# IV. *Uromyces viciae-fabae*

The objectives of the present study are to re-examine and evaluate the morphological variations of urediniospores and teliospores of *Uromyces viciae-fabae* on *Vicia*, *Lathyrus* and *Pisum* in Japan. Subsequently, sequences of large subunit rDNA (D1/D2) region and internal transcribed spacer (ITS) regions including 5.8S rDNA were determined and analyzed. Both of ribosomal DNA regions are reported that are commonly chosen because of their high genetic divergence. The two ribosomal DNA regions have already been applied to fungi by several research groups (O'Donnell 1993; Zambino and Szabo 1993; Kropp et al. 1997; Vogler and Bruns 1998; Pfunder et al. 2001; Virtudazo et al. 2001; Maier et al. 2003; Weber et al. 2003; Weber et al. 2003; Chung et al. 2004). In the present study, the specimens of *U. viciae-fabae* used for morphological observations were analyzed based on D1/D2 of LSU rDNA and ITS regions including 5.8 S rRNA gene. Correlations between morphological characteristics and molecular phylogeny were also discussed in the present study.

## 1. Morphological analyses

### Materials

Dry herbarium specimens were used for light microscopic (LM) and scanning electron microscopic (SEM) observations. 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, ninety-four specimens were collected from seven species of *Vicia*, three species of *Lathyrus* and one species of *Pisum* (Table 4.1). These host plants were 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.

## Results and discussion

**Morphological features of uredinia and urediniospores:** Morphology of uredinia and urediniospores on *Vicia, Lathyrus* and *Pisum* was not significantly different among specimens. Uredinia were amphigenous, scattered or aggregated and yellow or brownish. Uredinia often surrounded by yellow and yellowish lesion. Urediniospores were globose, subglobose or ellipsoid; the spore wall was pale yellow or yellow, and echinulate. Germ pores were 3-7 and scattered. Spore size was 15.9-36.2×12.0-30.5 μm and wall thickness was 1.1-2.8 μm.

The wall thickness was emphasized to distinguish *U. viciae-fabae* var. *viciae-fabae* (=*U. fabae*) from *U. viciae-fabae* var. *orobi* (=*U. orobi*) by some researchers (Gäumann 1934, Wilson and Henderson 1966, Hiratsuka 1973; Azbukina

1984; Hiratsuka et al. 1992). However, no discrete groups or correlation were detected among mean length, mean width and mean wall-thickness (Fig. 4. 1A, 4.1B).

**Morphological features of telia and teliospores:** Telia and teliospores of the specimens did not show morphological differences. Telia were amphigenous, scattered or aggregated and dark brown. Teliospores were subglobose, ovate or ellipsoid. Spore size was 22.0-44.9×14.2-36.6 μm; and the wall was 1.1-3.9 μm thick and brown or dark brown. The wall surface was smooth. The apex was rounded or truncate, and apex was 2.4-12.6 μm thick. Therefore, the specimens of *U. viciae-fabae* on *Vicia*, *Lathyrus* and *Pisum* were not sufficient morphological differences in their teliospore. Moreover, no discrete groups or correlation were detected among mean length, mean width, mean wall-thickness and mean apical thickness (Fig. 4.2, Fig. 4.3).

**Statistical analyses of *Uromyces viciae-fabae* on *Vicia, Lathyrus* and *Pisum*:** Minimum, mean and maximum values of measurement data were analyzed by principal component analysis methods. Principal component analyses were undertaken with various combinations of numerical variables in urediniospores and teliospores features. In the analysis here (Fig. 4.4) employed mean values of urediniospore length, width and the wall-thickness and teliospore length, width, the wall-thickness and the thickness of the apical papilla. After the Varimax rotation, the calculated factors 1 and 2 explained 34.5% and 28.5% of the total variance,

respectively. The scatter diagram with the factors one as the horizontal axis and the factor two as the vertical axis did not reveal discrete groups (Fig. 4.4). Analyses employed other combination of variables showed similar results. Based on principal component analyses, the specimens of *U. viciae-fabae* var. *viciae-fabae* could not form discrete group from the specimens of *U. viciae-fabae* var. *orobi* (Fig. 4.5).

**Morphological feature of *Uromyces viciae-fabae* in Japan:** No morphological group was detected based on principal component analyses. The specimens used for observations on *Vicia, Lathyrus* and *Pisum* were recognized as the same morphologically fungal group. Uredinia were amphigenous and scattered or aggregated. Urediniospores were globose, subglobose or ellipsoid. Spore wall was pale yellow or yellow, and wall surface was echinulate. Spore size was 15.9-36.2×12.0-30.5 µm and the wall was 1.1-2.8 µm thick. Germ pores were 3-7 and scattered. Telia were amphigenous, scattered or aggregated and dark brown. Teliospores were subglobose, obovoid or ellipsoid. Spore size was 22.0-44.9×14.2-36.6 µm and the wall was 1.1-3.9 µm thick and brown or brownish. The wall surface was smooth. The apex was rounded or truncate, and 2.4-12.6 µm thick (Fig. 4.6).

Table 4.1. Specimens of *Uromyces viciae-fabae* used for morphological observations

| Host plants | Locality (No. of specimens) |
|---|---|
| *Vicia amoena* Fisch. | Honshu (3) |
| *V. cracca* L. | Honshu (22), Hokkaido (3), Kyushu (1) |
| *V. faba* L. | Honshu (7), Hokkaido (4), Kyushu (4), Okinawa (1) |
| *V. japonica* A. Gray | Honshu (7), Hokkaido (6) |
| *V. nipponica* var. *capitata* Nakai | Honshu (1) |
| *V. pseudo-orobus* Fish. & C.A. Mey. | Honshu (2) |
| *V. unijuga* Al. Br. | Honshu (5), Kyushu (1) |
| *Lathyrus davidii* Hance | Honshu (2) |
| *L. maritimus* Bigel. | Honshu (5), Hokkaido (4), Kyushu (3) |
| *L. palustris* L. | Honshu (6), Hokkaido (1) |
| *Pisum sativum* L. | Honshu (3), Hokkaido (1), Kyushu (2) |
| Total | 94 |

