. nigra var. *italica*	Jilin	2001, Q. Wang	TSH-R16975	AB116784	AB116836
P. nigra var. *italica*	Jilin	2001, Q. Wang	TSH-R16978	AB116771	AB116837
P. nigra var. *italica*	Jilin	2001, Q. Wang	TSH-R16980	AB116783	AB116842
P. opera	Inner Mongolia	2001, C.M. Tian and Y.Z. Shang	HMNWFC-T002 (TSH-R16926)	AB116774	AB116827
P. opera	Inner Mongolia	1992, Y.Z. Shang	HMNWFC-T015	AB116787	AB116833
P. pseudoglauca	Tibet	1990, J.Y. Zhuang	HMAS67387	AB116798	—
P. pseudoglauca	Tibet	1990, J.Y. Zhuang	HMAS67388	AB116797	AB116868
P. purdomii	Shaanxi	1999, C.M. Tian	HMNWFC-T004 (TSH-R16928)	AB116779	AB116829
P. popularis	Shaanxi	2000, C.M. Tian and Y.M. Liang	HMNWFC-T001 (TSH-R16925)	AB116770	AB116826
P. simonii	Inner Mongolia	1994, Z.S. Hou	HMNWFC-T017	AB116785	AB116834
P. simonii	Jilin	2001, Q. Wang	TSH-R16977	AB116781	AB116838
P. simonii	Jilin	2001, Q. Wang	TSH-R16979	AB116782	AB116839
P. simonii	Jilin	2001, Q. Wang	TSH-R16981	AB116775	AB116841
P. simonii var. *shomliflia*	Inner Mongolia	1994, Y.Z. Ren	HMNWFC-T011	Ab116772	AB116831
P. tomentosa	Shaanxi	1973, J. Xu and T.Z. Wang	HMAS56276	AB116822	AB116847
P. tomentosa	Shaanxi	2003, C.M. Tian	HMNWFC-T075	AB116791	AB116851
P. tomentosa	Shaanxi	2000, C.M. Tian	HMNWFC-T023 (TSH-R16947)	AB116780	AB116848
P. tomentosa	Jilin	2001, Q. Wang	TSH-R16987	AB116806	AB116864
P. tomentosa	Shaanxi	1978, Y. Jing	HMNWFC-T025	AB116777	AB116845
P. talassica	Xinjiang	1981, Z.K. Liu	HMNWFC-T035	AB116802	AB116871
P. tremula	Xinjiang	1981, M. Shi	HMNWFC-T043	AB116789	AB116852
P. tremula	Xinjiang	1981, J. Lan	HMNWFC-T044	AB116790	AB116853
P. wilsonii	Shaanxi	1994, N. Zhang	HMAS55410	AB116799	AB116870
P. wilsonii	Shaanxi	1996, Z.M. Cao	HMNWFC-TR0009	—	AB116869
P. yunnanensis	Yunnan	1998, M. Kakishima	TSH-R20046	AB116821	AB116823
P. yunnanensis	Yunnan	1998, M. Kakishima	TSH-R20042	AB116820	AB116824

ITS, internal transcribed spacer

[a]Provinces of China

[b]HMAS, the Mycological Herbarium of Institute of Microbiology, Chinese Academy of Sciences; HMNWFC, the Mycological Herbarium of College of Forestry, Northwest Sci-Tech University of Agriculture and Forestry, China; TSH, the Mycological Herbarium of Institute of Agriculture and Forestry, University of Tsukuba, Japan

Table 3. Morphological groups of *Melampsora* species on *Populus* species

Groups	Position of uredinia	Urediospores				Smooth parts	Position of telia	Sect. of host plants	No. of specimens
		Shape	Size (average) (μm)	Wall equatorial part thickness (average) (μm)	Distance between spines (average) (μm)				
I	Hypophyllous	Ellipsoid Oblong	20.5–54.5 × 11.3–29.8 (34.0) (18.4)	1.3–12.4 (5.1)	1.1–4.6 (2.3)	Apex	Epiphyllous	*Tacamahaca* *Aigeiros* *Leucoides*	103
II	Amphigenous	Clavoid Oblong	20.7–40.1 × 10.7–23.7 (29.4) (15.9)	1.3–6.8 (2.8)	1.3–3.5 (2.2)	Apex	Amphigenous	*Tacamahaca*	9
III	Hypophyllous	Globose Ovate	13.3–33.3 × 12.2–25.6 (21.8) (16.7)	1.1–5.0 (2.8)	1.1–4.4 (2.7)	Absent	Hypophyllous	*Leuce* *Leuce*	69
IV	Hypophyllous	Globose Oblong Ovate	17.7–27.9 × 9.2–23.4 (22.7) (16.0)	0.8–2.7 (1.8)	0.9–2.4 (1.6)	Absent	Hypophyllous	*Leucoides* *Tacamahaca*	7
V	Amphigenous	Globose Ellipsoid	19.2–32.1 × 14.8–24.6 (24.3) (19.9)	2.4–5.7 (3.5)	0.7–1.4 (1.1)	Absent	Amphigenous	*Turanga*	8

Urediniospores mostly ellipsoid or oblong, 20.2–54.5 × 11.3–29.8 μm (average, 34.0 × 18.4 μm); walls strongly thickened laterally (average, 5.1 μm, up to 12.4 μm; Fig. 2-2), echinulate except smooth at apex (Fig. 2-3), distance between spines 1.1–4.6 μm (average, 2.3 μm). Host plants in sect. *Tacamahaca*, sect. *Leucoides*, and sect. *Aigeiros* of *Populus* (Table 3). This group differs from other groups in the laterally thickened urediniospore walls (Fig. 1B), longer urediniospores (average length, >30 μm), and the epiphyllous telia.

Group II. Telia amphigenous (Fig. 2-4), small, 0.5–1 mm, single, red-brown. Uredinia amphigenous, orange-yellow. Urediniospores mostly clavoid or oblong, 20.7–40.1 × 10.7–23.7 μm (average, 29.4 × 15.9 μm); walls usually uniformly thick or rarely irregularly thick, 1.3–6.8 μm (average, 2.8 μm; Fig. 2-5). echinulate, except smooth at apex (Fig. 2-6), distance between spines 1.3–3.5 μm (average, 2.2 μm). Host plants in sect. *Tacamahaca* and sect. *Leuce* of *Populus*. This group differs from others in the uniformly thick walls and smooth apex of urediniospores (Table 3).

Group III. Telia mainly hypophyllous (Fig. 2-7), golden to light brown initially, dark reddish-brown to black when mature. Uredinia mostly hypophyllous or rarely epiphyllous. Urediniospores globose, ovate, or elongate and 13.3–33.3 × 12.2–25.6 μm (average, 21.8 × 16.7 μm); walls uniformly thick, 1.1–5.0 μm (average, 2.8 μm; Fig. 2-8), distance between spines 1.1–4.4 μm (average 2.7 μm; Table 3). Host plants of this group are in sect. *Leuce* of *Populus*. This group differs from other groups in urediniospore shape and size (Fig. 2-8; Table 3).

Group IV. Telia hypophyllous (Fig. 2-10), single, red-brown. Uredinia hypophyllous, scattered, small, 0.1–0.5 mm, light yellow. Urediniospores globoid, ellipsoid, or oblong, 17.7–27.9 × 9.2–23.4 μm (average, 22.7 × 16.0 μm); walls uniformly thick, 0.8–2.7 μm (average, 1.8 μm; Table 3, Fig. 2-11), echinulate, distance between spines 0.9–2.4 μm (average, 1.6 μm). Host plants *P. wilsonii* Schneid and *P. pseudoglauca* Wang et Fu of sect. *Leucoides* and *P. yunnanensis* Dode of sect. *Tacamahaca*. This group differs from others in having a thin urediniospore wall.

Group V. Telia amphigenous (Fig. 2-13), single, reddish-brown. Uredinia amphigenous (Fig. 2-13), scattered or coalescing in groups, orange-yellow. Urediniospores globoid or ellipsoid, 19.2–32.1 × 14.8–24.6 μm (average, 24.3 × 19.9 μm); walls uniformly thick (Fig. 2-14), 2.4–5.7 μm (average, 3.5 μm), echinulate, distance between spines 0.7–1.4 μm (average, 1.1 μm). Host plant *P. euphratica* Oliv of sect. *Turanga*. This group differed from other groups in having a small echinulate (Fig. 2-15) and short distance between spines (see Table 3).

Phylogeny

The PCR products of the D1/D2 regions of the LSU rDNA of specimens on *Populus* ranged from 605 to 610 bp in

length. The alignment data matrix consists of 55 taxa and 614 characters, of which 563 were constant and 12 variable characters were parsimony uninformative, leaving 39 informative characters in the analyses. The specimens were separated into six clades (clades A, B, C, D, E, and F) based on the phylogenetic tree using the NJ method when *M. lini* (L20283) and *M. helioscopiae* (AF426197) were used as outgroups (Fig. 3). The consistency index (CI) was 0.791, retention index (RI) was 0.953, and rescaled consistency index (RC) was 0.754.

Clade A, supported by 89% of the bootstrap replicates, included 17 specimens on sections *Tacamahaca* and *Aigeiros* of *Populus*. Specimens belonging to morphological group I were included in this clade. Clade B, supported by 100% of the bootstrap replicates, included 6 specimens on *P. davidiana*, *P. adenopada* and *P. tomentosa* of sect. *Leuce*. Specimens belonging to this clade were included in morphological group III together with clade F. Clade C, supported by 98% of the bootstrap replicates, included 5 specimens on *P. wilsonii*, *P. pseudoglauca* of sect. *Leucoides*, and *P. yunnanensis* of sect. *Tacamahaca* of *Populus* (morphological group IV). Specimens on sections *Tacamahaca* and *Leuce* of *Populus* were included in clade D. Specimens belonging to morphological group II were included in this clade. Clade E, supported by 94% of the bootstrap replicates, included 5 specimens on *P. euphratica* (morphological group V). Specimens on *P. alba*, *P. alba* var. *pyramidalis*, *P. hopeiensis*, *P. tremula*, and *P. tomentosa* of sect. *Leuce* were included in clade F, and they belong to morphological group III.

The ITS rDNA amplification products of specimens on *Populus* ranged from 659 to 679bp in length, included ITS1–5.8S–ITS2 regions. The alignment data matrix consists of 690 characters, of which 558 were constant and 9 variable characters were parsimony uninformative, leaving 123 informative characters in the analyses. The CI was 0.791, RI was 0.968, and RC was 0.766. The NJ tree constructed from ITS and 5.8 S rDNA regions also separated the specimens into six clades (clades A, B, C, D, E, and F) with high bootstrap support when *M. occidentalis*

