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Taxonomy of two host specialized *Phakopsora* populations on *Meliosma* in Japan

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ABSTRACT

Phakopsora meliosmae, a macrocyclic autoecious rust fungus, is reported to occur on several *Meliosma* species widely distributed in Asia. Despite the apparent broad host range, a recent molecular phylogenetic study indicated that two rust populations on *Meliosma myriantha* and *Meliosma tenuis* respectively in Japan were biologically distinct. To clarify the biological and taxonomic relationships of these populations, cross inoculations and comparative morphological examinations were carried out. Cross inoculations using basidiospores and aeciospores confirmed the macrocyclic, autoecious nature of the life cycle in both rust populations and showed that the two populations were distinct in their host specificity. Furthermore, they were 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* is taxonomically separated from *P. meliosmae* originally proposed for the fungus on *M. myriantha*. A new name, *Phakopsora orientalis*, is proposed for the fungus on *M. tenuis*.

1. Introduction

Plants of the genus *Meliosma* are trees or shrubs of an evergreen or deciduous nature and about 50 species (or less according to Van Beusekom 1971) are distributed in Asia and the Americas (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* subsp. *oldhamii* (Maxim.) H. Ohba var. *oldhamii* [= *Meliosma oldhamii* Maxim., *Meliosma pinnata* subsp. *barbulata* (Cufod.) Beusekom var. *oldhami* (Maxim.) Beusekom, *Meliosma rhoifolia* Maxim.], *M. arnottiana* subsp. *oldhamii* var. *hachijoensis* (Nakai) H. Ohba [=*Meliosma hachijoensis* Nakai], *Meliosma myriantha* Siebold & Zucc., *Meliosma rigida* Siebold & Zucc. [=*Meliosma simplicifolia* subsp. *rigida* (Siebold & Zucc.) Beusekom], *M. squamulata* Hance [=*Meliosma lutchuensis* Koidz.], *Meliosma tenuis* Maxim. [*Meliosma dilleniifolia* subsp. *tenuis* (Maxim.) Beusekom]. These plant names are according to Satake et al. (1989), Wu et al. (2007), and Anonymous (2011).

Currently, eight rust species are recognized on various *Meliosma* plants: one *Goplana* (Raciborski 1909; Ono and Hennen 1983), four *Aecidium* (Hennings 1900; Cummins 1937,

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1941; Hosagoudar 1987), and three *Phakopsora* (Kusano 1904; Ono 2000) species are recognized on various *Meliosma* plants. Despite the broad geographic distribution of species of the host genus, records of their rust fungi are limited to Asia.

Among the *Phakopsora* species, *Phakopsora euvitis* Y. Ono with its uredinial/telial stage on *Vitis* plants and *Phakopsora vitis* P. Syd. with the uredinial/telial stage on *Parthenocissus* plants have been proven to form spermogonial/aecial stages on *M. myriantha* in Japan (Ono 2000). *Phakopsora meliosmae* Kusano is the only species for which macrocyclic autoecious life cycle is proven by experimental inoculations (Kakishima et al. 1983). This inoculation study was carried out with only a few populations on *M. myriantha* from a single locality in Japan, and no further life cycle and host range studies have been made for rust populations forming the uredinial/telial stages on other *Meliosma* plants. Therefore, taxonomic identity of phakopsoroid fungi with the uredinial/telial stage on various *Meliosma* species as *P. meliosmae* is determined by their host relationships and morphological similarities in sori and spores. Thus, *P. meliosmae* is now reported to occur on 7 species and one variety of *Meliosma* and distributed widely from East Asia through the Himalayas (Kusano 1904; Hiratsuka and Hashioka 1934; Hiratsuka 1935; Arthur and Cummins 1936; Tai 1979; Durrieu 1987; Zhuang and Wei 1994; Zhang et al. 1997; Cho and Shin 2004).

In Japan, *M. myriantha* is recorded as a frequent host of *P. meliosmae*, *M. tenuis* less frequent host, and *M. arnottiana* subsp. *oldhami* (*M. rhoifolia*) is a rare host (with only two records). Fungal populations on these hosts are described as having the same morphological features and assumed to possess the same parasitic nature on the three host plant species. 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 with voucher specimens IBAR-7612 and 7758). Furthermore, in a molecular phylogenetic study of Japanese *Phakopsora* populations parasitic on vitaceous plants, in which *Meliosma*-infecting autoecious *Phakopsora* rusts were included, the autoecious rust populations on *M. myriantha* and *M. tenuis* were shown to be genetically distantly related (Chatasiri and Ono 2008). These inoculation experiments and molecular phylogenetic study indicate the two autoecious *Phakopsora* populations on *M. myriantha* and *M. tenuis* are biologically and taxonomically distinct. We report cross-inoculation experiments and comparative morphological examination of the two *Phakopsora* populations and show these rust populations are distinct species.

2. Materials and methods

2.1. *Voucher specimens*

All the specimens examined morphologically and those resulting from artificial inoculations are deposited in the Herbarium of Systematic Mycology, Ibaraki University (IBAR, formerly designated as IBA) and are listed below. Letters, S, A, U and T preceding IBAR accession numbers, denote spermogonial, aecial, uredinial and telial stage, respectively. Vouchers used for and resulting from artificial inoculations are indicated following the IBAR accession numbers. IBAR-7797 and 7798 on *M. myriantha* collected by S. Kusano have been transferred from the Mycological Herbarium at the University of Tsukuba and formally registered in the Herbarium of Systematic Mycology, Ibaraki University.

