V. *Uromyces appendiculatus* and *U. vignae*

The objectives of the present study are to examine morphological features of urediniospores and teliospores in the *Uromyces appendiculatus* and *U. vignae* species complex for detecting morphologically discrete taxa and assess thus circumscribed taxa by phylogenetic divergence estimated from molecular divergence in the D1/D2 regions of LSU rDNA and the ITS regions including 5.8 S rRNA gene.

1. Morphological analyses

Materials

Dry herbarium specimens were used for light microscopic (LM) and scanning electron microscopic (SEM) observation. Specimens examined were loaned from the Hiratsuka Herbarium, Tokyo, Japan (HH); the National Fungus Collections, United States Department of Agriculture, Beltsville, USA (BPI); the Mycological Herbarium of the Institute of Agriculture and Forestry, the 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).

In the present study, two hundred and twenty-five specimens, including two species of *Phaseolus*, five species of *Vigna*, one species of *Apios*, one species of *Lablab* and one species of *Dunbaria*, were reported to be susceptible to *U. appendiculatus* and *U. vignae* and were examined for morphological characteristics in

teliospores and urediniospores (Table 5.1). The host identity and uniform application of their names that were mandatory for the study ascertained by Duke (1981) and Ohwi and Kitagawa (1992).

Results and discussion

Morphological features of uredinia and urediniospores: The uredinia *on Phaseolus, Vigna, Apios, Lablab* and *Dunbaria* were amphigenous, scattered or aggregated, early naked, brown or dark brown. Urediniospores of the specimens examined were globose, subglobose, ovoid, obovoid or ellipsoid and the urediniospore-wall ornamentation was uniformly echinulate (Fig. 5.1). Size and shape variations of the spores within individual specimens were as broad as those among the specimens. The mean urediniospore length of individual specimen ranged from 21.3 to 30.9 µm, width from 18.4 to 25.3 µm and wall thickness from 1.1 to 1.9 µm. The scatter diagram generated from these variables revealed no discrete morphological groups (Fig. 5.2, 5.3).

However, the number and position of *urediniospore germ-pores were steady* among the spores in each specimen. Germ pores were 2 (rarely 3) located on equatorial to superequatorial zone generally (Fig. 5.8A). Germ pores were 2 located on equatorial (rarely superequatorial) zone (Fig. 5.9A). Germ pores were 2 (rarely 3) located on superequatorial (rarely equatorial) zone (Fig. 5.10A). Thus, by the germ-pores position in the urediniospores, the specimens examined were sorted into three groups.

The rust fungi on *Phaseolus, Vigna* and related legumes had been reported morphological variations in their urediniospore (Fromme 1924; Arthur 1934). Cummins (1978), Guo and Wang (1986) and Hiratsuka et al. (1992) emphasized that position of urediniospore germ-pores was an important characteristic to distinguish *U. appendiculatus* from *U. vignae*. Moreover, Hiratsuka et al. (1992) emphasized that the size variations of urediniospores in *U. appendiculatus* were important to separate varieties. However, the quantitative characters were not sufficient to separate the specimens.

Morphological features of telia and teliospores: The specimens of telia and teliospores on *Phaseolus, Vigna, Apios, Lablab* and *Dunbaria* did not show distinct differences. Telia were amphigenous, scattered or aggregated and brown to dark. Teliospores of the specimens examined were subglobose, ovoid or ellipsoid and the wall ornamentation ranged from irregularly verrucose, slightly verrucose to completely smooth (Fig. 5.4). The degree of verrucouseness or smoothness of the wall surface varied both within each specimen and among the specimens. In the specimens identified as *U. appendiculatus*, the mean teliospore length ranged from 24.2 to 34.1 µm and width from 17.6 to 27.1 µm. In those identified *U. vignae*, the mean teliospore length ranged from 26.2 to 33.2 µm and width from 19.7 to 27.7 µm. The size variation in each specimen was as large as that among the specimens; thus, no discrete morphological groups were detected by the teliospore size (Fig. 5.5). On the other hand, teliospore wall-thickness varied narrowly within individual specimens and

differed among the specimens (Fig. 5.6A, 5.6B).

Cummins (1978) emphasized wall ornamentation was an important characteristic to distinguish *U. appendiculatus* from *U. vignae*, but the wall ornamentation was not steady characteristic among the specimens. Hirata (1952), Hiratsuka (1973) and Hiratsuka et al. (1992) emphasized that morphology of teliospores was different between *U. appendiculatus* var. *azukicola* (=*U. azukicola*) and *U. appendiculatus* var. *appendiculatus* and the characteristic could use to be a taxonomic characteristic.

Statistical analysis: Minimum, mean and maximum values of measurement data were analyzed by method of principal component analysis. Principal component analyses were undertaken with various combinations of numerical variables in urediniospores and teliospores features. In the analysis present here (Fig. 5.7A) employed mean values of urediniospore length, width and wall-thickness and teliospore length, width, wall-thickness and the thickness of the apical papilla. After the Varimax rotation, the calculated factors 1 and 2 explained 42.1 % and 21.0 % 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 (Fig. 5.7A). Analyses employed other combination of variables showed similar results. I combined the specimens of morphological group with principal component analysis and the specimens of morphological group I seem to form different group from the specimens of morphological group II and III, although they are still some overlap (Fig. 5.7B).

Morphological grouping: In combination of the germ pore position in urediniospores and the teliospore wall-thickness, the specimens examined were sorted into three morphological groups (Table 5.2).

Group I was commonly observed on *V. angularis* var. *angularis*, *V. angularis* var. *nipponensis*, *V. umbellata*, *A. fortunei*, *D. villosa*, *V. radiata* and *P. minimus*, and a few on *P. vulgaris*. Uredinia were amphigenous and scattered or aggregated, and surround by yellow or light yellow lesion. Uredinia were brown or brownish. Urediniospores were subglobose or obovioid. Spore size was 17.2-31.4×15.9-26.8 μm and the spore wall was 0.9-2.4 μm thick, brown or light brown and echinulate. Spore germ-pores were 2 (rarely 3) from equatorial to superequatorial position. Telia were amphigenous, scattered or aggregated and brown or brownish. Teliospores were ellipsoid or obovoid. Spore size was 19.4-37.1×14.6-27.3 μm, and the wall was light brown or brown. The mean wall thickness of individual specimens was 1.4-2.3 μm (rarely over 2.3 μm), and irregularly verrucose, slightly verrucose or smooth. Teliospores had apical papilla of 1.1-6.5 μm thick (Fig 5.8). In the scatter diagram with the mean teliospore wall-thickness and mean teliospore papilla-thickness, and the scatter diagram with the mean teliospore width and mean teliospore wall-thickness seems to reveal different group from other two groups (Fig. 5.11A, 5.11B).

Group II was commonly observed on *Phaseolus vulgaris* and a few on *V. angularis* var. *angularis*. Uredinia were amphigenous, scattered or aggregated and brown. Urediniospores were globose, subglobose or ovate. Spore size was 19.2-33.5 ×16.7-27.9 μm, and spore wall was 0.9-2.4 μm thick, brown or brownish and

echinulate. Urediniospore pores were 2 on equatorial (rarely superequatorial) position. Telia were amphigenous, scattered or aggregated and dark or dark brown. Teliospores were subglobose, ovate, obovoid or ellipsoid. Spore size was 21.4-42.1 × 16.8-32.1 μm, and walls were brown or dark brown. Walls mean in individual specimen was 2.4-3.3 μm thick, and irregular verrucose, slight verrucose or smooth (Fig. 5.9).

Group III was commonly observed on *V. unguiculata* ssp. *unguiculata, V. unguiculata* ssp. *sesquipedalis, V. marina* and *L. purpureus,* and only one on *V. angularis var. nipponensis* and *P. vulgaris*. Uredinia were amphigenous, scattered or aggregated and brown. Urediniospores were globose, subglobose, ellipsoid or ovate. Spore size was 20.7-35.1 × 15.3-27.9 μm thick, and spore wall was 0.9-1.7 μm, brown or light brown and echinulate. Spore pores were 2 (rarely 3) on superequatorial (rarely equatorial) position. Telia were amphigenous, scattered or aggregated and dark or dark brown. Teliospores were subglobose, ovate, obovoid or ellipsoid. Spore size was 21.4-37.5 × 16.8-31.0 μm, and walls were brown or dark brown. Walls mean in individual specimen was 2.6-3.4 μm thick, and irregular verrucose, slight verrucose or smooth (Fig. 5.10). Moreover, the scatter diagram with factor 1 and factor 2 generated from length, width, and wall thickness of urediniospores and length, width, wall thickness and papilla thickness, the specimens of group I seems to different from group II and group III (Fig. 5.7B).

Table 5.1. Specimens of rust fungi used for morphological observations

Host plants	Locality in Japan (No. of specimens)
V. angularis (Willd.) Ohwi & Ohashi var. *angularis*	Hokkaido (5), Honshu (30), Kyushu (10), Shikoku (1), Okinawa (4)
V. angularis var. *nipponensis* (Ohwi) Ohwi & Ohashi	Honshu (10), Kyushu (3), Okinawa (1)
V. umbellata (Thunb.) Ohwi & Ohashi	Honshu (6), Kyushu (3), Shikoku (1)
A. fortunei Maxim.	Honshu (5), Kyushu (3), Shikoku (3)
D. villosa (Thunb.) Makino	Honshu (1)
V. radiata (L.) Wilczek	Hokkaido (1), Kyushu (1), Shikoku (1), Japan (1)
P. minimus Roxb.	Okinawa (2)
P. vulgaris L.	Hokkaido (16), Honshu (49), Kyushu (5), Shikoku (4), Okinawa (2), Japan (3)
V. unguiculata (L.) Walp. ssp. *unguiculata*	Honshu (22), Kyushu (9), Shikoku (4), Okinawa (5)
V. unguiculata ssp. *sesquipedalis* (L.) Verdc.	Honshu (4), Kyushu (2), Shikoku (1)
V. marina Merr.	Okinawa (3)
L. purpureus (L.) Sweet	Honshu (1), Okinawa (3)
Total	225

Table 5.2. Morphological groups based on their characteristics of urediniospores and teliospores.

