Phylogenic and Taxonomic Studies of *Phakopsora* Species on *Meliosma* and *Vitis* Species

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Chapter 1. Introduction

1.1. Overview of rust fungi

Rust fungi are considered to constitute a monophyletic group (all having evolved from a common ancestor), classified in the Order Pucciniales (Uredinales), and in the Class Pucciniomycotina (Basidiomycota) (Aim et al. 2006). The name rust fungi comes from the fact that rust sori (sporulating structures) are often orange or reddish-orange that rupture the epidermis, thus making the plant "rusty" like the iron rust (Cummins and Hiratsuka 2003). The rust fungi are obligate biotrophic plant parasites that include many important plant pathogens, causing damage to many economic plants (Agrios 1997). In their natural habitat, rust fungi are ecologically obligate parasites on living plants, although a few species are now successfully cultured on artificial media (axenic culture) (Cummins and Hiratsuka 2003). Rust fungi parasitize a wide range of host plants, including ferns, conifers, and angiosperms (both mono and dicotyledonous). Many species need two unrelated groups of host plants to complete their life cycle (heteroecious life cycle), although others can complete their life cycles on a single kind of host plant (autoecious life cycle) (Cummins and Hiratsuka 2003). The rust fungi consist of 7798 species, in 166 genera and in 14 families (Kirk et al. 2008).

The rust fungi are unique in having complex life cycle patterns with up to five morphologically and functionally different spores in respective stages of the life cycle. Five kinds of spore-producing structures are generally recognized as basic spore states of the rust fungi: spermogonium (pl. spermogonia), aecium (pl. aecia), uredinium (pl. uredia), telium (pl. telia), and basidium (pl. basidia) (Cummins and Hiratsuka 2003).

The spermogonium is produced from a haploid mycelium, which is derived from a haploid basidiospore infection. They are minute, but occur in groups, often on discolored spots or hypertrophied tissues. The spermogonia produce spermatia in a sweetish exudate and the spermatia function as dikaryotizing elements. The morphological types of spermogonium have been considered to be dependable as characteristics useful in suprageneric taxonomy (Hiratsuka and Cummins 1963). Twelve morphological types were recognized in six groups by Hiratsuka and Hiratsuka (1980).

The aecium is a non-repeating spore stage usually produced as the result of the dikaryotization by the fusion of a sexually-compatible spermatium and a receptive hypha. After the dikaryotization of an aecial primordial cell with a haploid nucleus from a basidiospore. Aeciospores are produced commonly in chains (Cummins and Hiratsuka 2003).

The uredinium is a repeating vegetative state produced on a dikaryotic mycelium and produce dikaryotic urediniospores. The urediniospores are most commonly produced on a pedicel in the hymenium with or without paraphyses. When the urediniospores infect plants, either urediniospores or teliospores are produced. If the hosts are actively growing and climatic conditions are favorable, repeated urediniospore infections result in urediniospore production, which will increase the rust population in a short period (Cummins and Hiratsuka 2003).

The telium is formed from the same dikaryotic mycelium as is the uredinium. When the host plants undergo senescence or when climatic conditions become unfavorable for repeated production of urediniospores, the dikaryotic mycelium produces teliospores. Teliospores are the most important spore state in generic

distinctions. They produce basidia and, usually, basidiospores upon germination (Cummins and Hiratsuka 2003). The telial stage represents the teleomorph (perfect stage) of rust fungi under the International Code of Botanical Nomenclature (Greuter et al. 1994).

Depending on the number of spore states, three basic types of life cycle are recognized: macrocyclic, demicyclic and microcyclic. The macrocyclic life cycle has all spore states, the demicyclic lacks the uredinial state, and the microcyclic lacks both the aecial and the uredinial states, thus possessing only telial and sometimes spermogonia. Spermogonia may be absent from any type (Cummins and Hiratsuka 2003).

1.2. Taxonomic history of *Phakopsora* species on *Meliosma* and *Vitis* Species

The genus *Phakopsora* was established in 1895, and *Phakopsora punctiformis* (Barclay & Dietel) Ditel on *Galium aparine* (Rubiaceae) was treated as the type species (Dietel 1895). This genus contains at least 90 morphologically variable species (Ono et al. 1992; Cummins and Hiratsuka 2003) and several genera have been segregated from or synonymized with it (Cummins and Ramachar 1958; Ono et al. 1992; Burticá and Hennen 1994 cited in Aime 2006). This genus is well-described in the 'Illustrated Genera of Rust Fungi' (Cummins and Hiratsuka 2003), and it has the following morphological characteristics: spermogonia group VI (type 7), aecia are superepidermal, erumpent, *Calidion* or *Milesia* type, and aeciospores are borne singly and similar to the urediniospores; Uredinia are superepidermal, erumpent, with peripheral, incurved, unusually, dorsally thick-walled paraphyses surmounting peridial tissue, *Malupa*- and *Calidion*-type (*Phakopsora* sensu strictio), or without paraphyses, *Uredo*-type (*Bubakia*

when segregate), and urediniospores are borne singly with an echinulate wall, brownish or nearly colorless, and pores scattered or equatorial; Telia are subepidermal, non-erumpent, consisting of crusts of laterally adherent teliospores 2 or more cells deep, and teliospores are sessile, either catenulate or irregularly arranged, 1-celled, usually with a brownish wall, with 1 apical germ pore, presumably germinating after dormancy in most or all species: basidia external.

Currently, eight rust species are recognized on various *Meliosma* plants: one *Goplana* (Raciborski 1909; Ono and Hennen 1983), four *Aecidium* (Hennings 1900; Cummins 1937, 1941; Hosagoudar 1987), and three *Phakopsora* (Kusano 1904; Ono 2000). Despite the broad geographic distribution of the host genus, their rust records are limited in Asia. Among the three *Phakopsora* species on *Meliosma*, the grapevine leaf rust (GLR) fungi, *P. euvitis* Y. Ono with the uredinial/telial stage on *Vitis* plants and *P. vitis* P. Sydow with the uredinial/telial stage on *Parthenocissus* plants have been proven to form the spermogonial/aecial stage on *M. myriantha* in Japan (Ono 2000). *Phakopsora meliosmae* Kusano is a macrocyclic autoecious species on *Meliosma* plants (Kusano 1904). The *Aecidium* state of the common GLR pathogen was first reported on *Meliosma tenuis* (Kudo and Kaneko 1977) and on *M. myriantha* (Kudo and Kaneko 1978). The two fungal populations on these two different *Meliosma* species were considered to be one population that shared *M. tenuis* and *M. myriantha* as spermogonial and aecial hosts (Kudo and Kaneko 1977, 1978).

Plants of the genus *Meliosma* (Sabiaceae) are trees or shrubs that originate in Asia and the Americas (Van Beusekom 1971; Wu et al. 2007). In the Japanese archipelago, five species and one variety have been reported to occur either in evergreen or deciduous forests: *Meliosma arnottiana* (Wight) H. Ohba subsp. *oldhami* (Maxim.) H.

Ohba var. *oldhami* [=*M. oldhami* Maxim., *M. pinnata* (Roxb.) Maxim. subsp. *barbulata* Cufod. Beusekom var. *oldhamii* (Maxim.) Beusekom, *M. rhoifolia* Maxim.], *M. arnottiana* subsp. *oldhamii* var. *hachijoensis* Nakai H.ohba [=*M. hachijoensis* Nakai], *Meliosma myriantha* Sieb. & Zucc., *Meliosma rigida* Sieb & Zucc.[=*M. simplicifolia* subsp. rigida (Sieb. & Zucc.) Beusekom], *Meliosma squamulata* Hance [=*M. lutchuensis* Koidz], *Meliosma. tenuis* Maxim [=*M. dilleniifolia* subsp. *tenuis* (Maxim.) Beusekom] (Satake et al. 1989; Wu et al. 2007; Anonymous 2011).

Plants of the genus *Vitis* (Vitaceae: Vitales) harbor several rust fungi (Leu 1988). GLR is one of the most serious diseases of cultivated grapes in temperate regions and is often destructive in warm temperate or subtropical regions. The fungus causes yellowing on leaves (Fig. 1.) and premature defoliation of grapevines, by which fruit quality and yields are reduced. The disease normally becomes an epidemic near harvest time in cool temperate regions, but in warm temperate and subtropical regions, it is serious throughout the growing season (Ono 2000). Currently, leaf rust is a minor fungal foliar disease of commercial table wine and raisin grapes in temperate East Asia. It is important to realize, however, that the incidence and severity of major fungal foliar diseases of grapevines would change in the traditional viticulture regions under the global climate change (Hayman et al. 2009; Fraga et al. 2012) and in newly developing tropical viticulture regions like Southeast Asia (Possingham 2008; Commins et al. 2012), Australia (Weinert et al. 2003) and Brazil (Tessmann et al. 2004). The leaf rust is likely to become a major disease of grapevines grown under warm climate conditions, particularly in Southeast Asia where commercial cultivars and their root-stocks are often severely infected by a GLR fungus.

Before the work of Ono (2000), a fungus that causes leaf rust disease of cultivated grapes was first described and named as *Uredo vitis* Thüm. based on a specimen on *Vitis vinifera* collected in 1868 in South Carolina, USA (Thümen 1878). Cockerell (1891) cited *U. vialae* Lagerh. on *V. vinifera* collected in Jamaica (Lagerheim 1890) as the first rust described on grapes and did not consider Thümen`s material as the first rust described on grapes. Later, Massee compared the type material of both *U. vitis* and *U. vialae* and found the two identical (Cited from Dale 1955).

Ten years after *U. vitis* was described, two additional rust fungi collected on *Ampelopsis brevipedunculata* (as *A. leeoides* Planch.) and *Parthenocissus tricuspidata* (as *Vitis incostan* Miq.) in Tokyo, Japan were described as *P. ampelopsidis* (Dietel 1898) and *P. vits* (Sydow 1899), respectively. Hiratsuka (1900) compared Japanese isolates on *A. brevipedunculata*, *P. tricuspidata*, *V. coignetiae*, *V. flexuosa*, and *V. vinifera* and found that those isolates varied with morphology of paraphyses, size of urediniospores, and size and position of telia in relation to uredinia. However, he considered that those variations were insufficient to separate the fungal isolates on different hosts as distinct species. He concluded that all isolates on vitaceous plants in Japan belonged in *P. ampelopsidis* and treated *P. vitis* as a synomym. Hiratsuka (1900) also stated that *U. vitis* in North America was quite different from *P. ampelopsidis* without giving an explicit reason. In the monograph of the Japanese species of *Phakopsora*, Hiratsuka (1935) followed his father's taxonomic decisions (Hiratsuka 1900), but concluded that *U. vitis* and *U. vialae* were the synonyms of *P. ampelopsidis* without mentioning clear reason. He cited a specimen collected in 1882 in Tokyo as the oldest Japanese record of the cultivated grape leaf rust. Later, fungal isolates parasitic

on Vitaceous plants, including cultivated grapes, have been referred to as *P. ampelopsidis* (cited from Ono 2000).

Ono (2000) revised the *P. ampelopsidis* species complex (*P. ampelopsidis* sensu lato) on vitaceous hosts with morphological observation and host specificity. He determined a grape leaf rust fungus, *P. ampelopsidis s.l.*, distributed in Japan, consists of three populations differing in their life cycle and host specificity. One population forms the uredinial and telial stage on *Ampelopsis*, the second population on *Parthenocissus*, and the third population on *Vitis*. The three populations are well circumscribed by morphological characteristics: number and distribution of urediniospore germ pores, shape and size of uredinial paraphyses, arrangement of teliospores in the sorus, shape and size of basidiospores and apical wall thickness of aeciospores (Table 1). The number and distribution of urediniospore germ pores is as follows: 4 or 6, equatorial (rarely scattered) in *P. ampelopsidis*; 4, equatorial in *P. vitis*; and 6, scattered (rarely 4 at an equatorial zone) in *P. euvitis*. The shapes of the paraphyses are short, strongly incurved, dorsally thick-walled in *P. ampelopsidis* and *P. vitis*, whereas they are long, weakly or moderately incurved, and thin-walled in *P. euvitis*. The shapes of the teliospores and their organization in the sori are subglobose or oblong, and less randomly arranged in *P. ampelopsidis*; oblong in vertical rows in *P. euvitis* and *P. vitis*, with the uppermost layer of the sori consisting of narrow oblong or linear spores. The shape of the basidiospores is kidney–shaped in *P. ampelosidis* and *P. euvitis*, as opposed to being subglobose or broadly ellipsoid in *P. vitis* (Table 1). Ono (2000) concluded that the three fungal populations were distinct species. The population on *Ampelopsis* is *P. ampelopsidis* sensu Dietel & P. Sydow and the population on

Parthenocissus is *P. vitis* sensu P. sydow. The population on *Vitis* in Japan is described by its new name *P.euvitis* Y. Ono (Ono 2000).

The *Aecidium* state of the common GLR on *Meliosma tenuis* and on *M. myriantha* was considered to be one population which shared *M. tenuis* and *M. myriantha* as spermogonial and aecial hosts (Kudo and Kaneko 1977, 1978). However, the results of Ono (2000) showed that inoculation of the basidiospores derived from the teliospores formed on the *Vitis* did not result in the formation of spermogonia and aecia on *M. tenuis*. Furthermore, aeciospores formed by natural infection on *M. tenuis* did not infect all the vitaceous plants inoculated. Thus, Ono (2000) concluded that the spermogonial/aecial fungus on *M. tenuis* is different from the common GLR pathogen that forms the spermogonial/aecial stage on *M. myriahtha*. It was assumed that the *Aecidium* state on *M. tenuis* is the spermogonial/aecial stage of an undescribed *Phakopsora* species parasitic on a vitaceous plant because of the aeciospores-surface structure that is unique to the genus *Phakopsora,* as well as the close taxonomic relationships of host plants. (Ono 2000; Chatasiri and Ono 2008).

An additional macrocyclic, autoecious rust fungus, *Phakopsora meliosmae* Kusano was first described by Kusano in 1904 on the basis of its uredinial and telial stage on *M. myriantha.* It was collected from Mt. Takao, Tokyo (Musashi), Japan, and later, also recorded on *M. tenuis* and *M. rhoifolia* Maxim. (Hiratsuka 1935; Hiratsuka 1960; Ono and Kakishima 1982; Hiratsuka et al. 1992). However, the spermogonial and aecial stages of the rust had been unknown until Kakishima et al. (1983) found the *Aecidium* sp. on *M. myriantha* and then studied the life cycle and morphology of *P. meliosmae* on *M. myriantha*. Inoculation with basidiospores of *P. meliosmae* resulted in formation of spermogonia and aecia on *M. myriantha* (Kakishima et al. 1983). Thus, it

was clarified that *P. meliosmae* is an autoecious rust fungus and the description of *P. meliosmae* was emended by adding the description of the data on the aecial stage. This report was based on the sample from a single locality in Japan which included only specimens of *M. myriantha*, but is the only previous publication on the life cycle of this fungus. No further studies on life-cycle and host range ensued for rust populations forming the uredinail/telial stages on *M. myriantha* and other species were published. Phakopsoroid fungi with the uredinial/telial stage on various *Meliosma* species were identified as *P. meliosmae* based on the morphological similarities and host genus. As a result, *P. meliosmae* is now reported to occur on 7 species and two variety of *Meliosma* and distributed widely from East Asia through the Himalayas (Kusano 1904; Hiratsuka and Hashioka 1934; Hiratsuka 1935; Arthur and Cummins 1936; Ito 1938; Thiramalachar and Kern 1949; Shimabukuro 1961; Tai 1979; Durrieu 1987; Ono et al. 1990; Hiratsuka et al. 1992; Zhuang and Wei 1994; Zhang et al. 1997; Cao and Li 1999; Cao et al. 2000; Cho and Sin 2004; Chatasiri and Ono 2008).

In Japan, *M. myriantha* is recorded as a very common host of *P. meliosmae*, while *M. tenuis* is less common and *M. arnottiana* subsp. *oldhami* (*M. rhoifolia*) is a rare host (with only two records) of *P. meliosmae*. Morphological differences among the fungal populations on these hosts were not detected (Kusano 1904; Hiratsuka and Hashioka 1934; Hiratsuka 1935; Ito 1938; Hiratsuka and Shimabukuro 1955; Shimabukuro 1961; Hiratsuka et al. 1992; Ono 2000; Chatasiri and Ono 2008). However, a preliminary study showed that a *Phakopsora* population on *M. tenuis* was parasitic only on plants of the same host species, but not on *M. myriantha* plants (Ono, unpublished records). Furthermore, in a molecular phylogenetic study of Japanese *Phakopsora* populations parasitic on vitaceous plants and *Meliosma*, the autoecious rust

populations on *M. myriantha* and *M. tenuis* were shown to be genetically distant (Chatasiri and Ono 2008). Therefore, to clarify the taxonomic status of those two fungal populations, detailed comparative studies on host-specificity and morphology are required.

To detect cryptic species of rust fungi, the internal transcribed spacer (ITS) regions which separate the genes encoding the 18S, 5.8S and 28S ribosomal RNA are commonly chosen because of their high degree of variability. The sequences of conserved DNA region and transcribed spacer regions in the rDNA operon (ITS1 and ITS2) have been successfully used to identify distinct lineages of fungal species and resolve evolutionary relationships between closely related species by other authors (Zambino and Szabo 1993; Roy et al. 1998; Vogler and Bruns 1998; Pfunder et al. 2001; Weber et al. 2003). DNA-based phylogentic analyses have been used for taxonomy of rust fungi, such as species from genera *Puccinia, Pucciniastrum, Chrysomyxa, Melampsora, Uromyces, Gymnosporangium* and *Phakopsora*, along with morphological studies (Zambino and Szabo 1993; Virtudazo et al. 2001; Chung et al. 2004; Wingfield et al. 2004; Pei et al. 2005; Liang et al 2006; Maier et al. 2007; Chatasiri and Ono 2008; Vialle et al. 2011).

Chatasiri and Ono (2008) conducted molecular phylogenetic analyses of *Phakopsora* species on *Vitis* and *Meliosma* plants. They used the D1/D2 region of the nuclear, large subunit rDNA and the internal transcribed spacer 2 (ITS2) regions including 5.8S. Their results supported the biological and taxonomic distinctness of the three *Phakopsora* species, *P. ampelopsidis*, *P. vitis*, and *P. meliosmae-myriantha* (Ono 2000; Chatasiri and Ono, 2008). They concluded that the unconnected *Aecidium* is likely to be an aecial state of undescribed *Phakopsora* on vitaceous plants because of

the aeciospores-surface structure that is unique to the genus *Phakopsora* and its genetically similar to GLR in Japan. Moreover, they also have the close taxonomic relationships of the host plants in *Meliosma* species.

Regarding GLR, Chatasiri and Ono (2008) found 20 base positions of the ITS2 sequence of the Australian-East Timor samples of GLR that are different from Japanese samples. They suspected that the GLR distributed in tropical Asia is genetically different from, and may comprise a species distinct from, that which is distributed in temperate Asia (Fig. 2). However, the GLR sampled by Chatasiri and Ono (2008) were limited to Japan, Australia, and East Timor, and no sample from tropical Asia was included in the study. Therefore, GLR samples from tropical Asia, especially from Southeast Asia where GLR is the major disease in grapevine cultivation, needs to be further studied.

Regarding the autoecious rust fungus, *P. meliosmae* on *M. myriantha* and *M. tenuis*, the results of Chatasiri and Ono (2008) showed the genetic distinction between these two hosts. Therefore, a host-specificity study and morphological comparison between these two rusts is needed to confirm the taxonomic status.

1.3. Objective of the study

To recognize rust fungi at the species level, the life cycle, host specificity and morphological differences need to be elucidated (Ono 1994, 2000, 2003, 2005, 2008). The purpose of this study is to clarify the taxonomic status of *Phakopsora* species on *Meliosma* and *Vitis* species based on host specificity, morphology and molecular analyses. The studies shown in chapter 2 demonstrate that two macrocyclic autoecious rust fungi on *M. myriantha* and on *M. tenuis* are distinct species. The studies of

heteroecious *Phakopsora* species on *Vitis* and *Meliosma* species in Chapter 3 is to clarify the taxonomy of three grapevine leaf rust populations on East Asian, Southeast Asian and Australasian GLR.

Table 1. Morphological characteristics and host plants of *Phakopsora ampelopsidis*, *P. vitis* and *P. euvitis*, revised by Ono (2000).

Rust	Uredial-telial host	Spemogonial-aecial host	Aeciospore apical wall	Uredinial paraphyses	No. and distribution of germ pores	Telia organization	Basidiospore shape
P. ampelopsidis	*Ampelopsidis*	unknown	thin	strongly incurved	4 or 6 equatorial; rarely scattered	3-4 layered	kidney shaped
P. vitis	*Parthenocissus*	*M. myriantha*	thin	strongly incurved	4, equatorial	3-4 layered; uppermost spore elongated	subglobose-broadly ellipsoid
P. euvitis	*Vitis*	*M. myriantha*	thick	weakly-moderately incurved	6 scattered; rarely 4, equatorial	3-5 layered	kidney shaped

Fig. 1. The symptom of grapevine leaf rust.

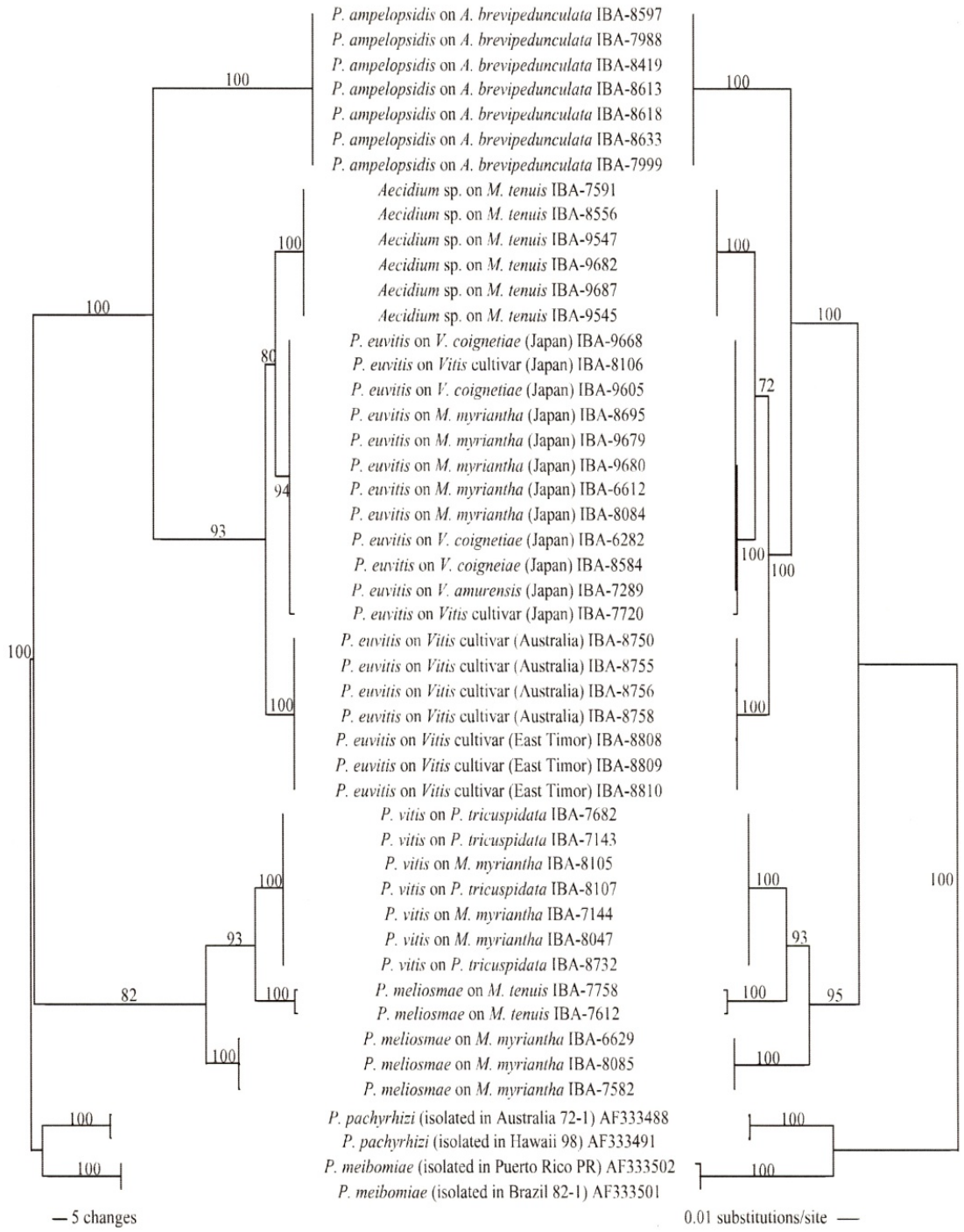


Fig. 2. Phylogenetic relationships among *P. euvitis* (=*P. meliosmae-myrianthae*), *P. vitis*, *P. ampelopsidis* and their allies estimated from the transcribed spacer region 2 sequence data of small ribosomal DNA (Chatasiri and Ono 2008).

Chapter 2. Autoecious species on *Meliosma* species

The *P. meliosmae*, autoecious rust populations on *M. myriantha* and *M. tenuis* were shown to be distantly related based on the molecular phylogenetic study conducted by Chatasiri and Ono (2008). In this chapter, cross-inoculation experiments and comparative morphological examination of the two *Phakopsora* populations were carried out to demonstrate that these rust populations are clearly distinguishable.

2.1. Materials and methods

2.1.1. Specimens

Forty-six dried specimens of the rust fungus on *M. myriantha* and fourteen of *M. tenuis* were used for morphological observations and statistical analyses. For inoculation, telial materials on *M. myriantha* were collected at five localities in Ibaraki in March 2009 and two localities in Tochigi in September 2008. Telial materials on *M. tenuis* were collected at Yunishigawa, Nikko, Tochigi in September 2008, and in Minowa-machi, Nagano in December 2009 (Table 2.1.). The telium-bearing leaves were preserved in a refrigerator at ca.5˚C until use. All specimens examined in this study were deposited in the herbarium of Systematic Mycology, Faculty of Education, Ibaraki University, Mito, Japan (IBAR). Additional specimens used in this study were loaned from IBA herbarium. All specimens, their host species, herbarium data, herbarium accession number and spore stages are listed in Table 2.1. Letters, S, A, U and T denote spermogonial, aecial, uredinial and telial stage, respectively.

2.1.2. Inoculation experiment

Juvenile trees of *M. myriantha* and *M. tenuis* obtained from the National Museum of Nature and Science in Tsukuba, Ibaraki, Japan were planted in a clay pot of 15 cm. diam. or larger, with loam soil, and maintained in a greenhouse to avoid possible spontaneous rust infection. The process for inducing teliospore germination and inoculation method proposed by Ono (1994) and Ono and Azbukina (1977) were used. The telium-bearing leaves were soaked in running water to induce germination of teliospores. The leaves with teliospores were cut into small pieces and incubated in a moist chamber over one or several nights. The small pieces of leaves with teliospores forming basidia and basidiospores were placed on the upper surface of healthy leaves of *M. myriantha* and *M. tenuis* (Fig. 2.1.). In each inoculation, five to ten apparently healthy leaves were used. The control plants were only sprayed with distilled water. The inoculated plants were sprayed with distilled water and placed in a dark, moist chamber at 20 ºC for 2 days, and then transferred into a greenhouse at 20 ºC with controlled artificial illumination.

2.1.3. Morphological studies

Dried herbarium specimens were observed by light microscope (LM) and scanning electron microscope (SEM). For LM observation, small pieces of the specimens were sectioned by free-hand with a razor blade, and spores were scraped from sori under a binocular dissecting microscope. Thin sections of spermogonia, aeciospores, urediniospores and teliospores were mounted by lactophenol solution onto glass slides (Ono 2000) and observed under an Olympus BX51 microscope (Olympus, Tokyo, Japan). For each specimen, thirty spores and paraphyses and five to ten

spermogonia were randomly selected, and measurements were made with a Leica Q-Win Image Analyzer (Leica Q-Win, Tokyo, Japan) with both bright-field and differential interference contrast (DIC). The selected morphological features as indicated in Fig. 2.2.