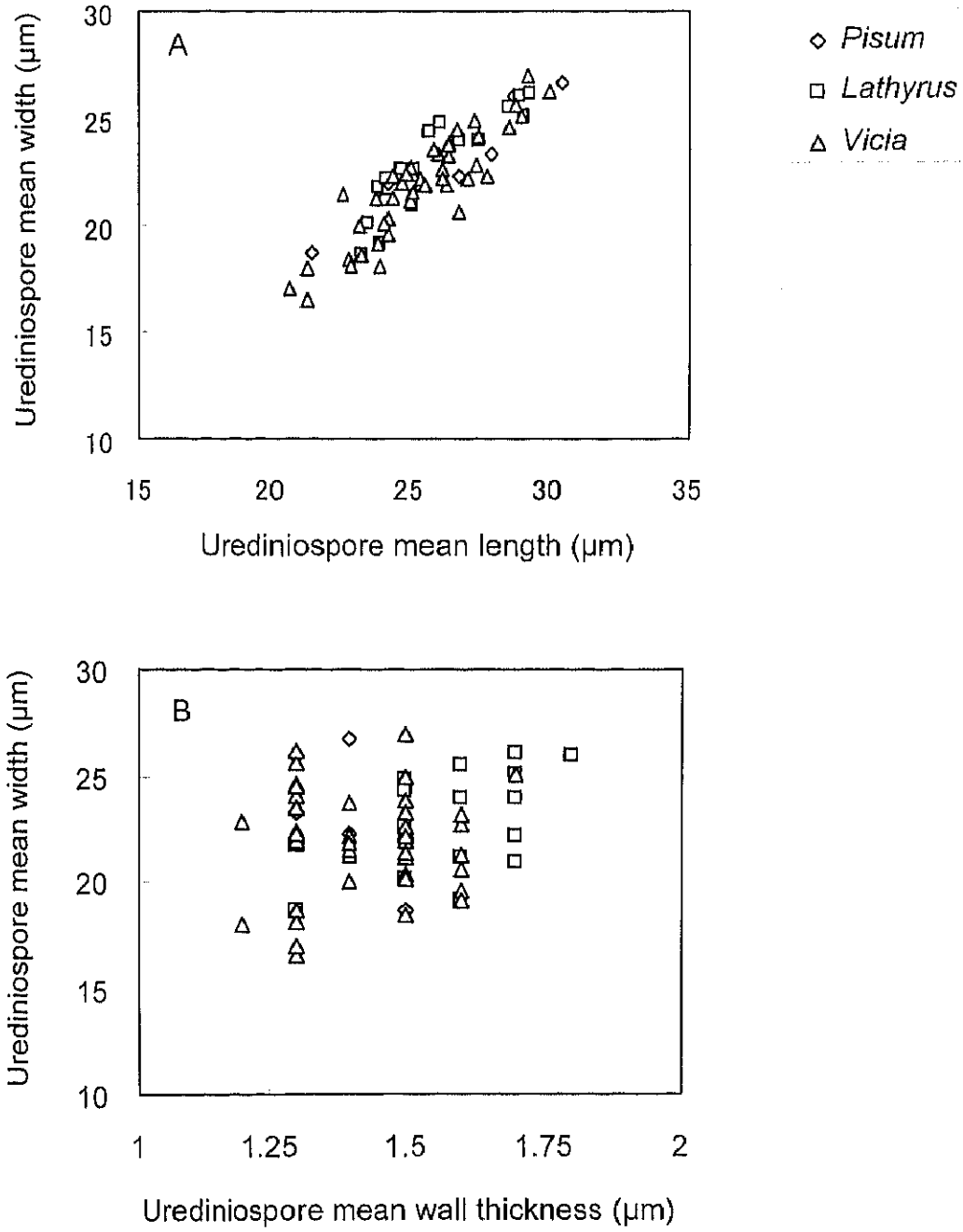


Fig. 4.1. Scatter diagram generated from urediniospores mean length against mean width (A) and mean wall thickness against mean width (B) on *Vicia*, *Lathyrus* and *Pisum*.

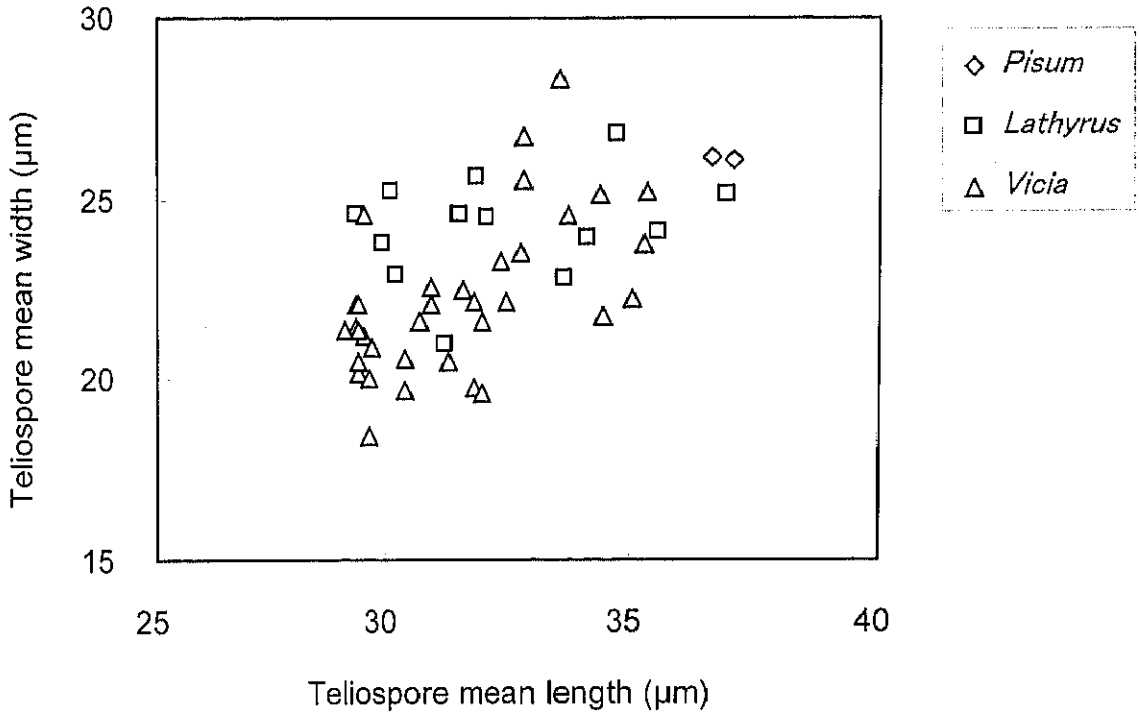


Fig. 4.2. A scatter diagram generated from teliospores mean length and mean width on *Vicia*, *Lathyrus* and *Pisum*.