On *M. myriantha* FUKUSHIMA: Futaba-machi, Mt. Goshayama, 27 Jun 2009, Y. Ono (YO) (S & A, IBAR-10078); Daigo-machi, Mt. Yamizo-san, 2 Jun 1995, YO (S & A, IBAR-7582); Iwaki, 11 Nov 2000, YO and H. Mori (U & T, IBAR-8640); 13 Oct 2007, YO (U & T, IBAR-9906); 24 May 2009, YO & S. Pota (SP) (T, IBAR-10067 & 10068; S & A, IBAR-10069); Saigo-mura, 19 Sep 2008, YO (U & T, IBAR-10045), GUMMA: Minakami-machi, Hoshi-onsen, 18 Jun 1973, YO (S & A, IBAR-1592). IBARAKI: Daigo-machi, Mt. Yamizo-san, 19 Nov 1990, YO (U & T, IBAR-5540); 24 Oct 1992, YO (U & T, IBAR-6360); 23 Oct 1993, YO (T, IBAR-7007); 17 Nov 2007, YO (T, IBAR-9945); Kitaibaraki, Hanazono, 9 Nov 1991, YO (T, IBAR-5997); 18 Sep 1992, YO (S, A & U, IBAR-6216); 4 Jul 1992, K. Higuchi et al. (S & A, IBAR-6629); 15 Dec 2007, YO et al. (T, IBAR-9977, used as an inoculum); 21 Mar 2009, YO & SP (T, IBAR-10059, used as an inoculum); Mito, Ibaraki Univ., 3 Jul 2008, YO (S & A, IBAR-10037 & 10038, result of basidiospore inoculation); 21 Mar 2009, YO & SP (T, IBAR-10058, used as an inoculum); 21 May 2009, YO & SP (S & A, IBAR-10064, result of basidiospore inoculation); 5 Jun 2009, YO & SP (S & A, IBAR-10065, result of basidiospores inoculation); 7 Jun 2009, YO & SP (S & A, IBAR-10066, result of basidiospore inoculation); 12 Jun 2009, YO & SP (S & A, IBAR-10074, result of basidiospore inoculation); Mito, Mito Forest Park, 6 Nov 1982, YO (U & T, IBAR-2694 & 2711); 27 Sep 1983, YO (U & T, IBAR-2878); Sakuragawa, Mt. Kaba-san, 20 May 2009, SP (T, IBAR-10072); Satomi-mura, 8 Jun 1990, YO (S & A, IBAR-4798); Takahagi, Hananuki, 21 Mar 2009, YO & SP (T, IBAR-10060, used as an inoculum); Shirosato-machi, Mt. Gozen-yama, 20 Oct 2007, YO (U & T, IBAR-9911, 9912); 16 Nov 2007, YO (T, IBAR-9946); 21 Mar 2009, YO & SP (T, IBAR-10057, used as an inoculum); Tsukuba, Mt. Tsukuba-san, Jun 1979, M. Kakishima (S & A, IBAR-1801); 17 Oct 1979, YO (T, IBAR-1887); 22 Apr 2009, SP (T, IBAR-10071, used as an inoculum); 15 Jul 2009, SP (S & A, IBAR-10087); Tsukuba, Univ. Tsukuba, 20 Jul 2009, SP (S & A, IBAR-10092, result of basidiospore inoculation); 6 Aug 2009, SP (S & A, IBAR-10095, result of basidiospore inoculation); 9 Jun 2009, YO & SP (S & A, IBAR-10073, result of basidiospore inoculation). MIYAGI: Sendai, 26 Aug 2009, SP (U, IBAR-10099); Matsushima, 29 Aug 2009, SP (U, IBAR-10102). NAGANO: Azumino, 30 Jul 2009, S. Yokosawa (U, IBAR-10094); 17 Aug 2009, S. Yokosawa (U, IBAR-10097). SAITAMA: Okutama-machi, 19 Sep 1954, N. Hiratsuka & T. Hiratsuka (U & T, IBAR-3601). TOCHIGI: Kanuma, 17 Oct 1981, YO (U & T, IBAR-2387); 16 Nov 2007, YO et al. (T, IBAR-9944); 28 Sep 2000, YO (U & T, IBAR-8581); 16 Nov 2007, YO et al. (T, IBAR-9943); Nikko, Hinata, 20 Sep 2008, YO (U & T, IBAR-10054, used as an inoculum); Nikko, Yunishigawa, 20 Sep 2008, YO (U & T, IBAR-10053, used as an inoculum); Nikko, Miyori. TOKYO: Hachioji, Mt. Takao-san, 18 Oct 1899, S. Kusano (U & T, IBAR-7797, probably part of the holotype of *P. meliosmae*). YAMANASHI: Enzan, 4 Dec 2009, Y. Yamaoka and SP (T, IBAR-10244). Specimen with no locality data: probably collected at Mt. Takao-san on 11 Jul 1899, S. Kusano (S & A, IBAR-7798, probably part of the designated holotype of *Aecidium meliosmae* (originally labeled as *meliosmatis* Dietel).

On *M. tenuis* IBARAKI: Mito, Ibaraki Univ., 10 Jun 1996, YO (S & A, result of basidiospore inoculation IBAR-7758); Tsukuba, Univ. Tsukuba, 19 Apr 2010, SP (S & A, result of basidiospore inoculation, IBAR-10253 & 10254); 21 Apr 2010, SP (S & A, result of basidiospore inoculation, IBAR-10255); 22 Apr 2010, SP (S & A, result of basidiospore inoculation, IBAR-10256) 25 Apr 2010, SP (S & A, result of basidiospore inoculation, IBAR-10257); 30 Aug 2009, SP (S & A, result of basidiospore inoculation, IBAR-10103); 30 Aug 2009, SP (U, IBAR-10104). MIYAZAKI: Ogata-machi, Mt. Sobosan, 25 Oct 1995, YO (T, IBAR-7662). NAGANO: Ina, Chusenji, 11 Jul 2009, Y. Yamaoka and SP (S, A, U, IBAR-10080); 5 Dec 2009, Y. Yamaoka and SP (T, IBAR-10248, used as an inoculum). TOCHIGI: Nikko, Yunishigawa, 22 Sep 1995, YO (U & T, IBAR-7612, used as an inoculum); 16 Nov 2007, YO et al. (T, IBAR-9942); 20 Sep 2008, YO (U & T, IBAR-10051, holotype of *Phakopsora orientalis*, used as an inoculum).

2.2. *Artificial inoculations*

Plants of *M. myriantha* and *M. tenuis* were planted in clay pots of 15 cm diam or larger with loam soil and maintained in a greenhouse to avoid possible spontaneous rust infections. Telial materials on *M. myriantha* were collected at six localities in Ibaraki in November and December 2007 and 2008, and in March 2009; and two localities in Tochigi in September 2008. Telial materials on *M. tenuis* were collected at Yunishigawa (two localities), Nikko, Tochigi in September 1995 and 2008, and in Minowa-machi, Nagano in December 2009.

Because no precise conditions were determined for the overwintering and germination of the teliospores, and basidiospore infection in nature, no effort of mimicking natural infection of basidiospores was made. The telium-bearing leaves were preserved in a refrigerator at ca. 5 °C until used. The leaves were processed and teliospore germination and basidiospore production were induced with the method described by Ono and Azbukina (1997). The leaves were soaked in running tap water for one to a few days followed by placing them in 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 those from *M. tenuis* leaves were inoculated once in 1996 and 2009 and five times in 2010. In each inoculation, five to ten apparently healthy leaves of *M. myriantha* and *M. tenuis* plants were inoculated with basidiospores from telium-bearing *M. myriantha* or *M. tenuis* leaves. Most inoculations were conducted at room temperature between 15 °C and 22 °C; however, whenever possible, the inoculated plants were placed in a growth chamber at ca. 20 °C with controlled artificial illumination for subsequent observations.

2.3. *Microscopic observations*

To examine sorus structure and spore morphology, small sorus-bearing pieces were cut from the herbarium specimens and thin-sectioned with a razor blade under a binocular dissecting microscope. To observe morphology and to measure sizes, aeciospores, urediniospores, and uredinial paraphyses, were scraped from sori on herbarium specimens. Thin-sections and scraped spores and/or paraphyses were mounted on microscope slides and treated as described by Ono (2000). The slide preparations were examined under an Olympus BH2 microscope (Olympus, Tokyo, Japan) 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) equipment. Fifty or twenty randomly selected spores and paraphyses were measured for each specimen and five to ten spermogonia were measured. To determine the number and distribution of urediniospore germ pores, the spores were mounted in lactic acid on a slide glass and heated to boiling for a few seconds, and a drop of lactophenol solution with aniline blue was then added onto the boiled spores.