Morphological groups	Urediniospores	Teliospores	Host plants (number of specimens)
	No. of germ pores and its position	Individual mean of wall-thickness	
I	2 (rarely 3), equatorial or superequatorial	1.4-2.3μm (rarely over 2.3μm)	*Vigna angularis* var. *angularis* (46) *V. angularis* var. *nipponensis* (14) *V. umbellata* (10), *V. radiata* (4) *Dunbaria villosa* (1), *Apios fortunei* (18), *Phaseolus minimus* (2) *Phaseolus vulgaris* (9)
II	2, equatorial (rarely superequatorial)	2.4-3.3μm	*P. vulgaris* (71) *V. angularis* var. *angularis* (3)
III	2(rarely 3), superequatorial (rarely equatorial)	2.6-3.4μm	*V. unguiculata* ssp. *unguiculata* (41) *V. unguiculata* ssp. *sesquipedalis* (7) *V. marina* (3), *Lablab purpureus* (4) *V. angularis* var. *nipponensis* (1) *P. vulgaris* (1)

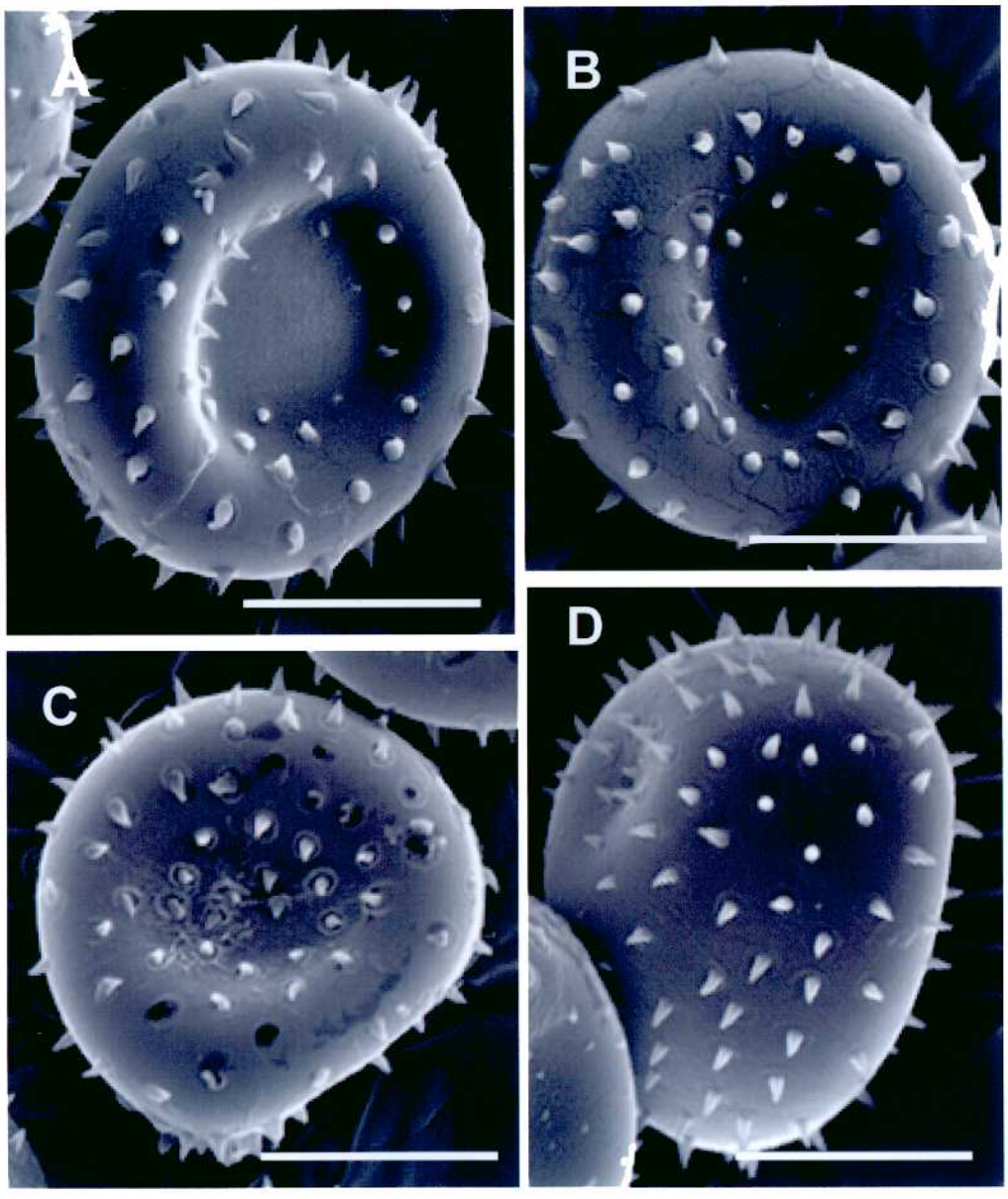

Fig. 5.1. Wall ornamentation of urediniospores from *Phaseolus vulgaris* (**A, B**), *Vigna angularis* var. *angularis* (**C**), *Vigna unguiculata* ssp. *unguiculata* (**D**). Bar=10 µm

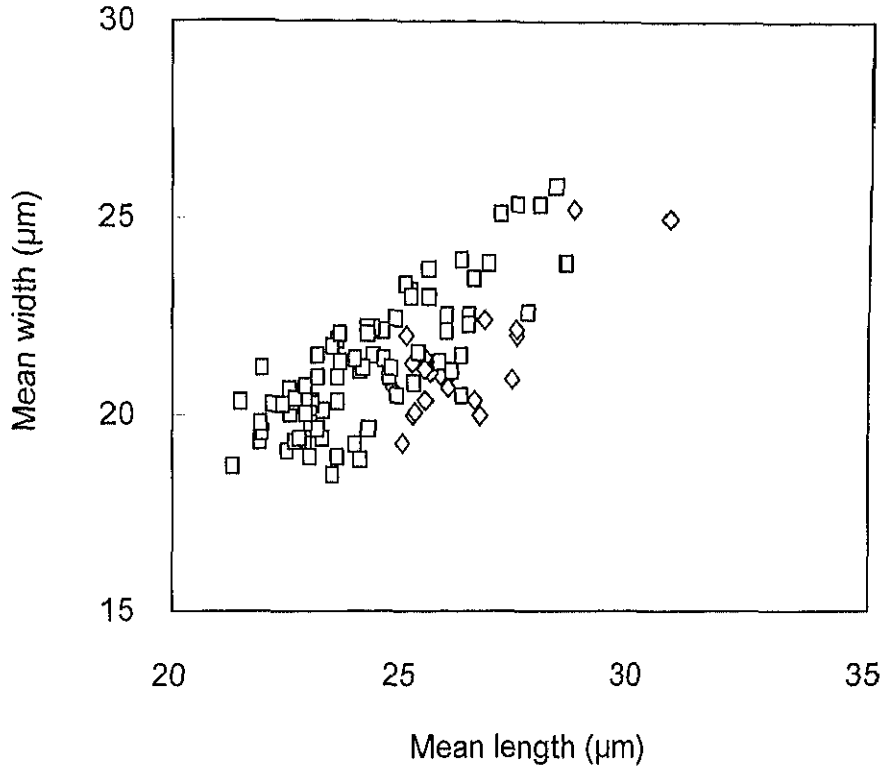

Fig. 5.2. A scatter diagram generated from urediniospores mean length and mean width of the specimen used for morphological analysis. □: the specimens of *Uromyces appendiculatus*; ◇: the specimens of *U. vignae*.

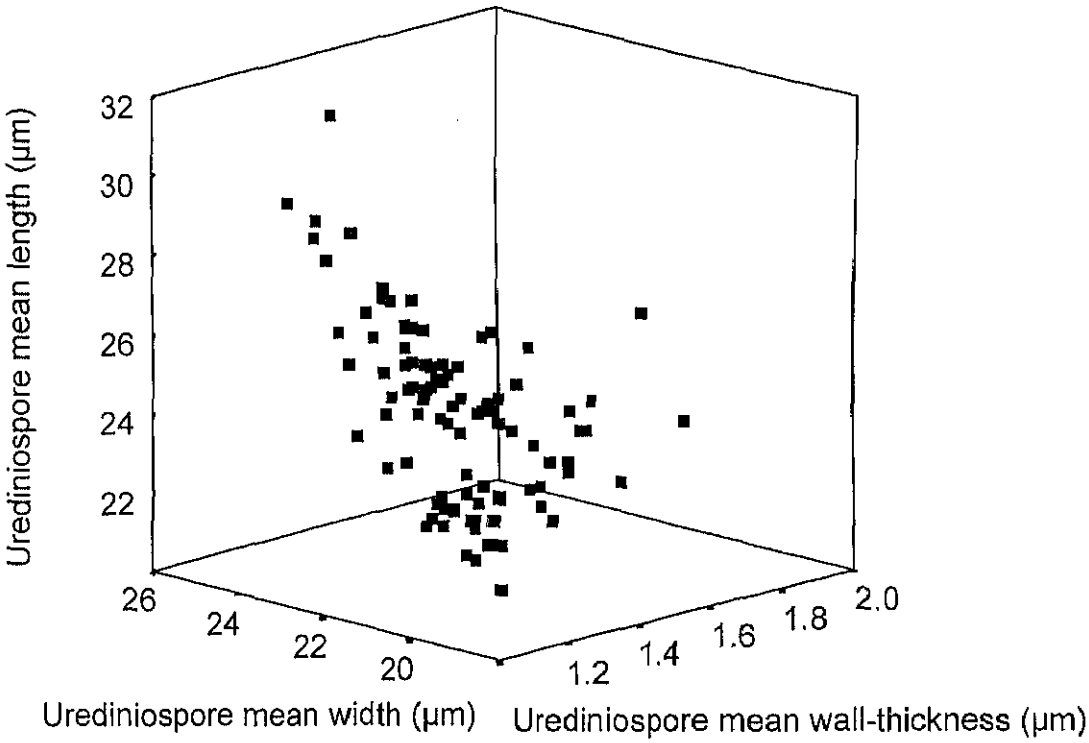

Fig. 5.3. A scatter diagram generated from urediniospores mean length, mean width and mean wall-thickness on *Phaseolus, Vignae, Apios, Lablab* and *Dunbaria*.

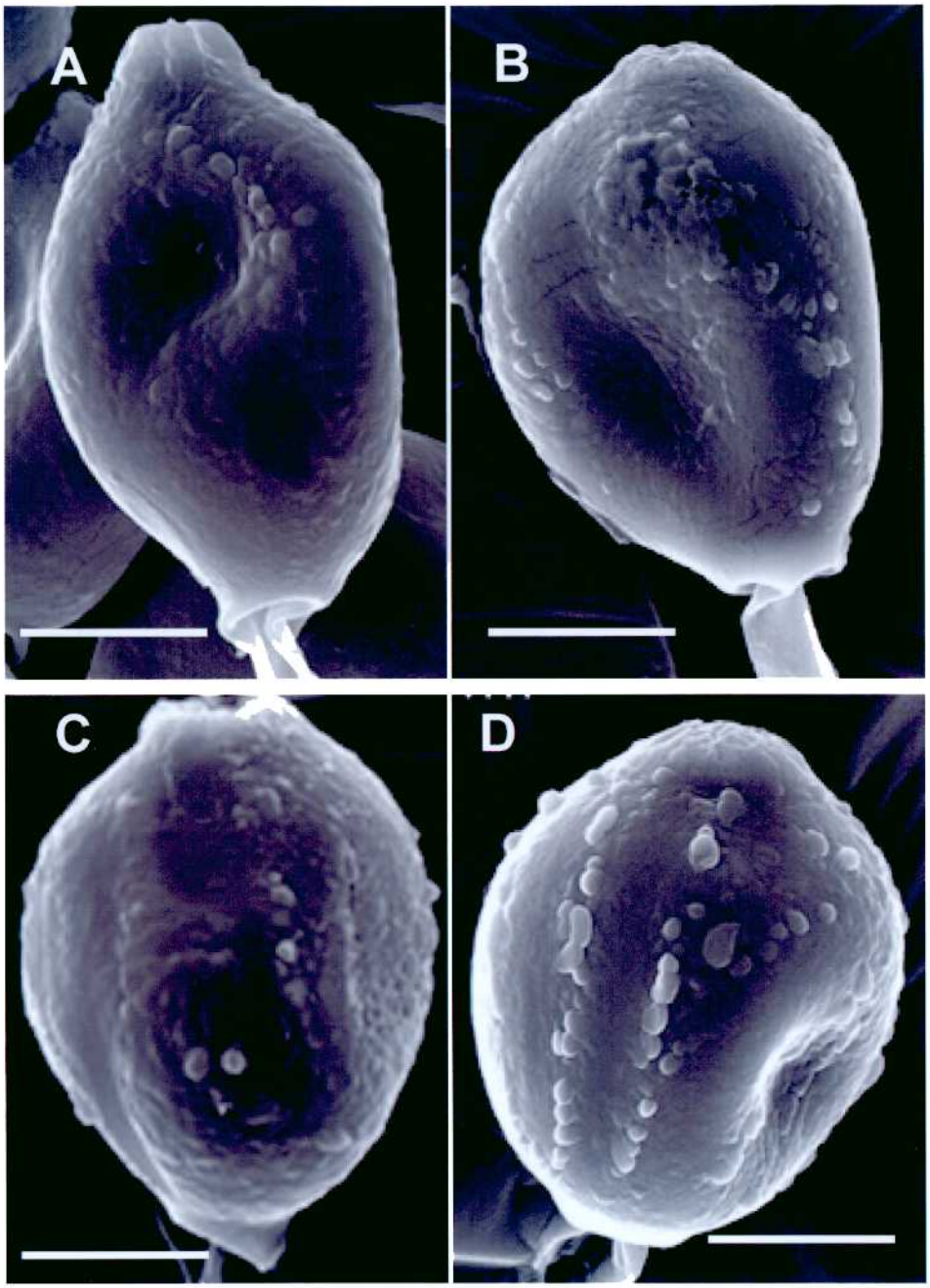

Fig. 5.4. Wall ornamentation of teliospores from *Vigna angularis* var. *angularis* (**A**), *Phaseolus vulgaris* (**B, D**), *Vigna unguiculata* ssp. *unguiculata* (**C**). Bar=10 µm