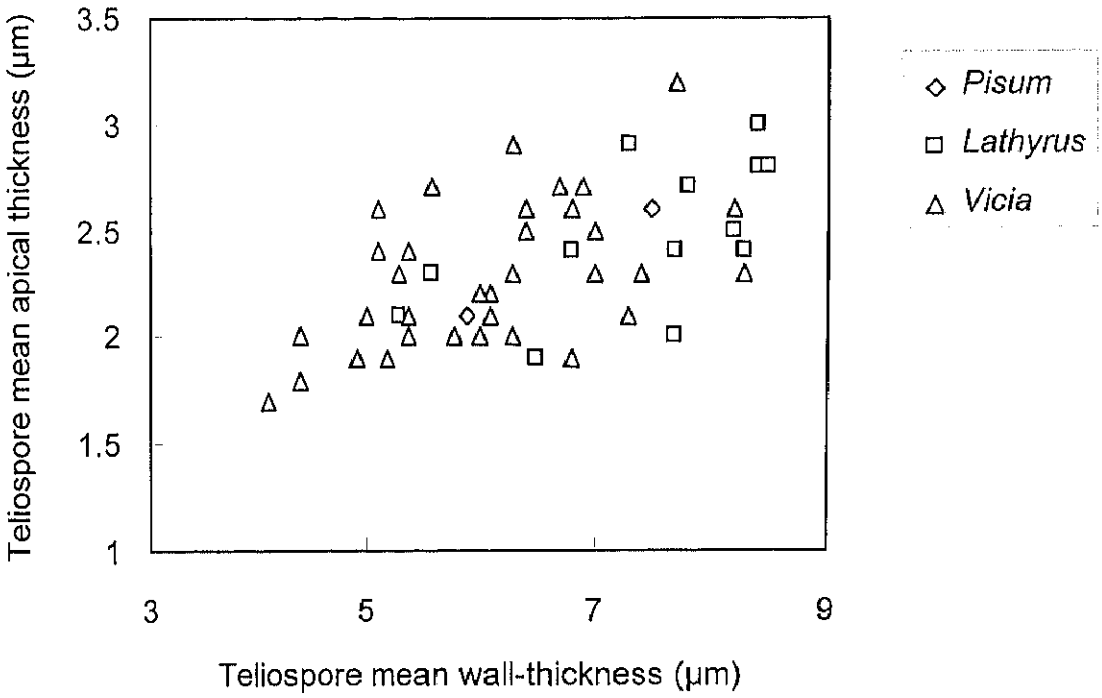


Fig. 4.3. A scatter diagram generated from teliospores mean wall-thickness and mean apical thickness on *Vicia*, *Lathyrus* and *Pisum*.

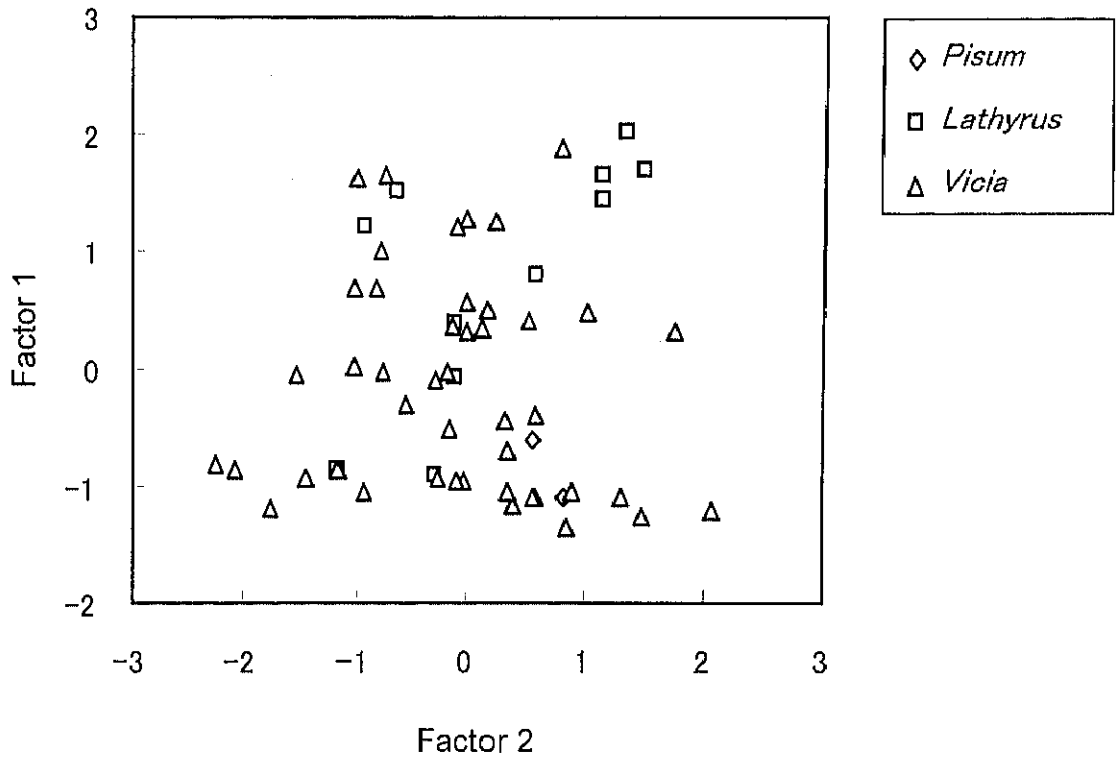


Fig. 4.4. A scatter diagram generated from the principal component analysis based on non-standardized data of length, width and wall-thickness of urediniospores and length, width, wall-thickness and apical thickness of teliospores.

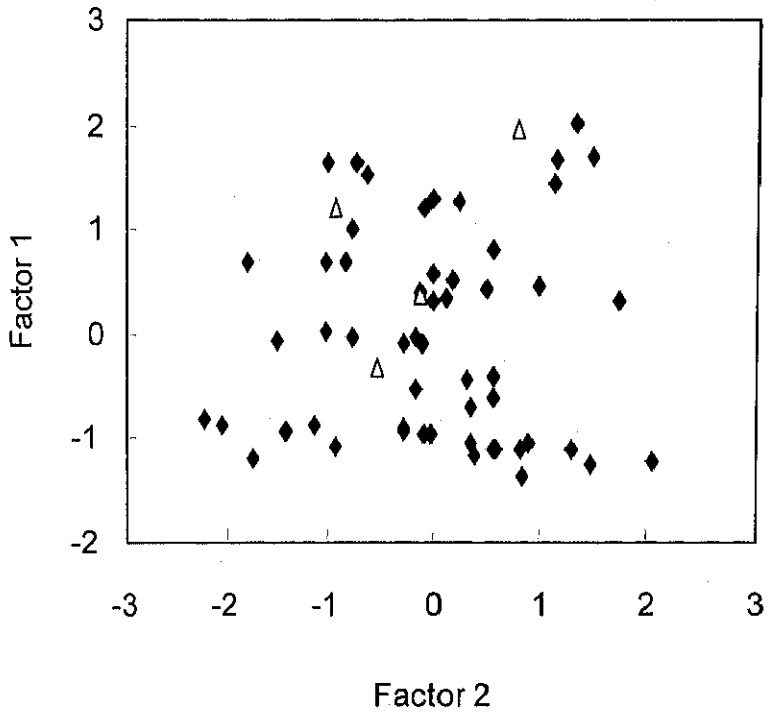


Fig. 4.5. A scatter diagram generated from the principal component analysis based on non-standardized data of length, width and wall-thickness of urediniospores and length, width, wall-thickness and apical thickness of teliospores.( Δ , the specimens of *Uromyces viciae-fabae* var. *orobi*; ♦,the specimens of *U. viciae-fabae* var. *viciae-fabae*)