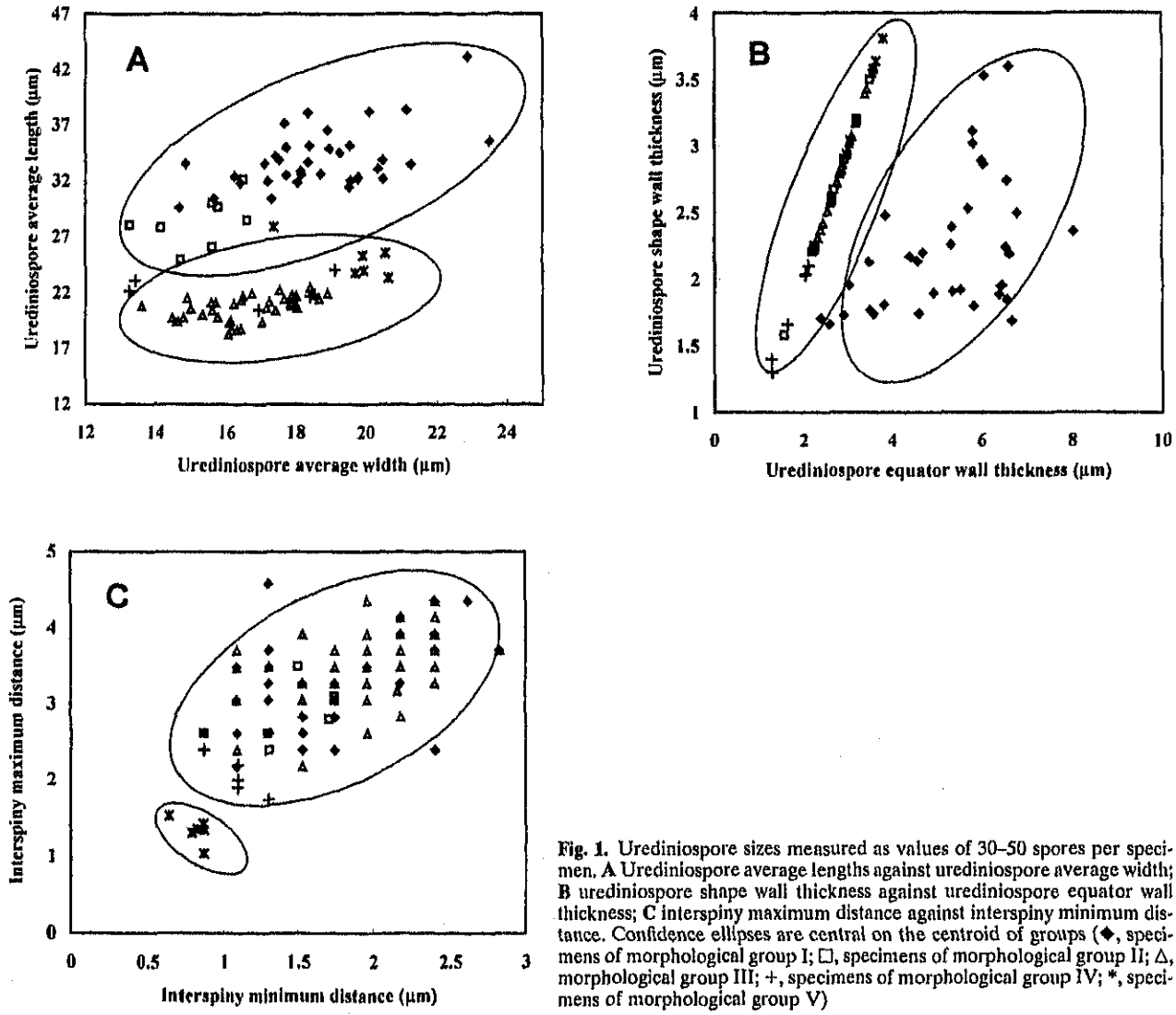

Fig. 1. Urediniospore sizes measured as values of 30–50 spores per specimen. A Urediniospore average lengths against urediniospore average width; B urediniospore shape wall thickness against urediniospore equator wall thickness; C interspiny maximum distance against interspiny minimum distance. Confidence ellipses are central on the centroid of groups (◆, specimens of morphological group I; □, specimens of morphological group II; △, morphological group III; +, specimens of morphological group IV; *, specimens of morphological group V)

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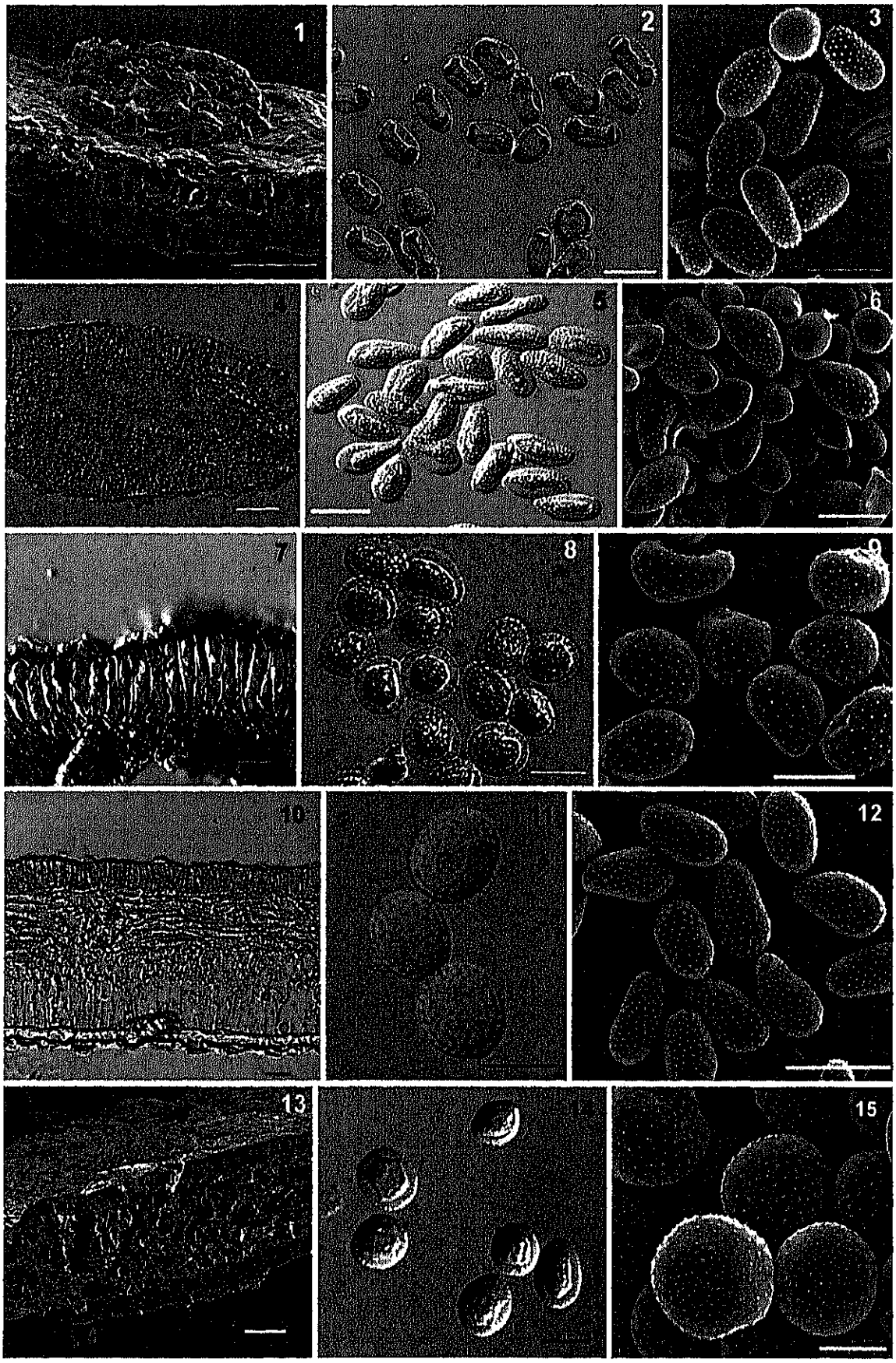

(AF087711) and *M. medusae* (AF087710) were used as outgroups (Fig. 4).

Specimens belonging to morphological group I were included in clade E; morphological group II was included in clade C; specimens belonging to morphological group III were included in clade D and clade F; morphological group IV was included in clade A; and morphological group V was included in clade B. However, phylogenetic trees showed high similarity of genetic variation between ITS region and LSU rDNA regions from the specimens of *Melampsora* on *Populus*.

Discussion

In this study, *Melampsora* specimens on *Populus* species were divided into five different groups by morphological observations. These specimens were separated into six clades by phylogenetic analyses of the LSU rDNA (D1/D2) and ITS1–5.8S–ITS2 regions (Table 4).

Specimens on sections *Tacamahaca*, *Aigeiros*, and *Leucoides* belonging to morphological group I were included in the same phylogenetic groups as D1/D2 and ITS sequences. Specimens of morphological II were included in the same group (D1/D2 clade D and ITS clade C).

Specimens on sect. *Leuce* belonging to morphological group III were separated into two phylogenetic groups. Based on D1/D2 regions, these specimens can be separated into two clades (D1/D2 clade B and F), and the same specimens were separated into two clades (ITS clade D and F) based on sequence analysis of the ITS regions. Specimens of D1/D2 clade F contain 15 collections of *P. alba*, *P. alba* var. *pyramidalis*, *P. hopeiensis*, *P. tomentosa*, and *P. tremula*; these same specimens were separated into ITS clade D. Specimens belonging to D1/D2 clade B and ITS clade F are the same, and included specimens on *P. tomentosa*, *P. davidiana*, and *P. adenopoda* belonging to morphological group III. Sequence analyses confirmed that genetic relationship of specimens of D1/D2 clade B and ITS clade F are closer to morphological group IV (D1/D2 clade C and ITS clade A) than clade F (D1/D2) and clade D (ITS). Although there are no obvious morphological differences within group III, the phylogenetic analyses strongly suggest that there are two distinct taxa within the group.

The specimens on *P. tremula*, *P. tomentosa*, *P. adenopoda*, and *P. davidiana* were usually identified as *M. laricis* based on characters of urediniospores in China (Wang 1949; Tai 1979; Yuan 1984; Zhuang 1986; Shang et al. 1990; Guo 1989). Cao and Li (1999) described specimens on *P. adenopoda* and *P. davidiana* as *M. populnea*, and *M. laricis* was identified as a synonym of *M. populnea*, as there is no morphological difference. However, these specimens were separated into two different groups based on our molecular analyses. Specimens on *P. tremula*, along with other specimens on sect. *Leuce*, were included in D1/D2 clade F and ITS clade D. Specimens on *P. davidiana* and *P. adenopoda* were included in D1/D2 clade B and ITS clade F.

In former various reports, specimens on *P. tomentosa*, *P. alba*, *P. alba* var. *pyramidalis*, and *P. hopeiensis* were identified as *M. magnusiana* (Tai 1979; Yuan 1984; Guo 1989; Cao and Li 1999), *M. aecidioides* (Liu and Wang 1936; Shang et al. 1990), or *M. rostrupii* (Ge et al. 1964; Tai 1979) in China. Our morphological and phylogenetical analyses showed no morphological and genetic variation among specimens identified as *M. aecidioides*, *M. rostupii*, and *M. magnusiana* (group III, D1/D2 clade F and ITS clade D).

All specimens on *P. euphratica* were morphologically and phylogenetically included in the same group (morphological group V, D1/D2 clade E and ITS clade B) and were clearly separated from other groups with high bootstrap support. Therefore, we consider that this group represents a distinct taxon from other groups.

Specimens belonging to morphological group IV were clearly included in the same genetic group (D1/D2 clade C or ITS clade A). Some specimens on *Populus* species were identified as *M. populnea* or *M. laricis* in China (Guo 1989; Zhuang and Wei 1994); however, in this study, these specimens can be placed in morphological group IV based on

Table 4. Relationship among morphological groups and phylogenetic clades

Morphological group[a]	D1/D2 clade[b]	ITS clade[c]
I	A	E
II	D	C
III	F	D
III	B	F
IV	C	A
V	E	B

[a] Table 4 and Fig. 1
[b] Fig. 3
[c] Fig. 4

Fig. 2. Urediniospores and teliospores of *Melampsora* spp. on poplars observed by light microscopy and SEM. **1–3** Group I. **1** Hypophyllous urediniospores and epiphyllous teliospores on *Populus opera* (HMNWFC-T002). **2** Ellipsoid or oblong urediniospores with wall thickened equatorially on *P. laurifolia* (HMNWFC-T040). **3** Urediniospores echinulate except for smooth apex on *P. simonii* × *P. nigra* var. *italica* (TSH-R16925). **4–6** Group II. **4** Amphigenous telia on *P. laurifolia* (HMAS4138). **5** Oblong urediniospores with uniform cell wall on *P. laurifolia* (HMAS4138). **6** Urediniospores with smooth apex on *P. talassica* (HMNWFC-T035). **7–9** Group III. **7** Teliospores on *P. alba* var. *pyramidalis* (HMAS4132). **8** Globose, or ovate urediniospores with uniform cell wall on *P. tomentosa* (HMNWFC-T024). **9** Urediniospores with echinulate surface on *P. tomentosa* (HMNWFC-T026). **10–12** Group IV. **10** Hypophyllous teliospores on *P. wilsonii* (HMNWFC-TR0009). **11** Globose, or ellipsoid urediniospores with a thin cell wall on *P. yunnanensis* (TSH-R20042). **12** Urediniospores with echinulate surface on *P. yunnanensis* (TSH-R20046). **13–15** Group V. **13** Amphigenous uredinia and telia on *P. euphratica* (HMNWFC-T046). **14** Globose, or ovate urediniospores with uniform cell wall. **15** Urediniospores surface with small echinulate on *P. euphratica* (HMNWFC-T037). *Bars* **1** 120 μm; **2** 35 μm; **3** 21 μm; **4** 55 μm; **5** 35 μm; **6** 20 μm; **7** 35 μm; **8** 20 μm; **9** 14 μm; **10** 30 μm; **11** 18 μm; **12** 20 μm; **13** 100 μm; **14** 20 μm; **15** 8.4 μm