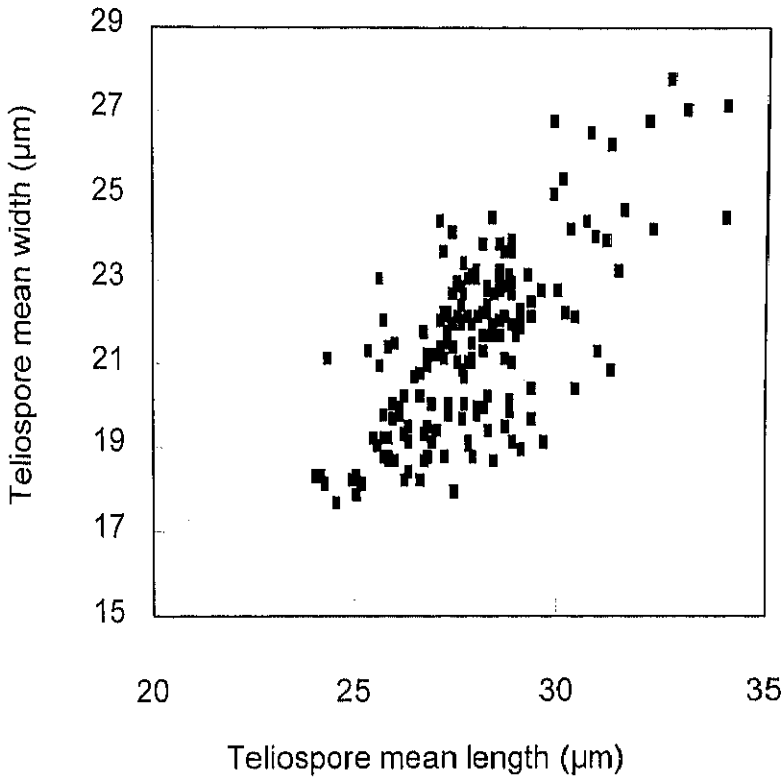

Fig. 5.5. A scatter diagram generated from teliospores mean length and mean width on *Phaseolus, Vignae, Apios, Lablab* and *Dunbaria*.

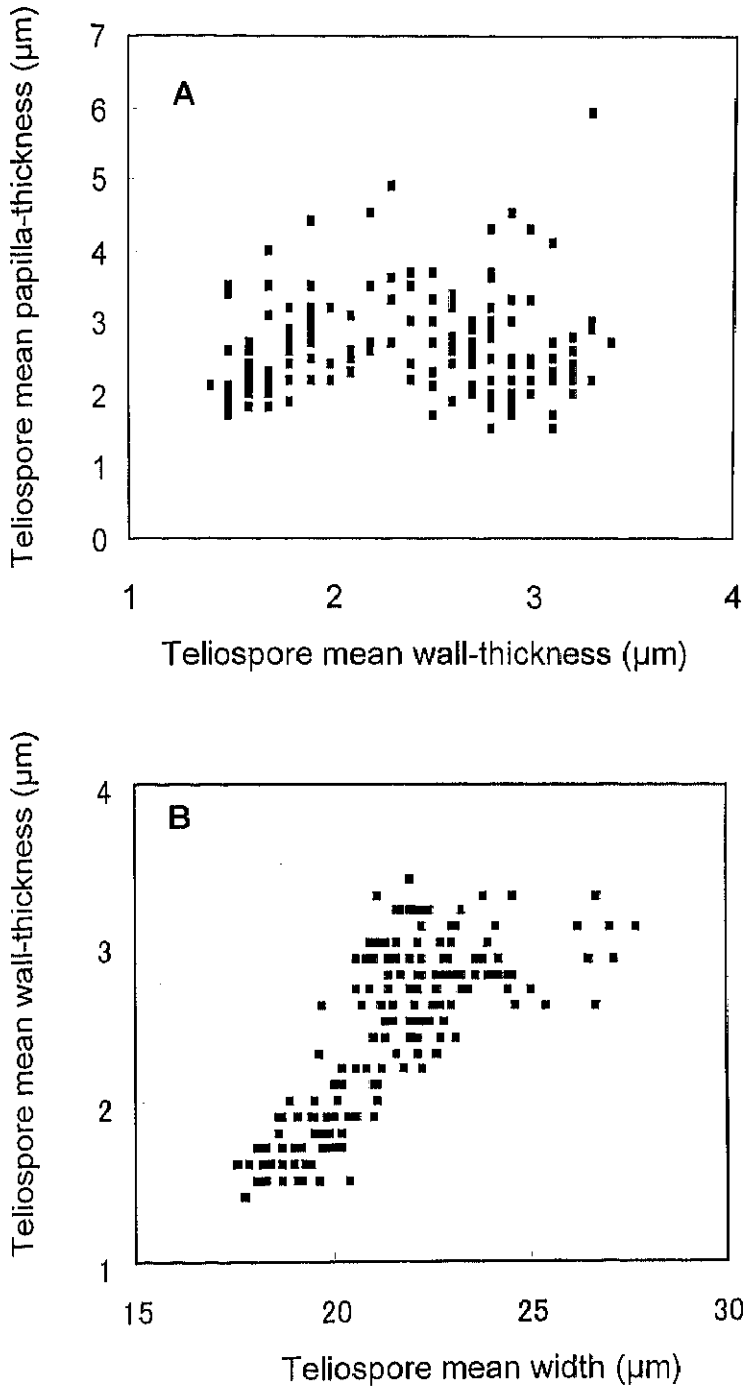

Fig. 5.6. Scatter diagram generated from teliospores mean width, mean wall-thickness and papilla-thickness of the specimens on *Phaseolus, Vignae, Apios, Lablab* and *Dunbaria*.

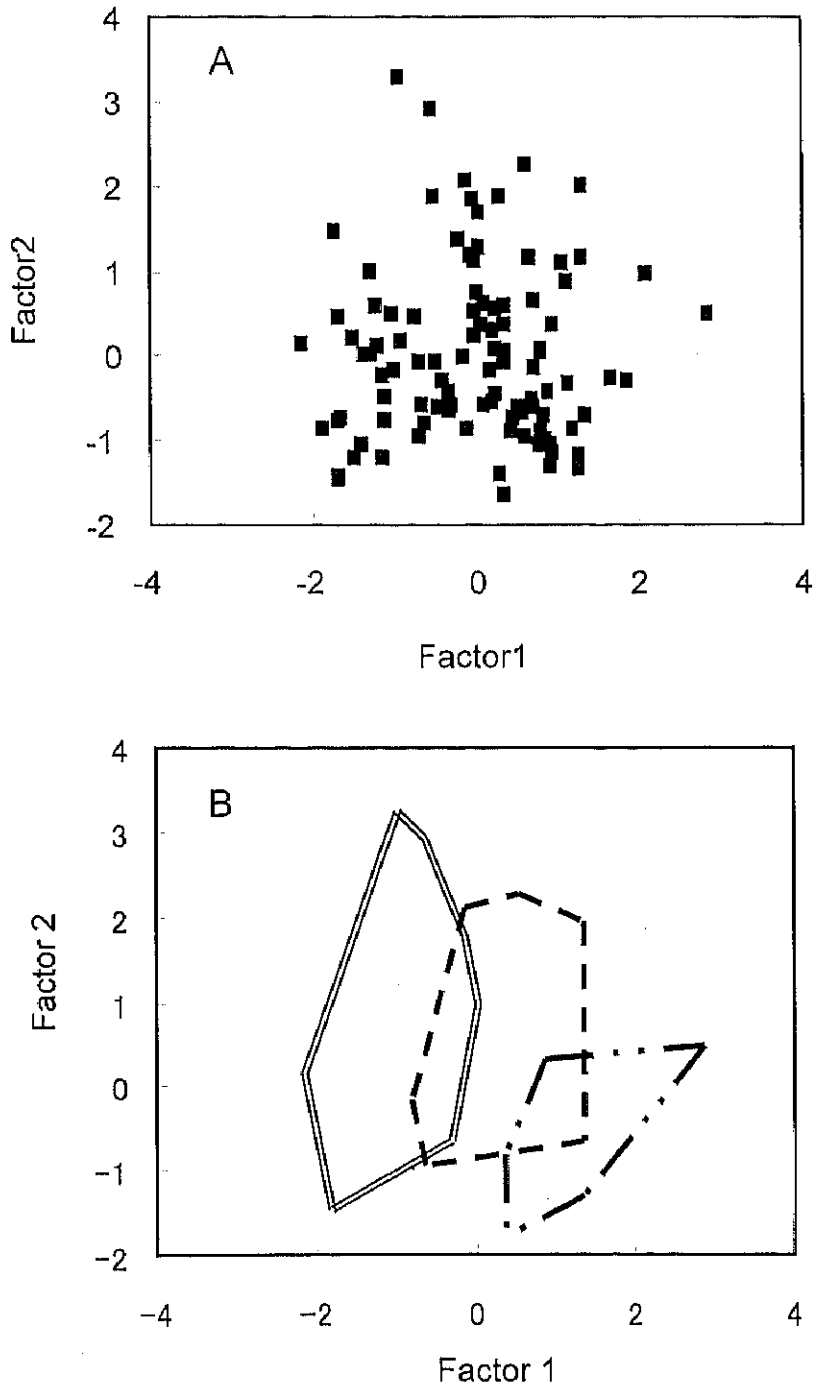

Fig. 5.7. Two-dimensional map of the specimens using principal component analysis. **A** A result of a principal component analysis. **B** Distribution of possible morphological groups in a diagram generated by a principal component analysis. ═══: morphological group I; ------ : morphological group II; ⁻·⁻·⁻ : morphological group III.