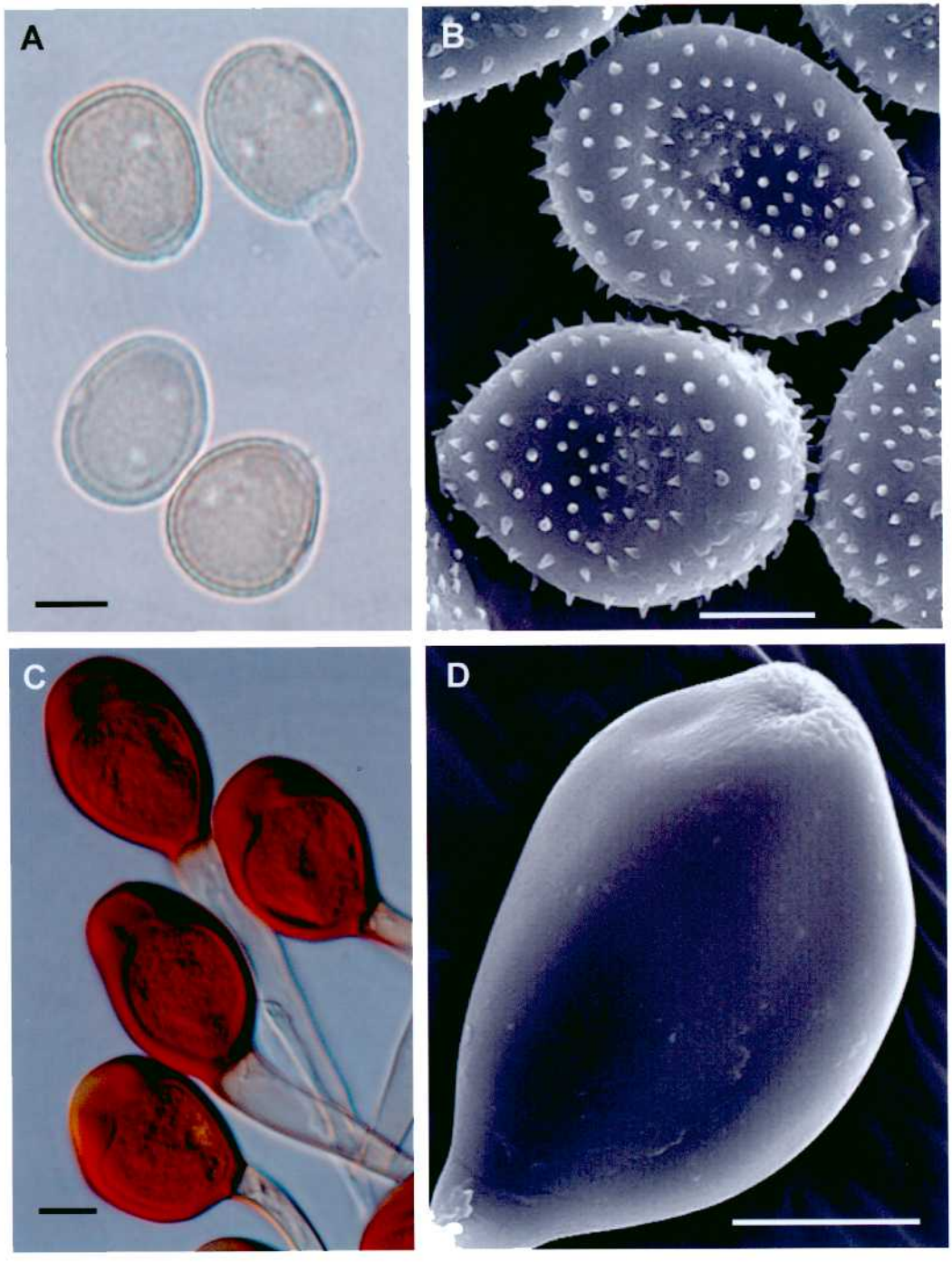


Fig. 4.6. Morphology of *Uromyces viciae-fabae*. A, B: Urediniospores. C, D: Teliospores. Bras=10 $\mu$ m

## 2. Molecular phylogenetic analyses

### Materials

Twenty-three specimens on *Vicia, Lathyrus* and *Pisum* (Table 4.2) were selected from those subjected to morphological analyses for large subunit rDNA (D1/D2) and ITS including 5.8S sequence analyses. 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. appendiculatus* (Pers.) Unger, *U. vignae* Barclay, *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 4.3.

### Results and discussion

The DNA sequences of the entire LSU rDNA (D1/D2) region of *U. viciae-fabae*, ranging from 604 to 607 bases, were used for phylogenetic analyses. Of the 617 aligned bases, 19 sites were variable and 24 sites were parsimony-informative characters. The neighbor-joining tree constructed from the LSU rDNA (D1/D2) regions showed that the rust fungi on *Vicia, Lathyrus* and *Pisum* formed a single clade with high bootstrap support (96%) (Fig. 4.7). The LSU rDNA (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 region including 5.8S rDNA of specimens

used for analyses, 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 neighbor-joining tree constructed from the ITS and 5.8 S rDNA regions, the rust fungi on *Vicia, Lathyrus* and *Pisum* also formed a single clade, with high bootstrap support (100%) (Fig. 4.8). The ITS including 5.8 S bootstrap phylogram has a consistency index (CI) of 0.809, a retention index (RI) of 0.667, retention consistency (RC) of 0.539 and a tree length of 251. Therefore, sequences analyses of these rust fungi on *Vicia, Lathyrus* and *Pisum* revealed that these rust fungi are in the same lineage based on the D1/D2 and ITS including 5.8 S regions.

In addition, the D1/D2 and ITS including 5.8 S sequences were analyzed by maximum-parsimony method and showed the similar genetic clades with neighbor-joining analysis (Fig. 4.9, 4.10). Although different sub-clades seem to form among the maximum-parsimony tree based on D1/D2 sequence, the bootstrap values (62% and 58%) are too low to support the sub-clades.

Based on D1/D2 and ITS including 5.8 S regions, the specimens on *Vicia unijuga* Al. Br. and *V. nipponica* var. *capitata* Nakai fell into same clade with other specimen on *Vicia, Lathyrus* and *Pisum* (Fig. 4.7-4.10). Although the *V. unijuga* and *V. nipponica* var. *capitata* were reported to be susceptible to *U. viciae-fabae* var. *orobi* in Japan (Hiratsuka et al. 1992), the specimens on the two host plants did not form different clade. Moreover, the specimens used for molecular phylogenetic analyses showed

morphological similarity. Each specimen used for molecular phylogenetic analyses was similar morphology based on urediniospores and teliospores. The mainly morphological characteristics of urediniospores and teliospores are listed in Table 4.4.

