

Rediscovery of Seven Long-Forgotten Species of *Peronospora* and *Plasmopara* (Oomycota)

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ABSTRACT

The family Peronosporaceae, an obligate biotrophic group of Oomycota, causes downy mildew disease on many cultivated and ornamental plants such as beet, cucumber, grape, onion, rose, spinach, and sunflower. To investigate the diversity of Peronosporaceae species in Korea, we performed morphological analysis for dried plant herbariums with downy mildew infections by two largest genera, *Peronospora* and *Plasmopara*. As a result, it was confirmed that there are five species of *Peronospora* and two species of *Plasmopara*, which have been so far unrecorded in Korea, as well as rarely known in the world; *Pl. angustiterminalis* (ex *Xanthium strumarium*), *Pl. siegesbeckiae* (ex *Siegesbeckia glabrescens*), *P. chenopodii-ambrosioidis* (ex *Chenopodium ambrosioides*), *P. chenopodii-ficifolii* (ex *Chenopodium ficifolium*), *P. clinopodii* (ex *Clinopodium cf. vulgare*), *P. elsholtziae* (ex *Elsholtzia ciliata*), and *P. lathyrina* (ex *Lathyrus japonicus*). In addition, their phylogenetic relationship was inferred by molecular sequence analysis of ITS, LSU rDNA, and *cox2* mtDNA. By rediscovering the seven missing species and barcoding their DNA sequences, this study provides valuable insights into the diversity and evolutionary studies of downy mildew pathogens.

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1. Introduction

The family Peronosporaceae is an obligate biotrophic pathogen group belonging to Oomycota. They cause downy mildew disease on a wide range of angiosperm plants [1]. Notably, it led to severe damages on many economically cultivated and ornamental plants, including beet, broccoli, cucumber, grape, onion, rose, spinach, sunflower, and tobacco [2]. The *Peronospora* is the largest genus in Peronosporaceae, including many notorious species, e.g. *P. belbahrii* (parasitic to sweet basil), *P. destructor* (onion), *P. effusa* (spinach), *P. sparsa* (rose), and *P. tabacina* (tobacco) [1]. The genus *Plasmopara* is the second largest group in Peronosporaceae and includes a historically important species *Plasmopara viticola*, responsible for grape downy mildew [3] that caused huge losses of grape production in Europe in the nineteenth century [4].

Morphological characteristics applied for modern taxonomy of oomycetes are often too variable to distinguish between close species of the Peronosporaceae. In addition, as they are unculturable in artificial media, cultural features are unavailable for classification and identification of this group. Instead, their high host specificity limited to a host genus or species is one of the most useful

characters, although there are some exceptional species with a broad host range, e.g. *Pseudoperonospora cubensis* clade 1 [5] and *Plasmopara viticola* clade 1 [6,7]. The recent advance of molecular phylogenetic analysis has led to marked changes and developments in taxonomy of two genera *Peronospora* and *Plasmopara* [3,8,9]. However, long-forgotten species, which have been previously recorded but neither collected again or analyzed, are key pieces for solving the puzzle of studying their diversity and taxonomy. In some cases, however, it is still uncertain if such species are present or wrongly recorded by misidentification of pathogen or/and host plant. However, since most of them have been on unusual host plants, their inclusion is essential to understand the evolutionary relationship between pathogen and host plants. Unfortunately, they are mostly based on a single or few collection, which is frequently hard to access and even if accessed, it is challenging to obtain sequence data from such old collections.

During the surveys of the species diversity of Peronosporaceae in Korea [10–14], we collected several rare species of *Peronospora* and *Plasmopara*, which have been previously recorded but not been subjected to modern taxonomic and phylogenetic approaches. The present study performed

Table 1. Information of Oomycetes specimens used in this study.

Oomycete species	Host plant	Geographic origin	Herb. No.	GenBank accession No.		
				ITS rDNA	cox2 mtDNA	LSU rDNA
<i>Peronospora cheonopodii-ambrosioides</i>	<i>Chenopodium ambrisioides</i>	Korea, Jeju	KUS-F20063	MT734673	MT731360	–
<i>P. cheonopodii-ficifolii</i>	<i>Chenopodium ficifolium</i>	Korea, Namyangju	KUS-F18857	AY211018	MT731363	–
<i>P. clinopodii</i>	<i>Clinopodium cf. vulgare</i>	Korea, Yangpeong	KUS-F20032	MT734670	MT731361	–
<i>P. elsholtziae</i>	<i>Elsholtzia ciliata</i>	Korea, Chuncheon	KUF-F20252	MT734671	MT731362	–
<i>P. lathyrina</i>	<i>Lathyrus japonicus</i>	Korea, Samcheok	KUS-F19431	MT734672	MT731359	–
<i>Plasmopara angustiterminalis</i>	<i>Xanthium strumarium</i>	Korea, Jeongseon	KUS-F24490	–	MT731364	MT29826
<i>Pl. siegesbeckiae</i>	<i>Siegesbeckia glabrescens</i>	Korea, Hoengseong	KUS-F21312	–	EU743805	EU743814

morphological and molecular phylogenetic analyses for the Korean specimens. Seven species of *Peronospora* and *Plasmopara* are recorded here; *Pl. angustiterminalis* (ex *Xanthium strumarium*), *Pl. siegesbeckiae* (ex *Siegesbeckia glabrescens*), *P. cheonopodii-ambrosioides* (ex *Chenopodium ambrosioides*), *P. cheonopodii-ficifolii* (ex *Chenopodium ficifolium*), *P. clinopodii* (ex *Clinopodium cf. vulgare*), *P. elsholtziae* (ex *Elsholtzia ciliata*), and *P. lathyrina* (ex *Lathyrus japonicus*).

2. Materials and methods

2.1. Oomycete samples

Plants with oomycete infection were collected from different sites of Korea. Information on the dried herbarium samples selected for morphological and molecular phylogenetic analyses is provided in Table 1.