To determine the number and distribution of the germ pores, urediniospores were mounted using lactic acid on a glass slide and heated at boiling point for a few seconds, and then lactophenol/aniline blue was added to the boiled spores (Kaneko and Hiratsuka 1982; Ono 2005). The slide preparations were observed under the Olympus BX51 microscope. The distribution pattern of the germ pores and type of spermogonium was categorized according to Kaneko and Hiratsuka (1982) and Hiratsuka and Sato (1982), respectively.

For SEM observations of surface structures, aeciospores and peridial cells were dusted onto double-sided adhesive tape on a specimen holder, and then coated with platinum-palladium at 25 nm thicknesses under a Hitachi E-1030 ion sputter (Hitachi, Tokyo, Japan). Those samples were observed with a Hitachi S-4200 SEM operating at 15 kV. Patterns of surface structures of aeciospores and peridial cells were categorized according to Lee and Kakishima (1999a, 1999b).

Totally, 16 morphological characteristics in the uredinial and telial stages were observed (Fig. 2.2.). Statistics including multivariate analyses of measured continuous numerical variables were performed using the software package SPSS (SPSS Japan, Tokyo, Japan) run on Microsoft Windows XP. One-way analysis of variance (one-way ANOVA) was conducted to detect statistical significance of morphological characteristics. The mean value of each morphological measurement was calculated and Tukey Multiple Means Comparison test was performed. Subsequently, in order to detect

possible group, principal component analyses (PCA) based on a correlation matrix was conducted.

2.2. Results and discussion

2.2.1. Host specificity and life cycle

Teliospores, both on *M. myriantha* and *M. tenuis* leaves, germinated well and formed abundant basidiospores after treating telium-bearing leaves in running tap water for one to three days followed by placing them in the dark at 18-20°C for 12-24 h. Basidiospores from *M. myriantha* leaves were inoculated once in 2008 and seven times in 2009 and the inoculations were successful only on *M. myriantha* (Table 2.2.), producing spermogonia 4-7 days after inoculation and aecia in subsequent 7-10 days. However, no sign of infection was observed on inoculated *M. tenuis* leaves. Contrarily, inoculations with basidiospores from teliospore on *M. tenuis* were successful only on *M. tenuis* leaves once in 2009 and five times in 2010 (Table 2.2.). Spermogonia were formed 5-7 days after inoculation, followed by aecial production in subsequent 7-10 days.

Kakishima et al. (1983) confirmed life cycle of the *M. myriantha* infecting rust population (MMR), but not of the *M. tenuis* infecting rust population (MTR). Later Ono (unpublished records with voucher specimens IBAR-7612 and 7758) demonstrated that MTR had an autoecious macrocyclic life cycle. In this study, cross inoculations using basidiospores confirmed that both populations had macrocyclic, autoecious nature of life cycles but showed that each population had different host specificity. Symptoms and spermogonial/aecial sori formed on infected plants of the two *Meliosma* species were similar (Fig. 2.3A, B). Lesions were small, pale yellowish or yellowish orange

with a relatively clear boundary. Dark-colored dots of spermogonia were produced in a dense cluster on both sides of the lesion; and columnar aecia were produced almost exclusively on the adaxial surface of the lesion.

2.2.2. Morphological observations

Spermogonial and aecial stage: Spermogonia and aecia on *M. myriantha* and *M. tenuis* were produced on the adaxial surface of the leaf. Spermogonia were subcuticular and conical, surrounded by conspicuous paraphyses (Type 7). Spermogonia are larger in MMR than in MTR. Spermogonia size was 63.8–157.9 × 60.9–156.2 μm (width × height) (Table 2.3.; Fig. 2.4A) in MMR and 45.7–120.9 × 50.0–117.9 μm (Fig. 2.5A) in MTR. Aecia of both MMR and MTR were aecidium-type surrounded by well-developed peridia, and appeared columnar or horn-shaped, later becoming cup shaped due to apical ruptured when sori matured (Figs. 2.4A, 3.4B). Peridial cells were oblong to broadly ellipsoid, smooth in MMR (Fig. 2.4C) and verrucose in MTR (Table 2.3.; Fig. 2.5C). No apparent differences between MMR and MTR are found in aeciospore morphology. Aeciospores were produced by basipetal succession from the basal sporogenous layer in sori. The aeciospores were subglobose to broadly ellipsoid, 14.3–25.8 × 17.7–40.9 μm in size with walls 0.9–2.1 μm thick in MMR (Table 2.4.; Fig. 2.4D) and 14.9–23.0 × 18.5–33.2 μm in size, with walls 0.9–1.9 μm thick in MTR (Fig. 2.5D). The aeciospore surface was verrucose with flattened heads (Table 2.4.; Figs. 2.4E, 2.5E). Nail-head projection subtended by buttress observed on the MMR and MTR aeciospores seems to be characteristic aeciospore ornamentation in the *Phakopsora* species.

Surface ornamentation of peridial cells has been treated as an important taxonomic character to distinguish some rust fungi e.g. the *Roestelia* stage of *Gymnosporangium* species (Kern 1973; Hiratsuka et al. 1992; Lee and Kakishima 1999b). This characteristic was useful to distinguish two populations, i.e., MMR has smooth peridial cells (Fig. 2.4C), which confirmed by Kakishima et al (1983) while MTR has verrucose surface of peridial cells (Fig. 2.5C).

Urenial stage: Uredia were minute, scattered or in loose groups on the abaxial surface of the leaf, and densely surrounded by strongly incurved paraphyses (Figs. 2.4F, 2.5F). Paraphyses were hyaline to brown, moderately to strongly incurved and basally united (Figs. 2.4G, 2.5G). Paraphyses are larger in MMR than in MTR. Because of the strong incurvature of the paraphyses, their precise length was not measured; therefore, the height from the sorus base to the paraphysis apex was measured. They were 7.5–23.0 × 22.6–73.5 μm (width × height) in MMR and 8.5–18.1 × 18.1–50.9 μm in MTR. The wall was hyaline to brown and the color appeared to change as the sori aged. The wall thickness was uneven, i.e., the dorsal wall was prominently thicker than the ventral wall. The dorsal and apical walls of paraphyses are prominently thickened in MMR than MTR. The dorsal walls were 2.6–11.9 μm thick with 3.0–17.5 μm ventral walls apical thickness in MMR (Table 2.4.; Fig. 2.4G) and 2.1–8.7 μm thick with 1.3–7.9 μm ventral walls thickness in MTR (Table 2.4.; Fig. 2.5G). Urediniospores were apedicellate, obovoid to obovoid-ellipsoid. Urediniospores are longer and narrower in MTR than in MMR as shown in the L:W ratio. Urediniospore size was 11.1–23.0 × 14.5–32.6 μm in MMR (Table 2.5.; Fig. 2.4H) and 13.2–19.2 × 20.2–32.8 μm in MTR (Table 2.5.; Fig. 2.5H). No apparent differences between MMR and MTR are found in urediniospore surface structures, wall thickness, number and distribution of germ pores. The walls

were evenly echinulate, colorless or pale yellowish, 0.6–1.9 μm thick. Germ pores numbered 2 or 4, observed at an equatorial zone (Table 2.4.; Figs. 2.4I, 2.5I).

Telial stage: Telia were subepidermal, scattered or in groups, composed of 2–7 layers of catenulated or irregularly arranged teliospores (Figs. 2.4J, 2.5J). No teliospore morphological differences between these two groups. Teliospores were ellipsoid to oblong, 6.4–20.0 × 9.6–29.6 μm with walls 0.6–2.3 μm thick and apical wall thickness was 0.6–4.1 μm in the top layer, and 6.2–20.2 × 8.5–28.3 μm with walls 0.6–2.1 μm at the side and the apical wall thickness was 0.6–2.6 μm in second layer and below in MMR (Table 2.5.; Fig. 2.4J). While in MTR, and in the top layer 7.5–15.8 × 11.5–23.0 μm with walls 0.6–1.9 μm thick and apical wall thickness was 1.3–3.2 μm in the top layer, and 6.4–16.6 × 8.7–21.3μm with walls 0.6–1.9 μm at the side and the apical wall thickness was 0.9–2.1 μm in second layer and below in MTR (Table 2.5.; Fig. 2.5J). Ono (2000) considered the shape of teliospores as a useful taxonomic characteristic to separate *P. vitis* from *P. ampelosidis* and *P. euvitis.* However, in the present study, we recognize that the shape of teliospores is not an important characteristic to separate these two populations of *P. meliosmae*.

Basidial stage: Shapes of basidiospores of two populations are morphologically similar and are subglobose to broadly ellipsoid. They were 4.7–7.7 × 6.6–9.6 μm in size in MMR (Fig. 2.4K) and 5.5–8.1 × 7.7–10.7 μm in size in MTR (Fig. 2.5K). Furthermore, shapes of basidiospores of *P. meliosmae*are also morphologically similar to those of *P. vitis.* However, these are distinguishable from those of *P. euvitis* because the latter are kidney-shaped even when they have the same aecial stage host (Ono 2000).

All specimens observed could be divided into two populations that are the *M. myriantha* population (MMR) and *M. tenuis* population (MTR). Comparisons of

statistical analyses of two populations of *P. meliosmae* showed significant differences in some morphological characteristics. Concerning length/width ratio of urediniospore, a significant difference (99% confidence level) was shown. Paraphyses also showed significant difference between two populations. The dorsal wall thickness and apical thickness of paraphyses of the fungal population on *M. tenuis* had smaller than those of the *M. myriantha* population. The scatter diagram was clearly separated the population on *M. myriantha* from those of on *M. tenuis* (Fig. 2.6.). The principal component analyses were undertaken with various combinations of urediniospores and teliospores. After the Varimax rotation, the calculated factor 1, 2 and 3 explained 59.1%, 15.9% and 9.2 % of the total variance, respectively. The three and two dimension scatter diagram generated by the principal component were separated into two groups (Figs. 2.7. and 2.8.). No significant difference was observed in teliospores, basidiospores and aeciospores.

Based on the molecular phylogenetic analyses results of Chatasiri and Ono (2008) and results of inoculation experiments and morphological analyses in this study, the rust fungus population on *M. tenuis* is taxonomically separated from *P. meliosmae* on *M. myriantha*. They had different host specificity and were also found to be distinct in the structure of the aecial peridium surface, the size and wall thickness of uredinial paraphyses, and the urediniospore size and shape. Consequently, the fungal population on *M. tenuis* was taxonomically separated from *P. meliosmae* originally described for the fungus on *M. myriantha.* The autoecious macrocyclic *Phakopsora* species on *M. tenuis* in Japan was described as a new species, *Phakopsora orientalis* Chatasiri, Pota & Y. Ono and the description of *P. meliosmae* on *M. myriantha* in Japan was amended (Pota et al. 2013).

Biological species in rust fungi can be well-resolved by the life cycle and host specificity studies and the analysis of morphological character variations (Ono 2008). Apparently strict host specificity between MMR, MTR and corresponding morphological character disjunction between them are a good indicator of their reproductive isolation. Consequently, I conclude that MMR and MTR are biologically-distinct species. Recognition of biological species by the host specificity, life-cycle difference and associated morphological character disjunction has been reported in the *P. ampelopsidis* Dietel & P. Syd. (Ono 2000), the *Puc. hemerocallidis* Thüm. (Ono 2003, 2005), *Ochropsora* species (Ono 2006) and *Puc. calystegiae-soldanellae* Z. Li, F. Nakai & Y. Harada (Li et al. 2004).

Table 2.1. The specimens of autoecious species on *Meliosma* used in morphological observation.

Rust/host	Herbarium accession no.	Specimen data			Stage[a]
		Location	Date	Collector	
Phakopsora meliosmae	IBAR-1592	Gumma, Tone-gun	18 Jun. 1973	Y. Ono	S, A
on *Meliosma myriantha*	IBAR-1887	Ibaraki, Tsukuba-gun	17 Oct. 1979	Y. Ono	T
	IBAR-2387	Tochigi	17 Oct. 1981	Y. Ono	U, T
	IBAR-2694	Ibaraki, Mito	6 Nov. 1982	Y. Ono	U, T
	IBAR-2711	Ibaraki, Mito	6 Nov. 1982	Y. Ono	U, T
	IBAR-3601	Saitama	19 Sep. 1954	Y. Ono	U, T
	IBAR-4798	Ibaraki, Kuji-gun	3 Jun. 1990	Y. Ono	S, A
	IBAR-5540	Ibaraki, Kuji-gun	19 Nov. 1990	Y. Ono	U
	IBAR-5997	Ibaraki, Kitabaraki	9 Nov. 1991	Y. Ono	U, T
	IBAR-6360	Ibaraki, Kuji-gun	24 Oct. 1922	Y. Ono	U
	IBAR-7797	Tokyo, Mt. Takaosan	18 Oct. 1899	S. Kusano	U, T
	IBAR-7798	Tokyo, Mt. Takaosan	11 Jul. 1899	S. Kusano	S, A
	IBAR-8581	Tochigi, Shioya-gun	28 Sep. 2000	Y. Ono	U, T
	IBAR-8640	Fukushima, Iwaki	11 Nov. 2000	Y. Ono and M. Mori	U, T
	IBAR-9906[c]	Fukushima, Iwaki	13 Oct. 2007	Y. Ono	U, T
	IBAR-9911[c]	Ibaraki, Higashiibaraki-gun	20 Oct. 2007	Y. Ono	T
	IBAR-9912[c]	Ibaraki, Higashiibaraki-gun	20 Oct. 2007	Y. Ono	T
	IBAR-9943[c]	Tochigi, Shioya-gun	16 Nov. 2007	Y. Ono	T
	IBAR-9944	Tochigi, Shioya-gun,	16 Nov. 2007	Y. Ono	T
	IBAR-9945	Ibaraki, Kuji-gun	17 Nov. 2007	Y. Ono	T
	IBAR-9946[c]	Ibaraki, Higashiibaraki-gun	16 Nov. 2007	Y. Ono	T
	IBAR-9977[c]	Ibaraki, Kitaibaraki	15 Dec. 2007	Y. Ono	T

Table 2.1. continued

Rust/host	Herbarium accession no.	Specimen data			Stage[a]
		Location	Date	Collector	
Phakopsora meliosmae	IBAR-10037[c]	Ibaraki, Mito	3 Jul. 2008	Y. Ono	S, A
on *Meliosma myriantha*	IBAR-10038[c]	Ibaraki, Mito Bunkyo	3 Jul. 2008	Y. Ono	S, A
	IBAR-10045	Fukushima, Nishishirakawa-gun	19 Sep. 2008	Y. Ono	U, T
	IBAR-10053[b]	Tochigi, Nikko	20 Sep. 2008	Y. Ono	U, T
	IBAR-10054[b]	Tochigi, Nikko	20 Sep. 2008	Y. Ono	U, T
	IBAR-10057[b,c]	Ibaraki, Higashiibaraki-gun	21 Mar. 2009	Y. Ono and S. Pota	T
	IBAR-10058[b,c]	Ibaraki, Kuji-gun	21 Mar. 2009	Y. Ono and S. Pota	T
	IBAR-10059[b,c]	Ibaraki, Kitaibaraki	21 Mar. 2009	Y. Ono and S. Pota	T
	IBAR-10060[b,c]	Ibaraki, Takahagi	21 Mar. 2009	Y. Ono and S. Pota	T
	IBAR-10064	Ibaraki, Mito Bunkyo	21 May 2009	S. Pota and Y. Ono	S, A
	IBAR-10065	Ibaraki, Mito Bunkyo	5 Jun. 2009	S. Pota and Y. Ono	S, A
	IBAR-10066	Ibaraki, Mito Bunkyos	7 Jun. 2009	S. Pota and Y. Ono	S, A
	IBAR-10069	Fukushima, Iwaki	24 May 2009	Y. Ono and S. Pota	S, A
	IBAR-10071[b]	Ibaraki, Tsukuba	2 May 2009	S. Pota	T
	IBAR-10073	Ibaraki, Tsukuba	9 Jun. 2009	S. Pota and Y. Ono	S, A
	IBAR-10074	Ibaraki, Mito Bunkyo	12 Jun. 2009	S. Pota and Y. Ono	S, A
	IBAR-10078	Fukushima, Iwaki	27 Jun. 2009	Y. Ono	S, A
	IBAR-10087	Ibaraki, Tsukuba	15 Jul. 2009	S. Pota	S, A
	IBAR-10092	Ibaraki, Tsukuba	20 Jul. 2009	S. Pota	S, A
	IBAR-10094	Nagano, Azumino	30 Jul. 2009	S. Yokosawa	U
	IBAR-10097	Nagano, Azumino, Karasu-gawa	17 Aug. 2009	S. Yokosawa	U
	IBAR-10099	Miyagi, Sendai	26 Aug. 2009	S. Pota	U
	IBAR-10102	Miyagi, Miyagi-gun	29 Aug. 2009	S. Pota	U

Table 2.1. continued

Rust/host	Herbarium accession no.	Specimen data			Stage[a]
		Location	Date	Collector	
Phakopsora meliosmae	IBAR-7612	Tochigi, Shioya-gun	22 Sep. 1995	Y. Ono	U, T
on *M. tenuis*	IBAR-7662	Miyazaki, Nishiusukine-gun	25 Oct. 1995	Y. Ono	T
	IBAR-7758	Ibaraki, Mito	10 Jun. 1996	Y. Ono	S, A
	IBAR-9942[c]	Tochigi, Shioya-gun	16 Nov. 2007	Y. Ono	T
	IBAR-10103	Ibaraki, Tsukuba	30 Aug. 2009	S. Pota	S, A
	IBAR-10051[b]	Tochigi, Nikko	20 Sep. 2008	Y. Ono	U, T
	IBAR-10080[c]	Nagano, Minamiminowa-mura	11 Jul. 2009	S. Pota	S, A, U
	IBAR-10104	Ibaraki, Tsukuba	30 Aug. 2009	S. Pota	U
	IBAR-10248[b]	Nagano, Ina	5 Dec. 2009	S. Pota and Y. Yamaoka	T
	IBAR-10253	Ibaraki, Tsukuba	19 Apr. 2010	S. Pota	S, A
	IBAR-10254	Ibaraki, Tsukuba	19 Apr. 2010	S. Pota	S, A
	IBAR-10255	Ibaraki, Tsukuba	21 Apr. 2010	S. Pota	S, A
	IBAR-10256	Ibaraki, Tsukuba	22 Apr. 2010	S. Pota	S, A
	IBAR-10257	Ibaraki, Tsukuba	25 Apr. 2010	S. Pota	S, A

[a]Letters, S, A, U and T denote spermogonial, aecial, uredinial and telial stages in the life cycle, respectively.

[b]Specimen used as an inocula in inoculation experiment.

[c]Specimen used for molecular phylogenetic analyses.

Table 2.2. Result of inoculation with basidiospores from teliospores on *M. myriantha* and *M. tenuis.*

Inoculum (telium)	Voucher specimens	Inoculate date	Date of first appearance of spemogonia	Date of first appearance of aecia	Infected and sporulated plants	Voucher specimens
on *Meliosma myriantha*	IBA-10058	9 Apr. 2009	14 Apr. 2009	24 Apr. 2009	*M. myriantha*	IBAR-10065
	IBA-10057	14 Apr. 2009	21 Apr. 2009	30 Apr. 2009	*M. myriantha*	IBAR-10064
	IBA-10071	28 Apr. 2009	8 May 2009	15 May 2009	*M. myriantha*	IBAR-10073
	IBA-10060	1 May 2009	8 May 2009	15 May 2009	*M. myriantha*	IBAR-10066
	IBA-10059	3 May 2009	9 May 2009	29 May 2009	*M. myriantha*	IBAR-10074
	IBA-10053	1 Jun. 2009	9 Jun. 2009	17 Jun. 2009	*M. myriantha*	IBAR-10095
	IBA-10054	3 Jun. 2009	11 Jun. 2009	20 Jun. 2009	*M. myriantha*	IBAR-10092
on *Meliosma tenuis*	IBA-10051	1 Jul. 2009	8 Jul. 2009	16 Jul. 2009	*M. tenuis*	IBAR- 10103
	IBA-10248	21 Mar. 2010	27 Mar. 2010	7 Apr. 2010	*M. tenuis*	IBAR- 10253
	IBA-10248	22 Mar. 2010	1 Apr. 2010	7 Apr. 2010	*M. tenuis*	IBAR- 10254
	IBA-10248	25 Mar. 2010	5 Apr. 2010	12 Apr. 2010	*M. tenuis*	IBAR- 10255
	IBA-10248	27 Mar. 2010	5 Apr. 2010	12 Apr. 2010	*M. tenuis*	IBAR- 10256
	IBA-10248	30 Mar. 2010	7 Apr. 2010	15 Apr. 2010	*M. tenuis*	IBAR- 10257

In all the inoculations, both *M. myriantha* and *M. tenuis* plants were inoculated, and only positive results are listed.

Table 2.3. Morphological characteristics of spermogonial and aecial stage of rust fungus population on *M. myriantha* and *M. tenuis.*

Rust population on	Voucher	Spermogonium		Aeciospore			Peridial cell
		Size width × height (µm)	Type	Size width × height (µm)	Wall thickness (µm)	Surface	surface
M. myriantha (MMR)	IBAR-10037	-[a]	type 7	-	-	verrucose with flat-headed	smooth
	IBAR-10038	-	type 7	17.0-24.5 × 21.5-34.1	1.3-1.9	verrucose with flat-headed	smooth
	IBAR-10064	63.8-128.8 × 68.7-145.7	type 7	16.4-21.9 × 20.0-31.1	0.9-1.9	verrucose with flat-headed	smooth
	IBAR-10065	88.3-144.9 × 79.2-156.2	type 7	18.1-21.3 × 20.5-27.5	1.1-1.9	verrucose with flat-headed	smooth
	IBAR-10066	89.2-140.5 × 73.5-139.5	type 7	16.6-21.5 × 19.0-26.0	1.1-2.1	verrucose with flat-headed	smooth
	IBAR-10069	66.6-136.6 × 60.9-122.7	type 7	16.6-23.9 × 21.1-27.7	1.1-2.1	verrucose with flat-headed	smooth
	IBAR-10073	-	type 7	14.3-20.2 × 21.5-33.0	0.9-1.9	verrucose with flat-headed	smooth
	IBAR-10074	90.0-149.9 × 86.6-145.7	type 7	14.9-21.9 × 20.9-29.2	1.1-1.9	verrucose with flat-headed	smooth
	IBAR-10078	74.8-138.8 × 74.0-141.4	type 7	16.6-23.2 × 24.7-35.8	1.1-2.1	verrucose with flat-headed	smooth
	IBAR-10087	95.7-126.6 × 87.4-116.2	type 7	14.9-25.8 × 21.5-29.8	1.1-2.1	verrucose with flat-headed	smooth
	IBAR-10092	101.8-157.9 × 115.7-147.9	type 7	15.6-20.5 ×17.7-26.0	1.1-1.7	verrucose with flat-headed	smooth
	IBAR-1592	-	type 7	18.3-24.1 × 21.8-29.2	1.1-1.7	verrucose with flat-headed	smooth
	IBAR-4798	90.9-126.1 × 92.2-122.7	type 7	18.3-23.4 × 24.1-36.9	1.1-1.7	verrucose with flat-headed	smooth
	IBAR-7798	-	type 7	14.3-22.8 × 26.6-40.9	1.1-1.9	verrucose with flat-headed	smooth
M. tenuis (MTR)	IBAR-10103	104.4-120.9 × 104.8-110.9	type 7	15.6-20.5 × 19.6-26.6	0.9-1.5	verrucose with flat-headed	verrucose inside
	IBAR-10253	57.4-70.9 × 52.2-68.3	type 7	16.0-21.1 × 21.3-29.6	0.9-1.7	verrucose with flat-headed	verrucose inside
	IBAR-10254	53.9-82.7 × 52.6-85.7	type 7	16.4-20.5 × 19.6-27.5	0.9-1.7	verrucose with flat-headed	verrucose inside
	IBAR-10255	60.0-88.3 × 66.6-86.6	type 7	17.7-23.0 × 21.9-32.2	1.1-1.7	verrucose with flat-headed	verrucose inside
	IBAR-10256	45.1-100.1 × 50.0-82.7	type 7	14.9-20.9 × 18.5-28.5	0.9-1.9	verrucose with flat-headed	verrucose inside
	IBAR-10257	60.9-89.2 × 60.5-99.2	type 7	15.8-22.2 × 20.7-31.3	0.9-1.7	verrucose with flat-headed	verrucose inside
	IBAR-7758	84.4-109.6 × 71.3-117.9	type 7	16.6-21.5 × 22.4-33.2	1.1-1.7	verrucose with flat-headed	verrucose inside

[a]Not measured.

