Taxonomic Evaluation of Morphologically Similar Species of *Pucciniastrum* in Japan Based on Comparative Analyses of Molecular Phylogeny and Morphology

May 2006

Ying Mei LIANG

Taxonomic Evaluation of Morphologically Similar Species of *Pucciniastrum* in Japan Based on Comparative Analyses of Molecular Phylogeny and Morphology

A Dissertation Submitted to
the Graduate School of Life and Environmental Sciences,
the University of Tsukuba
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Agricultural Science
(Doctoral Program in Biosphere Resource Science and Technology)

Ying Mei LIANG

Table of contents

1. Introduction ---- 1

1.1 Taxonomic status of *Pucciniastrum* ---- 1

1.2 Host plant and life cycle ---- 10

1.3 Species delimitation in *Pucciniastrum* ---- 12

1.4 Molecular phylogeny of fungi ---- 16

1.5 Objective ---- 18

2. Molecular phylogenetic analysis ---- 19

2.1 Materials and methods ---- 19

Materials ---- 19

DNA extraction ---- 20

PCR amplification and DNA sequencing ---- 20

Sequence alignment and analysis ---- 21

2.2 Results ---- 22

The D1/D2 region analysis ---- 22

ITS1-5.8S-ITS2 region analysis ---- 23

2.3 Discussion ---- 24

3. Morphological examination ---- 40

3.1 Materials and methods ---- 41

Materials ---- 41

Morphological observation ---- 41

Statistical analyses ---- 43

3.2 Results and discussion ---- 47
Ostiolar peridial cells ---- 47
Urediniospore-surface characteristics ---- 51
Germ pores of urediniospores ---- 54
Shape, size, and wall thickness of urediniospores ---- 61
Quantification characteristics within phylogenetic group A,
and within group Fb ---- 65
4. Taxonomic discussion ---- 81
Abstract ---- 88
Acknowledgements ---- 92
Literature cited ---- 94
Appendix 1: Specimens used for this study
Appendix 2: Sequence alignment of D1/D2 region
Appendix 3: Sequence alignment of ITS region

1. INTRODUCTION

1.1 Taxonomic status of *Pucciniastrum*

The genus *Pucciniastrum* was first established by Otth in 1861 with a type species, *Pucciniastrum epilobii* Otth on *Epilobium angustifolium* L. On the other hand, Magnus described a new genus *Phragmopsora* based upon a specimen on *Epilobium roseum* Retz in 1875, and named it as *Phragmopsora epilobii* Magn. Later, however, Magnus treated it as a synonym of *Pucciniastrum epilobii* (Hiratsuka 1936).

Taxonomic history of *Pucciniastrum* was shown in Fig 1.1. Its taxonomic treatment was different among researchers, especially, taxonomic consideration on the genus *Calyptospora* and *Thekopsora* were different among them. The genus *Thekopsora* was newly described by Magnus in 1875 based on the species, *Thekopsora areolata* (Fr.) Magn. It was distinguished from *Pucciniastrum* only by the position of the telia in host plants, namely in *Pucciniastrum* teliospores develop underneath the epidermis of plants; in *Thekopsora* teliospores are formed within the epidermal cells of plants. While, the genus *Calyptospora* was originally established by Kühn in 1869, consisting of a single species, *Calyptospora goeppertiana* Kühn found on stems of *Vaccinium vitis-idaea* L. This species was distinguished from *Thekopsora* by the absence of the uredinial stage and formation of teliospores on stems (Pady 1933, Faull 1938). Although these three genera could be distinguished by the position of telia, other morphological characteristics were similar to each other.

In earlier studies, some authors did not consider the position of telia as an important taxonomic characteristic and treated *Thekopsora* and *Calyptospora* as synonyms of *Pucciniastrum*, thus, *Pucciniastrum* as broad sense (Dietel 1900, Fischer 1904, Liro 1908, Trotter 1908-1914, Arthur 1907-1925). About 34 species were described under the broad sense of *Pucciniastrum* (Kuprevich and Tranzschel 1957). However, Bubák (1908), Grove (1913), Sydow (1912-1915), Fragoso (1925), and Pady (1933) separated *Pucciniastrum* from two other genera because they recognized the position of telia as an important taxonomic characteristic in the classification of genus level. Hiratsuka (1936) also followed this genus concept.

Pucciniastrum, *Thekopsora, Calyptospora, Melampsoridium, Milesina, Melampsorella*, *Hyalopsora*, *Uredinopsis* and *Naohidemyces* belong to the family Pucciniastreae (Hiratsuka 1958, Cummins and Hiratsuka 2003). Hiratsuka (1958) revised the taxonomy of Pucciniastreae, and emphasized the position of telia in plant tissue as an important taxonomic characteristic. As a result, he definitely separated *Thekopsora* and *Calyptospora* from *Pucciniastrum*, and included 23 species within *Pucciniastrum*. Gäumann (1959) also followed Hiratsuka. Although Cummins and Hiratsuka (1983) emphasized telia as the most important spore stage in generic distinction, but accepted *Pucciniastrum* in a broad sense, and treated *Thekopsora* and *Calyptospora* as synonyms of *Pucciniastrum.* Sato et al (1993) followed Hiratsuka's (1958) taxonomic treatment of *Pucciniastrum*, *Thekopsora* and *Calyptospora,* and further

established a new genus, *Naohidemyces*, based on the type of aecia and life-cycle. The most distinctive feature of *Naohidemyces* is the presence of *Uredo*-type aecia rather than *Peridermium*-type aecia on *Tsuga* host with spermogonia. This is the first genus having *Uredo*-type aecia in heteroecious species. Recently, Cummins and Hiratsuka (2003) made taxonomic revision, following the taxonomic opinion of Hiratsuka (1958) and Sato et al (1993), thus, they treated *Pucciniastrum, Thekopsora*, *Calyptospora* and *Naohidemyces* as separate genera, respectively.

According to Cummins and Hiratsuka (2003), the genus *Pucciniastrum* has the following morphological characteristics: spermogonia subcuticular, Group I (type 3). Aecia produced under the epidermis of host plant, erumpent, with cylindrical peridium, peridermium-type; aeciospores catenulate and verrucose. Uredinia also formed subepidermally on the abaxial surface of leaves, protected by hemispherical or conical peridia, rupturing at the apical pore, peridial ostiolar cells surrounding the orifice, large and sometimes roughened, *Milesia*-type; urediospores borne singly on very short pedicels; walls echinulate, colorless, pores scattered, obscure; contents orange-yellow. Telia occur on leaves, mostly hypophyllous (abaxial), sometimes amphigenous, telia produced under the epidermal cells of the host plant and 1 spore deep, consisting of laterally adherent teliospores. Teliospores sessile, 2-5, or more celled by vertical or oblique septa, germ pore 1 in the outer wall of each cell, walls yellowish brown to brown; germinate after dormancy, basidia external.

After Hiratsuka (1958), two additional species, *P*. *magnisporum* Laundon on *Acer davidi* Franch and *A. rubescens* Hayata and *Pucciniastrum hakkodaense* Y. M. Liang & Kakishima on *Enkianthus campanulatus* (Miq.) G. Nicholson were described by Laundon (1963) and Liang et al. (2005), respectively. Consequently, *Pucciniastrum* in the narrow sense consists of 25 species (Table 1.1).

Geographic distribution of 25 species belonging to the genus *Pucciniastrum* is also shown in Table 1.1. Among the 25 species, one species *P. agrimoniae* (Diet.) Tranzschel is distributed worldwide (Hiratsuka 1936, 1958). Thirteen species exist exclusively in Asia, of which 4 species, i.e., *P. corchoropsidis* (Diet.) Dietel ex P. et H. Sydow*, P. kusanoi* Dietel*, P. yoshinagai* f. Hiratsuka, and *P. hakkodaense*, are distributed only in Japan (Hiratsuka et al. 1992, Liang et al. 2005); *P. magnisporum* is known only in China Taiwan (Laundon 1963); five species, i.e., *P. actinidiae* f. Hiratsuka, *P. corni* Dietel, *P. fagi* Yamada, *P. hikosanense* f. Hiratsuka, and *P. styracinum* Hiratsuka, are commonly reported in Japan and China Taiwan, (Hiratsuka et al. 1992, Cao and Li 1999, Tai 1979, Hiratsuka and Chen 1991, Cummins and Ling 1950); species *P. aceris* H. Sydow is found in Japan, China, India and Himalaya (Hiratsuka et al. 1992, Cao and Li 1999, Hiratsuka 1958); *P. castaneae* Dietel is ubiquitous in Japan, China, Taiwan, India, Philippines and Korea (Hiratsuka 1958, Tai 1979); species *P. coriariae* Dietel is known in Japan, China, Taiwan, Philippines and Himalaya (Hiratsuka 1958, Hiratsuka et al. 1992, Tai 1979). *P. boehmeriae* P. et H. Sydow is known in New Guinea and Asia including Japan, Himalaya, Philippines and Taiwan

(Hiratsuka 1958, Arthur and Cummins 1936). Five species is common in Asia and Europe, of which *P. circaeae* (Winter) Spegazzini ex de Toni is widespread in Europe and Asia including Japan, China, Nepal, Taiwan and Korea (Tai 1979, Hiratsuka 1958, Kuprevich and Tranzschel 1957, Jørstad 1958, Tranzschel 1939); *P. coryli* Komarov is reported in Europe and Japan, China, Korea and India of Asia (Hiratsuka 1958, Kuprevich et al. 1957, Cummins and Ling 1950); *P. hydrangeae-petiolaris* f. Hiratsuka exists only in Japan, China, Taiwan, Nepal and Russia (Sakhalin) (Hiratsuka 1958, Cao and Li 1999, Hiratsuka and Chen 1991, Kuprevich and Tranzschel 1957); *P. miyabeanum* Hiratsuka only in Japan and Nepal of Asia and Russia (Sakhalin) (Hiratsuka et al. 1992, Kuprevich and Tranzschel 1957); *P. tiliae* Miyabe exclusively in Japan, China and Korea of Asia and Russia (Maritime provinces of Siberia) (Cummins and Ling 1950, Hiratsuka et al. 1992, Kuprevich and Tranzschel 1957). Three species is common in Asia, America and Europe, of which *P. epilobii* is widespread in New Zealand, Europe and America, also in Asia including Japan, China and Korea (Gäumann 1959, Savile 1962, Cummins and Ling 1950, Hiratsuka et al. 1992); *P. potentillae* Komarov is distributed in North America and Russia (Maritime provinces of Siberia, Sakhalin), and Asia including Japan, China, Korea and East Indies, and New Guinea (Conners 1967, Parmelee 1960, Arthur 1934, Kuprevich and Tranzschel 1957, Cummins and Ling 1950, Hiratsuka et al 1992, Cummins 1941); *P. pyrolae* Dietel ex Arthur is found in Japan, Taiwan, Korea, North America and Europe (Molnar and Sivak 1964, Gäumann 1959, Hiratsuka et al.

1992, Jørstad 1958, Kuprevich and Tranzschel 1957, Hiratsuka and Chen 1991). Two species are distributed only in America and Europe, of which *P. americanum* (Farl.) Arthur is found only in America (Figueiredo et al. 2003, Ziller 1974); *P. arcticum* Tranzschel is widespread in North America, South America and Central America and Europe including Finland and Russia (Anderson 1952, Kuprevich and Tranzschel 1957, Ziller 1974).

In Japan, a total of 22 species of *Pucciniastrum* have been reported by Hiratsuka and Hiratsuka (e.g.1897, 1926, 1930, 1936, 1940), Kamei (1931), Ito (1938), Hiratsuka (1958), Hiratsuka et al (1992) and Liang et al (2005) (Table 1. 2).

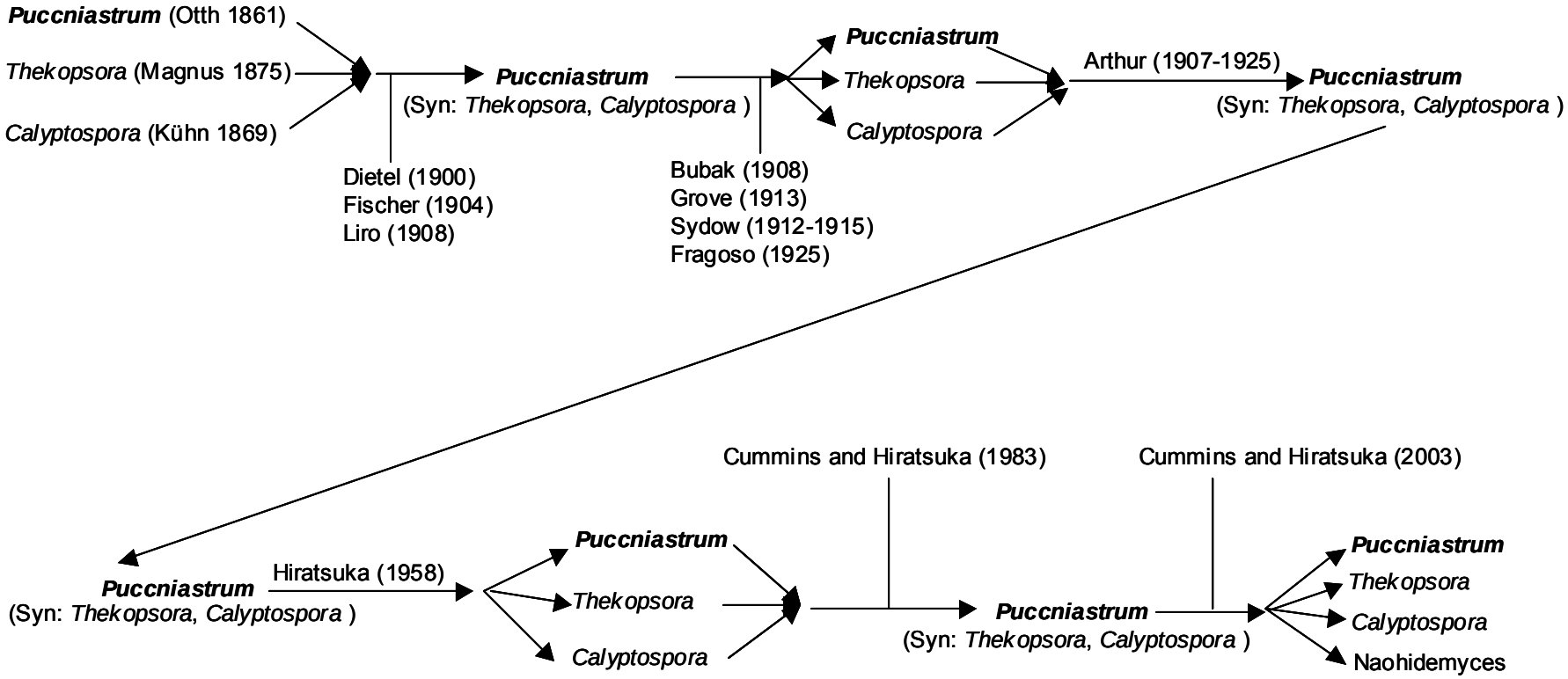


Fig. 1.1. Taxonomic history of *Pucciniastrum* and its relationship with *Thekopsora, Calyptospora,* and *Naohidemyces.*

Table1.1. The 25 species of *Pucciniastrum*, and their geographic distribution in the world.

Species	Morphological Group	Geographic distribution			
		Asia	Europe	America	Others
Pucciniastrum arcticum	I		Finland, Russia	America	
P. americanum				America	
P. pyrolae		Japan, Taiwan, Korea	Europe	North America	
P. hakkodaense		Japan			
P. agrimoniae	II	Worldwide			
P. potentillae		Japan, China, Korea, East Indies	Russia	North America	New Guinea
P. epilobii	III	Japan, China, Korea	Europe	America	New Zealand
P. circaeae		Japan, China, Taiwan, Korea, Nepal	Europe		
P. coryli		Japan, China, Korea, India	Europe		
P. hydrangeae-petiolaris		Japan, China, Taiwan, Nepal	Russia		
P. miyabeanum		Japan, Nepal	Russia		
P. tiliae		Japan, China, Korea	Russia		
P. coriariae		Japan, China, Taiwan, Philippines, Himalaya			
P. aceris		Japan, China, India, Himalaya			
P. actinidiae		Japan, China, Taiwan			
P. corni		Japan, China, Taiwan			
P. fagi		Japan, China, Taiwan			
P. styracinum		Japan, China, Taiwan			
P. hikosanense		Japan, Taiwan			
P. yoshinagai		Japan			
P. kusanoi		Japan			
P. magnisporum		China, Taiwan			
P. boehmeriae		Japan, Taiwan, Himalaya, Philippines			New Guinea
P. castaneae	IV	Japan, China, Taiwan, India, Korea, Philippines			
P. corchoropsidis		Japan			

Table1.2. Morphology and host plants of 22 species of *Pucciniastrum* distributed in Japan.（Hiratsuka et al. 1992, Liang et al. 2005）

Species	Group	Ostiolar cells of peridia		Uredinial and telial host plants (genus)
		Development degree	Surface characters	
Pucciniastrum pyrolae	I	Well-developed	coarsely or sparsely	Pirolaceae (*Piroa* and *Chimaphila*)
P. hakkodaense				Ericaceae (*Enkianthus*)
P. agrimoniae	II	Well-developed	Minutely echinulate or nearly smooth when wet	Rosaceae (*Agrimonia*)
P. potentillae				Rosaceae (*Potentilla*)
P. coryli	III	Well-developed	smooth	Betulaceae (*Corylus*)
P. fagi				Fagaceae (*Fagus*)
P. boehmeriae				Urticaceae (*Boehmeria*)
P. hydrangeae-petiolaris				Saxitragaceae (*Hydrangea*)
P. coriariae				Coriariaceae (*Coriaria*)
P. tiliae				Tiliaceae (*Tilia*)
P. actinidiae				Actinidiaceae (*Actinidia*)
P. yoshinagai				Theaceae (*Stewartia*)
P. corni				Cornaceae (*Cornus, Lynoxylon, Macrocarpium*)
P. kusanoi				Clethraceae *(Clethra*)
P. styracinum				Styraceae (*Pterostyrax, Styrax*)
P. miyabeanum				Caprifoliaceae (*Vibuvnum*)
P. circaeae				Oenotheraceae (*Circaea*)
P. epilobii				Oenotheraceae (*Epilobium*)
P. aceris				Aceraceae (*Acer*)
P. hikosanense				Aceraceae (*Acer*)
P. castaneae	IV	not well development		Fagaceae (*Castanea, Castanopsis*)
P. corchoropsidis				Tiliaceae (C*orchoropsis*)

1.2 Host plant and life cycle

Of the 25 species of *Pucciniastrum*, 12 species are known to have heteroecious and macrocyclic life cycles (Hiratsuka 1955, 1958, Hiratsuka et al. 1992). Their uredinial and telial stages mainly occur on dicotyledonous plants, including 17 families, such as Fagaceae, Betulaceae, Rosaceae, Tiliaceae, Oenotheraceae (Onagraceae), Theaceae, Pirolaceae, Ericaceae, Urticaceae, Saxitragaceae, Coriariaceae, Actinidiaceae, Cornaceae, Clethraceae, Styraceae, Caprifoliaceae and Aceraceae; spermogonial and aecial stages on the needles of Pinaceae (*Abies*, *Picea* and *Tsuga*) (Hiratsuka 1955, 1958; Ziller 1974; Hiratsuka and Kaneko 1976; Kaneko and Hiratsuka 1980, 1981, 1983; Sato et al. 1993).

The earliest inoculation experiments were done in the 19th century by Klebahn (1899) who successfully infected *Epilobium angustifolium* with aeciospores of *Pucciniastrum epilobii* Otth [= *Pucciniastrum pustulatum* (Pers.) Diet.] obtained from *Abies alba* Mill. (*A. pectinata* DC.). Klebahn also infected *A. alba* with basidiospores from *Epilobium angustifolium* to clarify its life cycle. Later, Fischer (1904), Bubák (1906) and Arthur (1907) also confirmed experimentally the life cycle connection between rusts on *Epilobium angustifolium* and *A. alba*. Fischer (1916) carried out inoculation experiments with basidiospores of *Pucciniastrum circaeae* and found its spermogonia and aecia produced on *A. alba*. Inoculation experiments of other *Pucciniastrum* species were subsequently made by Fraser (1912, 1913, 1914), Arthur (1925), Adams (1919), and Clinton

(1911, 1912, 1924). Darker (1929) successfully infected *Picea glauca* with basidiospores of *Pucciniastrum americanum* from *Rubus idaeus* subsp. *sachalinensis* and those of *Pucciniastrum arcticum* on *Rubus pubescens* in Canada, but failed to infect *Picea mariana*, *Abies balsamea* and *Tsuga canadensis*. Moreover, in 1970, Hiratsuka reported *Picea glauca*, *Picea mariana* (Mill) Bsp., and *Picea pungens* Engelm. as new aecial hosts of *Pucciniastrum sparsum* (Wint.) E. Fisch. by inoculation experiments in North America.

In Japan, Hiratsuka (1926, 1927, 1932) and Kamei (1932), extensively carried out inoculation experiments. Hiratsuka (1926) proved that aecia of *Pucciniastrum epilobii* occured on the leaves of *Abies sachalinensis* var. *mayriana* and the telia on *Epilobium angustifolium.* This was the first report of successful inoculation in Japan. In the following years, heteroecious life cycles of *Pucciniastrum kusanoi*, *P. miyabeanum*, *P. styracinum* and *P. tiliae* were reported by Hiratsuka (1932, 1933) and Kamei (1932). Hiratsuka and Kaneko (1976) proved that the aecia of *Pucciniastrum corni* Dietel occured on *Abies firma* Sieb. et Zucc., and uredinia and telia of *P. corni* occurred on *Cornus florida* L. Kaneko and Hiratsuka (1980) proved by inoculation experiments that the beech rust, *Pucciniastrum fagi* Yamada, produces the aecia on the current-year needles of *Tsuga diversifolia* (Maxim.) Masters, and the uredinia and telia on the leaves of *Fagus crenata* Blume and *F. japonica* Maxim. Subsequently, Kaneko and Hiratsuka (1981) experimentally determined the life cycle of *Pucciniastrum coryli* Komarov and showed the relationship between the telia on leaves of *Corylus*

sieboldiana and the aecia on needles of *Abies firma*, *A*. *homolepis* and *A*. *veitchii.* Moreover, Kaneko and Hiratsuka (1983) reported *Tsuga sieboldii* Carr. as a new aecial host plant of *P*. *fag*i. Likewise, Kaneko and Hiratsuka (1984) proved that aecia of *Pucciniastrum boehmeriae* were produced on needles *A. firma* by inoculation experiments.

Among 22 species of *Pucciniastrum* in Japan, the life cycle has been determined in 10 species by inoculation experiments (Table 1.3). However, aecial hosts of other 12 species remains unknown.

1.3 Species delimitation in *Pucciniastrum*

In the narrow sense of *Pucciniastrum*, species delimitation was mainly based upon the morphological characteristics of uredinial stage, i.e., the surface structure of ostiolar peridial cells, the shape and size of urediniospores, and host plant range (Hiratsuka 1936, 1958; Kuprevich and Tranzschel 1957, Arthur 1962). According to Hiratsuka (1936, 1958) and Hiratsuka et al. (1992), all species of *Pucciniastrum* can further be divided into four morphological groups (Table 1.1) based on the structure of the peridial ostiolar cells in the uredinia. Group I, including four species, is characterized by coarsely or sparsely aculeate ostiolar cells. Group II, two species are characterized by minutely echinulate ostiolar cells that sometimes became smooth under wet conditions. Group III has smooth ostiolar cells and contains 17 species. The ostiolar cells of group IV are obscure and two species are included in this group.

All of the 16 species of group III except *P. magnisporum* have been reported

Table 1.3. Life cycle of 10 species of *Pucciniastrum* which are distributed in Japan.

Species	Genera of host plants		Investigators (Year of publication)
	Uredinial and telial stages	Spermagonial and aecial stages	
Pucciniastrum epilobii	*Epilobium*	*Abies*	Klebahn (1899), Fischer (1904), Bubák (1906), Arthur (1907), Fraser (1912), Weir and Hubert (1916), Arthur et al. (1925), Hiratsuka (1926, 1927, 1932), Kamei (1932)
P. circaeae	*Circaea*	*Abies*	Fischer (1916)
P. kusanoi	*Clethra*	*Abies*	Hiratsuka, f. (1933)
P. miyabeanum	*Viburnum*	*Abies*	Hiratsuka, f. (1932)
P. styracinum	*Styrax*	*Abies*	Kamei (1932)
P. tiliae	*Tilia*	*Abies*	Kamei (1932)
P. corni	*Cornus*	*Abies*	Hiratsuka and Kaneko (1976)
P. coryli	*Corylus*	*Abies*	Kaneko and Hiratsuka (1981)
P. fagi	*Fagus*	*Tsuga*	Kaneko and Hiratsuka (1980, 1983)
P. boehmeriae	*Boehmeria*	*Abies*	Kaneko and Hiratsuka (1984)

Table 1.4. Morphological characteristics of 16 species of *Pucciniastrum* (group III) distributed in Japan.

Species	Urediniospores		Uredinial and telial host plants (genus)
	Shape	Size (μm)	
Pucciniastrum epilobii	ovate, oblong or subglobose	14-24 × 10-16	*Epilobium*
P. circaeae	ovate, ellipsoid or subglobose	16-24 × 12-16	*Circaea*
P. coryli	obovate, ellipsoid or oblong	18-27 × 10-16	*Corylus*
P. hydrangeae-petiolaris	ovate, ellipsoid, oblong or clavate	18-33 × 14-21	*Hydrangea*
P. fagi	obovate, ellipsoid or oblong	18-24 × 10-15	*Fagus*
P. tiliae	obovate, ellipsoid, oblong or subglobose	18-27 × 12-18	*Tilia*
P. boehmeriae	ovate, ellipsoid or subglobose	18-25 × 13-18	*Boehmeria*
P. miyabeanum	ovate, ellipsoid, pyriform or globose	18-30 × 12-20	*Viburnum*
P. corni	ovate, ellipsoid or pyriform	18-29 × 12-18	*Cornus*
P. styracinum	ovate, ellipsoid or subglobose	18-27 × 12-17	*Styrax*
P. kusanoi	ovate, ellipsoid or subglobose	18-25 × 14-20	*Clethra*
P. yoshinagai	ovate, ellipsoid or oblong	18-27 × 15-20	*Stewartia*
P. actinidiae	obovate, ellipsoid or oblong	18-27 × 12-16	*Actinidia*
P. hikosanense	obovate, ellipsoid or oblong	24-36 × 13-18	*Acer*
P. aceris	ovate, broadly ellipsoid or subglobose	17-22 × 14-17	*Acer*
P. coriariae	ovate, ellipsoid or oblong	21-26 × 14-20	*Coriaria*

.

in Japan (Hiratsuka et al. 1992) (Table 1.4). These species have similar shape of urediniospores, they are ovate, obovate, ellipsoid, oblong, or subglobose occasionally, broadly ellipsoid or pyriform, and the urediniospore size range of these species also closely resembles each other. Furthermore, they have extremely similar morphological characteristics in teliospores (Hiratsuka 1936, 1958, Hiratsuka et al. 1992). In fact, there are no definite circumscription between the 16 species except for their host plants of uredinia and telia. Consequently, taxonomy of the 16 species of *Pucciniastrum* group III is doubtful and requires reconsideration.

For revision of *Pucciniastrum* species, some authors tried to find other useful morphological characteristics. Cummins (1936) examined arrangements of germ pores in urediniospores and considered them as useful taxonomic characteristics in rust fungi. Kaneko and Hiratsuka (1982) also observed the urediniospore germ pores in the Pucciniastraceous and Melampsoraceous rust fungi, and suggested that related groups of the rust species tend to have the same pore arrangements in *Melampsoridium* and *Uredinopsis.* Therefore, they considered that the arrangement of germ pores in urediniospores was an important taxonomic characteristic in the species level. However, eight species of *Pucciniastrum* group III were included in their study, of which four species have typically bizonate and the number of germ pores ranges between 4-6 (rarely 7), three species contain some variation in their germ pore arrangement from the predominant type, i.e. from predominant bizonate arrangement to

scattered, or from scattered arrangement to bizonate. Only one species has equatorial arrangement. Thus, the germ pore arrangement to distinguish the species of *Pucciniastrum* group III is not a diagnostic feature.

1.4 Molecular phylogeny of fungi

Recent advances in molecular genetics have provided techniques that allow researchers to study relationships among organisms at the molecular level. DNA sequence analysis has been exploited extensively in recent years by mycologists for systematic and phylogenetic studies on the various groups of fungi. Molecular data are useful when morphological characters alone are insufficient for the delineation of clear taxonomic groups. Most of these studies have focused on the analysis of ribosomal RNA genes.

Ribosomal RNA genes exist in most genomes as multiple copies arranged in tandem repeat along one or more chromosomes. In eukaryotes, each repeat is composed of a transcription unit that codes for three RNAs: a small subunit RNA (SSU-18S), a large subunit RNA (LSU-28S) and 5.8S RNA. The three genes are separated by two transcribed spacers, ITS1 and ITS2. Each repeat is separated by a non-transcribed spacer, also called an intergenic spacer (IGS).

Several features of rDNA make it appropriate for systematic and phylogenetic studies. First, this region of the genome is well characterized and conserved. Many primers already developed are available to amplify regions of the rDNA repeat that would supply sequence data for a wide range of taxa (White et al. 1990). Secondly, substantial research has been done on rDNA for many fungi

(Guadet et al. 1989; Begerow et al. 2000; Taylor et al. 2000; Ko and Jung 2001; Almaraz et al. 2002), so ample datasets are available for reference. Additionally, different regions of rDNA evolve at variable rates, which can be used to investigate fungal relationships at different taxonomic levels (Bruns et al. 1991). For example, ITS regions are very suitable for phylogenetic analysis at the species level (Brown et al. 1972, White et al. 1990, Takamatsu 2005).

In recent years, there have been several molecular phylogenetic studies of rust fungi that have been very useful in establishing phylogenetic relationships. e.g. Sjamsuridzal et al. (1999) utilized molecular methods to determine relationships among the rusts that infect ferns. Maier et al. (2003) analyzed sequences of large subunit (LSU) rDNA to discuss suprageneric relationships of the rust fungi. Wingfield et al. (2004) employed sequence data from the small subunit (SSU) rRNA to infer phylogenetic relationships in the Uredinales. Moreover, molecular phylogenetic studies within single genera have also been successfully applied to determine the phylogenetic relationships among rust fungi that are morphologically similar, for example in the genera *Puccinia* (Zambino and Szabo 1993, Weber et al. 2003, Chung et al. 2004), *Melampsora* (Smith et al. 2004, Tian et al. 2004, Pei et al. 2005), *Melampsoridium* (Kurkela et al. 1999), and *Cronartium* (Vogler and Bruns 1998).

However, molecular phylogenetic studies on *Pucciniastrum* have not been reported until the recent study by Maier et al. (2003) based on LSU-28S sequences. She reported a phylogenetic analysis of genera of the Uredinales, which included two species of *Pucciniastrum* group III (*P. epilobii* and *P. circaeae*), one species of *Pucciniastrum* group I (*P. pylolae*) and one species of

Pucciniastrum group II (*P. agrimoniae*). The results showed that *P. epilobii* and *P. circaeae* were included in the same cluster and were phylogenetically distant from *P. pylolae* and *P. agrimoniae*. The latter two species were closely related to *Cronartium* and *Thekopsora*. The results suggest that relationships within *Pucciniastrum* may be more complicated. Moreover, species of *Pucciniastrum* were found to be polyphyletic (Maier et al. 2003). Consequently, a comprehensive phylogenetic study is required to clarity the phylogenetic relationship of species in *Pucciniastrum*.

1.5 Objective

This study was conducted to evaluate taxonomy of morphologically similar species of *Pucciniastrum* group III from Japan. For this purpose, first, the LSU rDNA (D1/D2) region of the 28S rDNA and the internal transcribed spacer (ITS) region including the 5.8 S rDNA were sequenced and the phylogenetic trees were constructed based on these sequence. Then, morphological comparisons were made to clarify the relationships with phylogenetic analyses. Lastly, taxonomy of these species was discussed based on the phylogenetic and morphological analyses.

2. Molecular phylogenetic analysis

In this section, phylogenetic analysis using DNA sequences from two gene regions was conducted to clarify the relationships among the morphologically similar species of *Pucciniastrum* group III in Japan. The D1/D2 region at the 5' end of the LSU rDNA gene and the internal transcribed spacers ITS1 and ITS2 and 5.8S gene of the nuclear ribosomal DNA transcriptional unit were used for analysis.

2.1 Materials and methods

Materials

Forty-nine specimens comprising 14 species of group III were used for the phylogenetic analysis. Two species were excluded because specimens were not available. Some specimens were freshly collected from different districts in Japan by the author, while most specimens were loaned from the following herbaria: the Hiratsuka Herbarium in Tokyo Japan (HH); the Herbarium of Systematic Mycology, the College of Education, Ibaraki University, Mito Japan (IBA); and the Mycological Herbarium of the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba Japan (TSH). Specimen voucher number and accession numbers of the DNA sequences (DDBJ, EMBL, and GenBank) are listed in Table 2.1.

DNA extraction

DNA was extracted from about 150-200 urediniospores obtained from a single uredinium. Spores were crushed between two sterile glass slides and suspended in 20 µl extraction buffer [10 mM Tris-HCI pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCI, 0.01% sodium dodecyl sulfate (SDS), 0.01% Proteinase K], and incubated at 37°C for 60 min and then at 95°C for 10 min, followed by a 4°C soak (Suyama et al. 1996, Virtudazo et al. 2001). From the crude extract, 1 to 3 µl sample was used directly for each polymerase chain reaction (PCR) amplification (Fig. 2.1).

PCR amplification and DNA sequencing

Double stranded DNA spanning the D1/D2 region of the LSU rDNA and the entire ITS1-5.8S- ITS2 (ITS) region of the rDNA were amplified by PCR, using the primer pairs NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O'Donnell 1993), and ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) (Gardes and Bruns 1993) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990), respectively (Fig. 2.2).

DNA was amplified using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA); 40-µl reaction mixtures comprised 1 unit of Taq DNA polymerase (TaKaRa, Tokyo, Japan), a commercial deoxynucleoside triphosphate (dNTP) mixture (containing 2.5 mM of each dNTP), Taq reaction buffer (containing 2 mM Mg^{2+}), and 0.2 µM of each primer. PCR was run under the following conditions: 3 min initial denaturation at 95°C, followed by 35 cycles

of 30 s at 95°C, 1 min at 55°C, and 1 min at 72°C; the reaction was terminated after a final extension at 72°C for 10 min.

PCR products were first purified with MicroSpin™ S-400 HR columns (Amersham Pharmacia Biotech, NJ, USA) and prepared for sequencing using a Big Dye™ Terminator ver.3.1 Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) with the same primers used for PCR amplification under the following conditions: 25 cycles of 96°C for 10 s, 50°C for 5 s, 60°C for 4 min. Cycle sequencing reaction products (20 µl) were finally purified by ethanol precipitation (Fig. 2.3), and then sequenced using an ABI PRISM 310 Automated DNA Sequencer (Applied Biosystems).

Sequence alignment and analysis

DNA sequence alignments were generated with CLUSTAL X multiple program ver.1.8 (Thompson et al. 1997). Then manual alignment was done in Se-Al ver.2.07a (Rambaut 2001). *Pucciniastrum pyrolae* Dietel ex Arthur (AF426233) and *P. goeppertianum* (Kühn) Klebahn (L76508, L76509) were included as outgroup in D1/D2 and ITS analyses, respectively (Table 2.1). In addition, for comparison, two sequence data of *P*. *circaeae* (Winter) Spegazzini ex de Toni (AF426227) and *P*. *epilobii* (Otth) (AF522179), from GenBank, were also included in the analysis (Table 2.1). The program PAUP ver.4.0b10 (Swofford 2002) was used to construct a neighbor-joining (NJ) topology under the Kimura 2-parameter model, with transition/transversion rate: 2.0 (Kimura 1980), excluding positions with gaps and correcting for multiple substitutions.

Maximum parsimony analysis was also performed using the heuristic search option with 100 random stepwise addition sequences to search for the most parsimonious tree. Bootstrap (Felsenstein 1985) values were determined using 1000 replicates to estimate support for clade stability of the consensus tree using the same program.

2.2 Results

The D1/D2 region analysis

The data for the D1/D2 region analysis comprised 47 samples, which included GenBank accessions AF522179 (*P. epilobii*) and AF426227 (*P. circaeae*) for comparison to original data, plus *P. pyrolae* (AF426233) as the outgroup.

All specimens produced a single fragment 545-566 bases long from the nuclear LSU rDNA region. The final LSU rDNA (D1/D2) sequence alignment included a total of 576 characters, of which 495 sites were constant, 20 sites were variable and parsimony uninformative, and 61 sites were parsimony informative. A parsimony analysis using PAUP* obtained one most parsimonious tree with 104 steps [consistency index (CI) = 0.846, retention index (RI) = 0.940 and rescaled consistency (RC) = 0.795, Fig. 2.4]. The NJ tree (Fig.2.5) was also obtained through the distance phylogenetic analysis using the NJ method, which was nearly the same as the parsimony tree (Fig. 2.8).

All specimens of *Pucciniastrum* except for the outgroup taxon *P. pyrolae* separated into six groups (see Fig. 2.5). Four specimens of *P. circaeae* and *P*.

epilobii on Oenotheraceae formed a distinct group (Group A), strongly supported by the 100% bootstrap values. Group B included four specimens of *P. hydrangeae-petiolaris* Hiratsuka, f. on the genus *Hydrangea*, Hydrangeaceae, which formed a well-supported group (100% in both the NJ tree and the MP tree). Likewise, group C also represented a distinct group (98% in the NJ tree, 99% in the MP), including three specimens of *P. coryli* Komarov on the genus *Corylus,* Betulaceae. Group D, supported by 62% of the bootstrap replicates and consisted of two species, *P. fagi* Yamada on the genus *Fugus*, Fagaceae and *P. hikosanense* Hiratsuka, f. on the genus *Acer*, Aceraceae. Group E only included specimens of *P. tiliae* Miyabe on the genus *Tilia*, Tiliaceae, but without bootstrap values. Group F consisted of twenty-four specimens from seven species, i.e. *P. corni* Dietel*, P. kusanoi* Dietel, *P. styracinum* Hiratsuka, *P. actinidiae* Hiratsuka, f., *P. boehmeriae* P. et H. Sydow, *P. miyabeanum* Hiratsuka and *P. yoshinagai* Hiratsuka, f., which supported by the bootstrap (66% in the NJ tree and 62% in the MP tree).

The ITS1- 5.8S - ITS2 region analysis

All samples produced a single fragment 724-729 bases long. The alignment data matrix consists of 40 samples, of which two sequences of *P. goeppertianum* (GenBank accession no. L76508 and L76509) were used as outgroup. The final sequence alignment of the ITS region included 752 characters, of which 575 sites were constant, 56 variable characters were parsimony uninformative, and 121 sites were parsimony informative. The parsimony analysis of the sequence

data resulted in a single most parsimonious tree with 237 steps (CI = 0.873, RI = 0.907 and RC = 0.792, Fig. 2.6). The neighbor-joining consensus tree (Fig. 2.7) by distance phylogenetic analysis was identical with the maximum parsimony tree (Fig. 2.9).

The phylogenetic trees constructed from ITS and 5.8S rDNA regions separated the specimens into 8 groups with high bootstrap support (see Fig. 2.7); these groups are similar to the groups from the D1/D2 region analysis. Group A (*P. circaeae*), group B (*P. hydrangeae-petiolaris,* both 100% in NJ and MP), group C (*P. coryli*), group E (*P. tiliae,* 85% in NJ and 73% in MP) were distinct, each composed of only one species. However, D1/D2 Group D was more clearly divided into two groups (ITS group Da and Db) with 72% bootstrap support; group Da included two specimens of *P. hikosanense* (100% in NJ and 98% in MP), while sister position group Db consisted of six specimens of *P. fagi* (84% in NJ and 74% in MP). Similarly, in contrast to the D1/D2 analysis, group F was also clearly divided into two groups (ITS group Fa and Fb), group Fa included four specimens of *P. boehmeriae* (86% in NJ, 83% in MP), and group Fb (64% in NJ, 96% in MP) consisted of six species: *P. kusanoi*, *P. styracinum*, *P. corni*, *P. actinidiae*, *P. miyabeanum* and *P. yoshinagai*.

2.3 Discussion

In the present section, two species of *P. circaeae* and *P. epilobii* formed a highly coherent cluster in NJ and maximum parsimony and were well-separated from other species of *Pucciniastrum*. Moreover, SEM observations based on a

large number of specimens on the family Oenotheraceae from Japan, showed that *P. circaeae* on *Circaea* and *P*. *epilobii* on *Epilobium* did not have the ostiolar cells (these taxonomic characters will be discussed in section 3). The combined results of our study suggest that ostiolar cell characteristics may be important taxonomically. Furthermore, our specimens of *P*. *circaeae* and *P*. *epilobii* clustered with *P*. *circaeae* (AF426227) and *P*. *epilobii* (AF522179) from America and placed in the basal position in the D1/D2 tree (bootstrap 100%). Likewise, *P. circaeae* was separated from other species by a long genetic distance based on ITS, though the sequence data of *P*. *epilobii* was not obtained for the ITS region. In addition, Maier et al (2003) examined phylogenetic relationship of four species of *Pucciniastrum* based on 28S and reported that *P*. *circaeae* and *P*. *epilobii* formed a highly supported cluster (100%), separating them from two other species of *Pucciniastrum*. Therefore, it is suggested that these two species have a common ancestral lineage. On the other hand, *P*. *circaeae* and *P*. *epilobii* have closely related hosts and have morphologically similar urediniospores and teliospores (Hiratsuka et al. 1992) as well as possess very similar gymnopedunculate haustoria (Berndt and Oberwinkler 1995). These features suggest that *P*. *circaeae* and *P*. *epilobii* belong to the same species.

Molecular phylogenetic trees show that *P*. *hydrangeae-petiolaris*, *P. coryli* and other species are distant from each other and separate from other species. Therefore, we regard these two species as distinct taxa.

Comparing the divergence variation between the D1/D2 region and the ITS

regions, the D1/D2 region showed low bootstrap support for the groups D and F (62% and 66%, see Fig. 2.5), respectively. Furthermore, group E did not received bootstrap values. In contrast, the ITS group Da and Db (they corresponded to D1/D2 group D), the ITS group Fa and Fb (they corresponded to D1/D2 group F) and group E each clearly received high bootstrap support in the ITS regions analysis (Fig. 2.7). However, the discordance between LSU (D1/D2) and ITS regions has been pointed out by many authors. Approximately 30 years ago, researchers first considered that rDNA genes from closely related species are highly evolutionally conserved, but that ITS and intergenic regions were much more variable (Brown et al. 1972). In the recent phylogenetic analysis, the findings regarding the genetic variability in ITS and 5.8S rDNA regions was repeatedly (frequently) argued. It is known that the mutation rate in the D1/D2 region of LSU rDNA is often slower than that in the ITS region. Therefore, the sequence variation in D1/D2 region is often insufficient to distinguish biological species (O'Donnell and Cigelnik 1997, Maier et al. 2003), whereas sequence variation in the ITS region is commonly large enough to separate taxa at the species level (White et al. 1990, Zambino and Szabo 1993, Takamatsu 2005). In this study, Both the NJ and MP trees of ITS region clearly revealed that *P*. *fagi* (ITS group Db) and *P*. *hikosanense* (ITS group Da) are phylogenetically separated from each other, though they are sister taxa. Similarly, *P. boehmeriae* (ITS group Fb 86%) is sister to the six other species (ITS group Fa, Fig. 2.7). Consequently, these results suggest that *P. fagi*, *P. hikosanense*, *P.*

tiliae and *P. boehmeriae* represent distinct taxa.

Twenty-four rust specimens from six host species, including *P. kusanoi* on *Clethra*, *P. styracinum* on *Styrax*, *P. corni* on *Cornus*, *P. actinidiae* on *Actinidia*, *P. miyabeanum* on *Viburnum* and *P. yoshinagai* on *Stewartia* constituted a single cluster in both NJ and maximum parsimony analyses based on both D1/D2 and ITS regions. In addition, they have identical nucleotide sequences in both regions. Although molecular data from wider regions of the rust genome and from a more rust species will provide a better understanding of evolutionary relationships among different taxa of rust in Uredinales (Maier et al 2003, Pei et al. 2005), the present results did not support that these six species were separate taxa in the species level.