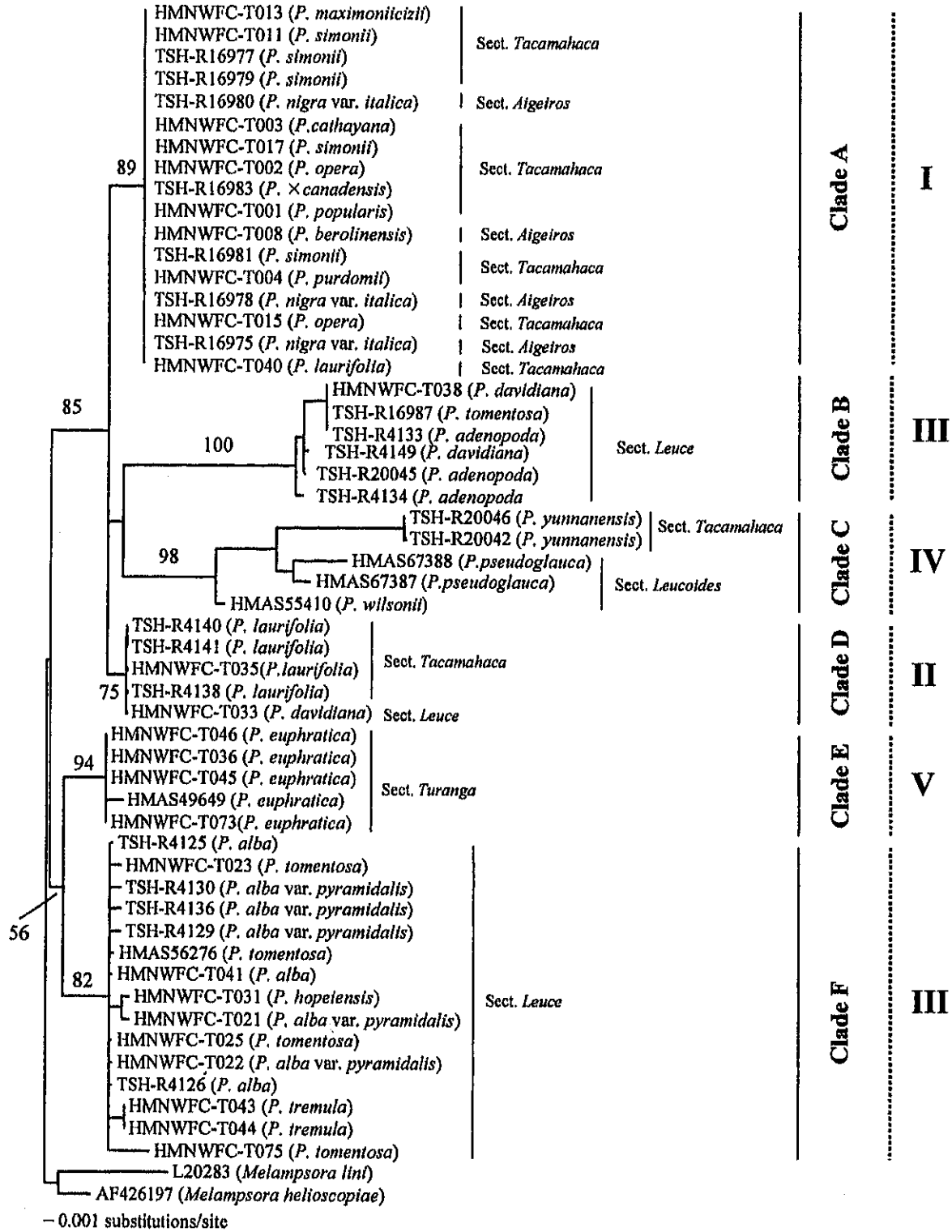

Fig. 3. Phylogenetic tree constructed by neighbor-joining method for 53 specimens of *Melampsora* on poplars based on nucleotide sequences of the LSU rDNA region. The values at the nodes are the confidence levels from 1000 replicate bootstrap samplings

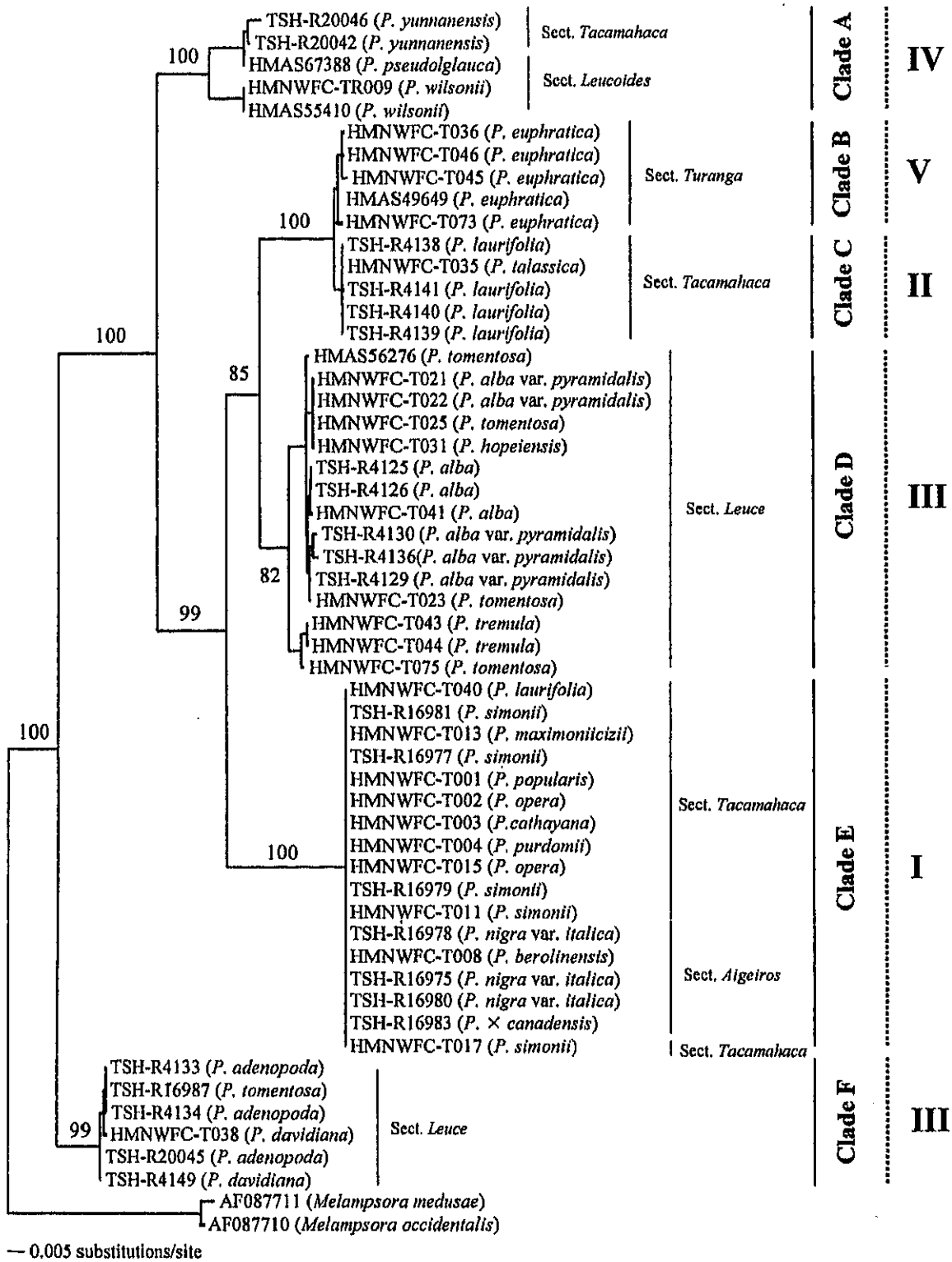

Fig. 4. Phylogenetic tree constructed by neighbor-joining method for 53 specimens of *Melampsora* on poplars based on nucleotide sequences of the ITS1–5.8S–ITS2 region of rDNA. The values at the nodes are the confidence levels from 1000 replicate bootstrap samplings

urediniospore wall thickenings and phylogenetical analyses. Therefore, we consider that group IV is a distinct taxon from others, and the thin urediniospore wall (average, <2 μm) is a consistently dependable character for identification of this group from other groups. We will discuss the taxonomic treatment of these taxa including examinations of authentic specimens (including type specimens) in another paper.

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References

Ayliffe MA, Dodds PN, Lawrence GJ (2001) Characterisation of a β-tubulin gene from *Melampsora lini* and comparison of fungal β-tubulin genes. Mycol Res 105:818–826

Bagyanarayana G (1998) The species of *Melampsora* on *Populus* (Salicaceae). In: Jalkanen R, Crane PE, Walla JA, Aalto T (eds) Proceedings of the first IUFRO rusts of forest trees working party conference, 2–7 Aug. 1998, Saariselkä, Finland, pp 37–51

Cao ZM, Li ZQ (1999) Rust fungi of Qinling Mountains (in Chinese). China Forestry Publishing House, Beijing, pp 29–41

Cellerino GP (1999) Review of poplar diseases. (3. Disease by fungi. 3.4.1. Rusts caused by *Melampsora* spp.) Grugliasco. (www.efor.ucl.ac.be/ipc/pub/celle01/celle01.htm)

Dai YC (1989) Study on poplar rusts (*Melampsora* spp) by numerical taxonomic method (in Chinese). Sci Silvae Sin 25:87–91

Edwards J, Ades PK, Parbery DG, Halloran GM, Taylor PWJ (1999) Morphological and molecular variation between Australian isolates of *Puccinia menthae*. Mycol Res 103:1505–1514

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Ge GP, Jing Y, Shen MM, Shi Q (1964) Observations on the development and morphology of *Melampsora rostrupii* Wagner on *Populus tomentosa*. Sci Silvae Sin 9:221–232

Guo L (1989) Uredinales of Shennongjia, China. In: Academia Sinica (ed) Fungi and lichens of Shennongjia (in Chinese). World Publ., Beijing, p 113

Hantula J, Kasanen R, Kaitera J, Moricca S (2002) Analyses of genetic variation suggest that pine rusts *Cronartium flaccidum* and *Peridermium pini* belong to the same species. Mycol Res 106:203–209

Hiratsuka N, Sato S, Katsuya K, Kakishima M, Hiratsuka Y, Kaneko S, Ono Y, Sato T, Harada Y (1992) The rust flora of Japan. Tsukuba Shuppankai, Tsukuba, Ibaraki, Japan, pp 271–272

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotides sequences. J Mol Evol 16:111–120

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth & Bisby's dictionary of the fungi, 9th edn. CABI, Wallingford, UK, p 311

Liu TN, Wang YZ (1936) Materials for study on rusts of China. VI. Chin J Bot 1:69

Maier W, Begerow D, Weiß M, Oberwinkler F (2003) Phylogeny of the rust fungi: an approach using nuclear large subunit ribosomal DNA sequences. Can J Bot 81:12–23

Nakamura H, Kaneko S, Yamaoka Y, Kakishima M (1998) Differentiation of *Melampsora* rust species on willows in Japan using PCR-RFLP analysis of ITS regions of ribosomal DNA. Mycoscience 39:105–113

Newcombe G, Stirling B, McDonald S, Bradshaw HD (2000) *Melampsora* × *columbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*. Mycol Res 104:261–274

O'Donnell K (1993) *Fusarium* and its near relatives. In: Reynolds DR, Taylor JW (eds) The fungal holomorph: mitotic, meiotic and pleomophic speciation in fungal systematics. CAB International, Wallingford, UK, pp 225–233

Rambaut A (2001) Se-Al: sequence alignment editor v2.0. Department of Zoology, University of Oxford, Oxford, UK

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Shang YZ, Pei MH, Yuan ZW (1986a) A new rust fungus on poplars (in Chinese). Acta Mycol Sin Suppl I:180–184

Shang YZ, Yuan XY, Hao JZ (1986b) Taxonomy of *Melampsora* on *Populus* (in Chinese). J Inner Mongolia For Coll 8:126–133

Shang YZ, Hao JZ, Yuan XY (1990) *Melampsora* on poplars in Nei Monggol (in Chinese). Acta Agric Bor-Sin 5:86–92

Suyama Y, Kawamuro K, Kinoshita I, Yoshimura K, Tsumura Y, Takahara H (1996) DNA sequence from a fossil pollen of *Abies* spp. from Pleistocene peat. Genes Genet Syst 71:145–149

Swofford DL (2002) PAUP* 4.0: phylogenetic analysis using parsimony, version 4.0b10. Computer program distributed by Sinauer Associates, Fitchburg

Tai F L (1979) Sylloge fungorum sinicorum (in Chinese). Science Press, Academia Sinica, Beijing, pp 537–539