For scanning electron microscopy, sori-bearing pieces of herbarium specimens or spores scraped from herbarium specimens were placed on double-sided adhesive tape on a specimen holder and then coated with platinum-palladium to a 25 nm thicknesses under a Hitachi E-1030 ion sputter (Hitachi, Tokyo, Japan). These samples were observed with a Hitachi S-4200 SEM (Hitachi, Tokyo, Japan) operating at 15 kV.

3. Results

3.1. *Host specificity and life cycle*

Teliospores, both on *M. myriantha* and *M. tenuis* leaves, germinated well and formed abundant basidiospores. Inoculations with basidiospores from *M. myriantha* were successful only on *M. myriantha* (Table 1), producing spermogonia 4–7 d after inoculation and aecia after another 7–10 d. However, no sign of infection was observed on inoculated *M. tenuis* leaves. Inoculations of basidiospores from *M. tenuis* were successful only on *M. tenuis* leaves (Table 1). Spermogonia were formed 5–7 d after inoculation, and followed by aecial production after another 7–10 d.

Cross inoculations on *M. myriantha* and *M. tenuis* plants with basidiospores either from the *M. myriantha* infecting rust population (MMR) or the *M. tenuis* infecting rust population (MTR) proved the autoecious nature of the life cycle and their restricted host preference. Although MMR and MTR were shown to be host specific, symptoms and spermogonial/aecial sori formed on infected plants of the two *Meliosma* species were similar (Fig. 1A and 1B). Lesions were small, pale yellowish or yellowish orange with a more or less clear boundary. Dark-colored dots of spermogonia were produced in a dense cluster on both sides of the lesion; and columnar aecia were produced more on the adaxial surface of the lesion than on the abaxial surface.

3.2. *Morphology of the* M. myriantha *infecting rust population (MMR)*

Spermogonia were subcuticular and conical surrounded with conspicuous paraphyses. The height ranged between 60 and 157 μm (mean: 108.3 μm), and the width between 68 and 158 μm (mean: 113.4 μm) (Fig. 2A; Table 2). Aecia were firmly surrounded by well-developed peridial cells and, thus, appeared columnar or horn-shaped (Fig. 1A and 2B), later becoming cup-shaped due to apical rupture of the peridium when sori

Table 1 – Results of artificial inoculations with basidiospores formed from teliospores on *M. myriantha* and *M. tenuis*.

Inoculum (telium)	Voucher specimens	Source locality	Collected date	Inoculated date	Infected and sporulated plants	Voucher specimens
on *M. myriantha*	IBAR-9977	Ibaraki, Kitaibaraki, Hanazono	15 Dec 2007	12 Apr 2008	*M. myriantha*	IBAR-10037, 10038
	IBAR-10058	Ibaraki, Daigo-machi, Yamizo-san	21 Mar 2009	9 Apr 2009	*M. myriantha*	IBAR-10065
	IBAR-10057	Ibaraki, Shirosato-machi, Gozen-yama	21 Mar 2009	14 Apr 2009	*M. myriantha*	IBAR-10064
	IBAR-10071	Ibaraki, Tsukuba, Tsukuba-san	22 Apr 2009	28 May 2009	*M. myriantha*	IBAR-10073
	IBAR-10060	Ibaraki, Takahagi, Hananuki	21 Mar 2009	1 May 2009	*M. myriantha*	IBAR-10066
	IBAR-10059	Ibaraki, Kitaibaraki, Hanazono	21 Mar 2009	3 May 2009	*M. myriantha*	IBAR-10074
	IBAR-10053	Tochigi, Nikko, Yunishigawa	20 Sep 2008	1 Jun 2009	*M. myriantha*	IBAR-10095
	IBAR-10054	Tochigi, Nikko, Hinata	20 Sep 2008	1 Jun 2009	*M. myriantha*	IBAR-10092
on *M. tenuis*	IBAR-7612	Tochigi, Nikko, Yunishigawa	22 Sep 1995	17 May 1996	*M. tenuis*	IBAR-7758
	IBAR-10051	Tochigi, Nikko, Yunishigawa	20 Sep 2008	1 Jul 2009	*M. tenuis*	IBAR-10103
	IBAR-10248	Nagano, Ina, Chusenji	5 Dec 2009	12 Mar 2010	*M. tenuis*	IBAR-10253
	IBAR-10248	Nagano, Ina, Chusenji	5 Dec 2009	22 Mar 2010	*M. tenuis*	IBAR-10254
	IBAR-10248	Nagano, Ina, Chusenji	5 Dec 2009	25 Mar 2010	*M. tenuis*	IBAR-10255
	IBAR-10248	Nagano, Ina, Chusenji	5 Dec 2009	27 Mar 2010	*M. tenuis*	IBAR-10256
	IBAR-10248	Nagano, Ina, Chusenji	5 Dec 2009	30 Mar 2010	*M. tenuis*	IBAR-10257

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

matured. The peridial cells were oblong to broadly ellipsoid and the inner surface was almost smooth (Fig. 2C). Aeciospores were produced in a basipetal succession from the basal sporogenous layer in the sori (Fig. 2B), subglobose to broadly ellipsoid, often angular, and 17–37 × 14–26 μm (mean: 25.6 × 19.6 μm) in size (Fig. 2D; Table 2). The length/width ratio ranged between 1:1.2 and 1:1.6. The wall was colorless and 0.9–2.1 μm thick with the surface evenly covered by nail-head verrucae (Fig. 2E). Uredinia were minute, scattered or in loose groups on the abaxial leaf surface, and densely surrounded by incurved paraphyses (Fig. 2F). The paraphyses were hyaline to brown, moderately to strongly incurved, and basally united (Fig. 2G). 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 22–60 μm high and 9–21 μm wide with means of 36.5 μm high and 13.8 μm wide (Table 3). The wall was hyaline to brown; and the color appeared to change as the sori aged. The wall thickness was uneven: the dorsal wall was prominently thicker than the ventral wall, and 3.0–11.9 μm thick (mean: 6.3 μm). The ventral wall was evenly 1–2 μm thick. The apical wall was 3.0–15.8 μm thick (mean: 7.7 μm) (Fig. 2G; Table 3). Urediniospores were short-pedicellate, appearing almost sessile, obovoid to obovoid-ellipsoid, and 18–31 × 12–21 μm (mean: 24.3 × 17.4 μm) in size (Fig. 2H; Table 3). The length/width ratio ranged between 1:1.0 and 1:2.0.