The present molecular phylogenetic analyses within the genus *Pucciniastrum* clearly suggested that species systematics based on host plants did not support their phylogeny (Table 2.2), and their revision is required.

Table 2.1. Specimens of *Pucciniastrum* species and their DNA database accession no. used for phylogenetic analysis.

Rust species	Host plant	Voucher specimens[a]	Locality in Japan	Collector and year	Database accession no. D1/D2	ITS
Pucciniastrum fagi						
	Fagus crenata	TSH-R10724	Tochigi	W.H.Chung and Y.Oguchi, 2001	AB221378	AB221425
	F. crenata	TSH-R21254	Akita	Liang, Tian and Kaneko, 2003	AB221375	AB221425
	F. crenata	TSH-R21242	Kitaibaraki	Liang, Tian and Kaneko, 2003	AB221374	AB221420
	F. japonica	TSH-R4238 (IBA6307)	Niigata	Y. Ono, 1992	AB221376	AB221421
	F. crenata	TSH-R4245 (IBA8447)	Shizuoka	Y.Ono and K.Ishimiya, 1999	AB221377	AB221423
	F. crenata	TSH-R4243 (IBA8372)	Gunma	Y.Ono and K.Ishimiya, 1999	–	AB221422
P. kusanoi						
	Clethra. barbinervis	TSH-R21252	Kitaibaraki	Liang, Tian and Kaneko, 2003	AB221401	AB221430
	C. barbinervis	TSH-R3847	Shizuoka	W. Asano et al., 1998	AB221398	AB221428
	C. barbinervis	TSH-R21299	Nagano	C.M.Tian and M.Imazu, 2003	AB221399	AB221427
	C. barbinervis	21509 (Hiroraki University)	Miyazaki	Y. Harada, 1992	AB221402	AB221426
	C. barbinervis	HH98635	Hyugo	N.Haratsuka and S.Kaneko, 1975	AB221400	AB221429
P. actinidiae						
	Actinidia rufa	TSH-R4267 (IBA7716)	Okinawa	Y. Ono, 1995	AB221404	AB221447
	A. arguta	TSH-R23801	Okinawa	Y. Ono, 2003	AB221403	AB221446
	A. rufa	TSH-R4266 (IBA7700)	Okinawa	Y. Ono, 1995	AB221405	AB221448
	A. rufa	TSH-R4268 (IBA8002)	Okinawa	Y. Ono, 1997	AB221407	AB221445
	A. rufa	HH102310	Okinawa	S. Sato and N. Hiratsuka, 1955	AB221406	–
P. coryli						
	Corylus sieboldiana	TSH-R4236 (IBA7603)	Tochigi	Y. Ono, 1995	AB221380	–
	C. heterophylla	TSH-R4233 (IBA2582)	Yamanashi	Y. Ono, 1982	AB221379	–
	C. sieboldiana	TSH-R4237 (IBA8641)	Fukushima	Y. Ono and H. Mori, 2000	AB221381	AB221419
P. corni						
	Cornus kuosa	TSH-R13510	Tottori	S.Kaneko and I. Ohira et al., 1971	AB221409	AB221437
	C. kuosa	TSH-R4273 (IBA7671)	Miyazaki	Y. Ono, 1995	AB221408	AB221436
P. hikosanense						
	Acer rufinerva	TSH-R4287 (IBA2565)	Yamanashi	Y. Ono, 1982	AB221388	AB221441
	A. rufinerva	TSH-R4289 (IBA8441)	Shizuoka	Y. Ono and K. Ishimiya, 1999	AB221389	AB221440
	A. rufinerva	TSH-R4288 (IBA2569)	Yamanashi	Y. Ono, 1982	AB221390	–
P. styracinum						
	Styrax japonica	TSH-R t015	Tsukuba	C.M. Tian and M. Kakishima, 2002	AB221416	AB221431
	S. japonica	TSH-R1527	Toyama	Y. Sato, 1995	AB221417	AB221433
	S. japonica	TSH-R1583	Toyama	T. Kobayashi and C. Nakajima, 1996	AB221418	AB221432
P. yoshinagai						
	Stewartia monadelpha	TSH-R4272 (IBA8430)	Nara	Y. Ono and K. Ishimiya, 1999	AB221411	AB221434
	S. monadelpha	TSH-R4270 (IBA8404)	Nara	Y. Ono and K. Ishimiya, 1999	AB221410	AB221435

Table. 2.1. (continued)

Rust species	Host plant	Voucher specimens[a]	Locality in Japan	Collector and year	Database accession no. D1/D2	ITS
P. miyabeanum						
	Viburnum furcatum	TSH-R4281 (IBA8721)	Yamagata	Y. Ono, 2001	AB221394	AB221442
	V. furcatum	TSH-R4279 (IBA7888)	Aomori	Y. Ono, 1997	AB221395	–
	V. furcatum	TSH-R4278 (IBA7659)	Miyazaki	Y. Ono, 1995	AB221396	–
	V. furcatum	TSH-R3849	Shizuoka	W. Asano et al., 1998	–	AB221444
	V. furcatum	TSH-R10202	Akita	Y. Yamaoka et al., 1997	AB221397	AB221443
P. boehmeriae						
	Boehmeria platanifolia	TSH-R4253 (IBA8481)	Tokyo	Y. Ono, 1999	AB221391	AB221451
	B. spicata	TSH-R4254 (IBA8571)	Tochigi	Y. Ono, 2000	AB221392	–
	B. tricuspis	TSH-R21289	Aomori	Y .M .Liang and C .M. Tian, 2003	AB221393	AB221450
	B. tricuspis	TSH-R21290	Aomori	Y .M .Liang and C .M. Tian, 2003	–	AB221449
	B. tricuspis	TSH-R21307	Nikko	Y .M .Liang and C .M. Tian, 2003	–	AB221452
P. tiliae						
	Tilia japonica	TSH-R12717	Tochigi	K. Sugimoto, 1963	AB221413	–
	T. mandshurica	TSH-R19878	Niigata	C. M. Tian ang M. Imasu, 2003	AB221412	AB221455
	T. japonica	TSH-R4295 (IBA7878)	Aomori	Y. Ono, 1997	AB221415	AB221454
	T. japonica	TSH-R4294 (IBA7670)	Miyazaki	Y. Ono, 1995	AB221414	AB221453
P. hydrangeae-petiolaridia						
	Hydrangea petiolaris	TSH-R4263 (IBA6660)	Kitaibaraki	K.Higuchi and K.Suganuma et al., 1992	AB221382	–
	H. petiolaris	TSH-R4264 (IBA7881)	Aomori	Y. Ono, 1997	AB221385	AB221439
	H. petiolaris	TSH-R4265 (IBA8377)	Gumma	Y. Ono and K. Ishimiya, 1999	AB221384	AB221438
	H. petiolaris	TSH-R4261 (IBA2367)	Yamanashi	Y. Ono, 1981	AB221383	–
P. circaeae						
	Circaea erubescens	TSH-R10187	Aomori	Y.Yamaoka and T.Kobayashi et al., 1997	AB221387	AB221456
	C. lutetiana[b]	RB2098		R. Bauer	AF 426227	–
P. epilobii						
	Epilobium cephalostigma	TSH-R4285 (IBA2253)	Nagano	Y. Ono, 1981	AB221386	–
	E. angustifolium[b]				AF 522179	–
P. pyrolae [b] (outgroup)						
	Pyrolae minor	HERB 4570		R. Berndt	AF 426233	–
P. goeppertianum [b, c] (outgroup)						
	Abies grandis (Aecial)	PgW-1			–	L 76508
	Abies grandis (Aecial)	PgW-2			–	L 76509

[a] TSH, Mycological Herbarium, University of Tsukuba, Japan; IBA, Herbarium of Systematic Mycology, Ibaraki University, Japan; HH, Hiratsuka Herbarium, Tokyo, Japan

[b] From GenBank database

[c] According to the Hiratsuka (1958), and Cummis and Hiratsuka (2003), this species is described as *Calyptospora goeppertiana*.

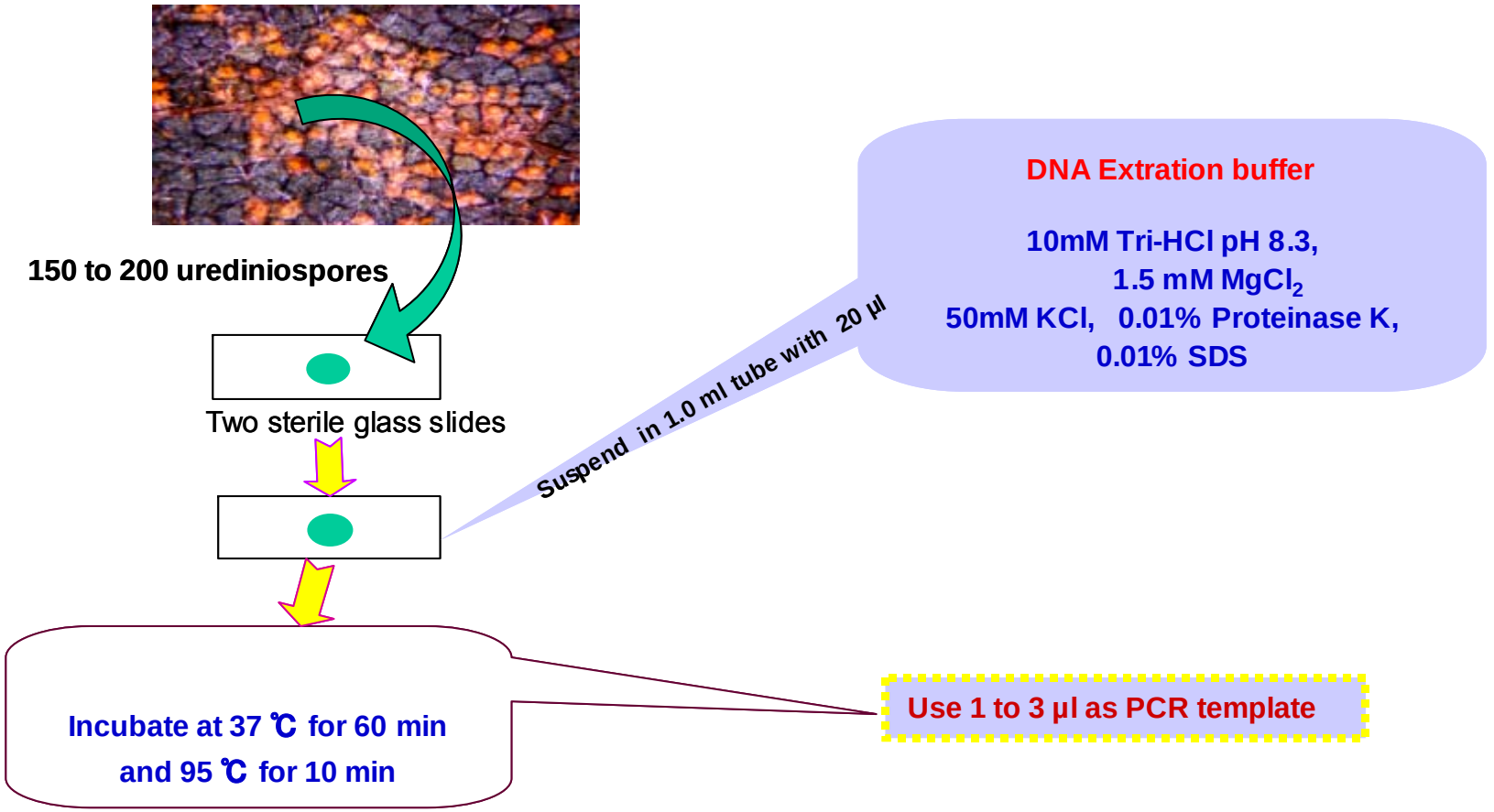
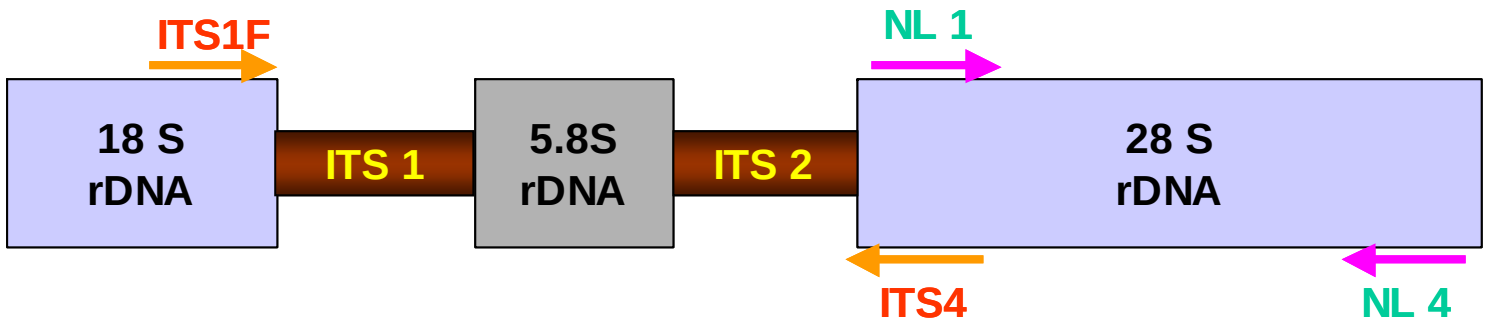


Fig. 2.1. DNA extraction method from urediniospores.



D1/D2 regions:

NL1 5'—GCATATCAATAAGCGGAGGAAAAG—3'

NL4 5'—GGTCCGTGTTTCAAGACGG—3' (O'Donnell, 1993)

ITS regions:

ITS1F 5'—CTTGGTCATTTAGAGGAAGTAA—3' (Gardes and Bruns, 1993)

ITS4 5'—TCCTCCGCTTATTGATATGC—3' (White et al., 1990)

Fig. 2.2. Diagram of a portion of the rDNA unit repeat of 28S (D1/D2) and ITS regions. D1/D2 region is amplified and sequenced by the primer pair of NL1 and NL4. ITS region is amplified and sequenced by the primer pair of ITS1F and ITS4.

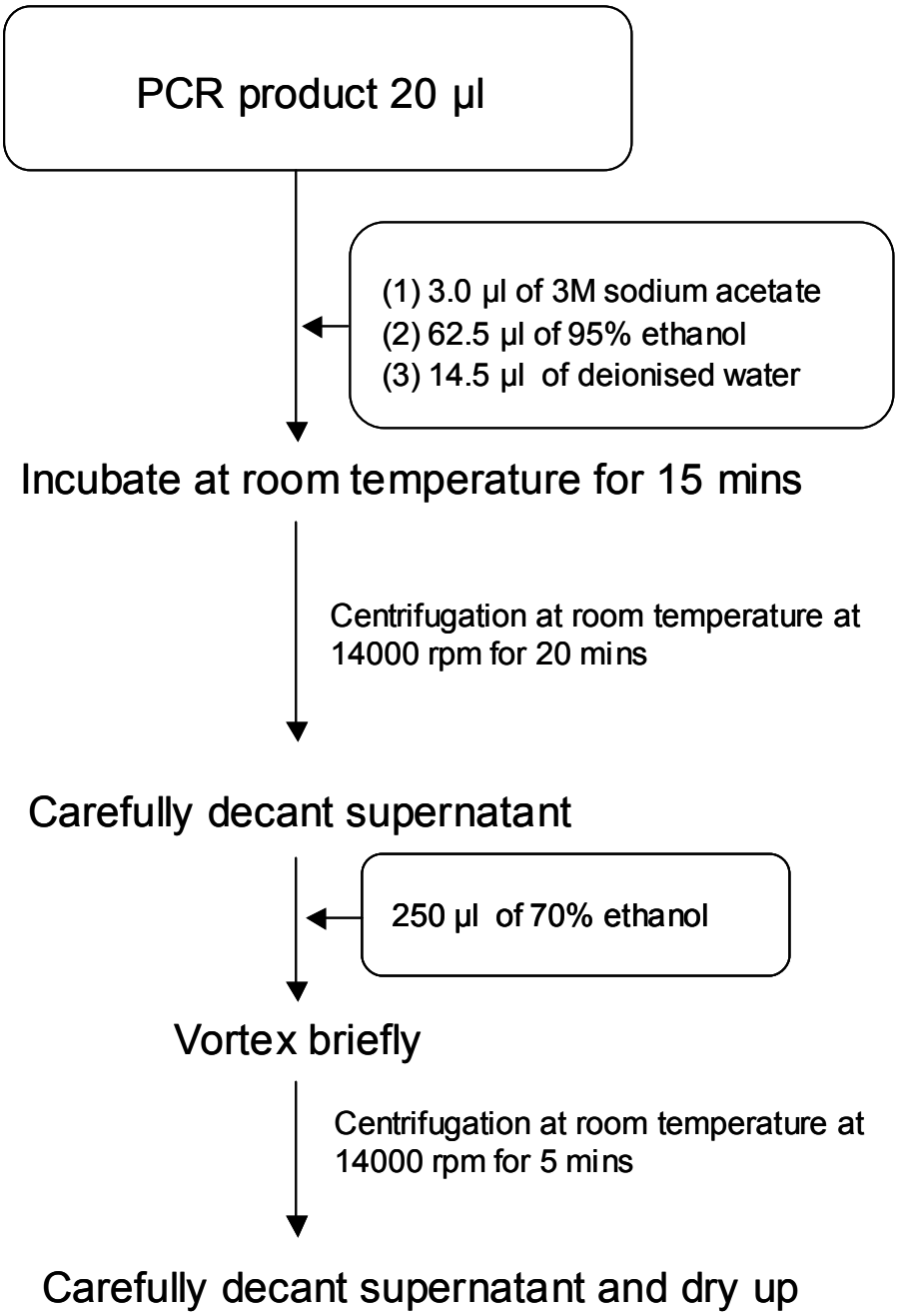


Fig. 2.3. The procedure of ethanol precipitation.

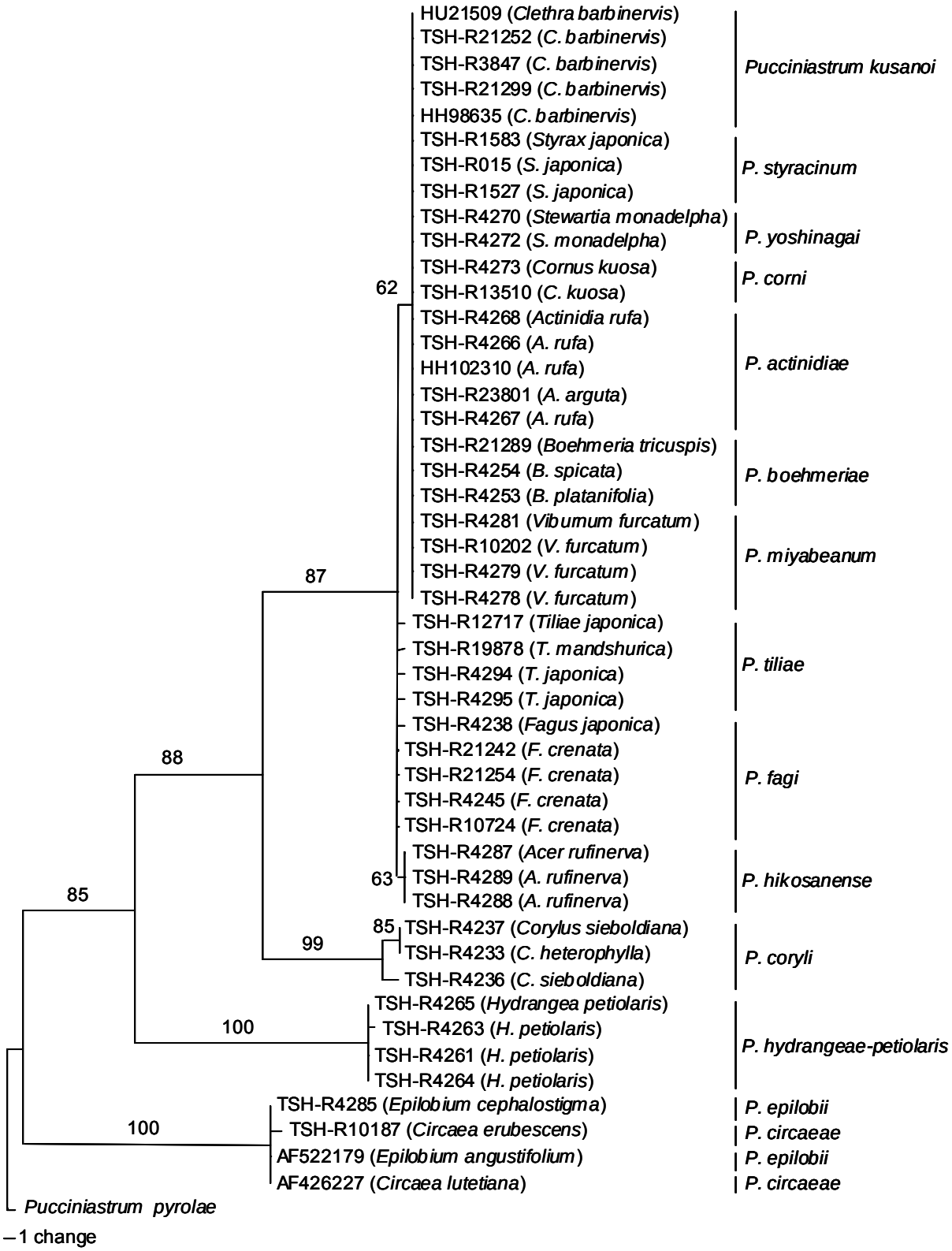


Fig. 2. 4. A maximum parsimony tree inferred from sequences of LSU rDNA (D1/D2) regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches.

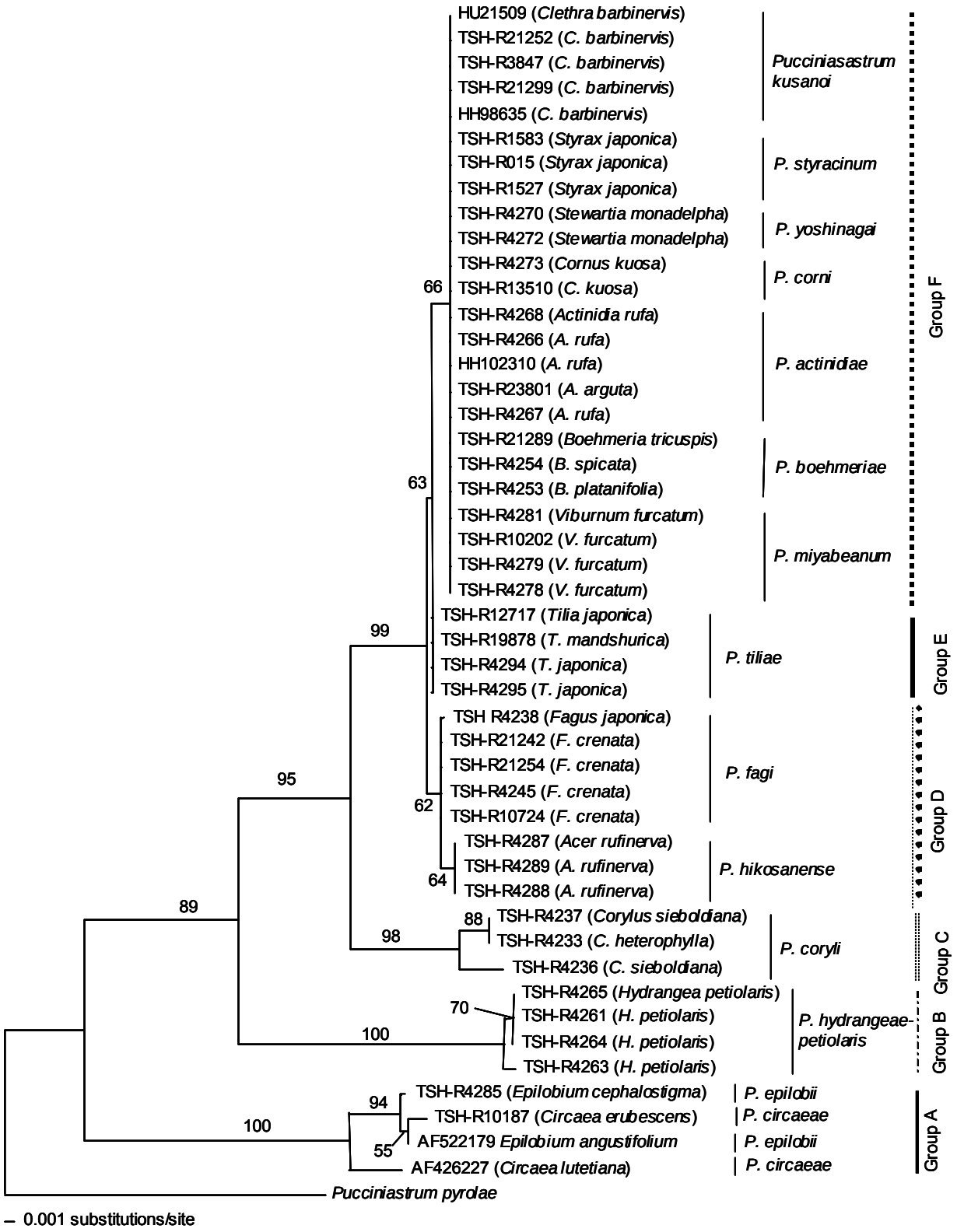


Fig. 2.5. A neighbor-joining tree inferred from sequences of D1/D2 regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches. Length of branches is proportional to number of base changes, indicated by the scale bottom.

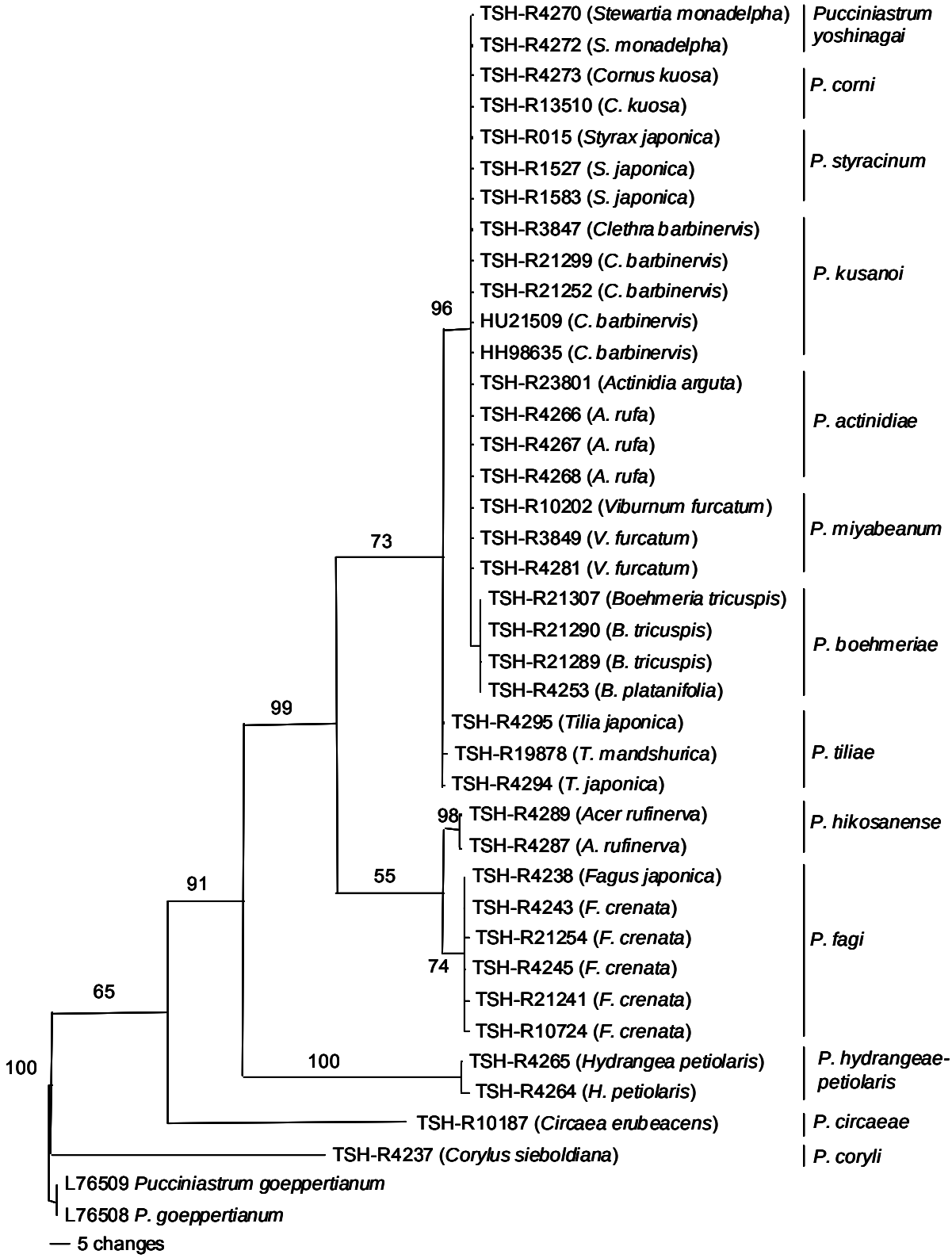


Fig. 2.6. A maximum parsimony tree inferred from sequences of ITS regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches.

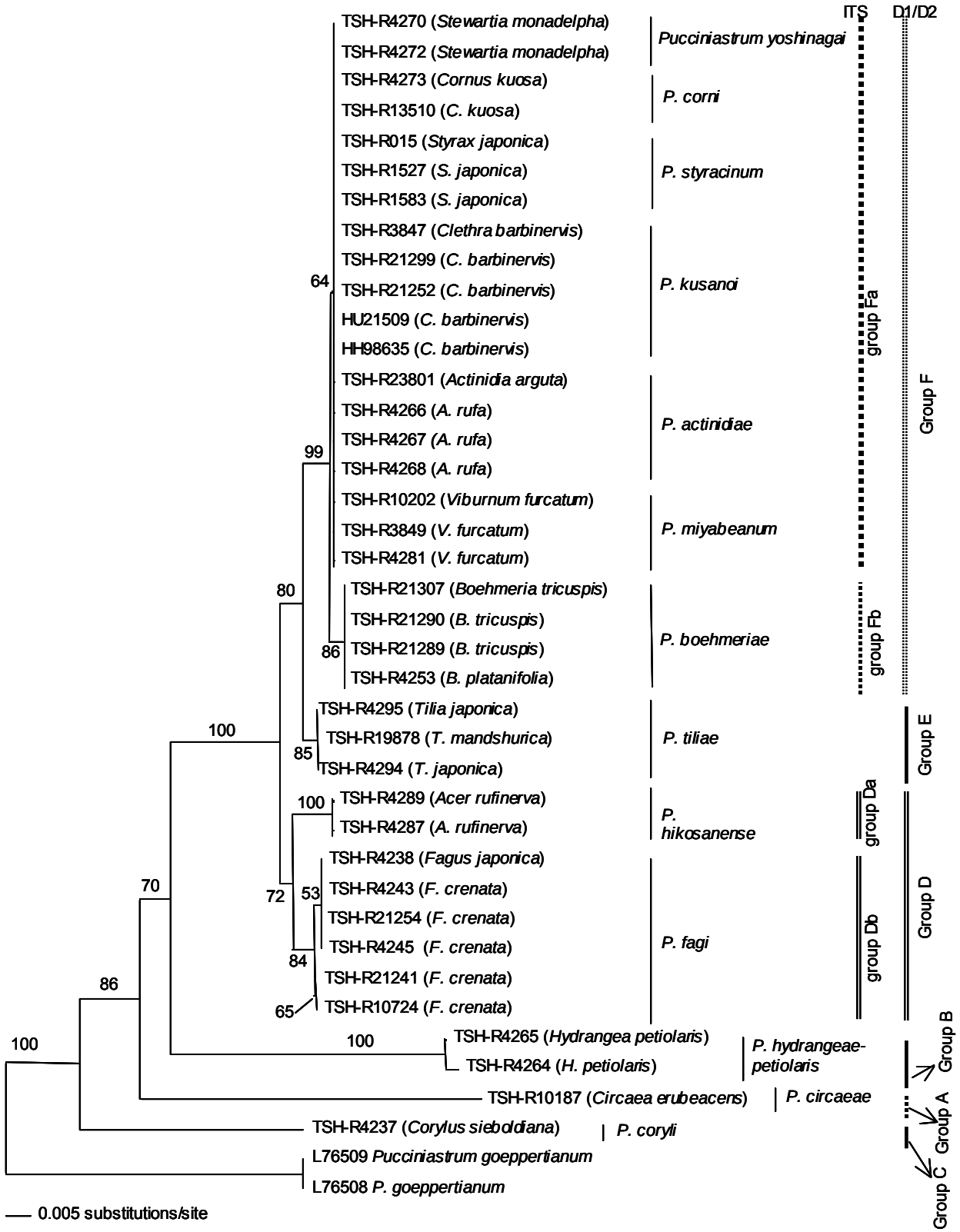


Fig. 2. 7. A neighbor-joining tree inferred from sequences of ITS and 5.8s regions using Clustal X. Bootstrap values above 50% from 1000 replicates are indicated for the corresponding branches. Length of branches is proportional to number of base changes, indicated by the scale bottom.

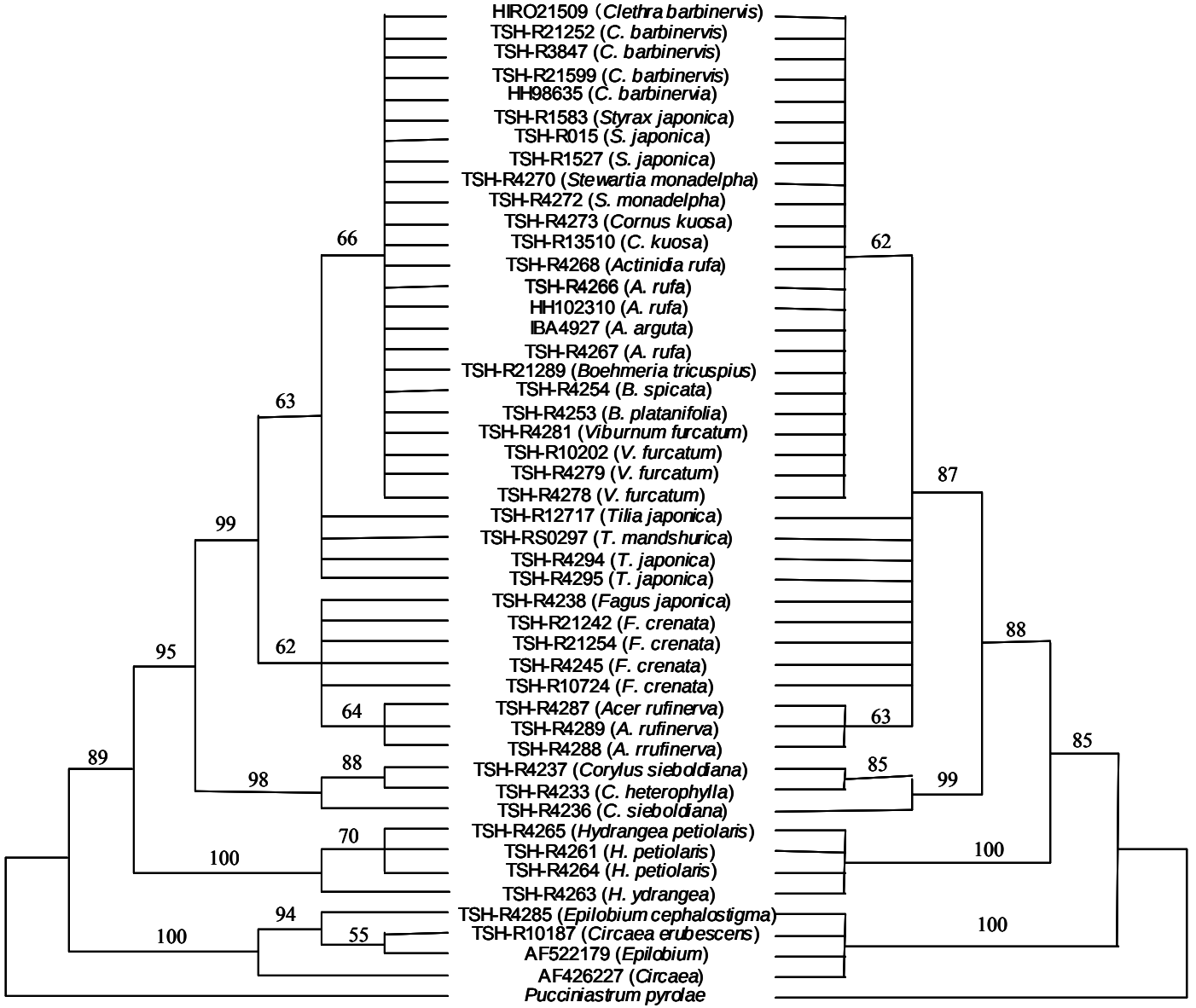


Fig. 2.8. Comparison of strict consensus trees from neighbor-joining (left) and maximum parsimony (right) analysis based on the sequences of LSU rDNA (D1/D2) region of 14 *Pucciniastrum* species.

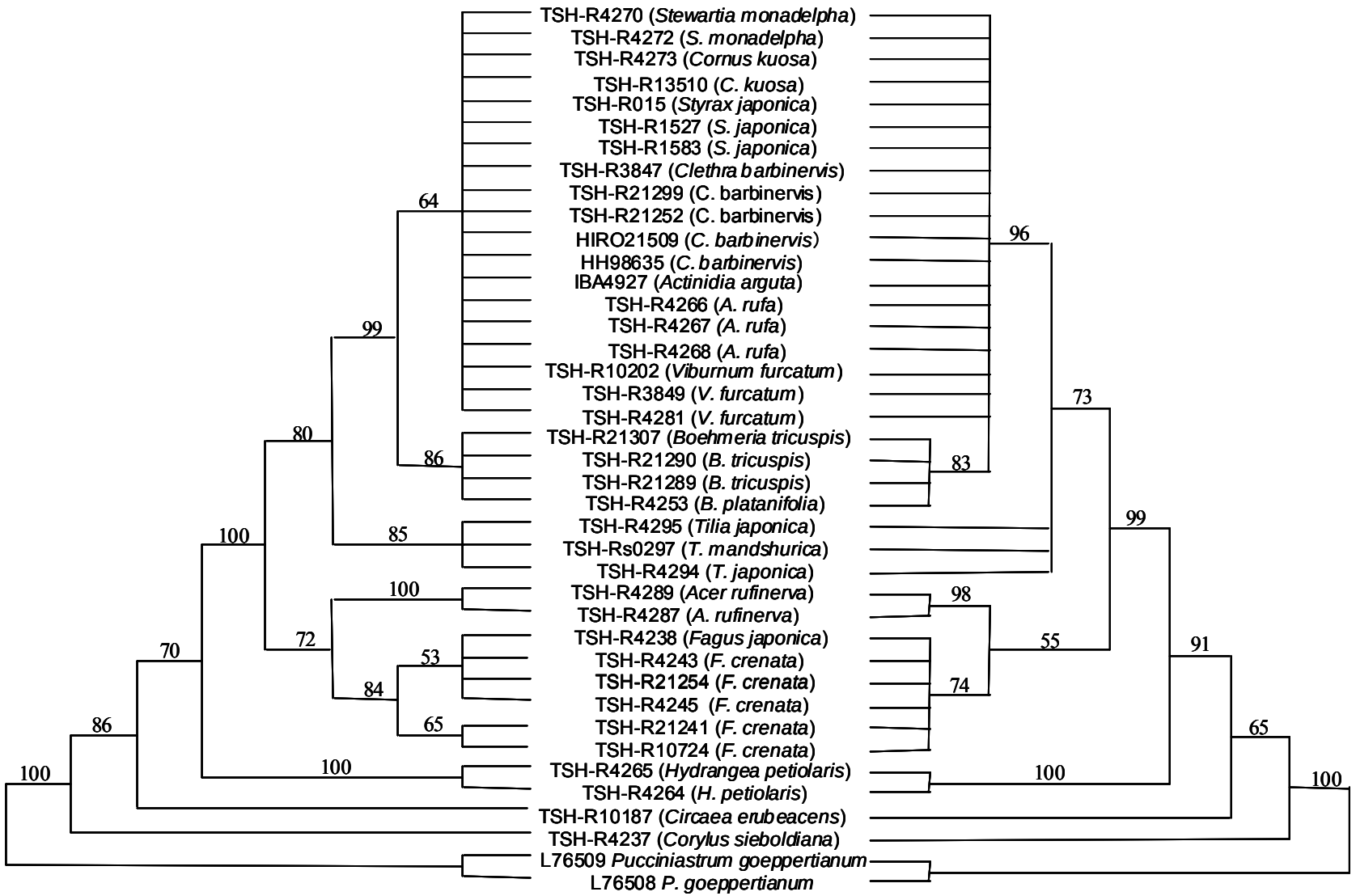


Fig. 2.9. Comparison of strict consensus trees from neighbor-joining (left) and maximum parsimony (right) analysis based on the sequences of ITS region of some *Pucciniastrum* species.

Table 2.2. Relationship between phylogenetic groups and host plants of *Pucciniastrum* species.

Phylogenetic group		Rust species	Host plant		
D1/D2	ITS		Genus	Family	Order
A	A	*Pucciniastrum epilobii*	*Epilobium*	Onagraceae	Myrtales
		P. circaeae	*Circaea*		
B	B	*P. hydrangeae-petiolaris*	*Hydrangea*	Saxifragaceae	Hamamelidales
C	C	*P. coryli*	*Corylus*	Betulaceae	Fagales
D	Da	*P. hikosanense*	*Acer*	Aceraceae	Sapindales
	Db	*P. fagi*	*Fagus*	Fagaceae	Fagales
E	E	*P. tiliae*	*Tilia*	Tiliaceae	Malvales
F	Fa	*P. boehmeriae*	*Boehmeria*	Urticaceae	Urticales
	Fb	*P. miyabeanum*	*Viburnum*	Caprifoliaceae	Rubiales
		P. corni	*Cornus*	Cornaceae	Apiales
		P. styracinum	*Styrax*	Styracaceae	Ebenales
		P. kusanoi	*Clethra*	Clethraceae	Ericales
		P. yoshinagai	*Stewartia*	Theaceae	Hypericales
		P. actinidiae	*Actinidia*	Actinidiaceae	Hypericales

3. Morphological examination

According to the previous morphological description, identifying the 16 species of *Pucciniastrum* group III is often difficult. The uredinia and telia provide few information for distinguishing these species. All species of *Pucciniastrum* group III except for *P. hikosanense* have similar shape and size of urediniospores, even though they are parasitic on different hosts. Because the teliospores are multicellular and are jointed laterally adherents, it is very difficult to measure the size of the teliospores accurately. Although the position and number of urediniospore germ pores are considered as useful and important taxonomic characteristics at the species level in many rust fungi (Cummins 1936; Wilson and Henderson 1966; Cummins and Hiratsuka 1983; Kaneko and Hiratsuka 1982; Jennings et al. 1989), these features are indistinct in the species of *Pucciniastrum* and are not easily observed (Cummins and Hiratsuka 1983, 2003). Because of the above reasons, the morphological circumscription of these species does not seem clear, and the taxonomic identity of each species is sometimes doubtful. Hence, careful microscopic examination is important and necessary to elucidate morphological differences within these species.

Molecular phylogenetic analysis on 14 species in *Pucciniastrum* group III (two other species of group III were not analyzed because specimens were not available) revealed that these species could be separated into six phylogenetic groups based on the sequences of the D1/D2 region of the LSU rDNA, but that

ITS and 5.8S rDNA regions clearly separated into eight phylogenetic groups.

Consequently, morphological characteristics in the uredinial stages were reexamined to compare with the results of phylogenetic analysis.

3.1 Materials and methods

Materials

Dried herbarium specimens were used for morphological observations (Table 3.1). Specimens were also freshly collected from the following prefectures in Japan: Nagano, Aomori, Akita, Fukushima, and Ibaraki. Most herbarium specimens examined were loaned from the following institutions: the Hiratsuka Herbarium, Tokyo, Japan (HH); the Herbarium of Systematic Mycology, the College of Education, Ibaraki University, Mito, Japan (IBA); the Mycological Herbarium of the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan (TSH); the Herbarium Hirosaki University (HU), Hirosaki, Japan; and the Forest Pathological Laboratory, Forestry and Forest Products Research Institute, Tsukuba, Japan. Among them, holotypes of some species were also included.