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Virtudazo EV, Nakamura H, Kakishima M (1998) A simple method of nucleic acid extraction from spores of rust fungi for PCR amplification. In: Progress in mycological science in Japan and United Kingdom: 6th international symposium of the Mycological Society of Japan, Chiba University, Japan, p 61

Virtudazo EV, Nakamura H, Kakishima M (2001) Phylogenetic analysis of sugarcane rusts based on sequences of ITS, 5.8 S rDNA and D1/D2 regions of LSU rDNA. J Gen Plant Pathol 67:28–36

Vogler DR, Bruns TD (1998) Phylogenetic relationships among the pine stem rust fungi (*Cronartium* and *Peridermium* spp.). Mycologia 90:244–257

Wang YC (1949) Uredinales of Shensi. Contrib Inst Bot Nat Acad 6:224–225

Weber RWS, Webster J, Engel G (2003) Phylogenetic analysis of *Puccinia distincta* and *P. lagenophorae*, two closely related rust fungi causing epidemics on *Asteraceae* in Europe. Mycol Res 107:15–24

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequence of fungal ribosomal RNA genes for phylogenetics. In: Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

Yuan Y (1984) On identification of some species of *Melampsora* infecting poplar trees in China and a test of host range of *M. pruinosae* (in Chinese). J Beijing For Coll 6:48–82

Zhuang JY (1986) Uredinales from East Himalaya. Acta Mycol Sin 5:75

Zhuang JY, Wei SX (1994) An annotated checklist of rust fungi from the Mt. Qomolangma region (Tibetan Everest Himalaya). Mycosystema 7:45

Zhang N, Zhuang JY, Wei SX (1997) Fungal flora of the Daba Mountains: Uredinales. Mycotaxon 61:59–61

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FULL PAPER

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Three species of *Exobasidium* causing Exobasidium leaf blight on subgenus *Hymenanthes*, *Rhododendron* spp., in Japan

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Abstract Three *Exobasidium* species causing Exobasidium leaf blight on *Rhododendron* of subgenus *Hymenanthes* in Japan are described and discussed. After examining the holotype and fresh materials of *E. shiraianum* on *R. degronianum*, its description is emended by the morphology of basidiospores that are ellipsoid to ovoid, or obovoid, 11–21 × 5–8μm, and with 1–3 septa. Culture of this species showed yeast-like growth. *Exobasidium woronichinii* on *R. brachycarpum* observed from Hokkaido Prefecture to Nagano Prefecture is described as a new species characterized by its ellipsoid to ovoid, 11–19 × 3–4.5μm, and 1–5(–6)-septated basidiospores. Culture of this species was gelatinous but obtrite, or thick and showed farinose appearance by conidiation. A fungus on *R. aureum* in Hokkaido and Nagano Prefectures is identified as *E. caucasicum*. This species is new to Japan and is characterized by its ellipsoid to ovoid, or obovoid, 11–19 × 3–6μm, and 0–2-septated basidiospores. Culture of this species showed yeast-like growth.

Key words Basidiomycetes · Culture · *Exobasidium* · Germination · Japan · Taxonomy

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Introduction

Exobasidium shiraianum Henn. was described in 1903 as a new species to accommodate a fungus that caused Exobasidium leaf blight on *Rhododendron metternichii* Siebold et Zucc. collected by Prof. S. Kusano in Mt. Shirane, Japan (Hennings 1903). *Exobasidium hemisphaericum* Shirai was also reported to infect several Rhododendrons in the subgenus *Hymenanthes* and caused leaf gall (Anonymous 2000). This species was described in 1896 based on a specimen on *R. metternichii* var. *hondoense* Nakai (Shirai 1896). This leaf gall was well documented in Japanese textbooks of plant pathology (Ideta 1901, 1903, 1929), whereas *E. shiraianum* was merely noted in a textbook (Ideta 1929). Morphology and host range of *E. shiraianum* were described by Ito (1955). Even if a picture of symptoms of Exobasidium leaf blight on *Rhododendron* sp. (Fig. 38a in Ito 1955) and line drawings of basidia and basidiospores (Fig. 38b in Ito 1955) were given, the size of basidiospores was exactly the same as the original description. He listed *R. fauriae* Franch var. *rufescens* Nakai (Shirobana-syakunage in Japanese) and *R. chrysanthum* Pall. (Kibana-syakunage in Japanese) as host plants of this species. In the latest monographic work (Davidian 1992), legitimate names of *Rhododendron* species in the subgenus *Hymenanthes* were proposed. *Rhododendron metternichii* was synonymized in *R. degronianum* Carrière, and *R. fauriae* var. *rufescens* and *R. chrysanthum* were assigned to *R. brachycarpum* D. Don ex G. Don and to *R. aureum* Georgi, respectively. The host plant originally described in *E. shiraianum* was *R. degronianum*, called "Azuma-syakunage" in Japanese. Thus, there is discrepancy in the record of host plants: *Rhododendron brachycarpum* and *R. aureum* have been noted as hosts of *E. shiraianum* (Ito 1955; Ezuka 1998; Anonymous 2000). Host range noted in these reports is evidently different from that in the original description by Hennings (1903).

In 1999, Dr. A. Ezuka found Exobasidium leaf blight on *R. degronianum* in Nagano Prefecture. We also collected several samples of Exobasidium leaf blight on *R.*

brachycarpum to compare the morphology and cultural characteristics of these fungi. Based on these results, we discuss the taxonomy of *E. shiraianum* and propose a new species to accommodate an *Exobasidium* specimen on *R. brachycarpum*.

The symptom caused by *E. caucasicum* Woron. on *R. aureum* described in Russia (Woronichin 1921) was yellowing of developing leaves and formation of farinose hymenium on the lower side (Exobasidium leaf blight), quite similar to that caused by *E. shiraianum* (Woronichin 1926). The lack of records of *E. caucasicum* in the Japanese mycological literature (Anonymous 2000; Ezuka 1998; Ito 1955) gives us to believe that the occurrence of *E. caucasicum* has not been recognized in Japan. However, in our recent survey of the herbarium materials, there are several specimens collected in Japan that have been sorted as *E. caucasicum* in the Herbarium of the Hokkaido University Museum (SAPA). Examining these specimens and another fresh material collected in Nagano Prefecture in 2002, we here describe morphological features of *E. caucasicum* in Japan.

Materials and methods

Morphological observations

Fresh materials of *Exobasidium* species on *R. aureum*, *R. brachycarpum* and *R. degronianum* collected in the field were used for morphological observations. Specimens examined are listed in the description of the species (Table 1). Materials for morphological observations were prepared and conducted by light (LM) and scanning electron microscopy (SEM) as described previously (Nagao et al. 2003). Samples for SEM were prepared and observed as mentioned previously (Nagao et al. 2001). All materials were deposited in the Mycological Herbarium of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba (TSH) and the Herbarium of the National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan (NIAES).

Culture of basidiospore isolate

Fresh materials were kept in a plastic bag until newly sporulating lesions were observed. Colonies from a single basidiospore were obtained as described previously (Nagao et al. 2003). Cultures were kept in the Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba, and some of the isolates of *Exobasidium* spp. obtained in this study were deposited in Genebank, National Institute of Agrobiological Sciences, Japan (MAFF).

Table 1. Morphological measurements of *Exobasidium* spp.

Species	Size of basidia (μm)	Size of sterigmata (μm)	Number of sterigmata	Size of basidiospores (μm)	Septal number of basidiospores
E. shiraianum					
Holotype S-F20843 (Hennings 1903)	nd	nd	4	7–11 × 2.5	nd
Holotype S-F20843	5–22 × 5–9	2–5 × 1–1.5	2–3	11–18 × 5–7	1–3
TSH-B 0023	nd	nd	nd	16–18 × 7–8	(1) 3
TSH-B 0025	nd	nd	nd	12–18 × 5.5–8	1–3
TSH-B 0026	nd	nd	nd	13–21 × 6–8	1–3
TSH-B 0027	15–30 × 5–7	2–6 × 1–2	2–3	14–18 × 6–8	1–3
E. woronichinii					
Holotype TSH-B 0081	22–40 × 4.5–8	4–5 (5.5) × 1.5–2	2–4	11–16 × 3–4.5	1–3 (5)
TSH-B 0085	11–50 × 6–8	4–5 × 1–2	2–3	11–16 × 3–4	(1) 2–5 (6)
TSH-B 0083	nd	nd		12–17 × 3–4	1–5
TSH-B 0114	13–60 × 6–7	2–6 × 1.5–2	2–3	11–17 × 3–4.5	1–5
TSH-B 0115	15–24 × 6–8	4–6 × 1–1.5	2	13–18 × 3–4.5	1–4
TSH-B 0116	20–30 × 6–12	3–5 × 1–1.5	2–3	12–19 × 3–4.5	2–4 (6)
TSH-B 0021	nd	nd		11–18 × 3–4	1–4
TSH-B 0022	10–40 × 5–8	2–6 × 1–2	2–4	12–17 × 3.5–4	1–3 (4)
NIAES20541	30 × 9–10	nd	3–4	12–19 (22) × 3–4	1–3
E. hemisphaericum					
(Shirai 1896)	nd	nd	4	15–19 × 3.5–4.5	nd
(Ito 1955)	nd	nd	4	15–19 × 3.5–4.5	3
TSH-B 0032	25–32 × 6–9	2–3 × 1–2	2–4	12–20 × 3–4	1–4
TSH-B 0033	22–35 × 7–8.5 (10)	2–3 × 1–1.5	2–3	20 × 7.5	nd
E. caucasicum					
Caucasus (Woronichin 1920)	39.6–42.9 × 6.6–8.8	nd	(2–3) 4	14–16.5 × 5	0–1
Kamchatka (Woronichin 1926)	42–52 × 7–10	nd	4	14–17.5 × 5–5.5	nd
NIAES20542	15–29 × 8–12	2–4 × 1–2	2–4	13–19 × 4–6	(0) 1–2
SAPA Aug. 5, 1925	nd	nd	nd	15 × 6	2
SAPA Aug. 4, 1903	20 × 6–7.5	nd	4	11–16 × 3–4.5	1–2
SAPA July 30, 1921	13–35 × 5–6	2–4 × 1.5–2	2–3	13–14 × 4	1
SAPA Aug. 1, 1895	nd	nd	nd	11–17 × 4–6	(0) 1

nd, not determined

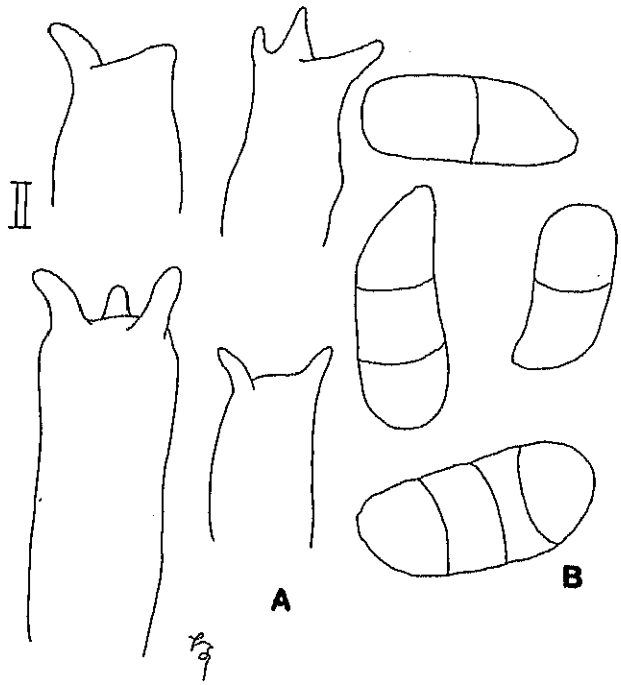


Fig. 1. Basidia (**A**) and basidiospores (**B**) of *Exobasidium shiraianum* holotype (S, F20843). *Bar* 3 μm