2.2. Morphological analysis

Conidiophores (or Sporangioophores) and conidia (or sporangia) formed under the infected leaves were transferred to a drop of lactic acid on a slide glass, covered with a coverslip, and gently warmed up using an alcohol lamp. The microscope preparations were examined under a DIC-light microscope (BX53F, Olympus, Tokyo, Japan) and photographed with a DigiRetina 16M digital camera (Tucsen, Fuzhou, China).

2.3. DNA extraction, PCR, and sequencing

Genomic DNA was extracted from the infected plant leaf using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The mitochondrial cytochrome c oxidase subunit 2 (*cox2*) gene was amplified with primers *cox2-F* [15] and *cox2-RC4* [16]. An additional marker for *Peronospora* species, the internal transcribed spacer (ITS) region was amplified with primers ITS1-O [17] and LR0 [18], and for *Plasmopara* species the D1-3 region of the 28S ribosomal large subunit (LSU) was amplified with primers LR0R [18] and LR6-O [19]. PCR products were purified and sequenced by a DNA sequencing

service (Macrogen Inc., Seoul, Korea), with the same primers used for amplification.

2.4. Phylogenetic analysis

Sequences of the ITS, LSU rDNA, and *cox2* mtDNA were edited using the DNASTAR software package ver. 5.05 (DNASTar Inc., Madison, WI). The sequences of each locus were aligned using the Q-INS-I algorithm [20] in MAFFT 7 [21], along with the reference sequences retrieved from GenBank. Maximum likelihood (ML) and minimum evolution (ME) methods were used to infer the phylogenetic trees. For ML analysis, 1000 rounds of random addition of sequences as well as 1000 fast bootstrap replicates were performed using RAXML 7.0.3 [22] with GTRCAT model. ME analysis was performed using MEGA 7.0 [23] with the default settings of the program, except for replacement with the Tamura-Nei model, and the robustness of the ME tree was evaluated with 1000 bootstrap replicates.

3. Results and discussion

Phylogenetic relationships of two *Plasmopara* species parasitic to *Xanthium strumarium* and *Siegesbeckia glabrescens* were inferred using ML and ME analyses of LSU rDNA and *cox2* mtDNA sequences. The reference sequences of other *Plasmopara* species were retrieved from a phylogenetic analysis of Choi et al. [24], and their GenBank accession numbers were shown in Table 2. As two topologies generated from ML and ME inferences were completely compatible (data not shown), except for minor differences in supporting values, only the ML tree is shown for each locus (Figure 1(A) for LSU and 1(B) for *cox2*), with ML and ME bootstrap values higher than 60% at the first and second position above the branches. In LSU and *cox2* trees, it was confirmed that *Plasmopara* specimen ex *X. strumarium* matches with a reference sequence of *Pl. angustiterminalis* in Bulgaria and that *Pl. siegesbeckiae* ex *S. glabrescens* is distinguished between other *Plasmopara* species.

For *Peronospora* species ML and ME trees were constructed using ITS rDNA and *cox2* mtDNA sequences (Figure 2(A) for ITS and 2(B) for *cox2*). Five *Peronospora* specimens in Korea, parasitic on

Table 2. Genbank accession numbers of the ITS, 28S rDNA, and *cox2* mtDNA sequences used for the phylogenetic study.

Oomycete species	Genbank accession number		
	ITS rDNA	28s rDNA	<i>cox2</i> mtDNA
<i>P. boni-henrici</i>	AY198286	–	DQ365720
<i>P. campestris</i>	AY608609	–	KJ654274
<i>P. chenopodii</i>	EF614955	–	KJ654201
<i>P. chenopodii-polyspermi</i>	AY198291	–	KJ654202
<i>P. chrysosplenii</i>	DQ643839	–	KJ654280
<i>P. corydalis</i>	AY211015	–	KJ654280
<i>P. effusa</i>	DQ643876	–	FJ649411
<i>P. lamii</i>	DQ643902	–	JF527436
<i>P. manshurica</i>	AY286222	–	AY286222
<i>P. rumicis</i>	AY465758	–	KJ654168
<i>P. trigonotidis</i>	AY608611	–	KJ654301
<i>P. vaiabilis</i>	EF614967	–	DQ365734
<i>Pl. angustiterminalis</i>	–	EU743802	EU743812
<i>Pl. baudysii</i>	–	AY250147	–
<i>Pl. chaerophylli</i>	–	AY250163	–
<i>Pl. densa</i>	–	AY035525	DQ365754
<i>Pl. epilobii</i>	–	AY250178	–
<i>Pl. geranii</i>	–	DQ148403	–
<i>Pl. geranii-sylvatici</i>	–	DQ148401	–
<i>Pl. halstedii</i>	–	AY035523	EU743813
<i>Pl. megasperma</i>	–	–	DQ365755
<i>Pl. nivea</i>	–	–	DQ365756
<i>Pl. obducens</i>	–	AY035522	DQ365757
<i>Pl. pimpinellae</i>	–	AY035519	DQ365758
<i>Pl. praetermissa</i>	–	DQ148395	–
<i>Pl. pusilla</i>	–	DQ148402	DQ365759
<i>Pl. sii</i>	–	AY035518	–
<i>Pl. solidaginis</i>	–	AY250144	–
<i>Pl. umbelliferarum</i>	–	AY250161	–
<i>Pl. viticola</i>	–	AY035524	DQ365760
<i>P. belbahrii</i>	KX228833	–	KT828759
<i>P. elsholtziae</i>	FJ527442	–	FJ527435
<i>P. swiglei</i>	–	–	FJ394338
<i>P. viciae</i>	MN620384	–	KJ654294
<i>P. lathyriana</i>	EU174896	–	–