Morphological observation

A total of 570 specimens of 14 *Pucciniastrum* species (Appendix 1) were observed by light microscope (LM) and scanning electron microscope (SEM). In some cases, specimens were also examined with a dissecting microscope for

observation of sori (uredinia and telia). For LM observations, urediniospores obtained from the specimens or hand sections of uredinia and telia were mounted in a drop of lactophenol solution on a microscopic slide. For each specimen, fifty urediniospores were randomly chosen and observed for the selected morphological features listed in Fig. 3.1 with a BH2 microscope (Olympus, Tokyo, Japan). The urediniospores dimensions were measured by an Image Analyzer (Leica Q-Win, Tokyo, Japan), a software connected to an Olympus BH 2 microscope. The shapes of urediniospores were categorized according to Stearn (1995). LM images were made by digitizing 35-mm color slides and converted them to black and white. Adobe photoshop 5.0 was used to adjust image contrast and to compose the plates.

The surface structure of ostiolar peridial cells in the uredinia and the surface feature of urediniospores were observed by SEM. For SEM, urediniospores were attached on aluminum stubs covered with double-sided adhesive tapes, and then coated with platinum-palladium at 25 nm thick by a Hitachi E-1030 Ion Sputter Coater. The coated specimens were observed under a Hitachi S-4200 scanning electron microscope (Hitachi, Tokyo, Japan) operated at 15 kV. SEM images were captured using Quartz PCI software, ver. 4.0.

For observing the position of the germ pores in urediniospores, the urediniospores on glass slides were stained with cotton blue in lactophenol and heated to the smoking point, and then a coverslip was placed while it was still warm. The coverslip was pressed hard on to the slide until the spore contents

were expelled and the spore walls were in one plane of focus. The stained spores were observed by a bright-field or Nomarski differential interference contrast microscopy (Kaneko and Hiratsuka, 1982; Jennings et al. 1989). The distribution pattern of germ pores was categorized according to Cummins and Hiratsuka (1983).

Statistical analyses

Four morphological characteristics of urediniospores were measured under a Leica Q-win image Analyzer and subjected to analyses. From a specimen, 50 spores were randomly chosen and each character was examined. Statistical analyses including multivariate analyses of measured continuous numerical variables was performed using the software package Systat^{TM} version 5.2 (Wilkinson, 1989) running 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 analysis to detect possible groups.

The histogram was presented using the mean of all specimens of each species by the software package Igor Pro. 4.02A. It was especially useful for observing the frequency distributions (presenting distributions) of continuous variables in each morphological characteristic, from examining urediniospore.

Table 3.1 Specimens of 14 morphologically similar species of *Pucciniastrum* examined for morphological characteristics.

Species	Host plants	Locality in Japan(no. of specimens)
Pucciniastrum fagi	*Fagus crenata*	Fukushima (26), Yamagata (4), Kanagawa (7), Yamaguchi (1), Yamanashi (7), Kagoshima (4), Aomori (7), Miyazaki (2), Fukuoka (1), Gifu (1), Niigata (4), Nagano (2), Gunma (2), Toyama (2), Saitama (2), Tochigi (6), Ibaraki (11), Chiba(13), Shizuoka (1), Hyogo (1), Ehime (2), Tottori (4), Nara (1), Iwate (3), Akita (10)
	F. japonica	Niigata (2), Saitama (3), Chiba (8)
P. kusanoi	*Clethra barbinervis*	Kagoshima (3), Shizuoka (5), Yamanashi (6), Kanagawa (1), Fukushima (16), Miyazaki (2), Ishikawa (2), Ibaraki (12), Nara (1), Tokyo (2), Aomori (2), Miyagi (3), Akita (2), Chiba (2), Nagano (10), Tochigi (6), Toyama (3), Fukuoka (3), Tottori (19), Kochi (2), Hyogo (6), Ehime (1), Ooita (1),
P. coryli	*Corylus sieboldiana*	Yamanashi (8), Fukushima (7), Saitama (2), Hyogo (1), Kanagawa (1), Fukuoka (1), Tochigi (1), Nagano (2), Yamagata (2), Tottori (2), Gunma (2), Niigata (1),
	C. rostrata	Tottori (2), Shimane (1), Hokaido (3)
	C. avellana	Tokyo (5)
	C. heterophyll	Yamanashi (1), Hirosaki (1)
	C. sp	Hiroshima (1)
P. boehmeriae	*Boehmeria spicata*	Ibaraki (1), Tochigi (5), Kagoshima (2), Fukushima (1), Tottori (3), Kanagawa (1), Fukui (1), Saitama (1), Okayama (1), Yamagata (1), Yamanashi (1),
	B. tricuspis	Tochigi (2), Aomori (2), Miyagi (3)
	B. platanifolia	Yamanashi (1), Tokyo (1), Niigata (1)
	B. holosericea	Hyogo (1), Chiba (1)
	B. longispica	Ibaraki (2), Kagoshima (1)
	B. biloba	Tokyo (1), Chiba (1)
	B. grandifolia	Ibaraki (1)
	B. splitzgerberas	Chiba (1)
	B. sp	Kumamoto (1)
P. actinidiae	*Actinidia rufa*	Okinawa (20)
	A. arguta	Kochi (Holotype1), Okinawa (1), Miyazaki (1)
	A. hypoleuca	Okinawa (1)
P. styracinum	*Styrax japonica*	Ibaraki (1), Kagoshima (2), Okinawa (1), Fukushima (1), Toyama (2), Niigata (1)
	S. obassia	Miyagi (7), Iwate(6), Hokkaidi (7), Aomori (2), Akita (4), Yamagata (1), hou gangshi (2)
P. hikosanense	*Acer rufinerva*	Yamanashi (3), Shizuoka (1), Buzen (5), Hyogo (1), Tochigi (2), Nagano (2), Kozuke (2), Iwashiro (2)
	A. insulare	Ryukyus (7), Okinawa (6)

Table 3.1. (Continued)

Species	Host plants	Locality in Japan(no. of specimens)
P. circaeae	*Circaea alpina*	Kuriles (1), Shinano (1), Shimotsuke (1), Yamanashi (2), Saitama (2), Hokkaido (5),
	C. erubescens	Hyogo (1), Gifu (1), Tottori (1), Kanagawa (1), Aomori (1), H31724 (1), Ibaraki (1)
	C. mollis	Chikuzen (2), Sado isl (1)
	C. quadrisulcata	Tottori (1)
	C. cardiophylla	Hokkaido (1)
	C. sp	Gunma (1), Tokyo (1)
P. epilobii	*Epilobium pyricholophum*	Yamanashi (2), Tochigi (2), Chiba (1), Hokkaido (2), Fukushima (1)
	E. angustifolium	Hokkaido (3), Saitama (1), Kushiro (1), Nagano (4),
	E. cephalostigma	Shinano (1), Nagano (1), Tochigi (1)
	E. amurense	Yamanashi (1)
	E. sp	Tochigi (1), Shinano (1)
P. hydrangeae-petiolaris	*Hydrangea petiolaris*	Fukushima (1), Nagano (1), Tochigi (6), Akita (1), Yamanashi (1), Ibaraki (2), Aomori (1), Gunma (2), Niigata (1), Tottori (1)
P. tiliae	*Tilia maximowicziana*	Niigata (3),
	T. japonica	Miyazaki (1), Aomori (1), Gunma (1), Hokkaido (8), Tochigi (1)
	T. mandshurica	Ibaraki (1)
P. yoshinagai	*Stewartia pseudo-camellia*	Tottori (2), Tosa (1), Iyo (2), Yamaguchi (1), Yamanashi (1)
	S. monadelpha	Nara (2)
	S. serrata	Hyogo (1),
P. corni	*Cynoxylon japonica*	Kanagawa (7), Yamanashi (2), Tottori (5), Hyogo (1), Fukushima (2), Fukuoka (1), Niigata (1), Kai (2)
	Cornus kousa	Miyazaki (1), Fukushima (1), Tottori (1), Tosa (1), Fukui (1), Rikuchu (1), Hyogo (1)
	C. florida	Tottori (1)
	C. brachypoda	Kiuga (1)
P. miyabeanum	*Viburnum furcatum*	Hokkaido (7), Iburi (1), Ishikari (5), Nagano (4), Mutsu (2), Toyama (1), Aomori (3), Tochigi (4), Saitama (3), Hoki (2), Fukushima (5), Hyogo (2), Kozuke (1), Kanagawa (2), Gifu (1), Miyazaki (1), Yamagata (2), Akita (2), Shizuoka (1), Tottori (1), Gunma (1)

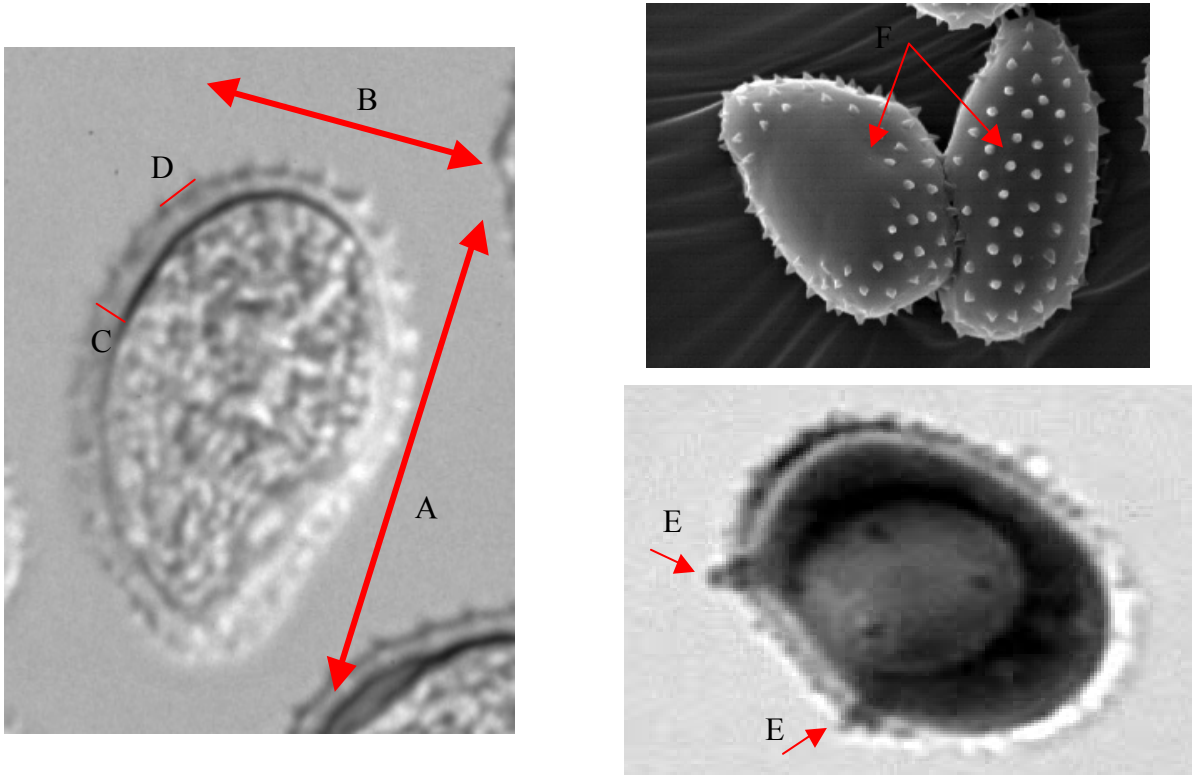


Fig. 3.1. Morphological characteristics of urediniospores observed in the present study. A. urediniospore length, B. urediniospore width, C. urediniospore wall thickness, D. distance between spines, E. position of urediniospore germ pores, F. urediniospores surface structure (SEM).

3.2 Results and discussion

Ostiolar peridial cells

Concerning the ostiolar peridial cell in the uredinia, Moss (1926) stated that at an early stage, the central peridial cells represent the sign of differentiation, compared with the adjoining cells, and that the central cells are not at all vacuolated, having nuclei and cytoplasm which stain deeply, as well as slightly thickened walls. Eventually these cells become separated from one another and encircle the narrow pore of the mature peridium.

Ostiolar peridial cells in the uredinia of these 14 species were examined, and the morphological characteristics of the ostiolar cells appeared to be very similar (uniform) within all specimens of a single species.

The 14 species could be classified into the following three types based on the developmental stage of the central peridial cell (Fig. 3.2 A-C). The first type of ostiolar peridial cells showed that the central peridial cells did not differentiate, in other words, ostiolar peridial cells in the uredinia are rudimentary or entirely lack ostiolar cells (Fig. 3.2. A). The second type of ostiolar peridial cells is characterized by well developing ostiolar cells. It is obvious, the ostiolar cells have been markedly differentiated, have very thickened wall with smooth (Fig. 3.2 B). These observations on the ostiolar cells are in accordance with the original description by Moss (1926). In contrast, the third type of ostiolar peridial cells showed that the central peridial cells are becoming differentiated only slightly with no markedly morphological change, in other words, the state of

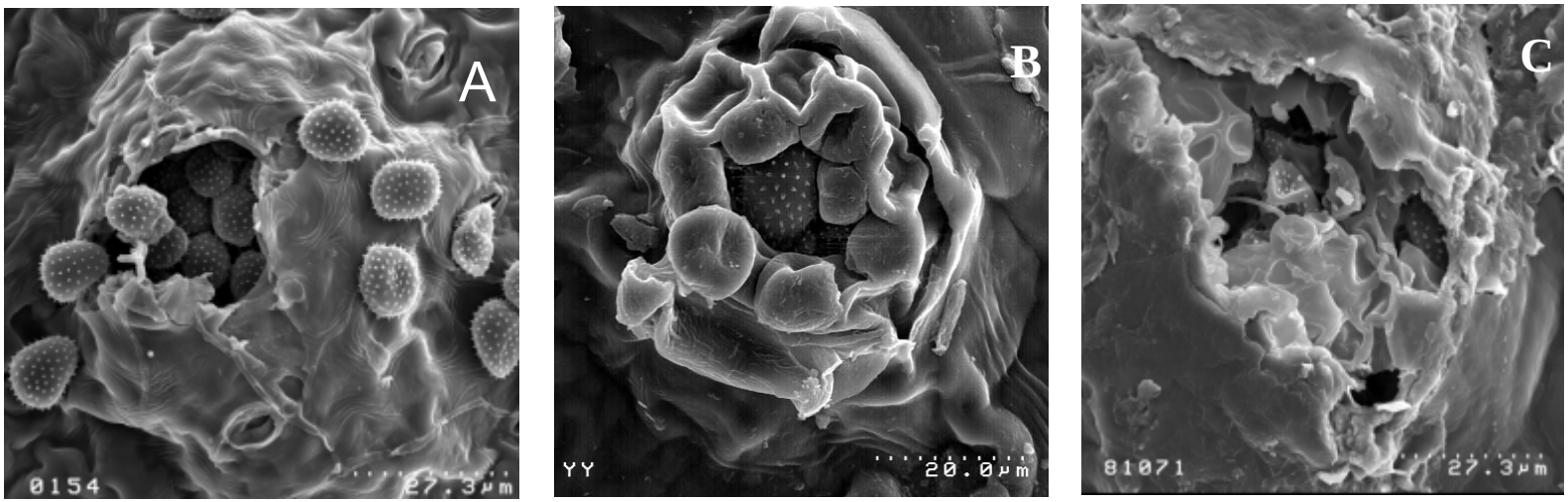


Fig. 3.2. Three types of ostiolar peridial cell based on their development degree. A: ostiolar cell absent; B: well developed; C: the development is in preliminary stages.

development is in a preliminary stage (Fig. 3.2 C).

Species (*P. epilobii* and *P. circaeae*) in both ITS and D1/D2 group A have the first type of ostiolar peridial cells (Fig. 3.3 I-j). Although the species *P. epilobii* and *P. circaeae* were described that they have well-developed ostiolar cells in previous taxonomic studies (Hiratsuka 1936, 1958, Hiratsuka et al. 1992, Kuprevich and Tranzschel 1957, Wilson and Henderson 1966), the present results based on SEM and light microscopy revealed that the specimens of *P. epilobii* on *Epilobium cephalostigma* and *E. angustifoliumand*, *P. circaeae* on *Circaea alpina*, *C. erubescens* and on *C. mollis* have no ostiolar cells. To confirm the morphological characteristic of ostiolar cells, specimens of the two species from Russia were also examined, likewise, those specimens have no ostiolar cells in uredinia (data, unpublished).

On the other hand, all species in ITS groups B, C, E, Db, Fa, Fb have the second type of ostiolar peridial cells (Fig. 3.3 d, f, g). The results of morphological examination of these species are identical with the previous descriptions (Hiratsuka et al. 1992). The well-developed ostiolar cells can easily be observed in all species, which belong to this type, by SEM and light microscopy. In contrast, only one species in ITS group Da, which is monophyletic by 100% bootstrap value support in ITS analysis, has the third type of ostiolar peridial cells (Fig. 3.3 a, c).

Every phylogenetic group contained one type of ostiolar peridial cells. From these results it was considered that characteristics of ostiolar peridial cells could

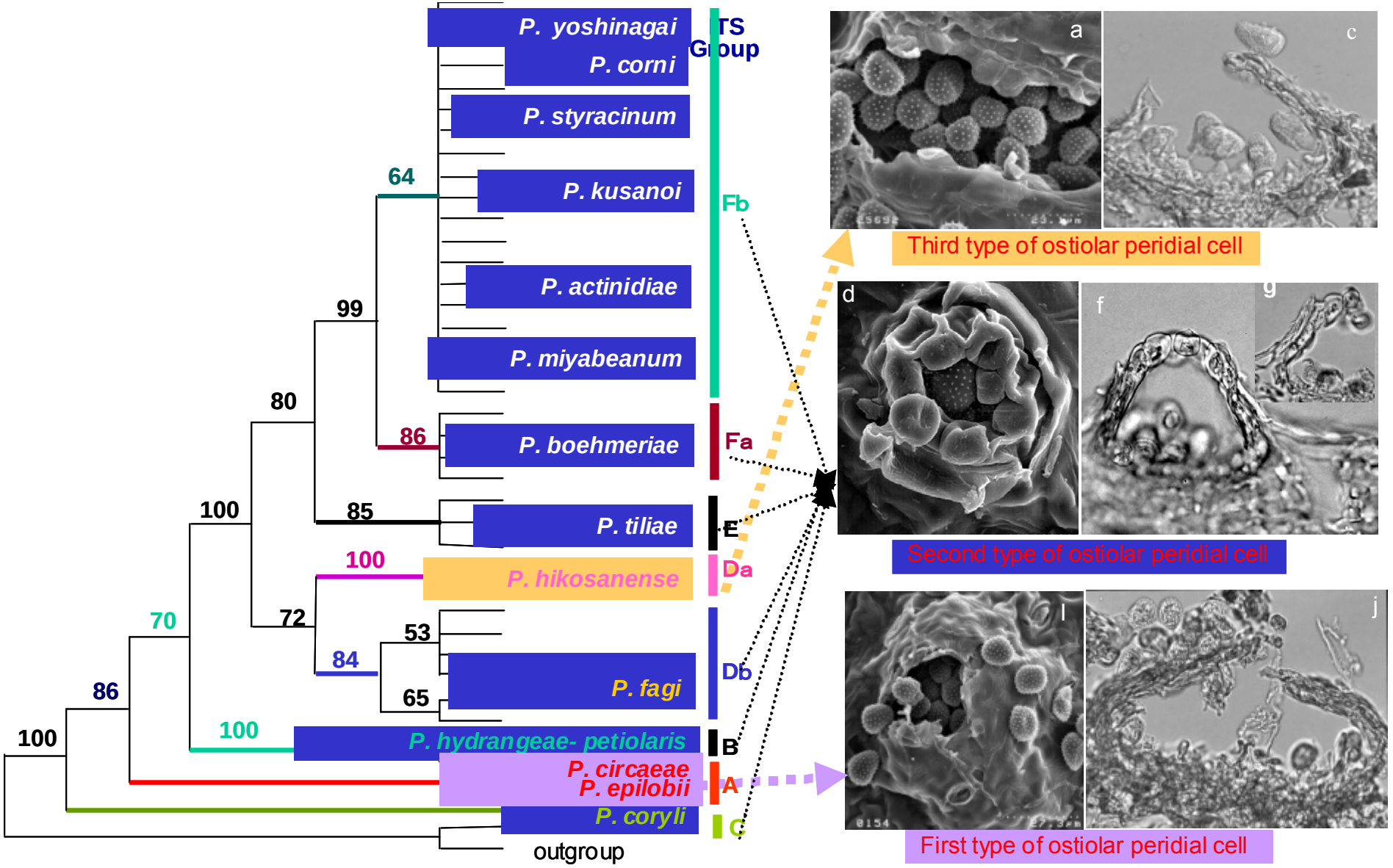


Fig. 3.3. The relationship of phylogenetic group and ostiolar peridial cells.

reflected the phylogeny.

Urediniospore-surface characteristics

Observations under SEM showed that urediniospores-surface structures of the specimens of 14 species examined could also be divided into the following three types, which were based on the urediniospore surface whether there is smooth area as well as smooth area size (Fig. 3.4). The first type is characterized by small smooth area on the urediniospore-surface. In this type, about 80% urediniospores commonly have a small smooth area, but occasionally, a few urediniospores were uniformly echinulate within the same uredinium (Fig. 3.4 e-f). Only this type was found in species *P. epilobii* and *P. circaeae* belonging to the D1/D2 and ITS group A.

Previous studies did not mention the presence of a smooth area on the urediniospore-surface of *P. epilobii* and *P. circaeae* (Arthur 1934, Hiratsuka 1936, Hiratsuka et al. 1992, Kuprevich and Tranzschel 1957, Wilson and Henderson 1966). However, many herbarium specimens of *P. epilobii* and *P. circaeae* collected from Russia and deposited in TSH were also observed with LM and SEM. The results showed that a part of spores of all these specimens also have a small smooth area in the urediniospore wall-surface.

The second type is characterized by uniformly echinulated on the urediniospore-surface. This characteristic is stable within the species. All species of the ITS groups B, C, and E as well as highly supported monophyletic

ITS groups Db, Fa, and Fb correspond to this type (Fig. 3.4 c-d).

The third type is characterized by the lateral surface of urediniospores with large smooth area compared to those found in urediniospores of species of the first type, and the large smooth area was observed in all urediniospores. Only species *P. hikosanense* of ITS group Da was included in this type ((Fig. 3.4 a-b). Hiratsuka (1940) first reported *P. hikosanense* and described “cellulis ostiolaribus rotundatis, levibus; uredosporis oblongis, ellipsoideis vel obovatis, echiunulatis”. However, observation of the holotype (HH 103463) by SEM showed that the ostiolar cells were indistinct (Fig 3.3 a) and urediniospore surface clearly had smooth area (Fig. 3.4. b).

Comparison of the phylogenetic group and the urediniospore surface structure showed that every phylogenetic group contained one type of urediniospore surface structure. The result indicated that urediniospore surface characteristic could reflect the phylogeny. And urediniospore surface structure was found to be an important morphological characteristic in *Pucciniastrum* to distinguish some species, although the characteristic was missed out in previous studies.in addition, the distance between spines of urediniospore surface was also examined. The result showed mean distance ranging from 1.20 to 1.43 μm, ranging mostly from 1.2 to1.3 μm. Although distance between spines somewhat varied within a spices and among species, all specimens of the 14 species examined did not exhibit any definite relationship with monophyletic group, except for the species *P. hydrangeae-petiolaris* which has somewhat longer

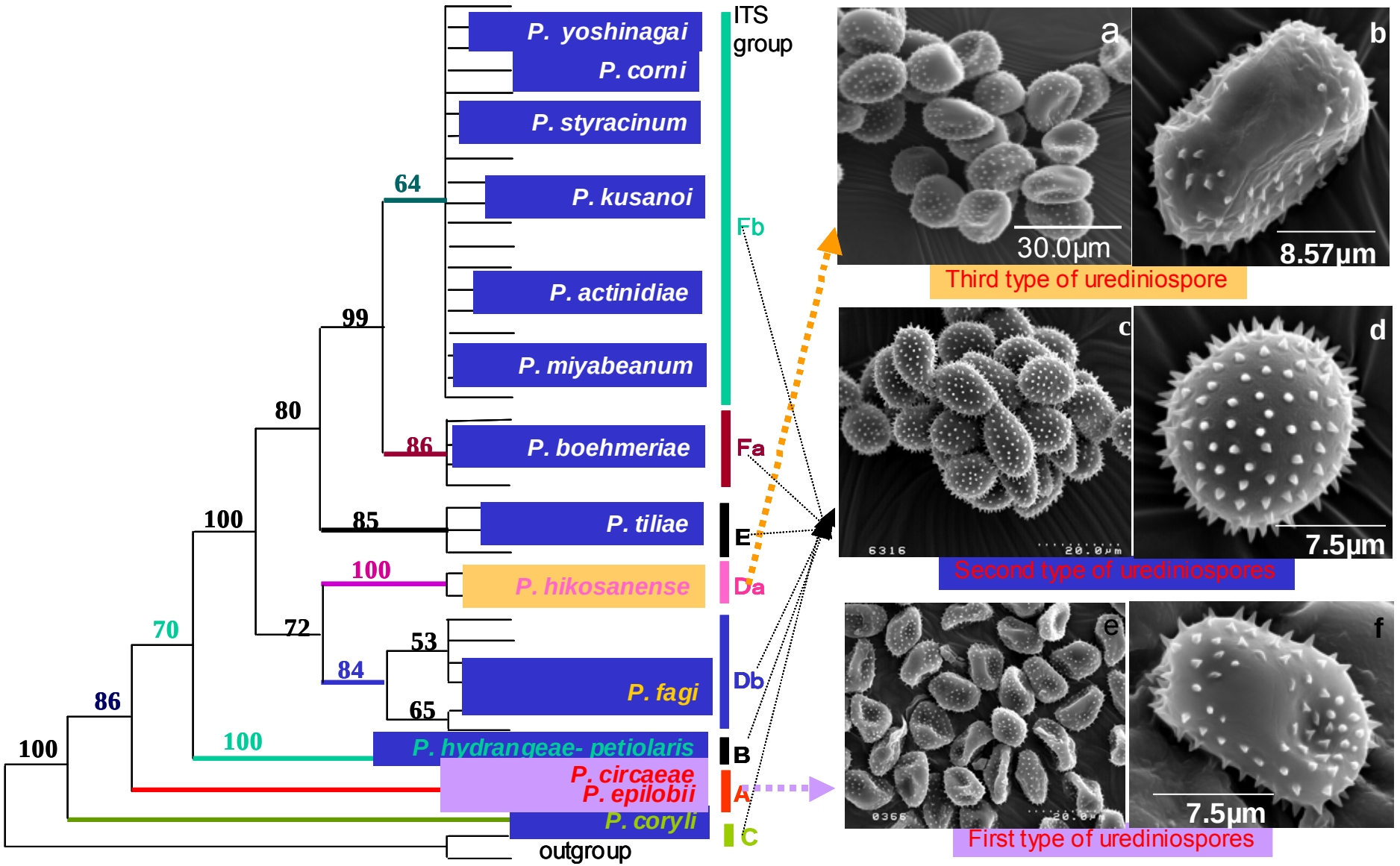


Fig. 3.4. The relationship of phylogenetic group and surface characteristics of urediniospores.

distance between spines (see Table 3.3 of page 64).

Germ pores of urediniospores

Visible germ-pores were found in the urediniospore of the species examined after staining and heating in cotton blue lactophenol. Result of observation showed the germ-pores were definite in position within a single species, although accurate germ-pore number was difficult to count in a certain species.

Based on the germ-pore position (Table 3.2), the specimens of the 14 species examined were divided into the following three types. Germ pores were scattered in the first type. Five (rarely 4) to 9 (rarely 10) germ-pores were scattered on the urediniospore wall (Fig. 3.5 A-C). This type of germ pore was observed in specimens of P. *epilobii* and *P. circaeae* which belonged to the group A, and specimens of *P. hydrangeae-petiolaris* which belonged to group B (Fig. 3.6 a). Kaneko and Hiratsuka (1982) first reported *P. epilobii* with scattered germ-pores, and it was consistent with the present study. *P. circaeae* was reported to have equatorial germ pores (Kaneko and Hiratsuka1982), however, the specimens of *P. circaeae* on *Circaea erubescens*, *C. alpina* and on *C. mollis* in this study showed scattered pores (Fig. 3.5-B).

The second type of germ pore was bizonate. Four to 8 germ pores in two fairly regular zones near the opposite ends of urediniospore were parallel to the equator (Fig.3.5 E-I). The bizonate type was observed in the specimens of two species (*P. yoshinagai* and *P. corni*) of ITS group Fb and *P. boehmeriae* of ITS

group Fa, and *P. fagi* of ITS group Db, and *P. coryli* of ITS group C (Fig. 3.6 c). Kaneko and Hiratsuka (1982) reported that *P. fagi*, *P. coryli* and *P. boehmeriae* had bizonate pores. The present results were identical with their report. The pores of *P. yoshinagai* and *P. corni* were first observed in the present study.

The third type had both bizonate and scattered germ pores in the same species. Urediniospores of certain species showed some variation in their germ pore position from the predominant type for their species, i.e. the arrangement of germ-pores was primary bizonate but sometimes scattered, or primary scattered but sometimes bizonate (Fig.3.5 J-O). The existence of such two types of pore arrangement is considered to be a specific characteristic (kaneko and Hiratsuka 1982). This case also applied to several species belonging to *Thekopsora*, *Melampsora*, *Cronartium* and *Phakopsora* (Kaneko and Hiratsuka 1982). In the present study, this type was observed in the specimens from four species (*P. styracinum, P. kusanoi, P. actinidiae*, and *P. miyabeanum*) of ITS group Fb and *P. tiliae* of group E, and *P. hikosanense* of ITS group Da (Fig. 3.6 b).

Comparison of phylogenetic group and germ pores arrangement of urediniospore indicated that two types of germ pore were intermingled within the same groups (Fig. 3.6 b). Therefore, the germ pore arrangement is considered to be the characteristic that does not reflect the phylogenetic group.

The pores of the urediniospores in the Pucciniastreae are usually considered to be absent or invisible. However, Moss (1926) studied the urediniospores of *Hyalopsora*, *Milesia*, *Pucciniastrum* and *Melampsorella*, and

found that germ pores were present and could be seen. He noticed, in *Pucciniastrum*, that prolonged heating was required to make them evident, and that the pores are quite minute and were considerably obscured by the projections of the echinulate spore. Hence it was difficult to determine their number and distribution with certainty, in many earlier reports, the position and number of urediniospore germ pores of *Pucciniastrum* were not described (Arthur 1934; Hiratsuka 1936, 1958; Ito 1938; Gaümann 1959; Wilson and Henderson 1966; Hawker and Madelin 1974).

Cummins (1936) studied the phylogenetic significance of the pores in urediniospores and indicated that there appeared to be some correlation between the arrangement of pores and the shape of urediniospores in the majority genera. The position and number of pores in the walls are reasonably constant for any given species and are useful characters for identification (Wilson and Henderson 1966). Kaneko and Hiratsuka (1982) observed the pores of a large number of species in the Pucciniastraceae and Melampsoraceae to conclude that the arrangement of germ pores in urediniospores is considered to be an important taxonomic characteristic at the specific level in the *Melampsoridium* and in other genera. Eleven *Pucciniastrum* species were included in their study, but they did not obtain clearly conclusion in *Pucciniastrum* species. Because several *Pucciniastrum* species exhibited variation in their pore arrangement.

Table 3.2. The arrangement of urediniospore germ pores in the 14 species of *Pucciniastrum*

Species	Host plants (no. of specimens)	Germ pores position
P. epilobii	*Epilobium cephalostigma* (2)	Scattered
	E. pyricholophum (2)	Scattered
	E. angustifolium (2)	Scattered
P. circaeae	*Circaea erubescens* (1)	Scattered
	C.alpina (1)	Scattered
	C. mollis (1)	Scattered
P. hydrangeae-petiolaris	*Hydrangea petiolaris* (3)	Scattered
P. fagi	*Fagus crenata* (2)	Bizonate
	F. japonica (1)	Bizonate
P. boehmeriae	*Boehmeria biloba* (1)	Bizonate
	B. spicata (2)	Bizonate
	B. tricuspis (1)	Bizonate
	B. platanifolia (1)	Bizonate
	B. longispica (1)	Bizonate
P. corni	*Cornus kuosa* (1)	Bizonate
	C. japonica (2)	Bizonate
P. yoshinagai	*Stewartia pseudo-camellia* (1)	Bizonate
	S. monadelpha (1)	Bizonate
P. coryli	*Corylus sieboldiana* (3)	Bizonate
	C. rostrata (2)	Bizonate
	C. heterophylla (2)	Bizonate
P. styracinum	*Styrax japonica* (3)	Bizonate (Scattered)
	S. obssia (1)	Bizonate (Scattered)
P. actinidiae	*Actinidia rufa* (1)	Bizonate (Scattered)
	A. arguta (2)	Bizonate (Scattered)
P. tiliae	*Tilia maximowicziana (*2)	Bizonate (Scattered)
	T. japonica (1)	Bizonate (Scattered)
P. kusanoi	*Clethra barbinervis* (3)	Scattered (Bizonate)
P. miyabeanum	*Vibuvnum furcatum* (5)	Scattered (Bizonate)
P. hikosanense	*Acer rufinerva* (3)	Bizonate (Scattered)

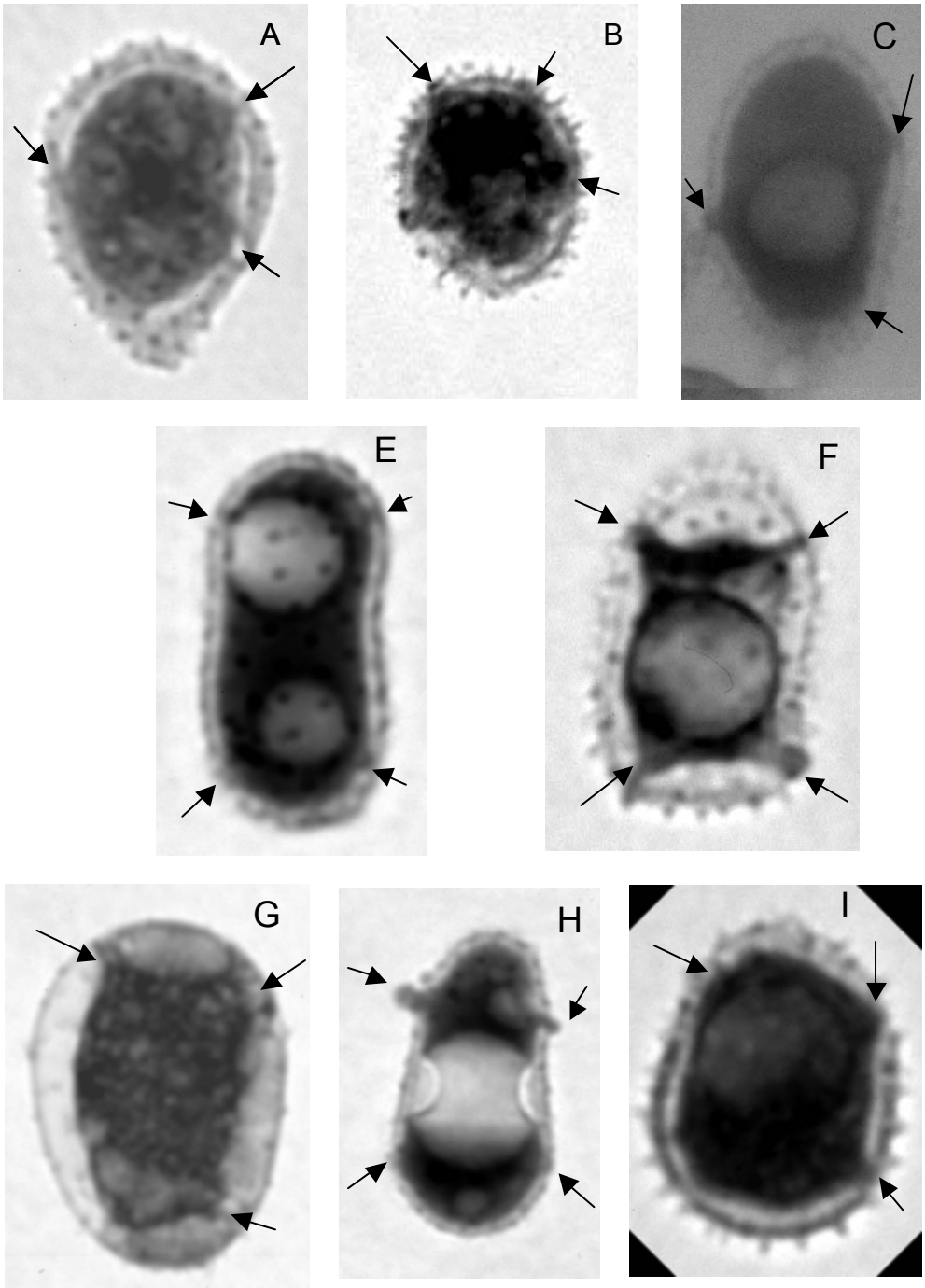


Fig. 3.5. Germ pore arrangement of urediniospores. A-C: scattered (A: *P. epilobii*; B: *P. circaeae*; C: *P. hydrangeae-petiolaris*); E-I: bizonate (E*: P. fagi*: F: *P. boehmeriae;* G: *P. corni*; H: *P. coryli*; I: *P. yoshinagai*).

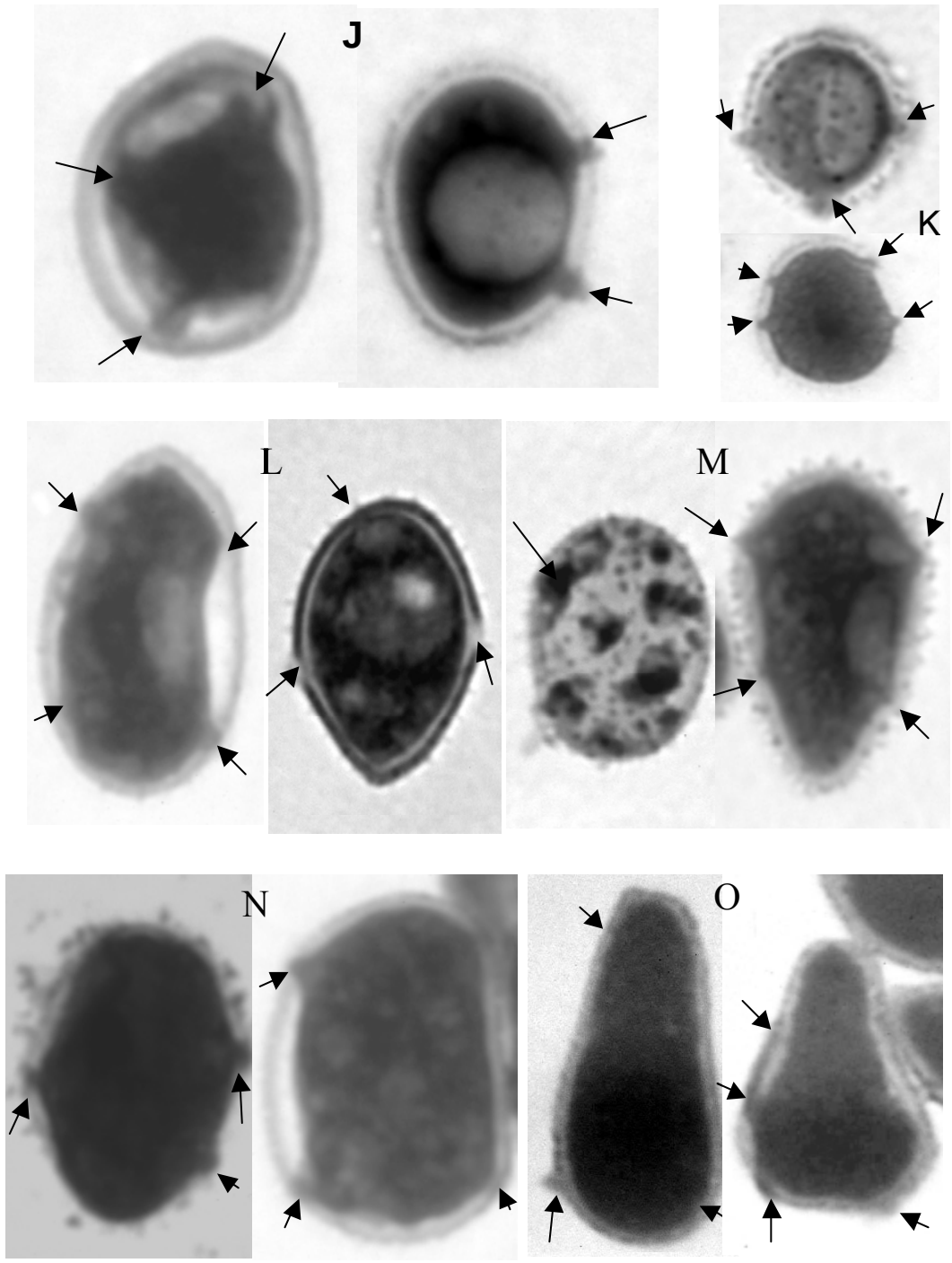


Fig. 3.5 (continued). Germ pore arrangement of urediniospores. J-O: biz. to sca. or sca. to biz. (J: *P. tiliae*; K: *P. kusanoi*; L: *P. styracinum*; M: *P. actinidiae*; N: *P. miyabeanum*; O: *P. hikosanense*).

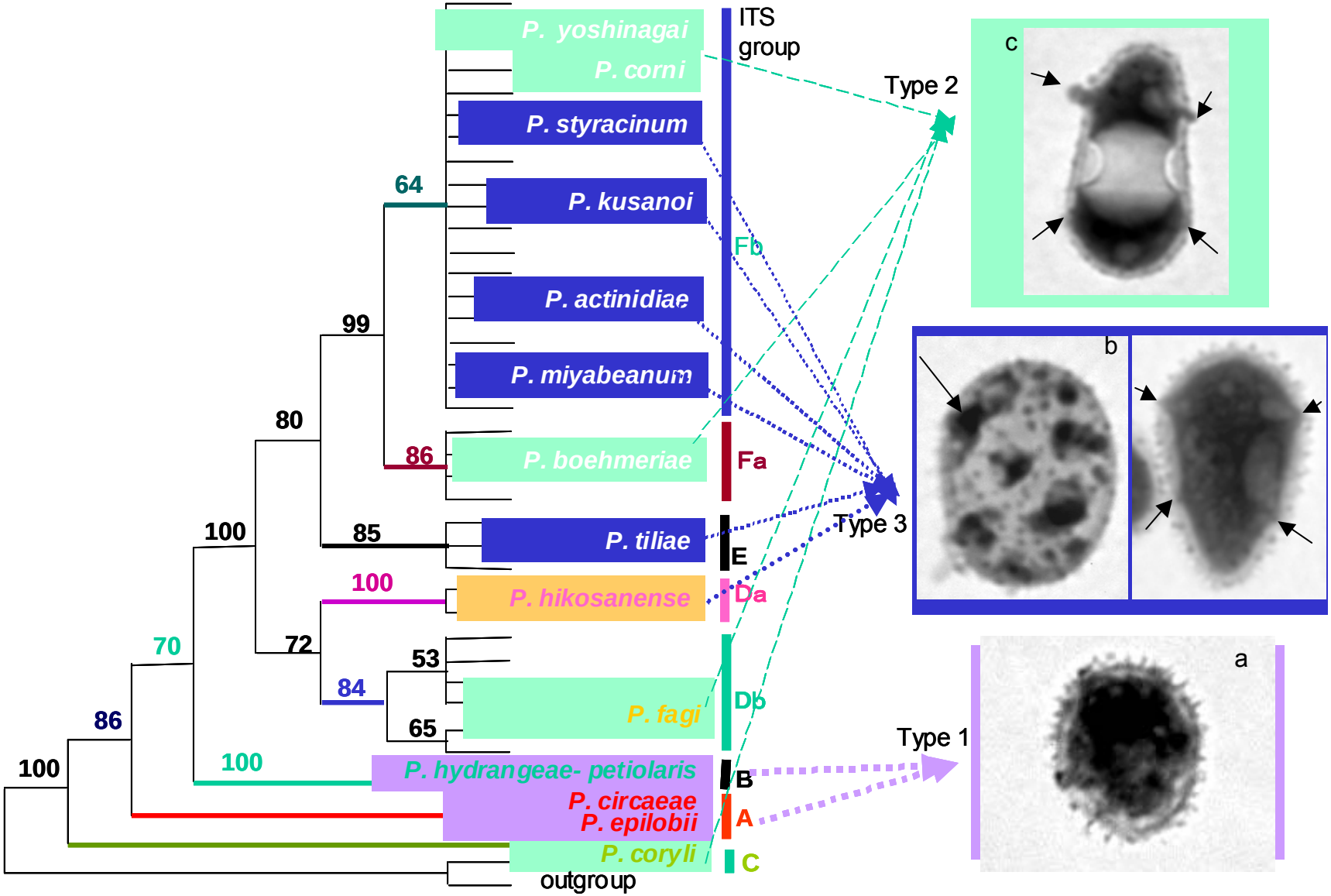


Fig. 3.6. The relationship of phylogenetic group and germ pore arrangement of urediniospores. a: Germ pores scattered; b: germ pores scattered or bizonate; c: germ pores bizonate.