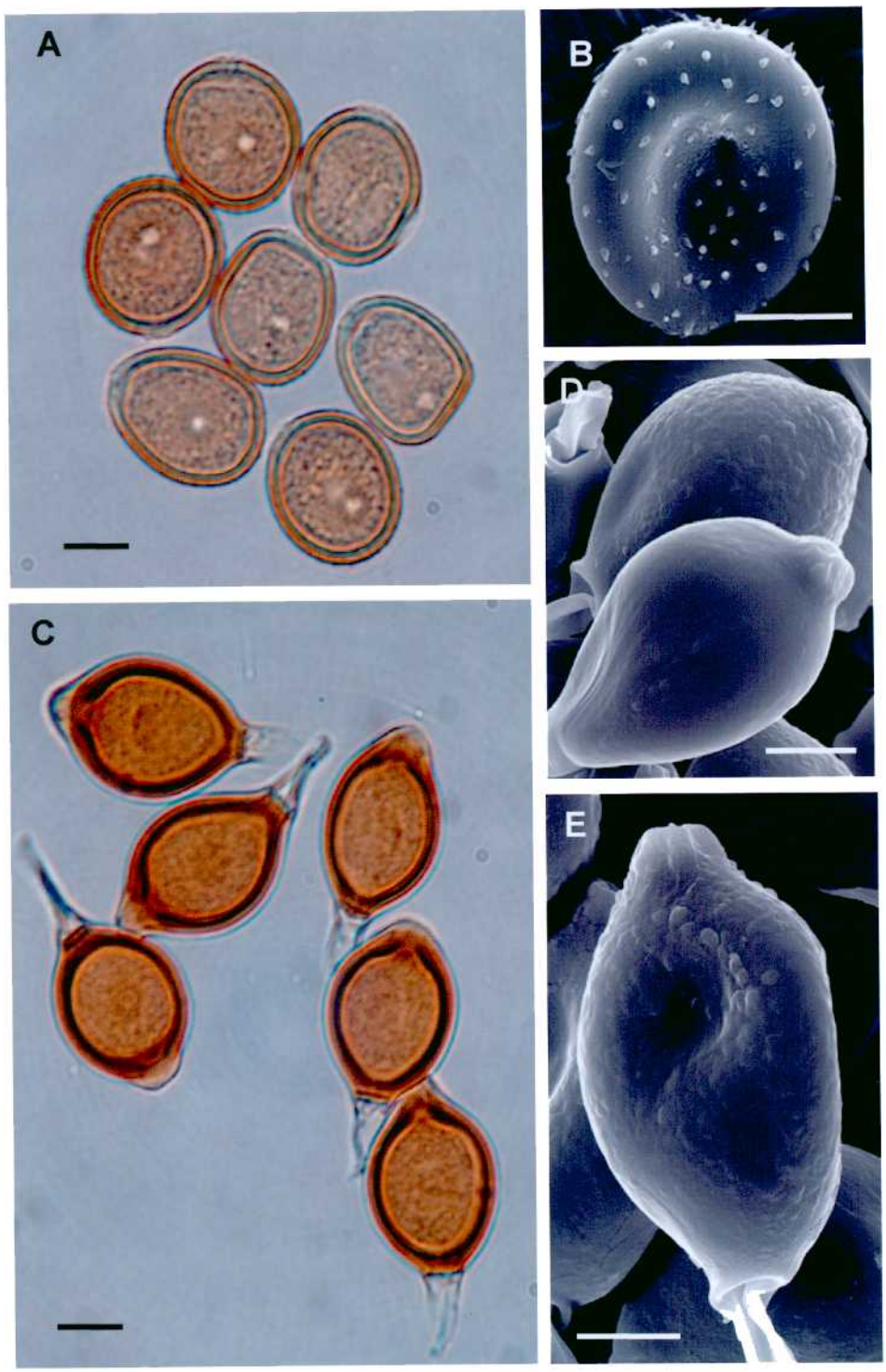

Fig. 5.8. Morphology of Group I. A, B: Urediniospores. C, D, E: Teliospores. Bras =10 μ m

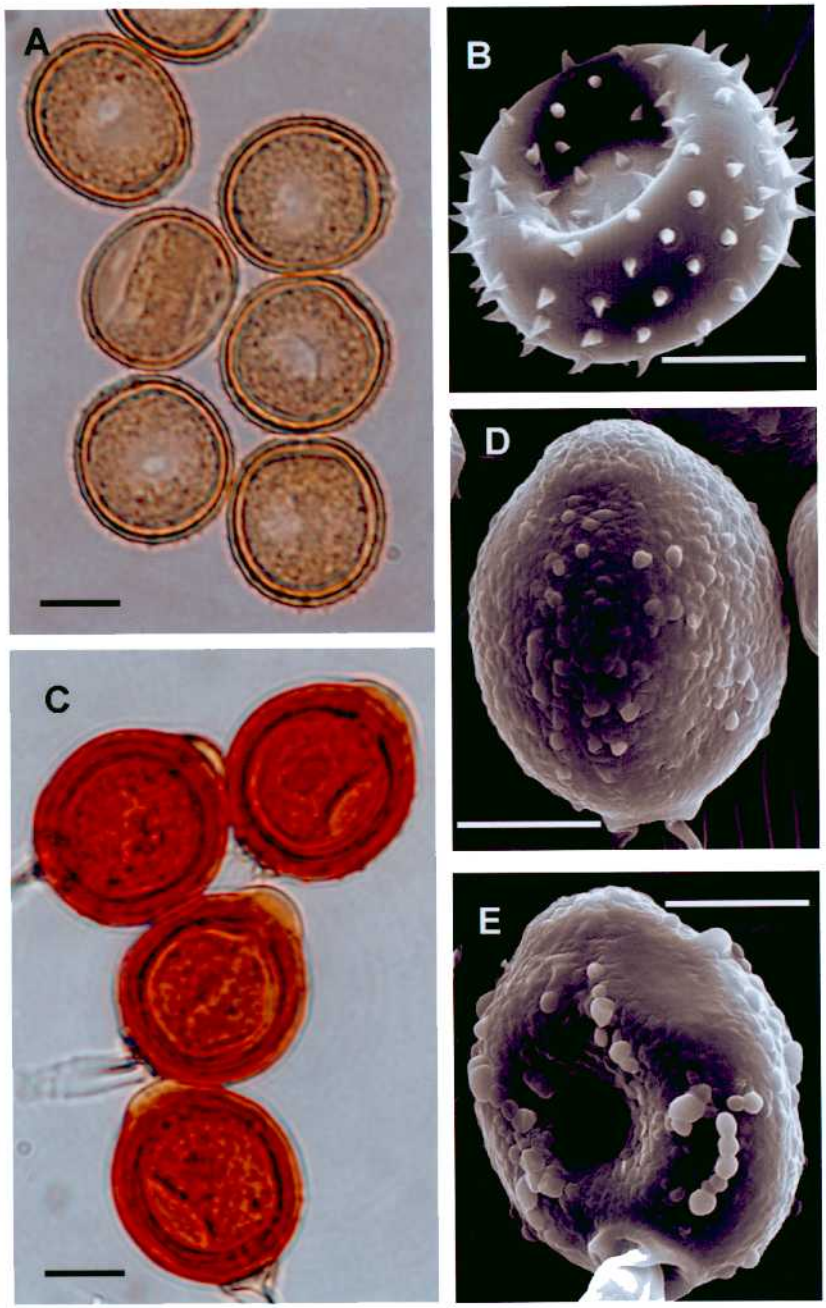

Fig. 5.9. Morphology of Group II. A, B: Urediniospores. C, D, E: Teliospores. Bras =10 μ m

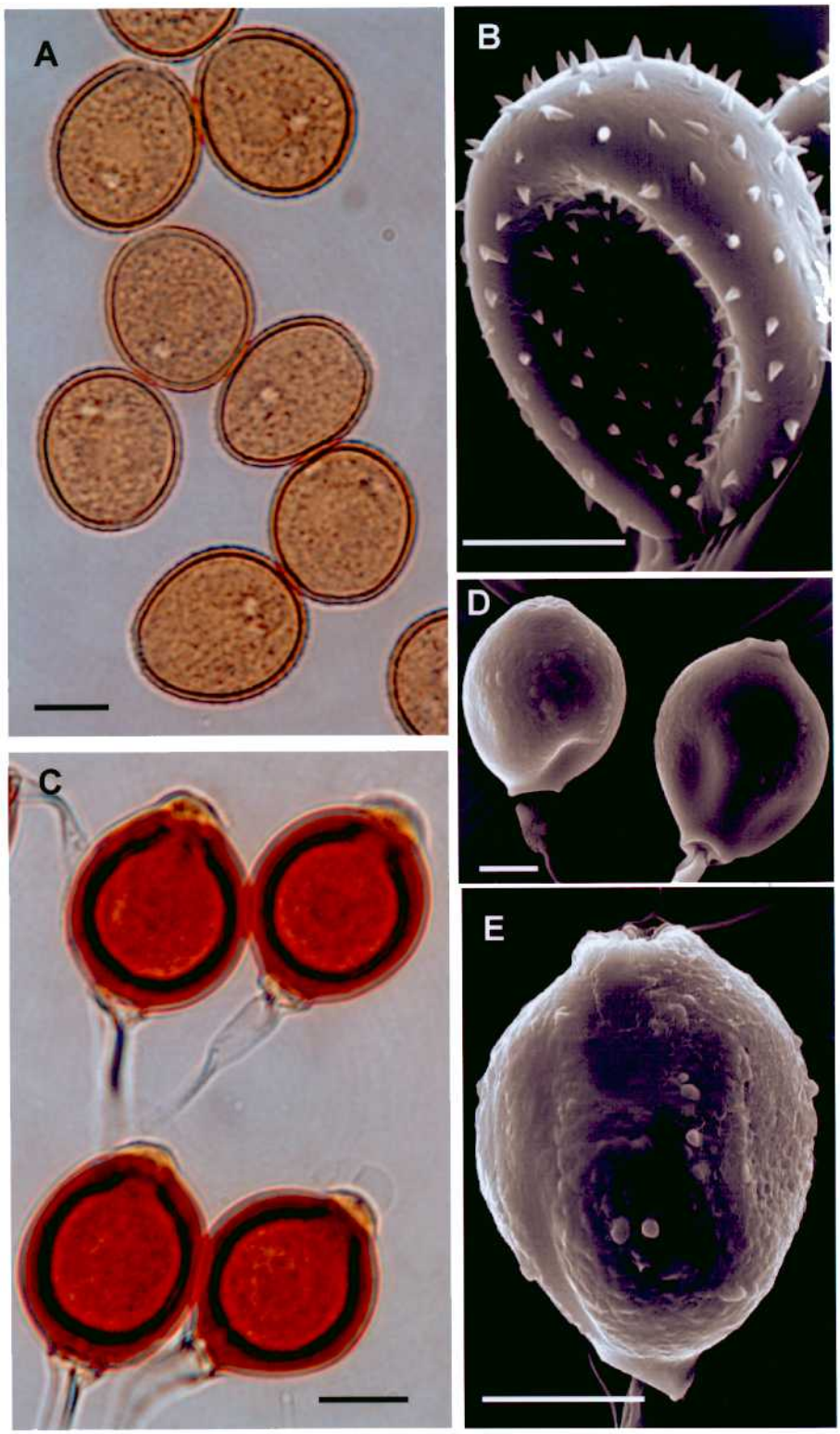

Fig. 5.10. Morphology of Group III. A, B: Urediniospores. C, D, E: Teliospores. Bras =10 μ m