Chenopodium ambrosioides, *Chenopodium ficifolium*, *Clinopodium* cf. *vulgare*, *Elsholtzia ciliata*, and *Lathyrus japonicus*, were compared with the reference sequences of other *Peronospora* species which were investigated in previous studies [9,12]. As this study initially provides the sequence data for these species, except for *P. elsholtziae*, there was no sequence available in GenBank. The GenBank accession numbers of the sequences used in this study were shown in Table 2. In both ITS and *cox2* trees, *P. chenopodii-ficifolii* ex *C. ficifolium* was closely related to *P. variabilis* ex *C. album*, with the maximum supporting value in ITS tree and high values of 99/98 in *cox2* tree, but other four species of *Peronospora*, *P. chenopodii-ambrosioidis* (ex *Chenopodium ambrosioides*), *P. clinopodii* (ex *Clinopodium* cf. *vulgare*), *P. elsholtziae* (ex *Elsholtzia ciliata*), and *P. lathyriana* (ex *Lathyrus japonicus*), formed each independent branch. In ITS tree, *P. lathyriana* was clearly distinguished from *P. viciae*, another downy mildew species on Fabaceae.

Through a microscopic examination, the morphological characteristics of two *Plasmopara* and five *Peronospora* species were described in detail below. Each species resembles ones of the original description. Based on phylogenetic and

morphological analyses, it was confirmed that there are five species of *Peronospora* (*P. chenopodii-ambrosioidis*, *P. chenopodii-ficifolii*, *P. clinopodii*, *P. elsholtziae*, and *P. lathyriana*) and two species of *Plasmopara* (*Pl. angustiterminalis* and *Pl. siegesbeckiae*), all of which have been unrecorded in Korea.

4. Taxonomy

Plasmopara angustiterminalis Novot., Bot. Zh. SSSR 47(7): 979 (1962) [MB#337101] (Figure 3(A–E)).

Description: Lesions commonly present on leaves, causing discoloration of the tissues and deformation of the attacked organs, polyangular, vein-limited, frequently covering larger areas by coalescing; infected tissues become necrotic. Down hypophyllous, whitish, consisting of agglomerated to scattered sporangiophores, felt-like, dense. Haustoria intracellular, not branched, flask-shaped vesicle, symmetrical, 11–20 µm diam., with a stalk at the part of entry into the host cell, surrounded by sheaths with 0.5–2 µm thickness. Sporangiophores emerging through stomata, subhyaline, straight to substraight, (200-)250-400(-550) µm long ($n = 50$); trunk straight, (150-)200-250(-400) µm long, 6–10 µm wide, base not or somewhat swollen, up to 11 µm wide, callose plugs commonly present; branches arising at a right angle to the main axis, monopodial, of 3–5 orders; callose plugs frequently present. Ultimate branchlets in pairs or three, straight, 4–14 µm long, 1–2.5 µm wide at the base; tip truncate or cup-like, rarely swollen. Sporangia subhyaline, broadly ellipsoidal, but sometimes ellipsoidal, (17-)19-22(-24) (av. 19.9) µm long, (11-)13-15(-17) (av. 14.6) µm wide, l/w ratio 1.2-1.5 (av. 1.37, $n = 50$). Resting organs not seen.

Habitat: on living leaves of *Xanthium strumarium*.

Specimen examined: Korea, Gangwon-do, Jeongseon-gun, 37°15'29 "N 128°21'35"E, 7 September 2009, H. D. Shin & Y. J. Choi, NIBRFG0000503192 (KUS-F24490).

Typus: on *Xanthium strumarium*; Ukraine, Zakarpatska, July 1960, M. K. Chochrjakov.

Note: The morphological features of the Korean specimen are consistent with the original description of *Pl. angustiterminalis* [25], except for the size of sporangia; in the Korean sample (av. 19.9 × 14.6 µm) it is smaller than original one (av. 25 × 16 µm), but close to its measurements of China (av. 19.39 × 15.0 µm) [26] and Austria (ca. 12–27 × 9–18 µm) [27]. Also, Komjáti et al. [28] recorded this species displays a high variation in sporangia, as av. 41.1 ± 7.8 × 27.0 ± 4.6 µm. In BLASTn search of LSU and *cox2* sequences, this