Shape, size, and wall thickness of urediniospores

Urediniospores were formed singly on a short pedicel, wall colorless, mostly ellipsoid, oblong or subglobose, ovate to obovate, sometimes pyriform or broadly ellipsoidal, occasionally clavate. All species observation showed great variation in urediniospore shape, which was observed within the same uredinium and among species (Fig. 3.7). Specimens of the 14 species examined could not be separated into any group based on the characteristics of shape. Therefore, it was considered that the shape of urediniospores was not related to phylogenetic group.

In previous studies, the 14 species of *Pucciniastrum* were distinguished from each other by urediniospore shape and size besides their host plants (Hiratsuka 1927, 1930, 1936, 1958; Arthur 1934; Kuprevich and Tranzschel 1957; Gäumann 1959; Wilson and Henderson 1966; Hiratsuka et al. 1992). In the current examination, however, the shape of urediniospores varied greatly within species, indicating that the shape of urediniospores was not consistent within a species. Therefore, I considered that the shapes are unstable characterstic of *Pucciniastrum*.

A large amount of specimens from the14 species were examined, and size of urediniospores of all specimens was listed in appendix 1. The variation range of the size of urediniospores was wide and continuous within individual species and among species.

Comparative examination on the size of urediniospores of the 14 species

showed (Table 3.3) that two species of both ITS and D1/D2 group A tend to be small, and the mean length (*P. epilobii* was19.1μm, *P. circaeae* was 20.6) of both two species are smallest in all species. In contrast, the size of urediniospores in *P. hikosanense* of ITS group Da, ranged from 19.4-38.4 × 12.4-24.5 μm, and the mean length and mean width (26.9 μm and 17.7 μm, respectivly) appear to be largest in all species. On the other hand, ITS groups B, C, E, Db and Fa, each monophyletic group contained only one species. Their average size were somewhat smaller or larger each other (Table 3.3).

Wall thickness of urediniospores was also examined in the 14 species, the mean range of individual species was 1.0-1.8 μm. Among them, *P. fagi* of ITS group Db had the mean thinnest walls (1.02 μm), whereas, *P. coryli* of group C had the mean thickest walls (1.42 μm), followed by *P. hikosanense* of ITS group Da (mean wall thickness was 1.34 μm). Between other species and monophyletic group did not show obvious difference in wall thickness (Table 3.3).

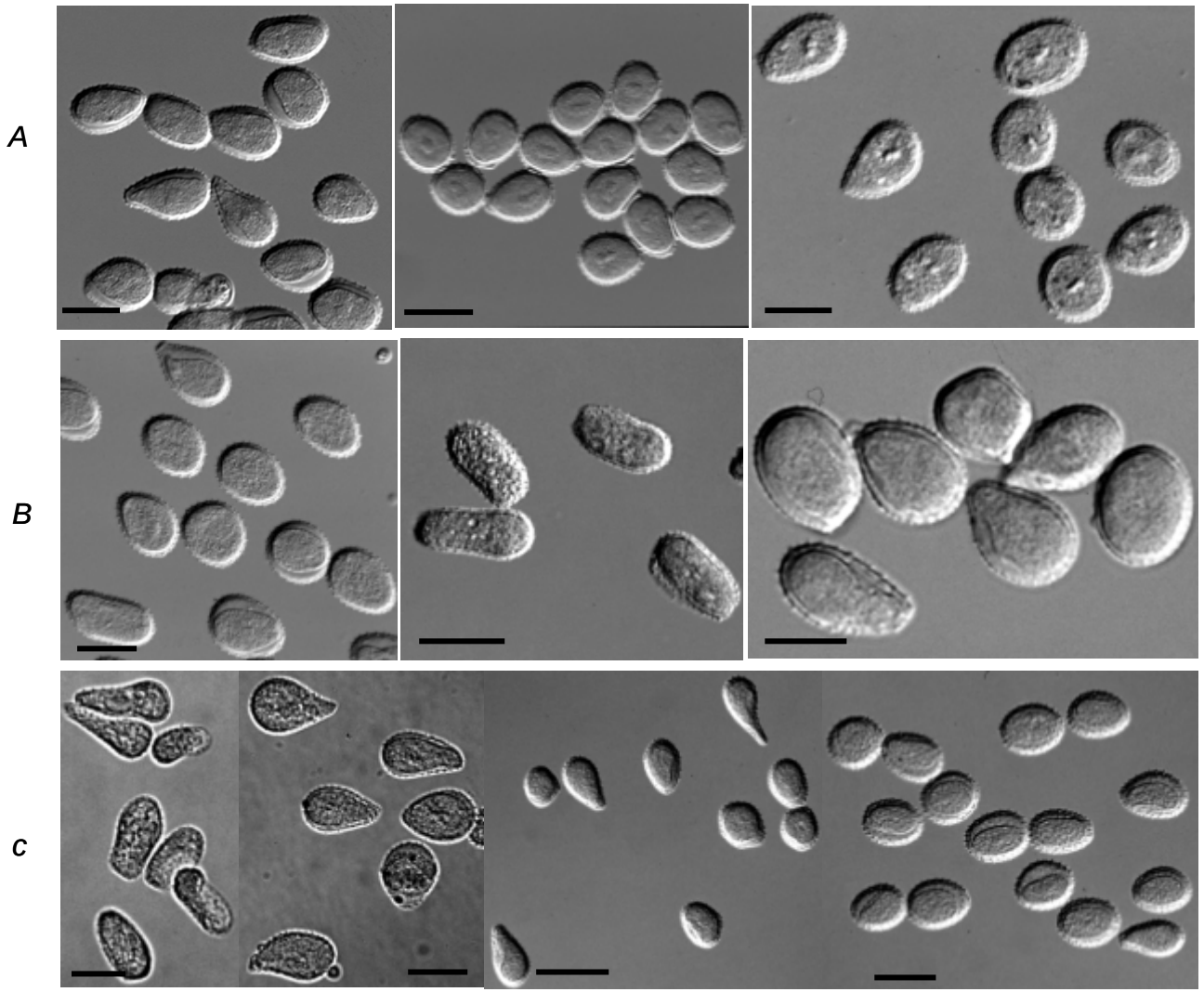


Fig. 3.7. Shape of urediniospores. All specimens showed variation in urediniospore shape which was observed within the same uredinium and among species. (e.g. A: *P. styracinum, B: P. tiliae, C: P. fagi*). Bars. A-C: 20 µm.

Table. 3.3 The relationship of phylogenetic group and size of urediniospores, wall thickness and distance between spines on urediniospore surface.

Phylogenetic group		species	Size of urediniospore(μm)		Mean wt* (μm)	Mean ds* (μm)
D1/D2	ITS		Length (Mean)	Width (Mean)		
A	A	*P. epilobii*	13.5 - 24.5 (19.1)	10.1 - 17.7 (14.4)	1.08	1.30
		P. circaeae	14.7 - 26.0 (20.6)	11.8 - 20.5 (15.7)	1.06	1.22
B	B	*P. hydrangeae -petiolaris*	17.2 - 32.4 (23.8)	12.6 - 20.7 (15.6)	1.20	1.43
E	E	*P. tiliae*	13.7 - 30.5 (21.8)	7.6 - 16.8 (13.7)	1.28	1.34
F	Fb	*P. kusanoi*	15.6 - 28.4 (22.0)	12.6 - 21.8 (16.9)	1.2	1.25
		P. miyabeanum	16.5 - 29.8 (22.5)	11.6 - 21.3 (17.1)	1.08	1.24
		P. styracinum	15.5 - 27.2 (22.0)	10.4 - 16.1 (14.1)	1.25	1.35
		P. actinidiae	16.2 - 30.9 (22.9)	11.4 - 21.5 (16.2)	1.30	1.26
		P. corni	16.5 - 31.3 (22.2)	11.6 - 22.5 (16.4)	1.11	1.24
		P. yoshinagai	17.8 - 27.7 (22.3)	13.0 - 21.9 (16.8)	1.06	1.2
	Fa	*P. boehmeriae*	16.1 - 27.8 (21.6)	10.8 - 20.0 (15.3)	1.06	1.2
C	C	*P. coryli*	15.7 - 28.9 (23.0)	10.4 - 22.3 (17.1)	1.42	1.3
D	Db	*P. fagi*	15.6 - 31.6 (22.1)	8.6 - 17.8 (12.8)	1.02	1.2
	Da	*P. hikosanense*	19.4 - 38.4 (26.9)	12.4 - 24.5 (17.7)	1.34	1.3

* wt: wall thickness of urediniospores; ds: distance between spines of urediniospore surface.

Within the monophyletic group A and ITS group Fb, because the each group contained two or more species, and there are not morphologically significant differences among species within each monophyletic group, statistical analyses on four continuous numerical attributes were undertaken to detect possible differences.

Quantification characteristics within phylogenetic group A, and within group Fb

Quantification characteristics, i.e. urediniospore length, width, thickness of wall, and distance between spines of urediniospore surface, as well as the minimum, mean and maximum values of measurement data were analyzed within phylogenetic group A and ITS Group Fb, respectively, by method of the principal component analyses (PCA). Principal component analyses were undertaken with various combinations of numerical variables in urediniospores features.

Mean values of measurement data of 49 specimens from two species (*P. epilobii* and *P. circaeae*) of group A were analyzed by PCA methods. In the analysis here (Fig. 3.8 - 3.9) mean values of urediniospore length, width, wall-thickness and distance between spines were employed. After the Varimax rotation, the calculated factors 1, 2 and 3 explained 24.9, 25.4 and 25.8 % of the total variance, respectively. The results of combination of the first three factors did not reveal a discrete distribution pattern among those examined specimens (Fig. 3.8). Likewise, combination of the first two factors also showed that those

examined specimens were continuously distributed in the scatter diagram (Fig. 3.9). The specimens of *P. epilobii* were not clearly separated from those of *P. circaeae.* Moreover, no discrete groups or correlation were detected based on mean length, width, wall thickness and distance between spines (Fig. 3.10-3.11). On the other hand, the histogram showed that there was a slight differences in length, width and distance between spines of urediniospores among specimens of *P. epilobii* and *P. circaeae* (Fig. 3.12 A-B, D) except for wall thickness which showed no difference (Fig. 3.12C). However, their size ranges overlapped and the limited number of specimens might have caused the slight difference. These results indicated that specimens of *P. epilobii* would not be separated from those of *P. circaeae* by the quantification characteristics of urediniospore.

Statistical analyses indicated that two species *P. epilobii* and *P. circaeae* of phylogenetic group A were not significantly different in quantification characteristics of urediniospore.

For phylogenetic ITS group Fb, mean values of 249 specimens consisting of six species [*P. kusanoi* (103 specimens), *P. actinidiae* (25), *P. corni* (30), *P. miyabeanum* (51), *P. yoshinagai* (10) and *P. styyracinum* (30)] were also analyzed by principal component analyses (PCA). In the analysis here (Fig. 13 – 3.14) quantification characteristics employed were mean values of urediniospore length, width, wall thickness and distance between spines. After the Varimax rotation, the calculated factors 1, 2 and 3 explained 25.0%, 25.2% and 25.1% of the total variance.

The scatter diagram with the factor one as the horizontal axis and the factor two as the vertical axis did not show discrete group, among those examined specimens of ITS group Fb (Fig. 3.13). Combination of the first three factors also revealed that those examined specimens were continuously distributed in the scatter diagram (Fig. 3.14). Specimens of six species still overlapped each other.

Combinations of urediniospore mean length and mean width, urediniospore mean length and mean wall thickness or mean length and mean distance between spines failed to detect discrete groups (Fig. 3.15 - 3.17). These scatter diagrams indicated that urediniospore length was not correlated with width, wall thickness, or distance between spines.

In addition, the frequency distribution was presented in a histogram, using the mean values of each morphological character of urediniospores measured. The results clearly demonstrated that there was not statistically significant difference in length, width, wall thickness or distance between spines among 249 specimens consisting of these six species (Fig. 3. 18).

Urediniospore dimensions had been considered to be useful to distinguish these six species of *Pucciniastrum*. All these species were discriminated each other by urediniospore size and (urediniospore) shape (Hiratsuka 1927, 1930, 1936, 1958; Arthur 1934; Kuprevich and Tranzschel 1957; Gäumann 1959; Wilson and Henderson 1966; Hiratsuka et al. 1992). However, the results of statistical analyses showed that those characteristics of urediniospore

dimensions did not separate the specimens examined. On the other hand, six species were found to constitute a single phylogenetic group, having identical gene sequence in both D1/D2 and the ITS regions. Consequently, I considered that the quantification characteristics of urediniospores reflect the identity of six species within group Fb.

Comparative studies on the phylogenetic group and morphological characteristics of uredinial stage showed that the characteristic of ostiolar peridial cells and the surface structure of urediniospore-wall could well reflect the molecular phylogeny. These characters coincided with each monophyletic clade. This congruence of phenotypic characters is precisely in harmony with Henning's proposition. Initially, Henning (1950) only using phenotypic characters constructed the cladistic, assuming that homologous clades share similar phenotypic characters. However, the resolving power cladistics has been enormously increased by including molecular characters. A consequence for fungi is that more objective phylogenetic analyses can replace phylogenetic conjectures, the majority of which were based on very hard facts (Burnett 2003).

However, the germ pores of urediniospores as well as shape, and dimensions of urediniospores did not reflect the molecular phylogeny. These characteristics may be good characters on the species level in other genera. In the 14 species of *Pucciniastrum*, however, the size range of urediniospores overlapped and the pores as well as shape of urediniospores were variable within a species and among species. Therefore, I considered that only using the

shape and dimensions of urediniospores are not enough to discriminate the species of *Pucciniastrum*. Further, the phenotypic character has great plasticity, responding to environmental conditions. Thus, differentiation can be suppressed or alterations are induced in structures or their dimensions (Burnett 2003). Hence, it is necessary to ascertain more stable characteristics for morphological classification of *Pucciniastrum*, such as the telial stage. Burnett (2003) considered that the teleomorphic phase tend to be more constant and provide many reliable taxonomically useful characters.

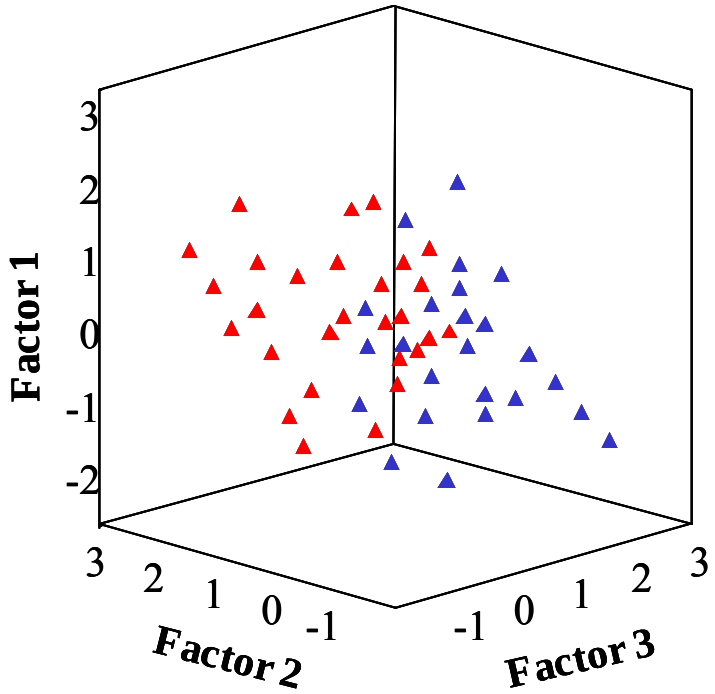


Fig. 3.8. Two dimensional scatter diagram generated by a principal component analysis based on non-standardized data of 4 characters of urediniospores of *P. epilobii* (blue) and *P. circaeae* (red) belonging to phylogenetic group A.

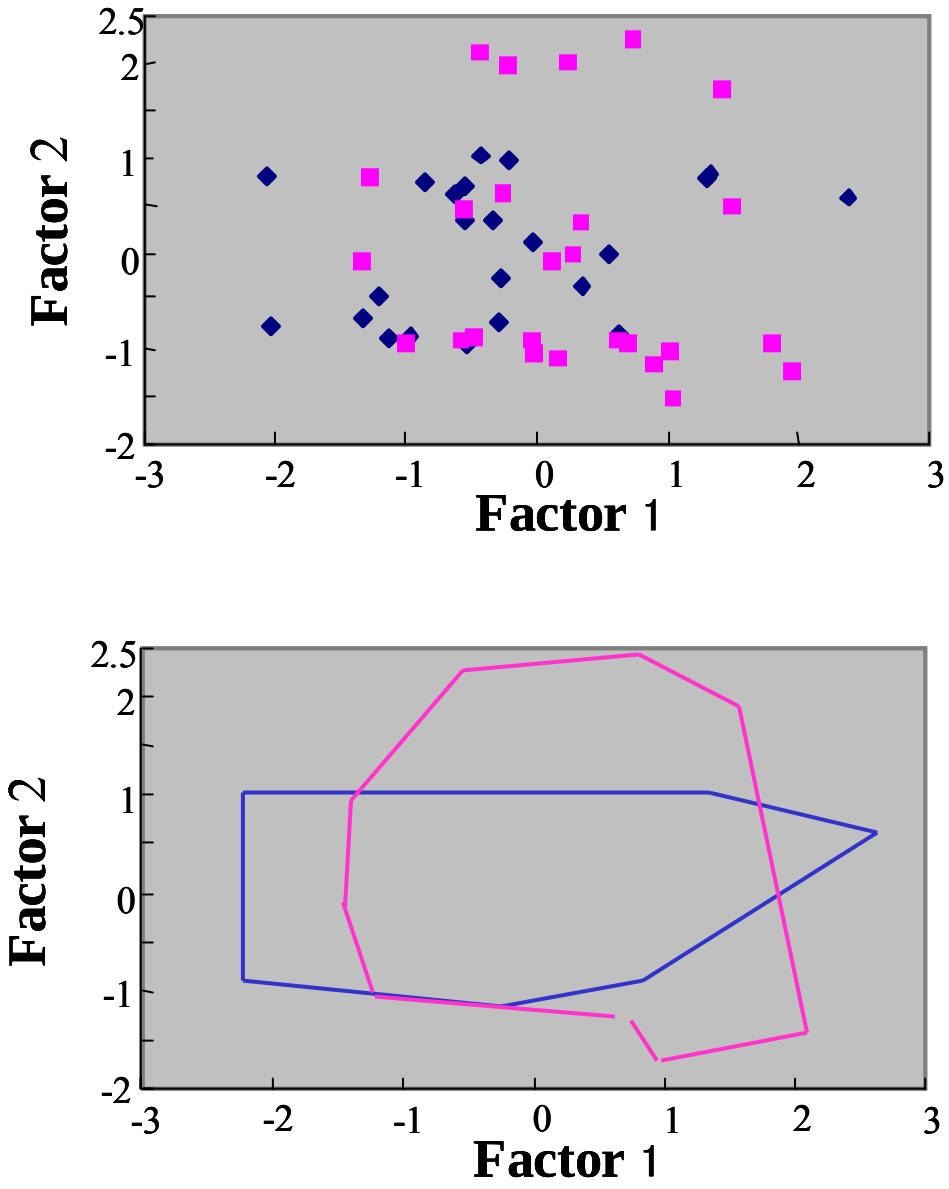


Fig. 3.9. Two dimensional scatter diagram generated by a principal component analysis based on non-standardized data of 4 characters of urediniospores of *P. epilobii* (◆) and *P. circaeae* (■).

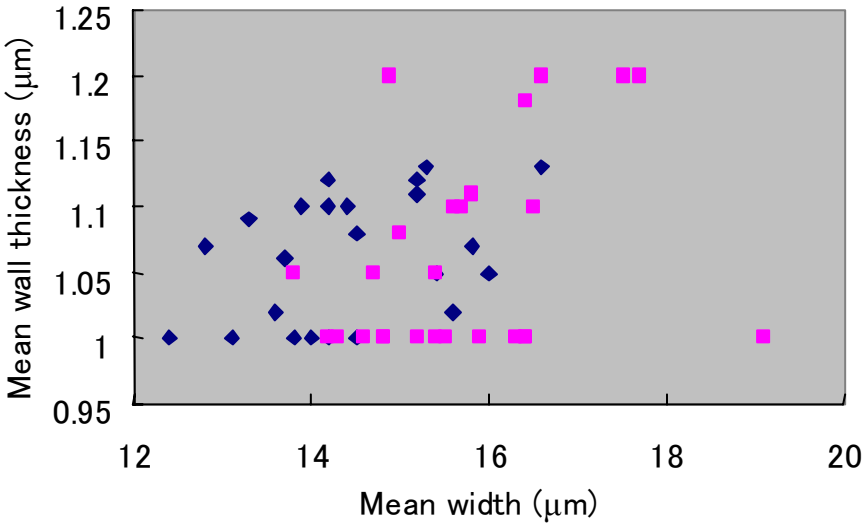


Fig. 3.10. Scatter diagram generated from mean urediniospore width against mean wall thickness of *P. epilobii* (◆) and *P. circaeae* (■).

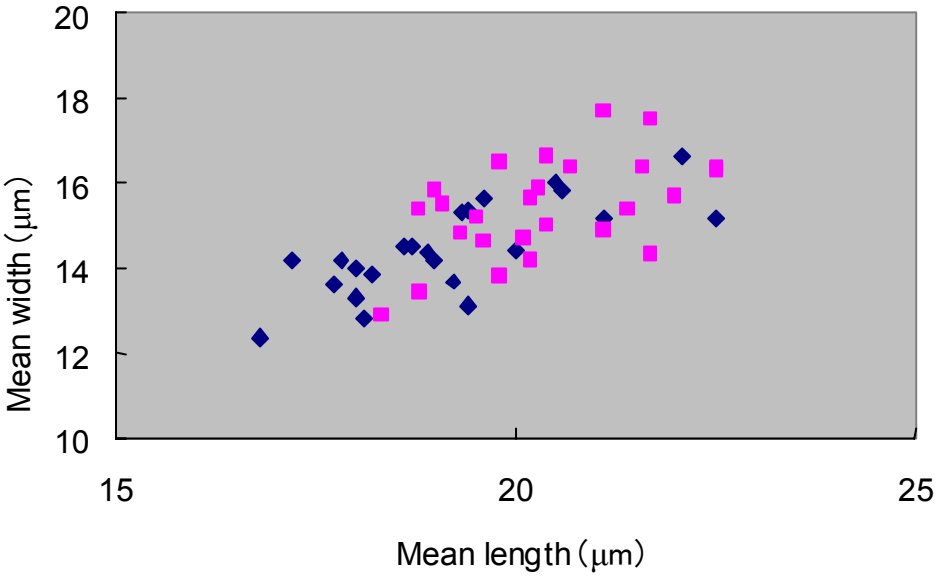


Fig. 3.11. Scatter diagram generated from mean urediniospore length against mean width of *P. epilobii* (◆) and *P. circaeae* (■).

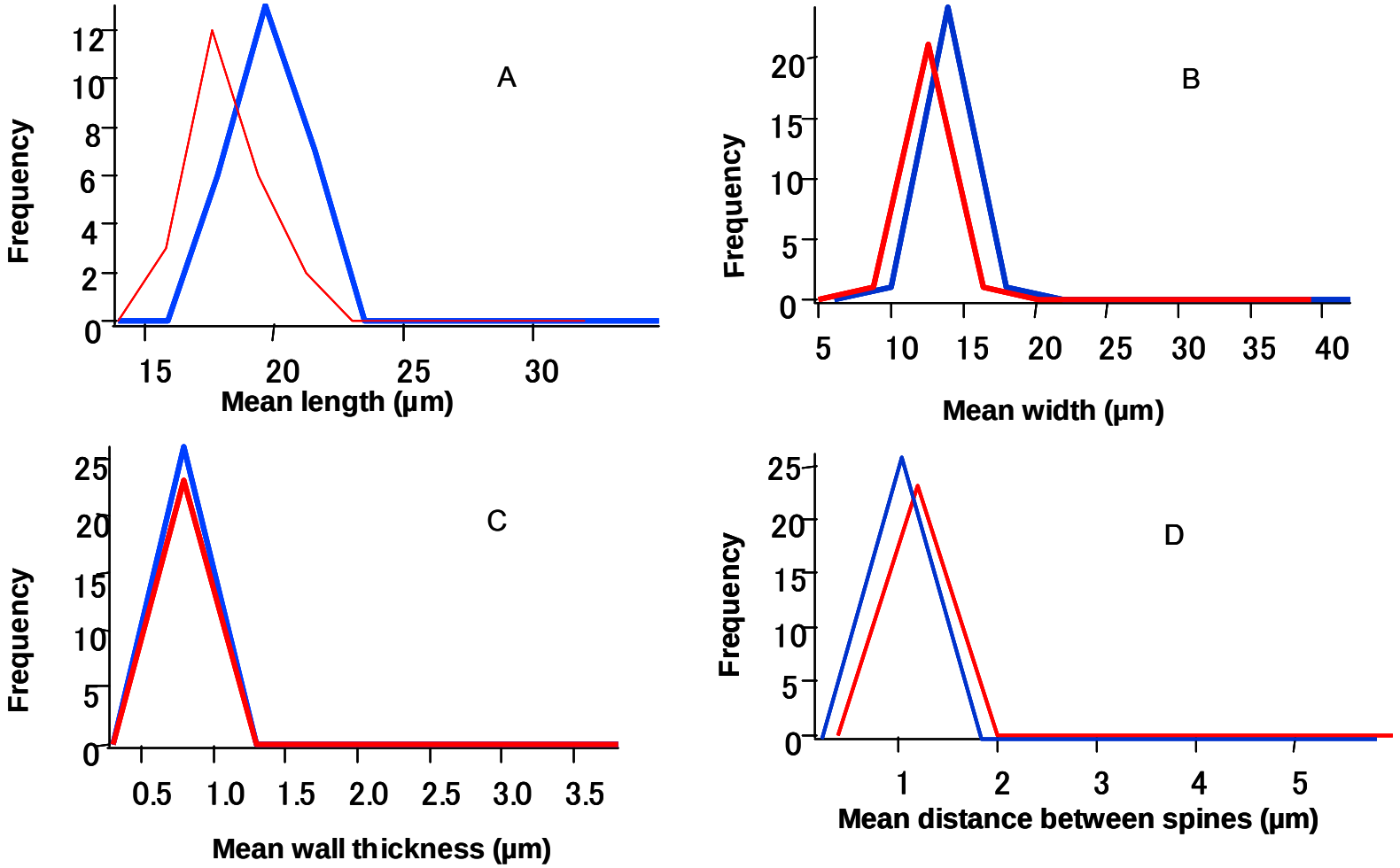


Fig. 3.12. The histogram of mean length (A), mean width (B), mean wall thickness (C) and mean distance between spines (D) of urediniospores of P. epilobii (—) and P. circaeae (—).

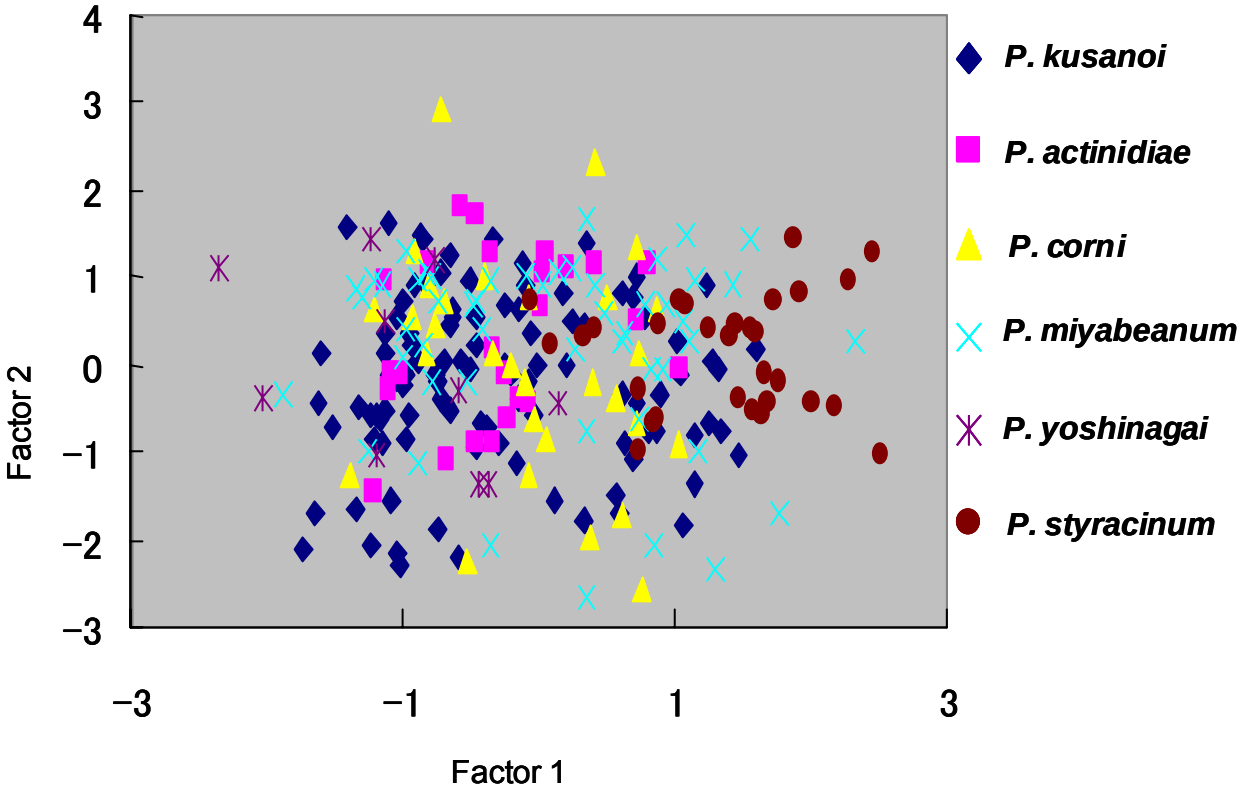


Fig. 3.13 A scatter diagram generated from the principal component analysis based on non-standardized data of length, width, wall thickness and distance between spines of urediniospores of six species belonging to ITS group Fb.

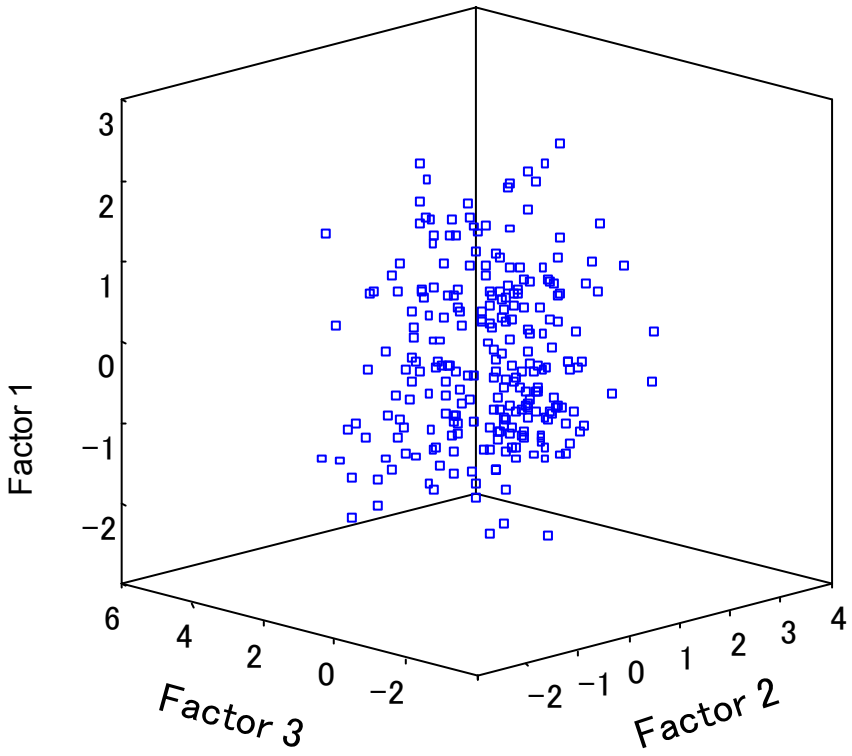


Fig. 3.14 A scatter diagram generated from the principal component analysis based on non-standardized data of length, width, wall thickness and distance between spines of urediniospores of six species belonging to ITS group Fb.

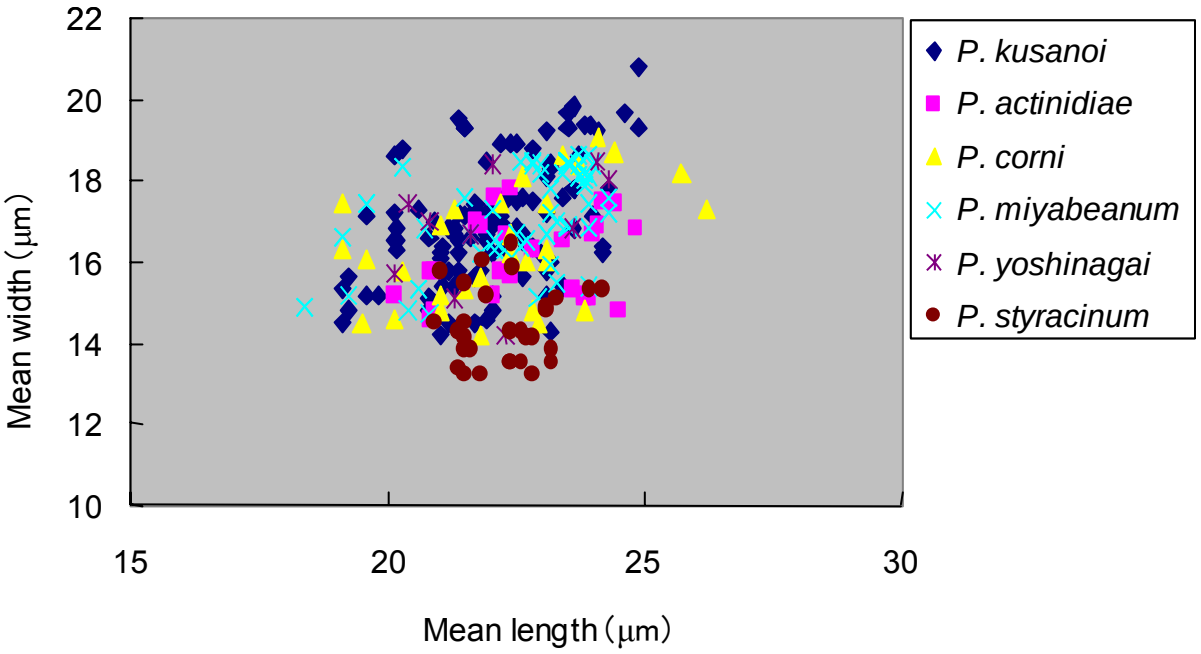


Fig. 3.15. Scatter diagram generated from urediniospores mean length against mean width of six species belonging to ITS group Fb.

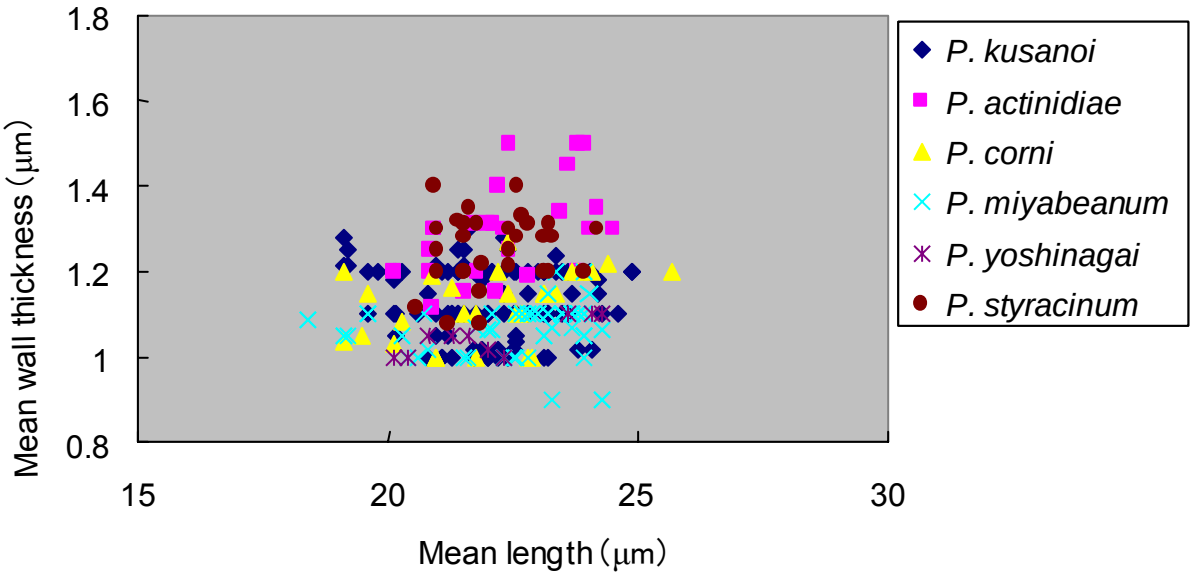


Fig. 3.16. Scatter diagram generated frommean urediniospore length against mean wall thickness of six species belonging to ITS group Fb.

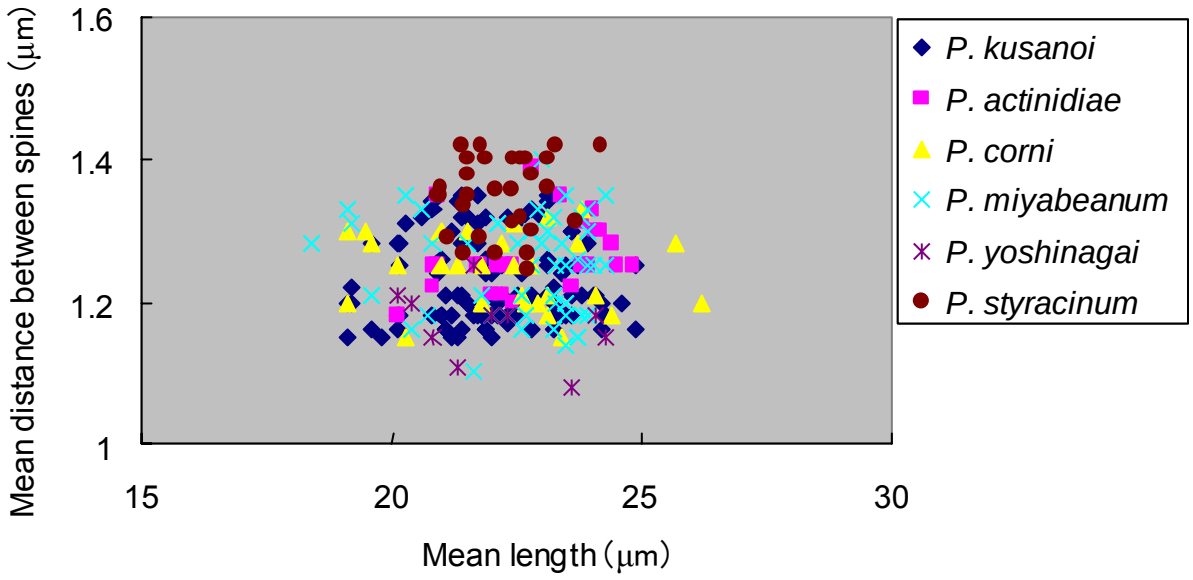


Fig. 3.17. Scatter diagram generated from urediniospore mean length against mean distance between spines of six species belonging to ITS group Fb.

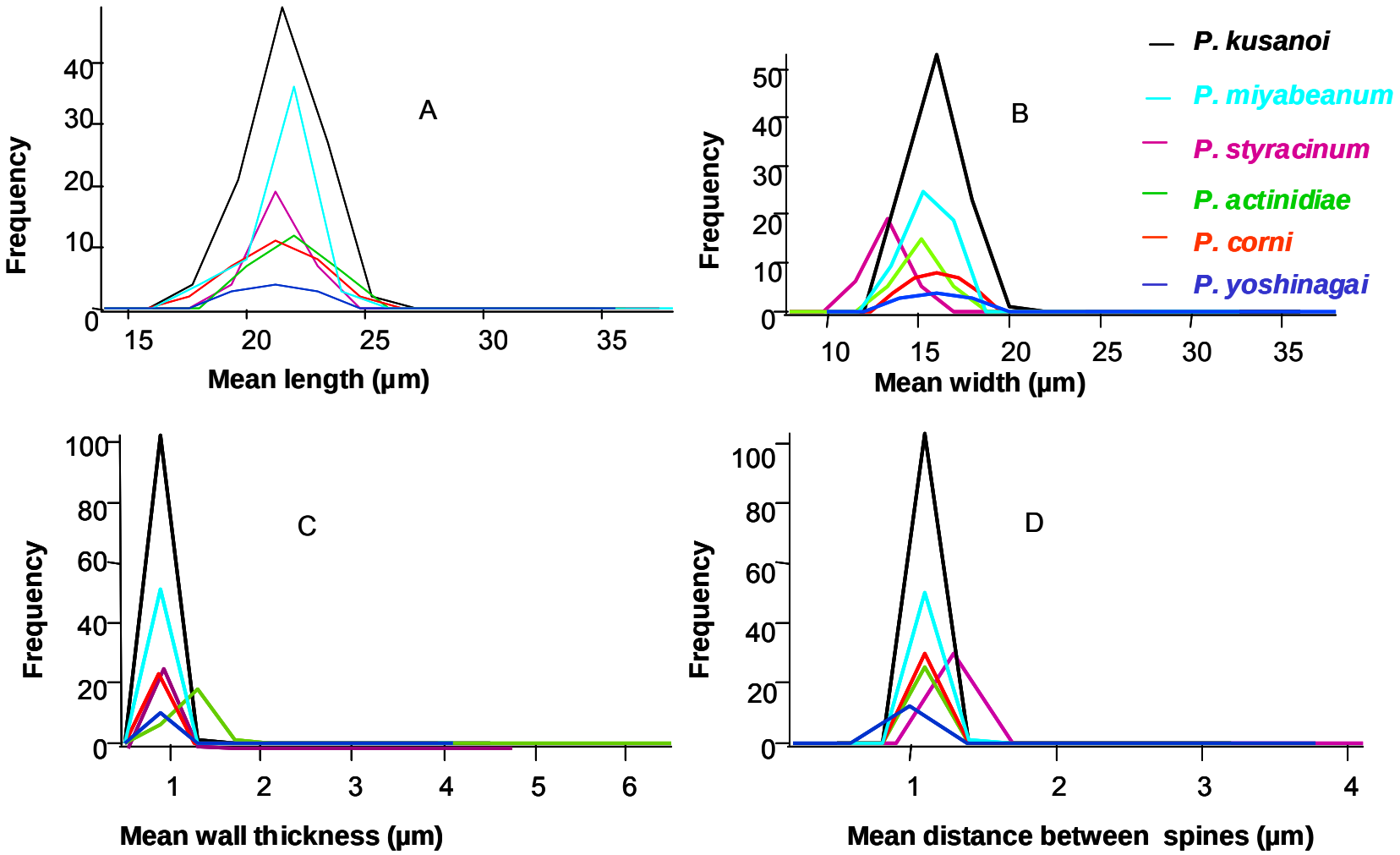


Fig. 3.18. The histogram of mean length (A), mean width (B), mean wall thickness (C) and mean distance between spines (D) of urediniospores from six species of ITS group Fb.