Table 4.2. Specimens of *Uromyces viciae-fabae* sequences used for phylogenetic analyses

| 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 | HH40928 | AB115605 | AB115661 |
| | Yamaguchi | HH67023 | 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 |

[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 4.3. 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. viciae-fabae* (Pers.)Schroet. | *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

Table 4.4. Morphological characteristics of urediniospores and teliospores of specimens from *Vicia*, *Lathyrus* and *Pisum* 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 (μm) | apex thickness (μm) |
| *Vicia amoena* Fisch. | Lake Yamanaka, Yamanashi Pref. | TSH-R13227 | II, III | 22.5-29.0× 21.2-25.9 | 3-5, scattered | echinulate | 1.3-2.0 | 26.4-37.1× 19.2-26.7 | smooth | 1.5-2.7 | 3.4-7.6 |
| *V. cracca* L. | Nobeyama, Nagano Pref. | TSH-R2986 | II | 25.3-34.4× 21.8-30.5 | 3-5, scattered | echinulate | 1.1-1.5 | | | | |
| *V. cracca* L. | Mt. Azuma, Nagano Pref. | TSH-R18187 | II, III | 21.2-26.6× 16.8-22.3 | 3-6, scattered | echinulate | 1.3-2.4 | 25.7-35.5× 16.1-23.3 | smooth | 1.1-2.6 | 2.6-7.2 |
| | Nobeyama, Nagano Pref. | TSH-R16998 | 0, I, II | 22.5-32.9× 18.8-22.3 | 3-5, scattered | echinulate | 1.3-2.0 | | | | |
| | Tsukuba, Ibaraki Pref. | TSH-R16999 | II, III | 20.7-27.5× 19.4-26.6 | 3-5, scattered | echinulate | 1.1-1.5 | 25.3-39.7× 19.8-25.9 | smooth | 1.3-2.6 | 2.4-7.0 |
| | Abashiri, Hokkaido Pref. | TSH-R16269 | II | 22.5-29.0× 18.1-24.4 | 3-4, scattered | echinulate | 1.1-1.3 | | | | |
| *V. faba* L. | Mitsuishi, Chiba Pref. | BPI-0005425 | II, III | 22.7-28.3× 20.1-26.2 | 3-5, scattered | echinulate | 1.1-2.0 | 31.2-37.5× 20.3-29.2 | smooth | 1.7-3.7 | 3.9-7.4 |
| | Mt. Wakasugi, Fukuoka Pref. | BPI-0005454 | II | 22.9-29.4× 20.3-24.0 | 3-5, scattered | echinulate | 1.1-1.3 | | | | |
| *V. pseudo-orobus* Fish. & C. A. Mey | Minamitsuru, Yamanashi Pref. | TSH-R1743 (IBA-2652) | II | 21.2-28.1× 19.8-25.3 | 3-7, scattered | echinulate | 1.1-1.3 | | | | |
| *V. unijuga* Al. Br. | Minamitsuru, Yamanashi Pref. | TSH-R1747 (IBA-3068) | III | | | | | 25.3-37.3× 19.6-26.8 | smooth | 1.7-3.5 | 5.5-12.6 |
| | Oyama, Kangawa Pref. | HH40928 | III | | | | | 28.1-36.4× 20.5-26.2 | smooth | 1.7-3.1 | 3.7-9.6 |
| | Ato, Yamaguchi Pref. | HH67023 | II, III | 22.2-26.2× 17.0-23.8 | 3-5, scattered | echinulate | 1.1-1.7 | 25.9-34.4× 18.3-23.8 | smooth | 1.7-2.8 | 3.3-10.9 |
| | Mt. Azuma, Nagano Pref. | TSH-R18185 | II, III | 22.2-28.6× 19.6-24.2 | 3-6, scattered | echinulate | 1.1-2.0 | 25.3-39.7× 19.8-25.9 | smooth | 1.3-2.6 | 2.4-7.0 |
| *V. japonica* A. Gray | Rebun, Hokkaido | TSH-R1738 (IBA-5836) | II | 27.0-35.5× 21.8-29.9 | 4-6, scattered | echinulate | 1.1-1.3 | | | | |
| | Lake Yamaka, Yamanashi Pref. | TSH-R13306 | II, III | 20.9-27.9× 16.8-23.1 | 3-7, scattered | echinulate | 1.1-2.0 | 23.8-36.4× 17.2-24.0 | smooth | 1.3-2.4 | 2.6-7.6 |
| *V. nipponica* Matsum. var. *capitata* Nakai | Inabayama, Tottori Pref. | BPI-0005541 | II, III | 23.1-32.5× 19.0-28.1 | 3-5, scattered | echinulate | 1.3-2.4 | 25.1-35.7× 19.1-26.2 | smooth | 1.7-3.7 | 3.5-7.8 |

Table 4.4. (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 (μm) | apex thickness (μm) |
| *L. palustris* L. | Morioka, Iwate Pref. | BPI-0005266 | II, III | 21.8-28.3× 19.4-26.8 | 3-6, scattered | echinulate | 1.1-2.0 | 28.3-41.4× 20.7-27.9 | smooth | 2.2-3.9 | 5.9-10.5 |
| | Lake Shirarutoro, Hokkaido | TSH-R16270 | II | 19.6-26.8× 16.6-20.9 | 3-5, scattered | echinulate | 1.1-1.5 | | | | |
| *L. maritimus* Bigel. | Tsukuba, Ibaraki Pref. | TSH-R6320 | II | 23.1-31.8× 18.8-27.3 | 3-6, scattered | echinulate | 1.1-2.2 | | | | |
| | Rebun, Hokkaido | TSH-R1736 (IBA-5842) | II, III | 25.9-31.6× 22.7-29.7 | 4-7, scattered | echinulate | 1.5-2.2 | 23.3-35.3× 20.3-29.4 | smooth | 1.5-3.1 | 3.7-7.9 |
| | Rebun, Hokkaido | TSH-R1739 (IBA-3004) | II, III | 25.9-33.8× 23.3-28.3 | 3-7, scattered | echinulate | 1.3-2.2 | 27.5-35.1× 20.5-29.0 | smooth | 1.5-2.8 | 5.0-10.5 |
| | Nakaminato, Ibaraki Pref. | TSH-R1744 (IBA-2894) | II | 24.6-32.3× 22.9-27.5 | 3-7, scattered | echinulate | 1.3-2.2 | | | | |
| *Pisum sativum* L. | Ishikari, Hokkaido | TSH-R16268 | II | 18.1-24.4× 14.8-21.2 | 3-5, scattered | echinulate | 1.1-1.3 | | | | |