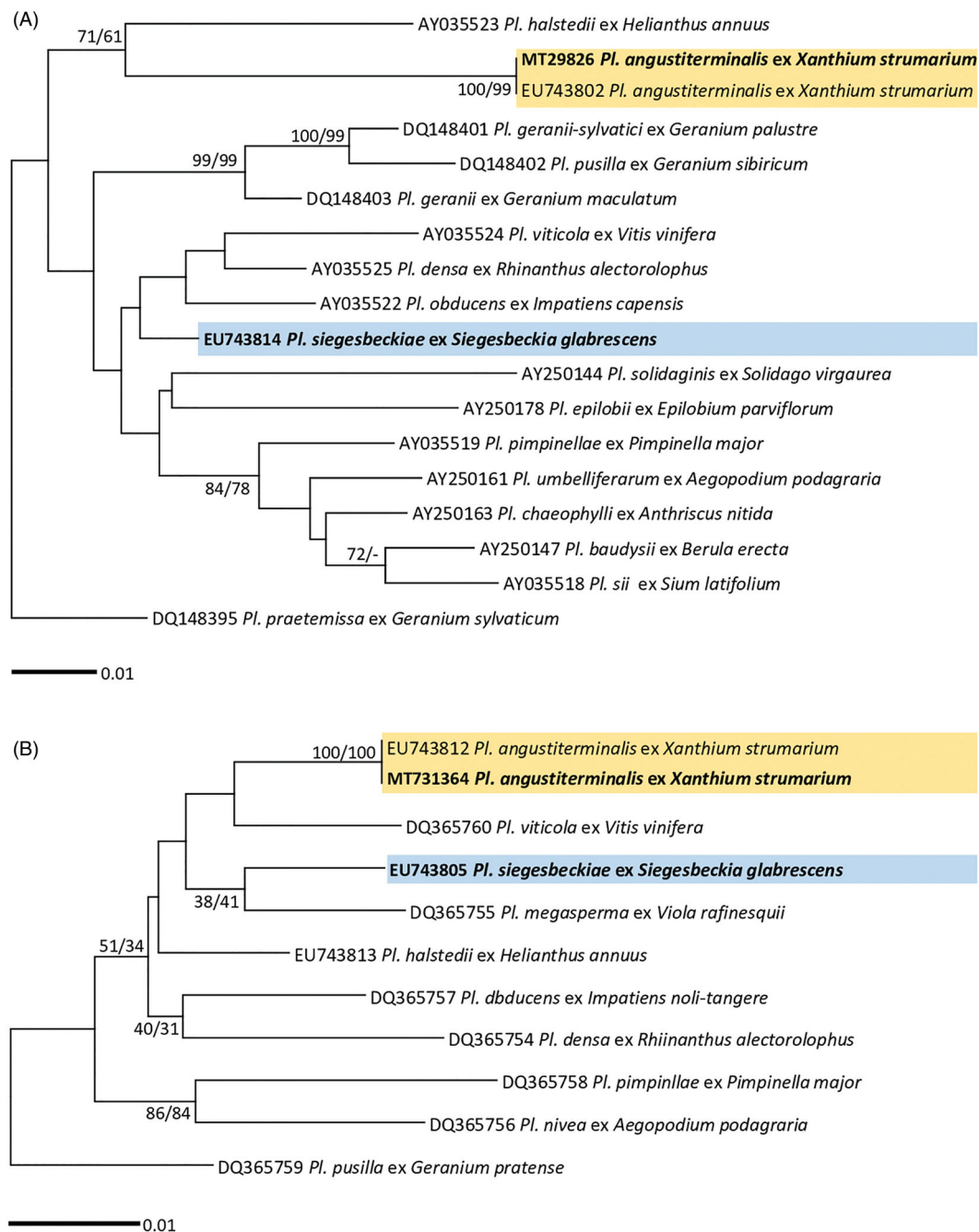


Figure 1. Maximum likelihood trees of *Plasmopara* species based on the LSU ribosomal DNA (D1/D2/D3) sequences (A) and the *cox2* mitochondrial DNA sequences (B), with support values in minimum evolution inference. Bootstrapping support values (minimum evolution/maximum likelihood) higher than 60% are given above the branches. The scale bar equals the number of nucleotide substitutions per site.

Korea specimen was identical to a Hungarian sample of *Pl. angustiterminalis* (SOMF07198). This species seems common in Europe, but rare in other continents [29]. In Asia, this species has been previously recorded in China [26], and this is the first report of *Pl. angustiterminalis* in Korea.

Plasmopara siegesbeckiae (Lagerh.) J. F. Tao, Acta Mycol. Sin. 6 (2): 69 (1987) [MB#131964] (Figure 3(F–J)).

Description: Lesions commonly present on leaves, causing discoloration of the tissues and deformation of the attacked organs, poly-angular, vein-limited, frequently covering larger areas by

coalescing; infected tissues become necrotic. Down hypophyllous, whitish, consisting of agglomerated to scattered sporangiophores, felt-like, dense. Haustoria intracellular, not branched, flask-shaped vesicle, symmetrical, 11–20 μm diam., with a stalk at the part of entry into the host cell, surrounded by sheaths with 0.5–2 μm thickness. Sporangiophores emerging through stomata, subhyaline, straight to substraight, (150–)200–350(–550) μm long ($n=50$); trunk straight, (100–)150–250(–400) μm long, 6–10 μm wide, base not or somewhat swollen, up to 11 μm wide, callose plugs commonly present; branches arising at a right angle to the main axis,