4. Taxonomic discussion

Comparative results of molecular phylogenetic group and morphological examination are listed in Table 4.1. Incorporation of molecular phylogenetic analysis into comparative morphological studies represents an approach to fungal phylogeny and taxonomy.

Two species *Pucciniastrum epilobii* and *P. circaeae* were monophyletic and clustered in the group A by high bootstrap support. They lack the ostiolar peridial cell, many urediniospores have a small smooth area on the surface and the germ pores arrangement are scattered. Urediniospore quantification characteristics did not show significantly difference. Their morphological identity coincided with results from the current phylogenetic analysis. And, based on those morphological characteristics, the two species could be clearly distinguished from other species.

Although two base pairs differ between *P. epilobii* and *P. circaeae* of monophyletic group, the two species were considered to have descended from a single lineage (Fig. 2.5). Burnett (2003) stated that a species descended from a single lineage that maintains its identity can be tested by investigating its phylogenetic tree. The two species possess similar gymnopedunculate haustoria. The haustorial ultrastructure is probably a conservative characteristic, which is not easily influenced by environmental factors and regarded as an important character (Berndt and Oberwinkler, 1995). Hence, I considered that the two

species may be a distinct taxa.

In a phylogenetic line of rust, the change of the pore arrangement is considered to be regular in sequence. Cummins (1936) indicated that the scattered arrangement of germ pores is phylogenetically primitive. In this study, *P. epilobii* and *P. circaeae* of group A, and *P. hydrangeae-petiolaris* of group B have scattered pores. Groups A and B were separated from other groups by a long genetic distance, respectively, based on both D1/D2 and ITS regions (Fig. 2.5, 2.7). These considerations suggest that above three species may be more primitive among the 14 *Pucciniastrum* species.

All specimens of *P. hydrangeae-petiolaris* formed a monophyletic group B (Fig. 2.5, 2.7). These specimens have well-developed ostiolar peridial cells, urediniospore wall–surface is uniformly echinulate and have scattered germ pores. All specimens determined have the consistent characteristics. Those characters support the hypothesis that *P. hydrangeae-petiolaris* is a distinct taxa.

Three species of *P. coryli*, *P. fagi* and *P. boehmeriae* have some similar morphological characteristics, i.e. the ostiolar peridial cells are markedly differentiated, which were developed very well, urediniospore wall-surface is uniformly echinulate, and the germ pores show bizonate arrangement. However, in the present phylogenetic analysis, the three species each belonged to independent monophyletic group C, ITS group Db and ITS group Fa, respectively, in phylogenetic trees constructed from the sequence data (Fig. 2.7, Table 4.1) and the phylogenetic tree shows that they are distant from each other.

In other words, they originated from different ancestors. In practice, the best way to resolve the taxonomic issues is to base phylogenetic trees, whenever possible, on combined information from genes that are both conservative and regulate essential fungal functions such as the rDNA or β–tubulin loci (Burnett 2003). Accordingly, I considered that *P. coryli*, *P. fagi* and *P. boehmeriae* are distinct taxa, respectively.

On the other hand, I did not examine the teliospores of the above three species. However, the three species differed in teliospore-cell numbers (Hiratsuka et al, 1992). Teliospores of *P. coryli* contain 2-8 cells, *P. fagi* contain 2-5 cells, in contrast, *P. boehmeria* contain 1-4 cells. Burnett (2003) emphasized that the teleomorphic phase was constant, providing many reliable taxonomically important characteristics. I think the telial stage may be (or become) important when combining with other characters to distinguish *Pucciniastrum* species. Unfortunately, the telial stage was not examined for classification in *Pucciniastrum*, because determination of these characteristics was more difficult.

Specimens of *P. tiliae* formed a monophyletic group B. They possess well-developed ostiolar peridial cells, urediniospore wall-surface is uniformly echinulate and the germ pores from bizonate to scattered arrangement. All specimens examined have the consistent characteristics. Although I did not examine the aeciospores or teliospores, Hiratsuka et al. (1992) reported that the teliospore length of *P. tiliae* was 20-45 μm (all other *Pucciniastrum* species had <

36 μm long teliospores) and the aeciospore-surface has a small and almost smooth area. Those characteristics support that *P. tiliae* is a distinct taxa.

All specimens of *P. kusanoi*, *P. miyabeanum*, *P. styracinum*, *P. actinidiae*, *P. corni* and *P. yoshinagai* formed a single monophyletic group, Fa in the ITS tree (Fig. 2.7). They have large ostiolar peridial cells and urediniospore wall-surface is uniformly echinulate. In addition, the results of statistic analysis showed that the urediniospore characteristics quantified were also similar, with no significant differences in length, width, wall thickness or distance between spines, except that the wall thickness of *P. actinitiae* was somewhat thicker. Likewise, according to Hiratsuka (1936, 1958) and Hiratsuka et al. (1992), the above six species also were similar in their teliospore size and teliospore-cell number.

The six species had a slight difference in germ pore arrangement, i.e. *P. kusanoi*, *P. miyabeanum*, *P. styracinum* and *P. actinidiae* were bizonate to scattered, two types of pore arrangement existed in the same specimen; on the other hand *P. corni* and *P. yoshinagai* only showed bizonate arrangement. The pore variation was considered that to be related with the shape of urediniospores (Cummins 1936). However, the six species formed a monophyletic group, which originated from a common ancestor according to the present phylogenetic trees based on both 28S rDNA and ITS regions (Fig. 2.5, 2.7). Berbee and Taylor (1994) emphasized that it was important to define a group as monophyletic based on common ancestry, not on characters that all members possess because an organism with shared common ancestry in a monophyletic group

could lack all the characters typical of the group. These considerations suggest that the above six species may be a distinct taxa or have a very close phylogenetic relationship.

Specimens of *P. hikosanense* formed a single monophyletic group Da. Their morphological characters were clearly different from other species. The ostiolar cells were not markedly developed. Urediniospore wall-surface has smooth area. Germ pores arrangement is bizonate or scattered. All specimens examined have the consistent characteristics. These significant morphological characters and phylogenetic analysis support that *P. hikosanense* is a distinct taxa.

Originally, Hiratsuka (1940) had not mentioned that the urediniospore surface of *P. hikosanense* had the smooth area, and he described that the ostiolar peridial cells are well developed. Thus, researchers afterwards (Hiratsuka 1958, Hiratsuka et al 1992) continued to use these primitive descriptions continuously. However, the holotype and others specimens of *P. hikosanens* were observed by SEM in the present studies, and results showed that the ostiolar peridial cell differentiation was not obvious, and the urediniospore wall-surface had the obvious smooth area. Therefore, I consider that these important morphological characteristics were missed out in the previous study.

According to the molecular phylogenetic analyses of LSU rDNA (D1/D2) region and ITS region including 5.8S rDNA combined with morphological comparative analyses, I concluded that *Pucciniastrum coryli, P. boehmeriae, P.*

hikosanense, P. hydrangeae-petiolaris, P. fagi and *P. tiliae* are distinct species. On the other hand *P. epilobii* and *P. circaeae* is a single taxon. In contrast, *P. kusanoi, P. styracinum, P. yoshinagai, P. actinidiae, P. corni* and *P. miyabeanum,* constitute a single taxon with a great probability. However, no inoculation tests have been conducted to determine if the six species belong to different biological species. Consequently, a more comprehensive study using specimens of *Pucciniastrum* from other countries or areas is important and required for further understanding of the phylogenetic relationship and classification of these species.

Table. 4.1. Relationship of phylogenetic group and morphological characteristics of urediniospore.

Phylogenetic group		*Pucciniastrum* species	Development of ostiolar cell	Urediniospore-surface	Germ pore	Mean size(μm)
D1/D2	ITS					Length × Width
A	A	*P. epilobii* *P. circaeae*	absence	small smooth area	scattered	19.1 × 14.4 20.6 × 15.7
B	B	*P. hydrangeae -petiolaris*	well	echinulate	scattered	23.8 × 15.6
E	E	*P. tiliae*	well	echinulate	bizonate	21.8 × 13.7
F	Fb	*P. kusanoi* *P. miyabeanum* *P. styracinum* *P. actinidiae*	well	echinulate	bizonate or scattered	22.0 × 16.9 22.5 × 17.1 22.0 × 14.1 22.9 × 16.2
		P. corni *P. yoshinagai*			bizonate	22.2 × 16.4 22.3 × 16.8
	Fa	*P. boehmeriae*	well	echinulate	bizonate	21.6 × 15.3
C	C	*P. coryli*	well	echinulate	bizonate	23.0 × 17.1
D	Db	*P. fagi*	well	echinulate	bizonate	22.1 × 12.8
	Da	*P. hikosanense*	not well-developed	big smooth area	bizonate or scattered	26.9 × 17.7

Abstract

Pucciniastrum is a large genus in Pucciniastraceae. About 25 species have been known in the world and all species whose life cycles are known as heteroecious, with aecia on needles of Pinaceae (*Abies*, *Picea* and *Tsuga*), and the uredinia and telia on many dicotyledonous plants belonging to a total of 17 families [e.g. Betulaceae, Fagaceae, Rosaceae, Ericaceae, Onagraceae, and Aceraceae etc. (26 genera) 17 families]. Among these species, 22 species have been reported in Japan and were classified into four morphological groups based on the structure of ostiolar peridial cells in the uredinia. One of these groups comprising 16 species has smooth ostiolar cells. Circumscription and identification of the 16 species have relied on host plants of telia stage and somewhat larger or smaller urediniospore size because they are extremely similar morphologically. However, morphological circumscription of the 16 species does not seem clear or distinct. In addition, phylogenetic relationships among them have not been explored adequately, and taxonomic identity is still unknown.

Therefore, I investigated the genetic diversity among these morphologically similar species and analyzed their phylogenetic relationships. To accomplish these purposes, isolates obtained from a total of 43 specimens that including14 species (two species were not included here) were collected from different areas in Japan and used for phylogenetic analysis. Sequence data were obtained from

D1/D2 region of nuclear large subunit rDNA, and from internal transcribed spacers, ITS1 and ITS2 regions including 5.8S rDNA. Then, phylogenetic trees were constructed from the D1/D2 and the ITS regions, respectively. Those species were separated into six groups by both NJ and parsimony trees in D1/D2 region, however, the analyses of ITS tree clearly distinguished eight groups. In addition, all specimens of single species belonged to the same group in D1/D2 or ITS tree.

The phylogenetic tree showed that *Pucciniastrum circaeae* and *P. epilobii* formed a monophyletic clade with 100% bootstrap value support. On the other hand, *P. fagi*, *P. hikosanense*, *P. boehmeriae, P. tiliae, P. hydrangeae-petiolaris* and *P. coryli* corresponded to each monophyletic group by high bootstrap value support in the ITS tree. The results indicated that they may be each distinct taxa. In contrast, six species of *P. kusanoi*, *P. actinidiae*, *P. corni*, *P. styracinum*, *P. yoshinagai* and *P. miyabeanum* constituted a single monophyletic clade in both NJ and maximum parsimony analyses based on both D1/D2 and ITS regions. The phylogenetic analysis results did not support that the six species were separate taxa at the species level. In addition, the present study did not find the clear relations between the phylogenetic group and host plant of telial stage in the phylogenetic line.

To define the relations between the phylogenetic group and morphological characteristics, and to evaluate the taxonomic the 14 morphologically similar species of *Pucciniastrum*, the morphological re-examination was carried out on a

large collection of specimens from Japan. Over 570 specimens including some holotypes were observed by light microscope (LM) and scanning electron microscope (SEM). Morphological examination showed that characteristics of ostiolar peridial cells and the urediniospore wall-surface structure reflected the phylogeny, each characteristic being identical within each phylogenetic group. In contrast, the germ pore arrangement of urediniospores and urediniospore quantification characteristics did not correspond to the phylogeny. On the other hand, urediniospores showed great variation in shape within the same uredinium and among species. It was considered that the shape of urediniospores is not related to phylogenetic group.

Comparison of phylogenetic group and morphological examinations showed that *P. epilobii* and *P. circaeae* originated from a single lineage in the phylogenetic tree. Further, they possess common consistent morphological characteristics. Therefore, the two species are suggested to belong to the same rust fungal taxon. Similarly, six species of *P. kusanoi*, *P. actinidiae*, *P. corni*, *P. styracinum*, *P. yoshinagai* and *P. miyabeanum* originated from a common ancestor based on the phylogenetic tree and morphology are extremely similar. In addition, the statistic analysis also failed to separate them based on the size of urediniospores. These results suggest that the six species may represent a single distinct taxon, however, further study is necessary to confirm in the future. In contrast, each species of *P. fagi*, *P. hikosanense*, *P. boehmeriae, P. coryli*, *P. hydrangeae-petiolaris* and *P. tiliae* has identical morphological characteristics

within specimens, each forming a clearly distinct phylogenetic group. Therefore, the morphological and molecular analyses suggest that each of these six species belongs to separate taxon at the species level.

Acknowledgements

First and foremost, I would like to express my gratitude to my academic adviser, Professor M. Kakishima, for his supervision, guidance and continued support throughout the study on fungal taxonomic and throughout my stay in the University of Tsukuba, Japan.

I wish to express my great appreciation to my committee members, Associate Professor Y. Yamaoka, Laboratory of Plant Parasitic Mycology, Institute of Agriculture and Forestry, University of Tsukuba; Professor S. Tokumatsu, Sugadaira Montane Research Center, University of Tsukuba; Professor Y. Kono, Laboratory of Applied Zoology, Institute of Agriculture and Forestry, University of Tsukuba; Professor H. Ishii, National Institute for Agro-Environmental Sciences, Tsukuba, for their numerous suggestion, comments, encouragement, and willingness to serve as my advisory committee members. Former Professor K. Katsuya, for his interest, encouragement in the conduct of the study.

I am also to express my deep appreciation to Hiratsuka Herbarium, Tokyo, Japan; Dr. Y. Ono, Laboratory of Biological Sciences, Faculty of Education, Ibaraki University; Dr. Y. Harada, Faculty of Agriculture and Life Science, Hirosaki University; Dr. M. Imazu, Faculty of Agricultural, Shinshu University; Dr. S. Kaneko and Dr. R. Kaneko, for invaluable assistance during the filed collections and for providing materials used in the study. I thank Dr. T. Nakamura,

University of Tsukuba, for the identification of host plants.

I thank Dr. N. Matsumoto and Dr. S. Tsushima, National Institute for Agri-Environmental Science, Tsukuba, for their helpful advices and encouragement in my life. I also thank Dr. W. H. Chung for technical advice and help.

I also thank the present and former members of the Laboratory of Plant Parasitic Mycology, Dr. C. P. Abe, Dr. J. Engkhaninum, Miss K. Kawasaki, Mr. M. Shiratori and Mr. T. Mahmood for their fruitful discussion and all the help extended and thing I learned from them about work in the laboratory and life in Japan.

I also thank to my friend Dr. J. J. Liu and his family for their friendship, help and support.

Finally, this thesis is dedicated to my husband, Dr. C. M. Tian, who supported me in many ways and thoughtful review of this work, and taught me regarding molecular phylogenetic analyses, PCR and sequencing methods. I also can not express my love and gratitude enough to my son and parents for their understanding and support in my life.

Literature cited

Adams, J. F. 1919. Rusts on conifers in Pennsylvania. Pennsylvania State Coll. Agric. Exper. Stat. Bull.

Almaraz, T., Roux, C., Maumont, S. and Durrieu, G. 2002. Phylogenetic relationships among smut fungi parasitizing dicotyledons based on ITS sequence analysis. Mycol. Res. 106: 541-548.

Anderson, J. P. 1952. The Uredinales of Alaska and adjacent parts of Canada. Iowa State Coll. Jour. Sci. 26: 507-526.

Arthur, J. C. 1907. Family 2. Uredinaceae. North American Flora 7: 97-127.

Arthur, J. C. 1907-1925. Order Uredinales. *In*: North American Flora, New York Botanical Gardens, N. Y. 7: 83-969 (parts 2, 9, 10).

Arthur, J. C. 1934. Manual of the Rusts in the United States and Canada. Purdue Research Foundation, Lafayette, Ind. Reprinted with a supplement by Cummins GB (1962). Hafner Publishing Co., New York.

Arthur, J. C. and Cummins, G. B. 1936. Philippines rusts in the Clemens collection, 1923-1926. II. Philippine Jour. Sci. 61: 463-488.

Arthur, J. C. 1962. Manual of the rusts in United States and Canada. Hafner Publishing Company, New York.

Begerow, D., Bauer, R. and Boekhout, T. 2000. Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences. Mycol. Res. 104: 53-60.

Berbee, M. L. and Taylor, J. W. 1994. 18S ribosomal DNA sequence data and dating, classifying, and ranking the fungi. – Ascomycete systematics: problems and perspectives in the nineties (ed. Hawksworth D. L.). New York and London. pp. 213-223.

Berndt, R. and Oberwinkler, F. 1995. Ultrastructure of the parasitic interface of

Pucciniastrum, *Thekopsora*, *Naohidemyces*, and *Calyptospora* (Uredinales, Pucciniastraceae) in the dikaryotic stage. Mycoscience 36: 51–59.

Brown, D., Wensink, P. and Jordan, E. 1972. A comparison of the ribosomal DNAs of *Xenopus laevis* and *Xenopus Mulleri*: Evolution of tandem genes. Jour. Mol. Biol. 63: 57-73.

Bruns, T. D., white, T. J. and Taylor J. W. 1991. Fungal molecular systematics. Ann. Rev. Ecol. Syst. 22: 525-564.

Bubák, F. 1906. Infektionsversuche mit einigen Uredineen, III. Bericht (1904 und 1905). Centralbl. f. Bakt., II. Abt. 16: 150-159.

Bubák, F. 1908. Die Pilze Böhmens, I. Rostpilze (Uredinales). Archiv. Nat. Landesdurchf. Böhmen. 8: 1-234.

Burnett, J. 2003. Fungal Populations and Species. Pub. In the United States. by Oxford University Press Inc., New York.

Cao, Z. M. and Li, Z. Q. 1999. Rust fungi of Qinling Mountains. China Fore. Publ. House, Bei Jing.

Chung, W. H., Tsukiboshi, T., Ono, Y. and Kakishima, M. 2004. Morphological and phylogenetic analyses of *Uromyces appendiculatus* and *U. vignae* on legumes in Japan. Mycoscience 45: 233-244

Clinton, G. P. 1911. Notes on plant diseases of Connecticut. Report of the Connecticut Agricultural Experiment Station. 1909-10, 715-738.

Clinton, G. P. 1912. Notes on some heteroecious rusts of Connecticut. Phytopathology 2: 94.

Clinton, G. P. 1924. Rust infection of leaves in Petri dishes. Conn. Agr. Expt. Sta. New Haven Bull. pp. 260, 475-501.

Conners, I. L. 1967. An annotated index of plant diseases in Canada and fungi recorded on plants in Alaska, Canada and Greeland. Can. Dep. Agr., Res. Br., Publ.

Cummins, G. B. 1936. Phylogenetic significance of the pores in Urediospores. Mycologia 28: 103-132.

Cummins, G. B. 1941. Uredinales of New Guinea. IV. Mycologia 33: 380-389.

Cummins, G. B. and Ling, L. 1950. An index of the plant rusts recorded for continental China and Manchuria. Pl. Dis. Rept., Suppl. 196: 520-556.

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

Cummins, G. B. and Hiratsuka, Y. 2003. Illustrated genera of rust fungi. Third Ed. Amer. Phytopathol. Soc., St. Paul, Minnesota.

Darker, G. D. 1929. Cultures of *Pucciniastrum americanum* (Farlow) Arthur and *P. arcticum* (Lagerheim) Tranzschel. J. Arnold Arbor. (Harv. Univ.) 10: 156-167.

Dietel, P. 1900. Uredinales (Nachträge). Engler-Prantl, Natürl. Pflanzenfam. I, 1. Abt. p 46 & 551.

Faull, J. H. 1938. Taxonomy and geographical distribution of the genus Uredinopsis. Contrib. Arnold arboretum, Harvard Univ. 11: 120 pp.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Figueiredo, M. B., Nogueira, E. M. de C., Ferrari, J. T., Aparecido, C. C. and Hennen, J. F. 2003. Ocorrência de ferrugem em framboesa no Estado de São Paulo. Arq. Inst. Biol., São Paulo 70: 199-201.

Fischer, E. 1904. Die Uredineen der Schweiz. Beiträge zur Kryptogamenflora der Schweiz, II, Heft 2, 94: 1-590.

Fischer, E. 1916. Der wirtswechsel der Uredineen *Thecopsora* sparsa und *Pucciniastrum Circaeae*. (Vorläufige Mitteilung). Centralbl. f. Bakt. II. Abt. 46: 333-334.

Fraser, W. P. 1912. Cultures of heteroecious rusts. Mycologia 4: 175-193.

Fraser, W. P. 1913. Further cultures of heteroecious rusts. Mycologia 5: 233-239.

Fraser, W. P. 1914. Notes on *Uredinopsis mirabilis* and other rusts. Mycologia 6: 25-28.

Fragoso, G. R. 1925. Flora Ibérica. Uredales. Roras de los vegetales, II. 7: 1-424

Fowler, J., Cohen, L. and Jarvis, P. 1998. Practical Statistics for Field Biology (1943). 2nd edition reprinted in 1998 by John Wiley and Sons Ltd, Baffins Lane, Chichester, England.

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.

Gäumann, E. 1959. Die Rostpilze Mitteleuropas. Büchler Co., Bern. (Beiträge zur Kryptogamenflora der Schweiz, Bd. 12).

Grove, W. B. 1913. The British rust fungi (Uredinales), their biology and classification. 7: 1-412.

Guadet, J., Julien, J., Lafay, J. F. and Brygoo, Y. 1989. Phylogeny of some *Fusarium* species, as determined by large- subunit rRNA sequence comparison. Mo. Biol. Evol. 6: 227-242.

Hawker, L. E., Madelin, M. F. 1974. The dormant spore. In The Fungal Spore (2nd International Fungal Spore Symposium) (ed. J. W. Darrel & W. M. Hess), New York: Wiley.

Henning, W. 1950. Grundzüge einer Theorie der Phylogenetischen Systematic. Deutscher Zentralverlag, Berlin.

Hiratsuka, N. 1897. Notes on some Melampsorae of Japan, I. Bot. Mag., Tokyo 6: 45-49.

Hiratsuka, N. 1926. On relationship between *Pucciniastrum* and *Uredinopsis*. Jour. Soc. Agric. & Forestr., Sapporo 18: 75-86. (in Japanese).

Hiratsuka, N. 1927. Studies on the Melampsoraceae of Japan. Jour. Facul. Agric., Hokkaido Imp. Univ. 41: 1-41.

Hiratsuka, N. 1930. *Pucciniastrum* of Japan. Bot. Mag., Tokyo 46: 261-284.

Hiratsuka, N. 1932. Inoculation experiments with some heteroecious species of the Melampsoraceeae in Japan. Jap. Jour. Bot. 6: 1-33.

Hiratsuka, N. 1933. Inoculation experiments with heteroecious species of the Japanese rust fungi, I. Bot. Mag., Tokyo 47: 710-714.

Hiratsuka N. 1936. A Monograph of the *Pucciniastreae.* Tottori Agricultural College, Tottori, Japan 1-374.

Hiratsuka N. 1940. A new species of *Pucciniastrum* on *Acer rufinerve*. Ann. Phytopath. Soc. Japan 10: 154-155. (In Japanese).

Hiratsuka, N. 1955. Uredinological Studies. Kasai Publishing Co. Tokyo, Japan.

Hiratsuka, N. 1958. Revision of Taxonomy of the Pucciniastreae. Tokyo, Japan.

Hiratsuka, Y. 1970. Identification and morphology of the Pucciniastrum sparsum in northwestern Canada. Canadian Journal of Bot. 48: 433-435.

Hiratsuka, N. and Kaneko, S. 1976. Aecial states of *Coleosporium horianum* Hennings and *Pucciniastrum corni* Dietel. Rept. Tottori Mycol. Inst. (Japan) 14: 79-84.

Hiratsuka, N. and Chen, Z. C. 1991. A list of Uredinales collected from Taiwan. Trans. Mycol. Soc. Japan 32: 3-22.

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, Ibaraki, Japan.

Ito, S. 1938. Mycological flora of Japan 2(2). Basidiomycetes. Uredinales – Melampsoraceae. Yokendo, Tokyo. (In Japanese).

Jennings, D. M., Ford-lloyd, B. V. and Butler, G. M. 1989. An aniline blue squash technique for observation of urediniospore germ pores. Mycol. Res. 92: 230-251.

Jørstad, I. 1958. Parasitic fungi, chiefly Uredinales, collected in the Rocky

Mountains by Johannes Lid. Nyt. Mag. Bot. 6: 141-153.

Kamei, S. 1931. A new species of *Milesina* parasitic on *Polypodium vulgare* L. Transact. Sapporo Nat. Hist. Soc. 7: 27-34.

Kamei, S. 1932. On new species of heteroecious fern rusts. Transact. Sapporo Nat. Hist. Soc. 7: 161-174.

Kaneko, S. and Hiratsuka, N. 1980. Fungi inhabiting on fagaceous trees II, Host alternation of the beech rust, *Pucciniastrum fagi*. Trans. Mycol. Soc. Japan 21: 417-421.

Kaneko, S. and Hiratsuka, N. 1981. Notes on four tree rusts from Japan. Trans. Mycol. Soc. Japan 22: 219-230.

Kaneko, S. and Hiratsuka, N. 1982. Taxonomic significance of the urediniospore germ pores in the pucciniastraceous and melampsoraceous rust fungi. Trans. mycol. Soc. Japan 23: 201-210.

Kaneko, S. and Hiratsuka, N. 1983. Notes on the host alternation of *Puccinia daisenensis* and *Pucciniastrum fagi*. Trans. Mycol. Soc. Japan 24: 437-440.

Kaneko, S. and Hiratsuka, N. 1984. Jour. Jap. For. Soc. 66: 280-284.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotides sequences. Jour. Mol. Evol. 16: 111-120.

Klebahn, H. 1899. Kulturversuche mit heterocischen Rostpilzen. VII. Bericht (1898). Zeitschr. f. Pflanzenkr. 4: 14-26, 88-89, 137-160.

Ko, K. S. and Jung, H. S. 2001. Phylogenetic relationships of *Hapalopilus* and related genera inferred from mitochondrial small subunit ribosomal DNA sequences. Mycologia 93: 270-276.

Kühn, J. V. 1869. *Calyptospora* nov. gen. Uredinearum. Hedwigia 8: 81-82.

Kuprevich, V. F. and Tranzschel, V. G. 1957. Cryptogamic plants of the USSR, Vol. IV. Rust Fungi (1), Rust Fungi, family Melampsoraceae. Akad. Nauk SSSR.

Bot. Inst. Komarova. (Translated from Russian by Israel Program for Scientific Translations, Jerusalem, 1970).

Kurkela, T., Hanso, M. and Hantula, J. 1999. Differentiating characteristics between *Melampsoridium* rusts infecting birch and alder leaves. Mycologia 91:987–992

Laundon, G. F. 1963. Rust Fungi II: on Aceraceae, Actinidiaceae, Adoxaceae and Aizoaceae. in Mycological Paper, C. M. I. 91: 1-17

Liang, Y. M., Tian, C. M. and Kakishima, M. 2005. A new species of *Pucciniastrum* on *Enkianthus campanulatus* from Japan. Mycotaxon 92: 371-376

Liro, J. I. 1908. Uredineae Fennicae. Finnlands Rostsvampar. 1-642.

Maier, W., Begerow, D., Wei, M. and Oberwinkler, F. 2003. Phylogenetic of the rust fungi: an approach using nuclear large subunit ribosomal DNA sequence. Can. Jour. Bot 81:12–23.

Molnar, A. C. and Sivak, B. 1964. *Melampsora* infection of pine in British Columbia. Can. Jour. Bot. 42: 145-158.

Moss, E. H. 1926. The uredo stage of the pucciniastrese. Ann. Bot. 40: 813-847.

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.). CAB International, Wallingford.

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.

Otth, G. 1861. Ueber die Brand-und Rostpilze. Mitteil. Naturforsch. Ges. Bern, N. F. pp. 57-88.

Pady, S. M. 1933. Teliospore development in the *Pucciniastraceae.* Can. Jour. Res. 9: 458-485.

Parmelee, J. A. 1960. The fungi of Ontario. I. Uredinales. Can. Dep. Agr., Res. Br.,

Publ.

Parmelee, J. A. 1989. The rusts (Uredinales) of arctic Canada. Can. Jour. Bot. 67: 3315-3365.

Pei, M. H., Bayon, C. and Ruiz, C. 2005. Phylogenetic relationships in some *Melampsora* rusts on Salicaceae assessed using rDNA sequence information. Mycol. Res. 109: 401-409.

Rambaut, A. 2001. Se-Al; sequence alignment editor. Department of Zoology, University of Oxford, Oxford, UK.

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

Savile, D. B. O. 1962. Some fungal parasites of Onagraceae. Can. Jour. Bot. 40: 1385-1398.

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

Smith, J. A., Blanchette, R. A. and Newcombe, G. 2004. Molecular and morphological characterization of the willow rust fungus, Melampsora epitea, from arctic and temperate hosts in North America. Mycologia 96: 1330–1338

Stearn, W. T. 1995. Botanical Latin. Timber Press Inc., Portland, Oregon.

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 preat. Genes Genet. Syst. 71: 145-149.

Sydow, P. & H. 1912-1915. Monographia Uredinearum seu specierum omnium ad hunc usque diem descriptio et adumbratio systematica. III.

Swofford, D. L. 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. Science Press, Peking. (in Chinese).

Takamatsu, S. 2005. Basics of molecular phylogenetic analysis. Plant Protection 59: 64-69.

Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibett, D. S. and Fisher, M. C. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31: 21-32.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876-4882.

Tian, C. M., Shang, Y. Z., Zhuang, J.Y., Wang, Q. and Kakishima, M. 2004. Morphological and molecular phylogenetic analysis of *Melampsora* species on poplars in China. Mycoscience 45: 56–66.

Tranzschel, W. 1939. Conspectus uredinalium URSS. 426 pp. (In Russian).

Trotter, A. 1908-1914. Flora Italica Cryptogama, Uredinales. Soc. Bot. Ital. Flora Ital. Cryptogama. Pars I, Funfi, fasc.

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 regions of LSU rDNA. Jour. Gen. Plant Pathol. 67: 28-36.

Vogler, D. R., Bruns, T. D. 1998. Phylogenetic relationships among the pine stem rust (*Cronartium* nd *Peridermium* spp.). Mycologia 90: 244–257.

Weber, R. W. S., Webster, J. and 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, T. J.; Bruns, T., Lee, S. B. and 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, San Diego.

Wilson, M. and Henderson, D. M. 1966. British Rust Fungi. Cambridge University Press, Cambridge.

Wilkinson, L. 1989. Systat: the system for statistics. Version 5.2. edition. Systat, Inc. Evanston, IIlinois.

Wingfield, B. D., Ericson, L, Szaro. T. and Burdon, J. J. 2004. Phylogenetic patterns in the Uredinales. Australian Plant Pathology 33: 327–335.

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.

Ziller, W. G. 1974. The tree rusts of western Canada. Environ. Can., Can. For. Serv., Victoria, BC. Publ.

Appendix 1: Specimens and morphologocal characteristics of urediniospores in *Pucciniastrum* spp.

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
Pucciniastrum fagi								
	TSH-R1355	*Fagus crenata*	Niigata	Sept. 13, 1991	17.4-(21.1)-24.4	10.9-(12.4)-13.5	1.09 / 1.15	II
	TSH-R1376	*F. crenata*	Niigata	Sept. 14, 1991	16.5-(19.1)-22.2	11.5-(13.2)-14.8	1.01 / 1.23	II
	TSH-R9544	*F. crenata*	Mt. Kinpoku-san	Oct. 8, 1996	20.5-(24.6)-29.6	10.9-(12.3)-15.1	1.06 / 1.08	II, III
	TSH-R10182	*F. crenata*	Aomori	Sept. 25, 1997	22.6-(24.4)-28.4	10.9-(13.6)-17.2	1.09 / 1.14	II, III
	TSH-R10197	*F. crenata*	Akita	Sept. 25, 1997	18.3-(23.1)-29.8	11.3-(13.5)-15.9	1.07 / 1.1	II, III
	TSH-R12051	*F. crenata*	Mt. Fuji	Oct. 6, 1981	19.4-(23.2)-27.8	11.5-(13.5)-15.2	1.07 / 1.32	II
	TSH-R12727	*F. crenata*	Tochigi	Sept. 11, 1963	17.8-(24.0)-28.4	10.6-(13.5)-15.9	1.05 / 1.18	II
	TSH-R12803	*F. crenata*	Fukushima	Oct. 10, 1959	16.8-(20.0)-24.5	11.3-(12.4)-15.1	1.04 / 1.15	II, III
	TSH-R12805	*F. crenata*	Fukushima	Sept. 25, 1959	14.8-(22.9)-27.7	7.9-(12.0)-16.2	1.06 / 1.08	II
	TSH-R12820	*F. crenata*	Toyama	Aug. 24, 1961	17.6-(21.5)-24.6	9.8-(12.7)-15.4	1.04 / 1.05	II
	TSH-R13408	*F. crenata*	Akita	Aug. 26, 1964	18.2-(22.4)-27.3	9.6-(11.9)-15.6	1.08 / 1.1	II
	TSH-R13548	*F. crenata*	Tottori	Oct. 13, 1971	20.4-(23.8)-29.9	11.5-(13.2)-17.1	1.00 / 1.1	II, III
	TSH-R13554	*F. crenata*	Tottori	Oct. 13, 1971	18.3-(22.9)-30.1	13.0-(14.7)-17.6	1.03 / 1.23	II, III
	TSH-R13597	*F. crenata*	Tottori	Oct. 13, 1971	20.7-(23.3)-28.4	11.3-(13.8)-17.0	1.04 / 1.35	II, III
	TSH-R10374	*F. crenata*	Tochigi	Oct. 12, 2001	18.9-(23.8)-27.2	10.9-(13.0)-14.3	1.07 / 1.32	II, III
	TSH-R10685	*F. crenata*	Tochigi	Oct. 10, 2000	17.0-(21.4)-28.7	11.9-(13.5)-16.1	1.05 / 1.15	II, III
	TSH-R10724	*F. crenata*	Tochigi	Oct. 11, 2001	17.3-(20.1)-24.6	10.2-(12.2)-15.3	1.05 / 1.28	II, III
	TSH-R17558	*F. crenata*	Fukushima	Oct. 10, 1959	16.3-(21.1)-25.7	12.2-(13.8)-15.0	1.06 / 1.13	II, III
	TSH-R17559	*F. crenata*	Fukushima	Oct. 10, 1959	21.1-(23.7)-30.9	11.7-(13.4)-15.2	1.06 / 1.2	II, III
	TSH-R17560	*F. crenata*	Fukushima	Oct. 10, 1959	20.4-(21.6)-27.4	10.6-(14.8)-19.8	1.00 / 1.18	II, III
	TSH-R17561	*F. crenata*	Fukushima	Oct. 10, 1959	18.5-(22.2)-26.6	11.3-(13.7)-15.9	1.00 / 1.15	II, III
	TSH-R17562	*F. crenata*	Fukushima	Oct. 10, 1959	20.7-(23.7)-27.9	12.2-(14.7)-17.2	1.03 / 1.21	II, III
	TSH-R17563	*F. crenata*	Fukushima	Sept. 25, 1959	16.8-(20.7)-26.3	10.9-(11.8)-16.8	1.05 / 1.25	II, III
	TSH-R17564	*F. crenata*	Fukushima	Oct. 10, 1959	18.9-(23.4)-25.7	11.4-(13.6)-15.3	1.05 / 1.21	II, III
	TSH-R17565	*F. crenata*	Fukushima	Oct. 10, 1959	17.4-(22.1)-27.0	9.5-(12.7)-15.4	1.08 / 1.33	II, III
	TSH-R17566	*F. crenata*	Fukushima	Oct. 10, 1959	18.4-(23.5)-28.7	10.4-(12.6)-15.9	1.05 / 1.23	II, III
	TSH-R17569	*F. crenata*	Fukushima	Sept. 25, 1959	17.6-(20.4)-25.4	9.8-(11.6)-16.9	1.06 / 1.25	II, III
	TSH-R17572	*F. crenata*	Gunma	Jul. 17, 1968	18.2-(21.8)-26.6	10.4-(12.4)-15.8	1.08 / 1.08	II
	IBA 6307	*F. japonica*	Niigata	Oct. 6, 1992	15.7-(19.4)-23.5	11.3-(13.4)-17.2	1.02 / 1.25	II, III
	IBA 6322	*F. japonica*	Niigata	Oct. 6, 1992	17.4-(20.9)-24.9	10.6-(11.9)-14.2	1.00 / 1.31	II, III
	IBA 6331	*F. japonica*	Fukushima	Oct. 6, 1992	14.0-(20.3)-25.8	11.8-(13.4)-17.8	1.03 / 1.25	II, III

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore			Spore State
					length	width	wall thc / ds *2	
P. fagi								
	IBA 8206	*F. crenata*	Tochigi	Oct. 10, 1998	18.9-(23.1)-27.6	9.6-(11.7)-15.3	1.04 / 1.21	II, III
	IBA 8384	*F. crenata*	Tochigi	Sept. 18, 1999	17.6-(21.9)-26.3	8.7-(12.5)-14.8	1.02 / 1.28	II
	IBA 8372	*F. crenata*	Gunma	Sept. 17, 1999	18.1-(23.3)-30.8	8.8-(11.2)-13.6	1.08 / 1.22	II
	IBA 8400	*F. crenata*	Nara	Sept. 30, 1999	18.7-(23.6)-31.6	9.5-(12.3)-14.4	1.08 / 1.31	II, III
	IBA 8447	*F. crenata*	Shizuoka	Oct. 4, 1999	18.3-(23.5)-27.9	9.2-(10.9)-12.2	1.06 / 1.13	II, III
	TSH-R21242	*F. crenata*	Kitaibaraki ogawa	Oct. 12, 2003	20.2-(23.1)-26.9	12.1-(14.3)-17.2	1.03 / 1.11	II, III
	TSH-R21243	*F. crenata*	Kitaibaraki ogawa	Oct. 12, 2003	18.3-(21.6)-24.2	12.2-(13.9)-15.9	1.04 / 1.15	II, III
	TSH-R21244	*F. crenata*	Kitaibaraki ogawa	Oct. 12, 2003	18.9-(20.9)-24.4	12.8-(14.6)-17.0	1.03 / 1.21	II, III
	TSH-R21250	*F. crenata*	Kitaibaraki ogawa	Oct. 12, 2003	18.5-(20.7)-31.1	8.9-(11.3)-13.1	1.03 / 1.15	II, III
	TSH-R21253	*F. crenata*	Tochigi Nikko	Oct. 24, 2003	18.7-(23.3)-26.1	11.1-(14.3)-16.4	1.02 / 1.35	II, III
	TSH-R21254	*F. crenata*	Akita Tasirodake	Oct. 21, 2003	17.8-(24.4)-29.8	11.1-(14.7)-18.2	1.1 / 1.13	II, III
	TSH-R21258	*F. crenata*	Aomori	Oct. 23, 2003	19.4-(23.9)-28.3	10.1-(11.9)-14.6	1.00 / 1.28	II, III
	TSH-R21259	*F. crenata*	Aomori Kudoji Mt.	Oct. 22, 2003	17.4-(21.2)-25.9	13.3-(14.0)-15.5	1.01 / 1.36	II, III
	TSH-R21262	*F. crenata*	Aomori	Oct. 22, 2003	20.9-(23.9)-30.7	11.7-(13.6)-15.7	1.03 / 1.31	II, III
	TSH-R21263	*F. crenata*	Aomori Kudoji Mt.	Oct. 22, 2003	19.6-(22.2)-25.1	12.8-(13.9)-16.4	1.03 / 1.21	II, III
	TSH-R21264	*F. crenata*	Tochigi Nikko	Oct. 24, 2003	19.6-(22.9)-24.4	11.3-(13.4)-15.0	1.03 / 1.26	II, III
	TSH-R21265	*F. crenata*	Aomori	Oct. 23, 2003	19.4-(22.3)-25.7	12.2-(14.4)-16.5	1.04 / .12	II, III
	TSH-R21272	*F. crenata*	Iwate Attupikougenn	Oct. 19, 2003	19.5-(22.3)-25.4	11.8-(13.1)-15.2	1.03 / 1.18	II, III
	TSH-R21280	*F. crenata*	Akita Tasirodake	Oct. 21, 2003	22.4-(24.1)-29.4	13.4-(14.9)-16.3	1.06 / 1.32	II, III
	HH77766	*F. crenata*	Tottori	Oct. 9, 1979	18.9-(22.4)-28.2	10.0-(12.3)-13.9	1.07 / 1.08	II
	HH72962	*F. crenata*	Ehime Ishizuchi Mt.	Sept. 14, 1975	19.8-(23.4)-28.6	6.7-(10.5)-14.1	1.03 / 1.15	II
	HH72986	*F. crenata*	Gifu	Sept. 10, 1979	21.1-(23.2)-27.2	9.5-(12.4)-14.8	1.08 / 1.14	II
	HH103891	*F. crenata*	Kozuke	Sept. 5, 1950	19.6-(23.6)-28.5	12.4-(14.0)-16.7	1.07 / 1.32	II
	HH103886	*F. crenata*	Chikugo	Sept. 23, 1951	18.5-(23.4)-28.5	10.4-(13.2)-15.2	1.08 / 1.11	II
	HH103882	*F. crenata*	Yamagata	Sept. 25, 1962	16.1-(22.8)-26.1	7.8-(12.4)-14.8	1.06 / 1.15	II, III
	HH103883	*F. crenata*	Yamagata	Sept. 25, 1962	19.9-(22.8)-30.8	9.1-(11.7)-13.7	1.08 / 1.24	II, III
	HH103884	*F. crenata*	Yamagata	Sept. 25, 1962	19.4-(23.5)-30.9	11.7-(13.6)-17.2	1.03 / 1.12	II
	HH103885	*F. crenata*	Yamagata	Sept. 25, 1962	20.0-(23.6)-28.5	13.1-(14.8)-16.7	1.08 / 1.35	II
	HH103919	*F. crenata*	Fukushima	Oct. 11, 1959	18.6-(23.6)-28.5	12.4-(14.0)-16.7	1.07 / 1.16	II, III
	HH103920	*F. crenata*	Fukushima	Oct. 11, 1959	18.4-(21.8)-30.4	9.5-(12.6)-15.7	1.06 / 1.14	II, III
	HH103921	*F. crenata*	Fukushima	Oct. 10, 1959	18.3-(23.5)-30.7	8.2-(10.7)-13.5	1.05 / 1.21	II
	HH103922	F. crenata	Fukushima	Oct. 10, 1959	19.6-(23.6)-30.3	8.7-(11.6)-13.3	1.02 / 1.24	II, III