Table 2.4. Morphological characteristics of uredinial stage of rust fungus population on *M. myriantha* and *M. tenuis.*

Rust populations on	Voucher specimens	Urediniospore			Paraphysis		
		Size width × length (µm)	Wall thickness (µm)	Germ pores	Size width × length (µm)	Dorsal wall thickness (µm)	Apical wall thickness (µm)
M. myriantha (MMR)	IBAR-10045	14.1-19.8 × 19.8-29.2	0.9-1.7	equatorial	9.8-15.1 × 24.7-45.2	2.6-7.7	3.8-12.8
	IBAR-10053	12.6-19.0 × 19.8-26.8	0.9-1.5	equatorial	7.5-20.9 × 22.6-44.1	3.0-7.9	3.2-11.7
	IBAR-10054	13.4-18.7 × 19.6-27.9	0.9-1.5	equatorial	10.7-17.5 × 32.2-73.5	3.0-8.7	3.8-15.8
	IBAR-10094	14.1-19.6 × 18.5-29.0	0.9-1.5	equatorial	10.0-19.0 × 31.1-59.4	2.8-6.8	3.6-7.9
	IBAR-10097	13.9-18.7 × 19.4-28.8	0.9-1.7	equatorial	10.7-18.3 × 32.0-50.5	4.7-10.9	3.6-12.8
	IBAR-10099	14.1-17.9 × 22.8-28.8	0.9-1.5	equatorial	11.1-18.7 × 27.9-51.1	3.4-9.8	5.5-14.5
	IBAR-10102	14.9-20.2 × 21.9-29.4	0.9-1.5	equatorial	12.6-18.7 × 23.9-45.4	5.8-10.2	6.6-17.5
	IBAR-2387	15.6-23.0 × 22.4-30.3	0.6-1.9	equatorial	11.9-18.3 × 24.5-48.8	3.8-8.3	3.8-9.8
	IBAR-2694	17.9-21.1 × 25.1-32.0	0.6-1.7	equatorial	9.6-18.5 × 23.9-52.0	4.3-8.7	3.6-9.4
	IBAR-2711	14.3-22.4 × 19.8-31.7	0.9-1.5	equatorial	11.9-23.0 × 29.2-53.9	3.6-11.9	4.1-10.4
	IBAR-3601	16.6-23.0 × 20.2-32.6	1.1-1.9	equatorial	9.8-18.5 × 28.3-44.1	3.8-9.2	4.3-14.9
	IBAR-5997	15.6-20.9 × 20.7-27.1	1.1-1.7	equatorial	9.8-18.1 × 26.8-49.2	3.4-11.9	5.1-16.8
	IBAR-7797	15.8-20.2 × 19.4-26.2	1.1-1.7	equatorial	7.9-23.0 × 28.8-50.9	4.7-11.9	3.0-10.9
	IBAR-8581	11.1-19.0 × 14.5-28.5	0.9-1.9	equatorial	9.6-20.5 × 29.2-44.3	4.1-9.6	5.3-15.3
	IBAR-8640	15.3-20.5 × 18.1-27.1	0.9-1.5	equatorial	9.6-16.4 × 26.4-43.7	3.0-10.0	4.5-10.2
M. tenuis (MTR)	IBAR-10051	13.4-19.0 × 21.1-32.8	0.6-1.5	equatorial	8.5-18.1 × 21.1-43.2	3.0-8.7	2.1-7.9
	IBAR-10080	13.9-19.2 × 24.3-32.2	0.9-1.9	equatorial	9.2-14.5 × 18.1-41.1	2.1-6.0	1.9-4.7
	IBAR-10104	13.2-17.5 × 21.3-32.2	0.6-1.5	equatorial	8.5-16.6 × 26.2-50.9	3.4-6.2	1.3-5.1
	IBAR-7612	13.2-19.2 × 20.2-29.2	0.9-1.7	equatorial	8.5-16.2 × 21.9-40.0	2.8-7.2	2.8-6.6

Table 2.5. Morphological characteristics of telial stage of rust fungus population on *M. myriantha* and *M. tenuis.*

Rust populations on	Voucher specimens	Uppermost cell teliospore			Second and below cell teliospore			Telial organization
		Size width × length (µm)	Lateral wall thickness (µm)	Apical wall thickness (µm)	Size width × length (µm)	Lateral wall thickness (µm)	Apical wall thickness (µm)	
M. myriantha (MMR)	IBAR-10045	7.7-12.4 × 13.9-26.2	0.9-1.7	1.5-3.0	8.3-13.0 × 11.7-19.4	0.9-1.9	0.9-1.9	2-4 layers
	IBAR-10053	6.6-13.0 × 13.4-22.8	1.1-1.9	1.5-3.8	8.7-14.7 × 11.7-21.5	0.9-1.9	1.1-1.9	2-6 layers
	IBAR-10054	7.2-12.5 × 12.6-25.4	0.9-2.1	1.5-3.2	6.6-11.9 × 11.5-22.6	0.9-1.9	0.9-1.7	3 layers
	IBAR-10057	9.0-14.1 × 14.9-24.7	1.1-2.3	1.7-3.8	7.9-13.4 × 11.3-21.3	0.9-1.7	0.6-2.3	4-7 layers
	IBAR-10058	7.7-13.2 × 13.0-23.2	0.9-1.7	1.7-3.6	7.9-13.4 × 10.9-17.9	0.9-1.7	0.9-2.1	3-7 layers
	IBAR-10059	8.7-14.1 × 12.1-21.7	0.6-1.7	1.5-3.0	6.8-15.8 × 10.2-21.9	0.9-1.5	0.9-1.9	3-6 layers
	IBAR-10060	8.1-14.1 × 14.7-24.7	0.6-1.7	1.5-2.8	7.5-14.9 × 11.1-19.4	0.9-1.5	0.9-1.5	3-5 layers
	IBAR-10071	7.0-14.7 × 11.5-19.2	0.9-1.5	1.3-2.6	6.6-13.4 × 10.0-18.5	0.9-1.5	0.9-1.5	2-5 layers
	IBAR-1887	9.2-20.0 × 11.5-29.6	0.9-1.7	1.5-3.2	8.1-20.2 × 9.4-28.3	0.9-2.1	0.9-2.3	2,3 layers
	IBAR-2387	7.9-15.3 × 13.4-24.3	0.9-1.9	1.7-3.4	8.3-12.8 × 10.2-24.1	0.6-1.5	0.9-2.1	3,4,5 layers
	IBAR-2694	8.7-13.4 × 12.6-21.7	0.9-1.9	1.3-3.4	7.7-14.9 × 9.4-19.4	0.9-2.1	0.9-1.9	3,4,5 layers
	IBAR-2711	6.8-13.0 × 13.4-26.8	1.1-1.9	1.5-3.2	7.9-14.7 × 11.1-21.3	1.1-2.1	0.9-2.3	3-6 layers
	IBAR-3601	7.5-16.0 × 13.0-24.3	0.9-1.9	1.3-2.8	7.9-16.6 × 9.4-21.1	0.9-1.9	0.9-1.9	3,4,5 layers
	IBAR-5540	8.3-14.3 × 11.9-21.5	1.1-1.7	1.1-2.8	8.7-14.5 × 10.9-22.8	1.1-1.7	0.9-2.1	3-8 layers
	IBAR-5997	7.9-14.7 × 12.4-20.5	0.6-1.5	0.6-2.3	8.3-15.8 × 9.0-20.7	0.9-1.5	1.3-2.6	3,4 layers
	IBAR-6360	7.9-13.0 × 11.7-20.9	0.9-1.7	1.1-2.3	7.7-15.8 × 9.0-19.4	0.9-1.7	0.9-1.7	4 layers
	IBAR-7797	8.1-12.8 × 13.6-23.6	0.6-1.5	1.3-2.8	7.7-13.4 × 8.5-18.5	0.6-1.5	0.9-1.5	3,4,5 layers
	IBAR-8581	6.4-12.8 × 9.6-21.7	0.9-2.1	1.3-3.2	7.2-13.2 × 9.8-19.2	0.9-1.7	0.9-1.9	3,4 layers
	IBAR-8640	6.4-13.0 × 12.6-21.9	0.9-1.9	0.9-3.0	6.2-12.8 × 10.4-19.0	0.9-1.7	0.9-1.7	3,4 layers
	IBAR-9906	7.5-14.1× 14.1-27.1	0.9-1.5	1.3-2.8	8.1-14.1 × 12.6-21.7	0.9-1.5	0.9-1.5	3-5 layers
	IBAR-9911	8.3-15.1 × 11.3-20.2	0.9-1.7	0.9-3.2	8.1-13.0 × 8.7-20.5	1.1-2.1	0.9-2.1	2-4 layers

Table 2.5. Continued

Rust populations on	Voucher	Uppermost cell teliospore			Second and below cell teliospore			Telial organization
		Size width × length (µm)	Lateral wall thickness (µm)	Apical wall thickness (µm)	Size width × length (µm)	Lateral wall thickness (µm)	Apical wall thickness (µm)	
M. myriantha	IBAR-9912	9.2-14.9 x 14.9-23.4	0.9-1.7	1.1-2.6	9.8-14.7 x 11.5-18.5	0.9-1.9	0.9-1.9	4,5 layers
(MMR)	IBAR-9943	6.8-14.1 x 12.4-22.4	0.9-1.9	0.9-2.8	6.4-11.3 x 11.3-19.6	0.9-1.5	0.6-1.7	3,4 layers
	IBAR-9944	7.0-13.0 x 14.5-25.6	0.9-1.7	0.9-3.4	8.3-15.6 x 11.3-24.5	0.9-1.9	0.9-1.9	3-7 layers
	IBAR-9945	7.0-14.1 x 10.2-21.5	0.9-1.7	1.3-4.1	6.6-14.5 x 10.0-19.4	0.6-1.9	0.6-1.7	3-5 layers
	IBAR-9946	8.7-13.2 x 13.2-19.8	0.9-1.5	1.1-2.3	8.3-15.1 x 11.3-20.2	0.9-1.9	0.9-1.9	4-6 layers
	IBAR-9977	7.7-14.1 x 12.1-22.8	0.6-1.7	1.3-2.8	8.7-12.6 x 11.5-20.0	0.9-1.7	0.9-2.3	4-6layers
M. tenuis	IBAR-10051	7.5-15.8 x 11.5-23.0	1.1-1.9	1.7-3.0	8.3-16.6 x 11.9-19.4	1.1-1.9	0.9-1.9	3-6 layers
(MTR)	IBAR-7612	7.7-13.0 x 11.9-20.2	0.6-1.5	1.3-3.2	7.9-16.2 x 8.7-21.3	0.6-1.7	0.9-1.5	3,4 layers
	IBAR-7662	8.3-14.1 x 13.2-21.7	0.9-1.5	1.3-2.6	6.4-14.9 x 10.0-18.3	0.9-1.7	0.9-2.1	3-7 layers

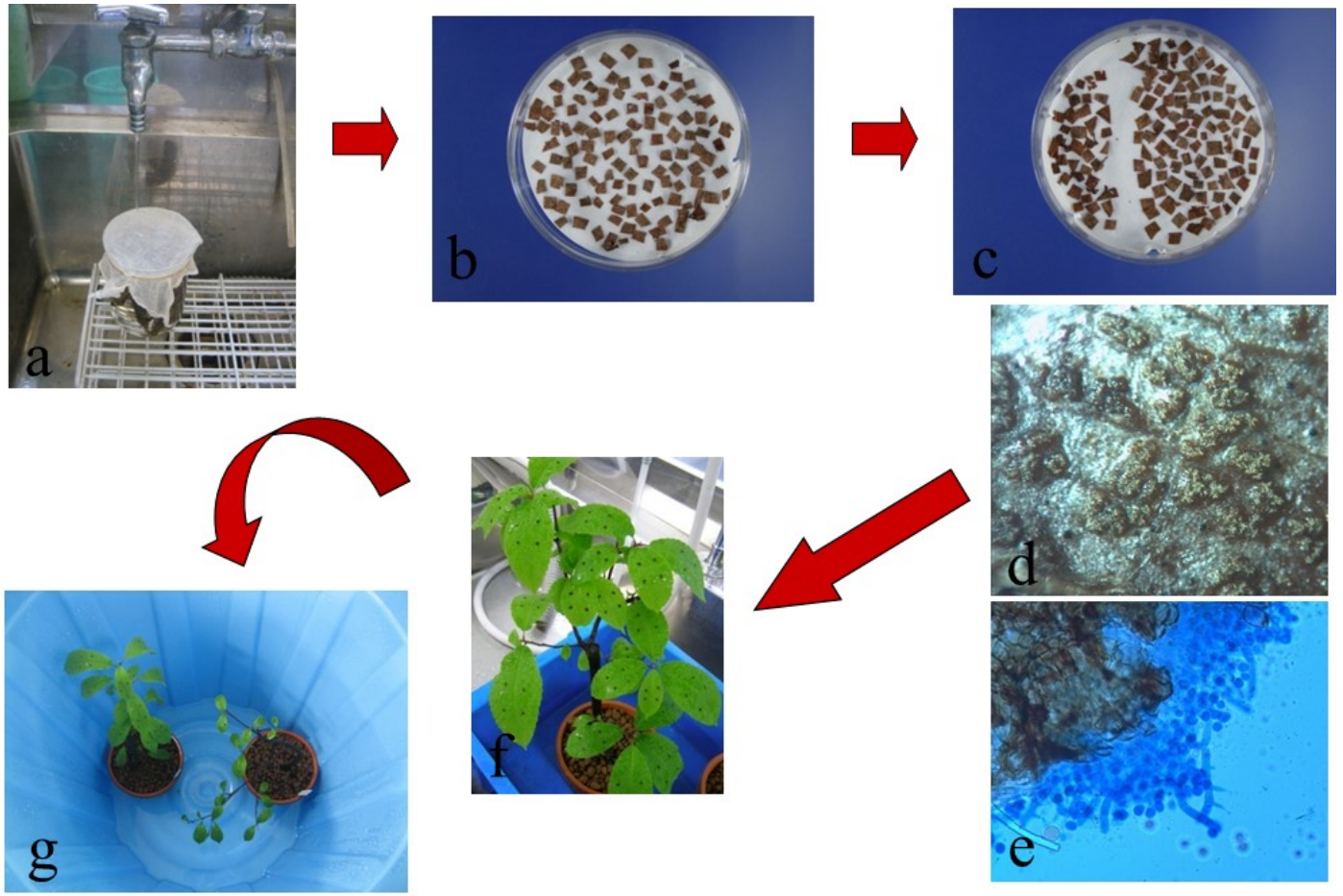


Fig. 2.1. Inoculation method. a: The telium-bearing leaves were soaked in running water. b: The leaves with teliospores were cut into small pieces and incubated in a moist chamber overnight. c: The small pieces with germinated teliospore were selected. d: Teliospores forming basidia and basidiospores under binocular dissecting microscope. e: Teliospores forming basidia and basidiospore under light microscope. f: The small pieces of teliospores forming basidia and basidiospores were place on the upper surface of healthy leaves of *M. myriantha* and *M. tenuis*. g: Inoculated plants were sprayed with distill water and place in dark.

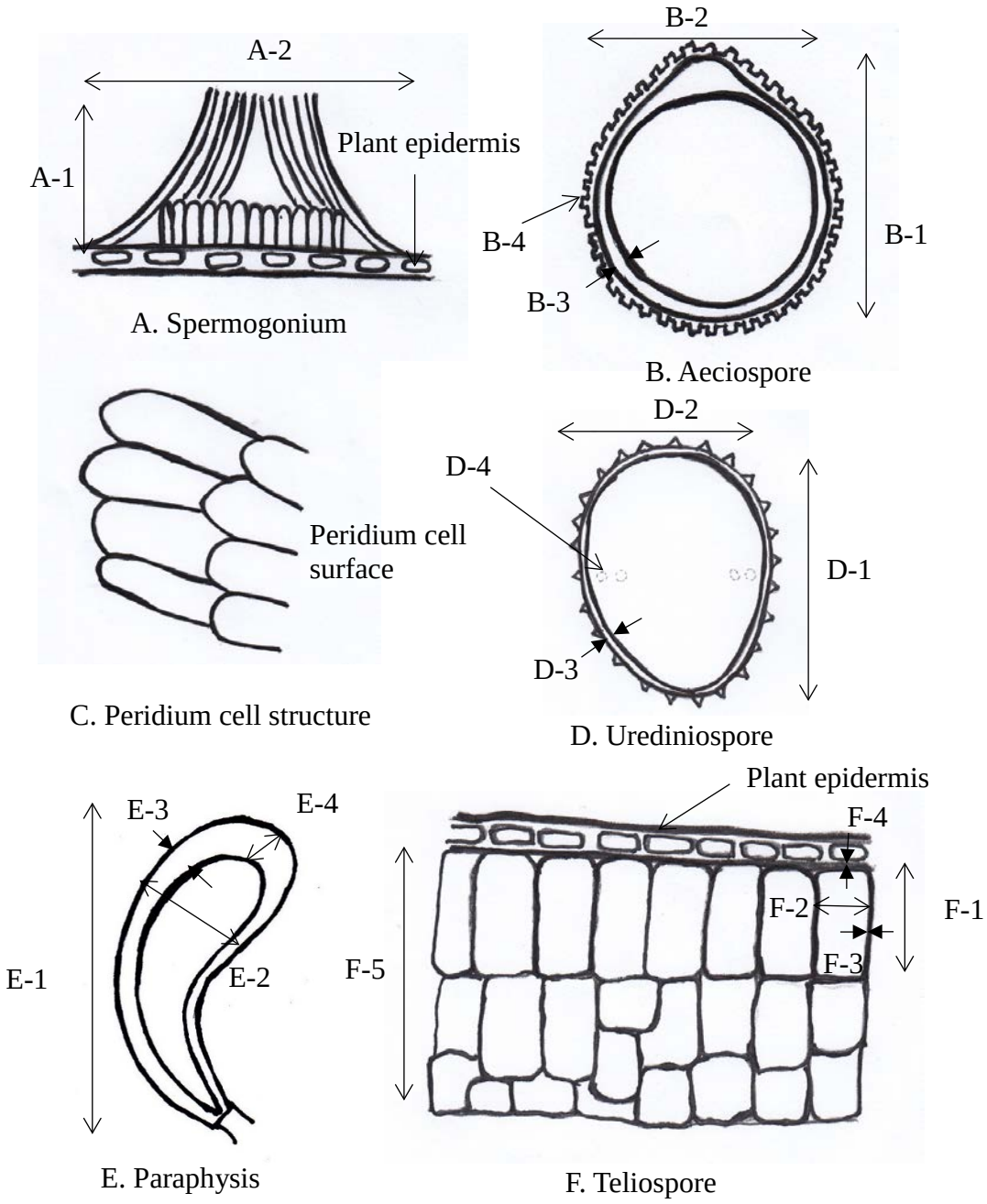


Fig. 2.2. Morphological characteristics of *Phakopsora* species observation in present study. A. Spermogonium: A-1 = spermogonium height, A-2 = spermogonium width. B. Aeciospore: B-1 = aeciospore length, B-2 = aeciospore width, B-3 = aeciospore wall thickness, B-4 = aeciospore surface structure (SEM). C. Peridium cell structure (SEM). D. Urediniospore: D-1 = urediniospore length, D-2 = urediniospore width, D-3 = urediniospore wall thickness, D-4 = number and position of germ pores. E. Paraphysis: E-1 = paraphysis height, E-2 = paraphysis width, E-3 = dorsal wall thickness, E-4 = apical wall thickness. F. Teliospore: F-1 = teliospore length, F-2 = teliospore width, F-3 = teliospore wall thickness, F-4 = teliospore apical thickness, F-5 = telial organization.

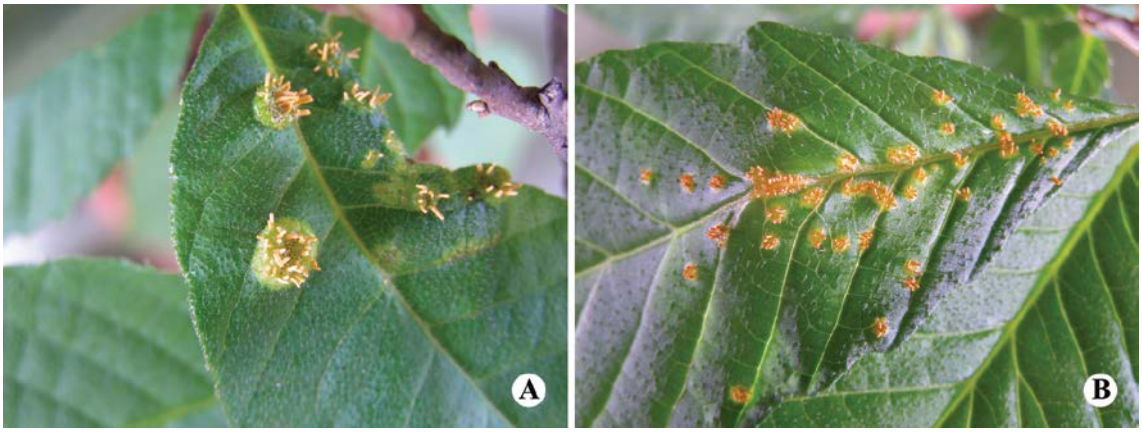


Fig. 2.3. Results of basidiospore inoculations. A: Aecia formed on the adaxial leaf surface of *M. myriantha* by inoculation of basidiospores derived from the same host species. B: Aecia formed on the adaxial leaf surface *M. tenuis* by inoculation of basidiospores derived from the same host species.

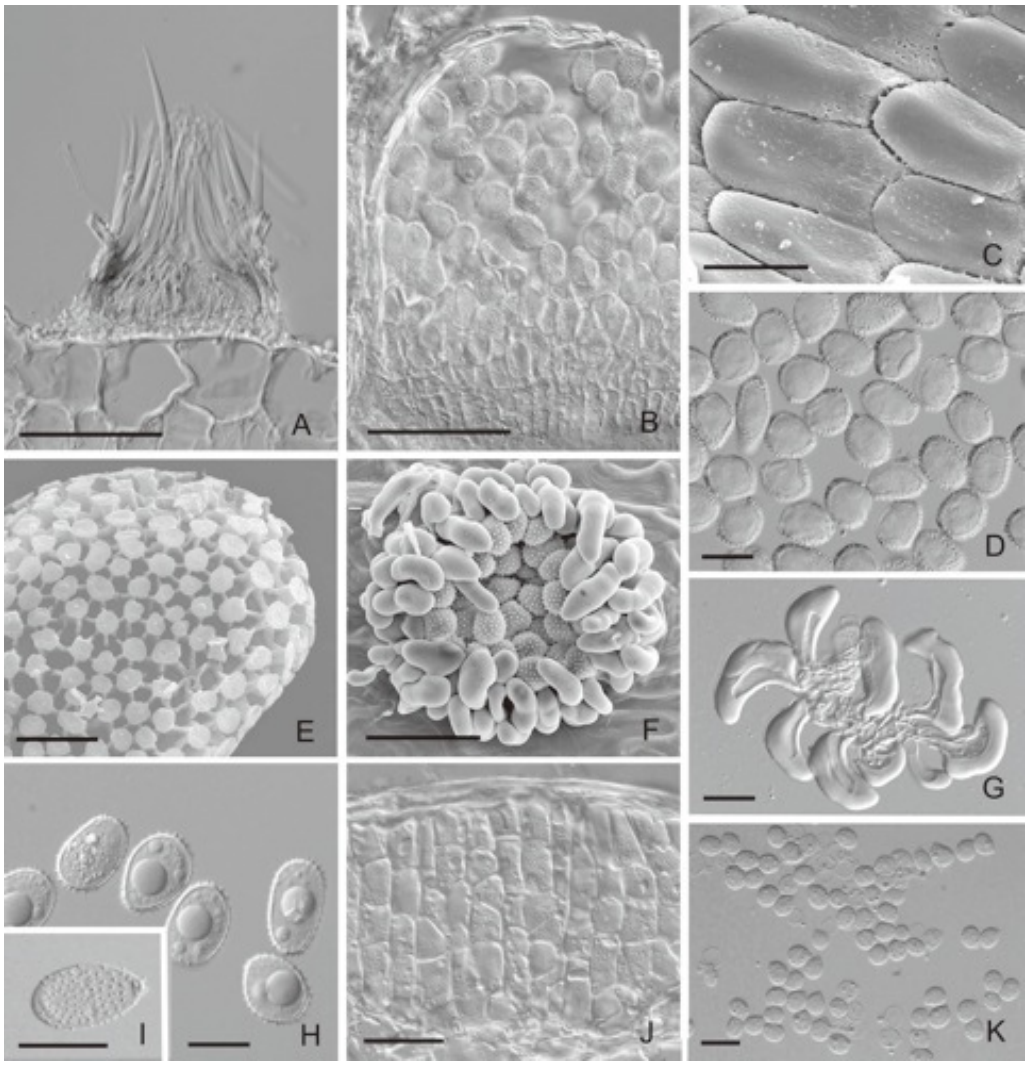


Fig. 2.4. Morphological features of the *Meliosma myriantha* infecting rust population. A: Spermogonium (IBAR-10065). B: A vertical section of an aecium (IBAR-4798). C: Almost smooth inner surface of peridial cells (IBAR-10038, SEM). D: Aeciospores (IBAR-10092). E: Nail-head verrucae on aeciospore wall surface (IBAR-10069, SEM). F: An overview of a uredinium (IBAR-10102, SEM). G: Uredinial paraphyses (IBAR-10102). H: Urediniospores (IBAR-10099). I: Urediniospore germ pores (IBAR-2387). J: A telium in vertical section (IBAR-5540). K: Basidiospores (IBAR-10244). Bars A, B 50 µm; C, D 20 µm; E 5 µm; F-K 20 µm (Pota et al. 2013).

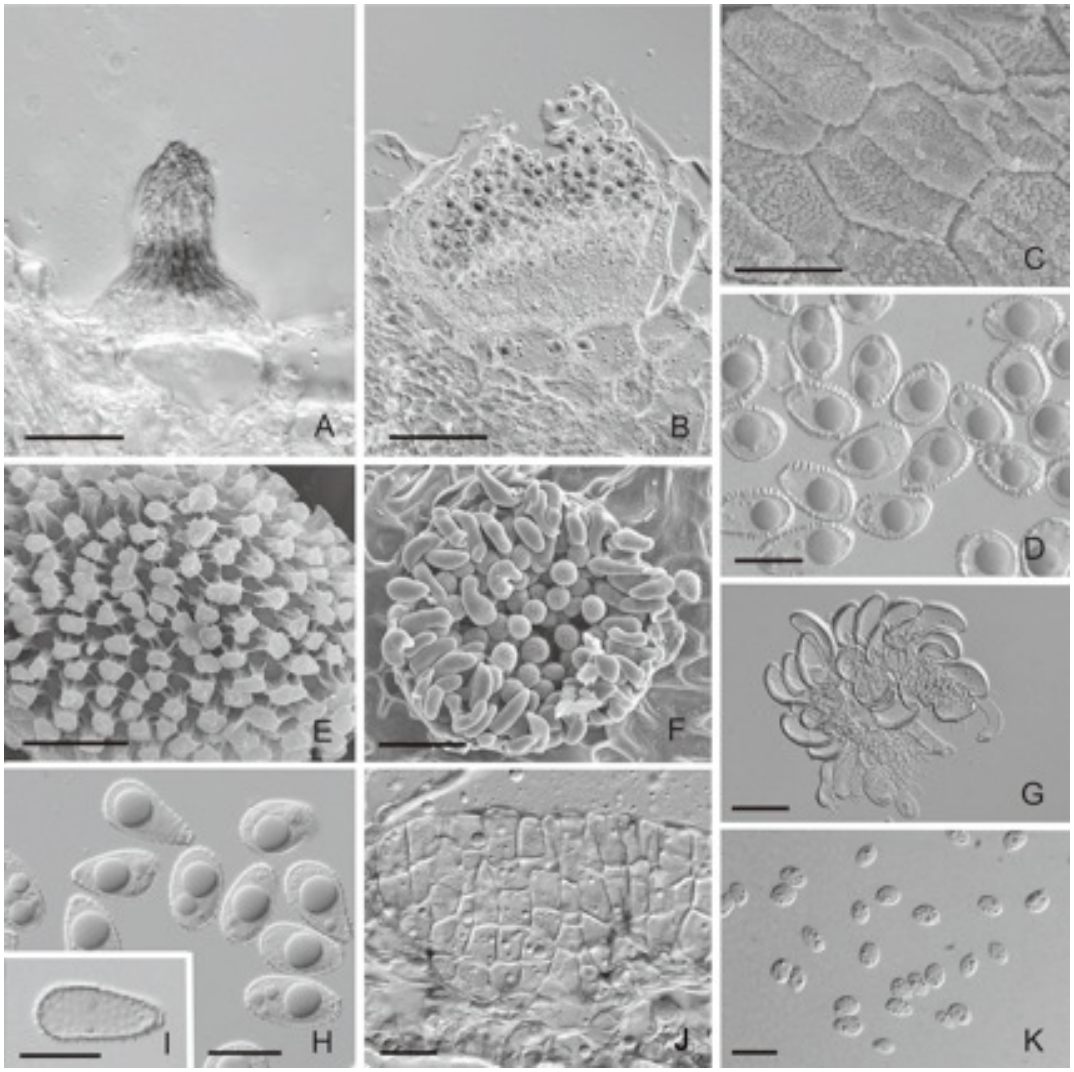


Fig. 2.5. Morphological features of *Meliosma tenuis* infecting rust population. A: Spermogonium (IBAR-10253). B: A vertical section of an aecium (IBAR-10256). C: Verrucose inner surface of peridial cells (IBAR-10253, SEM). D: Aeciospores (IBAR-10253). E: Nail-head verrucae on aecispore wall surface (IBAR-10253, SEM). F: An overview of a uredium (IBAR-10080, SEM). G: Uredinial paraphyses (IBAR-10104). H: Urediniospores (IBAR-10104). I: Urediniospore germ pores (IBAR-10104). J: A telium in vertical section (IBAR-10051). K. Basidiospores (IBAR-10248). Bars A, B 50 µm; C, D 20 µm; E 5 µm; F50 µm; G-K 20 µm (Pota et al. 2013).