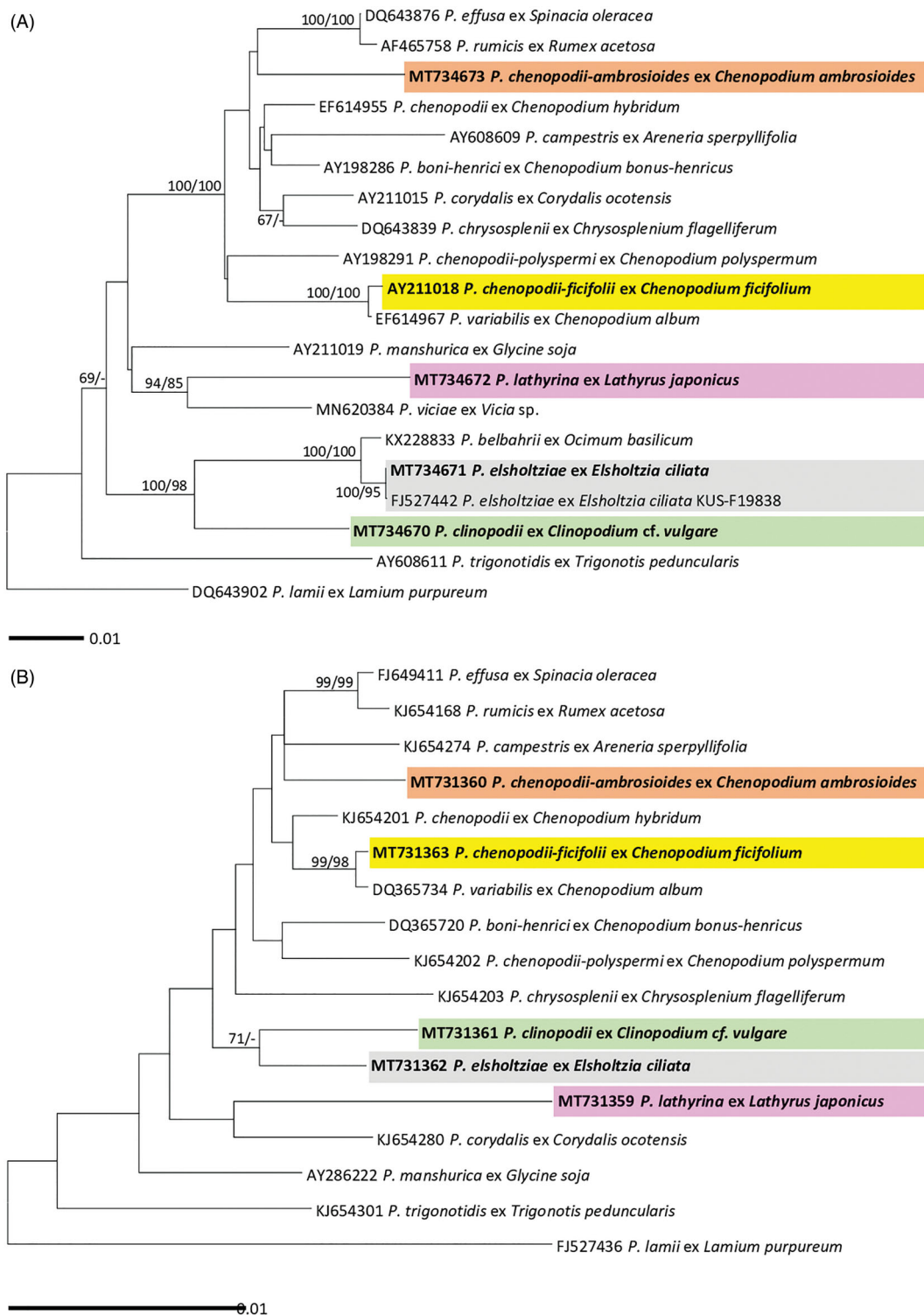


Figure 2. Maximum likelihood trees of *Peronospora* species based on the internal transcribed spacer (ITS) sequences (A) and the *cox2* mitochondrial DNA sequences (B), with support values in minimum evolution inference. Bootstrapping support values (minimum evolution/maximum likelihood) higher than 60% are given above the branches. The scale bar equals the number of nucleotide substitutions per site.

monopodial, of 3–5 orders; callose plugs frequently present. Ultimate branchlets in pairs or three, straight, 4–13 μm long, 1–2.5 μm wide at the base; tip truncate or cup-like, rarely swollen. Sporangia subhyaline, broadly ellipsoidal, but sometimes ellipsoidal, (17.1–)18–22(–24) (av. 19.9) μm long, (11–)13–

16(–19) (av. 14.6) μm wide, l/w ratio 1.2–1.5 (av. 1.37, $n = 100$). Resting organs not seen.

Habitat: on living leaves of *Siegesbeckia glabrescens*.