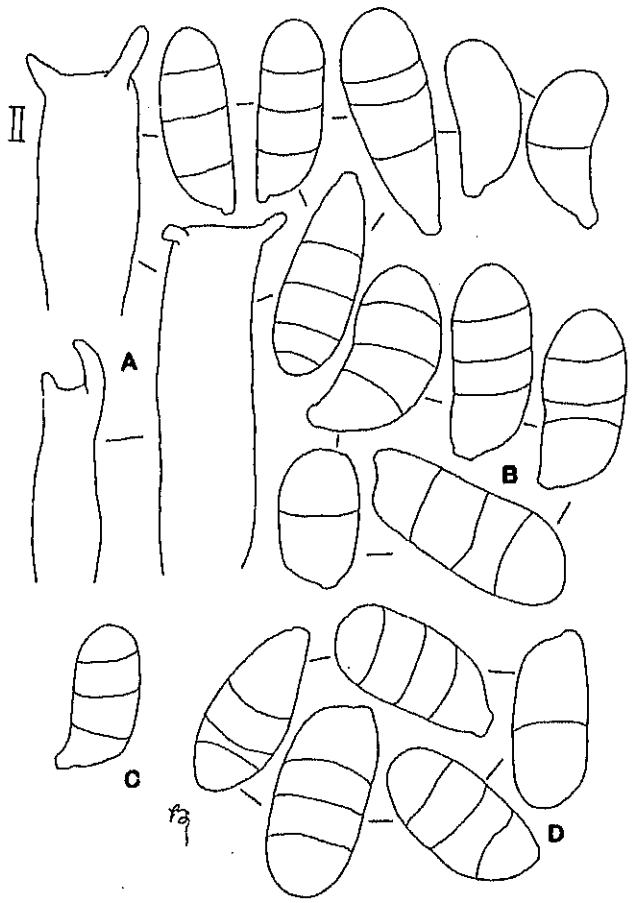


Fig. 2. Basidia and basidiospores of *E. shiraianum* TSH-B0027 (**A**), TSH-B0026 (**B**), TSH-B0025 (**C**), and TSH-B0023 (**D**). *Bar* 3 μm

Taxonomy

1. ***Exobasidium shiraianum*** Henn., Bot. Jahrb. 32: 38, 1903. emend Nagao Figs. 1, 2

Hymenium composed of basidia with 2 or 3 sterigmata and conidia. Hyphae not developing directly on the surface of epidermis. Basidia emerging directly from the host surface or through stomata, not fasciculate, clavate to cylindrical, 5–30 × 5–9 μm (Figs. 1A, 2A), obtuse at the apex. Sterigmata 1–2 μm in diameter at the base and 2–6 μm in height, tapering toward the tip (Fig. 11A–C). Basidiospores ellipsoid to oval, or obovoid, 11–21 × 5–8 μm, hyaline, smooth, with 1–3(–4) septa (Figs. 1B, 2, 11D, 12A). Septate basidiospores dropped on the agar surface germinating after 15 h (Fig. 3). Germ tubes or conidia emerging from the spore cells. Pseudohyphae not distinguishable from budded conidia. Conidia bacilliform or subfusiform (Fig. 9A–C), 2–10 × 1–2 μm, and budding polarly in culture (Figs. 3, 12A; Table 2) to produce daughter cells and also to develop pseudohyphae. Colonies on potato dextrose agar (PDA) growing gradually, reaching maximum 13 mm diameter in 21-day incubations at 22°C, and wrinkling irregularly at the periphery, with pale yellow and smooth surface not becoming farinose by conidial formation, glutinous and not fixed on the agar surface, composed of pseudohypha and conidia. The reverse of colonies dark yellow to pale pink. Dark pigmentation not exuded into PDA (Fig. 13A–C). Colonies from conidia showing the same morphological features as those from basidiospores.

Specimens examined: On *R. metternichii*, (syn. of *R. degranianum*) Prov. Shimozuke, Mt. Shirane, July 14, 1900, S. Kusano leg. (holotype S-F20843); on *R. degranianum*, Mt. Tengu-yama, Minamimaki-mura, Nagano Prefecture, July 26, 1999, A. Ezuka leg. (TSH-B0023, B0024, B0025, B0026)

Hennings (1903) briefly described the shapes of basidia and basidiospores, the number of sterigmata, and the size of basidiospores as 7–11 × 2.5 μm. He precisely described the symptom of infected leaves. As the hymenial appearance does not accompany gall formation, he again commented on this "atypical" symptom compared with the symptom caused by *E. pentasporium*. We examined the holotype and observed some basidiospores and basidia with sterigmata (Fig. 1). We noticed that the size of basidiospores (11–18 × 5–7 μm with 1–3 septa) was different from the description by Hennings (Fig. 1B). We complemented the morphological characteristics based upon the holotype and TSH-B 0027. This species is characterized by the width of basidiospores (5–8 μm) (Figs. 1B, 2, 11D, 12A). In our observations, size and shape of conidia of *E. shiraianum* (2–10 × 1–2 μm) were quite different from those of basidiospores of *E. shiraianum* (Tables 1, 2). We supposed that the size mentioned in the original description was that of conidia rather than that of basidiospores. Among 102 taxa of *Exobasidium* validly described so far, the following 8 taxa showed similarities to *E. shiraianum* in some morphological

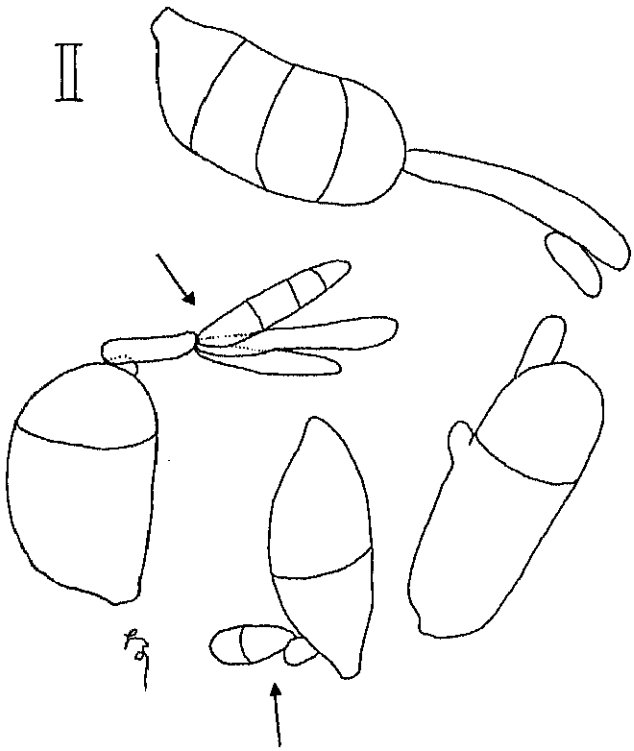


Fig. 3. Basidiospore germination of *E. shiraianum* (TSH-B 0025) on potato dextrose agar (PDA) after 12 h incubation. Some of the basidiospores produced conidia on the germ tube (*arrows*). *Bar* 3 μm

Table 2. Conidial morphology of *Exobasidium* spp.

Species	Size of conidia (μm)	Septal number of conidia
E. shiraianum		
MAFF238602	2–5 × 1–1.5	0
MAFF238603	3–10 × 1–2	0
MAFF238604	3–8 × 1–2	0
E. woronichinii		
MAFF238617	2–25 × 1–2	0
MAFF238618	3–19 × 1–2	0–1 (4)
MAFF238666	6–26 × 1–1.5	0
MAFF238667	2–17 × 1–2	0
MAFF238625	5–30 × 1–2	0–4
MAFF238622	4.5–18 × 1–2.5	0
MAFF238610	3–12 × 1–2	0
MAFF238825	5–21 × 1–2	0
E. hemisphaericum		
E-11*	5–24 × 1–1.5	0–2
E-13*	5–24 × 1–2	0
E. caucasicum		
Caucasus (Woronichin 1920)	5 × 1.5	nd
Kamchatka (Woronichin 1926)	7–10 × 1.75–3	nd
MAFF238830	3–7 × 1–2	0

nd, not determined
*Culture was deposited in the Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba

measurements (Table 3). *Exobasidium shiraianum* differed from *E. aequale* Sacc., *E. dimorphosporum* Savile, *E. dracophylli* McNabb, *E. splendidum* Nannf., and *E. vaccinii-uliginosi* Boud. in the septal number of basidiospores, from *E. bisporum* Sawada ex Ezuka in the septal number and mode of germination of basidiospores, and from *E. camelliae* Shirai and *E. nudum* S. Ito et Y. Otani in the mode of germination of basidiospores. *Exobasidium shiraianum* is surely proved to be valid species. Exobasidium leaf blight on *R. degronianum* is characterized by the chlorosis and powdery appearance on the lower surface of newly developed leaves (Fig. 14A–C). The host plant and distribution of *E. shiraianum* may be restricted. When we surveyed the occurrence of this fungus on *R. degronianum* at the Hanazono-jinja shrine, Kitaibaraki-shi, Ibaraki Prefecture in June 2001, at Mt. Sanbon-yariga-take, Nasu, Tochigi Prefecture in June 2001, and at Yuno-ko Lake and Mt. Ohmanako-yama, Nikko-shi, Tochigi Prefecture in August 2000 and July 2001, we could not find it.

2. ***Exobasidium woronichinii*** Nagao, sp. nov. Fig. 4

Misapplied name: *Exobasidium shiraianum* sensu S. Ito, Mycological Flora of Japan 2(4):51, 1955–pro parte, non Henn. 1903.

Hymenium hypophyllum, effusum, saepe totum infrasuperficiem folii tegens. Folia infecta flava vel albolutescentia, infra concoloria dein albofarinosa, leviter carnosa. Basidia hyalina, clavato-cylindracea, 10–60 × 4.5–12 μm, terminaliter cum 2–4 sterigmatibus longiconoideis 2–6 × 1–2 μm praedita. Basidiosporae hyalinae, leaves, unciformes vel reclinatae, ad apicem muticae, ad basim curratae et angustatae, 11–19(22) × 3–4.5 μm, primo continuae dein 1–5(6)-septatae, per hyphas germinantes. Conidia hyalina, continua, laevia, linearia, 2–30 × 1–2.5 μm, 0–1(4) septata. Coloniae in PDA restricte crescentes, post 21 dies maxime 22 mm diameter attingens, corrugatae, gelatinosae, ad ambitum irregulares, ex hyphis circa 1 μm latis et conidiis constantes, pallide primulinae vel pallide aurantiacae, in agaro non pigmentiferae; reversum concolor.

Etymology: Referring to Russian mycologist, N. Woronichin, who described several new species of *Exobasidium*.

Holotypus in folliis vivis *Rhododendri brachycarpi* D. Don ex G. Don, Me-akan-dake spa, Hokkaido Pref. in Japonia, 14 VI 2002, H. Nagao leg., in Herbario Instituti Agriculturae et Silviculturae, Universitatis Tsukubensis, Tsukuba, Japonia (TSH-B0081) conservatus.

Specimens examined: TSH-B0083 (on *R. brachycarpum*, Me-akan-dake spa, Hokkaido Prefecture, June 14, 2001, H. Nagao leg.); TSH-B0018, TSH-B0021, TSH-B0022 (on *R. brachycarpum*, Mt. Tengu-yama, Minamimaki-mura, Nagano Prefecture, June 26, 1999, A. Ezuka leg.); TSH-B0114, TSH-B0115 (on *R. brachycarpum*, Kumami-sone, Mt. Nasu-dake, Nasu, Tochigi Prefecture, June 14, 2001, H. Nagao leg.); TSH-B0116 (on *R. brachycarpum*, Mt. Sanbon-yariga-dake, alt. 1917 m, Yumoto, Nasu-machi, Nasu-gun, Tochigi Prefecture, June 14, 2001, H. Nagao leg.); NIAES 20541 (on *R. brachycarpum*, Mt. Hakkoda Botanical

Table 3. Comparison of morphological measurement among *Exobasidium* spp.

Species	Sizes of basidia (μm)	Sizes of sterigmata (μm)	Number of sterigmata	Sizes of basidiospores (μm)	Number of septa of basidiospores	References
E. shiraianum	15–30 × 5–7	2–6 × 1–2	2–3	14–18 × 6–8	1–3	
E. aequale	6–8 wide	4–6 long	2	(14) 15–22 × (6) 7–9 (10)	0	Symb. Bot. Ups. 23(1981):1–72
E. bisporum	40–60 × 6–8	4–6 × 2–3	2 (3)	14–24 (27) × 4–7	1–7	Trans. Mycol. Soc. Jpn 32(1991):169–185
E. bisporum	60–80 × 5–7	4–7 × 1.5–2.5	2	15–22 × 5–8	1–4	Trans. Mycol. Soc. Jpn 32(1991):169–185
E. camelliae	130–160 × 6–12	4–6 × 2–3	2–3 (4)	15–25 × 5–7.5	(1) 3 (7)	Trans. Mycol. Soc. Jpn 31(1990):375–388
E. dimorphosporum	26–40 × 5–9.5	5–7 × 1.5–2.5	2–3	13–18.5 × 6.5–8.5; 18.5–28.5 (32) × 5–7 (8)	0 (1)	Can. J. Bot. 37(1959):641–656
E. dracophylli	25–45 × 5–6.5	6–8.5 long	2 (3)	20–27 × 5–8	0 (1)	Trans. N-Z. Bot. 1 (1962):259–268
E. nudum	100 × 5–8	4–5 × 2	(2) 4	10–20 × 4.5–8	1–3	Trans. Mycol. Soc. Jpn 31(1990):375–388
E. splendidum	6–8 wide	nd	2	(15) 20–27 × 6–11.5	0	Symb. Bot. Ups. 23(1981):1–72
E. vaccinii-uliginosi	9–10 wide	7 long	2	16–23 (28) × 6.5–9 (12)	0	Symb. Bot. Ups. 23(1981):1–72
E. woronichinii	22–40 × 4.5–8	4–5 (5.5) × 1.5–2	2–4	11–16 × 3–4.5	1–5 (6)	
E. canadense	24–40 × 5.5–8	2.5–4.5 × 1.5–2	2–4 (5)	14–20 × 3–4.7	1–3	Can. J. Bot. 37 (1959):641–656

nd, not determined

Fig. 4. Basidia and basidiospores of *E. woronichinii*. Holotype TSH-B0081 (**A**), NIAES 20541 (**B**), TSH-B0022 (**C**), and TSH-B0014 (**D**). *Bar* 3 μm

Garden, Tohoku Univ., Sukayu, Aomori Prefecture, June 9, 2002, Y. Harada leg.)