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. fagi								
	HH103912	*F. crenata*	Chiba	Oct. 24, 1967	19.6-(23.2)-28.5	8.2-(11.9)-14.8	1.06 / 1.1	II, III
	HH103913	*F. crenata*	Chiba	Oct. 24, 1967	17.6-(20.7)-27.6	11.7-(13.5)-16.3	1.02 / 1.18	II, III
	HH103914	*F. crenata*	Chiba	Oct. 24, 1967	16.7-(19.7)-24.6	11.7-(13.4)-15.0	1.05 / 1.11	II, III
	HH103915	*F. crenata*	Chiba	Oct. 24, 1967	16.1-(21.8)-28.3	9.1-(11.9)-15.0	1.06 / 1.33	II, III
	HH103908	*F. crenata*	Toyama	Aug. 24, 1961	16.7-(23.4)-29.4	10.0-(12.5)-15.9	1.08 / 1.21	II
	HH103909	*F. crenata*	Fukushima	Sept. 24, 1959	18.1-(21.7)-25.7	10.4-(12.9)-15.2	1.08 / 1.22	II
	HH103910	*F. crenata*	Tochigi	Sept. 11, 1963	20.2-(22.5)-29.7	10.0-(12.7)-14.8	1.09 / 1.14	II, III
	HH103911	*F. crenata*	Tochigi	Sept. 11, 1963	17.8-(21.4)-26.6	11.1-(12.9)-15.1	1.08 / 1.34	II, III
	HH103907	*F. crenata*	Fukushima	Sept. 25, 1959	17.2-(22.1)-27.6	8.7-(10.8)-13.5	1.06 / 1.32	II, III
	HH103904	*F. crenata*	Osumi	Oct. 24, 1939	16.5-(21.6)-26.3	11.3-(13.2)-15.7	1.09 / 1.11	II
	HH103899	*F. crenata*	Chiba	Oct. 24, 1967	17.8-(20.1)-23.9	10.0-(11.6)-13.5	1.07 / 1.15	II, III
	HH103901	*F. crenata*	Tottori	Oct. 13, 1966	18.3-(23.8)-28.1	10.2-(12.0)-15.0	1.02 / 1.24	II, III
	HH103845Holotyp	*F. sieboldi*	Iwate Mt. Rikuchu	Oct. 2, 1904	16.7-(21.4)-27.0	9.6-(13.0)-16.5	1.03 / 1.15	II, III
	HH103847	*F. sieboldi*	Nanshozan, Rikuchu	Oct. 11, 1903	18.1-(21.1)-30.5	10.9-(13.9)-16.1	1.03 / 1.35	II, III
	HH103896	*F. sieboldi*	Nanshozan, Rikuchu	Oct. 111903	18.1-(23.9)-28.4	7.8-(11.1)-15.2	1.06 / 1.24	II, III
	HH103897	*F. sieboldi*	Hakogamine, Rikuchu	Oct. 11905	16.5-(19.7)-33.5	10.2-(12.8)-17.0	1.07 / 1.12	II, III
	HH60241	*F. crenata*	Hakkoda Mt., Mutsu	Aug. 29, 1953	16.1-(23.0)-29.2	9.8-(13.7)-16.5	1.06 / 1.11	II
	HH90185	*F. crenata*	Kanagawa	Oct. 2, 1970	16.7-(22.0)-29.6	9.1-(11.5)-14.1	1.01 / 1.14	II
	HH90186	*F. crenata*	Kanagawa	Oct. 2, 1970	18.7-(22.6)-27.2	8.9-(11.2)-14.1	1.02 / 1.18	II
	HH103878	*F. crenata*	Fukushima	Oct. 10, 1959	19.1-(23.8)-30.5	8.7-(11.9)-15.4	1.01 / 1.13	II, III
	HH103875	*F. crenata*	Akita	Aug. 26, 1964	16.5-(20.8)-25.0	11.1-(12.9)-15.2	1.05 / 1.12	II
	HH89386	*F. crenata*	Kanagawa	Oct. 12, 1969	20.1-(22.3)-28.0	11.5-(12.8)-15.1	1.01 / 1.2	II, III
	HH89387	*F. crenata*	Kanagawa	Oct. 12, 1969	18.7-(21.2)-24.6	12.2-(13.4)-15.0	1.00 / 1.2	II, III
	HH103873	*F. crenata*	Kirishima Mt., Hyuga	Sept. 24, 1946	17.6-(23.0)-26.8	9.8-(11.7)-13.9	1.00 / 1.11	II
	HH103872	*F. crenata*	Kirishima Mt., Hyuga	Sept. 24, 1946	16.9-(22.1)-27.3	10.3-(11.3)-14.6	1.04 / 1.15	II, III
	HH103871	*F. crenata*	Kirishima Mt., Osumi	Oct. 24, 1939	18.5-(21.3)-27.4	9.4-(12.3)-14.4	1.03 / 1.32	II, III
	HH103867	*F. crenata*	Hikosan Mt., Buzen	Oct. 11, 1939	20.2-(22.0)-28.7	11.1-(13.6)-15.7	1.02 / 1.14	II, III
	HH103868	*F. crenata*	Hikosan Mt., Buzen	Oct. 9, 1939	19.8-(21.2)-27.6	9.8-(11.3)-15.7	1.03 / 1.3	II, III
	HH103869	*F. sieboldi*	Hikosan Mt., Buzen	Sept. 2, 1934	19,6-(22.0)-29.0	12.2-(13.5)-15.2	1.05 / 1.28	II, III
	HH103848	*F. japonica*	Saitama	Aug. 27, 1958	16.5-(21.2)-25.2	11.7-(13.8)-15.4	1.03 / 1.11	II
	HH103849	*F. japonica*	Saitama	Aug. 16, 1956	13.0-(19.1)-25.5	8.5-(13.4)-15.9	1.00 / 1.11	II
	HH103850	*F. japonica*	Saitama	Aug. 20, 1955	18.3-(20.8)-24.2	11.3-(13.3)-16.9	1.00 / 1.31	II

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. fagi								
	HH103859	*F. japonica*	Chiba	Oct. 24, 1967	16.1-(19.5)-21.8	8.7-(10.5)-11.9	1.04 / 1.12	II
	HH103860	*F. japonica*	Chiba	Oct. 24, 1967	17.2-(20.1)-22.2	11.9-(13.5)-15.9	1.01 / 1.14	II
	HH103858	*F. japonica*	Chiba	Oct. 24, 1967	14.6-(19.9)-23.7	11.1-(12.9)-14.8	1.02 / 1.2	II
	HH103857	*F. japonica*	Chiba	Oct. 24, 1967	16.5-(20.2)-24.8	10.9-(11.8)-14.2	1.08 / 1.2	II
	HH103856	*F. japonica*	Chiba	Oct. 24, 1967	18.1-(21.7)-25.5	9.8-(13.0)-15.5	1.02 / 1.34	II, III
	HH103851	*F. crenata*	Chiba	Oct. 24, 1967	16.7-(19.4)-24.7	11.6(12.4)-15.0	1.06 / 1.2	II, III
	HH103852	*F. japonica*	Chiba	Oct. 24, 1967	17.6-(20.1)-23.8	10.0-(11.6)-13.6	1.07 / 1.11	II, III
	HH103854	*F. japonica*	Chiba	Oct. 24, 1967	15.6-(21.6)-24.7	11.1-(12.6)-14.8	1.03 / 1.09	II
	HH103876	*F. crenata*	Akita	Aug. 26, 1964	17.2-(22.3)-26.6	8.8-(10.8)-13.7	1.07 / 1.1	II, III
	HH103877	*F. crenata*	Akita	Aug. 26, 1964	16.6-(21.4)-25.8	11.4-(13.1)-15.3	1.05 / 1.08	II
	HH99719	*F. crenata*	Yamaguchi	Oct. 9, 1974	16.3-(20.1)-24.7	11.3-(12.5)-15.1	1.04 / 1.32	II, III
	HH93113	*F. crenata*	Fukuoka	Oct. 1, 1963	14.7-(22.5)-27.2	7.9-(12.0)-16.2	1.05 / 1.2	II, III
	HH61376	*F. crenata*	Yamanashi	Oct. 22, 1952	17.5-(21.5)-24.5	9.8-(12.6)-15.5	1.04 / 1.24	II, III
	HH61374	*F. crenata*	Yamanashi	Oct. 22, 1952	18.8-(23.5)-25.6	11.2-(12.8)-16.2	1.05 / 1.18	II, III
	HH61391	*F. crenata*	Yamanashi	Oct. 22, 1952	15.4-(23.2)-27.7	10.8-(13.2)-15.2	1.04 / 1.25	II, III
	HH103887	*F. crenata*	Yamanashi	Oct. 22, 1952	16.6-(21.4)-25.8	9.8-(11.6)-16.9	1.08 / 1.14	II, III
	HH103888	*F. crenata*	Yamanashi	Oct. 22, 1952	17.2-(22.5)-25.6	10.4-(12.4)-15.8	1.08 / 1.15	II
	HH103889	*F. crenata*	Yamanashi	Oct. 22, 1952	15.7-(19.5)-23.7	11.3-(13.4)-17.2	1.09 / 1.25	II, III
	TSH-R21245	*F. crenata*	Kitaibaraki ogawa	Oct. 12, 2003	17.9-(20.5)-24.2	11.8-(14.6)-16.8	1.02 / 1.21	II, III
	TSH-R21246	*F. crenata*	Kitaibaraki ogawa	Oct. 12, 2003	18.1-(21.6)-23.8	10.2-(13.6)-15.3	1.05 / 1.26	II, III
	TSH-R21247	*F. crenata*	Kitaibaraki ogawa	Oct. 12, 2003	16.9-(19.8)-25.2	10.5-(12.6)-15.7	1.03 / 1.21	II, III
	TSH-R21266	*F. crenata*	Aomori Hirosaki	Oct. 22, 2003	19.2-(22.4)-24.8	11.8-(13.7)-16.4	1.04 / 1.18	II, III
	TSH-R21271	*F. crenata*	Iwate Attupikougenn	Oct. 19, 2003	16.8-(21.5)-26.8	9.6-(12.2)-16.8	1.08 / 1.13	II, III
	TSH-R21276	*F. crenata*	Akita Tasirodake	Oct. 21, 2003	20.9-(23.9)-27.7	11.7-(13.6)-15.7	1.03 / 1.11	II, III
	TSH-R21277	*F. crenata*	Akita Tasirodake	Oct. 21, 2003	19.6-(22.8)-25.4	10.6-(12.8)-16.0	1.03 / 1.28	II, III
	TSH-R21260	*F. crenata*	Fukushima	Oct. 23, 2003	17.4-(22.3)-26.7	12.6-(14.4)-16.8	1.04 / 1.25	II, III
	TSH-R21261	*F. crenata*	Fukushima	Oct. 23, 2003	19.4-(21.3)-25.7	11.2-(13.4)-14.9	1.03 / 1.11	II, III
P. sp.								
	HH103894	*F. crenata*	Iwashiro	Sept. 5, 1950	23.8-(28.2)-33.3	15.0-(17.2)-20.2	1.08 / 0.9	II
	HH90187	*F. crenata*	Kanagawa	Oct. 2, 1970	23.3-(28.0)-31.6	14.6-(17.8)-20.5	1.09 / 0.85	II
	HH89388	*F. crenata*	Kanagawa	Oct. 12, 1969	25.3-(31.4)-37.7	16.1-(19.1)-26.3	1.04 / 0.95	II, III
	HH103874	*F. crenata*	Akita	Aug. 26, 1964	19.6-(26.7)-34.8	16.1-(18.7)-21.3	1.01 / 0.83	II
	HH89573	*F. crenata*	Kanagawa	Oct. 12, 1969	24.2-(29.3)-39.0	15.0-(17.1)-20.5	1.01 / 0.95	II

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. kusanoi								
	TSH-R21251	*Clethra barbinervis*	Kitaibaraki ogawa	Oct. 12, 2003	13.8-(21.4)-23.1	13.0-(16.2)-18.1	1.2 / 1.35	II, III
	TSH-R21252	*C. barbinervis*	Kitaibaraki ogawa	Oct. 12, 2003	20.0-(20.9)-22.0	16.1-(17.0)-17.6	1.2 / 1.24	II, III
	TSH-R21281	*C. barbinervis*	Tochigi Nikko	Oct. 24, 2003	19.4-(20.8)-27.9	14.3-(16.6)-18.5	1.00 / 1.34	II, III
	TSH-R21282	*C. barbinervis*	Tochigi Nikko	Oct. 24, 2003	18.6-(21.1)-26.8	14.8-(15.7)-19.6	1.00 / 1.16	II, III
	TSH-R21283	*C. barbinervis*	Tochigi Nikko	Oct. 24, 2003	18.3-(22.0)-25.9	14.3-(16.9)-20.0	1.00 / 1.18	II, III
	TSH-R21284	*C. barbinervis*	Akita Tasirodake	Oct. 21, 2003	17.4-(21.1)-25.6	15.1-(16.4)-18.2	1.00 / 1.21	II, III
	TSH-R21298	*C. barbinervis*	Nagano Sinnsyuu	Nov. 7, 2003	19.8-(24.2)-27.9	15.3-(16.2)-18.8	1.15 / 1.16	II, III
	TSH-R21299	*C. barbinervis*	Nagano Sinnsyuu	Nov. 7, 2003	18.6-(23.2)-26.8	14.8-(16.0)-18.2	1.00 / 1.18	II, III
	TSH-R21300	*C. barbinervis*	Nagano Sinnsyuu	Nov. 7, 2003	15.7-(20.6)-25.2	13.8-(17.3)-19.4	1.10 / 1.32	II, III
	TSH-R21306	*C. barbinervis*	Tochigi Nikko	Oct. 24, 2003	18.2-(21.7)-24.3	15.8-(17.4)-18.9	1.00 / 1.28	II, III
	TSH-R18019	*C. barbinervis*	Fukushima	Sept. 25, 1959	16.5-(22.0)-26.1	13.9-(16.2)-19.7	1.00 / 1.24	II
	TSH-R1458	*C. barbinervis*	Ibaraki Tsukuba	Nov. 17, 1993	15.1-(23.1)-26.8	12.8-(15.2)-17.9	1.00 / 1.34	II, III
	TSH-R1525	*C. barbinervis*	Toyama	Sept. 28, 1995	19.6-(21.3)-22.6	14.8-(16.7)-18.1	1.08 / 1.15	II
	TSH-R12673	*C. barbinervis*	Fukushima	Sept. 25, 1959	20.0-(23.2)-27.5	17.1-(18.3)-21.8	1.10 / 1.16	II
	TSH-R12674	*C. barbinervis*	Nagano	Sept. 3, 1963	15.3-(22.1)-26.5	14.8-(17.4)-21.3	1.20 / 1.30	II
	TSH-R12675	*C. barbinervis*	Yamanashi	Sept. 2, 1958	19.4-(23.8)-27.7	18.5-(19.4)-18.5	1.02 / 1.21	II
	TSH-R12685	*C. barbinervis*	Yamanashi	Sept. 2, 1958	16.3-(21.2)-25.3	13.8-(15.8)-19.6	1.05 / 1.18	II
	TSH-R12720	*C. barbinervis*	Toyama	Aug. 24, 1961	18.1-(21.9)-27.4	12.7-(16.5)-20.0	1.02 / 1.16	II
	TSH-R12812	*C. barbinervis*	Fukushima	Sept. 11, 1959	20.4-(23.0)-28.1	13.5-(16.2)-21	1.20 / 1.32	II, III
	TSH-R12813	*C. barbinervis*	Fukushima	Sept. 25, 1959	17.6-(21.7)-25.8	12.6-(14.5)-17.0	1.00 / 1.35	II, III
	TSH-R12815	*C. barbinervis*	Nagano	Sept. 3, 1963	22.3-(24.1)-27.5	16.1-(19.2)-21.7	1.02 / 1.21	II
	TSH-R12816	*C. barbinervis*	Nagano	Sept. 2, 1963	20.5-(23.2)-28.1	13.7-(14.3)-17.5	1.10 / 1.22	II
	TSH-R3846	*C. barbinervis*	Shizuoka	Sept. 6, 1998	16.1-(21.1)-24.2	13.5-(15.4)-17.4	1.25 / 1.16	II
	TSH-R3847	*C. barbinervis*	Shizuoka	Sept. 27, 1998	15.8-(22.0)-24.8	12.8-(14.8)-17.2	1.10 / 1.25	II, III
	TSH-R3848	*C. barbinervis*	Shizuoka	Nov. 8, 1998	16.6-(21.8)-25.6	14.2-(15.8)-18.2	1.15 / 1.18	II, III
	TSH-R13126	*C. barbinervis*	Tottori	Oct. 14, 1966	20.4-(22.6)-27.3	13.5-(16.5)-20.1	1.05 / 1.24	II, III
	TSH-R13903	*C. barbinervis*	Tottori	Oct. 13, 1971	18.8-(21.4)-23.7	15.7-(19.5)-21.8	1.20 / 1.28	II, III
	TSH-R14908	*C. barbinervis*	Ibaraki Tsukuba	Oct. 17, 1979	15.6-(22.3)-27.4	13.7-(17.1)-21.1	1.15 / 1.32	II, III
	TSH-R17552	*C. barbinervis*	Fukushima	Sept. 25, 1959	15.1-(20.8)-26.8	12.8-(15.1)-20.5	1.00 / 1.33	II, III
	TSH-R17553	*C. barbinervis*	Fukushima	Sept. 25, 1959	16.3-(21.4)-24.8	14.6-(15.8)-19.6	1.10 / 1.18	II, III
	HU21509	*C. barbinervis*	Miyagi	Oct. 4, 1992	21.5-(24.9)-28.1	17.6-(20.8)-22.8	1.20 / 1.25	II, III
	HU22616	*C. barbinervis*	Miyagi	Oct. 4, 1992	19.2-(23.6)-27.5	15.4-(19.8)-20.8	1.20 / 1.21	II, III
	IBA 5472	*C. barbinervis*	Tochigi Nikko	Aug. 26, 1990	18.9-(22.6)-26.6	15.4-(17.6)-20.7	1.04 / 1.20	II

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. kusanoi								
	IBA 6679	*C. barbinervis*	Kitaibaraki	Sept. 11, 1992	18.0-(22.5)-25.0	13.0-(18.9)-20.9	1.02 / 1.20	II
	IBA 7663	*C. barbinervis*	Miyazaki	Oct. 25, 1995	16.8-(23.6)-27.5	14.2-(16.8)-18.8	1.10 / 1.35	II, III
	IBA 8402	*C. barbinervis*	Nara	Oct. 1, 1999	17.1-(20.2)-24.3	13.8-(16.5)-18.6	1.05 / 1.28	II, III
	HH103864	*C. barbinervis*	Yamanashi	Aug. 24, 1950	18.7-(21.7)-24.2	15.0-(17.3)-18.9	1.08 / 1.25	II
	HH103685	*C. barbinervis*	Aki	Nov. 9, 1939	16.5-(20.2)-25.1	14.3-(16.8)-19.2	1.10 / 1.25	II, III
	HH103686	*C. barbinervis*	Sagami	Oct. 21, 1953	18.8-(21.3)-26.8	13.5-(15.4)-17.6	1.10 / 1.21	II, III
	HH103687	*C. barbinervis*	Hyuga	Nov. 11, 1951	17.8-(21.2)-26.1	12.8-(14.5)-16.1	1.10 / 1.15	II, III
	HH103672	*C. barbinervis*	Mayasaki	Oct. 20, 1938	19.2-(22.3)-27.8	15.2-(16.4)-19.1	1.10 / 1.28	II, III
	HH103673	*C. barbinervis*	Hoki Mt. Daisen	Aug. 20, 1930	16.5-(21.0)-26.1	13.9-(16.2)-19.9	1.10 / 1.18	II
	HH103674	*C. barbinervis*	Hoki Mt. Daisen	Nov. 9, 1930	16.8-(21.0)-25.8	12.3-(14.2)-17.1	1.05 / 1.26	II, III
	HH103675	*C. barbinervis*	Hoki Mt. Daisen	Nov. 10, 1929	15.8-(19.6)-24.6	11.7-(15.2)-17.8	1.15 / 1.28	II, III
	HH103680	*C. barbinervis*	Hiroshima	Oct. 6, 1947	18.3-(21.9)-28.3	11.9-(14.6)-16.7	1.20 / 1.26	II
	HH103681	*C. barbinervis*	Saitama	Nov. 10, 1956	15.7-(22.8)-27.2	13.7-(17.5)-21.1	1.10 / 1.16	II, III
	HH103682	*C. barbinervis*	Saitama	Nov. 10, 1956	18.1-(22.4)-25.1	13.1-(18.9)-20.8	1.00 / 1.18	II, III
	HH102230	*C. barbinervis*	Tottori	Jun. 12, 1933	20.4-(22.2)-23.7	17.4-(18.9)-20.4	1.15 / 1.21	II
	HH60837	*C. barbinervis*	Tottori	Aug. 27, 1976	18.6-(22.2)-24.6	15.3-(17.1)-18.9	1.02 / 1.25	II
	HH61234	*C. barbinervis*	Tottori	Oct. 26, 1976	20.4-(24.0)-28.9	15.0-(17.1)-20.0	1.20 / 1.21	II, III
	HH61235	*C. barbinervis*	Tottori	Oct. 21, 1976	20.8-(22.5)-27.8	13.5-(16.8)-20.1	1.10 / 1.18	II, III
	HH98858	*C. barbinervis*	Tottori	Oct. 1, 1975	20.2-(22.6)-27.2	13.5-(16.7)-20.0	1.18 / 1.32	II
	HH99646	*C. barbinervis*	Tottori	Oct. 3, 1975	16.8-(19.8)-25.4	12.8-(15.2)-18.8	1.20 / 1.15	II
	HH98677	*C. barbinervis*	Tottori	Oct. 11, 1975	22.2-(24.9)-27.6	17.4-(19.3)-21.1	1.25 / 1.16	II
	HH98678	*C. barbinervis*	Tottori	Oct. 11, 1975	23.3-(24.6)-28.1	16.1-(19.7)-22.6	1.10 / 1.34	II
	HH103664	*C. barbinervis*	Fukushima	Sept. 25, 1959	16.2-(21.5)-27.3	13.2-(17.1)-21.3	1.25 / 1.32	II
	HH103665	*C. barbinervis*	Fukushima	Sept. 25, 1959	22.4-(23.9)-27.0	16.1-(19.4)-21.3	1.20 / 1.28	II
	HH103666	*C. barbinervis*	Fukushima	Sept. 25, 1959	21.2-(23.1)-28.0	14.1-(15.8)-20.4	1.20 / 1.25	II, III
	HH103667	*C. barbinervis*	Fukushima	Sept. 25, 1959	20.4-(23.5)-27.5	15.7-(19.3)-21.8	1.15 / 1.18	II
	HH103660	*C. barbinervis*	Tottori	Oct. 16, 1959	17.2-(19.8)-23.6	17.8-(20.0)-22.8	1.10 / 1.20	II, III
	HH103661	*C. barbinervis*	Tottori	Oct. 17, 1959	18.2-(23.6)-27.2	15.2-(17.8)-21.0	1.25 / 1.30	II, III
	HH103662	*C. barbinervis*	Tottori	Oct. 17, 1959	18.8-(21.6)-27.5	14.8-(16.6)-19.8	1.30 / 1.18	II, III
	HH103663	*C. barbinervis*	Kagoshima	Jul. 1, 1953	18.9-(21.5)-27.5	15.7-(19.3)-21.8	1.21 / 1.20	II
	HH103656	*C. barbinervis*	Nagano	Sept. 12, 1963	19.8-(22.6)-18.2	12.1-(15.6)-18.8	1.20 / 1.35	II
	HH103657	*C. barbinervis*	Nagano	Sept. 12, 1963	17.8-(21.2)-25.3	14.2-(16.8)-18.5	1.20 / 1.30	II, III

(continued)

Species	Voucher specimen ∗1	Host plant	Locality	Date collected	urediniospore			Spore State
					length	width	wall thc / ds ∗2	
P. kusanoi								
	HH103658	*C. barbinervis*	Tottori	Oct. 16, 1963	18.1-(23.1)-25.6	15.2-(18.1)-20.8	1.10 / 1.26	II, III
	HH103659	*C. barbinervis*	Tokushima	Oct. 10, 1963	16.8-(21.4)-25.5	13.6-(16.5)-19.6	1.25 / 1.32	II, III
	HH103688	*C. barbinervis*	Kai Mt. Fuji	Aug. 24, 1950	19.1-(21.9)-25.7	15.7-(17.2)-18.7	1.18 / 1.24	II
	HH103689	*C. barbinervis*	Kai Mt. Fuji	Aug. 24, 1950	19.4-(21.9)-27.4	15.9-(18.5)-20.4	1.20 / 1.16	II
	HH103690	*C. barbinervis*	Suruga	Aug. 15, 1950	18.2-(20.8)-24.3	12.6-(14.8)-18.2	1.15 / 1.18	II
	HH103691	*C. barbinervis*	Hyugo	Sept. 24, 1946	16.2-(19.2)-23.0	12.1-(14.8)-19.6	1.25 / 1.2	II, III
	HH103696	*C. barbinervis*	Kouchi	Oct. 18, 1940	19.8-(23.2)-27.2	15.0-(18.4)-20.4	1.10 / 1.20	II, III
	HH103697	*C. barbinervis*	Ehime	Oct. 17, 1930	17.3-(21.4)-24.0	13.2-(17.2)-21.1	1.10 / 1.21	II, III
	HH103698	*C. barbinervis*	Kouchi	Sept. 22, 1930	18.5-(22.3)-24.5	15.4-(17.6)-18.8	1.20 / 1.18	II
	HH103692	*C. barbinervis*	Tottori	Oct. 14, 1929	19.8-(22.3)-26.5	14.2-(16.8)-19.2	1.28 / 1.25	II, III
	HH98679	*C. barbinervis*	Tottori	Oct. 11, 1975	18.5-(23.4)-28.4	12.2-(15.4)-17.6	1.24 / 1.24	II
	HH99601	*C. barbinervis*	Tottori	Nov. 8, 1975	17.8-(21.7)-24.1	14.0-(15.6)-19.2	1.20 / 1.31	II, III
	HH196092	*C. barbinervis*	Yamanashi	Sept. 2, 1960	15.8-(20.1)-26.6	13.4-(17.2)-21.5	1.10 / 1.28	II
	HH98629	*C. barbinervis*	Hyugo	Sept. 24, 1975	18.9-(21.9)-27.4	13.5-(16.7)-21.3	1.20 / 1.32	II
	HH98635	*C. barbinervis*	Hyugo	Oct. 22, 1975	20.1-(22.8)-27.2	15.1-(18.8)-21.4	1.20 / 1.33	II, III
	HH69387	*C. barbinervis*	Tottori	Sept. 21, 1979	18.9-(22.8)-25.0	14.6-(16.4)-18.7	1.15 / 1.21	II
	HH103700	*C. barbinervis*	Toyama	Aug. 24, 1961	19.4-(22.1)-25.7	13.9-(17.2)-19.1	1.20 / 1.20	II
	HH103701	*C. barbinervis*	Fukushima	Sept. 25, 1959	21.1-(23.6)-28.9	16.1-(17.8)-20.0	1.10 / 1.18	II
	HH103702	*C. barbinervis*	Chiba	Oct. 24, 1967	16.8-(20.1)-24.5	14.3-(18.6)-21.2	1.18 / 1.18	II, III
	HH103676Holotyp	*C. barbinervis*	Iwate	Sept. 4, 1901	17.4-(23.7)-28.3	15.6-(18.6)-20.0	1.15 / 1.25	II, III
	HH103678	*C. barbinervis*	Tokyo	Oct. 311902	15.6-(19.2)-23.8	13.2-(15.6)-20.1	1.21 / 1.22	II, III
	HH103608	*C. barbinervis*	Tottori	Oct. 1, 1929	21.8-(24.2)-28.1	14.1-(16.4)-20.4	1.18 / 1.20	II, III
	HH103653	*C. barbinervis*	Chiba	Oct. 24, 1967	17.1-(20.3)-25.2	16.4-(18.8)-22.1	1.20 / 1.31	II, III
	HH93119	*C. barbinervis*	Fukuoka	Aug. 3, 1963	16.4-(21.0)-26.4	14.0-(16.1)-19.8	1.21 / 1.18	II
	HH103648	*C. barbinervis*	Fukuoka	Nov. 5, 1939	17.2-(19.1)-24.2	12.8-(15.3)-18.8	1.28 / 1.20	II, III
	HH103650	*C. barbinervis*	Fukuoka	Oct. 21, 1939	18.3-(22.4)-17.1	14.2-(16.7)-20.5	1.25 / 1.20	II, III
	HH97067	*C. barbinervis*	Tochigi	Nov. 1, 1968	19.2-(23.1)-26.2	15.1-(17.2)-21.0	1.20 / 1.21	II, III
	HH98628	*C. barbinervis*	Hyogo	Sept. 24, 1975	19.8-(23.5)-27.2	18.5-(19.7)-21.8	1.20 / 1.25	II
	HH98627	*C. barbinervis*	Hyugo	Sept. 24, 1975	16.6-(19.1)-22.2	11.9-(14.5)-19.4	1.21 / 1.15	II
	HH103646	*C. barbinervis*	Kagoshima	Oct. 24, 1939	15.8-(19.6)-25.8	14.2-(17.1)-20.5	1.20 / 1.16	II, III
	HH103645	*C. barbinervis*	Kagoshima	Oct. 25, 1939	17.8-(21.3)-26.5	13.9-(16.8)-19.4	1.10 / 1.34	II, III
	HH103644	*C. barbinervis*	ooyita	Oct. 18, 1939	20.2-(23.1)-27.3	15.8-(19.2)-21.2	1.20 / 1.35	II, III
	HH103636	*C. barbinervis*	yishikawa	Oct. 19, 1931	16.2-(22.0)-25.8	12.3-(15.2)-18.1	1.10 / 1.21	II, III
	HH103637	*C. barbinervis*	yishikawa	Oct. 19, 1931	18.2-(23.4)-28.1	16.2-(17.6)-20.1	1.20 / 1.18	II, III

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. kusanoi								
	HH103632	*C. barbinervis*	Tokyo	Nov. 15, 1959	16.4-(20.2)-24.3	13.4-(16.3)-20.8	1.20 / 1.18	II, III
	HH103626	*C. barbinervis*	Nagano	Sept. 2, 1963	15.7-(22.5)-27.4	13.7-(17.5)-21.1	1.20 / 1.21	II
	HH103628	*C. barbinervis*	Nagano	Sep. 2, 1963	16.8-(20.1)-27.2	14.2-(16.5)-21.5	1.20 / 1.16	II
	HH103621	*C. barbinervis*		Sep. 23, 1933	21.8-(24.3)-27.3	15.4-(17.8)-20.7	1.10 / 1.18	II
P.actinidiae								
	IBA 7716	*Actinidia rufa*	Okinawa	Dec. 6, 1995	16.7-(20.9)-25.5	11.7-(14.8)-20.4	1.30 / 1.25	II
	IBA 7700	*A. rufa*	Okinawa	Dec. 4, 1995	18.53-(22.8)-27.9	13.7-(16.3)-23.5	1.40 / 1.39	II
	IBA 8002	*A. rufa*	Okinawa	Nov. 11, 1997	18.9-(23.4)-30.9	13.1-(16.5)-19.2	1.55 / 1.35	II, III
	HH98823	*A. rufa*	Okinawa	Jan. 10, 1975	19.8-(23.6)-32.4	11.7-(15.3)-17.8	1.42 / 1.22	II
	HH98824	*A. rufa*	Okinawa	Jan. 9, 1975	17.4-(22.2)-25.5	13.5-(15.8)-18.5	1.40 / 1.21	II
	HH98825	*A. rufa*	Okinawa	Jan. 9, 1975	19.4-(23.9)-28.9	12.9-(15.1)-17.2	1.28 / 1.25	II
	HH102319Holotyp	*A. arguta*	Tosa	Nov. 21, 1945	18.0-(23.8)-27.8	13.0-(15.1)-18.2	1.50 / 1.31	II, III
	HH102308	*A. rufa*	Okinawa	Sept. 22, 1954	20.1-(25.8)-29.4	11.9-(16.7)-20.27	1.30 / 1.33	II
	HH102309	*A. rufa*	Okinawa	Dec. 16, 1955	17.2-(22.1)-28.3	14.4-(17.6)-21.3	1.30 / 1.25	II
	HH102310	*A. rufa*	Okinawa	Dc. 17, 1955	18.1-(23.8)-28.7	10.7-(13.4)-16.1	1.15 / 1.18	II
	HH102313	*A. rufa*	Okinawa	Dec. 16, 1955	17.0-(21.8)-26.1	12.2-(16.9)-21.3	1.20 / 1.25	II
	HH102318	*A. arguta*	Hiuga	Nov. 10, 1951	17.0-(20.9)-25.9	13.5-(15.8)-18.8	1.30 / 1.35	II
	HH102322	*A. rufa*	Okinawa	Dec. 30, 1954	12.2-(18.7)-23.3	10.4-(14.6)-17.6	1.10 / 1.25	II
	HH102324	*A. rufa*	Okinawa	Jan. 7, 1955	13.3-(22.0)-27.5	11.9-(15.2)-19.4	1.15 / 1.21	II
	HH102329	*A. rufa*	Okinawa	Sept. 14, 1954	18.1-(21.7)-25.9	12.8-(17.0)-19.6	1.31 / 1.25	II
	HH102312	*A. rufa*	Okinawa	Dec. 22, 1955	16.6-(20.1)-25.3	12.4-(15.2)-20.5	1.20 / 1.18	II
	HH102307	*A. rufa*	Okinawa	Dec. 24, 1955	16.6-(19.1)-24.3	11.4-(15.8)-19.6	1.25 / 1.18	II
	IBA 4927	*A.. Arguta*	Okinawa	Oct. 30, 2003	21.9-(26.9)-31.3	12.8-(19.4)-24.7	1.20 / 1.25	II
	HH102311	*A. hypoleuca*	Okinawa	Oct. 30, 2003	18.6-(24.1)-27.9	14.1-(16.9)-18.6	1.45 / 1.30	II
	HH102515	*A. rufa*	Okinawa	Dec. 29, 1954	21.3-(26.9)-30.8	15.6-(18.4)-21.1	1.30 / 1.18	II
	HH102326	*A. rufa*	Okinawa	Sept. 23, 1954	17.3-(22.4)-27.9	11.9-(17.8)-21.5	1.50 / 1.20	II
	HH102325	*A. rufa*	Okinawa	Oct. 14, 1953	19.3-(22.4)-24.9	12.4-(15.6)-18.2	1.25 / 1.25	II
	HH102327	*A. rufa*	Osumi	Dec. 30, 1952	20.2-(24.2)-27.8	15.2-(17.5)-22.1	1.35 / 1.30	II
	HH102328	*A. rufa*	Okinawa	Jan. 6, 1955	16.3-(22.3)-26.0	13.6-(16.7)-20.6	1.30 / 1.25	II
	HH102330	*A. rufa*	Okinawa	Sept. 16, 1954	21.5-(26.8)-34.1	19.9-(22.8)-27.5	1.30 / 1.20	II
P. corni								
	IBA7671	*Cornus kuosa*	Miyazaki	Oct. 25, 1995	20.1-(21.0)- 22.7	15.3 -(16.9)- 19.8	1.00 / 1.30	II,III
	TSH-R12686	*C. kousa*	Fukushima	Sept. 25, 1959	18.3 -(20.1)- 22.45	12.4 -(14.6)- 16.6	1.03 / 1.25	II
	TSH-R13510	*C. kousa*	Tottori	Oct. 13, 1971	20.3 -(22.6)- 26.8	15.1 -(18.1)- 19.6	1.10 / 1.21	II,III

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. corni								
	HH97332	*C. florida*	Tottori	Jul. 7, 1975	18.7 -(22.2)- 25.5	15.3 -(17.4)- 20.0	1.20 / 1.28	II
	HH103927	*C. kousa*	Tosa	Sept. 22, 1930	18.9 -(24.1)- 29.4	15.7 -(19.1)- 22.7	1.20 / 1.221	II
	HH103951	*C. kousa*	Fukui	Oct. 17, 1931	20.9 -(23.1)- 25.5	13.7 -(15.5)- 16.3	1.15 / 1.32	II,III
	HH103953	*C. kousa*	Rikuchu	Oct. 2, 1910	18.5 -(22.4)- 24.2	15.3 -(16.7)- 20.3	1.27 / 1.25	II,III
	HH103961	*C. kousa*	Hyougo	Oct. 23, 1938	17.6 -(19.1)- 20.9	14.6 -(_16.3)- 17.2	1.04 / 1.20	II
	HH103963	*C. brachypoda*	Kiuga	Nov. 11, 1951	19.4 -(23.7)- 27.9	15.9 -(18.4)- 21.3	1.20 / 1.28	II,III
	HH89392	*Cynoxylon japonica*	Kanagawa	Oct. 12, 1969	20.7 -(24.4)- 26.8	14.4 -(18.7)- 21.8	1.22 / 1.18	II,III
	HH90023	*C. japonica*	Kanagawa	Oct. 12, 1969	18.7 -(23.8)- 27.2	7.4 -(14.8)- 19.2	1.20 / 1.33	II,III
	HH90195	*C. japonica*	Kanagawa	Oct. 2, 1970	18.9 -(23.1)- 27.7	13.1 -(16)- 18.9	1.15 / 1.21	II
	HH90199	*C. japonica*	Kanagawa	Oct. 2, 1970	19.2 -(22.8)- 27.2	12.2 -(14.8)- 17.0	1.00 / 1.25	II
	HH103929	*C. japonica*	Yamanashi	Aug. 24, 1950	16.6 -(21.3)- 23.7	15.3 -(17.3)- 20.1	1.16 / 1.25	II
	HH103930	*C. japonica*	Yamanashi	Aug. 24, 1950	16.6 -(19.6)- 22.7	14.4 -(16.1)- 17.6	1.15 / 1.28	II
	HH103931	*C. japonica*	Tottori	Oct. 17, 1959	17.2 -(21.0)- 25.1	11.1 -(15.2)- 17.8	1.00 / 1.30	II,III
	H103932	*C. japonica*	Tottori	Oct. 17, 1959	17.2 -(22.9)- 31.4	9.6 - (14.5)- 20.1	1.00 / 1.20	II,III
	HH103933	*C. japonica*	Tottori	Oct. 18, 1959	15.9 -(19.5)- 22.0	11.5 -(14.5)- 17.2	1.05 / 1.30	II,III
	HH103934	*C. japonica*	Tottori	Oct. 18, 1959	18.7 -(20.3)- 24.6	14.2 -(15.8)- 17.4	1.08 / 1.15	II,III
	HH103937	*C. japonica*	Tottori	Oct. 17, 1959	23.7 -(26.2)- 29.9	15.5 -(17.3)- 18.5	1.12 / 1.20	II,III
	HH103941	C. japonica	Hyuga	Sept. 6, 1951	20.5 -(23.1)- 26.6	13.5 -(17.4)- 20.1	1.15 / 1.18	II,III
	HH103943	*C. japonica*	Fukushima	Sept. 24, 1959	20.3 -(21.8)- 24.6	12.8 -(14.2)- 15.7	1.00 / 1.20	II,III
	HH103942	*C. japonica*	Fukushima	Oct. 11, 1959	19.2 -(21.0)- 22.9	14.6 -(14.8)- 15.0	1.00 / 1.25	II,III
	HH103947	*C. japonica*	Fukuoka	Sept. 23, 1951	22.4 -(25.7)- 31.3	15.7 -(18.2)- 20.9	1.20 / 1.28	II,III
	HH103948	*C. japonica*	Niigata	Oct. 24, 1911	19.4 -(21.5)- 23.2	14.0 -(15.3)- 18.2	1.10 / 1.30	II,III
	HH103949	*C. japonica*	Kai	Aug. 24, 1950	15.5 -(19.1)- 20.9	14.9 -(17.4)- 19.4	1.20 / 1.30	II,III
	HH103955	*C. japonica*	Kai	Aug. 24, 1950	16.6 -(22.4)- 28.3	13.5 -(16.2)- 19.8	1.15 / 1.31	II, III
	HH90196	*C. japonica*	Kanagawa	Oct. 2, 1970	17.3 -(22.7)- 26.8	13.2 -(16.0)- 18.3	1.10 / 1.20	II,III
	HH90197	*C. japonica*	Kanagawa	Oct. 2, 1970	17.6 -(21.8)- 24.3	14.0 -(15.6)- 17.3	1.10 / 1.25	II,III
	HH89579	*C. japonica*	Kanagawa	Oct. 12, 1969	18.1 -(23.4)- 26.9	14.7 -(18.6)- 22.3	1.15 / 1.15	II,III
P.yoshinagai								
	HH101589	*Stewartia serrata*	Hyoga	Jul. 25, 1953	17.8 -(20.8)- 24.1	15.2 -(17.0)- 19.7	1.08 / 1.00	II
	HH101583	*S. pseudo-camellia*	Tosa	Oct. 18, 1931	21.2 -(22.0)- 23.6	15.6 -(18.4)- 20.8	1.02 / 1.18	II, III
	HH101584	*S. pseudo-camellia*	Iyo	Oct. 19, 1930	19.6 -(20.1)- 25.5	14.3 -(15.7)- 18.6	1.00 / 1.21	II, III
	HH101585	Holotype *S. pseudo-camellia*	Iyo	Oct. 19, 1930	17.8 -(23.6)- 27.3	14.6 -(16.8)- 20.3	1.20 / 1.08	II, III
	HH101582	*S. pseudo-camellia*	Tottori	Oct. 17, 1959	23.2 -(26.8)- 28.2	13.0 -(18.0)- 21.4	1.10 / 1.15	II
	HH61128	*S. pseudo-camellia*	Tottori	Oct. 19, 1973	19.3 -(21.6)- 23.4	14.9 -(16.7)- 18.4	1.05 / 1.25	II, III

(continued)

Species	Voucher specimen ∗1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds ∗2	Spore State
P.yoshinagai								
	HH99715	*S. pseudo-camellia*	Yamaguchi	Sept. 29, 1974	13.9 -(17.4)- 20.4	13.6 -(17.4)- 21.9	1.00 / 1.28	II, III
	IBA 8404	*S. monadelpha*	Nara	Oct. 1, 1999	18.0- (22.3)- 27.3	11.5 -(14.2)- 16.3	1.00 / 1.18	II, III
	IBA 8430	*S. monadelpha*	Nara	Oct. 2, 1999	17.8 -(21.3)- 24.7	11.1 -(12.8)- 14.5	1.08 / 1.11	II, III
	IBA 2871	*S. pseudo-camellia*	Yamanashi	Sept. 5, 1983	19.1- (24.1)-27.7	14.7 -(19.3)- 21.5	1.10 / 1.26	II
P. coryli								
	IBA2582	*Corylus heterophylla*	Yamanashi	Sept. 18, 1982	14.6 -(21.4)- 28.5	13.1 -(16.1)- 19.6	1.50 / 1.35	II
	TSH-R4234	*C. sieboldiana*	Yamanashi	Sept. 29, 1982	15.8 -(22.7)- 25.6	13.5 -(15.6)- 19.8	1.60 / 1.31	II, III
	TSH-R4235	*C. sieboldiana*	Niigata	Oct. 6, 1992	21.9 -(25.7)- 28.9	17.5 -(20.1)- 22.3	1.40 / 1.33	II
	TSH-R4236	*C. sieboldiana*	Tochigi	Sept. 22, 1995	18.5 -(23.7)- 29.4	11.9 -(16.8)- 21.9	1.50 / 1.35	II
	TSH-R4237	*C. sieboldiana*	Fukushima	Nov. 11, 2000	20.8 -(24.9)- 29.1	12.5 -(16.6)- 20.6	1.60 / 1.33	II,III
	HU20859	*C. heterophylla*	Hirosaki	Oct. 30, 1991	18.8 -(21.4)- 23.4	12.4 -(15.0)- 17.6	1.30 / 1.31	II
	TSH-R12671	*C. sieboldiana*	Fukushima	Sept. 25, 1959	16.4 -(20.2)- 23.3	13.3 -(16.4)- 19.4	1.30 / 1.28	II
	TSH-R12672	*C. sieboldiana*	Yamanashi	Sept. 1, 1960	19.8- (23.2)-25.5	15.0 -(16.7)- 18.5	1.20 / 1.28	II
	TSH-R12708	*C. sieboldiana*	Yamanashi	Sept. 1, 1960	16.8 -(21.6)- 23.8	11.5 -(14.8)- 19.4	1.20 / 1.35	II
	TSH-R12711	*C. sieboldiana*	Fukushima	Sept. 25, 1959	19.2 -(22.7)- 26.2	13.5 -(15.6)- 17.9	1.20 / 1.35	II
	TSH-R12801	*C. sieboldiana*	Fukushima	Sept. 10, 1959	21.6 -(23.3)- 25.9	14.4 -(17.6)- 19.6	1.40 / 1.31	II
	TSH-R12806	*C. sieboldiana*	Fukushima	Sept. 25, 1959	15.5 -(19.3)- 23.1	12.6 -(14.6)- 16.1	1.30 / 1.28	II
	HH102959	*C. sieboldiana*	Fukushima	Sept. 25, 1959	17.9 -(23.6)- 26.7	14.9 -(17.2)- 18.8	1.40 / 1.28	II
	HH102953	*C. avellana*	Tokyo	Aug. 12, 1960	22.9 -(24.8)- 28.2	16.4 -(18.1)- 22.2	1.70 / 1.30	II
	HH102954	*C. avellana*	Tokyo	Aug. 12, 1960	21.7 -(23.8)- 28.2	16.0 -(18.5)- 20.8	1.60 / 1.25	II
	HH103013	*C. sieboldiana*	Yamagata	Oct. 12, 1961	19.3 -(22.2)- 27.9	16.2 -(18.1)- 22.3	1.45 / 1.28	II, III
	HH78264	*C. sieboldiana*	Yamanashi	Oct. 6, 1981	20.8 -(24.6)- 28.4	14.7 -(16.8)- 19.1	1.60 / 1.25	II, III
	HH102998	*C. sieboldiana*	Nagano	Sept. 3, 1963	18.4 -(21.8)- 25.3	14.9 -(17.1)- 18.6	1.60 / 1.30	II, III
	HH102972	*C. sieboldiana*	Chichibu-tama	Aug. 14, 1954	16.2 -(19.8)- 22.1	13.5 -(15.6)- 16.9	1.40 / 1.21	II
	HH102966	*C. sp*	Hiroshima	Oct. 6, 1947	20.8 -(22.7)- 25.8	16.7 -(18.2)- 19.5	1.70 / 1.25	II
	TSH-R12807	*C. sieboldiana*	Nagano	Sept. 3, 1963	18.2 -(20.9)- 25.2	14.9 -(17.5)- 20.4	1.50 / 1.33	II, III
	TSH-R12819	*C. sieboldiana*	Yamagata	Sept. 23, 1962	20.4 -(24.9)- 28.8	15.4 -(17.5)- 21.2	1.60 / 1.21	II
	TSH-R13588	*C. sieboldiana*	Tottori	Oct. 10, 1971	20.1 -(25.3)- 26.0	13.0 -(15.4)- 19.9	1.35 / 1.28	II, III
	TSH-R17881	*C. sieboldiana*	Fukushima	Oct. 10, 1959	15.7 -(20.2)- 24.9	13.2 -(15.6)- 19.7	1.30 / 1.28	II, III
	TSH-R18372	*C. sieboldiana*	Gunma	Oct. 3, 1968	16.7 -(21.9)- 27.3	14.5 -(16.3)- 20.5	1.40 / 1.25	II
	FPH01371	*C. avellana*	Tokuo		21.5 -(24.1)- 27.5	15.6 -(18.1)- 20.2	1.30 / 1.35	II
	FPH4770	*C. sieboldiana*	Fukuoka	Oct. 20, 1977	16.9 -(20.7)- 25.2	10.4 -(12.0)- 14.9	1.40 / 1.28	II