Specimen examined: Korea, Gangwon-do, Hoengseong-gun, 37°32'20"N 128°6'40"E, 4

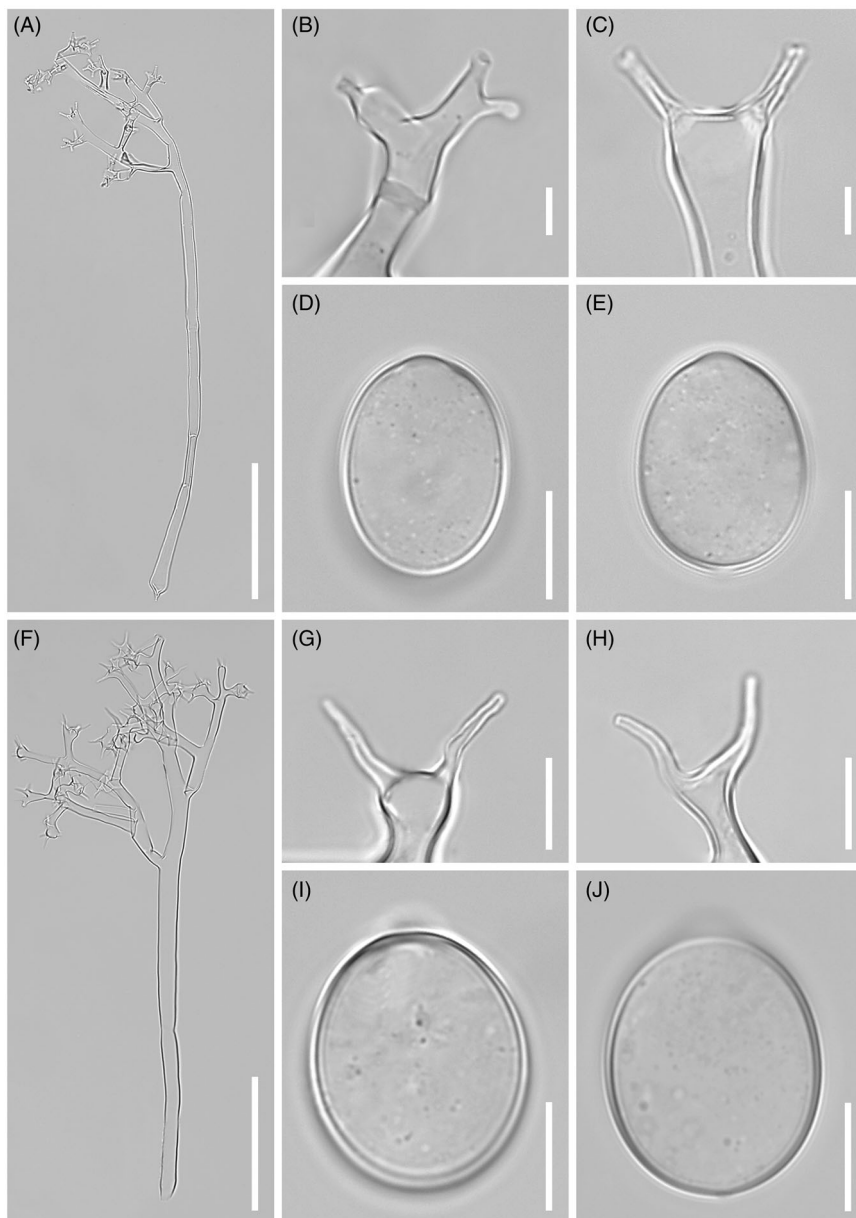


Figure 3. Morphological characteristics of two *Plasmopara* species, *Pl. angustiterminalis* ex *Xanthium strumarium* (A–E) and *Pl. siegesbeckiae* ex *Siegesbeckia glabrescens* (F–J). (A & F), Sporangiophores; (B, C, G, H), Ultimate branchlets; (D, E, I, J), Sporangia (scale bars: 100 μm for sporangiophores, 10 μm for ultimate branchlets, and 10 μm for sporangia).

September 2005, H. D. Shin & Y. J. Choi, NIBRFG0000503193 (KUS-F21312).

Typus: on *Siegesbeckia orientalis*; China, July 1979, J. F. Tao (Herb. No. 1373).

Note: This species was first reported as *Peronospora leptosperma* var. *siegesbeckiae* by Lagerh in 1895. Since then, Tao and Qin [30] have combined it under the genus *Plasmopara* and promoted to the specie level, *Plasmopara siegesbeckiae*. The morphological characteristics of the Korean specimen resemble the original description [30], except for the narrower sporangia (11–19 versus 16.6–20.6 μm) and two different host species (*Siegesbeckia glabrescens* versus *S. orientalis*). *Pl. siegesbeckiae* has been recorded previously in China

and presently in Korea, hinting at it as an East Asian species.

Peronospora chenopodii-ambrosioidis Golenia, Monogr. bot., Soc. bot. Polon. 13: 147 (1962) [MB#335900] (Figure 4(A, F, K, P)).

Description: At initial infection, lesions began yellowish then darken to become purplish, vein-delimited, resulting in a polyangular, mosaic appearance on leaves. Down present on the lower leaf surface of leaves, sparse to dense, felt-like, purplish, or beige. Conidiophores 220–500 μm long, colorless, straight; trunk 150–300 μm long, 5–10 μm wide, basal end slightly bulbous, up to 13 μm wide, callose plugs absent. Branches sub-dichotomously or