Hymenium composed of basidia with 2 to 4 sterigmata and conidia. Hyphae not developing directly on the surface of epidermis. Basidia clavate to cylindrical, 10–60 × 4.5–12 μm (Fig. 4A,C,D), with obtuse apex, emerging directly from leaf surface or through stomata, not fasciculate. Sterigmata 1–2 μm in diameter at the base and 2–6 μm in height, emerging outwardly and tapering toward the tip. Basidiospores ellipsoid to ovoid, 11–19(22) × 3–4.5 μm, hyaline, smooth, one-celled when formed, becoming septate with 1–5(6) septa (Figs. 4, 12B), slightly curved and tapering at the end. Septate basidiospores dropped on the agar surface germinated after 6 h (Fig. 5). Germ tubes of the basidiospores emerging from cells of both ends at first then from other cells and producing conidia at the tip or lateral of germ tubes 22 h after dropping (Fig. 5). Hyphae growing into pseudohyphae and branched. Conidia bacilliform, lacrimiform, subfusiform, and clavulate (Fig. 9D–G), 2–30 × 1–2.5 μm with 0–1(4) septa, and budding polarly (Table 2), to produce daughter cells polarly and also develop

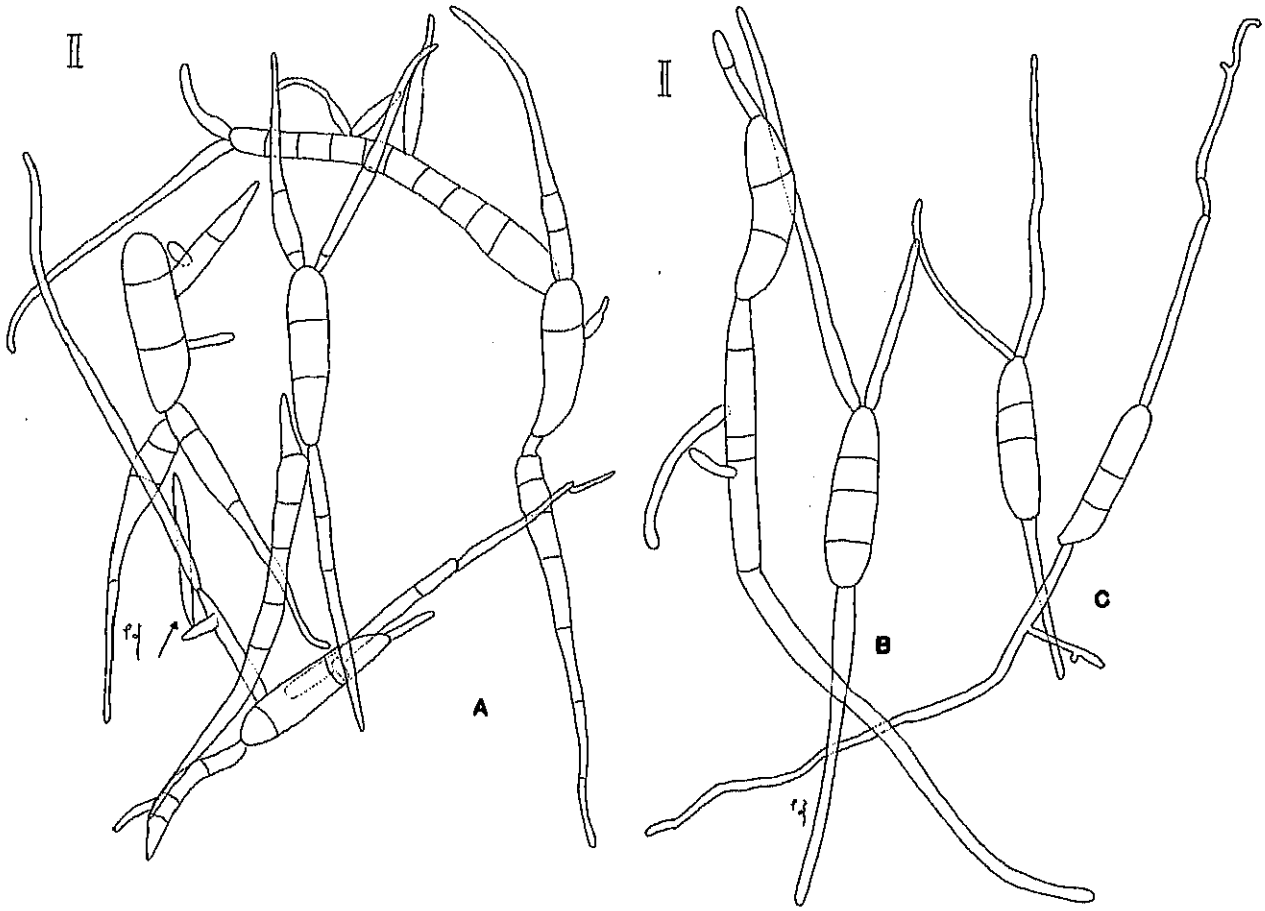


Fig. 5. Basidiospore germination of *E. woronichinii* (TSH-B 0040) on PDA after 22 h incubation. Some of the basidiospores produced conidia on the germ tube (*arrow*): TSH-B0116 (**A**), TSH-B0081 (**B**), and NIAES 14236 (**C**). *Bars* 3 μm

hyphae. Colonies on PDA growing gradually, reaching maximum 22 mm diameter in 21-day incubations at 22°C, and wrinkling irregularly at the periphery, with pale primrose-yellow to pale orange and corrugate surface, gelatinous but obtrite, or thick, often providing farinose appearance, composed of branching, intricate hypha and pseudohypha, and conidia. The reverse of colonies pale yellow to pale orange. Dark pigment not exuded on PDA (Fig. 13D,E). Colonies from conidia showing the same morphological features as those from basidiospores.

Among 102 taxa of *Exobasidium* that have been validly described, *E. canadense* Savile and *E. hemisphaericum* showed similarities in some morphological measurements (Tables 1, 3) to this new species, which, however, was distinguished from *E. canadense* in number of septa of basidiospores. The shape of basidiospores of *E. canadense* were musiform judged from the line drawing by Savile (1959) as well as that of *E. woronichinii*, whereas that of *E. hemisphaericum* was clavate to cylindrical. Especially, basidiospores of *E. woronichinii* showed strong curvature at the end of spores (Figs. 4A–D, 12B) and were distinguishable from *E. canadense* and *E. hemisphaericum* (see Fig. 10B; fig. 3 in Savile 1959). Exobasidium leaf blight on *R. brachycarpum* is characterized by the chlorosis and powdery appearance on the lower surface of newly developed leaves (Fig. 15A–F).

3. ***Exobasidium caucasicum*** Woron., Monit. Jard. Bot. Tiflis 51: 3, 1921. Figs. 6, 7

Hymenium composed of basidia with 2 to 4 sterigmata and conidia. Pseudohyphae not developing directly on the surface of epidermis. Basidia clavate to cylindrical, 13–35 × 5–12 μm, obtuse at the apex, not fasciculate. Sterigmata 1–2 μm in diameter at the base and 2–4 μm in height, emerging outwardly and tapering toward the tip (Figs. 6A,B, 7A). Basidiospores ellipsoid to oval, or obovoid, 11–19 × 3–6 μm, hyaline, smooth, one celled, or becoming septate with 1–2 septa (Figs. 6C, 7A–D). Septate basidiospores dropped on the agar surface germinating after 6 h (Fig. 8). Germ tubes or conidia emerging from the both end-cells and from the septal region of basidiospores or conidia produced at the tip of germ tubes 22 h after the dropping (Fig. 8). Conidia

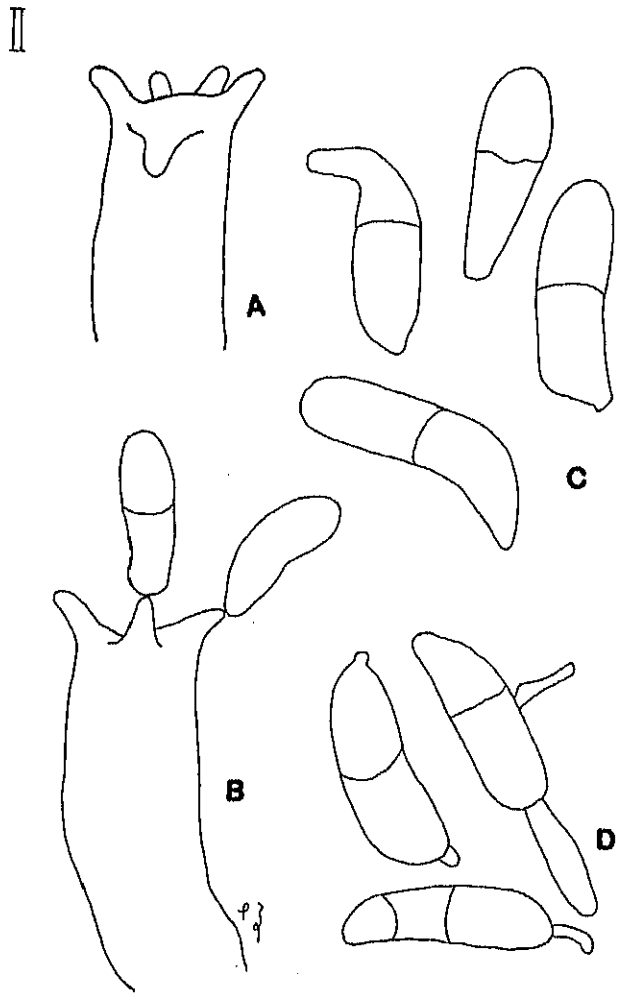


Fig. 6. Basidia and basidiospores of *E. caucasicum* NIAES 20542: basidium (**A**), basidium with basidiospores (**B**), basidiospores (**C**), and germlings in the hymenium (**D**). *Bar* 3 μm

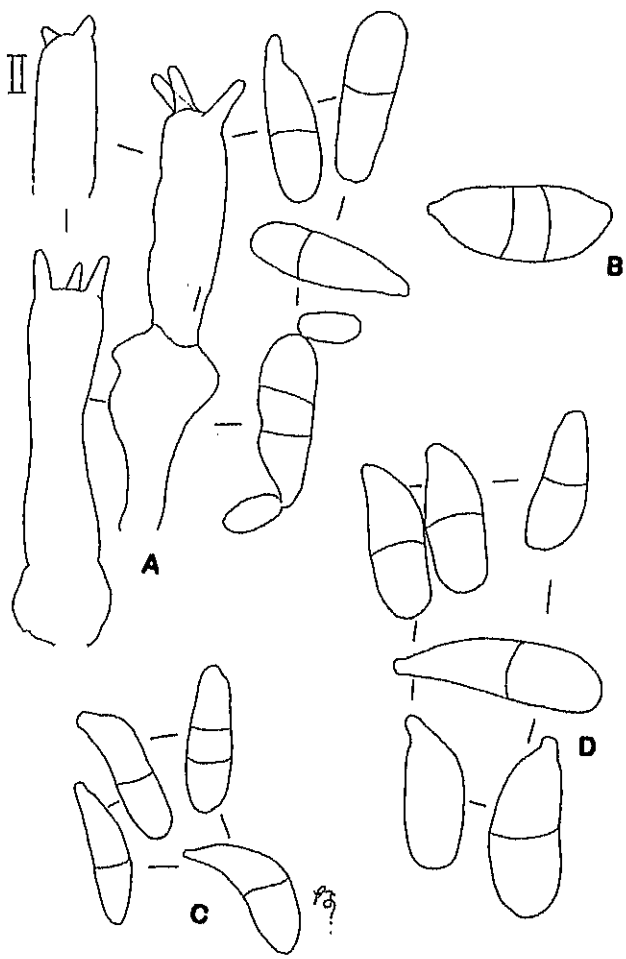


Fig. 7. Basidia and basidiospores of *E. caucasicum*: SAPA July 30, 1921 (**A**), SAPA Aug. 5, 1925 (**B**), SAPA Aug. 4, 1903 (**C**), and SAPA Aug. 1, 1895 (**D**). *Bar* 3 μm

bacilliform and lacrimiform (Fig. 9H), 3–7 × 1–2 μm, and budded polarly, to produce daughter cells and to develop pseudohyphae. Colonies on PDA growing gradually, reaching maximum 11 mm diameter in 21-day incubations, and wrinkling irregularly at the periphery. Colonies with pale orange and smooth surface not becoming farinose by conidial formation, glutinous and not fixed on the agar surface, composed of pseudohypha and conidia. The reverse of colonies pale yellow to pale pink. Dark pigment not exuded on PDA (Fig. 13G). Colonies from conidia showing the same morphological features as those from basidiospores.