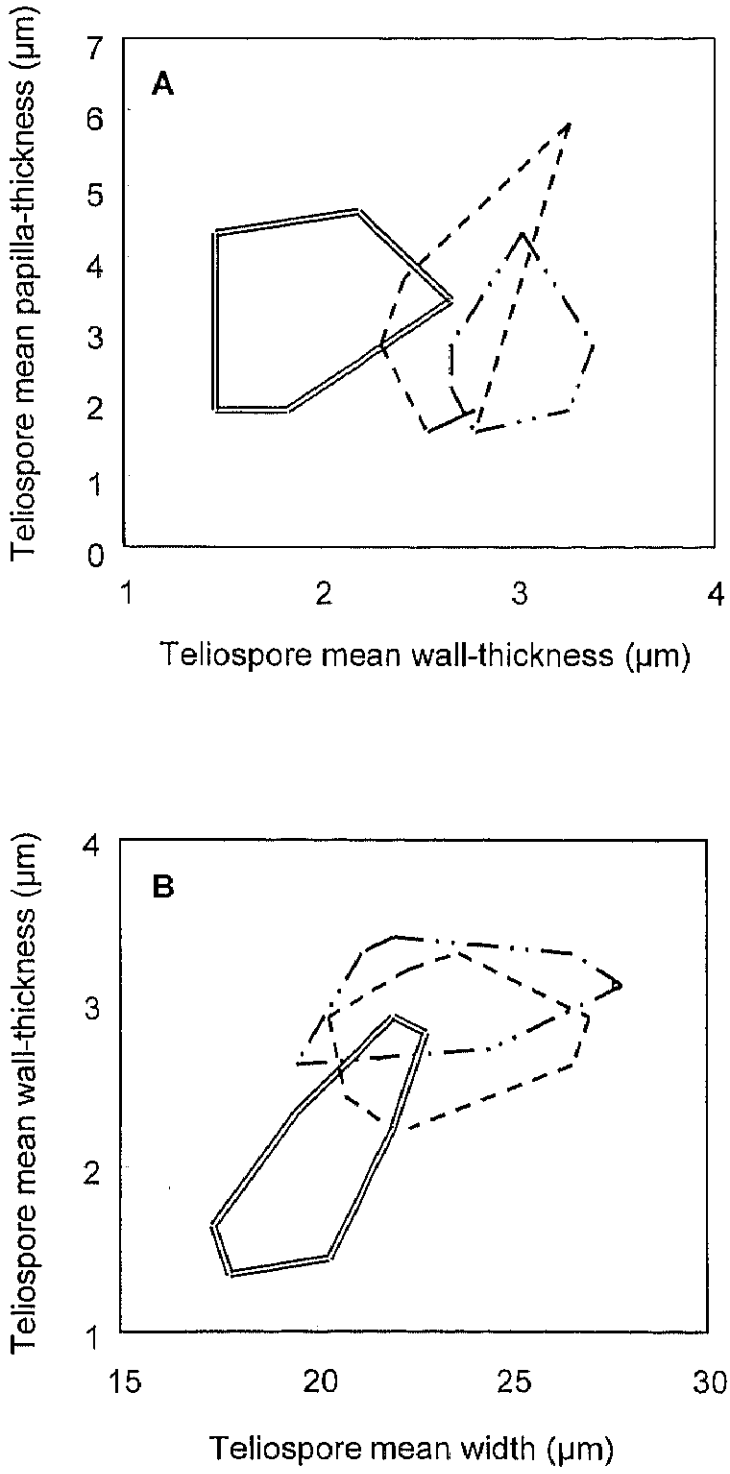

Fig. 5.11. Scatter diagram generated from teliospore side wall-thickness against teliospore papilla-thickness (A) and teliospore width against teliospore side wall-thickness (B) that superimposed with morphological groups on. Group I: ═══ ; Group II: - - - - ; Group III: — · · — .

2. Molecular phylogenetic analyses

Materials

Forty-five specimens on *Phaseolus, Vigna, Apios, Lablab* and *Dunbaria* (Table 5.3) were selected for sequence analyses at the D1/D2 region of LSU rDNA and ITS regions including 5.8 S rRNA gene. As outgroup taxa, non-fabaceous rust fungi were chosen *U. gageae* Beck on *Gagea lutea* (L.) Ker Gawl. and a *Puccinia miscanthi* Miura rust on *Miscanthus sinensis* Anderss. *Uromyces viciae-fabae* (Pers.) Schroet., *U. pisi* (DC.) Otth and *U. minor* Schroet. were also included in the analyses. These GenBank sequences of fungi used for phylogenetic comparison are listed in Table 5.4.

Results and discussion

The nucleotide sequences of the D1/D2 regions ranged from 597 to 617 bases, which were subjected to phylogenetic analyses. Of the 657 total characters included gaps, 43 sites were variable and 93 sites were parsimony informative characters. A neighbor-joining tree constructed from the D1/D2 region showed that the 45 specimens fell into two major clades with 84% bootstrap support (Fig. 5.12). The D1/D2 phylogram had a consistency index (CI) of 0.766, a retention index (RI) of 0.842, retention consistency (RC) of 0.645 and a tree length of 205. Between the two groups, the specimens grouped in the D1/D2 group I had higher T nucleotide repeats (10-11 bases) at D2 than the D1/D2 group II.

The ITS regions including 5.8 S rRNA gene were analyzed in the study ranged

from 625 to 640 bases. Of the 687 total characters including gaps, 107 sites were variable and 124 sites were parsimony informative. In a neighbor-joining tree constructed from the ITS regions including 5.8 S rRNA gene, the 45 specimens fell into three major clades with 100% bootstrap support (Fig. 5.13). The ITS phylogram had a consistency index (CI) of 0.678, a retention index (RI) of 0.802, retention consistency (RC) of 0.544 and a tree length of 398. The specimens constituting the ITS I, II and III groups possessed sequence homology at 98-99%, 95-100% and 97-100%, respectively. The ITS1 region revealed higher sequence divergence (32.4-35.1%) than the ITS2 region (27.2-29.3%). In the present study, the specimens of each three ITS groups showed morphological similarity in urediniospores and teliospores, and corresponded to each morphological group (Table 5.5). In addition, the D1/D2 and ITS including 5.8 S rRNA regions were analyzed by maximum-parsimony method and the results of maximum-parsimony analyses revealed similar topological tree with neighbor-joining analyses (Fig. 5.14, Fig. 5.15). Based on maximum-parsimony analyses, the two major D1/D2 clades were supported with high bootstrap value (84%) and three major ITS clades were supported with high bootstrap values (97%, 97%).

Morphological observations revealed that the specimens of D1/D2 group I and ITS group I showed same morphology as morphological group II. The D1/D2 group II included the specimens of morphological group III and I. In contrast D1/D2 region, the specimens of D1/D2 group II formed two major different clades inferred from ITS including 5.8 S rRNA gene regions (Table 5.5). The specimens of three ITS group are

corresponding to each three morphological groups. The specimens of ITS group I showed same morphology as morphological group II and urediniospore germ-pores were two being equatorial. The specimens of ITS group II showed same morphology as morphological group I and urediniospore germ-pores were two (rarely three) being constant mixture of equatorial and superequatorial, and teliospore wall-thickness was thinner. The specimens of ITS group III showed same morphology as morphological group III and urediniospore germ-pores were two (rarely three) being superequatorial. The specimens of mainly morphological characteristics of urediniospores and teliospores are listed in Table 5.6.

Table 5.3. Specimens of rust fungi sequences used for phylogenetic analysis.

Host plants	Locality in Japan	Voucher specimens[a]	GenBank accession no.	
			D1/D2	ITS
Apios fortunei	Kyoto	HH89813	AB115615	NA[b]
	Miyazaki	HH89814	AB115636	NA
	Tokyo	HH89673	AB115635	AB115727
	Miyazaki	HH89818	NA	AB115728
	Fukuoka	HH89819	NA	AB115729
Dunbaria villosa	Saitama	HH92766	AB115638	NA
Lablab purpureus	Okinawa	BPI-0019468	AB115614	AB115723
Phaseolus vulgaris	Ibaraki	TSH-R1734 (IBA-2460)	AB115644	AB115741
	Yamanashi	HH92821	AB115646	AB115739
	Tokushima	HH92832	AB115645	AB115740
	Tochigi	HH94638	AB115647	AB115738
	Okinawa	HH50721	AB115634	AB115726
Vigna angularis var. *angularis*	Nagano	HH92876	AB115642	AB115734
	Tokyo	HH92788	AB115640	NA
	Miyazaki	HH92802	AB115616	AB115732
	Yamaguchi	HH92014	NA	AB115730
	Hokkaido	TSH-R16262	NA	AB115705
	Hokkaido	TSH-R16263	AB115623	AB115706
	Hokkaido	TSH-R16264	AB115621	AB115707
	Hokkaido	TSH-R16265	NA	AB115708
	Ibaraki	TSH-R17960	AB115624	AB115714
	Tochigi	TSH-R16273	AB115623	NA
	Ibaraki	TSH-R17959	AB115620	AB115713
	Ibaraki	TSH-R17961	AB115625	AB115715
V. angularis var. *nipponensis*	Kagoshima	TSH-R16267	AB115622	AB115709
	Ibaraki	TSH-R16271	AB115619	AB115710
	Ibaraki	TSH-R1748 (IBA-7021)	NA	AB115712
V. umbellata	Hiroshima	HH92840	AB115641	AB115733
	Nagano	HH92772	AB115639	NA
V. marina	Okinawa	HH50717	AB115632	AB115724
	Okinawa	HH50718	AB115633	AB115725
V. unguiculata ssp. *sesquipedalis*	Fukuoka	HH92911	AB115618	NA
	Kagawa	HH92905	AB115617	AB115736
	Ibaraki	TSH-R17968	AB115630	AB115721

Table 5.3. (continued)

Host plants	Locality in Japan	Voucher specimens[a]	GenBank accession no. D1/D2	ITS
V. unguiculata ssp. *sesquipedalis*	Ibaraki	TSH-R17969	AB115631	AB115722
V. unguiculata ssp. *unguiculata*	Tokyo	HH92902	AB115643	AB115735
	Yamaguchi	HH92019	AB115637	AB115731
	Ibaraki	TSH-R17967	AB115629	AB115720
	Ibaraki	TSH-R17965	AB115613	AB115718
	Ibaraki	TSH-R17962	AB115626	AB115716
	Ibaraki	TSH-R17958	AB115612	AB115711
	Ibaraki	TSH-R17963	AB115627	AB115717
	Ibaraki	TSH-R17966	AB115628	AB115719
	Shizuoka	BPI-0000967	AB115648	AB085196
	Shizuoka	BPI-0000968	AB115649	AB085197

[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 analysis.

Table 5.4. Additional taxa selected for D1/D2 and ITS analyses.

Species	Host plants	GenBank accession no.	
		D1/D2	ITS
Uromyces minor Schroet.	*Trifolium lupinaster* L.	NA[a]	AB115737
U. viciae-fabae (Pers.) Schroet.	*Vicia pannonica* Crantz	AF426199	NA
U. viciae-fabae	*Vicia cracca* L.	AB115598	AB115655
U. viciae-fabae	*Pisum sativum* L.	AB115594	AB115651
U. viciae-fabae	*Lathyrus maritimus* Bigel.	AB115599	AB085193
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 analysis.

Table 5.5. Correlations between morphological groups and phylogenetic groups

Morphological groups	D1/D2 groups	ITS groups
I	II	II
II	I	I
III	II	III

Table 5.6. Morphological characteristics of urediniospores and teliospores of the specimens used for molecular phylogenetic analyses.

Host plants	Locality	Voucher specimen	Stage	Urediniospores				Teliospores			
				size (μm)	germ pores position and no.	wall sufrace	wall thickness (μm)	size (μm)	wall surface	wall thickness (mean) (μm)	papilla-thickness (μm)
Apios fortunei Maxim.	Kyoto, Kyoto Pref.	HH89813	III					22.5-31.4× 15.7-20.9	slight verrucose	1.3-2.6 (1.9)	1.1-5.2
	Mt. Shiraiwa, Miyazaki Pref.	HH89814	III					26.2-32.7× 17.4-23.1	slight verrucose	1.3-2.4 (1.9)	3.3-5.9
	Kogezawa, Tokyo Met.	HH89673	II, III	23.8-29.9× 22.2-28.8	2 or 3, equatorial or superequatorial	echinulate	1.1-1.5	24.4-34.0× 17.2-22.5	slight verrucose	1.5-2.6 (2.0)	2.2-5.5
	Sobosan, Miyazaki Pref.	HH89818	III					20.9-31.4× 17.0-22.5	slight verrucose	1.5-2.2 (1.9)	1.5-5.2
	Hikosan, Fukuoka Pref.	HH89819	III					22.9-30.5× 18.1-21.6	slight verrucose or smooth	1.5-2.6 (1.8)	1.7-4.4
Dunbaria villosa Makino	Tajimagahara, Saitama Pref.	HH92766	II, III	19.6-25.5× 15.9-23.5	2, equatorial or superequatorial	echinulate	1.1-1.5	21.4-31.6× 16.8-22.0	slight verrucos or smooth	1.5-2.0 (1.7)	2.2-5.9
Lablab purpureus (L.) Sweet	Oroku, Okinawa Pref.	BPI-0019468	II, III	23.1-29.0× 19.0-23.1	2, superequatorial	echinulate	1.1-1.5	24.2-31.0× 19.8-23.5	irregular verrucos or slight verrucose	2.7-3.6 (3.2)	2.0-4.8
Phaseolus vulgaris L.	Mito, Ibaraki Pref.	TSH-R1734 (IBA-2460)	II, III	20.5-25.7× 18.3-23.1	2, equatorial	echinulate	0.9-1.3	24.4-32.7× 20.7-27.5	irregular verrucos or slight verrucose	2.4-4.1 (3.3)	4.4-7.4
	Lake Sai, Yamanashi Pref.	HH92821	II, III	23.3-31.8× 20.7-27.3	2, equatorial	echinulate	0.9-1.7	26.2-34.2× 18.3-23.8	irregular verrucos or slight verrucose	2.0-3.5 (2.6)	1.3-3.6
	Hirai, Tokushima Pref.	HH92832	II, III	22.5-30.3× 19.4-25.5	2, equatoiral	echinulate	1.1-2.0	26.4-42.1× 21.2-26.6	irregular verrucos or slight verrucose	2.0-3.9 (2.8)	2.4-5.7
	Kuriyama, Tochigi Pref.	HH94638	II, III	19.6-25.9× 17.0-22.2	2, equatorial	echinulate	0.9-1.5	27.5-33.1× 23.3-27.7	irregular verrucos or slight verrucose	2.0-4.0 (2.6)	2.6-3.3
	Sonai, Okinawa Pref.	HH50721	II	22.7-30.5× 18.8-24.2	2, superequatorial	echinulate	0.9-1.5				
V. angularis (Willd.) Ohwi & Ohashi var. *angularis*	Nobeyama, Nagano Pref.	HH92876	II, III	21.8-25.9× 18.3-23.8	2 or 3, equatorial or superequatorial	echinulate	1.3-2.2	27.53-36.0× 18.6-23.3	irregular verrucose or slight verrucose or smooth	1.5-2.4 (2.0)	2.0-5.2