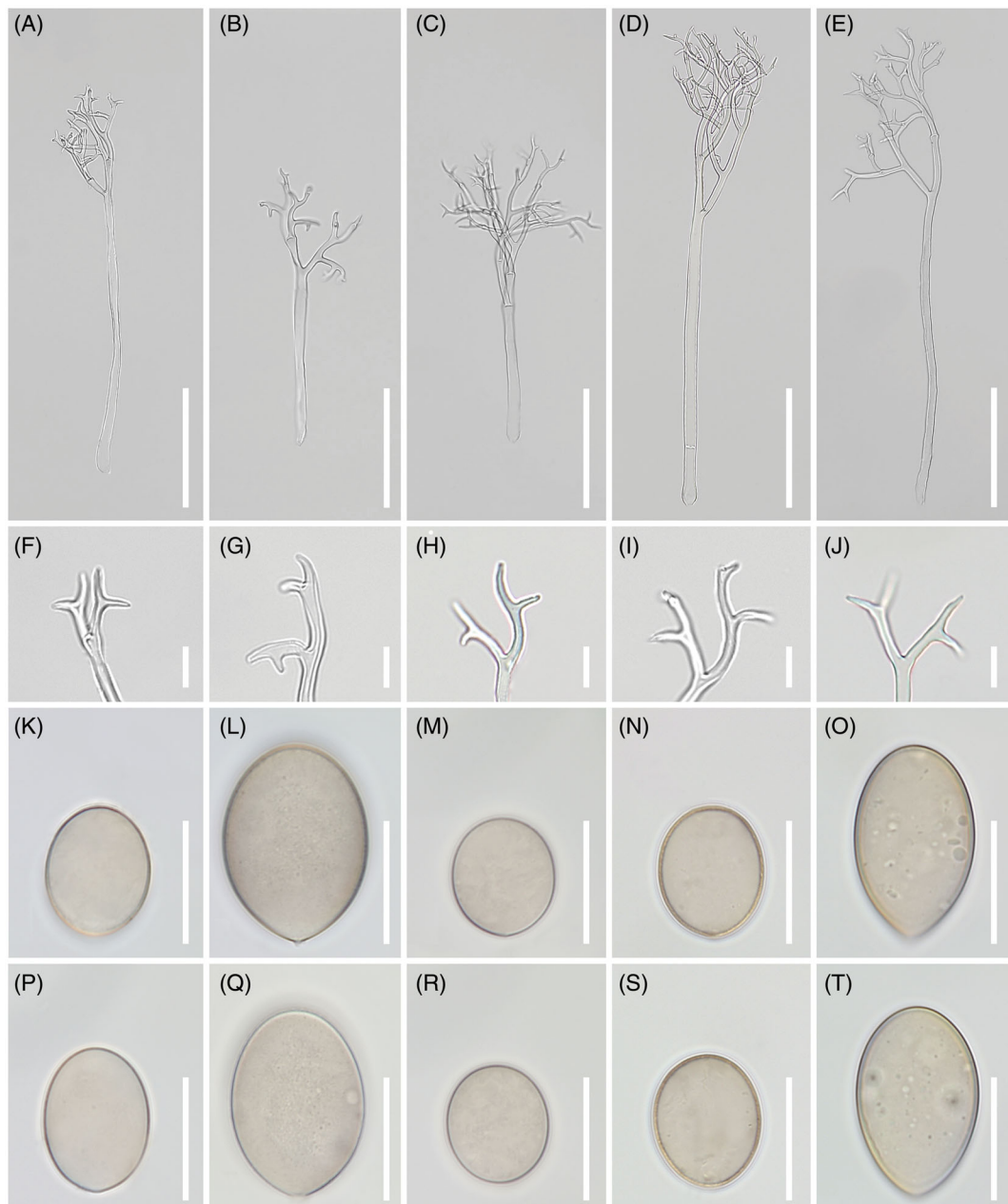


Figure 4. Morphological characteristics of five *Peronospora* species, *P. chenopodii-ambrosioidis* ex *Chenopodium ambrosioides* (A, F, K, P), *P. chenopodii-ficifolii* ex *Chenopodium ficifolium* (B, G, L, Q), *P. clinopodii* ex *Clinopodium* cf. *vulgare* (C, H, M, R), *P. elsholtziae* ex *Elsholtzia ciliata* (D, I, N, S), and *P. lathyirna* ex *Lathyrus japonicus* (E, J, O, T). (A–E), Conidiophores; (F–J), Ultimate branchlets; (K–T), Conidia (scale bars: 100 μm for conidiophores, 10 μm for ultimate branchlets, and 20 μm for conidia).

monopodially branched 4–6 orders, substraight to slightly curved; ultimate branchlets flexuous to curved, 5–11 μm long, 1.5–2 μm wide, apex obtuse. Conidia olivaceous with gray tint, broadly ellipsoidal to ellipsoidal, greatest width median in most conidia, (17–)18–23(–28) (av. 21.5) μm long, (13–)15–19(–22) (av. 17.1) μm wide, l/w ratio = 1.2–1.5 ($m = 1.32$), pedicel absent in most conidia, sometimes appeared protruding. Resting organs not seen.

Habitat: on living leaves of *Chenopodium ambrosioides*.

Specimen examined: Korea, Jeju-do, 33°27'34"N 126°33'50"E, 12 November 2003, H. D. Shin & Y. J. Choi, NIBRFG0000503198 (KUS-F20063).

Typus: on *Chenopodium ambrosioides*; Poland, Poznań, no date information, A. Golenia (Herb. Inst. For Medicinal Plants Research, Poznań).

Note: This is the first report of *P. chenopodii-ambrosioidis* in Asia. All morphological features of the present specimen are consistent with the original description of Golenia [31]; the size of conidia (av. 21.5 \times 17.1 versus 22.5 \times 17.2 μm), a length to width ratio of conidia (av. 1.32 versus 1.3), and the length of conidiophores (220–500 versus 192–640 μm). Although the downy mildew infections on *C. ambrosioides* have been recorded worldwide [29], the causal agents have been often mis-identified as either *P. farinosa* or *P. chenopodii*, due to the

complex nomenclatural history for *Peronospora* species infecting Chenopodiaceae plants [12,32,33].

Peronospora chenopodii-ficifolii Sawada, Taiwan, Sotokufu Noji Shikenjo (Agr. Exp. Stat. Formosa): 9 (1916) [MB#176653] (Figure 4(B, G, L, Q)).

Description: At initial infection, lesions began yellowish then darken to become purplish, vein-delimited, resulting in a polyangular, mosaic appearance on leaves. Down present on the lower leaf surface of leaves, sparse to dense, felt-like, purplish, or beige. Conidiophores (200-)250-350(-500) μm long, straight; trunk (100-)150-200(-300) μm long, 8–10 μm wide, basal end not differentiated, rarely bulbous, up to 15 μm wide. Branches sub-dichotomously branched 5–7 orders slightly curved to sigmoid; ultimate branchlets flexuous to curved, 6–20 μm long, apex obtuse. Conidia olivaceous to brown, broadly ellipsoidal to ellipsoidal, greatest width median in most conidia, (20-)26-35 μm long, 20–25 μm wide, l/w ratio = 1.2–1.5, pedicel present. Resting organs not seen.

Habitat: on living leaves of *Chenopodium ficifolium*.

Specimen examined: Korea, Namyangju-si, 37°54'44"N 127°2'0"E, 23 May 2016, H. D. Shin & Y. J. Choi, NIBRFG0000503199 (KUS-F29169).

Typus: on *Chenopodium ficifolium*; China (Taiwan), June 1916, no details about the collection were provided.

Note: All morphological characteristics fit well with the original description of *P. chenopodii-ficifolii* [34]. After the first description, this species has been long forgotten due to the same reason with *P. chenopodii-ambrosioidis*. The Korean specimens have been previously classified under the name *P. farinosa* [10] that has been recently rejected [33]. In the present morphological and phylogenetic analyses, *P. chenopodii-ficifolii* is distinguishable from other species of *Peronospora* parasitic to *Chenopodium* spp., and thus we treat it as a distinct species. This species seems common in Europe, as well as East Asia, including China, Japan, and Korea [29].

Peronospora clinopodii Terui, Ann. Phytopath. Soc. Japan: 524 (1978) [MB#319385] (Figure 4(C, H, M, R)).

Description: At initial infection, lesions began yellowish or light greenish, and then darken to become purplish, vein-delimited, resulting in a polyangular, mosaic appearance on leaves. Down present on the lower leaf surface of leaves, sparse to dense, felt-like, purplish or beige. Conidiophores protruding from stomata on the underside of leaves, erect, hyaline, sub-dichotomously to monopodially or dichotomously branched 5–8 orders, (150-)200-300(-400) μm long, 9–11 μm wide; basal end slightly swollen (av. 13 μm). Ultimate branchlets slightly

curved, with somewhat different lengths, 5–10 μm . Conidia subglobose to broadly ellipsoidal, olivaceous to light brownish, (17-)19-23(-25) μm long (av. 20.4), (15-)17-20(-23) (av. 18.7) μm wide, with a length to width ratio of 1.1–1.25 (av. 1.15, $n = 50$); pedicel absent or appeared as a scar.