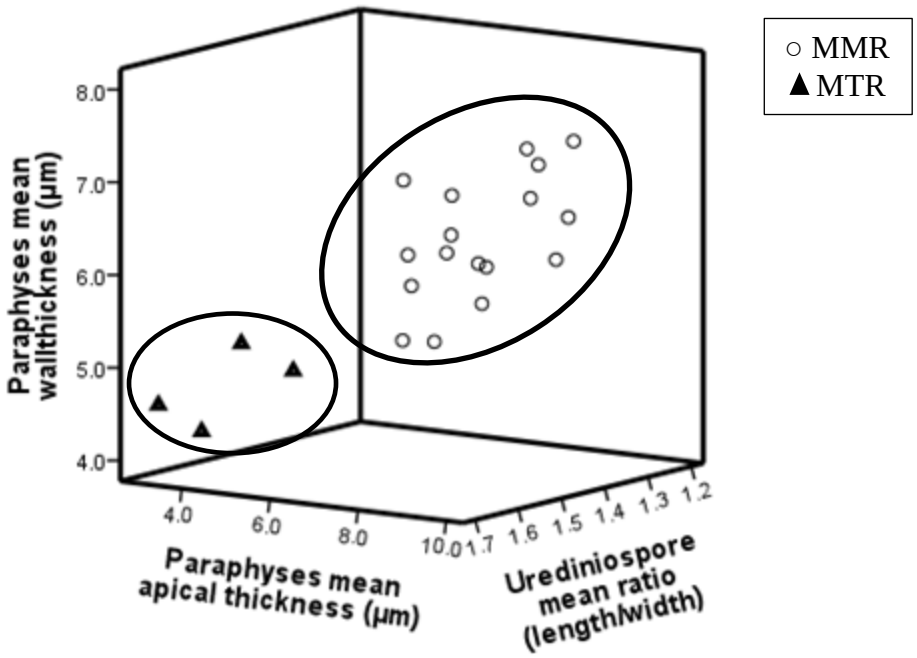


Fig. 2.6. A scatter diagram generated from urediniospore mean ratio (length/width), paraphyses mean wall thickness and paraphyses mean apical thickness of two rust population: (○) *M. myriantha* population (MMR) (▲) *M. tenuis* population (MTR).

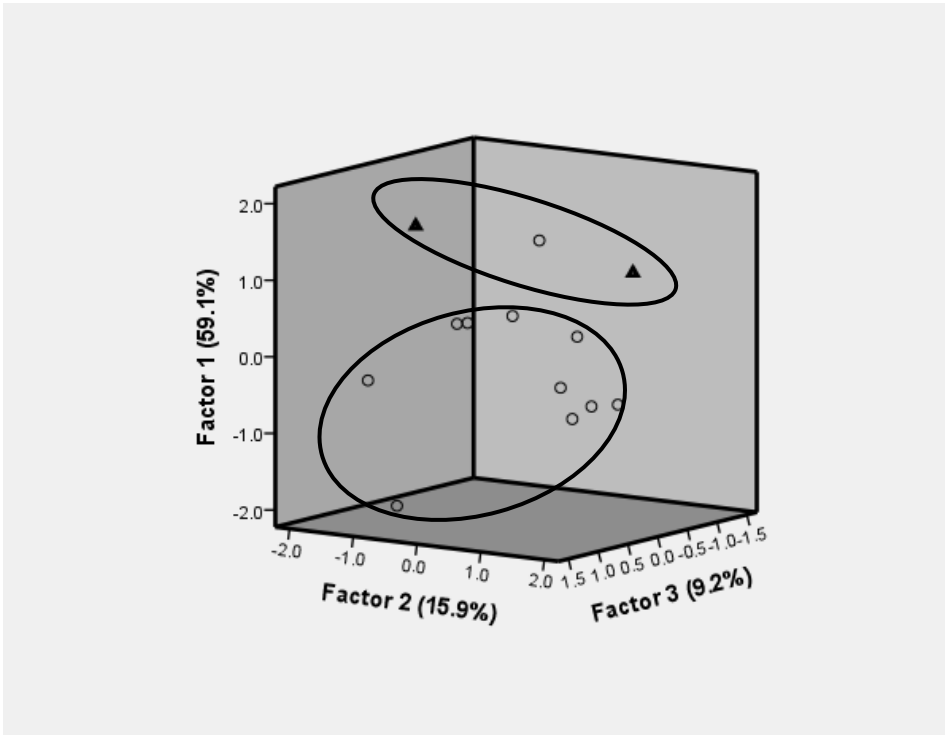


Fig. 2.7. A three dimensional scatter diagram generated by the principal component analysis based on non-standardized data of morphological characteristic of urediniospores and teliospores: (○) *M. myriantha* population (MMR) (▲) *M. tenuis* population (MTR).

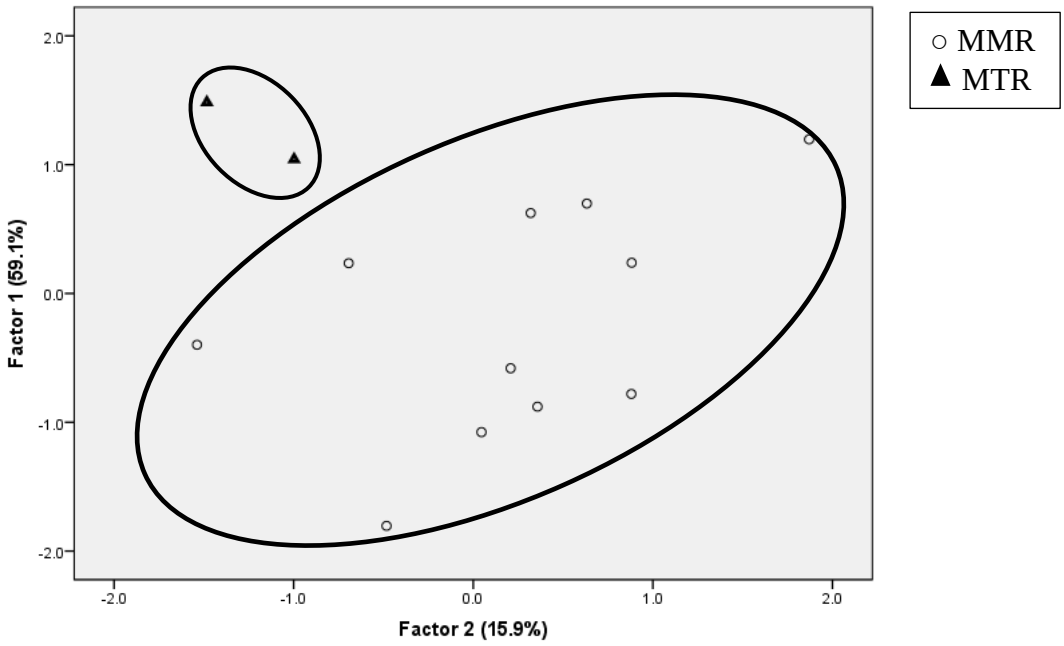


Fig. 2.8. A two dimensional scatter diagram generated by the principal component analysis based on non-standardized data of morphological characteristic of urediniospores and teliospores: (○) *M. myriantha* population (MMR) (▲) *M. tenuis* population (MTR).

Chapter 3. Heteroecious species on *Vitis* species

In this chapter, molecular phylogenetic analyses were conducted to clarify the relationships among the *Phakopsora* on *Meliosma* and *Vitis* species, particularly on the uncertain GLR populations found by Chatasiri and Ono (2008). Furthermore, comparative morphological examinations were carried out to clarify the taxonomic status of three GLR populations detected in the present studies. The inoculation experiment was conducted to confirm the heteroecious life cycle of undescribed grapevine leaf rust.

3.1. Materials and Methods

3.1.1. Specimens

According to the prospect of Chatasiri and Ono (2008), the GLR fungus distributed in tropical Asia may comprise a species distinct from temperate Asia. However, because of the limited sample, more GLR sampled in tropical Asia is required to study. Therefore, the GLR in Southeast Asia (where GLR is the major disease in grapevine cultivation) were used in this study and comparison with Japanese GLR.

Samples of rust fungi on *Vitis* and *Meliosma* were freshly collected from various areas in Japan and Thailand in 2009-2010. All the samples were deposited as dried specimens in the herbarium of Systematic Mycology, Faculty of Education, Ibaraki University, Mito, Japan (IBAR), except for samples used in the inoculation experiments, which were preserved in a refrigerator at ca.5°C until use. Additional specimens of GLR represented in Japan, Malaysia, Indonesia, East Timor and Australia

were taken from IBAR. Ninety-two specimens were used for the morphological study. All specimens, their host species, herbarium data, herbarium accession number and stages are listed in Table 3.1. Letters, S, A, U and T denote spemogonial, aecial, uredinial and Telial stage, respectively.

3.1.2. DNA extraction

DNA was extracted from about 100-200 urediniospores or teliospores obtained from a single uredium or telium. Spores from single uredium or telium were crushed between two sterile glass slides and suspended in 20 µl extraction buffer containing 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% SDS, and 0.01% ProteinaseK. The suspensions were incubated at 37°C for 60 min., and then at 95°C for 10 min. (Suyama et al. 1996; Virtudazo et al. 2001; Fig. 3.1.). From the crude extract, 1 to 3 µl samples were used directly for each polymerase chain reaction (PCR) amplification.

3.1.3. PCR amplification and sequencing

Two nuclear ribosomal RNA gene regions, rDNA D1/D2 region and the ITS regions were amplified by PCR. The rDNA D1/D2 region was amplified using primers NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') (O'Donnell 1993). For the complete ITS regions including 5.8S rDNA were amplified with primer set ITS5 (5'GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) (Fig. 3.2.). Amplification of D1/D2 region was done using 25 µl PCR reaction mixture each containing 2.5 µl of

each primer 2 μM, 2.5 μl of 10 × Ex Taq reaction buffer (20 mM Tris-HCl, pH8.0, 100 mM KCl), 0.125 TaKaRa Ex Taq DNA polymerase (Takara, Tokyo, Japan), and the commercial deoxymucleoside triphosphate (dNTP) mixture (containing 2.5 mM of each dNTP), 1 μl template DNA and 14.5 μl SDW. PCR was carried out using a Gene AmP PCR System 9700 (Applied Biosystems, Foster City, CA, USA) under the following condition: 94 °C for 10 min, then 40 cycles of 95 °C for 1 min, 55 °C for 90 s, and 72 °C for 2 min, and finally of 72 °C for 10 min (Chatasiri and Ono 2008). After amplification, 3 μl of the reaction product were run on 1% (w/v) agarose gels containing 0.5 μg/ml ethidium bromide in TAE buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 8.0) and visualized under UV light.

PCR products were purified using SV Minicolumns (WiZard® SV Gel and PCR Clean-Up System, Promega Corporation, Madison, USA) following the manufacturer's instruction and then used for sequence analysis. Purified products were subsequently prepared for sequencing using the BigDye Terminator V3.1 Cycle Sequencing Reaction Kit (Applied Biosystems) with the same primers used for PCR amplification under the following conditions: 96 ºC for 1 min, then 25 cycles of 96 °C for 30s, 50 °C for 1 min, 60 °C for 3 min (Chatasiri and Ono 2008). Cycle sequencing reaction products (20 μl) were purified by ethanol precipitation (Fig. 3.3.) and resuspended in 15 μl Hi-Di formamide (Applied Biosystem). Data were collected using an ABI PRISM 3130 automated sequencers (Applied Biosystems). Sequence with many ambiguous base calls were found in the ITS1 region, which made definite alignment impossible. Thus, further analysis did not include for the ITS1 sequence, leaving only the ITS2 region. All determined sequences were deposited in GenBank.

3.1.4. Phylogenetic analyses

All rust species used for molecular phylogenetic analyses are listed in Table 3.1. The sequences of *Phakopsora* species available from the GenBank were chosen and analyzed together with the sequences obtained in this study (Table 3.2.). Phylogenetic analyses were conducted with two individual loci (D1/D2 and ITS2 region) and a combined dataset of D1/D2 and ITS2 without missing data (visible only D1/D2 or ITS2). In these analyses, *P. pachyrhizi* Syd. & P. Syd. was used as the outgroup. DNA sequences were initially aligned using Clustal X v 1.8 (Thompson et al. 1997), followed by manual alignment in the data editor of BioEdit ver. 7.0.1 (Hall 1999). Ambiguously aligned regions and introns were excluded from analyses.

To test for incongruence between the two individual datasets, 70% bootstrap (BS) trees from parsimony analyses of individual loci were compared. First, 70% BS trees were calculated (100 BS replicates with five random addition sequences, TBR and Multrees options off) including only the taxa with sequences from the both loci.

Comparisons of 70% bootstrap trees between D1/D2 and ITS2 indicated no hard conflict between datasets. After confirming there were no conflicts, the individual gene datasets were combined and phylogenetic analyses were conducted with a combined dataset of two loci.

Phylogenetic trees were inferred with maximum parsimony (MP) and maximum likelihood (ML) analyses. MP analyses were conducted under the equally weighted parsimony criterion using PAUP version 4.0b10 (Swofford 2002). A two-step search approach was performed following the method of Hosaka et al. (2008). Briefly, in the first step, the heuristic search option with TBR, but no Multrees, and 1K replicates of random addition sequence were performed, keeping only up to two of the shortest trees

per replicate. In the second step, all of the shortest trees from the first step were used as starting trees for the heuristic search option (with TBR and Multrees on) with MAXTREES set to 10K. Support for the individual nodes was tested with bootstrap (BS) analysis under the equally-weighted parsimony criterion. BS analysis was based on 1000 BS replicates using the fast step option (with TBR option on, but Multrees option off), with five random addition sequences. ML analyses were performed in RAxML 8.0.2 software (Stamatakis 2014) using the GTR + Gamma model of evolution and 1000 bootstrap replicates.

3.1.5. Inoculation experiment

To demonstrate life cycle and host range of the population in clade II of the GLR fungus in Japan, the following inoculation experiments were conducted. Plant of *M. myriantha*, *M. tenuis*, *V. coignetiae*, *V. ficifolia* and *V. vinifera*×*V. labruscana* (Kyoho) were planted in a clay pot (15 cm in diam. or larger) with loam soil and maintained in a greenhouse to avoid possible spontaneous rust infection. Telial materials on *V. coignetiae* collected at Ina, Nagano in December 2009 (IBAR10249) and Koushu, Yamanashi in December 2009 (IBAR10243) were used as inocula. Inoculation method of *M. myriantha* and *M. tenuis* with basidiospores was the same as described in Chapter 2. Aeciospores formed by basidiospores inoculation were used to inoculate table grape cultivars *Vitis* spp., by the method described by Ono (1994) and Ono and Azbukina (1977). In each inoculation experiment, the control plants were only sprayed with distilled water. The inoculated plants were sprayed with distilled water and placed in the dark, moist chamber at 20 ºC for 2 days, and then transferred into a greenhouse at 20 ºC with controlled artificial illumination.

3.1.6. Morphological studies

Dried herbarium specimens were observed under light microscope (LM) and scanning electron microscope (SEM) by the same method described in Chapter 2. Data were statistically analyses by the method described in Chapter 2.

3.2. Results and Discussion

3.2.1. Phylogenetic analyses of *Phakopsora* species on *Meliosma* and *Vitis* species

Comparisons of 70% bootstrap trees from the individual gene analyses did not reveal any major conflicts among the datasets. The combined dataset after excluding the ambiguously aligned regions had an alignment length of 1045 bp, including 587 bp of D1/D2 sequence and 458 bp of ITS2 region. The number of parsimony informative characters was 163 for the combined dataset, including 34 for D1/D2 sequence and 129 for ITS2 region.

Parsimony analyses yielded 10 most parsimonious trees in the first step of the heuristic search for combined dataset and 4 for the individual D1/D2 sequence and ITS2 region sequence. The most parsimonious trees had 290 steps with a consistency index (CI) of 0.810, retention index (RI) of 0.984, rescaled consistency index (RC) of 0.798 for the combined dataset, 233 step with a consistency index (CI) of 0.850, retention index (RI) of 0.988, rescaled consistency index (RC) of 0.839 and 290 step with a consistency index (CI) of 0.810 for D1/D2 sequence, retention index (RI) of 0.984, rescaled consistency index (RC) of 0.798 for ITS2 region.

The results from the individual gene of D1/D2 and ITS2, and a combined dataset of both sequences of 91 taxa revealed a total of seven distinct clades with high bootstrap values, which were almost the same as the ones detected by Chatasiri and Ono

(2008) i.e., I: GLR fungus (*P. euvitis*) from Japan and Taiwan, II: *Aecidium* on *M. tenuis* and GLR fungus on *V. coignetiae* from Japan, III: Southeast Asian and Australasian GLR fungus from Thailand, Malaysia, Indonesia, East Timor and Australia, IV: *P. ampelopsidis* from Japan, V: *P. vitis* from Japan, VI: *P. meliosmae* on *M. tenuis* from Japan and VII: *P. meliosmae* on *M. myriantha* from Japan (Fig. 3.4.). Each of the seven clades was corresponded to the fungal collections circumscribed by life cycle, host specificity and geographic distribution range. Among them, two populations with autoecious life cycles (*P. meliosmae* and *P. orientalis*) were already discussed in Chapter 2 in this thesis. Three populations on *Ampelopsis*, *Parthenocissus* and *Vitis* were recognized as good species, *P. ampelopsidis*, *P. vitis* and *P. euvitis*, respectively, because of the difference of life cycle, host range and morphology (Ono 2000, Chatasiri and Ono 2008). The GLR fungi were composed to three distinct groups, i.e. clade I, II and III. Clade I and II included Japanese populations, but clade III included populations in Southeast Asia, i.e., Thailand, Malaysia, Indonesia, East Timor and Australia. The Southeast Asian and Australasian fungus (clade III) is composed of three subgroups different in the D1/D2 and ITS2 sequence. The three subgroups corresponded with the three distribution ranges, i.e., Thailand, Malaysia-Indonesia and East Timor-Australia.

In this study, clade II including specimens of *Aecidium* on *M. tenuis* and *V. coignetiae*. Therefore, the *Aecidium* on *M. tenuis* was expected as aecial stage of the GLR fungus on *V. coignetiae*. The inoculation experiments were needed to confirm the life cycle and host range of the rust fungus in clade II. Furthermore, comparative morphological examinations were carried out to clarify the taxonomic status of three GLR populations in following sections.

3.2.2. *Phakopsora* species on *Meliosma tenuis* and *Vitis* species

To demonstrate life cycle and host range of the population in clade II (*Aecidium* on *M. tenuis* and GLR fungus on *V. coignetiae* from Japan) of the GLR fungus in Japan, inoculation experiments were conducted. All inoculation with basidiospores, derived from telial on *V. coignetiae* collection from Yamanashi and Nagano, resulted in successful infection only on *M. tenuis* but not on *M. myriantha* (Table 3.3.; Fig. 3.5.). Five to 10 days after inoculation, pale yellowish, diffuse lesions appeared on leaf of inoculated plant on the inoculated sites of leaves (Fig. 3.5a). Spermogonia formed on adaxial surface of the lesion 7-14 days after inoculation, appeared dark dots in dense group (Fig. 3.5b). 7-16 days after the appearing of spermogonia, dome-shape or columnar aecia appeared on the abaxial surface of the lesion (Fig. 3.5c). On the other hand, no sign of infection was detected on basidiospore-inoculated *M. myriantha* leaves. Control plants sprayed only with distilled water also had no sign of infection. These results confirmed that the population on *V. coignetiae* was heteroecious and used *M. tenuis* as the acial/spermogonail and aecial host.

Inoculation with aeciospores formed on *M. tenuis* by basidiospore inoculation described above, successfully infected *V. vinifera*×*V. labruscana* (Kyoho) and *V. ficifolia* (Table 3.4.). Eight to 13 days after aeciospores inoculation, uredinia appeared on the leaves of inoculated plants. *V. vinifera*×*V. labruscana* (Kyoho) were highly susceptible and formed abundant urediniospores, while *V. ficifolia* had tiny often with necrotic lesions with less abundant spore production. Inoculation on *A. brevipedunculata* and *P. tricuspidata* and control plants sprayed only with distilled water were not infected. The inoculation experiments demonstrated that GLR

population on *V. coignetiae* was also able to infect table grape cultivars at least under the artificial condition.

Spermogonia were densely aggregated on the adaxial leaf surface, subcuticular and conical with peripheral paraphyses, and 51.3–129.2 μm high and 69.2–161.4 μm wide (Table 3.5.; Fig. 3.6a). Aecia formed on the abaxial leaf surface, covered with a well-developed perdium, becoming cupulate by rupture of the peridium (Figs. 3.6b, 3.6c). The inner surface of peridial cells was verrucose, the outer surface smooth (Fig. 3.6f). Aeciospores were produced in basipetal succession from a basal sporogenous layer, angularly subglobose to broadly ellipsoid and 12.8–18.3 × 13.9–20.7 μm in size; the wall was covered with nail-head verrucose and 0.6–1.7 μm thick (Table 3.5.; Fig. 3.6e).

Uredinia were hypophyllous, subepidermal in origin, densely surrounded by basally united paraphyses (Fig. 3.6i). The paraphyses were 17.3–79.5 μm high and 4.9–15.3 wide. The dorsall paraphysis wall was 0.9–4.9 μm and apical wall was 0.9–2.1 μm (Fig. 3.6l). Urediniospores were short-pedicellate, obovoid or broadly ellipsoid, and 12.6–23.6 × 14.5–27.3 μm in size. The urediniospore wall was hyaline, 0.6–1.5 μm thick, and evenly echinulate (Fig. 3.6k). Germ pores were scattered or distributing in equatorial zone (Table 3.6.; Fig. 3.6k).

Telia were hypophyllus, crustose and composed of 2–5 layers of more or less linearly arranged teliospores. The teliospores of the uppermost layer were ellipsoid to oblong, angular, and 6.1–17.0×10.2–23.6 μm in size: the apical wall was 0.8–2.3μm thick and lateral wall 0.6–2.3 μm thick. The teliospores of the second layer and below were 7.5–16.4×7.5–23.6 μm in size: the apical wall was 0.9–2.8 μm thick and lateral wall 0.6–2.3 μm thick thick (Table 3.7.; Fig. 3.7m).

Based on molecular phylogenetic analyses, morphological and host information resulted from specimens collected in nature and artificial inoculation, the GLR population in clade II was heteroecious species which alternates between *M. tenuis* and *V. coignetiae*. And also, it has the ability to infect table grape cultivars. Ono (2000) and Chatasiri and Ono (2008) assumed that the aecidium fungus on *M. tenuis* (= clade II in the present study) was an aecial anamorph of an underscribed *Phakopsora* on a vitaceous plants because of the aeciospores-surface structure that is unique to the genus *Phakopsora* and because of the close taxonomic relationships between the two *Meliosma* species. No clear differences in morphology of uredinial and telial state between the GLR clade I and II, but they were distinct in the aeciospore morphology (the spore apical wall is thicker in the former while thinner in the latter fungus) in addition to the difference in spermogonial-aecial host preference. The former species used *M. myriantha* while the later *M. tenuis*. The GLR population in clade II was described as a new species, *P. montana* Y. Ono & Chatasiri (Ono et al. 2012) combined with the data shown in the present thesis. Because the new International Code of Nomenclature of algae, fungi, and plants mandates only one correct name be accepted for fungi, the new name of *P. euvitis* in clade I was changed to *P. meliosmae-myrianthae* (Henn. & Shirai) Y. Ono (Ono et al. 2012).

Both *M. myriantha* and *M. tenuis* were considered as alternate host plants of GLR in Japan since Kudo and Kaneko (1977, 1978). Two populations on *M. myriantha* and *M. tenuis* are now recognized as two distinct species. Both species were able to parasitize table grape cultivars, so that they might infect the same leaves. Distribution of these species on the table grape cultivars have to be examined.

3.2.3. Southeast Asian and Australasian population of *Phakopsora* species on *Vitis* species

As the results of the previous section, the molecular phylogenetic of the Southeast Asian and Australasian GLR fungus were divided into three subclades i.e., Thailand, Malaysia-Indonesia and East Timor-Australia. The morphology of these three subclades was compared. Uredinial and telial morphology were similar between the samples from different localities in Southeast Asia and Australasia (Tables 3.6. and 3.7.).

Uredinia were produced on the abaxial leaf surface, minute, loosely or densely grouped, and surrounded by paraphyses (Figs. 3.7a, 3.7b). The paraphyses were cylindrical, weakly to moderately incurved, 23.9–63.3 μm high and 6.0–19.4 μm wide in Thai specimens, 24.9–66.2 μm high and 6.0–21.1 μm wide in Indonesia specimens, 29.4–62.2 μm high and 8.1–24.1 μm wide in Australia specimens and 30.5–66.0 μm high and 8.3–21.1 μm wide in East Timor specimens. The wall was colorless, dorsally 0.4–2.3 μm thick and apically 0.9–2.8 μm thick in Thai specimens, 0.9–3.0 μm thick and apically 0.9–3.6 μm thick in Indonesia specimens, 0.9–2.8 μm thick in Australia specimens, 0.9-2.1 μm in East Timor specimens and apically 0.9–2.3 μm thick in Australia and East Timor specimens (Table 3.6.). The size and shape of paraphyses varied within individual specimens as well as among specimens. Urediniospores were short-pedicellate, obovoid to ellipsoid, or pyriformand. The urediniospore size was 13.6–21.7 × 17.5–27.1 μm in Thai specimens, 13.9–24.1 × 15.1–25.1 μm in Indonesia specimens, 13.2–24.3 × 17.0–26.6 μm in Australia specimens and 14.1–21.3 × 16.2–27.7 μm in East Timor specimens (Table 3.6.; Fig. 3.7c). The wall was colorless to pale yellow, evenly 0.6–2.1 μm thick in Thai specimens, 0.9–1.7 μm in Indonesia and East

Timor and 0.6–1.7 μm in Australia specimens and completely echinulate. Urediniospore germ pores were hardly observable. When observed, they were six (rarely five or seven) and scattered over the wall (Figs. 3.7d, 3.7e); and rarely four germ pores were distributed on an equatorial zone.

Telia were observed only in the Thai specimens. They were scattered or grouped on the abaxial leaf surface, subepidermal and composed of 2–6 layers of more or less linearly arranged teliospores (Fig. 3.7f). The teliospores at the uppermost layer were ellipsoid to oblong, angular and 5.8–15.8 × 8.5–22.2 μm in size. The apical wall was 0.9–2.3 μm thick and light brown; and the lateral wall was 0.6–2.6 μm thick and almost colorless. The teliospores at the second layer and below were 6.2–14.3 × 9.2–22.8 μm in size. The apical wall was 0.9–2.1 μm thick and lateral wall was 0.9–2.3 μm thick (Table 3.7.).

Comparisons of statistical analyses of three populations of *Phakopsora* (clade I, II and III) showed morphology of uredia and urediniospore on *Vitis* species was not significantly different among populations. Even the dorsal and apical wall thickness of paraphyses showed slight difference between the GLR fungus in Japan (clade I and II) and Southeast Asian and Australasian population (Thai, Indonesia, Malaysia and Australia-East Timor: clade III) and the dorsal wall and apical thickness of paraphyses of the fungal population on tropical regions was smaller than those of the temperate region population, no significant differences were observed in urediniospore and other characteristic of paraphyses. The principal component analyses were undertaken with various combinations of urediniospores and teliospores. After the varimax rotation, the calculated factor 1, 2 and 3 explained 32.9 %, 16.7 % and 13.6 % of the total variance,

respectively. The scatter diagram of PCA of all specimens observed could not be divided into the group (Figs. 3.8., 3.9.).

The Southeast Asian and Australasian GLR fungus (clade III) was similar to *P. meliosmae-myrianthae* and *P. montana* in morphology of uredinial and telial. Uredinial paraphyses of the Southeast Asian and Australasian GLR fungus appeared thinner than those of the East Asian GLR fungi, but there was no significant difference among the populations. Furthermore, the Southeast Asian and Australasian fungus is composed of three subgroups different in the D1/D2 and ITS2 sequence. The three subgroups corresponded with the three distribution ranges, i.e., Thailand, Malaysia-Indonesia and East Timor-Australia. Results of the studies on *P. montana* and *P. meliosmae-myrianthae* indicated the importance of morphological differences of aecial state. However, the spermogonial-aecial state of these populations in clade III was unknown. Thus, the Southeast Asian and Australasian GLR population was treated as *Phakopsora* species for the present, until spermogonial-aecial state is found (Pota et al. 2014).