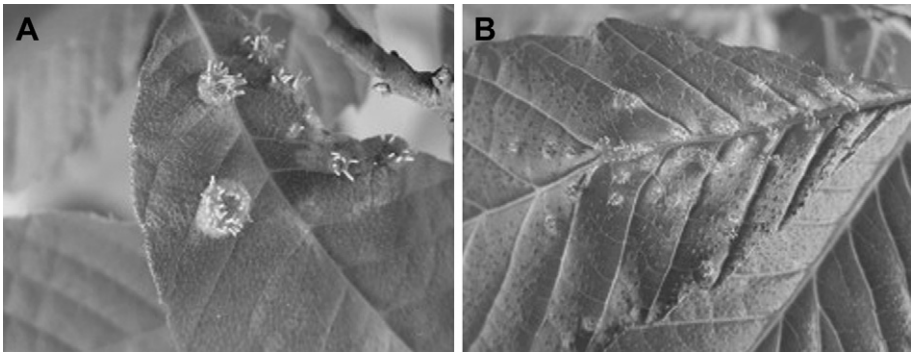

Fig. 1 – Results of basidiospore inoculations. A: Aecia formed on the adaxial leaf surface of *M. myriantha* by inoculation with basidiospores derived from the same host species. B: Aecia formed on the adaxial leaf surface of *M. tenuis* by inoculation with basidiospores derived from the same host species.

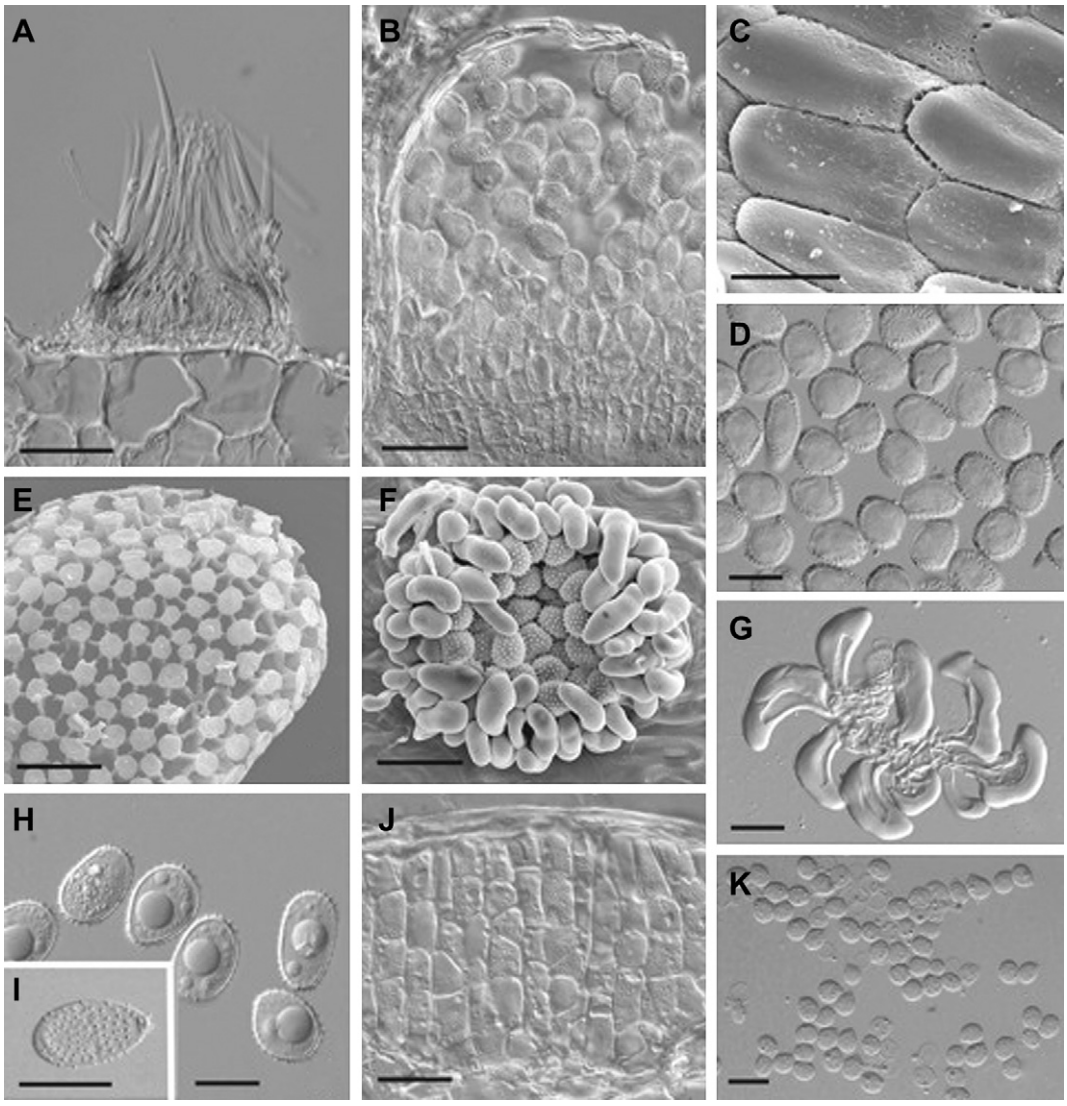

Fig. 2 – Morphological features of the *M. myriantha* infecting rust population. A: Spermogonium (IBAR-10065). B: Vertical section of an aecium (IBAR-4798). C: Almost smooth inner surface of peridial cells (IBAR-10038, SEM). D: Aeciospores (IBAR-10092). E: Nail-head verrucae on aeciospore wall surface (IBAR-10069, SEM). F: Overview of a uredinium (IBAR-10102, SEM). G: Uredinial paraphyses (IBAR-10102). H: Urediniospores (IBAR-10099). I: Urediniospore germ pores (IBAR-2387). J: 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.

The wall was 0.6–1.9 μm thick, colorless or pale yellowish, and evenly echinulate. Urediniospore germ pores were faint and appeared to be two to four distributed in an equatorial zone (Fig. 2I). Telia were scattered or in dense groups on the abaxial leaf surface, often surrounding uredinia, subepidermal, and composed of 2–7 layers of linearly arranged teliospores (Fig. 2J). The teliospores of the uppermost layer were ellipsoid to oblong, angular, and 9–25 × 6–16 μm (mean: 17.5 × 10.5 μm) in size. The apical wall was 0.9–4.1 μm thick and light brown; and the lateral wall was 0.6–2.3 μm thick and almost colorless. The teliospores of the second layer and below were 8–25 × 6–17 μm (mean: 15.0 × 10.7 μm) in size (Table 4). The wall was 0.6–2.3 μm thick and almost colorless. Basidiospores were observed in IBAR-10057 and 10244, subglobose to broadly ellipsoid, and 6.6–9.6 × 4.7–7.7 μm in size (Fig. 2K).