Habitat: on living leaves of *Clinopodium* cf. *vulgare*.

Specimen examined: Korea, Yangpyeong-gun, 37°30'10"N 127°43'24"E, 4 November 2003, H. D. Shin & Y. J. Choi, NIBRFG0000503197 (KUS-F20032).

Typus: on *Clinopodium sachalinense*; Japan, Aomori Pref., Mt. Ajara, 10 October 1974, M. Teuri

Note: *Peronospora clinopodii* was first reported by Terui [35] in Japan. The morphological features of the present specimen are similar to the original description, except for the length of conidiophores (150–400 μm versus 300–500 μm). In addition, it was parasitic on *Clinopodium* cf. *vulgare* in Korea, but on *Clinopodium sachalinense* in Japan. *P. clinopodii* seems to distribute only in East Asia, including Korea and Japan.

Peronospora elsholtziae T. R. Liu & C. K. Pai, Acta Mycol. Sin. 4(1): 5 (1985) [MB#105621] (Figure 4(D, I, N, S)).

Description: At initial infection, lesions began yellowish then darken to become purplish, vein-delimited, resulting in a polyangular, mosaic appearance on leaves. Down present on the lower leaf surface of leaves, sparse to dense, felt-like, purplish, or beige. Conidiophores protruding from stomata on the underside of leaves, erect, hyaline, monopodially to rarely subdichotomously branched 5–7 orders, (300-)350-500(-600) μm long, 8–12 μm wide; basal end not or slightly swollen at the base up to 13 μm . Ultimate branchlets straight to curved, mostly in pair, with somewhat different lengths, 5–10 μm . Conidia broadly ellipsoidal to ellipsoidal, olivaceous to light brownish, (18-)20-22.5(-24) μm long (av. 20.9), (16-)18-20(-21) μm wide (av. 18.4), with a length to width ratio of 1.05–1.25 (av. 1.13, $n = 50$). Resting organs not seen.

Habitat: on living leaves of *Elsholtzia ciliata*.

Specimen examined: Korea, Chuncheon-si, Sporangia, 37°56'8"N 127°39'35"E, 1 June 2004, H. D. Shin & Y. J. Choi, NIBRFG0000503196 (KUS-F20252).

Typus: on *Elsholtzia ciliata*; China, Heilongjiang, Mi-Shan Reg., June 1981, T. R. Liu.

Note: Before the present study, *P. elsholtziae* has been recorded only in China [29,36], and this is the first record of *P. elsholtziae* in Korea. The morphological features of the present specimen are consistent with the original description [36], although the conidia of the Korean sample (av. 20.9 \times 18.4 μm)

are somewhat smaller than the original ones (av. $23.9 \times 20.8 \mu\text{m}$).

Peronospora lathyrina Vienn.-Bourg., Bull. trimest. Soc. mycol. Fr. 66: 64 (1950) [MB#302539] (Figure 4(E, J, O, T)).

Description: Lesions commonly present on leaves, causing discoloration of the tissues and deformation of the attacked organs, poly-angular, vein-limited, frequently covering larger areas by coalescing; infected tissues become necrotic. Down hypophyllous, whitish, consisting of agglomerated to scattered conidiophores, felt-like, dense. Haustoria intracellular, not branched, flask-shaped vesicle, symmetrical, $11\text{--}20 \mu\text{m}$ diam., with a stalk at the part of entry into the host cell, surrounded by sheaths with $0.5\text{--}2 \mu\text{m}$ thickness. Conidiophores emerging through stomata, subhyaline, straight to substraight, $(200\text{--})250\text{--}400\text{--}(550) \mu\text{m}$ long ($n=50$); trunk straight, $(150\text{--})200\text{--}250\text{--}(400) \mu\text{m}$ long, $6\text{--}10 \mu\text{m}$ wide, base not or somewhat swollen, up to $11 \mu\text{m}$ wide, callose plugs commonly present; branches arising at a right angle to the main axis, monopodial, of 3–5 orders; callose plugs frequently present. Ultimate branchlets in pairs or three, straight, $4\text{--}13 \mu\text{m}$ long, $1\text{--}2.5 \mu\text{m}$ wide at the base; tip truncate or cup-like, rarely swollen. Conidia subhyaline, broadly ellipsoidal, but sometimes ellipsoidal, $(20\text{--})22\text{--}26\text{--}(30) \mu\text{m}$ long (av. 23.6), $(11\text{--})13\text{--}15\text{--}(17) \mu\text{m}$ wide (av. 14.5), l/w ratio $(1.2\text{--})1.3\text{--}1.6\text{--}(1.7)$ (av. 1.55 $n=100$). Resting organs not seen.

Habitat: on living leaves of *Lathyrus japonicus*.

Specimen examined: Korea, Samcheok-si, Miro-myeon, $37^{\circ}24'52''\text{N}$ $129^{\circ}6'2''\text{E}$, 8 May 2003, H. D. Shin & Y. J. Choi, NIBRFG0000503195 (KUS-F19431).

Typus: on *Lathyrus japonica*; France, Alpes Mts, Pelvoux Mt., Gironde valley, between the village Vollouise and Glacier Blanc shelter, 22 August 1948, G. Viennot-Bourgin.

Note: *Peronospora lathyrina* was first described by Viennot-Bourgin [37] in France. The conidial size and l/w ratio of the Korean sample are slightly larger than ones of the original description; av. $21.4 \times 16.3 \mu\text{m}$ versus $23.6 \times 14.5 \mu\text{m}$ in size and av. 1.31 versus 1.55 in l/w ratio. In addition, the host species of the Korean sample is different from the original specimen; *Lathyrus japonicus* versus *Lathyrus latifolius*. Taxonomy and nomenclature of Fabaceae-infecting downy mildews, including *P. lathyrina*, are still in confusion [38], and further study using additional specimens from different host plants is essential to their correct species definition and identification.

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