Aecidium meliosmae-myrianthae on *M. myriantha* is an aecial stage of *P. meliosmae-myrianthae* in Japan (Ono et al. 2012). The other two *Aecidium* species has been reported in tropical Asia. One is *A. hornotinum* Cummins (1937), was described on *M.* aff. *multivlora* Merr. from the Philippines. This fungus has apically thick-walled aeciospores and fragile peridia. Another is *A. wareoense* Cummins (1941), was described on *M. ferruginea* Blume and *M. fruticosa* Blume in Papua New Guinea and Indonesia (Boedijin 1959). This fungus has apically thick-walled aeciospores like *A. hornotinum* but separated from *A. hornotinum* by forming persistent peridia like *A. meliosmae-myrianthae*. However, *A. wareoense* was said to be “entirely different” from

A. meliosmae-myrianthae because the apically thickened aeciospore wall was not explicitly described for *A. meliosmae-myrianthae* by Hennings (1900) and *A. hornotinum* will prevent confusion with *A. awareoense* (Cummins 1941). Recent widespread occurrence of the grapevine leaf rust (GLR) in tropical/subtropical Asia may be caused by still undisclosed species, which can be connected to *A. wareoense* in the life cycle. Therefore, geographically more widespread samples representing tropical and temperate Asia should be studied to clarify the degree and extent of genetic and morphological differentiation, including host range and life cycle.

Artificial inoculations with urediniospores of the Australian GLR fungus (Daly et al. 2005) and of the Japanese *P. meliosmae-myrianthae* (Ono 2013) proved that *Ampelocissus* species native of Northern Territory, Australia, were susceptible to the GLR fungi. Daly and Hennesy (2006) found that an unidentified *Ampelocissus* plant was naturally infected and supported a low level of sporulation of the Australian GLR fungus in Darwin, Northern Territory, Australia. In northern Australia, where extended, dry winter exists, these potential host plants become dormant in the winter and would contribute little in the source of urediniospores for the persistent GLR incidences in this geographic region (Daly et al. 2005; Daly and Hennessy 2006). However, tropical Asian *Ampelocissus* species and its allies of evergreen nature, together with the above-mentioned *Meliosma* species, are likely to support the complete life cycle of the GLR fungus in the geographic region.

Table 3.1.The specimens used for molecular phylogenetic studies and morphological observation of *Phakopsora* species on *Vitis* species.

Rust/host	Herbarium accession no.	Specimen data			Stage[a]	Group	GenBank accession no.[b]	
		Location	Date	Collector			D1/D2	5.8+ITS2
Japanese GLR fungus, *Phakopsora euvitis* on *Vitis coignetiae*	IBAR-1817	Japan: Fukushima	28 Sep. 1979	Y. Ono	U, T			
	IBAR-2525	Japan: Yamanashi	16 Sep. 1982	Y. Ono	U, T			
	IBAR-2594	Japan: Yamanashi	18 Sep. 1982	Y. Ono	U, T			
	IBAR-2782	Japan: Yamanashi	6 Sep. 1983	Y. Ono	U, T			
	IBAR-2783	Japan: Yamanashi	6 Sep. 1984	Y. Ono	U, T			
	IBAR-3602	Japan: Yamagata	23 Sep. 1962	N. Hiratsuka et al.	U			
	IBAR-3603	Japan: Yamagata	23 Sep. 1963	N. Hiratsuka et al.	U			
	IBAR-3607	Japan: Niigata	13 Sep. 1964	S. Sato	U			
	IBAR-3608	Japan: Niigata	12 Sep. 1964	S. Sato	U			
	IBAR-3614	Japan: Fukushima	24 Sep. 1959	S. Wada	U, T			
	IBAR-3615	Japan: Nagano	2 Sep. 1963	K. Sugimoto	U			
	IBAR-3620	Japan: Yamagata	12 Oct. 1961	S. Sato et al.	U, T			
	IBAR-3622	Japan: Yamagata	16 Oct. 1961	T. Shimanuki	U, T			
	IBAR-3726	Japan: Gumma	16 Jul. 1963	K. Sugimoto	U			
	IBAR-3930	Japan: Tochigi	1 Oct. 1989	Y. Ono	U, T			
	IBAR-4123	Japan: Ibaraki	15 Oct. 1989	Y. Ono	T			
	IBAR-6073	Japan: Ibaraki	9 Jul. 1992	Y. Ono	U			
	IBAR-6081	Japan: Ibaraki	17 Jul. 1992	Y. Ono	U			
	IBAR-6218	Japan: Ibaraki	18 Sept. 1992	Y. Ono	U			
	IBAR-6279	Japan: Niigata	5 Oct. 1992	Y. Ono	U, T			
	IBAR-6282	Japan: Niigata	6 Oct. 1992	Y. Ono	U, T			
	IBAR-6375	Japan: Ibaraki	24 Oct. 1992	Y. Ono	U			

Table 3.1. Continued

Rust/host	Herbarium accession no.	Specimen data			Stage[a]	Group	GenBank accession no.[b]	
		Location	Date	Collector			D1/D2	5.8+ITS2
Japanese GLR fungus, *P. euvitis* on *V. coignetiae*	IBAR-6714	Japan: Ibaraki	12 Jun. 1993	Y. Ono	U			
	IBAR-6724	Japan: Ibaraki	28 Jun. 1998	Y. Ono	U			
	IBAR-8584[b]	Japan: Tochigi	28 Sep. 2000	Y. Ono	U, T	I	AB354748	AB354785
	IBAR-9546	Japan: Tochigi	18 Aug. 2005	Y. Ono	U			
	IBAR-9605[b]	Japan: Ibaraki	11Oct. 2005	Y. Ono and S. Kodato	U	I	AB354749	AB354786
	IBAR-9668[b]	Japan: Ibaraki	4 May 2006	Y. Ono and S. Kodato	U	I	AB354750	AB354787
	IBAR-9669	Japan: Tochigi	14 Apr. 2006	Y. Ono et al.	T			
	IBAR-10176	Japan: Nagano	24 Oct. 2009	Y. Yamaoka	U, T			
Japanese GLR fungus, *P. euvitis* on *V. flexuosa*	IBAR-4328	Japan: Tokyo	date not specified	S. Kusano	T			
	IBAR-4333	Japan: Tokyo	18 Oct. 1899	S. Kusano	U, T			
	IBAR-5996	Japan: Ibaraki	9 Nov. 1991	Y. Ono	T			
	IBAR-6217	Japan: Ibaraki	18 Sep. 1992	Y. Ono	U			
	IBAR-6729	Japan: Ibaraki	4 Aug. 1993	Y. Ono	U			
	IBAR-7008	Japan: Ibaraki	22 Oct. 1993	Y. Ono	T			
on *V. ficifolia*	IBAR-6246	Japan: Tochigi	21 Sep. 1992	Y. Ono	U, T			
Japanese GLR fungus, *P. euvitis* on *V. vinifera* × *V. labrusca* (Kyoho)	IBAR-5989	Japan: Ibaraki	3 Nov. 1991	Y. Ono	U, T			
	IBAR-6711	Japan: Ibaraki	12 Jun. 1993	Y. Ono	U			
	IBAR-6721	Japan: Ibaraki	28 Jun. 1998	Y. Ono	U			
	IBAR-7720[b]	Japan: Okinawa	8 Dec. 1995	Y. Ono	U	I	AB354752	AB354789
	IBAR-7288	Japan: Ibaraki	26 Sep. 1994	Y. Ono	U			
	IBAR-8036	Japan: Ibaraki	13 Apr. 1998	Y. Ono	T			
	IBAR-8106[b]	Japan: Ibaraki	10 Jun. 1998	Y. Ono	U	I	AB354751	AB354788
	IBAR-10118	Japan: Saitama	12 Sep. 2010	Y. Ono and S. Pota	U, T	I	KC815599	KC815540

Table 3.1. Continued

Rust/host	Herbarium accession no.	Specimen data			Stage[a]	Group	GenBank accession no.[b]	
		Location	Date	Collector			D1/D2	5.8+ITS2
Japanese GLR fungus, *P. euvitis* on *V. vinifera* × *V. labrusca* (Kyoho)	IBAR-10119	Japan: Saitama	12 Sep. 2010	Y. Ono and S. Pota	U, T	I	KC815600	KC815541
	IBAR-10120	Japan: Yamanashi	12 Sep. 2010	Y. Ono and S. Pota	U, T	I	KC815601	KC815542
	IBAR-10121	Japan: Yamanashi	12 Sep. 2010	Y. Ono and S. Pota	U, T	I	KC815602	KC815543
	IBAR-10122	Japan: Yamanashi	12 Sep. 2010	Y. Ono and S. Pota	U, T	I	KC815603	KC815544
	IBAR-10123	Japan: Yamanashi	12 Sep. 2010	Y. Ono and S. Pota	U, T	I	KC815604	KC815545
Taiwanese GLR fungus, *P. euvitis* on *V. vinifera* cultivar	IBA-10239	Taiwan: Taichung	16 Nov. 2009	C. Kajanaphachoat	U	I	KC815605	KC815546
	IBA-10240	Taiwan: Taichung	18 Nov. 2009	M. Kakishima and W. Chung	U	I	KC815606	KC815547
Thai GLR fungus on *V. vinifera* cultivar	IBAR-10217	Thailand: Chonburi	Nov. 2009	S. Chatasiri	U, T	III	KC815607	KC815548
	IBAR-10218	Thailand: Chiang Mai	9 Jan. 2010	S. Pota and W. Anuthep	U	III	KC815608	KC815549
	IBAR-10219	Thailand: Chiang Mai	9 Jan. 2010	S. Pota and W. Anuthep	U	III	KC815609	KC815550
	IBAR-10220	Thailand: Chiang Mai	9 Jan. 2010	S. Pota and W. Anuthep	U	III	KC815610	KC815551
	IBAR-10221	Thailand: Chiang Mai	16 Jan. 2010	S. Pota and W. Anuthep	U	III	KC815611	KC815552
	IBAR-10222	Thailand: Nakhon Pathom	19 Jan. 2010	J. Phetdaew	U			
	IBAR-10223	Thailand: Nakhon Pathom	21 Jan. 2010	J. Engkhaninum	U	III	KC815607	KC815548
	IBAR-10224	Thailand: Bangkok	22 Jan. 2010	S. Pota	U			
	IBAR-10225	Thailand: Bangkok	22 Jan. 2010	S. Pota	U	III	KC815608	KC815549
	IBAR-10226	Thailand: Saraburi	23 Jan. 2010	Y. Ono et al.	U, T	III	KC815609	KC815550
	IBAR-10227	Thailand: Nakhon Ratchasima	23 Jan. 2010	Y. Ono et al.	U	III	KC815610	KC815551

Table 3.1. Continued

Rust/host	Herbarium accession no.	Specimen data			Stage[a]	Group	GenBank accession no.[b]	
		Location	Date	Collector			D1/D2	5.8+ITS2
Thai GLR fungus on *V. vinifera* cultivar	IBAR-10228	Thailand: Nakhon Ratchasima	23 Jan. 2010	Y. Ono et al.	U	III	KC815611	KC815552
	IBAR-10229	Thailand: Nakhon Ratchasima	23 Jan. 2010	Y. Ono et al.	U, T	III	KC815617	KC815558
	IBAR-10230	Thailand: Nakhon Ratchasima	23 Jan. 2010	Y. Ono et al.	U, T	III	KC815618	KC815559
	IBAR-10231	Thailand: Nonthaburi	24 Jan. 2010	S. Pota and W. Iambuntharik	U	III	KC815619	KC815560
Malaysian GLR fungus on *V. vinifera* cultivar	IBA-9657[c]	Malaysia: Penang	24 Nov. 2005	Y. Ono	U	III	KC815620	KC815561
Indonesian GLR fungus, on *V. vinifera* cultivar	IBAR-10041	Indonesia: Sleman	4 Sep. 2008	S. Subandiyah	U	III	KC815621	KC815562
	IBAR-10042	Indonesia: Sleman	4 Sep. 2009	S. Subandiyah	U	III	KC815622	KC815563
	IBAR-10043	Indonesia: Central Java	4 Sep. 2010	S. Subandiyah	U	III	KC815623	KC815564
	IBAR-10044	Indonesia: Yogyakarta	4 Sep. 2011	S. Subandiyah	U	III	KC815624	KC815565
	IBAR-10250	Indonesia: West Java	14 Feb. 2010	D. Wayuno	U			
East Timor GLR fungus on *V. vinifera* cultivar	IBAR-8807	East Timor: Dali	7 May 2002	Dili M. Weinert	U			
	IBAR-8808[b]	East Timor: DesaBecora	9 May 2002	M. Weinert	U	III	AB354756	AB354794
	IBAR-8809	East Timor: Dali	9 May 2002	M. Weinert	U			
	IBAR-8810[b]	East Timor: Dali	9 May 2003	M. Weinert	U	III	AB354757	AB354796
Australian GLR, on *V. vinifera* cultivar	IBAR-8755[b]	Australia: Northern Territory	25 Jan. 2002	M. Weinertand Y. Ono	U	III	AB354754	AB354791
	IBAR-8756	Australia: Northern Territory	25 Jan. 2002	M. Weinertand Y. Ono	U			
	IBAR-8757	Australia: Northern Territory	25 Jan. 2002	M. Weinertand Y. Ono	U			
	IBAR-8758[b]	Australia: Northern Territory	25 Jan. 2002	M. Weinertand Y. Ono	U	III	AB354755	AB354793

Table 3.1. Continued

Rust/host	Herbarium accession no.	Specimen data			Stage[a]	Group	GenBank accession no.[b]	
		Location	Date	Collector			D1/D2	5.8+ITS2
Japanese GLR fungus *Phakopsora* fungus on *M. tenuis*	IBAR-3600	Japan: Gumma	14 Jul. 1963	N. Hiratsuka and K. Sugimoto	S, A			
	IBAR-7591	Japan: Tochigi	14 Jun. 1955	Y. Ono	S, A			
	IBAR-7790	Japan: Tottori	25 Jul. 1976	N. Hiratsuka and S. Okubo	S, A			
	IBAR-7791	Japan: Tottori	5 Jul. 1982	S. Kaneko and A. Kudo	S, A			
	IBAR-8556[b]	Japan: Niigata	29 Jul. 2000	Y. Ono	S, A	II	AB354767	AB354810
	IBAR-9545[b]	Japan: Tochigi	18 Aug. 2005	Y. Ono et al.	S, A	II	AB354768	AB354811
	IBAR-9547[b]	Japan: Tochigi	18 Aug. 2005	Y. Ono et al.	S, A	II	AB354769	AB354812
	IBAR-9682[b]	Japan: Tochigi	21 Jul. 2006	Y. Ono et al.	S, A	II	AB354770	AB354813
	IBAR-9861	Japan: Tochigi	7 Jul. 2007	Y. Ono et al.	S, A	II		
	IBAR-10082	Japan: Nagano	11 Jul. 2009	S. Pota	S, A	II	KC815636	KC815577
	IBAR-10085	Japan: Nagano	11 Jul. 2009	S. Pota	S, A	II	KC815637	KC815578
	IBAR-10089	Japan: Gumma	17 Jul. 2009	S. Pota	S, A	II	KC815638	KC815579
Japanese GLR fungus, *Phakopsora* fungus on *V. coignetiae*	IBAR-9548	Japan: Tochigi	18 Aug. 2005	Y. Ono et al.	U			
	IBAR-10052	Japan: Tochigi	20 Sep. 2008	Y. Ono	T			
	IBAR-10124	Japan: Yamanashi	13 Sep. 2009	Y. Ono and S. Pota	U, T	II	KC815639	KC815580
	IBAR-10168	Japan: Ibaraki	1 Oct. 2009	S. Pota	U			
	IBAR-10243[d]	Japan: Yamanashi	4 Dec. 2009	Y. Yamaoka and S. Pota	T			
	IBAR-10249[d]	Japan: Nagano	5 Dec. 2009	Y. Yamaoka and S. Pota	T			
	IBAR-10252	Japan: Tochigi	16 Apr. 2000	Y. Ono	T			
	IBAR-10301	Japan: Tottori	13 Sep. 2010	Y. Ono	U, T			
	IBAR-10303	Japan: Tottori	13 Sep. 2010	Y. Ono	U, T	II	KC815640	KC815581

Table 3.1. Continued

Rust/host	Herbarium accession no.	Specimen data			Stage[a]	Group	GenBank accession no.[b]	
		Location	Date	Collector			D1/D2	5.8+ITS2
Japanese GLR fungus, *Phakopsora* fungus on *V. coignetiae*	IBAR-10309	Japan: Tottori	13 Sep. 2010	Y. Ono	U, T	II	KC815641	KC815582
	IBAR-10312	Japan: Tottori	13 Sep. 2010	Y. Ono	U, T	II	KC815642	KC815583
	IBAR-10314	Japan: Tottori	13 Sep. 2010	Y. Ono	U, T	II	KC815643	KC815584
Japanese *P. ampelopsidis* on *Ampelopsidis brevipedunculata*	IBAR-10115[c]	Japan: Saitama	12 Sep. 2009	Y. Ono and S. Pota	T	IV	KC815625	KC815566
	IBAR-10116[c]	Japan: Saitama	12 Sep. 2010	Y. Ono and S. Pota	T	IV	KC815626	KC815567
	IBAR-10126[c]	Japan: Ibaraki	19 Sep. 2009	Y. Yamaoka	T	IV	KC815627	KC815568
	IBAR-10127[c]	Japan: Ibaraki	22 Sep. 2009	S. Pota and S. Nagata	T	IV	KC815628	KC815569
	IBAR-10128[c]	Japan: Ibaraki	22 Sep. 2009	S. Pota and S. Nagata	T	IV	KC815629	KC815570
Japanese *P. vitis Parthenocissus tricuspidata*	IBAR-9894[c]	Japan: Ibaraki	8 Oct. 2007	Y. Ono	T	V	KC815630	KC815571
	IBAR-10098[c]	Japan: Ibaraki	25 Aug. 2009	S. Pota	T	V	KC815631	KC815572
	IBAR-10101[c]	Japan: Yamagata	28 Aug. 2009	S. Pota	T	V	KC815632	KC815573
	IBAR-10108[c]	Japan: Fukushima	5 Sep. 2009	Y. Ono	T	V	KC815633	KC815574
	IBAR-10117[c]	Japan: Saitama	12 Sep. 2010	Y. Ono and S. Pota	T	V	KC815634	KC815575
	IBAR-10125[c]	Japan: Tokyo	13 Sep. 2010	Y. Ono and S. Pota	T	V	KC815635	KC815576
Japanese *P. meliosmae* on *M. myriantha*	IBAR-9906	Japan: Fukushima	13 Oct. 2007	Y. Ono	T	VII	KC815644	KC815585
	IBAR-9911	Japan: Ibaraki	20 Oct. 2007	Y. Ono	T	VII	KC815645	KC815586
	IBAR-9912	Japan: Ibaraki	20 Oct. 2007	Y. Ono	T	VII	KC815646	KC815587
	IBAR-9943	Japan: Tochigi	16 Nov. 2007	Y. Ono	T	VII	KC815647	KC815588
	IBAR-9946	Japan: Ibaraki	16 Nov. 2007	Y. Ono	T	VII	KC815648	KC815589
	IBAR-9977	Japan: Ibaraki	15 Dec. 2007	Y. Ono	T	VII	KC815649	KC815590
	IBAR-10037	Japan: Ibaraki	3 Jul. 2008	Y. Ono	T	VII	KC815650	KC815591
	IBAR-10038	Japan: Ibaraki	3 Jul. 2008	Y. Ono	T	VII	KC815651	KC815592
	IBAR-10057[d]	Japan: Ibaraki	21 Mar. 2009	Y. Ono and S. Pota	T	VII	KC815652	KC815593

Table 3.1. Continued

Rust/host	Herbarium accession no.	Specimen data			Stage[a]	Group	GenBank accession no.[b]	
		Location	Date	Collector			D1/D2	5.8+ITS2
Japanese *P. meliosmae* on *M. myriantha*	IBAR-10058[d]	Japan: Ibaraki	21 Mar. 2009	Y. Ono and S. Pota	T	VII	KC815653	KC815594
	IBAR-10059[d]	Japan: Ibaraki	21 Mar. 2009	Y. Ono and S. Pota	T	VII	KC815654	KC815595
	IBAR-10060[d]	Japan: Ibaraki	21 Mar. 2009	Y. Ono and S. Pota	T	VII	KC815655	KC815596
Japanese *P. meliosmae* on *M. tenuis*	IBAR-9942	Japan: Tochigi	16 Nov. 2007	Y. Ono	T	VI	KC815656	KC815597
	IBAR-10080	Japan: Nagano	11 Jul. 2009	S. Pota	A,U	VI	KC815657	KC815598

[a]Letters, S, A, U and T denote spermogonial, aecial, uredinial and telial stages in the life cycle, respectively.

[b]GenBank accession numbers with an "AB" acronym are cited from Chatasiri and Ono (2008).

[c]Morphology not examined.

[d]Specimens used as inocula in inoculation experiments.

Table 3.2. GenBank accession numbers and references of taxa used in this study.

Species/host	Herbarium accession no.	GenBank accession no.		Reference
		D1/D2	5.8+ITS2	
Phakopsora meliosmae				
on *Meliosma myriantha*	IBAR-7582	AB354759	AB354798	Chatasiri and Ono, 2008
	IBAR-8085	AB354760	AB354799	Chatasiri and Ono, 2008
on *M. tenuis*	IBAR-7612	AB354758	AB354800	Chatasiri and Ono, 2008
P. vitis				
on *M. myriantha*	IBAR-7143	AB354761	AB354802	Chatasiri and Ono, 2008
	IBAR-8047	AB354762	AB354804	Chatasiri and Ono, 2008
	IBAR-8105	AB354763	AB354805	Chatasiri and Ono, 2008
on *Parthenocissus tricuspidata*	IBAR-7682	AB354764	AB354806	Chatasiri and Ono, 2008
	IBAR-8107	AB354765	AB354807	Chatasiri and Ono, 2008
	IBAR-8732	AB354766	AB354808	Chatasiri and Ono, 2008
P. ampelopsidis				
on *Ampelopsidis brevipedunculata*	IBAR-8597	AB354738	AB354771	Chatasiri and Ono, 2008
	IBAR-7988	AB354739	AB354772	Chatasiri and Ono, 2008
	IBAR-8613	AB354740	AB354773	Chatasiri and Ono, 2008
	IBAR-8618	AB354741	AB354774	Chatasiri and Ono, 2008
	IBAR-8633	AB354742	AB354775	Chatasiri and Ono, 2008
	IBAR-8419	AB354743	AB354777	Chatasiri and Ono, 2008
P. meliosmae-myrianthae				
on *M. myriantha*	IBAR-8084	AB354744	AB354779	Chatasiri and Ono, 2008
	IBAR-8695	AB354745	AB354780	Chatasiri and Ono, 2008
	IBAR-9679	AB354746	AB354781	Chatasiri and Ono, 2008
	IBAR-9680	AB354747	AB354782	Chatasiri and Ono, 2008
P. pachyrhizi on *Glycine max*		DQ354537		Aime, 2006
P. pachyrhizi on *Glycine max*			AF333488	Frederick et al., 2002

Table 3.3. Results of inoculation of *Meliosma tenuis* with basidiospores from teliospores on *Vitis coignetiae.*

Voucher for inoculum from *V. coignetiae*	Origin of inoculum	Date of inoculation	Date of first appearance of spemogonia	Date of first appearance of aecia	Voucher
IBAR10243	Yamanashi, Koshi	2 Apr. 2010	13 Apr. 2010	10 Apr. 2010	IBAR10280
		11 Apr. 2010	21 Apr. 2010	29 Apr. 2010	IBAR10283
		10 May. 2010	24 May. 2010	10 Jun. 2010	IBAR10286
		24 May. 2010	3 Jun. 2010	12 Jun. 2010	IBAR10285
IBAR10249	Nagano, Ina	9 Jun. 2010	17 Jun. 2010	27 Jun. 2010	IBAR10288
		2 Aug. 2010	6 Aug. 2010	27 Aug. 2010	IBAR10295

Table 3.4. Results of *Vitis* species with aeciospores obtained from the inoculation experiments with basidiospores.

Voucher of inoculum on *Meliosma tenuis*	Date of inoculation	Inoculated plants Date of first appearance of uredinial (voucher)[b]				
		V.vinifera × *V. labrusca* (Kyoho)	*Vitis coignetiae*	*Vitis ficifolia*	*Ampelopsidis brevipedunculata*	*Parthenocissus tricuspidata*
IBAR10288	25 Jul. 2010	5 Aug. 2017 (IBAR10289)	-[a]	-	×[c]	×
IBAR10285	3 Aug. 2010	15 Aug. 2010 (IBAR10291)	-	-	×	×
	2 Sep. 2010	11 Sep. 2010 (IBAR10297)	-	-	-	-
IBAR10286	17 Aug. 2010	-	-	1 Sep. 2010 (IBAR10298)	×	×

[a] Not inoculate.

[b] Successfully infected.

[c] Unsuccessfully infected.

Table 3.5. Morphological characteristics of spermogonial and aecial stage of *Phakopsora* species on *Vitis coignetiae.*

Voucher	Spermogonium		Aeciospore			Peridial cell surface	
	Size width × height (µm)	**Type**	**Size width × length (µm)**	**Wall thickness (µm)**	**Surface**	Inner	Outer
IBA3600	97.9-143.1 × 62.2-119.6	type 7	14.3-18.1 × 16.6-20.7	0.9-1.5	verrucose with flat-headed	verrucose	smooth
IBA7591	103.1-135.3 × 57.4-111.4	type 7	14.1-18.3 × 15.6-20.2	0.6-1.5	verrucose with flat-headed	verrucose	smooth
IBA7790	100.9-144.4 × 76.6-117.9	type 7	13.6-16.8 × 15.3-19.4	0.6-1.5	verrucose with flat-headed	verrucose	smooth
IBA7791	86.6-124.9 × 80.5-128.8	type 7	15.3-18.1 × 16.4-20.2	0.9-1.5	verrucose with flat-headed	verrucose	smooth
IBA8556	89.2-128.3 × 57.0-94.0	type 7	14.5-17.7 × 15.6-19.6	0.9-1.5	verrucose with flat-headed	verrucose	smooth
IBA9545	84.8-119.6 × 62.2-129.2	type 7	13.2-17.9 × 15.1-19.2	0.9-1.7	verrucose with flat-headed	verrucose	smooth
IBA9547	69.2-115.7 × 51.3-78.3	type 7	13.9-16.6 × 15.6-19.8	0.9-1.5	verrucose with flat-headed	verrucose	smooth
IBA9682	76.1-120.5 × 63.9-100.5	type 7	13.0-16.6 × 14.9-18.7	0.9-1.5	verrucose with flat-headed	verrucose	smooth
IBA9861	94.8-138.3 × 56.1-112.2	type 7	13.2-16.2 × 13.9-17.5	0.9-1.5	verrucose with flat-headed	verrucose	smooth
IBA10082	97.4-157.9 × 74.0-125.3	type 7	12.8-16.2 × 14.7-19.0	0.6-1.5	verrucose with flat-headed	verrucose	smooth
IBA10085	96.6-149.6 × 70.0-104.4	type 7	13.9-16.6 × 14.7-18.3	0.9-1.5	verrucose with flat-headed	verrucose	smooth
IBA10089	103.1-161.4 × 62.6-87.9	type 7	12.8-15.8 × 14.5-19.2	0.9-1.3	verrucose with flat-headed	verrucose	smooth

Table 3.6. Morphological characteristics of uredial stage of *Phakopsora* species on *Vitis* species.