Table 5.6. (continued)

Host plants	Locality	Voucher specimen	Stage	Urediniospores				Teliospores			
				size (μm)	germ pores position and no.	wall sufrace	wall thickness (μm)	size (μm)	wall surface	wall thickness (mean) (μm)	papilla-thickness (μm)
	Lake Yamaka, Yamanashi Pref.	HH92788	II, III	22.3-26.3× 18.8-24.5	2, equatorial or superequatorial	echinulate	1.1-1.7	23.3-30.5× 14.8-20.3	irregular verrucose or slight verrucose or smooth	1.3-2.0 (1.6)	1.5-4.1
	Miyazaki, Miyazaki Pref.	HH92802	II, III	22.4-30.1× 18.2-24.8	2, equatorial or superequatorial	echinulate	1.1-1.7	24.6-33.4× 16.6-20.9	irregular verrucose or slight verrucose or smooth	1.5-2.4 (1.9)	1.7-4.6
	Shimonoseki, Yamaguchi Pref.	HH92014	II, III	21.7-28.8× 18.3-24.1	2 or 3, equatorial or superequatorial	echinulate	1.1-1.9	22.9-30.3× 15.5-20.7	irregular verrucose or slight verrucose or smooth	1.1-2.0 (1.5)	1.5-3.1
	Atsuma, Hokkaido Pref.	TSH-R16262	II, III	20.3-24.4× 16.8-20.1	2 or 3, equatorial or superequatorial	echinulate	1.1-1.5	25.9-34.9× 17.4-20.7	irregular verrucose or slight verrucose or smooth	1.3-2.4 (1.9)	2.2-4.1
	Atsuma, Hokkaido Pref.	TSH-R16263	II, III	19.0-25.7× 18.1-23.8	2, equatorial or superequatorial	echinulate	0.9-1.5	24.2-35.8× 15.0-21.2	slight verrucose or smooth	1.5-2.6 (2.0)	1.7-4.8
	Atsuma, Hokkaido	TSH-R16264	II, III	19.4-25.9× 16.6-23.8	2, equatorial or superequatorial	echinulate	0.9-1.3	22.7-31.6× 16.4-23.5	irregular verrucose or slight verrucose or smooth	1.5-2.6 (2.1)	2.4-4.8
	Mugawa, Hokkaido	TSH-R16265	II, III	19.0-24.6× 16.6-22.5	2 or 3, equatorial or superequatorial	echinulate	0.9-1.3	21.8-31.2× 15.7-24.6	slight verrucose or smooth	1.3-2.6 (1.9)	2.0-4.8
	Mizukaido, Ibaraki Pref.	TSH-R17960	II, III	20.5-27.7× 16.1-22.0	2 or 3, equtorial or superequatoiral	echinulate	0.9-1.5	22.0-29.0× 17.9-20.5	slight verrucose or smooth	1.3-2.2 (1.7)	2.6-5.0
	Funsento, Tochigi Pref.	TSH-R16273	II, III	19.4-24.4× 17.0-21.8	2, equatorial or superequatorial	echinulate	1.1-1.7	23.8-30.1× 15.1-21.4	slight verrucose	1.3-2.2 (1.7)	1.5-3.5
	Tsuchiura, Ibaraki Pref.	TSH-R17959	II, III	21.0-26.8× 17.0-23.2	2 or 3, equatorial or superequatorial	echinulate	1.1-1.5	23.4-30.0× 18.2-24.3	irregular verrucose or sligh verrucose or smooth	1.3-2.0 (1.5)	2.8-4.6
	Mizukaido, Ibaraki Pref.	TSH-R17961	II, III	19.0-25.1× 16.4-22.1	2 or 3, equatorial or superequatorial	echinulate	0.9-1.5	21.8-28.8× 16.4-20.1	slight verrucose or smooth	1.1-2.0 (1.5)	2.0-4.8
V. angulairs var. *nipponensis* (Ohwi) Ohwi & Ohashi	Kinpo, Kagoshima Pref.	TSH-R16267	II, III	22.2-28.1× 18.3-24.0	2, superequatorial	echinulate	0.9-1.5	23.3-30.3× 17.0-23.3	slight verrucose or smooth	2.4-3.7 (3)	3.1-6.1
	Daigo, Ibaraki Pref.	TSH-R16271	II, III	20.5-27.3× 16.8-23.1	2 or 3, equatorial or superequatorial	echinulate	0.9-1.5	24.2-33.6× 17.2-21.8	irregular verrucose or sligh verrucose or smooth	1.1-2.0 (1.6)	2.0-5.2

Table 5.6. (continued)

Host plants	Locality	Voucher specimen	Stage	Urediniospores				Teliospores			
				size (μm)	germ pores position and no.	wall sufrace	wall thickness (μm)	size (μm)	wall surface	wall thickness (mean) (μm)	papilla-thickness (μm)
V. angulairs var. *nipponensis* (Ohwi) Ohwi & Ohashi	Daigo, Ibaraki Pref.	TSH-R1748 (IBA-7021)	II, III	19.6-24.2× 15.7-20.5	2, equatorial or superequatorial	echinulate	1.1-1.5	24.4-31.8× 18.8-25.1	irregular verrucose or slight verrucose or smooth	1.5-2.4 (2.1)	2.8-7.0
V. umbellata (Thunb.) Ohwi & Ohashi	Kameyama, Hiroshima Pref.	HH92840	II, III	20.1-25.3× 17.7-22.0	2, equatorial or superequatorial	echinulate	1.1-1.3	25.7-36.8× 17.7-23.3	irregular verrucose or slight verrucose or smooth	1.7-2.8 (2.2)	2.0-5.7
	Shiga, Nagano Pref.	HH92772	II, III	23.3-31.4× 18.1-25.7	2 or 3, equatorial or superequatorial	echinulate	1.1-1.7	23.1-29.7× 15.7-22.5	slight verrucose or smooth	1.3-2.4 (1.8)	1.3-3.3
V. marina Merr.	Sonai, Okinawa Pref.	HH50717	II	23.8-32.2× 18.2-25.2	2, superequatorial	echinulate	1.1-1.7				
	Maesato, Okinawa Pref.	HH50718	II	24.6-31.4× 18.3-24.4	2, superequatorial	echinulate	0.9-1.7				
V. unguiculata ssp. *sesquipedalis* (L.) Verdc.	Omuta, Fukuoka Pref.	HH92911	II, III	22.0-27.7× 18.5-23.3	2 or 3, superequatorial	echinulate	0.9-1.5	24.2-32.9× 19.2-27.3	slight verrucose or smooth	2.4-4.4 (3.1)	1.5-4.1
	Takamatsu, Kagawa Pref.	HH92905	II, III	20.9-28.1× 16.8-22.7	2, superequatorial	echinulate	1.1-1.7	22.2-35.3× 19.0-24.4	slight verrucose	2.6-3.7 (2.9)	1.7-3.9
	Tsuchiura, Ibaraki Pref.	TSH-R17968	II, III	23.1-30.3× 16.8-23.8	2 or 3, superequatorial	echinulate	0.9-1.5	23.8-30.7× 18.8-25.7	irregular verrucose or slight verrucose or smooth	2.6-4.1 (3.4)	1.7-3.9
	Tsuchiura, Ibaraki Pref.	TSH-R17969	II, III	21.4-28.8× 15.3-21.4	2 or 3, superequatorial	echinulate	1.1-1.5	24.6-32.7× 18.1-23.1	irregular verrucose or slight verrucose or smooth	2.6-4.6 (3.3)	1.5-3.9
V. unguiculata (L.) Walp. ssp. *unguiculata*	Oshima, Tokyo Met.	HH92902	II, III	22.7-29.2× 16.4-25.7	2, superequatorial	echinulate	0.9-1.3	25.3-35.3× 17.7-26.6	smooth	2.6-3.9 (3.2)	1.7-4.1
	Shimonoseki, Yamaguchi Pref.	HH92019	III					27.7-35.8× 22.0-25.5	smooth	2.0-3.5 (2.7)	1.5-4.4
	Mitsukaido, Ibaraki Pref.	TSH-R17967	II, III	24.9-32.1× 22.9-27.5	2, superequatorial	echinulate	1.1-1.5	24.9-30.1× 21.6-26.6	slight verrucose or smooth	2.2-3.3 (2.7)	1.5-4.1
	Tsukuba, Ibaraki Pref.	TSH-R17965	II, III	23.5-29.7× 19.0-24.4	2 or 3, superequatorial	echinulate	0.9-1.3	25.9-34.0× 24.4-30.5	slight verrucose or smooth	2.8-3.7 (3.3)	1.7-4.1

Table 5.6. (continued)

Host plants	Locality	Voucher specimen	Stage	Urediniospore				Teliospore			
				size (μm)	germ pores position and no.	wall sufrace	wall thickness (μm)	size (μm)	wall surface	wall thickness (mean) (μm)	papilla-thickness (μm)
V. unguiculata (L.) Walp. ssp. *unguiculata*	Tsukuba, Ibaraki Pref.	TSH-R17962	II, III	20.7-30.1× 18.1-23.1	2, superequatorial	echinulate	0.9-1.5	24.6-30.7× 21.8-26.4	slight verrucose or smooth	2.4-3.5 (3.1)	1.5-3.7
	Tsuchiura, Ibaraki Pref.	TSH-R17958	II, III	23.3-29.7× 19.2-22.9	2, superequatorial	echinulate	0.9-1.5	24.4-29.9× 21.4-26.4	slighth verrucose or smooth	2.2-3.7 (2.9)	1.1-3.3
	Mitsukaido, Ibaraki Pref.	TSH-R17963	II, III	23.5-29.7× 19.0-24.4	2 or 3, superequatorial	echinulate	0.9-1.3	25.9-34.0× 24.4-30.5	smooth	2.8-3.7 (3.3)	2.0-4.2
	Tsukuba, Ibaraki Pref.	TSH-R17966	II, III	22.5-29.1× 19.8-22.7	2, superequatorial	echinulate	0.9-1.5	24.6-34.4× 20.9-28.1	smooth	2.6-4.6 (3.3)	1.5-3.1
	Shizuoka Pref.	BPI-0000967	III					29.7-35.8× 18.8-25.7	slight verrucose	2.6-3.9 (3.1)	2.2-6.8
	Shizuoka Pref.	BPI-0000968	III					29.7-37.5× 22.7-31.0	slight verrucose	2.4-3.7 (3.1)	2.2-6.3