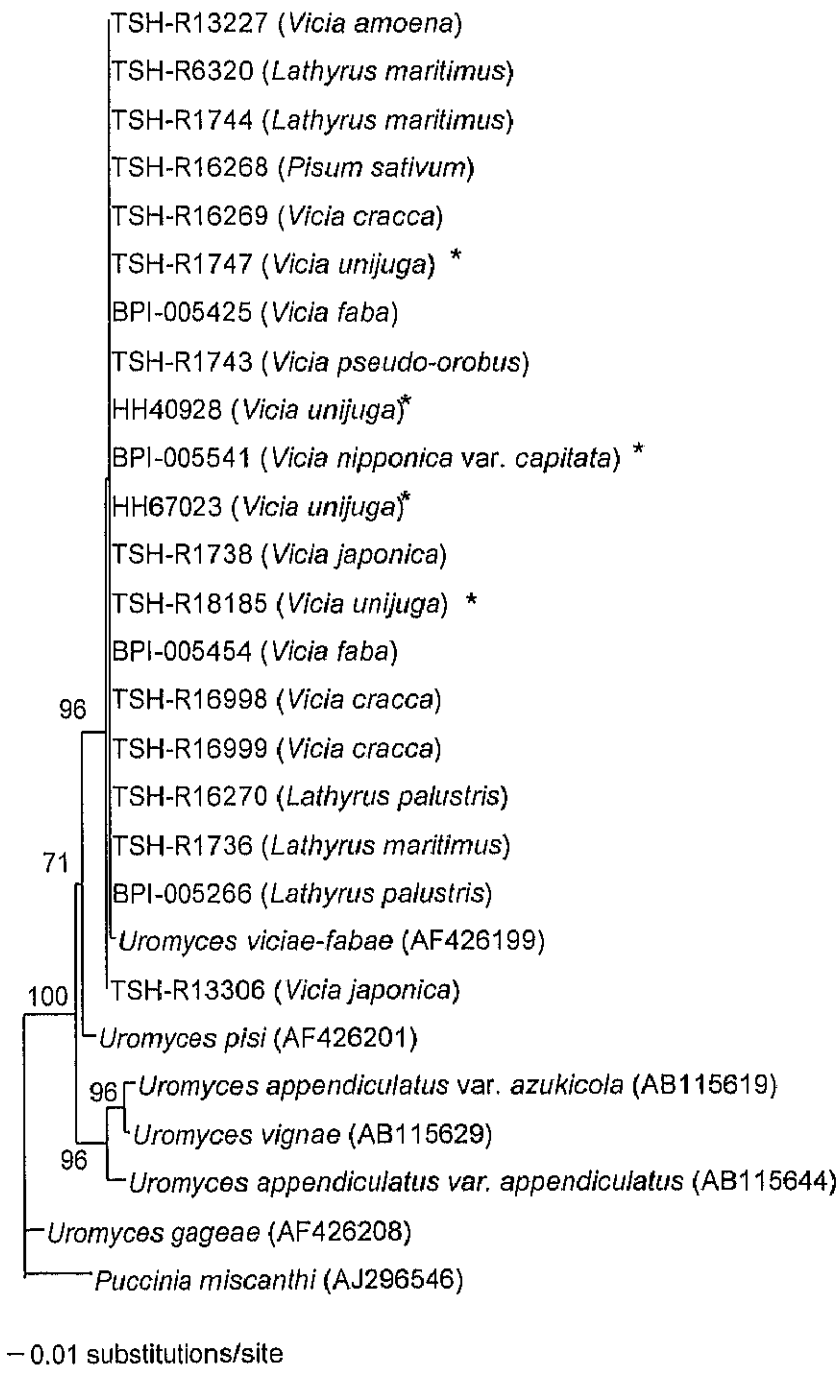


Fig. 4.7. A neighbor-joining tree inferred from sequences of LSU rDNA (D1/D2) regions using Clustal X. Values above the branches indicate percentage bootstrap support for 1000 replications. Length of branches is proportional to number of base changes, indicated by the scale bottom. * : host plants of *Uromyces viciae-fabae* var. *orobi* in Japan.

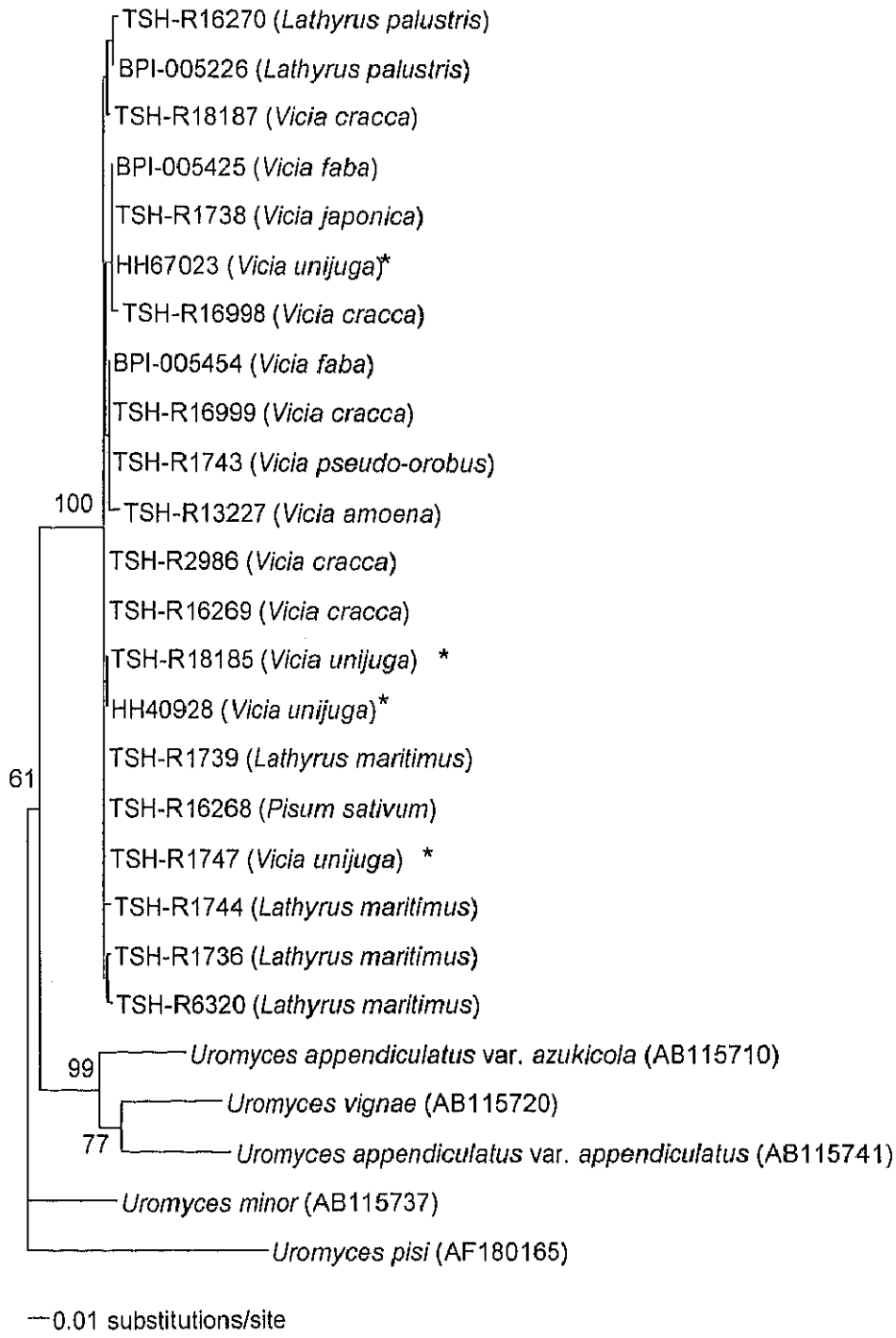


Fig. 4.8. A neighbor-joining tree inferred from sequences of ITS and 5.8S regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches. Length of branches is proportional to number of base changes, indicated by the scale bottom. * : host plants of *Uromyces viciae-fabae* var. *orobi* in Japan.