(continued)

Species	Voucher specimen ∗1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds ∗2	Spore State
P. coryli								
	FPH01317	*C. sieboldiana*	Yamanashi	Sept. 21, 1959	17.1 -(22.0)- 28.6	13.2 -(15.4)- 17.4	1.50 / 1.21	II
	HH103034	*C. sieboldiana*	Yamanashi	Sept. 13, 1962	20.18 -(23.2)- 26.3	16.9 -(18.8)- 20.4	1.50 / 1.25	II
	HH103036	*C. sieboldiana*	Yamanashi	Sept. 14, 1962	22.5 -(24.7)- 28.5	16.4 -(18.4)- 20.1	1.38 / 1.33	II, III
	HH103037	*C. sieboldiana*	Yamanashi	Sept. 14, 1962	22.1 -(24.3)- 28.8	15.9 -(18.6)- 22.8	1.60 / 1.35	II, III
	HH103026	*C. rostrata*	Inaba	Oct. 19, 1941	19.3 -(24.3)- 27.7	14.7 -(18.9)- 20.8	1.70 / 1.38	II, III
	HH103027	*C. rostrata*	Inaba	Oct. 17, 1941	18.5 -(22.6)- 27.4	15.8 -(17.2)- 19.4	1.50 / 1.31	II, III
	HH103025	*C. rostrata*	Izumo	Sept. 20, 1930	22.1 -(24.7)- 28.8	17.1 -(19.2)- 23.0	1.60 / 1.33	II, III
	HH103017	*C. sieboldiana*	Gunma	Oct. 3, 1968	16.1 -(24.1)- 28.8	15.2 -(17.4)- 21.1	1.70 / 1.28	II
	HH89389	*C. sieboldiana*	Kanagawa	Oct. 12, 1969	21.2 -(23.1)- 25.6	15.8 -(17.6)- 19.3	1.60 / 1.25	II, III
	HH98856	*C. sieboldiana*	Hyogo	Sept. 24, 1975	19.7 -(24.6)- 28.1	16.7 -(18.9)- 20.8	1.48 / 1.31	II
	HH97623	*C. sieboldiana*	Tottori	Oct. 19, 1973	14.5 -(20.6)- 25.1	16.2 -(18.6)- 22.3	1.60 / 1.33	II, III
	HH40527	*C. rostrata*	Sapporo	Nov. 10, 1924	23.4 -(24.9)- 28.9	15.8 -(18.0)- 20.4	1.60 / 1.24	II, III
	HH103004	*C. avellana*	Tokyo	Aug. 12, 1960	18.2 -(23.7)- 27.0	14.1 -(18.4)- 22.7	1.50 / 1.28	II
	HH103005	*C. avellana*	Tokyo	Aug. 12, 1960	21.2 -(23.5)- 27.1	16.5 -(19.0)- 22.3	1.70 / 1.36	II
	HH102974	*C. sieboldiana*	Chichibu-Tama Nat. Park	Oct. 3, 1962	20.2 -(21.8)- 24.7	15.6 -(17.5)- 19.7	1.70 / 1.31	II, III
P. epilobi								
	HH30730	*Epilobium sp.*	Shinano	July. 30, 1930	13.5 -(18.0)- 20.9	11.5 -(14.0)- 16.5	1.00 / 1.25	II
	HH32823	*E. cephalostigma*	Shinano	Aug. 23, 1932	18.5 -(19.4)- 20.7	11.1 -(13.1)- 15.5	1.00 / 1.20	II
	HH558201	*E. pyricholophum*	Yamanashi	Aug. 20, 1955	14.9 -(16.8)- 19.4	11.1 -(12.4)- 15.5	1.00 / 1.35	II
	HH629142	*E. pyricholophum*	Yamanashi	Sept. 9, 1962	17 -(18.9)- 21.2	12.6 -(14.4)- 16.6	1.10 / 1.22	II
	HH63912	*E. pyricholophum*	Tochigi	Sept. 12, 1963	15.2 -(18.7)- 20.7	13.1 -(14.5)- 16.1	1.08 / 1.20	II
	HH6710241	*E. pyricholophum*	Chiba	Oct. 24, 1967	17.1 -(20.6)- 22.7	13.7 -(15.8)-17.1	1.07 / 1.25	II
	HH7297	*E. amurense*	Yamanashi	Sept. 7, 1972	17.8 -(19.6)- 21.9	14.3 -(15.9)- 17.4	1.02 / 1.31	II
	HH5306	*E. angustifolium*	Sapporo	Aug. 19, 1924	14.6 -(17.8)- 21.2	12.5 -(14.2)- 16.8	1.00 / 1.36	II
	HH5399	*E. angustifolium*	Sapporo	Sept. 20, 1925	17.4 -(18.0)- 18.7	11.7 -(13.3)- 14.4	1.09 / 1.40	II, III
	HH88032	*E. pyrocholophum*	Hokkaido	Aug. 25, 1967	14.8 -(19.2)- 21.8	10.3 -(13.7)- 16.1	1.06 / 1.30	II
	HH88035	*E. pyrocholophum*	Hokkaido	Aug. 25, 1967	18.0 -(19.4)- 22.0	13.1 -(15.4)- 17.4	1.05 / 1.35	II
	IBA 2253	*E. cephalostigma*	Nagano	Jul. 30, 1981	14.7 -(17.7)- 19.1	11.5 -(13.6)- 15.4	1.02 / 1.32	II
	IBA 2301	*E. sp.*	Tochigi	Aug. 24, 1981	15.8 -(17.2)- 24.1	12.3 -(14.2)- 16.4	1.10 / 1.20	II
	TSH-R12804	*E. pyricholophum*	Tochigi	Sept. 12, 1963	16.7 -(18.2)- 25.3	12.8 -(13.9)- 16.5	1.10 / 1.25	II
	TSH-R10240	*E. cephalostigma*	Tochigi	Aug. 26, 1981	15.3 -(18.6)- 24.8	12.3 -(14.5)- 16.9	1.00 / 1.30	II
	HH102356	*E. angustifolium*	Satama	Aug. 17, 1924	18.9 -(21.1)- 24.8	13.9 -(15.2)- 17.4	1.12 / 1.32	II
	HH111598	*E. angustifolium*	Kushiro	Sept. 10, 1925	18.8 -(22.5)- 26.1	14.3 -(15.2)- 17.7	1.11 / 1.20	II
	HH5893	*E. angustifolium*	Nagano	Sept. 3, 1958	14.8 -(16.8)- 21.5	12.4 -(13.8)- 16.3	1.00 / 1.25	II

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. epilobi								
	HH5892	*E. angustifolium*	Nagano	Sept. 2, 1958	18.3 -(20.5)- 22.8	14.6 -(16.0)- 17.0	1.05 / 1.28	II
	HH5894	*E. angustifolium*	Nagano	Sept. 4, 1958	17.0 -(19.3)- 20.9	13.3 -(15.3)- 17.2	1.13 / 1.30	II
	HH64913	*E. angustifolium*	Hokkaido	Sept. 13, 1964	16.7 -(19.0)- 21.3	12.6 -(14.2)- 15.4	1.12 / 1.41	II
	HH591011	*E. pyricholophum*	Fukushima	Oct. 11, 1959	14.9 -(18.1)- 22.4	10.2 -(12.8)- 15.0	1.07 / 1.40	II, III
	HH55829	*E. angustifolium*	Nagano	Aug. 29, 1955	19.8 -(22.1)- 25.3	14.7 -(16.6)- 18.4	1.13 / 1.22	II, III
P. miyabeanum								
	HH103299	*Viburnum furcatum*	Sapporo	Sept. ,1898	19.6 -(20.7)- 22.6	15.9 -(16.8)- 18.9	1.10 / 1.18	II, III
	HH103300	*V. furcatum*	Iburi	Oct. 10, 1927	19.6 -(23.0)- 30.1	16.7 -(18.4)- 20.1	1.10 / 1.40	II, III
	HH103301	*V. furcatum*	Ishikari	Oct. 17, 1925	19.4 -(22.8)- 25.3	11.6 -(18.4)- 21.2	1.00 / 1.30	II, III
	HH103302Holotyp	*V. furcatum*	Ishikari	Oct. 17, 1925	19.8 -(21.6)- 23.1	15.2 -(16.7)- 18.1	1.00 / 1.10	II
	HH103315	*V. furcatum*	Ishikari	Sept. 26, 1926	21.4 -(23.3)- 24.9	15.7 -(17.0)- 17.8	1.00 / 1.20	II
	HH103297	*V. furcatum*	Sapporo	Aug. 29, 1924	21.5 -(23.7)- 25.7	15.2 -(18.1)- 20.8	1.10 / 1.15	II
	HH103298	*V. furcatum*	Ishikari	Sept. 12, 1926	21.8 -(23.8)- 26.9	16.3 -(18.2)- 20.9	1.10 / 1.18	II
	HH103288	*V. furcatum*	Nagano	Aug. 26, 1954	21.5 -(23.1)- 27.3	14.1 -(16.7)- 20.0	1.05 / 1.30	II
	HH103364	*V. furcatum*	Kozuke	Sept. 5, 1950	20.3 -(23.5)- 27.4	14.6 -(16.9)- 19.2	1.10 / 1.25	II
	HH90189	*V. furcatum*	Kanagawa	Oct. 3, 1970	21.6 -(23.7)- 28.9	15.2 -(18.6)- 21.1	1.10 / 1.15	II, III
	HH90190	*V. furcatum*	Kanagawa	Oct. 3, 1970	21.3 -(23.9)- 28.3	14.2 -(16.8)- 21.3	1.10/1.18	II, III
	HH98862	*V. furcatum*	Hyogo	Sept. 24, 1975	20.1 -(23.2)- 27.2	12.6 -(17.2)- 20.1	1.10 / 1.21	II
	HH73009	*V. furcatum*	Nagano	Sept. 10, 1979	21.3 -(24.0)- 29.0	15.7 -(18.4)- 21.3	1.15 / 1.25	II, III
	HH72950	*V. furcatum*	Gifu	Sept. 10, 1979	20.2 -(23.8)- 29.8	14.6 -(17.9)- 21.1	1.10 / 1.18	II
	HH60148	*V. furcatum*	Mutsu	Sept. 2, 1953	18.9 -(23.5)- 26.8	16.5 -(18.5)- 20.1	1.10 / 1.18	II
	IBA 7659	*V. furcatum*	Miyazaki	Oct. 23, 1995	17.4 -(22.1)- 25.8	15.2 -(16.3)- 18.6	1.10 / 1.31	II
	IBA7888	*V. furcatum*	Aomori	Sept. 7, 1997	18.8 -(22.9)- 26.0	13.6 -(15.1)- 17.6	1.10 / 1.33	II
	IBA 7899	*V. furcatum*	Aomori	Sept. 9, 1997	19.3 -(23.5)- 27.3	14.3 -(16.8)- 19.1	1.10 / 1.35	II
	IBA 8721	*V. furcatum*	Yamagata	Sept. 28, 2001	18.8 -(22.1)- 25.4	13.9 -(16.5)- 19.5	1.06 / 1.31	II
	TSH-R12535	*V. furcatum*	Fukushima	Oct. 11, 1959	17.8 -(22.6)- 26.9	13.5 -(16.4)- 18.2	1.00 / 1.21	II, III
	TSH-R12712	*V. plicatum*	Saitama	Oct. 30, 1957	19.3 -(22.5)- 25.8	15.2 -(16.7)- 19.1	1.00 / 1.28	II, III
	HH60148	*V. furcatum*	Mutsu	Sept. 2, 1953	18.9 -(23.5)- 26.8	16.5 -(18.5)- 20.1	1.10 / 1.20	II
	HH103285	*V. furcatum*	Toyama	Aug. 23, 1961	16.1 -(19.6)- 23.1	14.8 -(17.4)- 20.3	1.10 / 1.21	II
	HH103286	*V. furcatum*	Hokkaido	Sept. 6, 1969	19.2 -(20.3)- 21.4	16.5 -(18.3)- 20.5	1.05 / 1.35	II, III
	HH103282	*V. furcatum*	Hokkaido	Sept. 6, 1969	20.1 -(23.8)- 26.9	15.9 -(18.0)- 20.9	1.10 / 1.32	II, III
	HH103276	*V. furcatum*	Hokkaido	Oct. 1, 1957	18.7 -(21.5)- 24.2	16.5 -(17.6)- 18.9	1.00 / 1.28	II, III
	HH103344	*V. furcatum*	Aomori	Sept. 2, 1953	16.3 -(19.1)- 23.3	13.7 -(16.6)- 19.1	1.05 / 1.33	II
	HH103347	*V. furcatum*	Hokkaido	Oct. 1, 1957	16.5 -(22.7)- 27.5	14.8 -(16.5)- 19.2	1.10 / 1.18	II, III

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. miyabeanum								
	HH103336	*V. furcatum*	Nagano	Sept. 3, 1963	20.5 -(22.8)- 26.2	17.4 -(18.5)- 20.1	1.10 / 1.25	II
	HH103335	*V. furcatum*	Yamagata	Sept. 3, 1963	21.5 -(23.0)- 25.4	15.2 -(18.2)- 20.9	1.10 / 1.28	II
	HH103331	*V. furcatum*	Tochigi	Sept. 11, 1963	21.1 -(23.9)- 27.2	15.0 -(17.4)- 19.4	1.05 / 1.30	II, III
	HH103311Holotyp	*V. furcatum*	Sapporo	Sept. 22, 1896	20.9 -(22.6)- 24.0	17.2 -(18.5)- 19.1	1.10 / 1.16	II, III
	HH103314	*V. furcatum*	Ishikari	Aug. 24, 1898	21.8 -(23.5)- 26.1	17 -(18.4))- 20.8	1.10 / 1.14	II
	HH103354	*V. furcatum*	Saitama	Aug. 27, 1958	21.5 -(23.9)- 27.0	16.8 -(18.6)- 21.2	1.20 / 1.18	II
	HH103321	*V. furcatum*	Hoki	Aug. 24, 1931	19.2 -(23.3)- 27.1	14.2 -(15.5)- 18.3	1.07 / 1.25	II
	HH103322	*V. furcatum*	Hoki	Aug. 20, 1930	21.3 -(23.4)- 26.2	16.6 -(18.2)- 19.5	1.20 / 1.28	II
	HH103306	*V. furcatum*	Chichibu-Tama Nat. Park	Aug. 27, 1958	18.7 -(23.2)- 26.6	13.9 -(15.9)- 18.3	1.10 / 1.16	II
	HH103373	*V. furcatum*	Saitama	Aug. 27, 1958	18.3 -(20.6)- 24.8	13.5 -(15.3)- 17.2	1.00 / 1.33	II
	HH103371	*V. furcatum*	Fukushima	Sept. 25, 1959	20.9 -(23.2)- 26.8	14.2 -(17.8)- 21.0	1.15 / 1.32	II
	HH103366	*V. furcatum*	Hyuga	Sept. 23, 1948	15.5 -(18.4)- 21.2	14.4 -(14.9)- 15.7	1.09 / 1.28	II
	TSH-R12713	*V. plicatum*	Fukushima	Sept. 25, 1959	18.6 -(24.3)- 27.8	12.6 -(17.2)- 19.9	1.05 / 1.25	II, III
	TSH-R10202	*V. furcatum*	Akita	Sept. 25, 1997	19.5 -(22.0)- 24.5	14.3 -(17.3)- 20.2	1.06 / 1.18	II,III
	TSH-R9545	*V. furcatum*	Mt. Kinpoku-san, Sado is	Oct. 1, 1995	18.4 -(24.3)- 28.0	15.2 -(17.6)- 19.3	1.06 / 1.35	II,III
	TSH-R12716	*V. furcatum*	Nagano	Sept. 3, 1963	18.0 -(21.8)- 27.1	14.3 -(16.2)- 18.4	1.07 / 1.21	II
	TSH-R12811	*V. furcatum*	Fukushima	Sept. 25, 1959	20.2 -(22.3)- 24.3	13.4 -(16.3)- 18.8	1.00 / 1.18	II
	TSH-R3849	*V. furcatum*	Shizuoka	Sept. 6, 1998	18.8 -(20.4)- 22.7	13.0 -(14.8)- 16.3	1.00 / 1.16	II
	TSH-R13124	*V. furcatum*	Tottori	Oct. 14, 1966	20.8 -(23.9)- 28.1	15.8 -(18.1)- 19.9	1.05 / 1.25	II, III
	TSH-R10243	*V. furcatum*	Tochigi	Aug. 26, 1981	16.9 -(20.8)- 24.5	13.0 -(14.7)- 16.3	1.02 / 1.28	II
	TSH-R10290	*V. furcatum*	Tochigi	Aug. 17, 2000	21.0 -(23.7)- 27.4	16.6 -(18.4)- 20.5	1.07 / 1.26	II
	TSH-R10308	*V. furcatum*	Tochigi	Oct. 11, 2000	17.1 -(19.2)- 21.1	13.2 -(15.2)- 16.5	1.05 / 1.31	II, III
	TSH-R10606	*V. furcatum*	Gunma	Oct. 12, 2000	21.9 -(23.9)- 26.0	14.5 -15.4)- 17.1	1.00 / 1.33	II, III
P. hydrangeae-petiolaridia								
	IBA 1841	*Hydrangea petiolaris*	Fukushima	Sept. 29, 1979	19.1 -(22.4)- 27.7	14.1 -(15.2)- 17.1	1.25 / 1.45	II, III
	IBA 2207	*H. petiolaris*	Nagano	Jul. 27, 1981	20.8 -(22.5)- 26.7	12.6 -(14.9)- 17.3	1.28 / 1.55	II
	IBA 2342	*H. petiolaris*	Tochigi	Aug. 26, 1981	22.7 -(24.0)- 25.8	13.4 -(14.4)- 15.6	1.10 / 1.43	II, III
	IBA 2367	*H. petiolaris*	Yamanashi	Oct. 6, 1981	20.8 -(24.1)- 27.3	13.0 -(15.2)- 17.6	1.35 / 1.38	II
	IBA 6637	*H. petiolaris*	Kitaibaraki	Jul. 4, 1992	24.0 -(24.7)- 27.9	13.2 -(16.0)- 18.8	1.00 / 1.51	II
	IBA 6660	*H. petiolaris*	Kitaibaraki	Aug. 11, 1992	22.1 -(24.9)- 27.8	12.4 -(14.9)- 17.3	1.10 / 1.45	II
	IBA 7881	*H. petiolaris*	Aomori	Sept. 7, 1997	18.6 -(23.8)- 26.3	13.2 -(15.5)- 17.6	1.25 / 1.40	II
	IBA 8377	*H. petiolaris*	Gunma	Sept. 18, 1999	19.1 -(23.3)- 27.5	14.3 -(16.1)- 17.9	1.18 / 1.40	II
	TSH-R21293	*H. petiolaris*	Tochigi	Oct. 24, 2003	23.2 -(28.4)- 35.4	15.2 -(17.3)- 20.1	1.26 / 1.38	II, III
	TSH-R21294	*H. petiolaris*	Akita	Oct. 21, 2003	18.8 -(22.3)- 25.3	14.5 -(16.6)- 19.1	1.18 / 1.45	II

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. hydrangeae–petiolaridia								
	TSH-R21301	*H. petiolaris*	Niigata	Nov. 8, 2003	19.2 -(23.6)- 26.8	14.2 -(15.7)- 18.1	1.15 / 1.52	II, III
	TSh-R13194	*H. petiolaris*	Tottori	Oct. 13, 1971	17.2 -(22.6)- 27.5	12.8 -(15.0)- 17.6	1.30 / 1.48	II
	TSH-R10521	*H. petiolaris*	Tochigi	Aug. 6, 2001	19.2 -(22.4)- 26.8	11.9 -(14.6)- 18.1	1.33 / 1.35	II
	TSH-R10661	*H. petiolaris*	Gunma	Oct. 11, 2001	18.1 -(22.7)- 26.6	14.4 -(16.1)- 20.7	1.27 / 1.38	II
	TSH-R10674	*H. petiolaris*	Tochigi	Aug. 18, 2000	19.2 -(23.5)- 26.3	12.8 -(15.5)- 17.4	1.15 / 1.40	II
	TSH-R10682	*H. petiolaris*	Tochigi	Oct. 10, 2000	16.2 -(25.1)- 28.2	15.7 -(16.8)- 20.3	1.23 / 1.42	II, III
	TSH-R10720	*H. petiolaris*	Tochigi	Oct. 11, 2001	17.8 -(23.7)- 26.1	13.7 -(15.3)- 18.8	1.18 / 1.45	II
P. hikosanense								
	HH103459	*A. rufinerve*	Buzen	Aug. 27, 1941	21.1 -(24.2)- 26.9	12.4 -(17.6)- 20.6	1.30 / 1.28	II
	HH103464	*A. rufinerve*	Kozuke	Sept. 5, 1950	23.0 -(26.7)- 29.7	15.2 -(16.9)- 19.7	1.30 / 1.25	II
	HH103465	*A. rufinerve*	Iwashiro	Sept. 6, 1950	23.8 -(28.4)- 33.6	15.6 -(18.3)- 20.2	1.30 / 1.33	II
	HH103466	*A. rufinerve*	Iwashiro	Aug. 8, 1950	26.3 -(30.1)- 36.8	16.5 -(18.2)- 22.1	1.30 / 1.35	II
	HH103449	*A. insulare*	Ryukyus	Dec. 30, 1954	22.6 -(26.0)- 31.9	14.5 -(17.0)- 21.7	1.20 / 1.40	II
	HH103450	*A. insulare*	Ryukyus	Oct. 6, 1954	24.9 -(30.1)- 33.8	17.1 -(21.2)- 24.5	1.30 / 1.40	II
	HH103451	*A. insulare*	Ryukyus	Oct. 6, 1954	20.5 -(24.8)- 31.0	14.7 -(18.3)- 20.6	1.30 / 1.35	II
	HH103452	*A. insulare*	Ryukyus	Jan. 6, 1955	24.5 -(28.7)- 36.2	17.1 -(19.3)- 22.7	1.30 / 1.34	II
	HH103446	*A. insulare*	Ryukyus	Dec. 31, 1954	23.4 -(27.4)- 32.9	13.4 -(16.5)- 19.3	1.30 / 1.28	II
	HH103447	*A. insulare*	Ryukyus	Dec. 30, 1954	23.6 -(30.7)- 38.4	16.7 -(20.6)- 23.2	1.20 / 1.30	II
	HH103448	*A. insulare*	Ryukyus	Dec. 30, 1954	23.8 -(27.0)- 29.7	16.3 -(18.7)- 21.9	1.20 / 1.32	II
	HH103442	*A. insulare*	Okinawa	Dec. 17, 1955	26.0 -(27.7)- 30.8	16.7 -(20.3)- 23.4	1.20 / 1.28	II
	HH103443	*A. insulare*	Okinawa	Dec. 16, 1955	21.9 -(27.7)- 33.4	15.6 -(17.8)- 19.7	1.20 / 1.25	II
	HH103444	*A. insulare*	Okinawa	Dec. 18, 1955	22.7 -(25.0)- 29.1	15.2 -(17.8)- 19.5	1.30 / 1.28	II
	HH103445	*A. insulare*	Okinawa	Dec. 18, 1955	24.1 -(27.9)- 31.2	15.6 -(17.8)- 20.8	1.30 / 1.30	II
	HH103438	*A. insulare*	Okinawa	Dec. 17, 1955	24.3 -(29.2)- 35.8	13.6 -(18.6)- 21.9	1.30 / 1.40	II
	IBA 2565	*Acer rufinerva*	Yamanashi	Sept. 17, 1982	22.6 -(27.5)- 36.2	13.7 -(18.1)- 21.6	1.20 / 1.30	II, III
	IBA 8441	*A. rufinerve*	Shizuoka	Oct. 4, 1999	19.4 -(24.4)- 32.1	12.6 -(16.5)- 20.9	1.20 / 1.30	II, III
	IBA 2569	*A. rufinerve*	Yamanashi	Sept. 17, 1982	20.1 -(26.1)- 33.5	13.5 -(16.5)- 18.5	1.20 / 1.25	II, III
	FPH 6097	*A. rufinerve*	Yamanashi	Sept. 17, 1983	22.3 -(26.1)- 33.6	14.5 -(17.1)- 21.3	1.50 / 1.40	II, III
	HH103460	*A. rufinerve*	Buzen	Oct. 11, 1939	21.7 -(25.4)- 33.4	15.8 -(17.8)- 21.5	1.40 / 1.35	II, III
	HH103461	*A. rufinerve*	Hyuga	Oct. 17, 1946	22.7 -(24.9)- 34.1	15.9 -(17.9)- 20.3	1.40 / 1.28	II, III
	HH103462	*A. rufinerve*	Buzen	Oct. 16, 1938	21.0 -(24.2)- 29.3	15.6 -(17.4)- 18.4	1.30 / 1.35	II
	HH103463Holotyp	*A. rufinerve*	Buzen	Oct. 16, 1938	21.5 -(28.5)- 32.9	13.0 -(15.4)- 17.4	1.50 / 1.25	II, III
	HH103434	*A. rufinerve*	Tochigi	Sept. 12, 1963	21.9 -(25.7)- 31.0	13.7 -(15.7)- 18.0	1.40 / 1.25	II
	HH103435	*A. rufinerve*	Tochigi	Sept. 12, 1963	23.4 -(27.7)- 32.5	14.1 -(15.4)- 18.0	1.40 / 1.35	II

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. hikosanense								
	HH103431	*A. rufinerve*	Nagano	Sept. 3, 1963	19.3 -(23.5)0 27.3	15.4 -(18.2)- 20.2	1.50 / 1.30	II, III
	HH103432	*A. rufinerve*	Nagano	Sept. 3, 1963	23.6 -(29.0)- 34.9	15.2 -(20.2)- 23.8	1.50 / 1.40	II, III
	HH10315	*A. rufinerve*	Kozuke	Sept. 5, 1950	20.4 -(27.1)- 30.8	14.3 -(17.4)- 19.9	1.50 / 1.45	II, III
	HH103456	*A. rufinerve*	Buzen	Nov. 5, 1939	20.8 -(26.9)- 34.3	14.1 -(15.9)- 18.4	1.30 / 1.32	II, III
	HH103441	*A. insulare*	Okinawa	Dec. 17, 1955	21.1 -(24.3)- 28.6	14.1 -(16.5)- 19.5	1.20 / 1.35	II
P.circaeae								
	HH6290	*C. alpina*	Hokkaido	Sept. 12, 1926	14.7 -(18.4)- 22.3	14.5 -(15.8)- 17.6	1.00 / 1.20	II, III
	HH88030	*C. alpina*	Hokkaido	Aug. 25, 1967	!5.8 -(19.3)- 23.8	13.4 -(14.8)- 18.1	1.00 / 1.21	II, III
	HH3734	*C. alpina*	Hokkaido	Aug. 4, 1925	17.7 -(21.6)- 25.1	14.7 -(16.4)- 18.8	1.00 / 1.18	II
	HH2602	*C. alpina*	Hokkaido	Jul. 21, 1922	16.0 -(18.8)- 22.1	12.8 -(15.4)- 16.9	1.15 / 1.20	II
	HH55824	*C. erubescens*	Hyuga	Aug. 24, 1955	20.8 -(22.7)- 26.2	15.8 -(19.1)- 22.5	1.00 / 1.24	II
	HH72961	*C. erubescens*	Gifu	Sept. 10, 1979	20.4 -(22.5)- 24.3	15.4 -(16.3)- 17.7	1.00 / 1.28	II, III
	HH30829	*C. erubescens*	Inaba	Aug. 29, 1930	17.3 -(19.8)- 21.1	14.5 -(16.5)- 17.8	1.10 / 1.31	II
	HH31727	*C. erubescens*		Jul. 27, 1931	16.3 -(19.6)- 22.7	12.6 -(14.6)- 16.5	1.00 / 1.25	II
	HH90182	*C. erubescens*	Kanagawa	Oct. 2, 1970	20.6 -(22.0)- 24.9	14.7 -(15.7)- 17.6	1.10 / 1.23	II, III
	HH189999	*C. cardiophylla*	Hokkaido	Sept. 9, 1899	15.4 -(20.2)- 23.4	11.3 -(14.2)- 18.0	1.00 / 1.25	II
	HH39927	*C. mollis*	Chikuzen	Sept. 27, 1939	17.5 -(20.4)- 26.0	12.1 -(15.0)- 19.3	1.20 / 1.22	II
	HH39108	*C. mollis*	Chikuzen	Oct. 8, 1939	16.9 -(20.8)- 25.2	12.7 -(14.8)- 17.8	1.00 / 1.25	II, III
	HH69389	*C. quadrisulcata*	Tottori	Sept. 21, 1979	17.1 -(21.7)- 23.8	12.6 -(14.3)- 17.1	1.00 / 1.20	II, III
	TSH-R9543	*C. mollis*	Sado isl.	Sept. 15, 1995	17.8 -(21.4)- 25.9	13.3 -(15.4)- 17.6	1.00 / 1.20	II
	TSH-R10187	*C. erubescens*	Aomori	Sept. 25, 1997	17.5 -(20.3)- 26.2	12.3 -(15.9)- 17.4	1.25 / 1.08	II
	HH58827	*C. alpina*	Saitama	Aug. 27, 1958	15.9 -(20.1)- 22.8	12.8 -(14.7)- 16.8	1.00 / 1.18	II
	HH8011	*C. alpina*	Hokkaido	Aug. 12, 1927	17.8 -(20.7)- 23.4	14.9 -(16.4)- 18.2	1.00 / 1.20	II
	IBA 6672	*Circaea erubescens*	Kitaibaraki	Aug. 24, 1992	15.6 -(19.8)- 23.4	11.4 -(13.8)- 15.6	1.20 / 1.22	II
	IBA 1589	*C. sp.*	Gunnma	Jun. 18, 1973	16.2 -(19.5)- 24.1	12.4 -(15.2)- 16.8	1.00 / 1.15	II
	IBA 1772	*C. sp.*	Tokyo	Oct. 7, 1974	16.7 -(20.2)- 22.3	13.1 -(15.6)- 16.9	1.15 / 1.15	II
	HH7015	*C. alpina*	Kuriles	Jul. 16, 1924	15.3 -(21.1)- 24.9	12.8 -(14.9)- 18.0	1.20 / 1.18	II
	HH30730	*C. alpina*	Shinano	Jul. 30, 1930	16.0 -(20.4)- 25.1	14.3 -(16.6)- 20.1	1.20 / 1.30	II
	HH04829	*C. alpina*	Shimotsuke	Aug. 29, 1904	16.8 -(21.1)- 24.5	16.4 -(17.7)- 19.1	1.30 / 1.21	II
	HH61718	*C. alpina*	Yamanashi	Jul. 18, 1961	18.6 -(22.5)- 26.9	14.5 -(16.4)- 18.6	1.30 / 1.25	II
	HH62914	*C. alpina*	Yamanashi	Sept. 14, 1962	17.1 -(21.7)- 24.7	14.9 -(17.9)- 21.2	1.30 / 1.28	II
	HH58828	*C. alpina*	Saitama	Aug. 27, 1958	15.4 -(19.1)- 22.3	13.9 -(15.5)- 17.1	1.00 / 1.20	II

(continued)

Species	Voucher specimen ∗1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds ∗2	Spore State
P. boehmeriae								
	IBA 1664	*Boehmeria biloba*	Tokyo	Jul. 25, 1974	17.6 -(21.5)- 23.4	14.1 -(15.7)- 18.0	1.10 / 1.30	II
	IBA 2586	*B. platanifolia*	Yamanashi	Sept. 18, 1982	17.8 -(21.4)- 24.9	12.8 -(15.4)- 17.8	1.10 / 1.25	II, III
	IBA 5428	*B. tricuspis*	Tochigi	Aug. 16, 1990	18.6 -(22.9)- 27.3	14.1 -(15.8)- 18.0	1.08 / 1.00	II
	IBA 5611	*B. grandifolia*	Ibaraki	Nov. 18, 1990	18.2 -(19.4)- 20.8	11.9 -(12.7)- 14.1	1.10 / 1.13	II, III
	IBA 6213	*B. spicata*	Kitaibaraki	Sept. 18, 1992	19.3 -(24.4)- 26.7	14.9 -(16.3)- 20.0	1.08 / 1.00	II
	HU22618	*B. tricuspis*	Miyagi	Oct. 4, 1992	18.6 -(21.0)- 24.3	13.6 -(15.4)- 16.7	1.05 / 1.25	II, III
	HU22617	*B. tricuspis*	Miyagi	Oct. 4, 1992	17.1 -(20.3)- 23.4	10.8 -(14.4)- 17.6	1.00 / 1.21	II, III
	HH61140	*B. spicata*	Tottori	Sept. 29, 1976	18.6 -(21.1)- 24.9	11.9 -(15.6)- 18.2	1.04 / 1.25	II, III
	HH90221	*B. spicata*	Kanagawa	Oct. 3, 1970	20.1 -(22.6)- 25.8	11.9 -(14.3)- 16.2	1.06 / 1.15	II, III
	HH311018	*B. spicata*	Echizen	Oct. 18, 1931	17.9 -(20.9)- 24.9	12.1 -(15.6)- 17.6	1.07 / 1.13	II
	HH30829	*B. spicata*	Inaba	Aug. 29, 1930	20.7 -(23.8)- 27.7	15.4 -(17.1)- 19.0	1.08 / 1.34	II
	HH541014	*B. spicata*	Saitama	Oct. 14, 1954	17.8 -(23.2)- 27.4	12.4 -(14.9)- 16.7	1.02 / 1.32	II, III
	HH291026	*B. spicata*	Inaba	Oct. 26, 1929	18.8 -(22.5)- 26.0	12.8 -(16.5)- 19.8	1.05 / 1.18	II, III
	HH65864	*B. spicata*	Okayama	Sept. 2, 1976	18.2 -(21.8)- 26.0	12.6 -(14.9)- 17.1	1.05 / 1.14	II
	HH53118	*B. sp .*	Kumamoto	Nov. 8, 1953	20.4 -(23.2)- 26.2	13.6 -(15.8)- 17.8	1.08 / 1.25	II, III
	HH97765	*B. holosericea*	Hyogo	Oct. 22, 1975	175 -(20.3)- 23.2	10.8 -(14.2)- 16.5	1.08 / 1.21	II
	HH631122	*B. holosericea*	Chiba	Nov. 22, 1963	17.5 -(22.1)- 26.0	11.5 -(16.4)- 19.9	1.00 / 1.25	II
	HH64913	*B. platanifolia*	Niigata	Sept. 13, 1964	18.0 -(20.2)- 22.7	12.3 -(15.2)- 16.7	1.05 / 1.28	II
	HH611013	*B. spicata*	Yamagata	Oct. 13, 1961	16.2 -(20.7)- 24.3	11.2 -(14.1)- 17.3	1.00 / 1.21	II, III
	HH62914	*B. spicata*	Yamanashi	Sept. 14, 1962	16.2 -(22.5)- 27.9	13.0 -(15.0)- 17.3	1.02 / 1.20	II
	HH625	*B. spicata*	Satsuma	Oct. 16, 1930	16.1 -(20.7)- 23.0	12.3 -(14.6)- 16.7	1.00 / 1.18	II
	HH111348Holotyp	*B. biloba*	Chiba	Oct. 25, 1967	18.3 -(22.4)- 27.6	12.8 -(14.3)- 17.1	1.06 / 1.17	II,III
	HH111351	*B. splitzgerberas*	Chiba	Oct. 25, 1967	16.5 -(21.8)- 25.1	12.4 -(14.8)- 16.3	1.08 / 1.20	II, III
	IBA 6682	*B. longispica*	Kitaibaraki	Sept. 11, 1992	16.9 -(20.3)- 24.1	13.2 -(15.5)- 16.5	1.09 / 1.15	II.III
	IBA 8481	*B. platanifolia*	Tokyo	Nov. 5, 1999	13.4 -(19.5)- 26.2	11.7 -(14.2)- 16.3	1.05 / 1.17	II
	IBA 8571	*B. spicata*	Tochigi	Sept. 28, 2000	20.4 -(22.9)- 24.6	11.7 -(14.1)- 16.3	1.08 / 1.21	II
	IBA 8627	*B. longispica*	Kagoshima	Oct. 31, 2000	18.8 -(22.2)- 27.4	12.6 -(15.1)- 19.5	1.10 / 1.25	II, III
	IBA 8632	*B. spicata*	Kagoshima	Oct. 31, 2000	19.1 -(22.8)- 27.1	13.8 -(16.9)- 19.0	1.08 / 1.17	II
	IBA 8643	*B. spicata*	Fukushima	Nov. 11, 2000	17.3 -(21.2)-25.7	13.6 -(15.8)- 17.9	1.10 / 1.15	II, III
	TSH-R21285	*B. spicata*	Tochigi	Oct. 24, 2003	17.6 -(22.1)- 27.8	10.8 -(13.1)- 16.3	1.06 / 1.28	II
	TSH-R21286	*B. spicata*	Tochigi	Oct. 24, 2003	16.7 -(19.2)- 22.1	11.7 -(14.3)- 16.7	1.09 / 1.14	II
	TSH-R21287	*B. spicata*	Tochigi	Oct. 24, 2003	18.6 -(21.3)- 26.0	13.2 -(15.3)- 17.1	1.10 / 1.13	II
	TSH-R21288	*B. spicata*	Tochigi	Oct. 24, 2003	17.4 -(21.5)- 23.6	14.1 -(15.8)- 17.8	1.05 / 1.28	II

(continued)

Species	Voucher specimen *1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds *2	Spore State
P. boehmeriae								
	TSH-R21289	*B. tricuspis*	Aomori	Oct. 22, 2003	18.0 -(22.1)- 26.4	13.2 -(15.1)- 17.3	1.03 / 1.32	II
	TSH-R21290	*B. tricuspis*	Aomori	Oct. 22, 2003	18.1 -(21.2)- 23.3	16.0 -(17.4)- 19.9	1.00 / 1.17	II
	TSH-R21307	*B. tricuspis*	Tochigi	Oct. 24, 2003	18.3 -(22.6)- 26.4	15.4 -(16.9)- 19.3	1.05 / 1.17	II, III
	HU21508	*B. tricuspis*	Miyagi	Oct. 4, 1992	15.6 -(20.3)- 22.8	13.6 -(15.7)- 16.9	1.06 / 1.14	II
P. tiliae								
	HU13358	*T. japonica*	Hokkaido	Sept. 26, 1982	13.7 -(20.4)- 26.6	10.5 -(11.9)- 13.3	1.45 / 1.46	II
	HU14392	*T. japonica*	Hokkaido	Sept. 21, 1983	14.6 -(18.2)- 20.7	11.3 -(14.6)- 16.1	1.15 / 1.39	II
	HU23389	*T. maximowicziana*	Akita	Oct. 14, 1995	16.8 -(23.7)- 26.5	12.4 -(15.1)- 16.6	1.25 / 1.56	II
	HU20982	*T. maximowicziana*	Hokkaido	Sept. 30, 1991	18.2 -(23.3)- 28.8	11.8 -(13.7)- 15.4	1.35 / 1.43	II
	HU20983	*T. maximowicziana*	Hokkaido	Sept. 30, 1991	17.0 -(22.2)- 27.7	11.7 -(14.4)- 16.2	1.25 / 1.45	II
	HU20984	*T. maximowicziana*	Hokkaido	Sept. 30, 1991	16.4 -(21.9)- 26.4	12.1 -(13.8)- 16.3	1.42 / 1.28	II
	HU20981	*T. maximowicziana*	Hokkaido	Sept. 30, 1991	18.6 -(23.5)- 25.8	13.6 -(14.8)- 15.7	1.25 / 1.3	II
	HU19571	*T. maximowicziana*	Hokkaido	Otc. 20, 1989	16.6 -(22.8)- 27.1	12.6 -(15.3)- 16.8	1.33 / 1.45	II
	HU19572	*T. maximowicziana*	Hokkaido	Oct. 20, 1989	15.3 -(21.5)- 24.6	11.5 -(14.2)- 15.9	1.30 / 1.35	II
	HU19573	*T. maximowicziana*	Hokkaido	Oct. 20, 1989	18.2 -(24.0)- 29.1	13.8 -(15.1)- 16.2	1.28 / 1.44	II
	HU19574	*T. maximowicziana*	Hokkaido	Oct. 20, 1989	16.1 -(22.1)- 26.3	10.3 -(12.8)- 14.6	1.30 / 1.28	II
	IBA6284	*T. maximowicziana*	Niigata	Oct. 5, 1992	17.8 -(23.2)- 25.5	12.6 -(13.6)- 16.3	1.25 / 1.25	II
	IBA6317	*T. maximowicziana*	Niigata	Oct. 6, 1992	15.9 -(20.8)- 23.7	10.9 -(12.6)- 14. 8	1.35 / 1.3	II
	IBA6324	*T. maximowicziana*	Niigata	Oct. 6, 1992	16.6 -(23.7)- 26.5	12.2 -(13.8)- 14.4	1.45 / 1.31	II
	IBA7670	*T. japonica*	Miyazaki	Oct. 25, 1995	17.0 -(22.3)- 27.6	11.7 -(12.5)- 13.9	1.40 / 1.33	II, III
	IBA7878	*T. japonica*	Aomori	Sept. 7, 1997	15,4 -(19.7)- 24.5	11.8 -(13.2)- 15.7	1.30 / 1.3	II
	IBA8353	*T. japonica*	Hokkaido	Sept. 26, 1994	16.5 -(21.8)- 25.1	13.3 -(14.1)- 15.5	1.25 / 1.28	II
	HU23148	*T. japonica*	Hokkaido	Sept. 22, 1995	13.7 -(20.1)- 25.2	7.6 -(12.0)- 15.5	1.20 / 1.38	II
	TSH-R19878	*T. mandshurica*	Niigata	Oct. 20, 2003	15.0 -(19.5)- 23.5	13.9 -(14.9)- 15.9	1.18 / 1.55	II, III
	HU23342	*T. Japonica*	Hokkaido	Sept. 26, 1994	17.0 -(21.3)- 26.6	10.0 -(12.2)- 14.6	1.33 / 1.22	II, III
	HU21974	*T. japonica*	Hokkaido	Sept. 17, 1993	18.4 -(24.1)- 30.5	13.5 -(14.7)- 16.4	1.35 / 1.48	II, III
	HU14326	*T. japonica*	Hokkaido	Sept. 20, 1983	15.4 -(20.1)- 25.3	10.3 -(12.8)- 14.6	1.15 / 1.52	II, III
	HU23315	*T. japonica*	Hokkaido	Sept. 27, 1994	17.1 -(22.0)- 27.6	11.7 -(12.7)- 13.9	1.40 / 1.24	II
	HU23185	*T. japonica*	Hokkaido	Sept. 21, 1995	15.4 -(21.1)- 24.8	12.6 -(13.8)- 16.1	1.45 / 1.46	II
P. styracinum								
	TSH-R1527	*Styrax japonica*	Toyama	Sept. 4, 1995	15.5 -(18.1)- 21.2	10. 7 -(13.2)- 15.0	1.46 / 1.38	II
	TSH-R1583	*Sty. Japonica*	Toyama	Oct. 2, 1996	15.6 -(21.4)- 24.4	11.3 -(13.4)- 16.1	1.32 / 1.42	II
	TSH-Rt015	*Sty. Japonica*	Tsukuba	Oct. 15, 2002	17.8 -(22.8)- 28.1	10.5 -(14.1)- 16.0	1.5 / 1.43	II
	HU21498	*Sty. obssia*	Miyagi	Oct. 4, 1992	15.7 -(20.7)- 25.2	11.5 -(13.3)- 14.8	1.2 / 1.53	II

(continued)

Species	Voucher specimen ＊1	Host plant	Locality	Date collected	urediniospore length	urediniospore width	urediniospore wall thc / ds ＊2	Spore State
P. styracinum								
	HU22615	*Sty. obssia*	Miyagi	Oct. 4, 1992	20.8 -(23.09)- 26.7	12.6 -(14.9)- 16.1	1.2 / 1.52	II
	HU23948	*Sty. obssia*	Iwate	Oct. 22, 1996	18.0 -(23.9)- 25.1	12.1 -(14.3)- 15.8	1.15 / 1.45	II
	HU20816	*Sty. Japonica*	Akita	Aug. 22, 1991	15.6 -(22.4)-27.2	10.8 -(13.5)- 15.9	1.3 / 1.4	II, III
	HU20292	*Sty. Japonica*	Yamagata	Oct. 6, 1990	18.8 -(21.5)- 23.8	11.4 -(13.2)- 14.6	1.28 / 1.35	III
	HU21466	*Sty. Japonica*	Akita	Sept. 26, 1992	19.4 -(24.2)- 27.0	13.0 -(14.8)- 16.0	1.30 / 1.42	II
	HU18398	*Sty. Japonica*	Yamagata	Oct. 8, 1988	17.8 -(23.1)- 26.3	11.7 -(14.8)- 15.7	1.28 / 1.36	II, III
	HU22755	*Sty. Japonica*	Iwate	Sept. 27, 1993	16.6 -(21.8)- 25.7	11.2 -(13.2)- 15.6	1.30 / 1.42	II
	HU20815	*Sty. Japonica*	Akita	Aug. 22, 1992	16.4 -(23.2)- 26.9	11.1 -(13.5)- 15.9	1.25 / 1.44	II
	HU21478	*Sty. Japonica*	Akita	Sept. 26, 1992	16.2 -(21.5)- 24.8	10.6 -(12.1)- 14.8	1.30 / 1.45	II
	HU21279	*Sty. obssia*	Iwate	Oct. 22, 1991	18.0 -(23.2)- 25.6	12.4 -(13.8)- 16.0	1.2 / 1.35	II, III
	HU18947	*Sty. Japonica*	Yamagata	Oct. 8, 1988	17.2 -(21.6)- 24.8	12.0 -(13.8)- 15.8	1.35 / 1.42	II
	IBA8630	*Sty. Japonica*	Kagoshima	Oct. 31, 2000	18.2 -(20.9)- 24.5	13.1 -(14.5)- 16.1	1.4 / 1.45	III
	IBA8642	*Sty. Japonica*	Fukushima	Nov. 11, 2000	16.8 -(21.4)- 25.4	11.8 -(14.3)- 15.9	1.33 / 1.41	II, III
	IBA2877	*Sty. Japonica*	Ibaraki, Mito	Sept. 27, 1983	17.3 -(23.3)- 26.4	12.6 -(14.1)- 15.4	1.28 / 1.42	II, III
	IBA7646	*Sty. Japonica*	Kagoshima	Oct. 22, 1995	15.9 -(20.6)- 24.1	11.3 -(14.5)- 15.9	1.20 / 1.5	II
	IBA7730	*Sty. Japonica*	Okinawa	Dec. 9, 1995	17.2 -(21.5)- 25.5	12.5 -(14.8)- 16.0	1.20 / 1.48	II
	TSH-R1526	*Sty. Japonica*	Toyama	Sept. 28, 1995	19.4 -(22.7)- 25.6	11.2 -(13.1)- 15.5	1.30 / 1.4	II
	HU21996	*Sty. obssia*	Iwate, Morioka	Sept. 13, 1993	18.9 -(22.4)- 26.1	12.0 -(13.5)- 14.8	1.20 / 1.4	II, III
	HU21280	*Sty. obssia*	Iwate, Morioka	Oct. 22, 1991	16.7 -(22.8)- 27.0	11.8 -(13.2)- 15.1	1.30 / 1.3	II
	HU23947	*Sty. obssia*	Iwate, Morioka	Oct. 22, 1996	17.8 -(21.9)- 26.5	12.8 -(15.2)- 16.1	1.18 / 1.52	II
	HU14398	*Sty. obssia*	Hokkaido	Sept. 21, 1983	15.7 -(20.7)- 23.9	13.4 -(15.5)- 16.0	1.18 / 1.45	II
	HU14397	*Sty. obssia*	Hokkaido	Sept. 21, 1983	18.4 -(22.4)- 17.1	11.1 -(14.3)- 15.4	1.25 / 1.31	II
	HU14381	*Sty. obssia*	Hokkaido	Sept. 21, 1983	17.8 -(21.5)- 26.5	12.0 -(14.8)- 15.9	1.30 / 1.4	II

＊1 : TSH, Mycological Herbarium, University of Tsukuba, Japan; IBA, Herbarium of Systematic Mycology, Ibaraki University, Japan; HH, Hiratsuka Herbarium, Tokyo, Japan; HU, Mycolocal Herbarium, Hirosaki University, Japan; FPH, Mycological Herbarium, Laboratory of Forest Pathology Herbarium, Government Forest Experiment Station.