Rust/host	Voucher	Urediniospore			Paraphysis			Shape
		Size width × length (µm)	Wall thickness (µm)	Germ pores	Size width × height (µm)	Dorsal wall thickness (µm)	Apical wall thickness (µm)	
Japanese GLR fungus, *Phakopsora euvitis* on *Vitis coignetiae*	IBAR-1817	13.0-15.6 × 19.2-23.6	0.9-1.5	scatter, 6/ equatorial, 4	6.6-12.1 × 24.5-43.7	1.7-3.4	0.9-2.3	weakly, moderately incurved
	IBAR-2525	15.6-20.7 × 19.4-26.6	0.9-1.5	scatter, 6	6.0-10.7 × 20.2-32.6	1.5-3.8	1.1-5.8	strongly incurved, dorsally thick-walled
	IBAR-2594	15.6-19.6 × 17.5-25.4	0.9-1.5	scatter, 6	6.0-15.8 × 30.3-50.1	0.9-2.8	0.9-2.3	weakly incurved
	IBAR-2782	16.4-19.8 × 19.8-25.1	0.9-1.5	scatter, 6	6.6-17.0 × 44.5-78.6	0.9-3.0	0.9-2.1	long, cylindrical, weakly incurved
	IBAR-2783	15.6-20.7 × 17.7-24.1	0.9-1.5	scatter, 6	6.6-16.6 × 24.1-56.7	0.9-2.3	1.1-2.1	weakly incurved
	IBAR-3602	16.0-19.4 × 17.5-24.9	0.9-1.7	scatter	5.8-10.9 × 22.4-49.6	1.1-3.8	1.1-2.1	weakly, moderately incurved
	IBAR-3603	14.7-18.7 × 15.6-21.5	0.9-1.5		5.3-12.8 × 27.3-49.6	1.1-3.6	1.1-2.6	weakly, moderately incurved
	IBAR-3607	17.9-22.6 × 19.8-26.0	0.9-1.5		6.2-12.4 × 26.4-54.5	0.9-1.9	1.1-1.9	weakly, moderately incurved
	IBAR-3608	16.6-22.6 × 20.2-28.3	0.9-1.5		7.0-20.2 × 28.5-63.5	0.9-2.1	0.9-1.9	weakly, moderately incurved
	IBAR-3614	14.3-20.9 × 19.0-25.8	0.9-1.3	scatter	6.8-12.8 × 34.3-65.8	1.3-3.6	1.3-2.1	weakly, moderately incurved
	IBAR-3615	12.4-18.1 × 16.4-23.6	0.6-1.5	scatter, 6	8.3-16.0 × 35.6-69.0	1.1-3.0	1.3-2.1	weakly, moderately incurved
	IBAR-3620	13.9-19.0 × 16.8-24.7	0.9-1.5					weakly, moderately incurved
	IBAR-3622	16.0-19.6 × 19.2-25.1	0.9-1.7		10.9-19.0 × 37.1-75.0	0.9-2.3	1.1-2.3	weakly, moderately incurved
	IBAR-3726	14.9-18.3 × 19.4-25.1	0.9-1.5		5.1-13.0 × 19.8-56.0	1.5-3.6	0.9-1.7	weakly, moderately incurved
	IBAR-3930	16.0-20.2 × 20.5-26.2	0.9-1.5	scatter, 6	7.7-16.0 × 35.4-61.6	1.1-3.0	0.9-2.1	weakly, moderately incurved

Table 3.6. continued

Rust/host	Voucher	Urediniospore			Paraphysis			Shape
		Size width × length (µm)	Wall thickness (µm)	Germ pores	Size width × height (µm)	Dorsal wall thickness (µm)	Apical wall thickness (µm)	
Japanese GLR fungus, *P. euvitis* on *V. coignetiae*	IBAR-4123	13.2-19.2×17.5-26.8	0.9-1.7	scatter, 6/ equatorial, 4	9.6-16.8 × 34.3-57.9	1.1-2.3	0.9-2.8	weakly, moderately incurved
	IBAR-6073	14.1-17.7 × 18.7-24.3	0.6-1.5	equatorial	6.8-15.1 × 32.0-61.3	1.1-3.0	0.9-1.9	weakly, moderately incurved
	IBAR-6081	12.8-18.5 × 18.0-22.0	0.7-1.7		6.2-11.8 × 24.1-39.7	2.0-4.9	0.7-2.5	
	IBAR-6218	14.1-17.7 × 18.7-24.3	0.6-1.5	equatorial	6.8-15.1 × 32.0-61.3	1.1-3.0	0.9-1.9	long, cylindrical, weakly, moderately incurved
	IBAR-6282	13.2-16.2 × 16.4-24.7	0.9-1.5	equatorial, 4/ scatter				moderately incurved, dorsally thick-walled
	IBAR−6375	12.3-16.4 ×17.5-23.0	0.6-1.5		7.6-12.5 × 26.4-46.6	2.1-4.9	1.2-4.4	
	IBAR−6714	12.9-17.9 × 15.9-25.5	0.8-1.6	scatter, 6/ equatorial, 4	7.6-11.4 × 27.1-46.8	1.4-4.0	0.8-2.0	
	IBAR−6724	12.6-16.2 × 17.1-24.3	0.8-1.3	scatter, 6/ equatorial, 4	7.1-11.4 × 24.9-44.9	1.6-4.0	0.8-2.1	
	IBAR-8584	12.8-20.2 × 18.5-23.4	0.9-1.3	scatter, 6/ equatorial, 4	7.5-14.5 × 31.7-59.6	0.6-2.6	1.1-1.7	weakly, moderately incurved
	IBAR-9546	16.2-19.4 × 18.5-23.4	1.1-1.5	scatter, 6/ equatorial, 4	5.5-10.9 × 34.3-77.1	1.1-2.8	1.1-2.1	cylindrical, weakly incurved
	IBAR-9605	11.5-15.1 × 16.2-24.3	0.7-2.0		7.6-16.4 × 27.8-60.3	1.4-3.2	1.0-2.1	
	IBAR-9668	12.4-15.1 × 17.3-23.0	0.9-1.5		6.4-14.3 × 21.1-49.8	1.1-2.6	0.9-2.6	moderately incurved, dorsally thick-walled
Japanese GLR fungus, *P. euvitis* on *V.flexuosa*	IBAR-4333	14.3-17.9 × 17.5-27.3	0.9-1.7	scatter	6.2-16.0 × 20.7-35.6	1.1-3.4	1.1-3.2	moderately incurved, dorsally thick-walled
	IBAR-6217	12.4-16.0 × 15.8-21.9	0.9-1.5	scatter, 6	7.7-15.1 × 24.3-38.3	2.1-5.1	1.3-3.4	strongly incurved, dorsally thick-walled
	IBAR-6729	13.0-16.0 × 17.0-22.6	0.9-1.5	equatorial, 4	7.5-15.1 × 33.0-52.6	1.9-4.1	0.9-2.1	moderately, strongly incurved, dorsally thick-walled
on *V. ficifolia*	IBAR-6246	13.0-16.8 × 15.3-22.2	0.9-1.5	scatter, 6/ equatorial,4	6.8-14.9 × 22.4-40.0	2.3-6.6	0.9-2.1	moderately incurved, dorsally thick-walled

Table 3.6. continued

Rust/host	Voucher	Urediniospore			Paraphysis			Shape
		Size width × length (µm)	Wall thickness (µm)	Germ pores	Size width × height (µm)	Dorsal wall thickness (µm)	Apical wall thickness (µm)	
Japanese GLR fungus, *P. euvitis*, on *V.vinifera* × *V. labrusca* (Kyoho)	IBAR-5989	13.4-18.1 × 19.6-25.2	0.8-1.4		7.2-13.0 × 31.7-66.2	1.1-3.1	0.6-2.2	
	IBAR-6711	12.1-18.0 × 16.4-23.6	0.6-1.7	scatter, 6	7.1-11.6 × 33.1-58.1	1.7-4.1	0.8-3.0	moderately incurved
	IBAR-6721	11.8-16.8 × 15.3-25.3	0.6-1.5	scatter, 6/ equatorial, 4	6.2-11.7 × 21.4-43.8	1.5-3.7	0.9-1.9	moderately incurved
	IBAR-7720	14.8-18.8 × 20.0-25.0	0.8-1.5	scatter, 6/ equatorial, 4	8.1-14.7 × 27.2-42.7	1.7-5.2	1.1-2.4	moderately incurved
	IBAR-7288	12.4-18.8 × 16.4-24.4	0.8-1.4	scatter, 6/ equatorial, 4	6.9-11.9 × 21.6-40.4	1.6-3.4	0.8-2.8	moderately incurved
	IBAR-8106	12.1-16.0 × 15.8-24.0	0.6-1.4	scatter, 6/ equatorial, 4	7.1-11.5 × 28.4-55.5	1.1-3.1	0.9-1.9	moderately incurved
	IBAR-10118	11.7-15.9 × 19.8-25.3	0.6-1.2	scatter, 6/ equatorial, 4	7.8-13.5 × 26.9-62.9	1.8-4.8	1.1-2.5	moderately incurved
	IBAR-10119	11.1-15.0 × 17.3-24.7	0.6-1.5		6.7-14.6 × 32.6-53.4	0.9-2.3	0.9-2.0	moderately incurved
	IBAR-10120	10.9-15.7 × 18.5-26.2	0.6-1.6	scatter, 6/ equatorial, 4	7.8-14.4× 30.7-50.9	1.9-5.4	0.9-3.1	weakly, moderately incurved
	IBAR-10121	12.1-16.0 × 17.5-23.9	0.7-1.5	scatter, 6/ equatorial, 4	7.9-13.7 × 26.3-56.0	1.7-5.5	0.7-2.2	weakly, moderately incurved
	IBAR-10122	12.1-14.9 × 19.2-24.7	0.7-1.3	equatorial, 4	5.5-11.9 × 23.8-44.5	1.8-4.3	0.7-2.0	weakly, moderately incurved
	IBAR-10123	11.7-15.3 × 19.5-26.0	0.7-1.6	equatorial,4	7.9-13.3 × 29.5-67.9	2.0-4.6	0.7-1.9	weakly, moderately incurved
Taiwanese GLR fungus, on *V. vinifera* cultivar	IBAR-10239	13.2-17.7 × 17.9-25.8	0.8-1.6	scatter, 6	6.8-12.6 × 23.3-49.4	1.4-4.9	0.6-1.7	weakly incurved
	IBAR-10240	14.2-17.6 × 18.3-24.3	0.7-1.4	Scatter,6	8.2-11.8 × 28.7-48.8	1.1-3.3	0.8-1.9	weakly incurved
Thai GLR fungus on *V. vinifera* cultivar	IBAR-10217	14.3-17.7 × 18.7-24.7	0.9-1.3	scatter, 6	6.6-15.1 × 25.6-60.7	0.9-2.3	1.1-2.3	weakly incurved
	IBAR-10218	16.8-21.7 × 19.4-27.1	0.9-1.5	scatter, 5-6	8.5-13.6 × 29.2-62.8	1.3-2.3	1.1-2.6	weakly incurved
	IBAR-10219	14.9-20.0 × 20.7-25.8	0.9-1.5	scatter, 6	7.2-15.1 × 29.4-50.7	1.1-1.9	0.9-2.1	weakly incurved

Table 3.6. continued

Rust/host	Voucher	Urediniospore			Paraphysis			Shape
		Size width ×length (µm)	Wall thickness (µm)	Germpores	Size width ×height (µm)	Dorsal wall thickness (µm)	Apical wall thickness (µm)	
Thai GLR fungus on *V. vinifera* cultivar	IBAR-10220	13.6-17.5 × 17.5-22.8	0.9-1.5	scatter, 6	7.0-19.4 × 31.1-58.2	0.4-2.1	1.1-2.6	weakly incurved
	IBAR-10221	14.1-17.5 × 19.2-24.7	0.9-1.3	scatter, 6/ equatorial, 4	8.1-17.5 × 28.8-57.5	0.9-1.9	1.1-2.3	weakly incurved
	IBAR-10222	14.7-19.0 × 18.7-26.2	0.9-1.7	scatter, 6	7.0-15.1 × 30.5-52.4	0.9-1.9	1.1-2.1	weakly incurved
	IBAR-10223	15.3-19.0 × 18.3-22.2	0.6-1.7	scatter, 6/ equatorial, 4				weakly incurved
	IBAR-10224	16.0-19.0 × 19.4-23.9	0.9-1.5	scatter, 6	7.7-15.1 × 23.9-46.2	0.9-2.1	0.9-1.9	weakly incurved
	IBAR-10225	14.9-18.1 × 19.2-24.1	0.9-2.1	scatter, 5-7	8.5-15.8 × 32.8-63.3	0.9-1.9	0.9-2.1	weakly incurved
	IBAR-10226	14.1-18.5 × 21.3-26.0	0.9-1.5	scatter, 6/ equatorial, 4	7.9-14.5 × 30.5-59.2	0.9-1.7	0.9-1.9	weakly incurved
	IBAR-10227	13.9-18.3 × 19.0-26.8	0.9-1.5	scatter, 6	7.7-16.6 × 30.5-51.6	1.1-1.7	1.1-2.1	weakly incurved
	IBAR-10228	16.0-18.7 × 19.0-25.6	0.9-1.5	scatter, 6	8.5-14.7 × 29.0-52.2	0.9-1.9	1.1-2.3	weakly incurved
	IBAR-10229	14.5-19.2 × 19.4-26.0	0.9-1.5	scatter, 6	6.0-15.8 × 31.7-52.2	0.9-1.7	1.1-2.1	weakly incurved
	IBAR-10230	15.6-19.6 × 18.3-23.2	0.6-1.5	scatter, 6	8.7-14.7 × 29.0-58.2	0.9-1.9	0.9-2.8	weakly incurved
	IBAR-10231	15.8-19.4 × 19.0-26.2	0.9-1.5	scatter, 6	6.6-14.1 × 28.1-57.9	0.9-1.7	1.1-1.7	weakly incurved
Indonesian GLR fungus on *V. vinifera* cultivar	IBAR-10041	13.9-18.3 × 16.2-20.9	0.9-1.5	scatter, 6	8.1-17.9 × 33.0-59.4	0.9-2.1	0.9-2.6	weakly incurved
	IBAR-10042	13.9-18.3 × 16.6-21.9	0.9-1.7	scatter, 6	7.5-21.1 × 32.6-56.9	0.9-2.3	1.3-2.3	weakly incurved
	IBAR-10043	13.9-19.0 × 15.6-21.3	0.9-1.5	scatter, 6	7.9-20.7 × 36.0-58.6	1.1-3.0	1.3-3.6	weakly incurved

Table 3.6. continued

Rust/host	Voucher	Urediniospore			Paraphysis			Shape
		Size width × length (µm)	Wall thickness (µm)	Germ pores	Size width × height (µm)	Dorsalwall thickness (µm)	Apical wall thickness (µm)	
Indonesian GLR fungus on *V. vinifera* cultivar	IBAR-10044	14.5-19.4 × 15.8-25.1	0.9-1.5	equatorial, 4/ scatter,6	7.2-16.2 × 35.8-66.2	1.1-2.1	0.9-2.1	weakly incurved
	IBAR-10250	18.1-24.1 × 15.1-18.7	0.9-1.5	scatter, 4-6	6.0-13.2 × 24.9-42.8	0.9-1.5	0.9-1.7	weakly incurved
East Timor GLR fungus on *V. vinifera* cultivar	IBAR-8807	14.9-20.2 × 17.0-21.9	0.9-1.5	scatter, 6	8.7-16.2 × 31.5-59.9	0.9-2.1	1.1-2.1	weakly incurved
	IBAR-8808	14.1-19.4 × 17.9-23.4	0.9-1.7	scatter	10.9-21.1 × 30.5-66.0	1.1-2.1	0.9-1.9	weakly incurved
	IBAR-8809	15.8-21.3 × 18.1-27.7	0.9-1.7	scatter, 6	8.7-15.3 × 32.0-55.4	0.9-1.9	0.9-2.3	weakly incurved
	IBAR-8810	14.5-18.1 × 16.2-22.2	0.9-1.5	equatorial, 4/ scatter?	8.3-20.0 × 32.8-56.8	1.1-2.1	1.1-2.1	weakly in curved
Australian GLR on *V. vinifera* cultivar	IBAR-8755	13.2-18.1 × 17.0-21.7	0.6-1.3	scatter, 6	8.1-18.5 × 30.0-57.5	0.9-2.1	1.1-1.9	weakly incurved
	IBAR-8756	16.4-19.8 × 18.3-23.9	0.9-1.7	scatter	9.4-17.7 × 32.2-55.8	1.1-2.8	1.1-1.9	weakly incurved
	IBAR-8757	15.8-21.7 × 19.0-25.6	0.9-1.7	scatter, 6	9.6-17.5 × 29.4-48.4	0.9-1.7	0.9-2.3	weakly incurved
	IBAR-8758	16.6-24.3 × 19.8-26.6	0.9-1.7	scatter, 6	11.7-24.1 × 40.5-62.2	0.9-2.1	1.1-1.9	weakly incurved
Japanese GLR fungus, *Phakopsora* fungus on *V. coignetiae*	IBAR-9548	12.6-18.1 × 14.5-20.9	0.6-1.3	scatter, 6/ equatorial, 4				
	IBAR-10124	16.4-19.4 × 19.0-24.3	0.9-1.5	scatter, 6	6.0-13.0 × 29.6-69.2	0.9-2.1	0.9-2.1	cylindrical, weakly incurved
	IBAR-10168	12.8-15.8 × 17.5-21.3	0.9-1.3	equatorial, 4/ scatter 6	7.7-14.5 × 17.3-39.0	1.5-4.9	1.1-2.1	moderately incurved, dorsally thick-walled
	IBAR-10301	14.3-18.1 × 16.2-21.5	0.9-1.5	scatter, 6	4.9-11.7 × 27.5-59.4	1.1-2.6	1.1-2.1	cylindrical, weakly incurved
	IBAR-10303	12.8-18.1 × 16.2-25.4	0.6-1.5	scatter, 6	6.4-11.7 × 24.3-54.1	0.9-1.7	1.1-1.9	cylindrical, weakly incurved
	IBAR-10309	15.1-18.5 × 16.8-23.2	0.9-1.5	scatter, 6	8.5-15.3 × 36.4-79.5	1.1-2.3	1.5-1.9	cylindrical, weakly incurved

Table 3.6. continued

Rust/host	Voucher	Urediniospore			Paraphysis			Shape
		Size width × length (µm)	**Wall thickness (µm)**	**Germpores**	**Size width × height (µm)**	**Dorsal wall thickness (µm)**	**Apical wall thickness (µm)**	
Japanese GLR fungus, *Phakopsora* fungus on *V. coignetiae*	IBAR-10312	18.1-23.6 × 20.5-27.3	0.9-1.5	scatter, 6/ equatorial, 4	5.5-11.5 × 33.4-63.3	1.1-2.3	0.9-2.1	cylindrical, weakly incurved
	IBAR-10314	13.4-17.0 × 16.2-22.8	0.6-1.5	scatter, 6	6.8-14.9 × 32.0-69.4	0.9-3.0	1.1-1.9	cylindrical, weakly incurved

Table 3.7. Morphological characteristics of telial stage of *Phakopsora* species on *vitis* species.

Rust/host	Voucher	Uppermost cell teliospore			Second and below cell teliospore		
		Size width × length (µm)	**Lateral wall thickness (µm)**	**Apical wall thickness (µm)**	**Size width × length (µm)**	**Lateral wall thickness (µm)**	**Apical wall thickness (µm)**
Japanese GLR fungus,	IBAR-1817	7.2-12.1 × 11.3-20.5	0.9-1.5	1.1-2.6	7.5-11.9 × 10.0-17.9	0.9-1.5	0.9-1.7
Phakopsoa euvitis	IBAR-2594	8.1-14.1 × 13.4-19.8	0.9-1.7	0.9-2.1	8.3-13.6 × 10.7-20.2	0.9-1.7	0.9-2.1
on *Vitis coignetiae*	IBAR-2783	6.8-14.7 × 9.6-21.7	0.9-1.9	0.9-2.3	7.5-14.5 × 10.4-19.0	0.9-1.9	0.9-2.1
	IBAR-3602	8.7-13.6 × 12.4-24.3	0.9-2.6	1.1-2.6	8.3-14.1 × 11.1-20.9	1.1-2.1	1.1-2.1
	IBAR-3603	7.9-14.1 × 12.6-20.0	0.9-2.1	1.3-2.3	6.8-16.0 × 9.4-20.7	1.1-2.3	1.1-2.1
	IBAR-3614	7.5-12.4 × 13.6-21.5	1.1-1.9	1.3-2.6	7.7-13.6 × 12.4-21.9	0.9-1.9	1.1-2.6
	IBAR-3620	9.0-13.9 × 10.7-21.3	1.1-1.9	0.9-2.1	8.3-14.1 × 11.1-18.7	1.1-1.9	0.9-2.6
	IBAR-3622	7.9-14.9 × 9.2-19.2	1.1-2.6	1.1-2.1	9.2-16.0 × 12.8-18.3	0.9-2.3	1.1-2.1
	IBAR-3930	8.5-14.7 × 12.8-20.2	0.9-1.9	1.1-2.3	6.6-13.4 × 12.8-21.1	0.9-1.7	0.9-1.7
	IBAR-4123	7.7-11.3 × 10.9-19.2	0.9-2.1	1.1-2.1	7.7-12.1 × 10.0-16.2	0.9-1.9	0.9-1.9
	IBAR-6279	8.1-12.6 × 10.7-18.3	1.1-1.9	1.1-2.6	7.5-11.7 × 9.8-19.0	0.9-1.9	0.9-1.9
	IBAR-6282	7.2-11.3 × 10.7-17.3	1.1-1.9	0.9-1.9	8.1-11.3 × 8.7-16.6	0.9-2.1	1.1-2.3
	IBAR-8584	8.1-13.2 × 10.9-19.0	0.9-1.7	0.9-2.3	8.7-13.6 × 10.7-19.2	0.9-1.7	0.9-2.1
	IBAR-9669	8.7-14.9 × 11.9-18.5	0.9-2.1	1.1-1.9	7.2-14.1 × 11.5-20.5	1.1-2.3	1.1-2.3
	IBAR-10176	7.0-11.5 × 9.6-17.5	1.1-2.1	1.1-2.1	6.0-11.3 × 10.4-17.0	0.9-1.5	0.9-1.7
Japanese GLR fungus,	IBAR-4328	6.6-9.8 × 8.5-19.2	0.9-1.9	0.9-2.3	6.0-12.4 × 8.5-15.8	0.9-1.7	0.9-2.1
P. euvitis	IBAR-4333	6.8-12.4 × 11.7-19.6	0.9-1.7	1.1-1.7	7.0-12.1 × 10.7-17.0	0.9-1.7	1.1-1.9
on *V. flexuosa*	IBAR-5996	7.0-10.9 × 7.9-16.2	1.1-1.7	1.3-2.3	7.5-13.2 × 9.2-14.3	0.9-1.7	1.1-2.1
	IBAR-7008	6.0-11.5 × 9.6-15.1	0.9-1.7	1.1-2.1	5.5-11.3 × 8.1-15.3	0.9-1.5	0.9-1.9
on *V. ficifolia*	IBAR-6246	6.0-10.7 × 9.2-17.9	1.1-1.7	0.9-1.9	6.4-10.7 × 9.6-16.0	0.9-1.9	1.1-2.1

Table 3.7. continued

Rust/host	Voucher	Uppermost cell teliospore			Second and below cell teliospore		
		size width × length (µm)	**Lateral wall thickness (µm)**	**Apical wall thickness (µm)**	**Size width × length (µm)**	**Lateral wall thickness (µm)**	**Apical wall thickness (µm)**
Japanese GLR fungus,	IBAR-5989	7.1-12.2 × 11.4-18.9	0.9-2.2	1.0-3.1	8.0-12.7 × 11.3-18.2	0.9-2.1	1.2-2.1
P. euvitis on	IBAR-8036	8.1-13.1 × 11.6-20.0	1.0-2.7	1.0-2.2	7.4-12.4 × 9.8-19.2×	0.9-1.8	0.8-1.6
V. vinifera × *V. labrusca*	IBAR-10118	6.5-12.5 × 12.4-21.7	0.5-.7	1.0-2.2	6.9-12.1 × 10.2-19.2	0.7-1.5	0.6-12.1
(Kyoho)	IBAR-10119	6.6-11.5 × 12.5-19.6	0.8-2.0	1.2-2.4	7.5-12.9 × 10.7-19.8	0.8-1.6	0.9-1.9
	IBAR-10120	6.1-11.8 × 12.4-23.0	0.9-1.9	1.0-2.7	8.0-11.6 × 11.3-20.4	0.8-1.9	1.0-1.6
	IBAR-10121	6.6-11.9 × 12.2-20.1	1.1-2.4	1.3-2.7	7.5-11.2 × 10.0-17.7	0.9-2.2	0.9-2.2
	IBAR-10122	8.2-12.0 × 12.1-20.1	0.6-2.2	1.0-2.3	7.1-12.3 × 10.1-17.2	0.9-1.9	0.9-2.3
	IBAR-10123	6.9-11.8 × 14.3-21.8	0.9-1.8	1.0-3.1	6.9-12.1 × 12.9-19.7	0.5-1.8	0.9-1.7
Taiwanese GLR fungus,	IBAR-10239	8.1-13.1 × 13.0-19.3	0.9-2.1	1.1-2.4	7.4-13.1 × 10.8-19.1	0.8-1.8	1.0-1.9
on *V. vinifera*	IBAR-10240	7.4-11.7 × 10.5-16.5	0.8-1.7	0.8-1.8	7.7-12.2 × 11.2-19.2	0.7-1.6	0.8-1.6
Thai GLR fungus on	IBAR-10217	7.2-15.8 × 11.3-18.5	1.1-2.6	0.9-2.1	6.2-14.3 × 11.1-21.3	0.9-2.3	1.1-2.1
V. vinifera cultivar	IBAR-10219	6.6-14.5 × 10.4-18.3	1.1-2.1	0.9-2.1	7.2-13.2 × 9.4-16.0	0.9-1.9	0.9-2.1
	IBAR-10220	7.9-11.9 × 11.3-20.5	1.1-1.7	1.1-2.1	7.2-13.6 × 9.2-17.9	0.9-1.9	0.9-1.7
	IBAR-10226	7.7-11.9 × 10.4-22.2	0.9-2.1	1.1-2.1	7.9-12.4 × 9.4-17.9	0.9-2.1	0.9-1.7
	IBAR-10227	5.8-11.5 × 11.3-19.4	0.9-2.1	0.9-2.3	6.2-10.7 × 10.0-17.0	1.1-1.7	1.1-1.9
	IBAR-10228	7.9-12.1 × 11.3-20.7	0.6-2.1	0.9-2.1	7.5-11.9 × 11.3-22.8	0.9-1.9	1.1-2.1
	IBAR-10230	7.7-14.5 × 8.5-18.7	0.9-1.7	1.1-2.3	6.2-13.0 × 10.0-17.0	1.1-1.9	1.1-2.1
Japanese GLR fungus,	IBAR-1872	7.0-14.9 × 13.2-21.5	0.9-1.9	1.3-2.3	7.9-13.9 × 10.2-22.6	0.9-1.7	0.9-1.9
Phakopsora fungus on	IBAR-10052	8.1-14.5 × 10.7-19.0	0.9-2.3	1.3-2.1	8.1-13.6 × 9.6-19.8	1.1-1.7	0.9-2.1
V. coignetiae	IBAR-10124	8.3-14.7 × 14.3-21.3	0.9-2.1	1.3-2.1	7.5-14.1 × 12.1-23.6	0.9-1.9	0.9-2.1
	IBAR-10243	8.3-14.9 × 12.4-22.2	0.6-2.1	1.1-2.1	7.5-14.5 × 12.4-21.7	1.1-1.9	1.1-2.1