3.3. *Morphology of the* M. tenuis *infecting rust population (MTR)*

Spermogonia were subcuticular and conical with surrounding paraphyses (Fig. 3A). The height ranged between 50 and 118 μm (mean: 76.7 μm) and the width between 45 and 121 μm (mean: 80.3 μm) (Table 2). Aecia and aeciospores were similar to those of MMR (Figs. 1B and 3B and 3D). However, unlike MMR, the inner surface of the peridial cell was prominently verrucose (Fig. 3C). The aeciospore size was 18–34 × 14–23 μm (mean: 24.8 × 18.6 μm) (Fig. 3D; Table 2). The length/width ratio ranged between 1:1.2 and 1:1.4. The wall was colorless, 0.9–1.9 μm thick (mean: 1.3 μm), and evenly covered with nail-head verrucae (Fig. 3E). Uredinia and telia were similar to those of MMR (Fig. 3F and 3J). The paraphysis was 21–44 μm

Table 2 – Comparison of spermogonial/aecial morphologies between the *M. myriantha* infecting rust population and *M. tenuis* infecting rust population.

Rust populations on	Spermogonium size height × width (μm)	Aeciospore size length × width (μm)	Aeciospore length/width ratio	Wall thickness (μm)	Number of specimens measured
M. myriantha	60–(108.3)–157 × 68–(113.4)–158	17–(25.6)–37 × 14–(19.6)–26	1.2–(1.3)–1.6	0.9–(1.4)–2.1	12
M. tenuis	50–(76.7)–118 × 45–(80.3)–121	18–(24.8)–34 × 14–(18.6)–23	1.2–(1.3)–1.4	0.9–(1.3)–1.9	7

Mean value is shown in parentheses inserted between smallest and largest values measured.

high and 8–19 μm wide (means: 30.5 μm high and 12.0 μm wide) (Fig. 3G; Table 3). The dorsal and apical walls were colorless to brown, thickened: 2.1–8.7 μm thick (mean: 4.7 μm) dorsally and 1.9–7.9 μm thick (mean: 3.9 μm) apically (Fig. 3G; Table 3). The ventral wall was evenly 1–2 μm thick. Urediniospores were short-pedicellate, obovoid or obovoid-ellipsoid, and 20–33 × 13–20 μm (mean: 26.3 × 16.7 μm) in size (Fig. 2H; Table 3). The length/width ratio of the spores ranged between 1:1.2 and 1:2.4. The wall was 0.6–1.9 μm thick, colorless or pale yellowish, and evenly echinulate. Urediniospore germ pores were faint and appeared to be two to four distributed in an equatorial zone (Fig. 3I). Telia were subepidermal and composed of 3–7 layers of linearly arranged teliospores (Fig. 3J). The teliospores were ellipsoid to oblong, angular, and 11–23 × 6–16 μm (mean: 16.1 × 10.7 μm) for the uppermost layer. The apical wall was 1.3–3.2 μm thick (mean: 2.0 μm) and light brown; and the lateral wall was 0.6–1.9 μm thick and almost colorless. The teliospores of the second layer and below were 8–22 × 6–17 μm (mean: 13.7 × 11.4 μm) thick; and the wall was 0.6–1.9 μm thick and almost colorless. Basidiospores were observed only in IBAR-10248, and were subglobose to broadly ellipsoid, and 7.7–10.7 × 5.5–8.1 μm in size (Fig. 3K).

4. Discussion

4.1. *Host specificity and morphology of* M. myriantha *and* M. tenuis *infecting populations*

Artificial inoculations confirmed the autoecious macrocyclic life cycle of MMR (Kakishima et al. 1983). Similarly, MTR was proven to have an autoecious macrocyclic life cycle (Ono, unpublished records with voucher specimens IBAR-7612 and 7758). Cross inoculations with basidiospores from MMR and MTR in this study proved that MMR and MTR have strict host preference. Because a living rust fungus on *M. arnottiana* subsp. *oldhami* was not available in this study, the life cycle and host specificity of the rust population on *M. arnottiana* subsp. *oldhami* was not determined.

Adaptation of the two closely related fungi to different *Meliosma* species might have reduced a chance for mutual transfer of spermatia and cross fertilization in nature. The morphological character disjunctions (Figs. 2 and 3; Tables 2 and 3) suggest possible genetic separation due to no cross fertilization between MMR and MTR. The most prominent difference is observed on the inner surface structure of the peridial cell, i.e., smooth in MMR vs. verrucose in MTR. Spermogonia and paraphyses are larger in MMR than in MTR. The dorsal and apical walls of paraphyses are prominently thickened in MMR. Urediniospores are longer and narrower in MTR than in MMR as shown in the L:W ratio. No apparent differences between MMR and MTR are found in aeciospore morphology, urediniospore wall thickness, number and distribution of germ pores, and basidiospores shape and size. Nail-head projections subtended by buttresses observed on the MMR and MTR aeciospores seem characteristic of aeciospores of *Aecidium*-type aecia in *Phakopsora* species (cf. *P. euvitis* and *P. vitis*, Ono 2000). Sato and Sato (1982) reported that aeciospore surface of *Phakopsora ampelopsidis sensu lato* on *M. tenuis* is typical verrucose. They did not cite the specimen(s) examined; therefore, we were unable to confirm their report. However, all specimens of *Aecidium* on *M. tenuis* that we have examined exhibited nail-head type surface structure. The specimens included one from Mt. Daisen collected by N. Hiratsuka and S. Okubo on 25 July 1976 (BAR-7790), which might be the one Sato and Sato (1982) examined. No differences are apparent in telial features.

Both MMR and MTR form strongly incurved, dorsally thick-walled uredinial paraphyses and subglobose or broadly ellipsoid basidiospores. These morphological properties in MMR

Table 3 – Comparison of uredinial morphologies between the *M. myriantha* infecting rust population and *M. tenuis* infecting rust population.

Rust populations on	Paraphysis size height × width (μm)	Apical wall thickness (μm)	Dorsal wall thickness (μm)	Urediniospore size (μm)	Urediniospore length/width ratio	Wall thickness (μm)	Number of specimens measured
M. myriantha	22–(36.5)–60 × 9–(13.8)–21	3.0–(7.7)–15.8	3.0–(6.3)–11.9	18–(24.3)–31 × 12–(17.4)–21	1.0–(1.4)–2.0	0.6–(1.2)–1.9	15
M. tenuis	21–(30.5)–44 × 8–(12.0)–19	1.9–(3.9)–7.9	2.1–(4.7)–8.7	20–(26.3)–33 × 13–(16.7)–20	1.2–(2.2)–2.4	0.6–(1.2)–1.9	3

Mean value is shown in parentheses inserted between smallest and largest values measured.

Table 4 – Comparison of telial morphologies between the *M. myriantha* infecting rust population and *M. tenuis* infecting rust population.