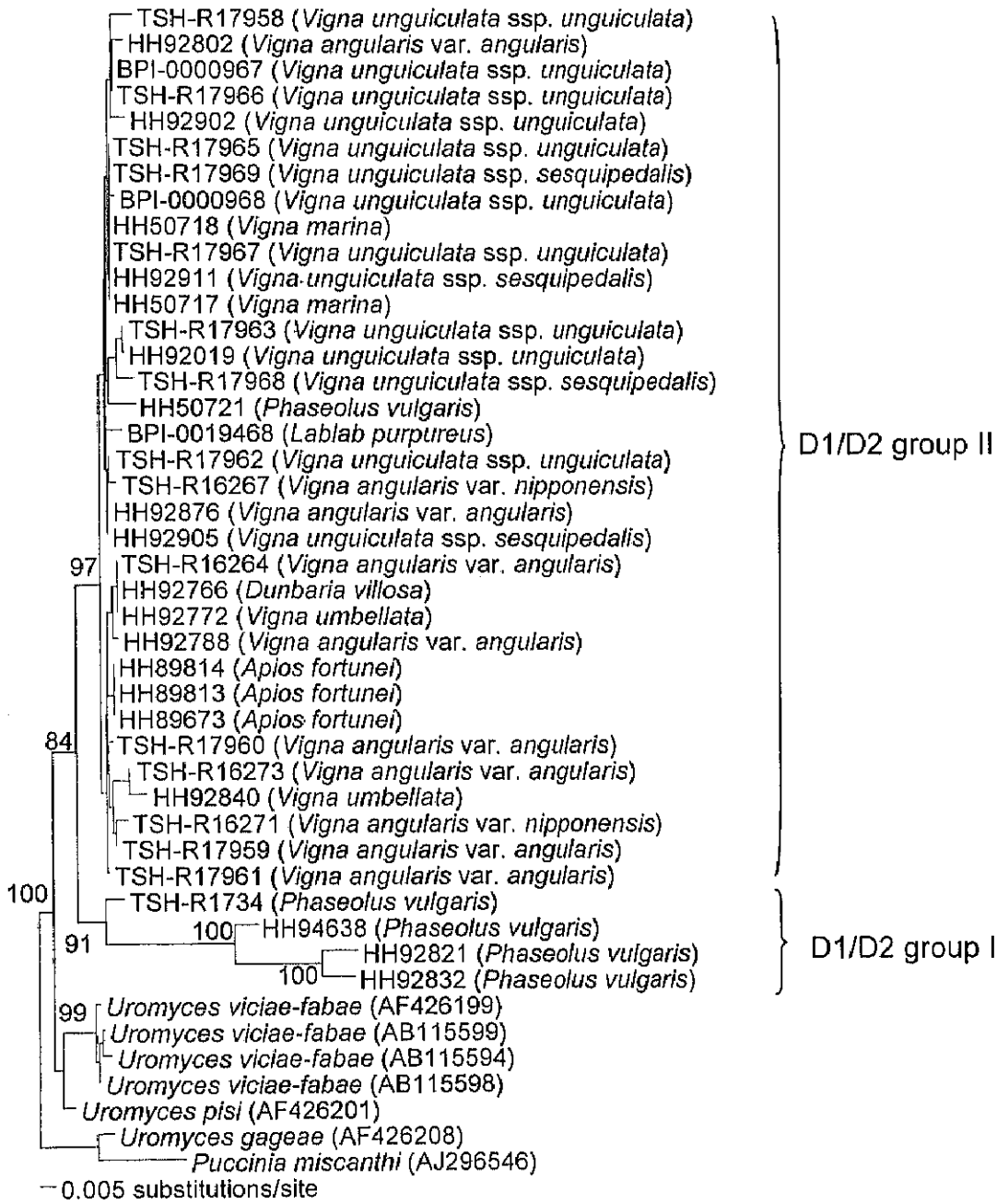

Fig. 5.12. A neighbor-joining tree inferred from sequences of LSU rDNA (D1/D2) regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches. Length of branches is proportional to number of base changes, indicated by the scale bottom.

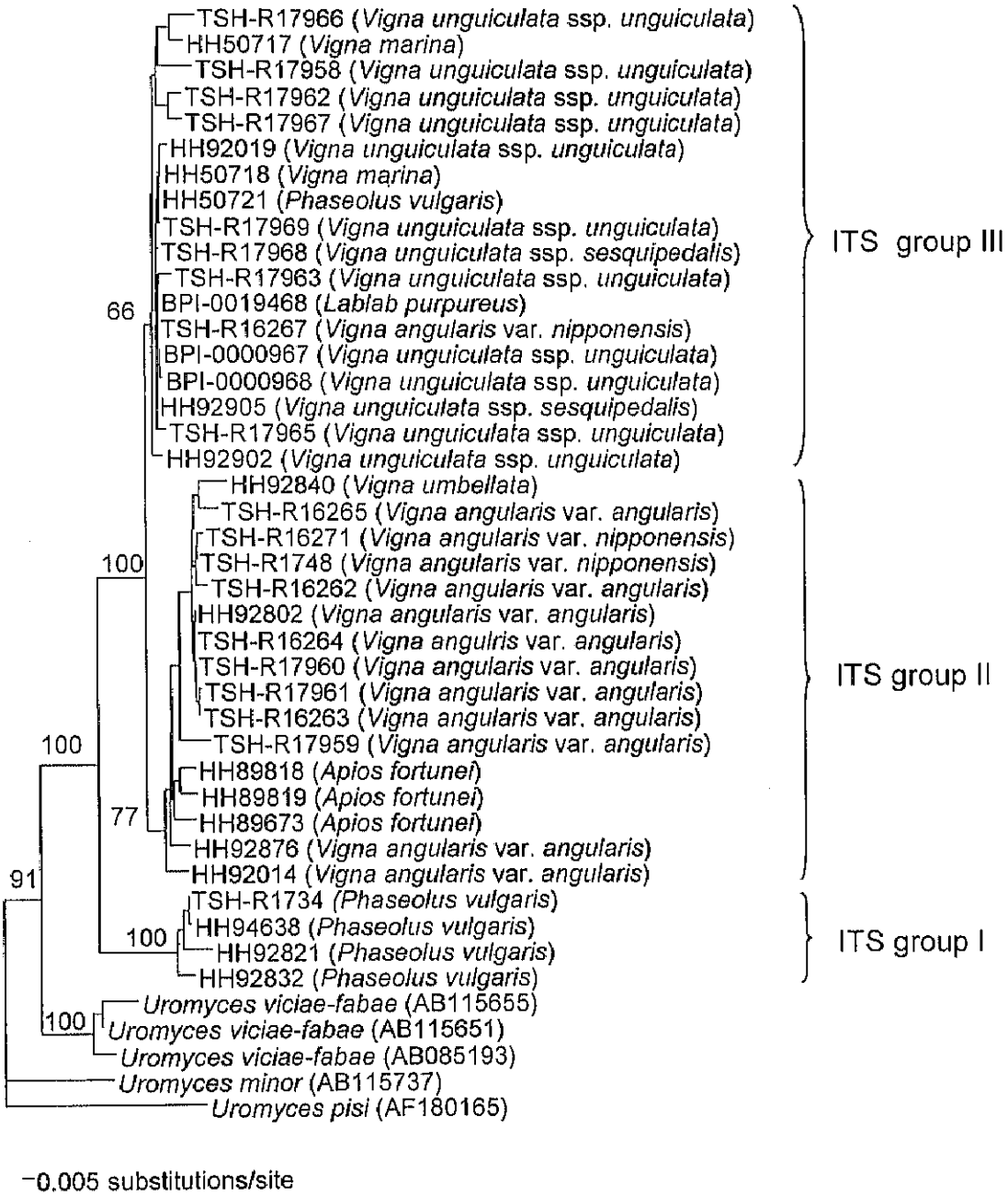

Fig. 5.13. A neighbor-joining tree inferred from sequences of ITS and 5.8S regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches. Length of branches is proportional to number of base changes, indicated by the scale bottom.

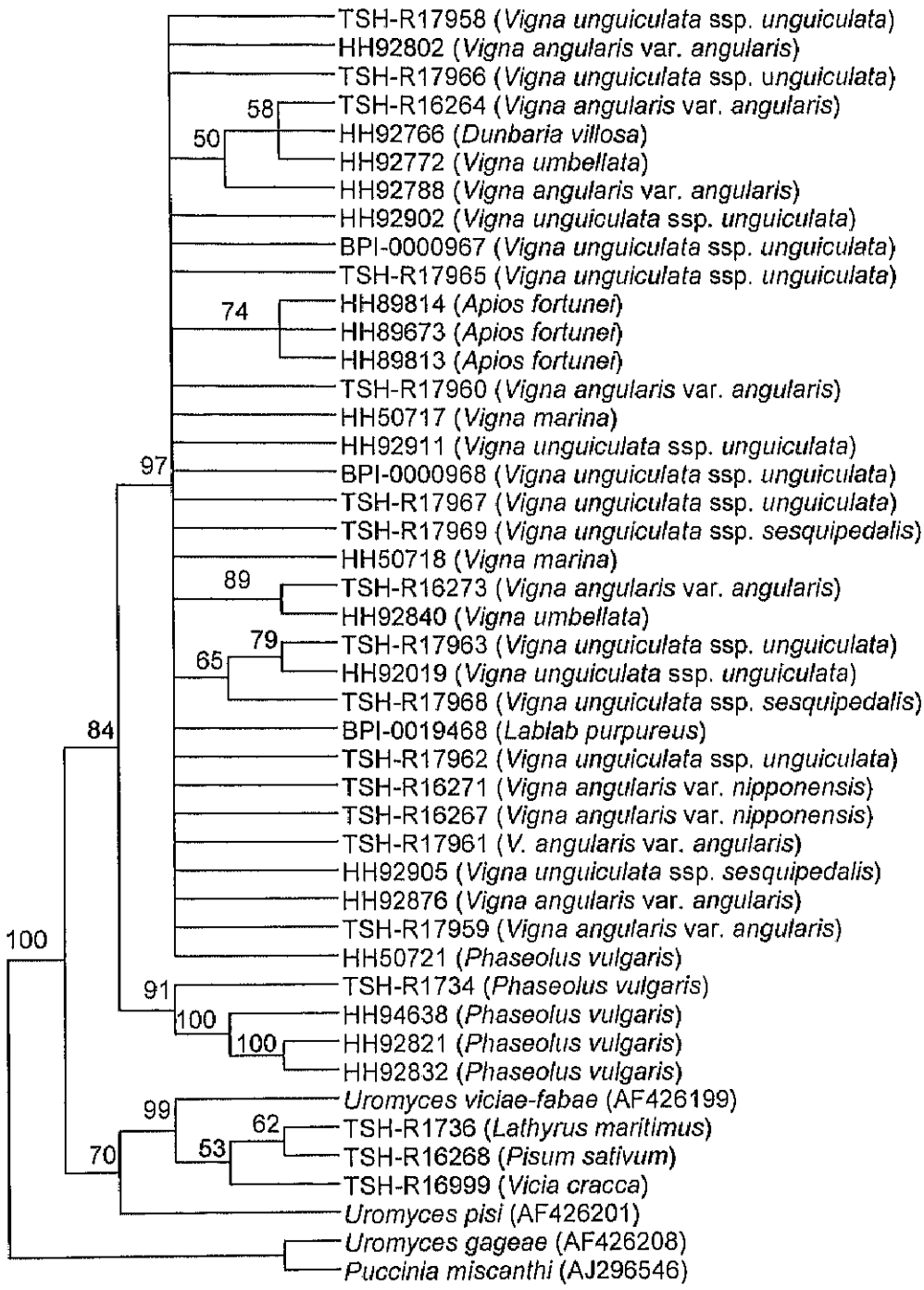

Fig. 5.14. A maximum-parsimony tree inferred from sequences of LSU rDNA (D1/D2) regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches.