Table 3.7. continued

Rust/host	Voucher	Uppermost cell teliospore			Second and below cell teliospore		
		Size width × length (µm)	**Lateral wall thickness (µm)**	**Apical wall thickness (µm)**	**Size width × length (µm)**	**Lateral wall thickness (µm)**	**Apical wall thickness (µm)**
Japanese GLR fungus	IBAR-10249	8.1-14.7×12.1-22.6	1.1-2.1	1.1-2.3	9.0-14.5 × 13.0-21.9	1.1-1.9	1.1-2.3
Phakopsora fungus	IBAR-10252	8.3-13.0 × 13.2-19.4	0.9-1.9	1.1-2.3	7.7-12.8 × 11.5-21.5	0.9-1.9	0.9-1.9
on *V. coignetiae*	IBAR-10301	8.3-15.1 × 11.7-23.6	1.1-1.9	1.1-2.3	7.9-16.4 × 10.0-19.4	1.1-2.1	0.9-2.8
	IBAR-10303	9.4-17.0 × 10.2-19.2	0.9-1.7	1.1-1.9	8.7-14.1 × 11.3-20.0	0.9-1.9	1.1-1.9
	IBAR-10309	8.7-15.8 × 14.1-21.5	0.9-1.9	1.1-2.6	9.0-14.9 × 13.6-23.4	1.1-2.3	1.1-2.3
	IBAR-10314	8.5-15.1×11.7-21.7	0.9-1.7	1.1-2.1	8.1-16.4 × 10.7-23.2	0.6-2.1	0.9-1.7
	IBAR-10355	6.1-11.4 × 12.1-19.1	0.8-2.2	0.8-1.8	11.1-15.8 × 7.5-12.0	0.8-1.9	0.9-2.2

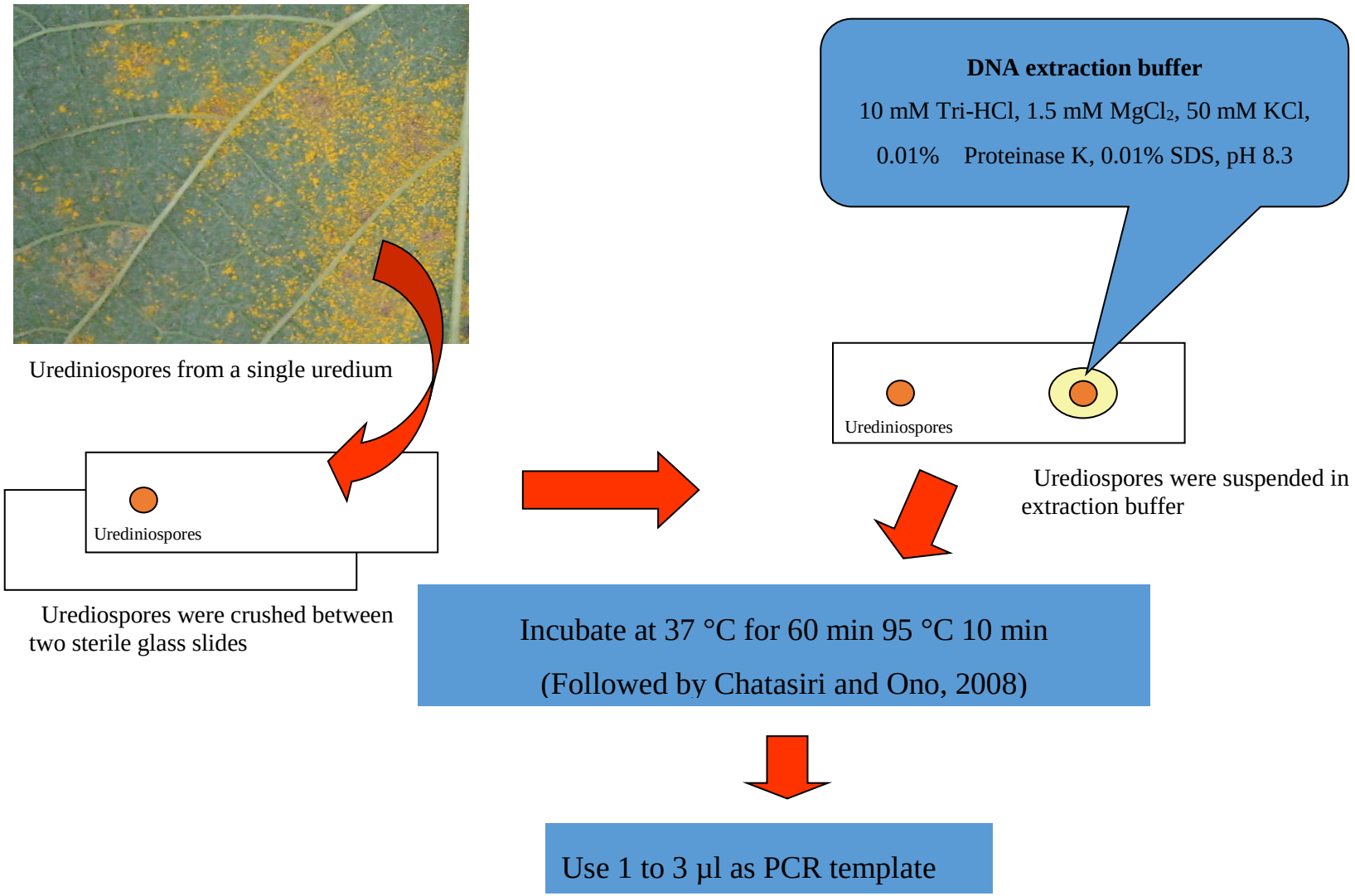
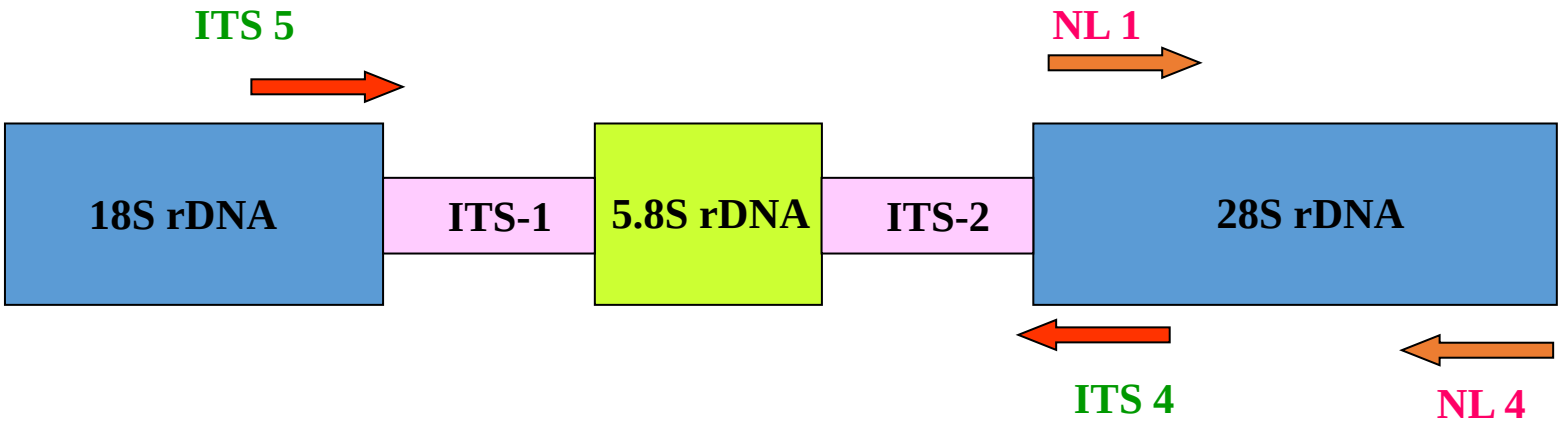


Fig. 3.1. DNA extraction method.



D1/D2 regions:

NL1 : 5’- GCATATCAATAAGCGGAGGAAAAG -3’

NL4 : 5’- GGTCCGTGTTTCAAGACGG -3’

(O’Donnell 1993)

ITS regions:

ITS5 : 5’- GGAAGTAAAAGTCGTAACAAGG- 3’

ITS4 : 5’ –TCCTCCGCTTATTGATATGC- 3’

(White et al. 1991)

Fig. 3.2. Diagram of a portion of the rDNA unit repeat of 28S (D1/D2) and IIS 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 ITS5 and ITS4.

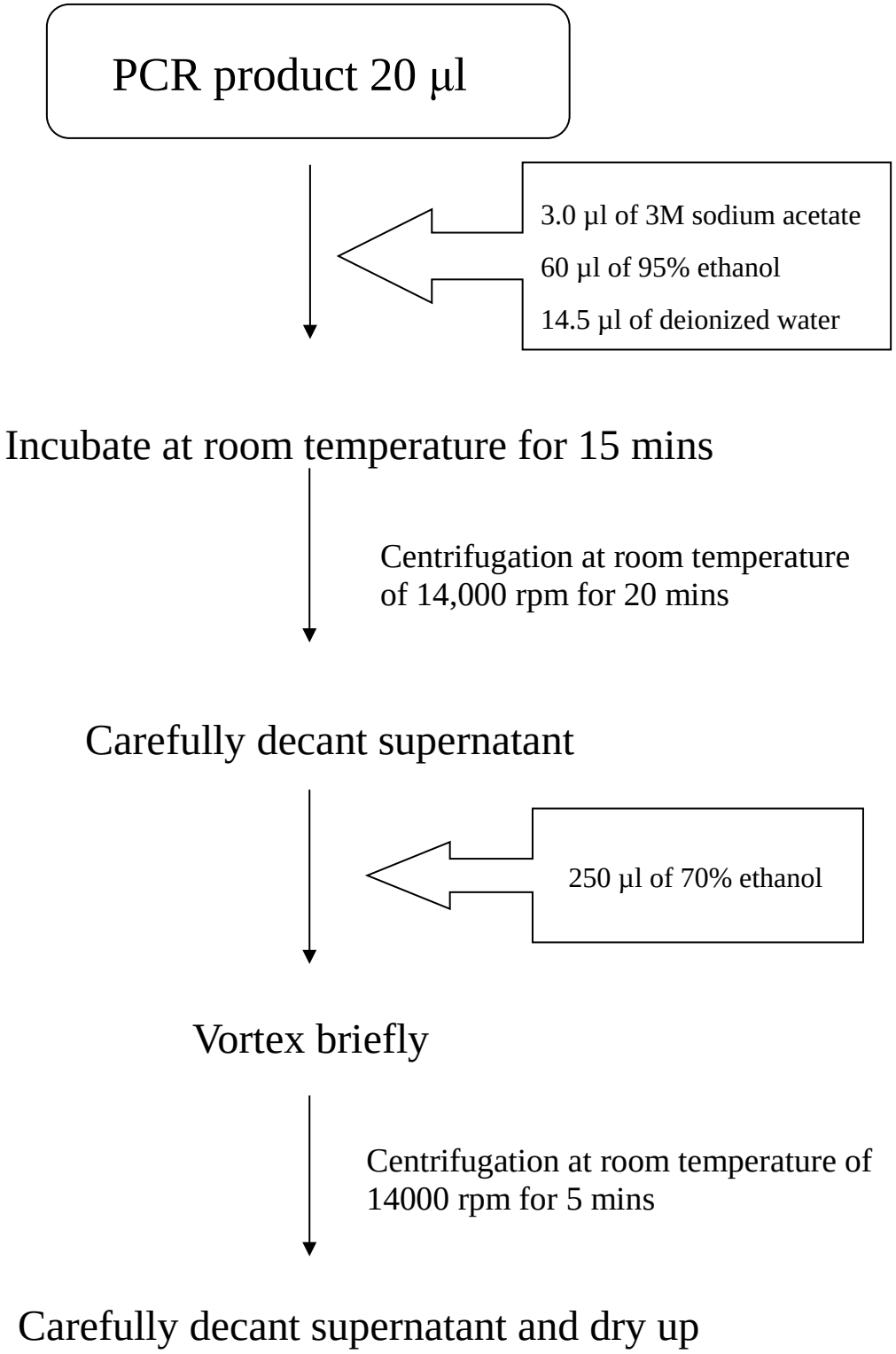


Fig. 3.3. The procedure of ethanol precipitation.

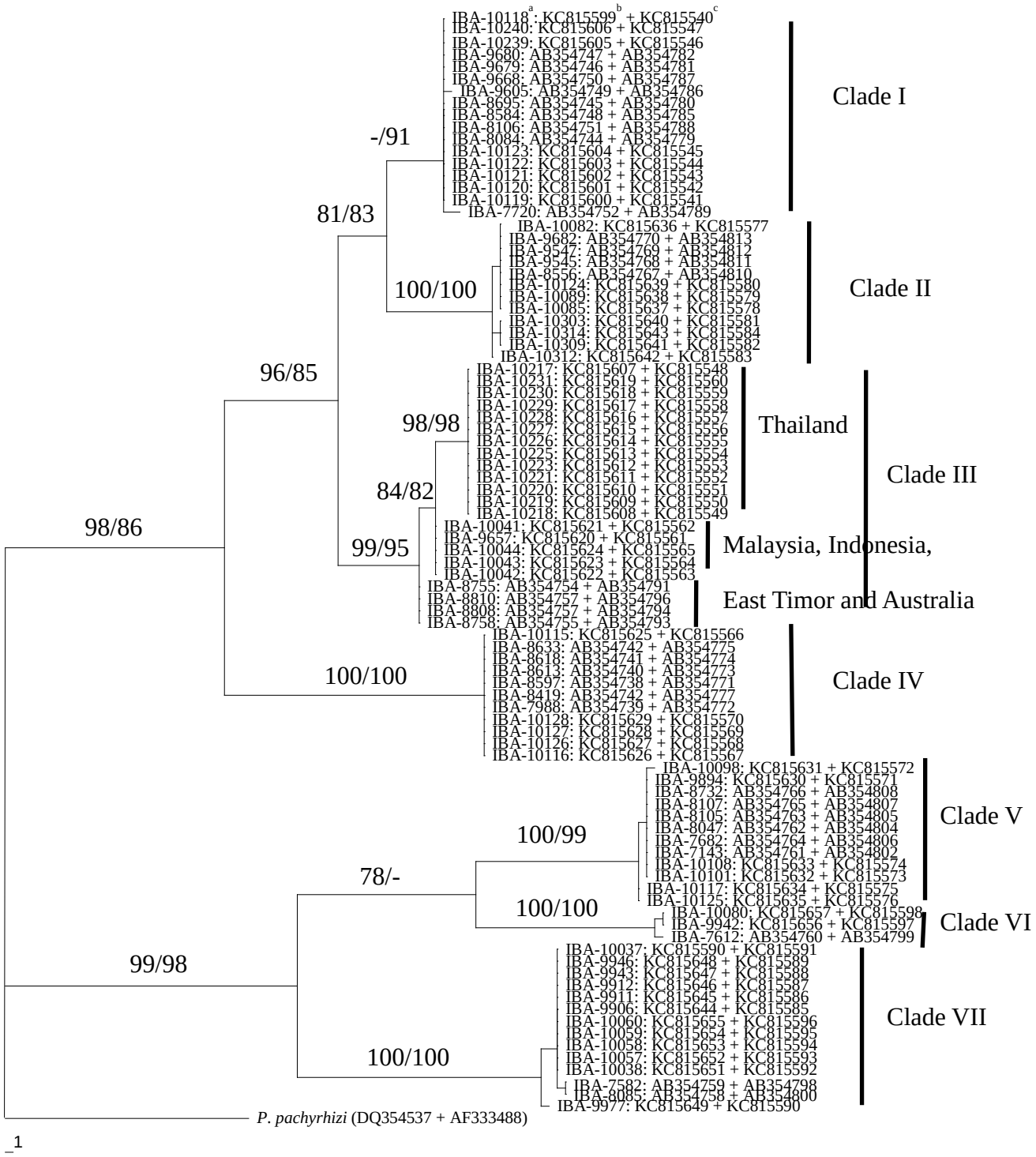


Fig. 3.4. One of ten most parsimonious trees of *P. meliosmae-myrianthae* complex from parsimony analysis of a combined dataset of D1/D2 and ITS2 regions of 91 taxa. Bootstrap values MP and ML above 70% from 1000 replicates are indicate for corresponding branches. The most parsimonious trees have a consistency index (CI) of 0.810, retention index (RI) of 0.984, rescaled consistency index (RC) of 0.798 and a tree length of 290.

Fig. 3.5. Symptom and sori of *Phakopsora montana* on *Meliosma tenuis*. a: Symptom after inoculation with basidiospores. b: Spermogonia. c: Aecia on abaxial leaf surface.

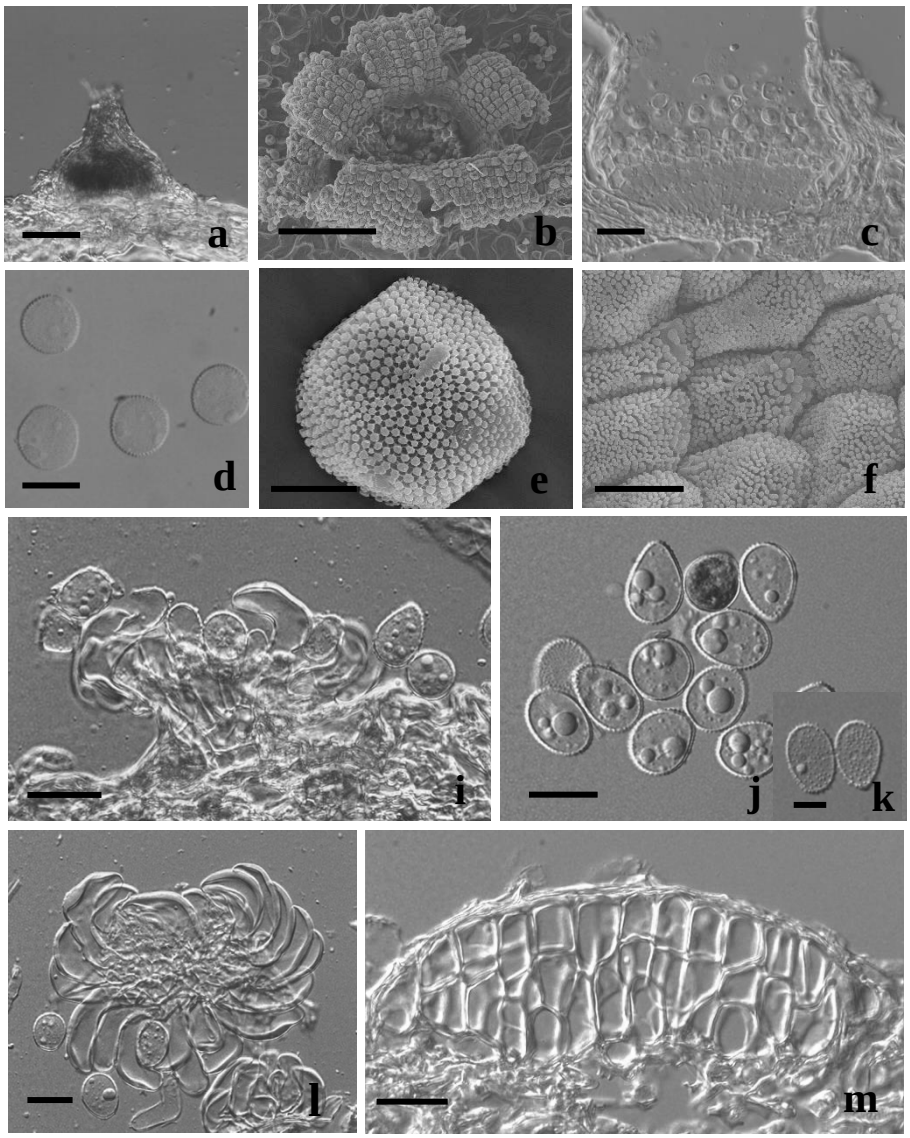


Fig. 3.6. Morphological features of the *Phakopsora montana* in Japan. a: Spermogonium (IBAR-9682). b: An overview of Aecidium (IBAR-10089). c: A vertical section of an aecium (IBAR-9547). d: Aeciospores (IBAR-8556). e: Nail-head verrucae on aeciospore wall surface (IBAR-9547). f: Verrucose surface of peridial cells (IBAR-8556). i: A vertical section of a uredium (IBAR-10168). j: Urediniospores (IBAR-10309). k: Urediniospore germ pores (IBAR-10168). l: Uredinial paraphyses (IBAR-10168). m: Teliospores. *Scale bars* a, c, 50 μm, b 120 μm, d, i-m 20 μm, e 5 μm, f 10 μm.

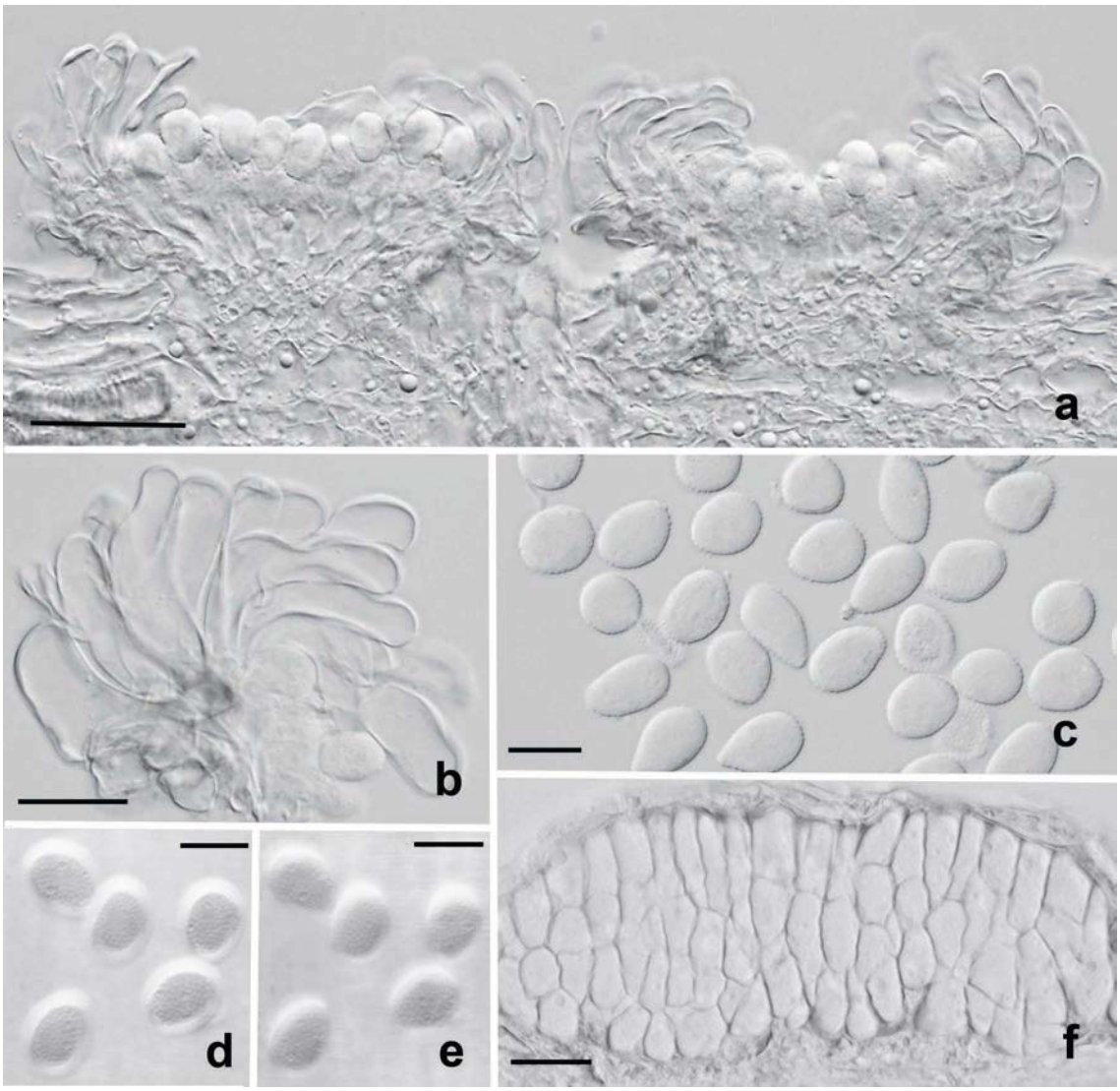


Fig 3.7. Uredinial and telial morphology of Southeast Asian grapevine leaf rust fungus. a: Peripherally paraphysateuredinia formed on the abaxial leaf surface. b: Basally united, thin-walledparaphyses. c: Urediniospores. d: Urediniospore germ poresappearing as small hollows, focused on the upper surface. e: the same spores as “d” focused on the lower surface. f: A telium with more or less linearly arranged teliospores formed beneath the host epidermis. a–c & f from IBAR10226; d & e from IBAR4073. Scale bar = 50 μm in a, 20 μm in b–f. (Pota et al. 2015).

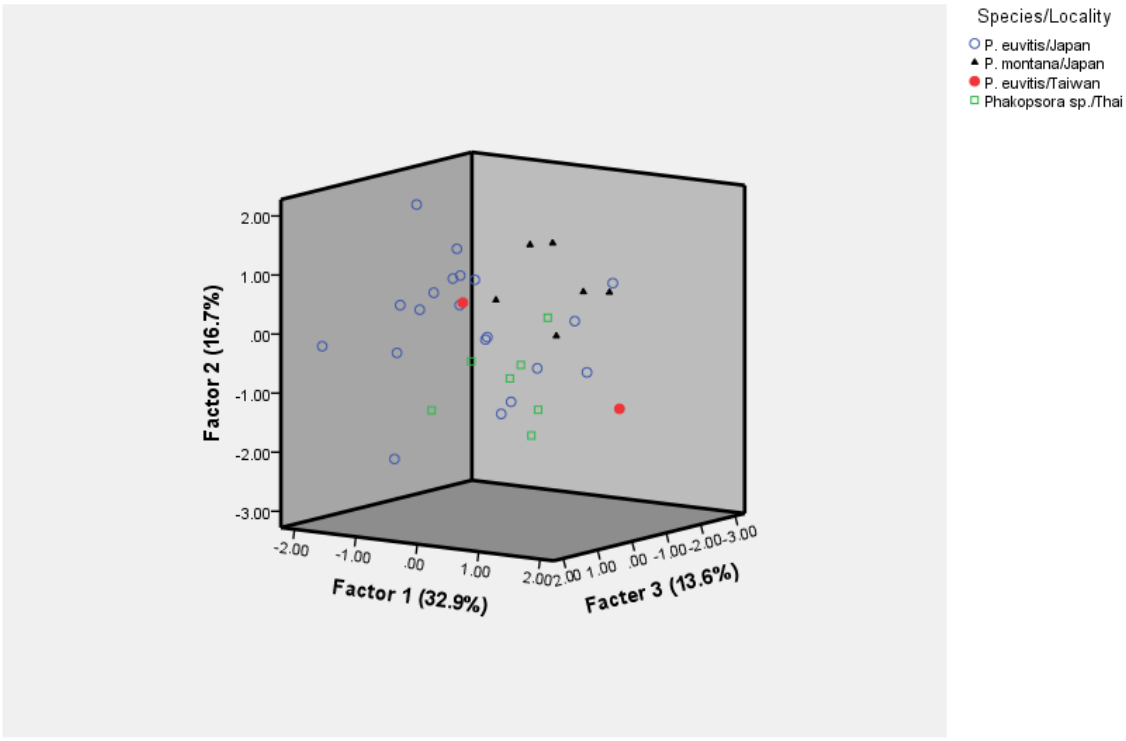


Fig. 3.8. A three dimensional scatter diagram generated by the principal component analysis based on non-standardized data of morphological characteristic of urediniospores and teliospores. : (○) specimens of *Phakopsora euvitis* in Japan (clade I), (▲) specimens of *P. montana* in Japan (clade II), (●) specimens of Taiwan grapevine leaf rust (clade II), (▭) specimens of Thai grapevine leaf rust (clade III).