Specimens examined: NIAES 20542 (on *R. aureum*, Shirakoma-ike, Yachiyo-mura, Minamisaku-gun, Nagano Prefecture, June 30, 2002, M. Kakishima et C.-m. Tian leg.), SAPA Aug. 1, 1895 (on *R. aureum*, Mt. Matsukarinupuri, Abuta-gun, Shiribeshi subprefecture, Hokkaido Prefecture, Aug. 1, 1895, T. Tozu leg.), SAPA Aug. 4, 1903 (on *R. aureum*, Mt. Yatsuga-take, Nagano Prefecture, Aug. 4, 1903, T. Miyake leg.), SAPA July 30, 1921 (on *R. aureum*, Mt. Matsukarinupuri, Abuta-gun, Shiribeshi subprefecture, Hokkaido Prefecture, July 30, 1921, Y. Homma leg.), SAPA Aug. 5, 1925 (on *R. aureum*, Mt. Hakuun-dake, Kamikawa-gun, Kamikawa subprefecture, Hokkaido Prefecture, Aug. 5, 1925, N. Hiratsuka leg.).

Exobasidium caucasicum was described based on the leaf blight of *R. aureum* collected in Caucasus, Russia (Woronichin 1921). Woronichin (1926) also reported its distribution in Kamchatka. An Exobasidium leaf blight of *R. aureum* (=*R. chrysanthum*) collected by Dr. N. Hiratsuka at Mt. Hakuun-dake, Hokkaido Prefecture in 1925 has been deposited as *E. caucasicum* in SAPA. The specimen mentioned above together with three others have been arranged in the same sheet in SAPA. As there was no record of *E. caucasicum* in the Japanese literature (Anonymous 2000; Ezuka 1998; Ito 1955), we reexamined these four specimens in SAPA comparing with the description of *E. caucasicum*.

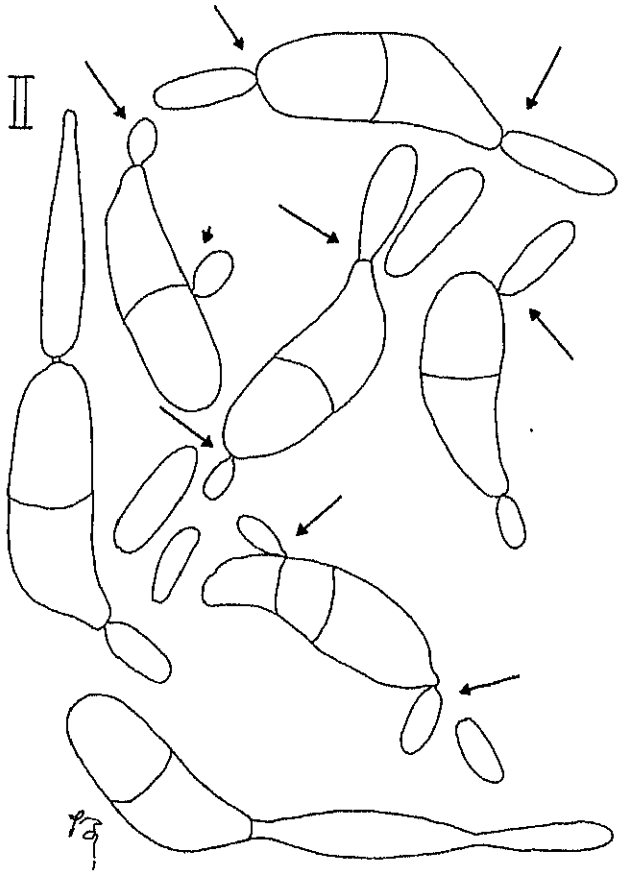


Fig. 8. Basidiospore germination of *E. caucasicum* (NIAES 20542) on PDA after 22h incubation. Basidiospores budded conidia (*arrows*). *Bar* 3μm

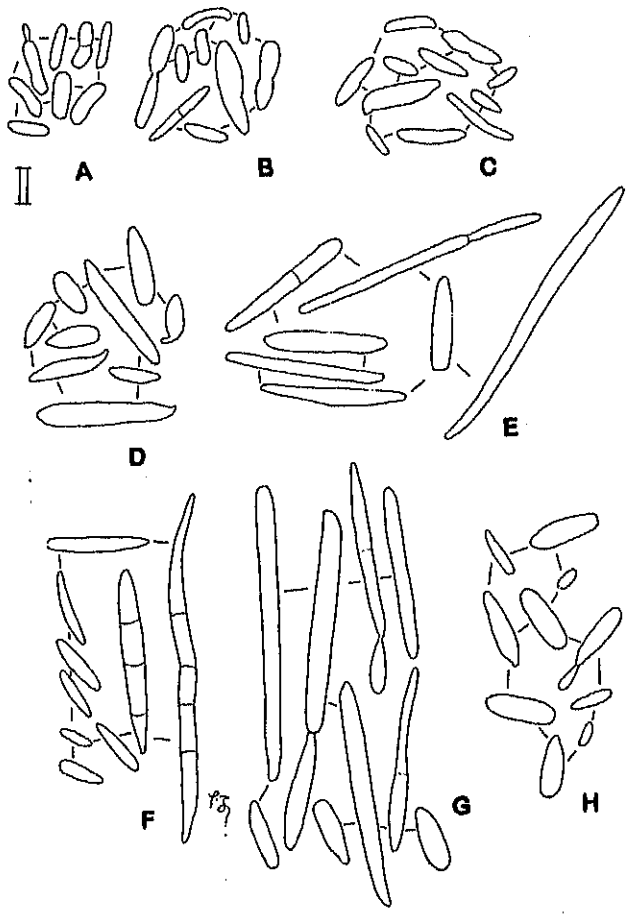


Fig. 9. Conidia of *E. shiraianum* MAFF 238602 (**A**), MAFF 238603 (**B**), MAFF 238604 (**C**), *E. woronichinii* MAFF 238610 (**D**), MAFF 238666 (**E**), MAFF 238617 (**F**), MAFF 238825 (**G**), and *E. caucasicum* MAFF 238830 (**H**) produced on PDA in 21-day incubations at 22°C. *Bar* 3μm

Lengths of basidia were shorter than the original description, whereas widths of basidia and the number of sterigmata matched with the same ranges of the original description. The feature of basidiospores showed high similarity each other. The fresh material (NIAES 20542) collected in 2002 was identified as *E. caucasicum* after examination of its morphological characteristics. The difference of morphological features of three *Exobasidium* species treated here was in their basidiospores; i.e., *E. shiraianum* had wider and *E. woronichinii.* had more septated basidiospores than *E. caucasicum*. Colonies of *E. caucasicum* on PDA were glutinous and showed yeast like growth. In this cultural characteristic *E. caucasicum* was similar to *E. shiraianum* but not to *E. woronichinii*. Color of colonies of *E. caucasicum* was pale orange, and that of *E. shiraianum* was pale primrose-yellow to pale orange with farinose appearance. Anamorphic culture of *E. caucasicum* produced bacilliform and lacrimiform conidia (Fig. 9H) and pseudohyphae, whereas *E. woronichinii* produced variously shaped conidia (Fig. 9D–G) and pseudohyphae. Exobasidium leaf blight on *R. aureum* by *E. caucasicum* is characterized by chlorosis and powdery appearance on the lower surface of newly developed leaves (Fig. 16A–C). Woronichin (1926) discussed the distribution of *E. caucasicum* to be limited to high altitude or high latitude, and it might be due to adaptation to the climate in the glacial period. In Japan, *R. aureum* is distributed in the alpine area of Hokkaido and Nagano Prefectures, and *E. caucasicum* also followed or accompanied the distribution of *R. aureum*. Three *Exobasidium* species treated here may have host specificity on *Rhododendron* spp. (Figs. 14B, 15B,C,E, 16B), although inoculation tests are needed to demonstrate the host specificity.

Acknowledgments We thank Dr. A. Ezuka for collecting the specimens of *R. degronianum* and *R. brachycarpum*, Ms. M. Myrdal in the Swedish Museum of Natural History for sending type specimens on loan, and Prof. H. Takahashi in the Herbarium of the Hokkaido University Museum for loaning materials. We profoundly appreciate the cooperation of Dr. V. Melnik for providing Russian papers and Dr. L. Vasilyeva for translating them into English; Dr. M. Akimoto, Donan Branch, Hokkaido Forestry Research Institute, Prof. Y. Harada, Hirosaki University, and Dr. W. Abe, Graduate School of Science, University of Hokkaido, for kind help with the sampling of *R.*

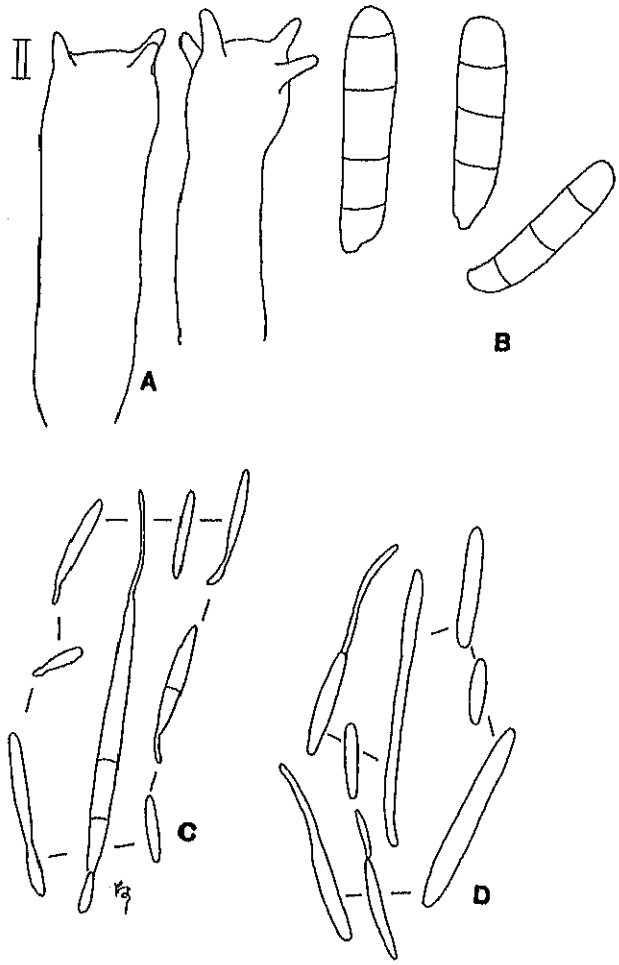


Fig. 10. Basidia and basidiospores of *E. hemisphaericum* TSH-B 0033. Basidium (**A**), basidium with basidiospores (**B**), and basidiospores (**C**) on *R. degronianum*. Conidia produced on PDA in 21-day incubations at 22°C (**D**, E11; **E**, E13). *Bar* 3 μm