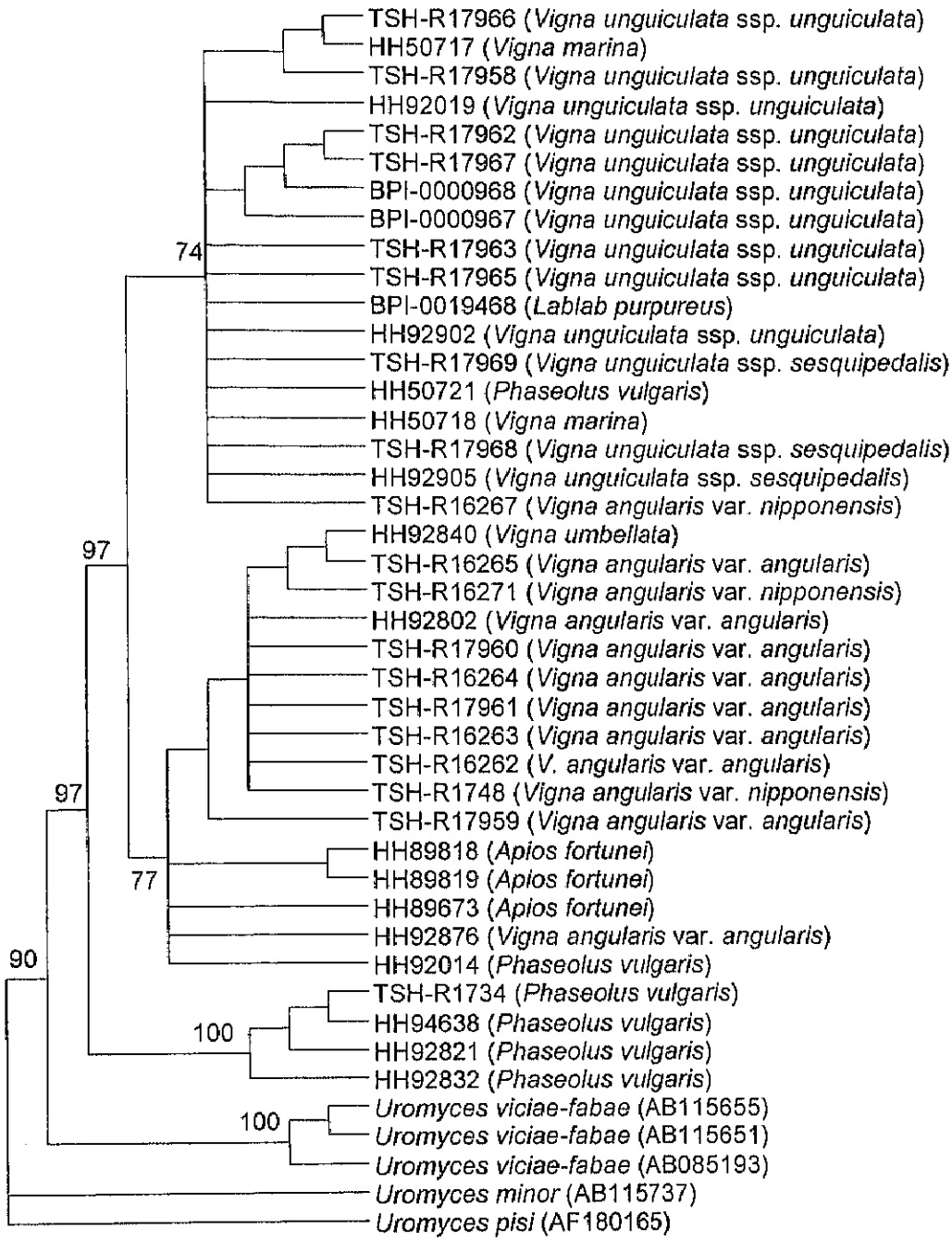

Fig. 5.15 A maximum-parsimony tree inferred from sequences of ITS and 5.8S regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches.

3. Taxonomic discussion

The specimens on *Phaseolus*, *Vigna*, *Apios*, *Lablab* and *Dunbaria* that were examined in this study were sorted by the position of urediniospore germ-pores and the teliospore-wall thickness into three morphological groups. Taxonomic importance of the position of urediniospore germ-pores in the *U. appendiculatus* and *U. vignae* species complex has been well recognized (Cummins 1978; Guo and Wang 1986; Hiratsuka et al. 1992). In addition, the degree of verrucoseness of the teliospore wall was stressed to differentiate the two species (Cummins 1978). The teliospore-wall thickness was considered as important to separate varieties in *U. appendiculatus* (Hiratsuka et al. 1992). Disagreements and confusions in the taxonomy of the *U. appendiculatus* and *U. vignae* species complex stemmed from the evaluation and judgment whether these morphological features can be a good taxonomic character to circumscribe the species or subspecific taxa. Thus, Cummins (1978) recognized *U. appendiculatus* and *U. vignae* as distinct species while Arthur (1934) and Hiratsuka (1972) merged the two species under the name of *U. phaseoli* with two varieties, var. azukicola and var. *dispersus* (cf. Hirata 1952, Ito and Maruyama 1943). In this study, the rust fungi on *Phaseolus*, *Vigna*, *Apios*, *Lablab* and *Dunbaria* that were classified either *U. appendiculatus* and its varieties or *U. vignae* were confirmed to be distinguished in the three morphologically circumscribed groups regardless of their position in the taxonomic hierarchy.

No other morphological features, either singly or in combinations, detected discrete groups in the specimens examined. The teliospore-wall ornamentation has

often been considered to be a good taxonomic character to differentiate between *U. appendiculatus* and *U. vignae* as employed by Cummins (1978). However, this study showed that the teliospore-wall ornamentation was highly variable both within a specimen and among the specimens examined. Guo and Wang (1986) and Hiratsuka et al. (1992) also considered that the teliospore-wall thickness cannot be a taxonomic character to separate *U. appendiculatus* from *U. vignae*.

It has been a long belief that the rust fungi have high host specificity and, thus, that *U. appendiculatus* and its varieties and *U. vignae* can be separated by their host genera and species. This view is well expressed in the most recent rust flora of Japan (Hiratsuka et al. 1992), in which *U. appendiculatus* var. *appendiculatus* is restricted on *P. vulgaris*, *U. appendiculatus* var. *azukicola* (Hirata) Hiratsuka, f. on *V. angularis* var. *angularis*, *V. angularis* var. *nipponensis* and *V. umbellata*, *U. appendiculatus* var. *dispersus* (Hiratsuka, f.) Hiratsuka, f. on *A. fortunei* and *U. vignae* on *V. unguiculata* and *L. purpureus*. In this study, however, showed that three morphologically circumscribed groups of the specimens were not host-limited (Table 5.2). Thus, the morphological group II occurs on *V. angularis* var. *angularis* and *Phaseolus vulgaris*. The morphological group I occurs on *V. angularis* var. *angularis*, *V. angularis* var. *nipponensis*, *V. radiata*, *V. umbellata*, *A. fortunei*, *D. villosa*, *P. vulgaris* and *P. minimus*. The morphological group III occurs on *V. unguiculata* var. *unguiculata*, *V. unguiculata* var. *sesquipedalis*, *V. marina*, *V. angularis* var. *nipponensis* and *P. vulgaris*. These results disagree with the long-belief of the strict restriction of the rust species under discussion to particular host genera.

This indicates that *U. appendiculatus* and *U. vignae* may parasitize both *Phaseolus* and *Vigna* species although no extensive cross inoculation experiments have been conducted to prove this perspective. Chung et al. (2003) recently reported that a rust fungus from *V. angularis* var. *angularis* infected and sporulated on *V. unguiculata* ssp. *unguiculata* and a rust fungus from *V. unguiculata* ssp. *unguiculata* infected and sporulated on *V. angularis* var. *angularis*. These results indicate that the host specificity of the fungi classified under *U. appendiculatus* var. *azukicola* and *U. vignae* is not genetically fixed at the level that the two species can be distinguished in Japan. Eckenwalder and Heath (2001) discussed the evolutionary significance of the infection process and host specificity of *U appendiculatus* and *U. vignae* on species of *Phaseolus* and *Vigna*.

A single or a few morphological features alone cannot be judged by themselves as a good taxonomic character to designate rust fungus taxa to a specific rank in the taxonomic hierarchy, even though a few morphological features can circumscribe the taxa. An appropriate assessment of taxonomic characters can be done by comparing independent characters that are not causally related. In this study, I compared the specimen groups circumscribed by morphological features and those in phylogenetic trees inferred by rDNA regions. The three morphological groups corresponded to the three distinct clades generated from the analysis of nucleotide sequences at the ITS region including 5.8S rRNA gene (Fig. 5.13, 5.15). In contrast, the specimens in the morphological groups I and III scattered over the D1/D2 group II while the morphological group II corresponded well with the D1/D2 group I (Fig. 5.12, 5.14).

Topologies of the D1/D2 trees generated by a neighbor-joining method (Fig. 5.12) and by a maximum parsimony method (Fig. 5. 14) were similar. The level of divergence between the D1/D2 groups I and II was not high while the D2 sequences showed high T nucleotide repeat at the sites from 546th to 556th base. The high T nucleotide repeat at the D2 sequence distinguished the clade I from the clade II. As with the D1/D2 trees, the topology of the ITS trees were similar both in a neighbor-joining method (Fig. 5.13) and a maximum parsimony method (Fig. 5.15). The ITS sequences were more diverse than were the D1/D2 sequences. The ITS tree and the D1/D2 tree are different in that the specimens in the ITS groups II and III scatter over the D1/D2 group II while the same specimens constitute both the ITS group I and the D1/D2 group I (Fig. 5.12-5.15). The discordance between the two inferred trees may indicate that either one or neither of them reflects the true phylogeny. It is argued, however, that sequence variations in the D1/D2 region is often insufficient to distinguish biological species (O'Donnell and Cigelnik 1997; Maier et al. 2003), whereas sequence variations in the ITS region is usually large enough to separate taxa at a species level (White et al. 1990). Furthermore, the agreement of morphological groups I, II and III to the ITS groups II, I and III is highly indicative that the three groups are distinct at a species level.

It is also appropriate to take a practical view in plant pathology into the consideration of this taxonomic/phylogenetic results. The ITS group I/morphological group II occurs on *P. vulgaris* (Fig. 5.13, 5.15) and shows morphological features of what has been recognized as *U. appendiculatus*. The ITS group II/morphological

group I occurs on *V. angularis* var. *angularis*, *V. angularis* var. *nipponensis*, *V. umbellata* and *Apios fortunei* (Fig. 5.13, 5.15) and shows morphological features of what has been recognized as *U. appendiculatus* var. *azukicola* and var. *dispersus*. The ITS group III/morphological group III occurs mostly on *V. unguiculata* and its variety with a few on *V. marina*, *L. purpureus* and *P. vulgaris* (Fig. 5.13, 5.15) and shows the morphological features of what has been recognized as *U. vignae*.

When considering the specimens of three morphological groups that have not been subjected to molecular analyses, the ITS groups/morphological groups are not restricted to particular host genus as discussed previously. Thus, it is concluded that three rust fungus species occur on *Phaseolus*, *Vigna*, *Apios*, *Lablab* and *Dunbaria* in Japan and the three species are referable to *U. appendiculatus*, *U. azukicola* and *U. vignae*. Before drawing the taxonomic and nomenclatural conclusion, however, it is mandatory to examine the nomenclatural types of the three species and allied taxa.