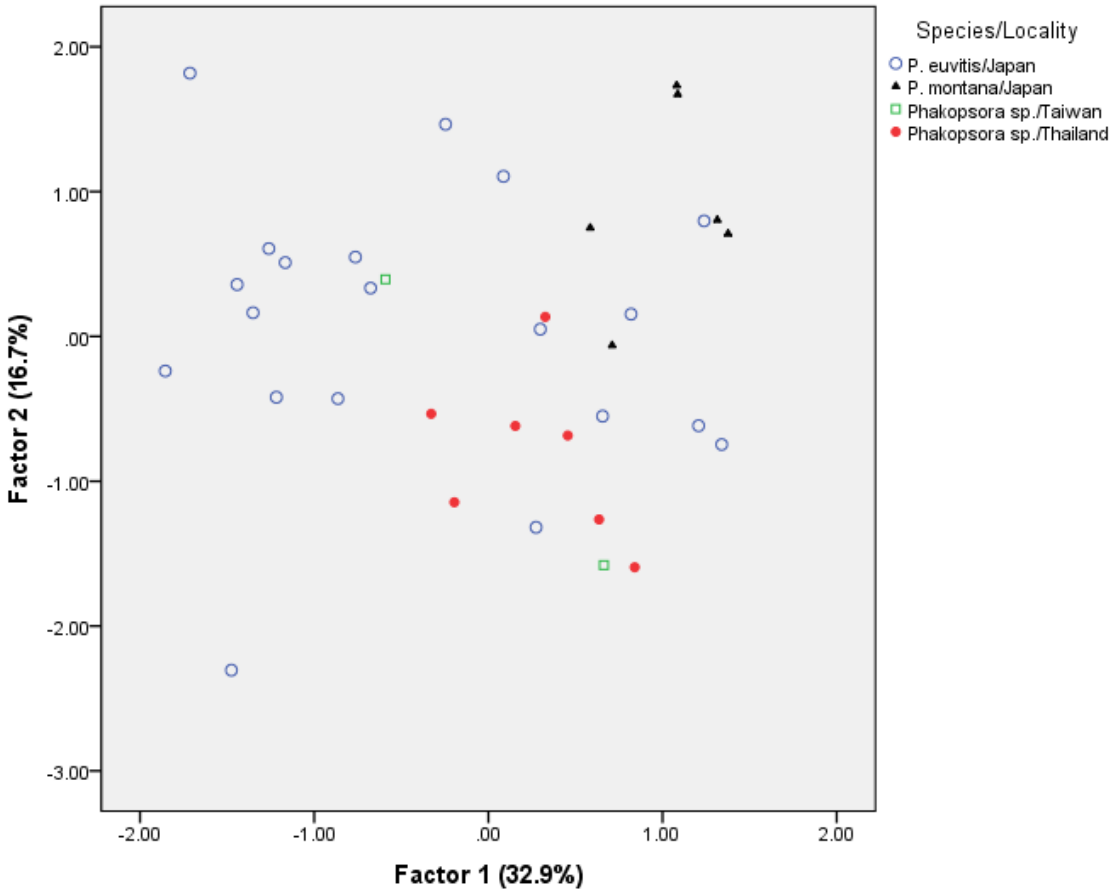


Fig. 3.9. A two dimensional scatter diagram generated by the principal component analysis based on non-standardized data of morphological characteristic of urediniospores and teliospores. : (○) specimens of *Phakopsora euvitis* in Japan (clade I), (▲) specimens of *P. montana* in Japan (clade II), (●) specimens of Taiwan grapevine leaf rust (clade II), (▭) specimens of Thai grapevine leaf rust (clade III).

Chapter 4. General discussion

4.1. Taxonomic conclusion of *Phakopsora* species on *Meliosma* and *Vitis* Species

In this study, morphology, host specificity, and molecular phylogenetic analyses were used to clarify the taxonomic status of *Phakopsora* species on *Meliosma* and *Vitis* species. In conclusion, four out of five populations, previously recognized as *P. euvitis* or *P. meliosmae*, detected by molecular phylogenic analyses were clearly distinguishable by life cycle, host specificity and morphology, particularly in aecial state. They were recognized as distinct species.

In morphological studies, a morphological difference in the aecial state of autoecious, *P. meliosmae* and *P. orientalis* was found in inner surface ornamentation of peridial cells, which is smooth in the former, while verrucose in the latter fungus. These morphological characteristics were considered as important taxonomic characteristics to recognize these two macrocyclic, autoecious *Phakopsora* species on *M. myriantha* and on *M. tenuis* populations (Pota et al. 2013). Similarly, heteroecious GLR fungi, *P. montana* and *P. meliosmae-myrianthae*, which are not separable in the uredinial and telial morphology, have distinct apical wall thickness of the aeciospore, which is thinner in *P. montana* versus thicker in *P. meliosmae-myrianthae* (Ono et al. 2012).

The morphologically similar fungal populations may be recognized as distinct and placed in different taxa (taxa being species, subspecies, varieties or formae speciales) if they exhibit distinct host specificity, depending on how well their biology is understood. Morphologically indistinguishable rust fungi populations may be recognized as distinct species if they have different life cycles (Ono et al. 2001).

The Ehrlich and Raven hypothesis suggests that parasites should often shift to closely related hosts (Ehrlich and Raven 1964). Roy et al. (1998) and Roy (2001), having worked with various geographic populations of three, apparently, closely related "life-cycle species," *Puc. monoica* Arthur, *Puc. consimilis* Ellis & Everhert and *Puc. thlaspeos* C. Schubert on crucifers, showed a complex phylogenetic pattern among populations and species with different life cycles. They proposed that the term "host shift" should be used only for events that are likely to be mediated by close host relationships and that the term "host jumps" should be used for associations with unrelated or distantly related hosts (Roy 2001). Because *M. myriantha* and *M. tenuis* belong to the same genus, *P. meliosmae* in present study showed the host shift pattern of host-pathogen association that the rust fungi on *M. myriantha* shift to new host (*M. tenuis*) that are ecogeographically available for new genetic variants. Similar to *P. meliosmae, Phakopsora montana* resembles *P. meliosme-myrianthae* in the life-cycle pattern (alternating between hosts *Meliosma* and *Vitis*) and morphology of all spore stages. Only the apical wall thickness of aeciospores was different as described above. However, *P. montana* has its spermogonial/aecial host specificity on *M. tenuis*. Because these two rust fungi are morphologically similar and form spermogonial/aecial stages on different host, but are closely related, *Meliosma* species and uredinial/aecial stages on the same grapevine species suggest that the two fungi have speciated by ecological means in the same habitat, a host shift in spermogonial/aecial stages followed by reproductive isolation (Ono et al. 2012).

Similar to the present study, the studies of the *Uromyces pisi* complex (Pfunder et al. 2001), the *Puc. andropogonis* complex, the *Puc. coronata* complex (Szabo 2006), and the *Puccinia* species parasitic on *Bellis* and *Senecio* (Weber et al. 2003) possessed

congruence between clades recognized by a molecular phylogeny and host/life cycle of specific rust populations (either species or cryptic species), which are documented. Similarly, in molecular phylogenetic studies of the *P. ampelopsidis* species complex and the *Puc. hemerocallidis* species complex, fungal samples (specimens) belong to the species recognized by the life cycle and host specificity constituted distinct clades in phylograms constructed from partial nucleotide sequences of SSU rDNA and LSU rDNA (Chatasiri et al. 2006; Chatasiri and Ono 2008). These life cycle and host specificity studies combined with molecular phylogenetic analyses indicated that the biological species of rust fungi recognized by the life cycle and host specificity may correspond to phylogenetic species recognized by a concordance of multiple gene genealogy (Avise and Ball 1900; Avise and Wollenberg 1997; Taylor et al. 2000; Dettman et al. 2003; Ono 2008).

The Southeast Asian and Australasian fungus is composed of three different subgroups in the D1/D2 and ITS2 sequence. The three subgroups corresponded with the three distribution ranges, i.e., Thailand, Malaysia-Indonesia and East Timor-Australia. The results of the studies on *P. montana* and *P. meliosmae-myrianthae* indicated importance of morphological differences of the aecial state. However, the spermogonial-aecial state of these populations was unknown. Thus, the Southeast Asian and Australasian GLR population was treated as *Phakopsora* species for the present, until the spermogonial-aecial state is found (Pota et al. 2014). The Southeast Asian and Australasian GLR population seemed to be a distinct species, but information on the spermogonial-aecial state of the population, which is expected to be produced on *Meliosma* species, is considered to be essential to conclude its taxonomic condition.

4.2. Relationships of unconnected *Aecidium* on *Meliosma* plants to *Phakopsora* fungi

When first confirming the autoecious, macrocyclic life cycle of *P. meliosmae* on *M. myriantha*, Kakishima et al. (1983) referred *A. meliosmae-pungentis* P. Hennings & Shirai as the aecial anamorph of *P. meliosmae* with no reference to the original material. This aecial fungus was reported to occur on *M. pungens* (Wight & Arn.) Walp. (*M. simplicifolia* (Roxb.) Walp. subsp. *pungens* (Wight & Arn.) Beusekom) and described as "…aecidiis petiolicolis vel foliicolis hypophyllis, effuses in villo nidulantibus, eos deformantibus curvulisque …" in Hennings (1900). The described nature of the aecial stage of *A. meliosmae-pungentis* is different from that described by Kakishima et al. (1983). *Aecidium meliosmae-pungentis* is likely to be an aecial state of an undescribed heteroecious *Phakopsora* species on vitaceous plants (Ono 2000).

Two or more *Aecidium* fungi on *Meliosma* species have been reported under the name of *A. meliosmae-myrianthae* P. Hennings & Shirai, e.g. on *M. cuneifolia* in China (Tai 1979); on *M. myriantha* in China (Spaulding 1961; Tai 1979; Teng 1996), Japan (Ito 1950) and Korea (Cho and Shin 2004); on *M. myriantha* var. *stewardii* (Merr.) Beusekom [= *M. stewardii* Merr.] in China (Tai 1979); on *M. parvifolia* Lecomte in China (Spaulding 1961; Tai 1979; Teng 1996); on *M. pinnata* subsp. *barbulata* var. *oldhamii* [= *M. oldhami*] in China (Spaulding 1961; Tai 1979), and Korea (Cho and Shin 2004); on *M. simplicifolia* (Roxb.) Walp. in India (Hosagoudar 1988); and on *M. tenuis* in Japan (Ito 1950). *Aecidium meliosmae-myrianthae* on *M. myriantha* in Japan was proven, by life cycle connection and morphology, to be the aecial anamorph of *P. euvitis* (Ono 2000). Another *Aecidium* fungus on *M. myriantha*,

for which no name has been given, was also found to be the aecial anamorph of *P. vitis* (Ono 2000).

Aecidium meliosmae Dietel can be an aecial anamorph of *P. meliosmae* on *M. myriantha*. However, Dietel's (1900) diagnosis indicates the presence of two aecial fungi on the type material on *M. myriantha*, i.e., one with loosely aggregate aecia on a diffused hypophyllous lesion and another with gregarious aecia on a small epiphyllous lesion. An aecial fungus specimen (IBAR-7798) on *M. myriantha* collected by S. Kusano bore no label; however, Kusano's collection number "90" is hand-written on the packet together with identification as "*Aecidium meliosmae-myrianthae* P. Hennings et Shirai." This collection number matches with Dietel's (1900) specimen citation in the protologue of *A. meliosmatis*. Specimen IBAR-7798 is, therefore, likely to be part of the specimen collected at Mt. Takao on 11 July 1899 by S. Kusano, which was designated as the holotype by Dietel (1900). However, the specimen apparently bears two kinds of aecial sori, i.e., *A. meliosmae-myrianthae* (now *P. meliosmae-mayrianthae*, Ono et al. 2012) and what we observed for *P. meliosmae*. *Aecidium meliosmae* Dietel is, therefore, treated as nomen ambiuum (Pota et al. 2013).

The following *Aecidium* fungi are known from only type material or a few additional collections with limited geographic distribution information and, therefore, no assumption for possible life cycle connection to their teleomorphic state is tenable. *Aecidium hornotinum* Cummins (1937) was originally described for a fungus on *M.* aff. *multiflora* Merr. in the Philippines. This fungus is characterized by fragile peridia and apically thick-walled aeciospores. Spermogonial infection on *M. pendula* Merr. was assumed to be caused also by this *Aecidium* fungus (Cummins 1937). An *Aecidium* fungus on *M. arnottiana* subsp. *oldhami* reported as *A. hornotinum* in the Ryukyus

(Hiratsuka and Shimabukuro 1955; Shimabukuro 1961) may need detailed examination for correct identification. *Aecidium painavuense* Hosagoudar (1987, 1988) was described for a fungus on *M. pinnata* subsp. *arnotiana* in Kerara, India. This fungus is characterized by systemic infection on shoots resulting in witches' broom. *Aecidium wareoense* Cummins (1941) was described for a fungus on *M. ferruginea* Blume in Papua, New Guinea and also reported on *M. fruticosa* Blume from Indonesia (Boedijn 1959). This fungus is characterized by apically thick-walled aeciospores like *A. hornotinum*, however, this fungus was separated from *A. hornotinum* by forming persistent peridia. Because the apically thickened aeciospore wall was not explicitly described for *A. meliosmae-myrianthae* (the anamorph of *P. euvitis*) by Hennings (1900), *A. hornotinum* was said to be "entirely different" from *A. meliosmae-myrianthae* (Cummins 1941). However, Ono (2015) studied the host specificity, life cycle and morphology of the rust on *M. arnottiana* subsp. *oldhamii* in Ryukyu Islands, Japan. He compared them with the type specimen of *A. hornotinum* on *M.* aff. *multiflora* in Philippines and concluded that the fungus on *M. arnottiana* subsp. *oldhamii* is taxonomically identical with *A. hornotinum* on *M.* aff. *multiflora.* Thus, *P. hornotina* (Cummins) Y. Ono has proposed a new holomorphic name for this fungus (Ono 2015).

4.3. Taxonomic conclusion used for agricultural

Currently, leaf rust is a minor fungal foliar disease of commercial table, wine and raisin grapes in temperate East Asia. It is important to realize, however, that the incidence and severity of major fungal foliar diseases of grapevines would change in the traditional viticulture regions under the global climate change (Hayman et al. 2009;

Fraga et al. 2012) and in newly developing tropical viticulture regions like Southeast Asia (Possingham 2008; Commins et al. 2012; Truong 2012; FAO database, http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E accessed on 20 May 2013). The leaf rust is likely to become a major disease of grapevines grown under the warm climate condition, particularly in Southeast Asia where commercial cultivars and their root-stocks are often severely infected by a GLR fungus (Pota et al. 2014).

Effective, integrated disease control of GLR disease requires understanding of the identity and biology of the causal agents. Thus, it is very important to have a good understanding of morphological features, life cycles, and host-rust relationships in the study of rust fungi as important plant pathogens or interesting biological agents. Knowing how many species are involved in plant diseases with similar symptoms is important for understanding disease etiology and developing practical controls (Ono 2000).

Research on taxonomy, classification, or identification is important for the agricultural sector. It is becoming even more important lately. Due to the Phytosanitary measures and Plant Health, the member countries that agreed on the import and export of agricultural products need to declare pests (weeds, insects, plant pathogens) that plague and quarantine pest in order to prevent those that are not present in the country by not allowing them to enter into the country. Therefore, it is essential to determine both the classification taxonomy and accurate diagnosis. The scholars at the Department of Plant Pathology under the Department of Agriculture perform surveillance on major pests in our households. Additionally, they track emerging and reemerging diseases to see if they have spread to new hosts. For example, the corn rust fungi, *P. polysora* (southern rust) and *P. sorghi* (common rust) have been reported on the Plant Pest list of

Thailand. Unartngam (2011) studied the genetic diversity of corn rust fungi in Thailand using inter simple sequence repeat (ISSR) markers. In this study, genetic diversity of southern rust from various locations of corn plantation in Thailand was investigated. Morphologically-based identification determined that all of the rust specimens were *P. polysora*. Moreover, *P. sorghi* (common rust) could be removed from the Plant Pest list of Thailand. It is a benefit to some countries that this fungus may be their quarantine pest. These countries will not import agriculture products from Thailand because they are afraid this fungus might also be imported to their country (Unarthgam 2016).

Results of the studies revealed that molecular phylogenic analysis is a useful tool for recognizing cryptic species in rust fungi, but the studies on their life cycle and morphology are also essential to define each species. Several species with different life cycles and host ranges are present on table grape cultivars and on *Meliosma* species. *Phakopsora meliosmae-myrianthe* (=*P. euvitis*) was believed to be a cause fungal of GLR in Asia (Ono 2000). The results of morphology, host specificity, and molecular phylogenetic analyses in the present study showed that at least three species have been reported on *Vitis* species: *P. meliosmae-myrianthae*, *P. montana* and Southeast Asian and Australasian fungus. *P. meliosmae-myrianthae* and *P. montana* are distinct in spermogonial/aecial host and aeciospore morphology. The former fungus had a host specificity on *M. myriantha* and on *M. tenuis* in the latter fungus. Similarly, the autoecious *P. meliosmae* and *P. orienthalis* also had a host specificity on *M. myriantha* and *M. tenuis*, respectively. Therefore, precise identification of GLR and information on their life cycle and host plants provided in this study will contribute to control of GLR. Removal of the alternate host disrupts the life cycle of the GLR fungi, preventing the formation of basidiospores, which infect the *Vitis* plants. A broad

understanding of the susceptibility of cultivated *Vitis* species and other species of Vitaceae to GLR and climatic conditions favorable for disease development need to be evaluated.

Chapter 5. Taxonomy

As a result of the present studies, autoecious macrocyclic species on *M. tenuis* in Japan was described as a new species, *Phakopsora orientalis*, and consequently, the description of *P. meliosmae* on *M. myriantha* in Japan was amended (Pota et al. 2013).

Phakopsora orientalis Chatasiri, Pota & Y. Ono, sp. nov. Figs 2.2. and 2.5.

MycoBank No.: MB 563058

Holotype: on *Meliosma tenuis* Maxim. JAPAN: Tochigi, Nikko, Yunishigawa, 20 Sep 2008, Y. Ono (IBAR-10051)

Etymology: Orientalis, from geographic distribution in eastern Asia

Spermogonia amphigenous, densely aggregate, subcuticular, conical with surrounding paraphyses, and 50–118 µm high and 45–121 µm wide. Aecia mostly epiphyllous, subepidermal in origin, surrounded by peridium, and becoming cupulate by apical rupture of the peridium; inner surface of peridial cells verrucose. Aeciospores catenulate, angularly subglobose to broadly ellipsoid, 18–34 × 14–23 µm; wall hyaline, 0.9–1.9 µm thick, evenly covered by nail-head verrucae. Uredinia hypophyllous, subepidermal in origin, becoming erumpent, and densely surrounded by basally united paraphyses at periphery; paraphysis irregularly cylindrical, moderately to strongly incurved, 21–44 × 8–19 µm; dorsal wall 2.1–8.7 µm thick, apical wall 1.9–7.9 µm thick. Urediniospores short-pedicellate, obovoid or ellipsoid, 20–33 × 13–20 µm; wall hyaline

or pale yellowish, 0.6–1.9 µm thick, evenly echinulate, with 2 to 4 germ pores in equatorial zone. Telia hypophyllous, crustose, subepidermal, with 3- or 7-layers of linearly-arranged spores. Teliospores at the uppermost layer ellipsoid to oblong, angular, 11–23 × 6–16 µm; apical wall 1.3–3.2 µm thick, light brown; lateral wall 0.6–1.9 µm thick, almost colorless. Teliospores at the second layer and below 8–22 × 6–17 µm thick; wall 0.6–1.9 µm thick, almost colorless. Basidiospores subglobose to broadly ellipsoid, 7.7–10.7 × 5.5–8.1 µm.

Phakopsora melisomae Kusano, Bot Mag (Tokyo) 18: 148. 1904. emend. S. Chatasiri, S. Pota & Y. Ono Figs. 2.2 and 2.4.

Spermogonia amphigenous, subcuticular, conical with surrounding paraphyses, 60-157 µm high, 68-158 µm wide. Aecia mostly epiphyllous, subepidermal in origin, surrounded by peridium, becoming cupulate by apical rupture of peridium; inner surface of peridial cells smooth. Aeciospores catenulate, subglobose to broadly ellipsoid, often angular, 17–37 × 14–26 µm, wall hyaline, 0.9–2.1 µm thick, evenly covered by nail-head verrucae. Uredinia subepidermal in origin, becoming erumpent, densely surrounded by basally united paraphyses; paraphysis hyaline to brown, moderately to strongly incurved, 22–60 µm high, 9–21 µm wide, wall hyaline to brown, 3.0–11.9 µm thick dorsally, 3.0–15.8 µm thick apically. Urediniospores short-pedicellate, obovoid to obovoid-ellipsoid, 18–31 ×12–21 µm wall 0.6–1.9 µm thick, hyaline or pale yellowish, evenly echinulate, with two to four germ pores distributed in equatorial zone. Telia hypophyllous, subepidermal with 2–7 layers of linearly arranged teliospores. Teliospores at the uppermost layer ellipsoid to oblong, angular, 9–25 × 6–16 µm, apical wall 0.9–4.1 µm thick, light brown, lateral wall 0.6–2.3 µm thick and almost hyaline;

teliospores at the second layer and below 8–25 × 6–17 µm, wall 0.6–2.3 µm thick, and almost hyaline. Basidiospores subglobose to broadly ellipsoid, 6.6–9.6 × 4.7–7.7 µm.

Specimen examined Holotype: on *M. myriantha* Sieb. & Zucc. JAPAN: Tokyo, Hachioji, Mt. Takao-san, 18 Oct 1899, S. Kusano (IBAR-7797, probably part of the holotype)

Summary

The rust fungi (Pucciniales) is one of the largest plant parasitic fungi that include economically important plant pathogens (Ono et al. 1992, Cummins and Hiratsuka 2003). One of the important species is *Phakopsora euvitis* causing grapevine leaf rust (GLR). This species spends its uredinial/telial state on *Vitis* plants and its spermogonial/aecial state on *Meliosma myriantha.* There are several more heteroecious and autoecious species using vitaceous plants and *Meliosma* plants (Sabiaceae).

Currently, four *Aecidium* and three *Phakopsora* species are recognized on various *Meliosma* plants (Sabiaceae). Despite the broad geographic distribution of the host genus, their rust records are limited in Asia. Among three *Phakopsora* species, *Phakopsora euvitis* Y. Ono, with the uredinial/telial stage on *Vitis* plants, and *P. vitis* P. Sydow, with the uredinial/telial stage on *Parthnocissus* plants, have been proven to form the spermogonial/aecial stage on *M. myriantha* in Japan. An additional macrocyclic, autoecious rust fungus, *P. meliosmae* Kusano, was reported to occur on several *Meliosma* species widely distributed in Asia. Chatasiri and Ono (2008) conducted molecular phylogenetic analyses of *Phakopsora* species on vitaceous and *Meliosma* plants using the D1/D2 region of nuclear, large subunit rDNA and the internal transcribed spacer 2 (ITS2) regions including 5.8S. Their results revealed the presence of cryptic species among these populations. The purpose of this study was to clarify the taxonomic status of *Phakopsora* species on *Vitis* and *Meliosma* plants based on host specificity, morphology and molecular analyses.

The autoecious rust populations on *M. myriantha* and *M. tenuis* were shown to be distantly related based on the molecular phylogenetic study conducted by Chatasiri and Ono (2008). In this study, cross-inoculation experiments and comparative morphological examination of the two *Phakopsora* populations was carried out to confirm that these rust populations are distinct species. As a result, cross-inoculations using basidiospores confirmed the macrocyclic, autoecious nature of the life cycles in both rust populations and showed that the two populations were different in their host specificity. They were also found to be distinct in the structure of the aecial peridium surface, the size and wall-thickness of uredinial paraphyses, and the urediniospore size and shape. Consequently, the fungal population on *M. tenuis* was taxonomically separated from *P. meliosmae*, originally described for the fungus on *M. myriantha.* The autoecious, macrocyclic *Phakopsora* species on *M. tenuis* in Japan was described as a new species, *Phakopsora orientalis* Chatasiri, Pota & Y. Ono and, consequently, description of *P. meliosmae* on *M. myriantha* in Japan was amended (Pota et al. 2013).

To clarify the taxonomic status of the heteorecious species of GLR, which alternates hosts between *Vitis* and *Meliosma* plants, inoculation experiments, morphological observations and molecular analyses were carried out. The results of molecular phylogenetic analyses showed the seven distinct groups with high bootstrap values, which were almost the same as the ones detected by Chatasiri and Ono (2008). Each of the seven clades corresponded with the fungal population circumscribed by life cycle, host specificity and geographic distribution range. Among them, three distinct groups, i.e. clade I, II and III were composed of the GLR fungi. Clade I and II included Japanese populations, but clade III included populations in Thailand, Malaysia, Indonesia, East Timor and Australia.

To demonstrate life cycle and host range of the population in clade II (*Aecidium* on *M. tenuis* and GLR fungus on *V. coignetiae* from Japan) of the GLR fungus in Japan, inoculation experiments were conducted. As for the results, the GLR population in clade II was a heteroecious species that alternates between *M. tenuis* and *V. coignetiae.* There were no clear differences in morphology of the uredinial and telial state between GLR clade I and II, but they were distinct in the aeciospore morphology (the spore apical wall is thicker in the former, while thinner in the latter fungus), in addition to the difference in the spermogonial-aecial host preference. The former species used *M. tenuis*, while the latter used *M. myriantha*. The GLR population in clade II was described as a new species, *P. montana* Y. Ono & Chatasiri (Ono et al. 2012) combined with the data shown in the present thesis. The GLR population in clade I was named *P. meliosmae-myrianthae* (= *P. euvitis*).

The Southeast Asian and Australasian GLR fungus (clade III) was similar to *P. meliosmae-myrianthae* and *P. montana* in morphology of the uredinial and telial state. Uredinial paraphyses of the Southeast Asian and Australasian GLR fungus appeared thinner than those of the East Asian GLR fungi, but there was no significant difference among the populations. Furthermore, the Southeast Asian and Australasian fungus is composed of three subgroups that are different in the D1/D2 and ITS2 sequence. The three subgroups corresponded with the three distribution ranges, i.e., Thailand, Malaysia-Indonesia and East Timor-Australia. Results of the studies on *P. montana* and *P. meliosmae-myrianthae* indicated importance of morphological differences of the aecial state. However, the spermogonial-aecial state of these populations in clade III was unknown. Thus, the Southeast Asian and Australasian GLR population was treated

as *Phakopsora* species for the present, until a spermogonial-aecial state is found (Pota et al. 2014).

In conclusion, four out of five populations previously recognized as *P. euvitis* or *P. meliosmae*, detected by molecular phylogenic analyses, were clearly distinguishable by life cycle, host specificity and morphology, particularly in the aecial state. They were recognized as distinct species. The other population, the Southeast Asian and Australasian GLR population, seemed to be a distinct species, but information on the spermogonial-aecial state of the population, which is expected to be produced on *Meliosma* species, is considered to be essential to conclude its taxonomic condition. Results of the studies revealed that molecular phylogenic analysis is a useful tool for recognizing cryptic species in rust fungi, but the studies on their life cycle and morphology are also essential to define each species. Several species with different life cycles and host ranges are present on table grape cultivars and on *Meliosma* species. Precise identification of GLR and information on their life cycle and host plants provided in this study will contribute to the control of GLR.

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