＊2 : wall thc: wall thickness of urediniospores; ds: distance between spines of urediniospore surface.

Appendix 2 aliangment　LSU

```
TSH-R4261              ----------------------TTCGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4265              ----------------------TTCGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4264              ----------------------TTCGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4263              ----------------------TTCGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4233              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4237              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4236              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R21242             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4238              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R21254             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4245              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R10724             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R1527              AAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R1583              AAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-Rt015              AAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4272              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4270              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R13510             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4273              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4268              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
HH102310               ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4266              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4267              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R23801             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
HU21509                ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R21252             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R3847              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R10202             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4278              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4279              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4281              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R21289             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4254              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4253              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R21299             ----------------------TTCGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
HH98635                ----------------------TTCGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R19878             AAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R12717             AAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4294              AAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4295              AAACTAACAAGGATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4287              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4289              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4288              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R4285              ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
TSH-R10187             ----------------------AACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
AF522179               ----------GATTCCCCTAGTAACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
AF426227               ----------------------ACGGCGAGTGAAGAGGGAAAAGCCCAAATTTGTAAT
AF426233Ppyrolae       ----------------------ACGGCGAGTGAAGAGGGAACAGCCCAAATTTGTAAT
                                               ****************** ***************

TSH-R4261              CTGGCTCTTTT------AGAGTCCGAGTTGTAATTTTGAGAACTGTTTTCCGTGCTGGAC
TSH-R4265              CTGGCTCTTTT------AGAGTCCGAGTTGTAATTTTGAGAACTGTTTTCCGTGCTGGAC
TSH-R4264              CTGGCTCTTTT------AGAGTCCGAGTTGTAATTTTGAGAACTGTTTTCCGTGCTGGAC
TSH-R4263              CTGGCTCTTTT------AGAGTCCGAGTTGTAATTTTGAGAACTGTTTTCCGTGCTGGAC
TSH-R4233              CTGGCTCTTTT------AGAGTCCGAGTTGTAATTTTGAGAACTGTTTTCCGTGCTGGAC
TSH-R4237              CTGGCTCTTTT------AGAGTCCGAGTTGTAATTTTGAGAACTGTTTTCCGTGCTGGAC
TSH-R4236              CTGGCTCTTTT------AGAGTCCGAGTTGTAATTTTGAGAACTGTTTTCCGTGCTGGAC
TSH-R21242             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4238              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R21254             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4245              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R10724             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R1527              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R1583              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-Rt015              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4272              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4270              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R13510             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4273              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4268              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
HH102310               CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4266              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4267              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R23801             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
HU21509                CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R21252             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
```

```
TSH-R3847              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R10202             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4278              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4279              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4281              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R21289             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4254              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4253              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R21299             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
HH98635                CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R19878             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R12717             CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4294              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4295              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4287              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4289              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4288              CTGGCTCTTTC------AGAGTCCGAGTTGTAATTTTAAGAACTGTTTTCCGTGCTGGAC
TSH-R4285              CTGGCCTTTCTT--GAAAAGGTCCGAATTGTAATTTTGAGAACTGTTTTCTGTGCTGGAC
TSH-R10187             CTGGCCCTTTCTTCAAAAGGGTCCGAATTGTAATTTTGAGAACTGTTTTCTGTGCTGGAC
AF522179               CTGGCCTTTCTT--GAAAGGGTCCGAATTGTAATTTTGAGAACTGTTTTCTGTGCTGGAC
AF426227               CTGGCCCTTTCTT-GAAAGGGTCCGAATTGTAATTTTGAGAACTGTTTTCTGTGCTGGAC
AF426233Ppyrolae       CTGGCTCTTTTTT---AAGGGTCCGAGTTGTAATTTTGAGAACTGTTATCTGTGCTGGAC
                      *****  **        *  ****** ********** ********* ** *********

TSH-R4261              CATTTACAAGTCTGTTGAAAAGCAGCATCATTGAGGGTGAGAATCCCGTACATGATATGG
TSH-R4265              CATTTACAAGTCTGTTGAAAAGCAGCATCATTGAGGGTGAGAATCCCGTACATGATATGG
TSH-R4264              CATTTACAAGTCTGTTGAAAAGCAGCATCATTGAGGGTGAGAATCCCGTACATGATATGG
TSH-R4263              CATTTACAAGTCTGTTGAAAAGCAGCATCATTGAGGGTGAGAATCCCGTACATGATATGG
TSH-R4233              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGACAATCCCGTTCATGATATGG
TSH-R4237              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGACAATCCCGTTCATGATATGG
TSH-R4236              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGACAATCCCGTACATGATATGG
TSH-R21242             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4238              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R21254             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4245              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R10724             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R1527              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R1583              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-Rt015              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4272              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4270              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R13510             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4273              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4268              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
HH102310               CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4266              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4267              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R23801             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
HU21509                CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R21252             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R3847              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R10202             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4278              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4279              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4281              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R21289             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4254              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4253              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R21299             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
HH98635                CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R19878             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R12717             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4294              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4295              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4287              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4289              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4288              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTACATGATATGG
TSH-R4285              CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTAGATGATATGG
TSH-R10187             CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTAGATGATATGG
AF522179               CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTAGATGATATGG
AF426227               CATGTACAAGTCTGTTGAAAAACAGCATCATTGAGGGTGAAAATCCCGTAGATGATATGG
AF426233Ppyrolae       CATGTACAAGTCTGTTGAAAAACAGCATCGTTGAGGGTGAAAATCCCGTAAATGATATGG
                      *** ***************** ******* ********** ********  *********

TSH-R4261              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4265              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4264              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4263              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
```

```
TSH-R4233              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4237              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4236              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R21242             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4238              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R21254             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4245              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R10724             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R1527              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R1583              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-Rt015              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4272              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4270              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R13510             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4273              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4268              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
HH102310               ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4266              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4267              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R23801             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
HU21509                ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R21252             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R3847              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R10202             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4278              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4279              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4281              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R21289             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4254              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4253              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R21299             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
HH98635                ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R19878             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R12717             ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4294              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4295              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4287              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4289              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4288              ACTACCAGTGCAAT-GTGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R4285              ACTACCAGGGCAAT-ATGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
TSH-R10187             ACTACCAGGGCAAT-ATGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
AF522179               ACTACCAGGGCAAT-ATGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
AF426227               ACTACCAGGGCAAT-ATGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
AF426233Ppyrolae       ACTACCAGGGCAATTATGATACAGTCTCTAAGAGTCGAGTTGTTTGGGAATGCAGCTCAA
                       ******** *****  ****************************************

TSH-R4261              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4265              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4264              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4263              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4233              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4237              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4236              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R21242             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4238              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R21254             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4245              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R10724             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R1527              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R1583              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-Rt015              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4272              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4270              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R13510             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4273              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4268              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
HH102310               AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4266              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4267              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R23801             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
HU21509                AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R21252             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R3847              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R10202             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4278              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4279              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4281              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R21289             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
```

```
TSH-R4254              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4253              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R21299             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
HH98635                AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R19878             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R12717             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4294              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4295              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4287              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4289              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4288              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R4285              AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
TSH-R10187             AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
AF522179               AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
AF426227               AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
AF426233Ppyrolae       AGTGGGTGGTAAATTCCATCTAAGGCTAAATATAGGTGAGAGACCGATAGCAAACAAGTA
                       ************************************************************

TSH-R4261              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4265              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4264              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4263              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4233              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4237              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4236              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R21242             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4238              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R21254             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4245              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R10724             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R1527              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R1583              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-Rt015              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4272              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4270              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R13510             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4273              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4268              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
HH102310               CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4266              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4267              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R23801             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
HU21509                CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R21252             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R3847              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R10202             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4278              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4279              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4281              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R21289             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4254              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4253              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R21299             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
HH98635                CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R19878             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R12717             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4294              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4295              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4287              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4289              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4288              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R4285              CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
TSH-R10187             CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
AF522179               CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
AF426227               CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
AF426233Ppyrolae       CCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAACAGTACGTGAAATTGTTAA
                       ************************************************************

TSH-R4261              AAGGGAAACATTTAAAGTTAGACTTGTTATTATTGGTTCAGCTTCTTTTTT-GAGGTGTA
TSH-R4265              AAGGGAAACATTTAAAGTTAGACTTGTTATTATTGGTTCAGCTTCTTTTTT-GAGGTGTA
TSH-R4264              AAGGGAAACATTTAAAGTTAGACTTGTTATTATTGGTTCAGCTTCTTTTTT-GAGGTGTA
TSH-R4263              AAGGGAAACATTTAAAGTTAGACTTGTTATTATTGGTTCAGCTTCTTTTTT-GAGGTGTA
TSH-R4233              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACCTTTTT----AAGGTGTA
TSH-R4237              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACCTTTTT----AAGGTGTA
TSH-R4236              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACCTTTTTTTTGAAGGTGTA
TSH-R21242             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4238              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R21254             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
```

```
TSH-R4245              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R10724             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R1527              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R1583              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R                  AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4272              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4270              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R13510             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4273              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4268              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
HH102310               AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4266              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4267              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R23801             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
HU21509                AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R21252             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R3847              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R10202             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4278              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4279              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4281              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R21289             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4254              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4253              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R21299             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
HH98635                AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R19878             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R12717             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4294              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4295              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4287              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4289              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4288              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACTTCTTTATT-GAGGTGTA
TSH-R4285              AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGATCAGCTTCTTTATT-GAGGTGTA
TSH-R10187             AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGATCAGCTTCTTTATT-GAGGTGTA
AF522179               AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGATCAGCTTCTTTATT-GAGGTGTA
AF426227               AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGATCAGCTTCTTTATT-GAGGTGTA
AF426233Ppyrolae       AAGGGAAACATTTGAAGTTAGACTTGTTATTGTTGGTTCAACCTTTTTTTA-AGGGTCTA
                       ************* ***************** **** *** * * ***     *** **

TSH-R4261              TTCCGGTGATTAACAGACCAGCATCAATTTTTGAGTGTCAGATAAGGGTTTAGGAAATGT
TSH-R4265              TTCCGGTGATTAACAGACCAGCATCAATTTTTGAGTGTCAGATAAGGGTTTAGGAAATGT
TSH-R4264              TTCCGGTGATTAACAGACCAGCATCAATTTTTGAGTGTCAGATAAGGGTTTAGGAAATGT
TSH-R4263              TTCCGGTGATTAACAGACCAGCATCAATTTTTGAGTGTCAGATAAGGGTTTAGGAAATGT
TSH-R4233              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTCTTGGAAATGT
TSH-R4237              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTCTTGGAAATGT
TSH-R4236              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTCTTGGAAATGT
TSH-R21242             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4238              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R21254             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4245              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R10724             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R1527              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R1583              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-Rt015              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4272              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4270              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R13510             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4273              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4268              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
HH102310               TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4266              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4267              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R23801             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
HU21509                TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R21252             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R3847              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R10202             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4278              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4279              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4281              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R21289             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4254              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4253              TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R21299             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
HH98635                TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R19878             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R12717             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
```

```
TSH-R4294             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4295             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4287             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4289             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4288             TTCCAATGGTTAACAGACCAGCATCAATTTTTGGGTGTTGGATAAGGGTTTAGGAAATGT
TSH-R4285             TTCTGATGATTAACAGACCAGCATCAATTTTTGAGTGCCGGAAAAAGGTATTGGGAATGT
TSH-R10187            TTCTGATGATTAACAGACCAGCATCAATTTTTGAGTGCCGGAAAAAGGTATTGGGAATGT
AF522179              TTCTGATGATTAACAGACCAGCATCAATTTTTGAGTGCCGGAAAAAGGTATTGGGAATGT
AF426227              TTCTGATGATTAACAGACCAGCATCAATTTTTGAGTGCCGGAAAAAGGTATTGGGAATGT
AF426233Ppyrolae      TTCCGATGATTAACAGACCAACGTCAATTTTTGAGTGTCAGAAAAGGGTCTTGAGAATGT
                      ***   ** ********** * ********* ***   ** ** *** * *  *****

TSH-R4261             AGCAATT--TCGGTTGTGTTATAGTCCTTTACT---TTGATATGATGCTTAGGA-TTGAG
TSH-R4265             AGCAATT--TCGGTTGTGTTATAGTCCTTTACT---TTGATATGATGCTTAGGA-TTGAG
TSH-R4264             AGCAATT--TCGGTTGTGTTATAGTCCTTTACT---TTGATATGATGCTTAGGA-TTGAG
TSH-R4263             AGCAATT--TCGGTTGTGTTATAGTCCTTTACT---TTGATATGATGCTTAGGA-TTGAG
TSH-R4233             GGCAACC--TTGGTTGTGTTATAGTCCTTGACT---TTGATACAATGCTTAAGA-TTGAG
TSH-R4237             GGCAACC--TTGGTTGTGTTATAGTCCTTGACT---TTGATACAATGCTTAAGA-TTGAG
TSH-R4236             GGCAACC--TTGGTTGTGTTATAGTCCTTGACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R21242            GGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4238             GGCAACC--TTGGTTGTGTTATAGTCCTTTACTC--TTGATACAATGCTTAGGAATTGAG
TSH-R21254            GGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4245             GGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R10724            GGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R1527             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R1583             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-Rt015             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4272             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4270             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R13510            AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4273             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4268             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
HH102310              AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4266             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4267             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R23801            AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
HU21509               AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R21252            AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R3847             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R10202            AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4278             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4279             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4281             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R21289            AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4254             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4253             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R21299            AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
HH98635               AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R19878            AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R12717            AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4294             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4295             AGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4287             GGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4289             GGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4288             GGCAACC--TTGGTTGTGTTATAGTCCTTTACT---TTGATACAATGCTTAGGA-TTGAG
TSH-R4285             AGCATCCCTCTGGTTGTGTTATAGACCTTTACTTCA-GAATACGGTGCTTAGGA-TTGAG
TSH-R10187            AGCATCC-TCTGGTTGTGTTATAGACCTTTACTTCA-GAATACGGTGCTTAGGA-TTGAG
AF522179              AGCATCC-TCTGGTTGTGTTATAGACCTTTACTTCA-GAATACGGTGCTTAGGA-TTGAG
AF426227              AGCATCC-TCTGGTTGTGTTATAGACCTTTACTTCA-GAATACGGTGCTTAGGA-TTGAG
AF426233Ppyrolae      AGCAACT--TTGGTTGTGTTATAGCTCTTGATTAAACGATTATGATGCTTAGGA-TTGAG
                      ***       *************  *** * *       **   ****** ** *****

TSH-R4261             GAACGCGGTAAGCTTTGTA-----GCAGATCTTTTTCGAAAGATCTCCTTACTA------
TSH-R4265             GAACGCGGTAAGCTTTGTA-----GCAGATCTTTTTCGAAAGATCTCCTTACTA------
TSH-R4264             GAACGCGGTAAGCTTTGTA-----GCAGATCTTTTTCGAAAGATCTCCTTACTA------
TSH-R4263             GAACGCCGTAAGCTTTGTA-----GCAGATCTTTTTCGAAAGATCTCCTTACTA------
TSH-R4233             GAACGCAGTAAGCTTTGTA-----GCAGATCTTTTTT--AGA--TCTCCTTACTA------
TSH-R4237             GAACGCAGTAAGCTTTGTA-----GCAGATCTTTTTT--AGA--TCTCCTTACTA------
TSH-R4236             GAACGCAGTAAGCTTTGTATTAA-GCAGATCTTTTTT--AGA--TCTCCTTACTA------
TSH-R21242            GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH-R4238             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH-R21254            GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH-R4245             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH-R10724            GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH-R1527             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R1583             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-Rt015             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4272             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
```

```
TSH-R4270              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R13510             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4273              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4268              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
HH102310               GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4266              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4267              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R23801             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
HU21509                GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R21252             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R3847              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R10202             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4278              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4279              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4281              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R21289             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4254              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R4253              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R21299             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
HH98635                GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAAA--TCTTCTTACTA------
TSH-R19878             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH R12717             GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH R4294              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH R4295              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGA--TCTTCTTACTA------
TSH R4287              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGG--TCTTCTTACTA------
TSH R4289              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGG--TCTTCTTACTA------
TSH-R4288              GAACGCAGTAAGCTTTGTA-----GCAGATTTCTTTGGAGG--TCTTCTTACTA------
TSH-R4285              GAACGCGGTAAGCTTTGTTGCAGAACCTTGTTAAACACAGGGGTCTCCTTACTA------
TSH-R10187             GAACGCGGTAAGCTTTGTTGCAGAACCTTGTTAAACACAGGGGTCTCCTTACTA------
AF522179               GAACGCGGTAAGCTTTGTTGCAGAACCTTGTTAAACACAGGGGTCTCCTTACTACGGATG
AF426227               GAACGCGGTAAGC-----------------------------------------------
AF426233Ppyrolae       GTACGCTGTGAGC-----------------------------------------------
                       * **** ** ***
```

Appendix 3 aliangment　ITS

```
TSHR4237            GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTCAA-TGAG
TSHR4264            ----------TTTCCGTAGGGGAACCTGCGGAAGGATCATTATTAAAAGTCAA-CAAG
TSHR4265            GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTCAA-CAAG
TSHR21241           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR10724           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4238            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4241            ----------TTTCAGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4243            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR21254           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAATGAG
TSHR4245            ----------TTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4287            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAATGAG
TSHR4289            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAATGAG
TSHR21298           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR21299           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4275            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR21252           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4248            GTCGTATCAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR3847            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
HH98635             ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4273            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4272            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR13510           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR21289           ----------TTTCCGTAGGTGACCCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4253            ----------TTTCCGTAGGTGACCCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR21290           ----------TTTCCGTAGGTGACCCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR21307           GTCGTACCAAGGTTTCCGTAGGTGACCCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4281            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR10202           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR23801           ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4254            GTCGTACCAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR3849            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4279            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4278            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4280            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4268            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4267            ----------TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4266            GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4270            GTCGTAACAAGGTT--CGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR1583            GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR1527            GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHRt015            GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAATGAG
TSHR4291            GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR19878           GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR4295            GTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTAAAAGTTAAACGAG
TSHR10187           ----------TTTCCGTAGGTGAACTTGCGGAAGGATCATTATTAGAA-TCAA--GGG
L76509              ------------------------------------ATCATTATTAAAAAGTCAATGAG
L76508              ------------------------------------ATCATTATTAAAAAGTCAATGAG

                                                         ********** **    *    *

TSHR4237            TGCACTTTATTGTGGCTCAAAAACCTTTA--AATCTTCACCCATT-----AACCCAAAGC
TSHR4264            CGCACTTTAGTGTGGCTCAAACCCTTTTAATTAAATTAACCCATTT---AAACCCAAAGC
TSHR4265            CGCACTTTAGTGTGGCTCAAACCCTTTTAATTAAATTAACCCATTT---AAACCCAAAGC
TSHR21241           TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR10724           TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR4238            TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR4241            TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR4243            TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR21254           TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR4245            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR4287            TGCACTTTATTGTGGCTCAAA-CCTCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR4289            TGCACTTTATTGTGGCTCAAA-CCTCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR21298           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR21299           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4275            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR21252           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4248            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR3847            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
HH98635             TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4273            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4272            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR13510           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR21289           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4253            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR21290           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
```

```
TSHR21307           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4281            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR10202           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR23801           TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4254            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR3849            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4279            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4278            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4280            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4268            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4267            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4266            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4270            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR1583            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR1527            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHRt015            TGCACTTTATTGTGGCTCAAAACATCTTT-TAACATACACCCATTTTTTAAACCCAAAGC
TSHR4291            TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR19878           TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR4295            TGCACTTTATTGTGGCTCAAA-CATCTTT-TAACATCAACCCATTTTTTAAACCCAAAGC
TSHR10187           TGCACTTTATTGTGACTC----TCTTTTA--TAATTTAAACCATCTTT-AAACCCAAAAC
L76509              TGCACTTTATTGTGGCTCAAA-CCTTTTAATATCTTTAACCCATTAT--AAACCCAAAAC
L76508              TGCACTTTATTGTGGCTCAAA-CCTTTTAATATCTTTAACCCATTAT--AAACCCAAAAC

                     ******** **** ***        **       *  * ****      ******** *

TSHR4237            TGTCTTGTGTACTCTTATCTTGTATAAGGGTATAGCATTTCAGTAGTACGCATCATGGAA
TSHR4264            TGTCTTGTGTACTTTTTTTA------AAAGTATAGCATTTCAGTAGTACGCATCACAAA-
TSHR4265            TGTCTTGTGTACTTTTTTTA------AAAGTATAGCATTTCAGTAGTACGCATCACAAA-
TSHR21241           TGTCTTGTGTGCTTTCTTTAT---TGAAAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR10724           TGTCTTGTGTGCTTTCTTTAT---TGAAAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4238            TGTCTTGTGTGCTTTCTTTAT---TGAAAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4241            TGTCTTGTGTGCTTTCTTTAT---TGAAAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4243            TGTCTTGTGTGCTTTCTTTAT---TGAAAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR21254           TGTCTTGTGTGCTTTCTTTAT---TGAAAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4245            TGTCTTGTGTGCTTTCTTTAT---TGAAAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4287            TGTCTTGTGTGCTTTCTTGAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4289            TGTCTTGTGTGCTTTCTTGAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR21298           TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR21299           TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4275            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR21252           TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4248            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR3847            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
HH98635             TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4273            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4272            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR13510           TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR21289           TGTCTTGTGTACTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4253            TGTCTTGTGTACTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR21290           TGTCTTGTGTACTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR21307           TGTCTTGTGTACTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4281            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR10202           TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR23801           TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4254            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR3849            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4279            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4278            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4280            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4268            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4267            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4266            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4270            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR1583            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR1527            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHRt015            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4291            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR19878           TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR4295            TGTCTTGTGTGCTTTCTTTAT---TGAGAGTATAGCATTTCAGTAGTACGCATCAGGGA-
TSHR10187           TGTCTGGTGTGCTTTAAC---------GAGTATAGCTTTTCAGTAGTACGCATCAGGGA-
L76509              TGTCTTGTGTACATTTTAT--------GAGTATAGCATTTCAGTAGTACGCATCATGAG-
L76508              TGTCTTGTGTACATTTTAT--------GAGTATAGCATTTCAGTAGTACGCATCATGAG-

                    ***** **** *  *              ******* ******************

TSHR4237            ATTGCCTTCATTGGAATGTTCAAGTTGCATTAACCCCCC-TTATAAGTTACCCTTA--AT
TSHR4264            --------C--TCTTTTGTTTGTGTTGCATTACCCCCCC-TTATAAGTGACCCTTT--AT
TSHR4265            --------C--TCTTTTGTTTGTGTTGCATTACCCCCCC-TTATAAGTGACCCTTT--AT
```

```
TSHR21241           --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TCATAAGTGACCCCCCC-AT
TSHR10724           --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TCATAAGTGACCCCCCC-AT
TSHR4238            --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TTATAAGTGACCCCCCC-AT
TSHR4241            --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TTATAAGTGACCCCCCC-AT
TSHR4243            --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TTATAAGTGACCCCCCC-AT
TSHR21254           --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TTATAAGTGACCCCCCC-AT
TSHR4245            --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TTATAAGTGACCCCCCC-AT
TSHR4287            --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TTATAAGTGACCCCCCC-AT
TSHR4289            --------CAATTTATTGTTCAGGTTGCATTACCCCCCC-TTATAAGTGACCCCCCC-AT
TSHR21298           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR21299           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4275            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR21252           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4248            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR3847            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
HH98635             --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4273            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4272            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR13510           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR21289           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4253            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR21290           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR21307           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4281            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR10202           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR23801           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4254            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR3849            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4279            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4278            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4280            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4268            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4267            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4266            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4270            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR1583            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR1527            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHRt015            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4291            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR19878           --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR4295            --------CAATTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCC--AT
TSHR10187           --------C--TTTATTGTTCAAGTTGCATTACCCCCCC-TCATAAGTGACCCCCCTTAT
L76509              --------C--TTTATTGTTCAAGTTGCATTACCCCCCCCTTATAAGTGACCCCTTT-AT
L76508              --------C--TTTATTGTTCAAGTTGCATTACCCCCCCCTTATAAGTGACCCCTTT-AT

                             *  *    ****   ********* ****** * ****** ****     **

TSHR4237            TTTT----ATTATTAAC---CCTATAAA--GTTTTAAGAATGTAAACCCCC-TTTAAATT
TSHR4264            TCTCTGAAATCATTAACCACAATAAAAG---TTTTAAGAATGTAAACCCCC-CTTAAATT
TSHR4265            TCTCTGAAATCATTAACCACAATAAAAG---TTTTAAGAATGTAAACCCCC-CTTAAATT
TSHR21241           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR10724           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR4238            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR4241            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR4243            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR21254           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR4245            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4287            TTTTTGAAGTCATTAAC-ACATTAAAAAAAGTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR4289            TTTTTGAAGTCATTAAC-ACATTAAAAAAAGTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR21298           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR21299           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4275            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR21252           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4248            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR3847            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
HH98635             TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4273            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4272            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR13510           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR21289           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4253            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR21290           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR21307           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4281            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR10202           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR23801           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4254            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR3849            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
```

```
TSHR4279            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4278            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4280            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4268            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4267            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4266            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4270            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR1583            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR1527            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHRt015            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCCCTTTAAAAT
TSHR4291            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR19878           TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR4295            TTTTTGAAATCATTAAC-ACATTAAAAA--GTTTTAAGAATGTAAACCCCC-TTTAAAAT
TSHR10187           TTTTTGAAACCATTACCCTTACAAGAAA--GTTTTAAGAATGTGAAAAAA---TGGAAAT
L76509              TTTTTGAAATCATTAAACGTTTTATGTAAAGTTTTAAGAATGTAAACCATG--TGGAATT
L76508              TTTTTGAAATCATTAAACGTTTTATGTAAAGTTTTAAGAATGTAAACCATG--TGGAATT

                    * *       ****       *       ********** **       *  ** *

TSHR4237            AA-----TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4264            ---AA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACGCAGTG
TSHR4265            ---AA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACGCAGTG
TSHR21241           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR10724           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4238            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4241            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4243            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR21254           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4245            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4287            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4289            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR21298           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR21299           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4275            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR21252           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4248            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR3847            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
HH98635             GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4273            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4272            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR13510           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR21289           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4253            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR21290           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR21307           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4281            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR10202           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR23801           GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4254            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR3849            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4279            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4278            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4280            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4268            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4267            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4266            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4270            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR1583            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR1527            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHRt015            GAAAA--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4291            GAAA---TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR19878           GAAA---TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR4295            GAAA---TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
TSHR10187           ----A--TATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACGCAGTG
L76509              AAATATATATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG
L76508              AAATATATATAACTTTTAACAATGGATCTCTTGGCTCTCACATCGATGAAGAACACAGTG

                          ****************************************** *****

TSHR4237            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCAAATCTTTGAACGCACC
TSHR4264            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4265            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR21241           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR10724           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4238            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4241            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4243            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR21254           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
```

```
TSHR4245            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4287            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4289            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR21298           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR21299           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4275            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR21252           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4248            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR3847            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
HH98635             AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4273            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4272            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR13510           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR21289           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4253            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR21290           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR21307           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4281            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR10202           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR23801           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4254            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR3849            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4279            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4278            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4280            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4268            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4267            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4266            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4270            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR1583            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR1527            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHRt015            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4291            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR19878           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR4295            AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
TSHR10187           AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
L76509              AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC
L76508              AAATGTGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACC

                    ************************************** ***************

TSHR4237            TTGCACCTTTTGGT-ACTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4264            TTGCGCCTTTTGGT-ATTCCAAAAGGCACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4265            TTGCGCCTTTTGGT-ATTCCAAAAGGCACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR21241           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR10724           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4238            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4241            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4243            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR21254           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4245            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4287            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4289            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR21298           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR21299           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4275            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR21252           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4248            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR3847            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
HH98635             TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4273            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4272            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR13510           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR21289           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4253            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR21290           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR21307           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4281            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR10202           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR23801           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4254            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR3849            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4279            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4278            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4280            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4268            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4267            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4266            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
```

```
TSHR4270            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR1583            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR1527            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHRt015            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4291            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR19878           TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR4295            TTGCACCTTTTGGT-ATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
TSHR10187           TTGCACCTCTTGGTTATTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
L76509              TTGCACCTTTTGGT-ACTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT
L76508              TTGCACCTTTTGGT-ACTCCAAAAGGTACACCTGTTTGAGTGTCATGAAACCCTCTCATT

                    **** *** ***** * ******** **********************************

TSHR4237            CCAATT-CTTTATTAAAT-----TAAGGAAATGTG-TAATGGATTTTGAGTGTTGCTGTT
TSHR4264            CTAATTTCTTCATT--TT-----TGAAGACGT-TGGTAATGGATGTTGAGCGTT-GCCGT
TSHR4265            CTAATTTCTTCATT--TT-----TGAAGACGT-TGGTAATGGATGTTGAGCGTT-GCCGT
TSHR21241           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR10724           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4238            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4241            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4243            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR21254           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4245            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4287            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4289            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR21298           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR21299           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4275            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR21252           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4248            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR3847            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
HH98635             CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4273            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4272            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR13510           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR21289           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4253            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR21290           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR21307           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4281            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR10202           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR23801           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4254            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR3849            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4279            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4278            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4280            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4268            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4267            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4266            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4270            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR1583            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR1527            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHRt015            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4291            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR19878           CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR4295            CCAATT-CTTTATTAATT-----TGAAGATGT-TGGTAATGGATGTTGAGTGTTTGCTGT
TSHR10187           CTGATT-CTTTATTAAGT-----TAAAGG-GT-TGGTGATGGATGTTGAGTGTT-GCCGT
L76509              CCAGTCTTTTTTTTATATATATATAAAGGATTCTGGTGATGGATGTTGAGTGTTGCCGGT
L76508              CCAGTCTTTTTTTTATATATATATAAAGGATTCTGGTGATGGATGTTGAGTGTTGCCGGT

                    *   *   **  **   *     * * *   * ** * ****** ***** ***     *

TSHR4237            ATT----GGCTCACTTTAAATATATAAGTACCTTTATTGAATAAAT--AAATGGAG-AAA
TSHR4264            GTTGAATGGCTCGCTTTAAATATATAAGTACGTCTATT-GATAAGT--AAATGGAGCAAA
TSHR4265            GTTGAATGGCTCGCTTTAAATATATAAGTACGTCTATT-GATAAGT--AAATGGAGCAAA
TSHR21241           GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAATGGAG-AAA
TSHR10724           GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAATGGAG-AAA
TSHR4238            GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAATGGAG-AAA
TSHR4241            GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAATGGAG-AAA
TSHR4243            GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAATGGAG-AAA
TSHR21254           GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAATGGAG-AAA
TSHR4245            GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAATGGAG-AAA
TSHR4287            GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAGTGGAG-AAA
TSHR4289            GTTAAACAGCTCACTTTAAATATATAAGTACATTTCTTTGATAAAT--AAGTGGAG-AAA
TSHR21298           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR21299           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4275            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
```

```
TSHR21252           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4248            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR3847            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
HH98635             GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4273            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4272            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR13510           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR21289           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4253            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR21290           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR21307           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4281            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR10202           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR23801           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4254            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR3849            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4279            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4278            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4280            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4268            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4267            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4266            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4270            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR1583            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR1527            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHRt015            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4291            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR19878           GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR4295            GTTAAACAGCTCACTTTAAATATATAAGTACATTTATTTGATAAAT--AAGTGGAG-AAA
TSHR10187           ATT---TGGCTCGCTTTAAATACATAAGTACTTTTGTT-GACAAATGAAAATGGAT-ATA
L76509              GTT----GGCTCACTTTAAATACATAAGTACTTTTATT-GATAAAT--AAATGGAG-AAA
L76508              GTT----GGCTCACTTTAAATACATAAGTACTTTTATT-GATAAAT--AAATGGAG-AAA

                     **     **** ********* ******* * * **  * ** *  ** ****  * *

TSHR4237            TACTTGGTGTAATA--TTTATTAT-TCATCAAGGAATGT------GGTTTACCA------
TSHR4264            AACTTGATGTGATA--ATTATCAT-TCATTGAGGAATGTTGGGGTAGTAAAATACTTTGA
TSHR4265            AACTTGATGTGATA--ATTATCAT-TCATTGAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR21241           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTAGAGTAGTAAAATACTTTGA
TSHR10724           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTAGAGTAGTAAAATACTTTGA
TSHR4238            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTAGAGTAGTAAAATACTTTGA
TSHR4241            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTAGAGTAGTAAAATACTTTGA
TSHR4243            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTAGAGTAGTAAAATACTTTGA
TSHR21254           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTAGAGTAGTAAAATACTTTGA
TSHR4245            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTAGAGTAGTAAAATACTTTGA
TSHR4287            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGAAGTAGTAAAATACTTTGA
TSHR4289            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGAAGTAGTAAAATACTTTGA
TSHR21298           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR21299           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4275            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR21252           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4248            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR3847            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
HH98635             TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4273            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4272            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR13510           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR21289           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4253            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR21290           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR21307           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4281            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR10202           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR23801           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4254            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR3849            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4279            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4278            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4280            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4268            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4267            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4266            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4270            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR1583            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR1527            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHRt015            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGGAGTAGTAAAATACTTTGA
TSHR4291            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGAAGTAGTAAAATACTTTGA
TSHR19878           TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGAAGTAGTAAAATACTTTGA
```

```
TSHR4295            TACTTGGTGTGATA--ATTATCAT-TCATCAAGGAATGTTGAAGTAGTAAAATACTTTGA
TSHR10187           TACTTGGTGTGATATTATTANCAAATCATTGAGGAGTGT------AGTTAACTCTGCAGC
L76509              TACTTGGTGTGATA--TTTATTAT-TCATTGAGGAATGT--GAATAGCTTGCTACTTA--
L76508              TACTTGGTGTGATA--TTTATTAT-TCATTGAGGAATGT--GAATAGCTTGCTACTTA--

                     ***** *** ***   ***  *  ****  **** ***       *

TSHR4264            CGGCCATTTGACCTTTAA--TAAATAGCTTCCTAACCCTA-TTCATTTTGA----ACTTT
TSHR4265            CGGCCATTTGACCTTTAA--TAAATAGCTTCCTAACCCTA-TTCATTTTGA----ACTTT
TSHR21241           CAGCCATTTGACTTTCAA-GTTAATAGCTTCCTAACCCCA-ATATTATATA--AACTTTT
TSHR10724           CAGCCATTTGACTTTCAA-GTTAATAGCTTCCTAACCCCA-ATATTATATA--AACTTTT
TSHR4238            CAGCCATTTGACTTTCAA-GTTAATAGCTTCCTAACCCCA-ATATTATATA--AACTTTT
TSHR4241            CAGCCATTTGACTTTCAA-GTTAATAGCTTCCTAACCCCA-ATATTATATA--AACTTTT
TSHR4243            CAGCCATTTGACTTTCAA-GTTAATAGCTTCCTAACCCCA-ATATTATATA--AACTTTT
TSHR21254           CAGCCATTTGACTTTCAA-GTTAATAGCTTCCTAACCCCA-ATATTATATA--AACTTTT
TSHR4245            CAGCCATTTGACTTTCAA-GTTAATAGCTTCCTAACCCCA-ATATTATATA--AACTTTT
TSHR4287            CAGCCATTTGACTTTCAAAGTTAATAGCTTCCTAACCCCA-ATTTTATATATAAACTTTT
TSHR4289            CAGCCATTTGACTTTCAAAGTTAATAGCTTCCTAACCCCA-ATTTTATATATAAACTTTT
TSHR21298           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR21299           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR4275            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTATT---ACCTTT
TSHR21252           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR4248            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR3847            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
HH98635             CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR4273            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR4272            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR13510           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR21289           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR4253            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR21290           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR21307           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR4281            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTT-
TSHR10202           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTT-
TSHR23801           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTTT
TSHR4254            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR3849            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTT-
TSHR4279            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTT-
TSHR4278            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTT-
TSHR4280            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTT-
TSHR4268            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTTT
TSHR4267            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTTT
TSHR4266            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTT-AAT---ACCTTT
TSHR4270            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR1583            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR1527            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHRt015            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTTTAAT---ACCTTT
TSHR4291            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTATAAA---CTTTTT
TSHR19878           CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTATAAA---CTTTTT
TSHR4295            CAGCCATTTGAATTTCAA-GTTGATAGCTTCCTAACCCCA-ATTTTATAAA---CTTTTT
TSHR10187           CATTTTTGTATATGTTGA--TGGATAGCTTCCTAACCCCA-TTTAATTTAA---TTATTT
L76509              CAGCCATTTGAGTTTTGA--TAAATAGCTTCCTAACCCAAATTCATTTTGA----CCTTT

                    *     * *     *  *  *  ************** *                **

TSHR4237            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA-
TSHR4264            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4265            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR21241           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR10724           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4238            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4241            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4243            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR21254           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4245            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4287            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4289            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR21298           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA-
TSHR21299           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA-
TSHR4275            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR21252           AGACCTCAAATCAGGTGGGATT-CCCGCTGAACTTAA----------------------
TSHR4248            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCATAAGCGGAGGAA-
TSHR3847            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
HH98635             AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4273            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR4272            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR13510           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA----------------------
TSHR21289           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA
```

```
TSHR4253            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA
TSHR21290           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATA----------------
TSHR21307           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCA-TAAGCGGAGGAA
TSHR4281            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR10202           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR23801           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA
TSHR4254            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA
TSHR3849            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR4279            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR4278            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR4280            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR4268            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR4267            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR4266            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA-
TSHR4270            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR1583            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR1527            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHRt015            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR4291            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR19878           AGACCTCAAATCAAGTGGGATTACCCGCTGAACTTAA--------------------
TSHR4295            AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
TSHR10187           AGACCTCAAATCAGGTGGGATTACCCGCTGAACTTAA--------------------
L76509              AGACCTCAAATCAGGTGGGA------------------------------------
L76508              AGACCTCAAATCAGGTGGGA------------------------------------

                    ************ ******
```