Rust populations on	Number of teliospore layers	Teliospores size of the uppermost layer (μm)	Apical wall thickness (μm)	Teliospores size below the uppermost layer (μm)	Number of specimens measured
M. myriantha	2–7	9–(17.5)–25 × 6–(10.5)–16	0.9–(2.0)–4.1	8–(15.0)–25 × 6–(10.7)–17	27
M. tenuis	3–7	11–(16.1) –23 × 6–(10.7)–16	1.3–(2.0)–3.2	8–(13.7)–22 × 6–(11.4)–17	4

Mean value is shown in parentheses inserted between smallest and largest values measured.

and MTR are also characteristic of *P. vitis*, whose spermogonial/aecial stages occurs on *M. myriantha* (Ono 2000). It is interesting to note that MMR, MTR and *P. vitis* constitute a clade separate from a clade of *P. euvitis*, whose uredinial paraphyses are weakly incurved, moderately thick-walled and basidiospores are kidney-shaped, in phylograms constructed from partial nucleotide sequences of ITS2 and D1/D2 regions of ribosomal DNA (Chatasiri and Ono 2008). Detailed analyses of morphological properties previously poorly examined specimens may help to interpret a pattern of host-limited rust populations in a DNA-based phylogram, and vice versa, even if a single or a few DNA-fragment (or gene) genealogy does not necessarily reflect the organismal phylogeny (Avise and Ball 1990; Maddison 1995; Avise and Wollenberg 1997).

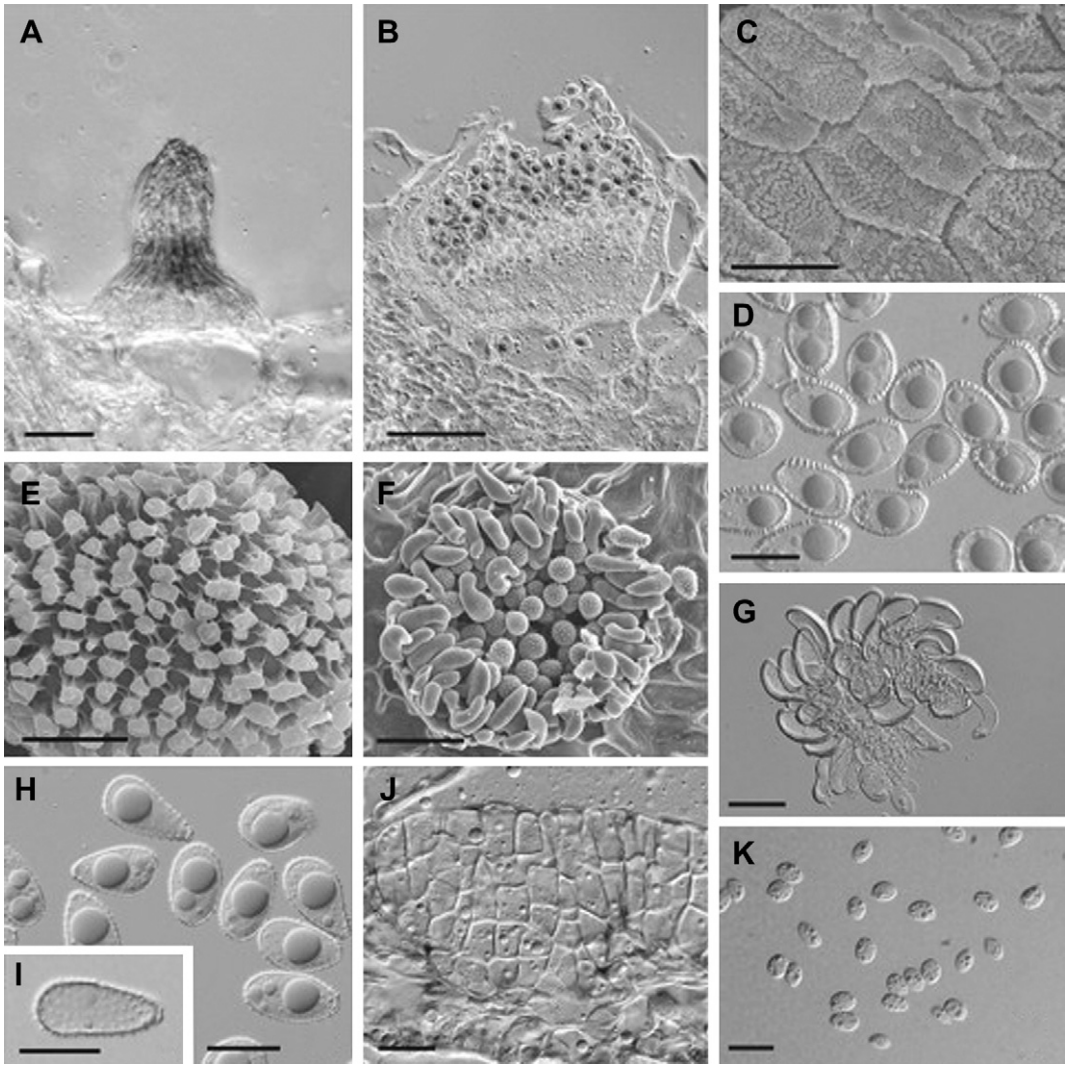

Fig. 3 – Morphological features of *Meliosma tenuis* infecting rust population. A: Spermogonium (IBAR-10253). B: Vertical section of an aecium (IBAR-10256). C: Verrucose inner surface of peridial cells (IBAR-10253, SEM). D: Aeciospores (IBAR-10253). E: Nail-head verrucae on aeciospore wall surface (IBAR-10253, SEM). F: Overview of a uredinium (IBAR-10080, SEM). G: Uredinial paraphyses (IBAR-10104). H: Urediniospores (IBAR-10104). I: Urediniospore germ pores (IBAR-10104). J: Telium in vertical section (IBAR-10051). K: Basidiospores (IBAR-10248). *Bars* A, B 50 μm; C, D 20 μm; E 5 μm; F 50 μm; G-K 20 μm.

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). Apparent strict host specificity between MMR and MTR and corresponding morphological character disjunction between them are a good indicator of their reproductive isolation. Consequently, we 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 *P. ampelopsidis* Dietel & P. Syd. and its allies (Ono 2000), *Puc. hemerocallidis* Thüm. and its allies (Ono 2003, 2005), *Ochropsora* species (Ono 2006), and *Puc. calystegiae-soldanellae* Z. Li, F. Nakai & Y. Harada (Li et al. 2004).