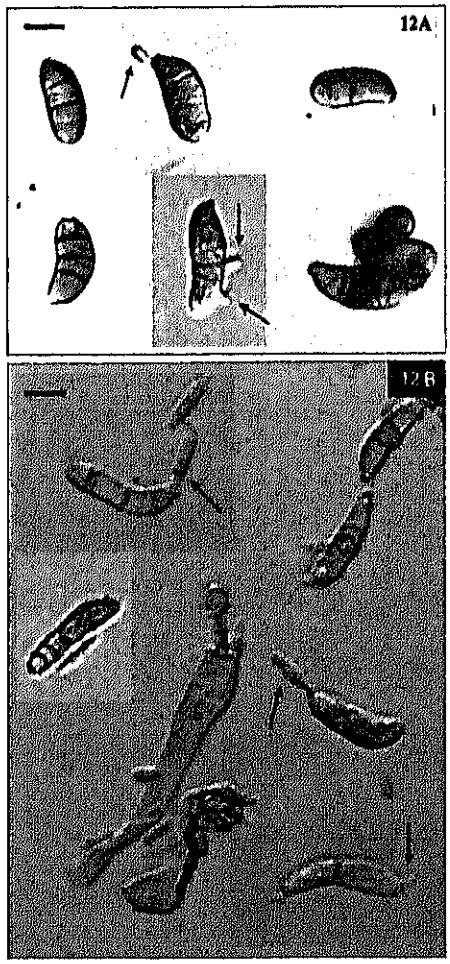
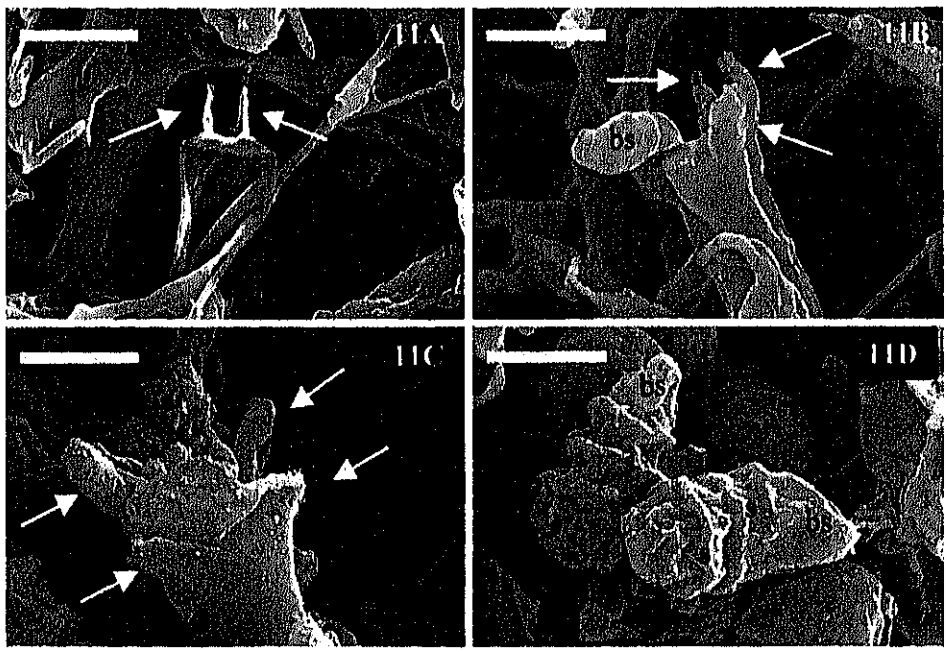


Fig. 12. Basidiospores of *E. shiraianum* and *E. woronichinii*: TSH-B0026 (**A**); TSH-B0114 (**B**). *b*, basidium; *arrows*, germination. *Bars* 3 μm

Fig. 11. Hymenium of *Exobasidium* spp. observed by scanning electron microscopy (SEM). **A** Basidia of *E. shiraianum* holotype (S, F20843) on infected leaf of *R. degronianum*. **B** Basidium and basidiospores of *E. shiraianum* holotype (S, F20843) on infected leaf of *R. degronianum*. **C** Basidium of *E. shiraianum* TSH-B0023 on infected leaf of *R. degronianum*. **D** Basidiospores of *E. shiraianum* TSH-B0023 on infected leaf of *R. degronianum*. There were 2–3 septa. *bs* and *s*, basidiospore and septum, respectively; *arrows*, sterigmata. *Bars* **A** 8.6 μm; **B** 5.0 μm; **C** 4.3 μm; **D** 6.0 μm



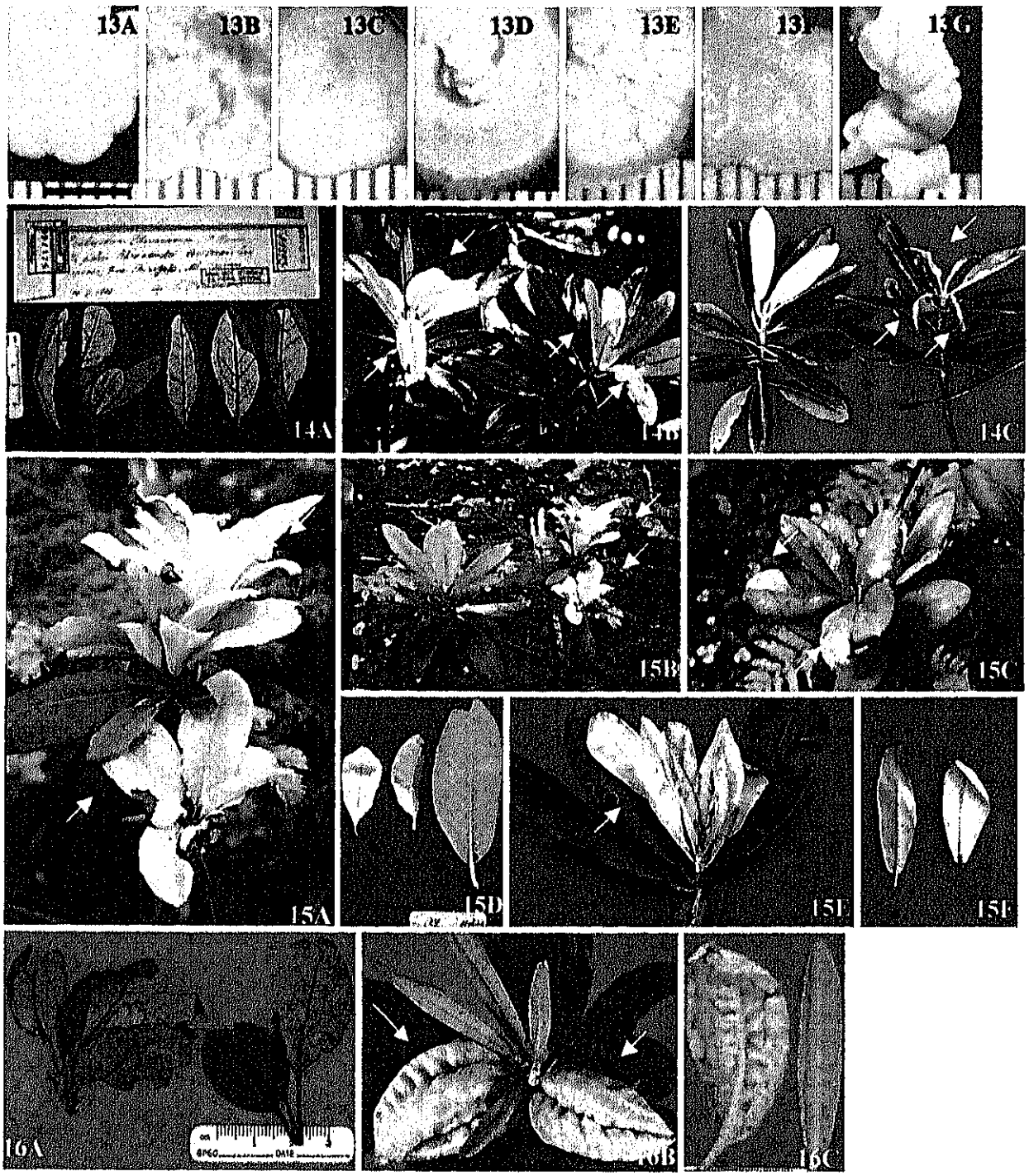


Fig. 13. Morphology and coloration of *Exobasidium* spp. on PDA. Surface of colonies of *E. shiraianum* MAFF 238602 (**A**), MAFF 238603 (**B**), 238604 (**C**); surface of colonies of *E. woronichinii* MAFF 238822 (**D**), MAFF 238825 (**E**); surface of colonies of *E. caucasicum* MAFF 238830 (**F**); surface of colonies of *E. hemisphaericum* E13 (**G**). *Bar* 5 mm

Fig. 14. Symptoms of Exobasidium leaf blight on *R. degronianum* by *E. shiraianum*. **A** Holotype collected on July 1900 in Gunma Prefecture. **B** Appearance of Exobasidium leaf blight on July 1999 in Nagano Prefecture. **C** Comparison of infected lower leaves (*right*) and healthy leaves (*left*). *Arrows*, chlorotic leaf

Fig. 15. Symptoms of Exobasidium leaf blight on *R. brachycarpum* by *E. woronichinii*. **A** Appearance of Exobasidium leaf blight on June 2001 in Hokkaido Prefecture. **B** Field appearance of Exobasidium leaf blight on June 2001 in Hokkaido Prefecture (*left*, healthy seedling; *right*, infected one). **C** Scratch lesions observed in some seedlings. **D** Comparison of infected lower leaf (*left*), partially infected lower leaf (*center*), and healthy leaf (*right*). **E** Appearance of Exobasidium leaf blight on June 1999 in Nagano Prefecture. **F** Comparison of infected lower leaf (*right*) and healthy leaf (*left*). Infected leaves were smaller than healthy ones. *Arrows*, chlorotic leaf

Fig. 16. Symptoms on Exobasidium leaf blight on *R. aureum* by *E. caucasicum*. **A** SAPA July 30, 1921, collected in Hokkaido Prefecture. **B** Appearance of Exobasidium leaf blight, June 2002, in Nagano Prefecture. **C** Comparison of infected lower leaf (*left*) and healthy leaf (*right*). *Arrows*, chlorotic leaf

brachycarpum; Dr. T. Yukawa, National Science Museum, Tokyo, for identifying the host plants including the host plant species, *R. degronianum*, of the holotype material of *E. shiraianum*; and Ms. H. Nakamura, for preparing the medium and helping with the experiments. This study was supported in part by a Grant-in-Aid for Scientific Research (B) (No.13460019), Japan Society for Promotion of Science (JSPS).

References

Anonymous (2000) Common names of plant diseases in Japan, 1st edn (in Japanese). Phytopathological Society of Japan, Tokyo

Davidian HH (1992) The *Rhododendron* species, vol III. Elepidotes continued: Neriiflorum-Thomsonii, Azaleastrum and Camtschaticum. Timber Press, Portland, OR, pp 151–174

Ezuka A (1991) Notes on some species of *Exobasidium* in Japan (IV). Trans Mycol Soc Jpn 32:169–185

Ezuka A (1998) Syakunage-kona-mochi-byo. In: Kishi K (ed) Plant disease in Japan (in Japanese). Zenkoku Noson Kyoiku Kyokai, Tokyo, pp 944–945

Hennings P (1903) Fungi japonici. III. Bot Jahrb 32:34–46

Ideta A (1901) Exobasidiineae. In: Jitsuyo syokubutsu-byori-gaku (in Japanese). Shokwabo, Tokyo, pp 202–204

Ideta A (1903) Basidiomycetes, Hymenomycetes, *Exobasidium*. In: Nippon syokubutsu-byorigaku (in Japanese). Shokwabo, Tokyo, pp 416–417

Ideta A (1929) Exobasidiineae. In: Nippon syokubutsu-byorigaku, 10th edn (in Japanese). Shokwabo, Tokyo, pp 594–601

Ito S (1955) Mycological flora of Japan, vol II. Basidiomycetes no 4: Auriculariales, Tremellaes, Dacrymycetales, Aphyllophorales (Polyporales) (in Japanese). Yokendo, Tokyo, pp 46–55

Nagao H, Ezuka A, Ohkubo H, Kakishima M (2001) A new species of *Exobasidium* causing witches' broom on *Rhododendron wadanum*. Mycoscience 42:549–554

Nagao H, Akimoto M, Kishi K, Ezuka A, Kakishima M (2003) *Exobasidium dubium* and *E. miyabei* sp nov causing Exobasidium leaf blisters on *Rhododendron* spp. in Japan. Mycoscience 44:1–9

Savile DBO (1959) Notes on *Exobasidium*. Can J Bot 37:641–656

Shirai M (1896) Descriptions of some new Japanese species of *Exobasidium*. Bot Mag Tokyo 10:53

Woronichin N (1921) Notes mycologiques 2–4 (in Russian). Monit Jard Bot Tiflis 51:1–6

Woronichin N (1926) *Exobasidium caucasicum* Woronich. in Transcaucasia and Kamchatka. Phytopathology 16:293–297