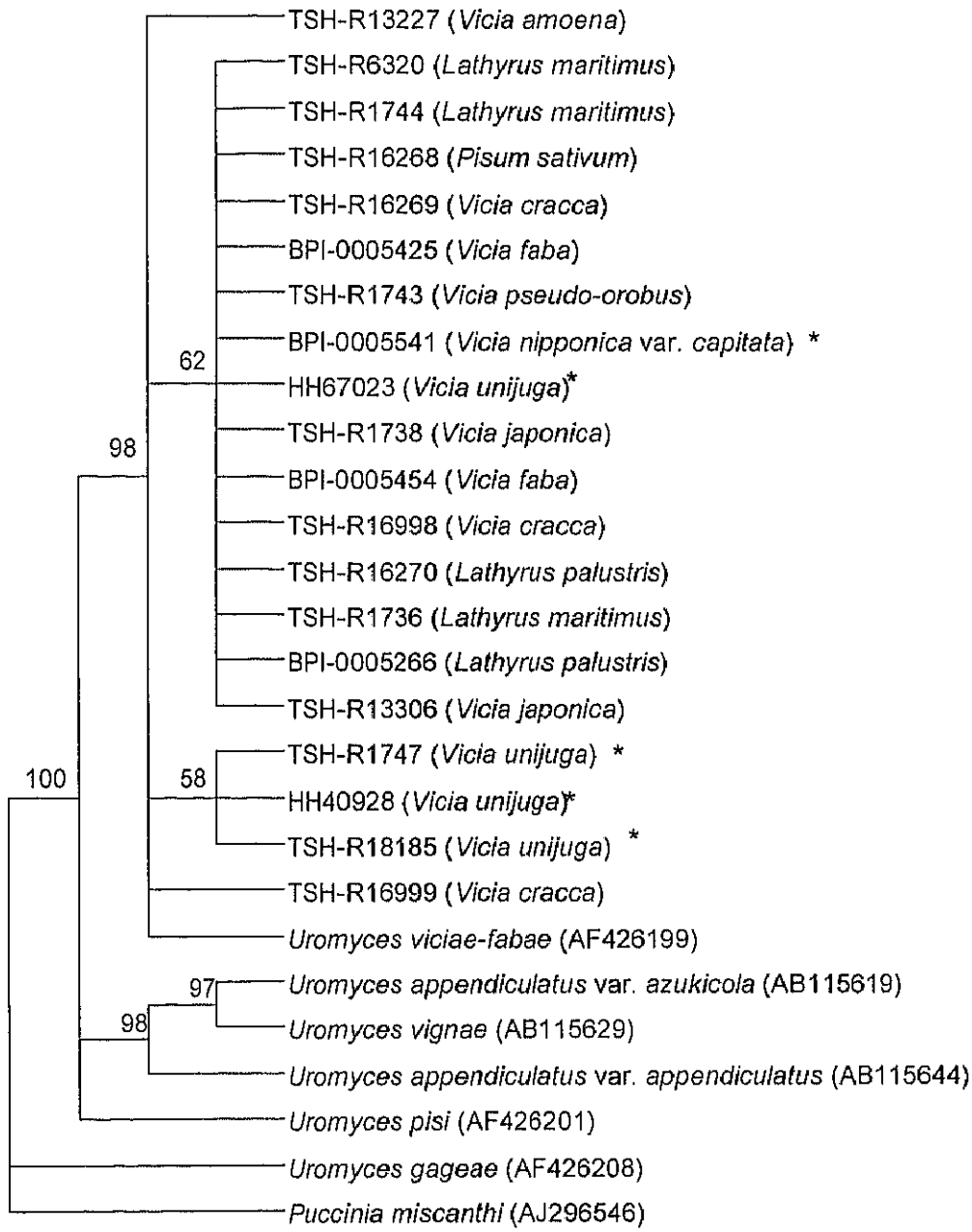


Fig. 4.9. A maximum-parsimony tree inferred from sequences of LSU (D1/D2) rDNA regions using Clustal X. Values above the branches indicate percentage bootstrap support for 1000 replications. * : host plants of *Uromyces viciae-fabae* var. *orobi* in Japan.