In studies of the species complexes of *Uromyces pisi* (F. Strauss) J. Schröt. (Pfunder et al. 2001), *Puc. andropogonis* Schwein. (Szabo 2006) and *Puc. coronata* Corda (Szabo 2006), congruence between clades recognized by a molecular phylogeny and host/life cycle specific rust populations (either species or cryptic species) are documented. Similarly, in molecular phylogenetic studies of *P. ampelopsidis*, *Puc. hemerocallidis* and their allies, fungal samples (specimens) belong to species recognized by the life cycle and host specificity constituted distinct clades in phylograms constructed from partial nucleotide sequences (entire ITS region in the former, Chatasiri et al. 2006; ITS 2 and D1/D2 regions in the latter, Chatasiri and Ono 2008). These life cycle and host specificity studies combined with molecular phylogenetic analyses indicate that biological species of rust fungi recognized by their life cycle and host specificity may correspond to phylogenetic species recognized by concordance of multiple gene genealogy (Avise and Ball 1990; Avise and Wollenberg 1997; Taylor et al. 2000; Dettman et al. 2003; Ono 2008). The biological and taxonomic distinction of MTR from MMR was suggested by a molecular phylogenetic study (Chatasiri and Ono 2008) and confirmed in this study. A significantly large proportion of rust fungi, which exceed over 7000 species (even 14 times more species expected) on the globe (Rossman 1994), are still unknown for their life cycle and host specificity. In Japan, rust fungi are traditionally well studied, but the full life cycle is known for only 46% (351) of all 763 recorded species (Ono 2008). The combined study of life cycle, host specificity, morphology and molecular phylogeny should advance the species recognition in rust fungi; such recognized species subsequently provide a better basis for the study of evolutionary processes and mechanisms in host switches and life cycle alterations.

4.2. *Taxonomy*

As described above, we conclude that MTR is a species distinct from MMR. Comparative morphological examination of MTR and MMR specimens including the holotype of *P. meliosmae* (Kusano 1904; IBAR-7797) confirmed the identity of MMR as *P. meliosmae*. Therefore, we propose a new name for MTR. In addition, the description of MMR as *P. meliosmae* is emended, because the spermogonial and aecial morphologies of *P. meliosmae* has not been precisely described (Kakishima et al. 1983) and because the currently available description of *P. meliosmae* is a mixture of characteristics observed for both MMR and MTR.

P. orientalis Chatasiri, Pota & Y. Ono, sp. nov. Figs. 1 and 3
MycoBank No.: MB 563058

This species is specialized on *Meliosma tenuis* and characterized by larger spermogonia, verrucose surface of peridial cells, shorter paraphyses with thinner dorsal wall, and longer and narrower urediniospores, by which it is separated from allied *P. meliosmae*.

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, subcuticular, conical, surrounded by paraphyses, 50–118 μm high and 45–121 μm wide. Aecia *Aecidium*-type; 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, *Physopella*-type; peripheral paraphyses irregularly cylindrical, moderately to strongly incurved, 21–44 × 8–19 μm; dorsal wall 2.1–8.7 μm thick; ventral wall evenly 1–2 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 2–4 equatorial germ pores. Telia hypophyllous, crustose, subepidermal, with 3- or 7-layers of linearly arranged spores. Teliospores of the uppermost layer ellipsoid to oblong, angular, 11–23 × 6–16 μm; apical wall 1.3–3.2 μm thick and light brown; lateral wall 0.6–1.9 μm thick and almost colorless. Teliospores of the second layer and below 8–22 × 6–17 μm; wall 0.6–1.9 μm thick and 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. Chatasiri, Pota & Y. Ono

Spermogonia amphigenous, subcuticular, conical, surrounded by paraphyses, 60–157 μm high and the 68–158 μm wide. Aecia *Aecidium*-type; inner surface of peridial cells smooth. Aeciospores catenulate, subglobose to broadly ellipsoid, 17–37 × 14–26 μm; wall hyaline, 0.9–2.1 μm thick, evenly covered by nail-head verrucae. Uredinia *Physopella*-type; peripheral paraphyses 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, evenly 1–2 μm thick ventrally, 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 equatorial germ pores. Telia hypophyllous, subepidermal, with 2–7 layers of linearly arranged teliospores. Teliospores of 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, almost hyaline; teliospores of the second layer and below 8–25 × 6–17 μm, wall 0.6–2.3 μm thick, almost hyaline. Basidiospores subglobose to broadly ellipsoid, 6.6–9.6 × 4.7–7.7 μm in size.

Specimen examined: on *M. myriantha* Siebold & Zucc. JAPAN: Tokyo, Hachioji, Mt. Takao-san, 18 Oct 1899, S. Kusano

(IBAR-7797, probably part of the holotype) and other specimens listed in the Material and method section.

4.3. *Relationships of* Aecidium *on* Meliosma *plants to* Phakopsora

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

Two or more *Aecidium* species on *Meliosma* species have been reported under the name of *A. meliosmae-myrianthae* Henn. & Shirai on *Meliosma 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 [=*Meliosma stewardii* Merr.] in China (Tai 1979); on *Meliosma parvifolia* Lecomte in China (Spaulding 1961; Tai 1979; Teng 1996); on *M. pinnata* subsp. *barbulata* var. *oldhamii* [=*M. arnottiana* subsp. *oldhami* var. *oldhami*, *Meliosma 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* species on *M. myriantha*, for which no name had been given, was also found to be the aecial anamorph of *P. vitis* (Ono 2000). The other *Aecidium* species on various *Meliosma* species, however, need detailed studies to determine their correct taxonomic identity and connection with the teleomorphic species.

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. meliosmae*. 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. euvitis*, Ono 2000) and what we observed for *P. meliosmae*. *Aecidium meliosmae* Dietel is, therefore, treated as a *nomen ambiguum*.

The following *Aecidium* fungi are known from only type material or a few additional collections with limited geographic distribution information, and therefore no assumptions of possible life cycle connection to teleomorphic states are 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 *Meliosma pendula* Merr. was assumed to be caused also by this *Aecidium* species (Cummins 1937). An *Aecidium* on *M. arnottiana* subsp. *oldhami* reported as *A. hornotinum* in the Ryukyus (Hiratsuka and Shimabukuro 1955) may need detailed examination for correct identification. *Aecidium painavuense* Hosag. (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 *Meliosma ferruginea* Blume in Papua New Guinea and also reported on *Meliosma 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). Life cycle connection of the fungus named *A. hornotinum* to *P. euvitis* remains to be studied.

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REFERENCES

Anonymous, 2011. *Arctos multi-Institution, multi-collection museum database*. Retrieved 11 July 2011. http://arctos.database.museum/TaxonomyResults.cfm?full_taxon_name=Meliosma.

Arthur JC, Cummins GB, 1936. Philippine rusts in the Clemens collection 1923–1926 II. *Philippine Journal of Science* 61: 463–488.

Avise JC, Ball JR, 1990. Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology* 7: 45–67.

Avise JC, Wollenberg K, 1997. Phylogenetics and the origin of species. *Proceedings of the National Academy of Sciences USA* 94: 7748–7755; http://dx.doi.org/10.1073/pnas.94.15.7748.

Boedijn KB, 1959. The Uredinales of Indonesia. *Nova Hedwigia* 1: 463–496.

Chatasiri S, Kitade O, Ono Y, 2006. Phylogenetic relationships among *Puccinia hemerocallidis*, *P. funkiae*, and *P. patriniae*

(Uredinales) inferred from ITS sequence data. *Mycoscience* 47: 123–129; http://dx.doi.org/10.1007/s10267-006-0283-y.
Chatasiri S, Ono Y, 2008. Phylogeny and taxonomy of the Asian grapevine leaf rust fungus, *Phakopsora euvitis*, and its allies (Uredinales). *Mycoscience* 49: 66–74; http://dx.doi.org/10.1007/s10267-007-0390-4.
Cho W-d, Shin H-d (eds), 2004. *List of plant diseases in Korea*, 4th edn. Korean Soc Pl Pathol.
Cummins GB, 1937. Studies in the Uredinales of the Philippines. *Ann Myc* 35: 98–105.
Cummins GB, 1941. Uredinales of New Guinea IV. *Mycologia* 33: 380–389.
Dettman JR, Jacobson DJ, Taylor JW, 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution* 57: 2703–2720; http://dx.doi.org/10.1554/03-073.
Dietel P, 1900. Uredineae japonicae. II. *Botanische Jahrbücher* 28: 281–290.
Durrieu G, 1987. Uredinales from Nepal. *Mycologia* 79: 90–96.
Hennings P, 1900. Fungi japonici I. *Botanische Jahrbücher* 28: 259–280.
Hiratsuka N, 1935. *Phakopsora* of Japan II. *Botanical Magazine (Tokyo)* 49: 853–860.
Hiratsuka N, Hashioka Y, 1934. Uredinales collected in Formosa II. *Botanical Magazine (Tokyo)* 48: 233–240.
Hiratsuka N, Shimabukuro S, 1955. Uredinales of the Southern Ryukyu Islands. *Science Bulletin of the Agriculture & Home Economics Division, University of the Ryukyus* 2: 1–15.
Hosagoudar VB, 1987. *Aecidium painavuensis* sp. nov. from Idukki, Kerala, India. *Current Science* 56: 94–95.
Hosagoudar VB, 1988. Uredinales of Kerala. *Journal of Economic and Taxonomic Botany* 12: 265–272.
Ito S, 1950. *Mycological Flora of Japan*. In: *Basidiomycetes. No. 3. Uredinales-Pucciniaceae. Uredinales Imperfecti*, vol. II. Yokendo, Tokyo (in Japanese).
Kakishima M, Sato T, Sato S, 1983. Life-cycle and morphology of *Phakopsora meliosmae* (Uredinales). *Transactions of the British Mycological Society* 80: 77–82.
Kusano S, 1904. Notes on the Japanese fungi. II. Some species of Uredineae. *Botanical Magazine (Tokyo)* 18: 147–149.
Li Z, Sano T, Fujita T, Nakai F, Harada Y, 2004. *Puccinia calystegiae-soldanellae*, a new rust species on *Calystegia soldanella* from Japan. *Mycoscience* 45: 200–205; http://dx.doi.org/10.1007/s10267-003-0178-0.
Maddison WP, 1995. Phylogenetic histories within and among species. In: Hoch PC, Stevenson AG (eds), *Experimental and molecular approaches to plant biosystematics*. Missouri Botanical Garden, St. Louis, pp 273–287.
Ono Y, 2000. Taxonomy of the *Phakopsora ampelopsidis* species complex on vitaceous hosts in Asia including a new species, *P. euvitis*. *Mycologia* 92: 154–173; http://dx.doi.org/10.2307/3761460.
Ono Y, 2003. Does *Puccinia hemerocallidis* regularly host-alternate between *Hemerocallis* and *Patrinia* plants in Japan? *Journal of General Plant Pathology* 69: 240–243; http://dx.doi.org/10.1007/s10327-003-0047-x.
Ono Y, 2005. Plantainlily rust fungus is distinct from daylily rust fungus. *Journal of General Plant Pathology* 71: 99–106; http://dx.doi.org/10.1007/s10327-004-0169-9.
Ono Y, 2006. Taxonomic implications of life cycle and basidium morphology of *Ochropsora ariae* and *O. nambuana* (Uredinales). *Mycoscience* 47: 145–151; http://dx.doi.org/10.1007/s10267-006-0285-9.
Ono Y, 2008. Why life-cycle studies?: Implications in the taxonomy of rust fungi (Uredinales) (in Japanese with English abstract). *Nippon Kingakukai Kaiho* 49: 1–28.
Ono Y, Azbukina ZM, 1997. Heteroecious life cycle of two graminicolous *Puccinia* (Uredinales). *Mycoscience* 38: 281–286.
Ono Y, Hennen JF, 1983. Taxonomy of the Chaconiaceous genera (Uredinales). *Transactions of the Mycological Society of Japan* 24: 369–402.
Pfunder M, Schürch S, Roy BA, 2001. Sequence variation and geographic distribution of pseudoflower-forming rust fungi (*Uromyces pisi* s. lat.) on *Euphorbia cyparissias*. *Mycological Research* 105: 57–66; http://dx.doi.org/10.1017/S0953756200003208.
Raciborski M, 1909. Parasitische und Epiphytische Pilze Javas. *Bulletin International de l'Académie des Sciences de Cracovie* 1909: 346–394.
Rossman AY, 1994. A strategy for an all-taxa inventory of fungal diversity. In: Peng C-i, Chen C-h (eds), *Biodiversity and terrestrial ecosystem*. Monograph Series 14. Institute of Botany, Academia Sinica, Taipei, pp 169–194.
Satake Y, Hara H, Watari S, Tominari T, 1989. *Wild flowers of Japan* (in Japanese). In: *Woody plants II*. Heibonsha, Tokyo.
Sato T, Sato S, 1982. Aeciospore surface structure of the Uredinales. *Transactions of the Mycological Society of Japan* 23: 51–63.
Spaulding P, 1961. Foreign diseases of forest trees of the world. *USDA Agriculture Handbooks* 197: 1–361.
Szabo LJ, 2006. Deciphering species complexes: *Puccinia andropogonis* and *Puccinia coronata*, examples of differing mode of speciation. *Mycoscience* 47: 130–136; http://dx.doi.org/10.1007/s10267-006-0287-7.
Tai FL, 1979. *Sylloge Fungorum Sinicorum*. Science Press, Academia Sinica, Peking.
Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fischer MC, 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology* 31: 21–32; http://dx.doi.org/10.1006/fgbi.2000.1228.
Teng S-c, 1996. *Fungi of China*. Mycotaxon, Ltd., Ithaca.
Van Beusekom CF, 1971. Revision of *Meliosma* (Sabiaceae), section *Lorenzanea* excepted, living and fossil, geography and phylogeny. *Blumea* 19: 355–529.
Wu Z-y, Raven PH, Hong D-y (eds), 2007. *Flora of China. Hippocastanaceae through Theaceae*, vol. 12. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis.
Zhang N, Zhuang J-y, Wei S-x, 1997. Fungal flora of the Daba Mountains: Uredinales. *Mycotaxon* 61: 49–79.
Zhuang J-y, Wei S-x, 1994. An annotated checklist of rust fungi from the Mt. Qomolangma region (Tibetan Everest Himalaya). *Mycosystema* 7: 37–87.