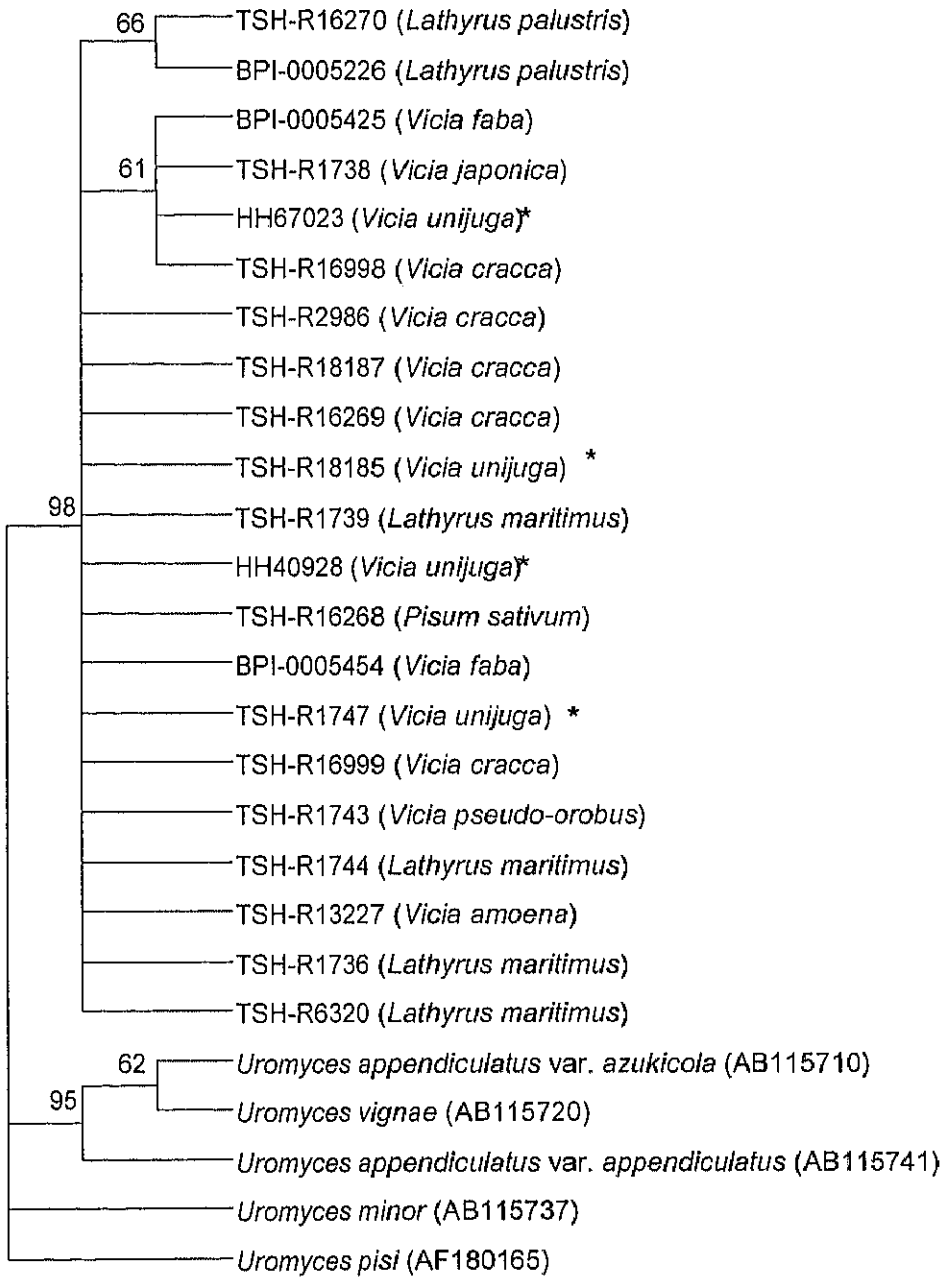


Fig. 4.10. A maximum-parsimony tree inferred from sequences of ITS and 5.8S regions using Clustal X. Values above the branches indicate percentage bootstrap support for 1000 replications. * : host plants of *Uromyces viciae-fabae* var. *orobi* in Japan.

## 3. Taxonomic discussion

*Uromyces viciae-fabae* (Pers.) Schroet. is said to be composed of var. *viciae-fabae* and var. *orobi* (Schumach.) Jørst. in Japan (Hiratsuka 1973; Hiratsuka et al. 1992). However, morphological and molecular phylogenetic analyses revealed that the specimens used in the present study did not show sufficient morphological differences in urediniospores and teliospores and high molecular divergence on D1/D2 and ITS including 5.8S regions. It suggests that the *U. viciae-fabae* on *Vicia*, *Lathyrus* and *Pisum* are a single taxon in Japan and no variety is detected in this study.

Historically, the rust fungi on *Vicia, Lathyrus* and *Pisum* were classified into three species, *U. fabae* de Bary (=*U. viciae-fabae* var. *viciae-fabae*), *U. orobi* Lév. (=*U. viciae-fabae* var. *orobi* (Schumach.) Jørst.) and *U. ervi* West., based on morphological characteristics of urediniospores and putative host specificity (Gäumann 1934; Ito 1922, 1950; Hiratsuka 1933, 1973; Wilson and Henderson 1966; Azbukina 1984; Hiratsuka et al. 1992). *Uromyces ervi* was different from *U. fabae* and *U. orobi* because the latter has urediniospore with 2 germ pores on an equatorial zone (Wilson and Henderson 1966; Cummins 1978; Guo and Wang 1986; Hiratsuka et al. 1992). However, *Uromyces orobi* was considered to be different from *U. fabae* because the former has urediniospores with thick wall and shows a narrow host range (Gäumann 1934; Wilson and Henderson 1966; Azbukina 1984; Hiratsuka et al. 1992).

In the present study, uredinial and telial characteristics are similar among the specimens and their variations ranges overlap. Based on the shape, position and

number of germ pores and wall ornamentation of urediniospores, these specimens are not distinguishable. Similarly, shape and wall ornamentation of teliospores were not sufficient morphological differences among these specimens. The results showed that qualitative morphologically characteristics could not be recognized to distinguish the two varieties of *U. viciae-fabae* in Japan.

In a scattered diagram of spore size and a principal component analysis, the specimens of *U. viciae-fabae* did not show discrete groups. According to Wilson and Henderson (1966), urediniospore wall-thickness of *U. viciae-fabae* var. *viciae-fabae* was from 1.2 to 2.5 µm (less frequently 3 or 4 µm) and *U. viciae-fabae* var. *orobi* was from 3 to 4 µm. In the present study, the urediniospores wall-thickness of the specimens ranged from 1.1 to 2.8 µm. Moreover, the urediniospore wall-thickness of the specimens on *V. unijuga*, *V. nipponica* var. *capitata* and *L. davidii* ranged from 1.1 to 2.8 µm, and the specimens on other *Vicia*, *Lathyrus* and *Pisum* ranged from 1.1 to 2.5 µm. In the present study, the thick urediniospore wall was not observed; especially the urediniospore wall of each specimen did not exceed 3 µm thick. The rust fungi on *V. unijuga*, *V. nipponica* var. *capitata* and *L. davidii* were reported that urediniospore wall was thicker than the rust fungi on other *Vicia*, *Lathyrus* and *Pisum* (Ito 1922; Ito 1950; Hiratsuka 1933; Hiratsuka 1973; Hiratsuka et al. 1992). However, the principal component analyses showed that the specimens of *U. viciae-fabae* on these three host plants could not form discrete groups based on the morphological characteristics of urediniospores and teliospores.

Molecular phylogenetic analyses revealed that specimens on *Vicia*, *Lathyrus* and

*Pisum* in Japan showed highly sequence homology based on D1/D2 (99-100%) and ITS (97-100%) regions, and a phylogram pattern was supported by high bootstrap values. The results revealed that molecular phylogeny of the specimens on *Vicia*, *Lathyrus* and *Pisum* from a highly genetically congruent clade, suggesting those constituting a monophyletic taxon. In molecular phylogenetic analyses, *U. viciae-fabae* (GenBank: AF426199) on *V. pannonica* Crantz was analyzed with these specimens. The results revealed that these specimens formed a single clade with *U. viciae-fabae* inferred from D1/D1 region.

In addition, the specimen of *U. viciae-fabae* on *V. unijuga, V. nipponica* var. *capitata* and *L. davidii* also did not show genetic variation based on D1/D2 and ITS regions. The molecular phylogenetic analyses, however, did not support the host specificity in this study. Although Hiratsuka (1933) reported that *U. viciae-fabae* var. *viciae-fabae* and *U. viciae-fabae* var. *orobi* could not infect other hosts except their host plants, I considered the rust fungi used by Hiratsuka (1933) might be different formae speciales. In Europe, the host plants of *U. viciae-fabae* var. *orobi* in Japan were host plants of *U. viciae-fabae* var. *viciae-fabae* (Gäumann 1934). Similarly, El-Gazzar (1981) also stated *U. viciae-fabae* var. *viciae-fabae* and *U. viciae-fabae* var. *orobi* had overlap in host range. Recently, Chung et al. (2004b) also reported that *U. viciae-fabae* from *V. unijuga* could infect and sporulate onto cultivated *P. sativum*. These results suggest that host specificity could not be used to be taxonomic character to separate *U. viciae-fabae* var. *viciae-fabae* and *U. viciae-fabae* var. *orobi* in Japan.

According to morphological and molecular phylogenetic analyses, specimens of *U. viciae-fabae* are a single fungal taxon in Japan. In the present study, these specimens used for morphological analyses showed morphological similarity with the *U. viciae-fabae* var. *viciae-fabae* that described by Wilson and Henderson (1966). Therefore, the morphological and molecular analyses suggest that *U. viciae-fabae* var. *orobi* may not be distributed in Japan. However, I did not observe the type specimens of *U. viciae-fabae* var. *orobi* in my study. It is necessary to confirm the morphological and molecular phylogenetic variations between the two varieties in future, and the species or variety delimitation also need to be discussed.