Taxonomy and Phylogeny of Peronospora Species (Oomycota) Parasitic to Stellaria and Pseudostellaria in Korea, with the Introduction of Peronospora casparyi sp. nov.

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Abstract The genus Peronospora, an obligate biotrophic group belonging to Oomycota, causes serious damage to a variety of wild and ornamental plants, as well as cultivated crops, such as beet, rose, spinach, and tobacco. To investigate the diversity of Peronospora species parasitic to Stellaria and Pseudostellaria (Caryophyllaceae) plants in Korea, we performed a morphological analysis on dried herbarium specimens and molecular phylogenetic inferences based on internal transcribed spacer rDNA and cox2 mitochondrial DNA sequences. As a result, it was confirmed that there are four species of Peronospora parasitic to specific species of Stellaria and Pseudostellaria, all of which were hitherto unrecorded in Korea: P. alsinearum (ex Stellaria media), P. stellariae-aquatica (ex Stellaria aquatica), P. stellariae-uliginosae (ex Stellaria alsine), and P. pseudostellariae (ex Pseudostellaria palibiniana). In addition, Peronospora specimens parasitic to Pseudostellaria davidii differed morphologically from P. pseudostellariae owing to the large and ellipsoidal conidia; this morphological discrepancy was also validated by the high genetic divergence between the two species. Peronospora casparyi sp. nov. is described and illustrated here.

Keywords Barcoding, Caryophyllaceae, cox2, Diversity, Host specificity

The Oomycota is a distinct phylogenetic lineage of fungus-like microorganisms. The genus Peronospora (Peronosporaceae) is one of the most diverse and economically important oomycete groups, which causes the notorious downy mildew disease on a wide range of cultivated and ornamental plants. They are likely the most widespread and potentially destructive pathogens in the cultivations of sugar beet (Beta vulgaris), roses (Rosa hybrida), berries (Rubus spp.), tobacco (Nicotiana tabacum), quinoa (Chenopodium quinoa), basil (Ocimum basilicum), and spinach (Spinacia oleracea) [1].

The Caryophyllaceae plants form a clade of approximately 70 genera and 2,200 species of herbs and subshrubs [2]. Although their centre of diversity is in the Mediterranean and Middle Eastern regions, they also include genera endemic to North America, South America, Africa, and Asia [3]. To date, 44 names of Peronospora, the third highest number following ones introduced on Fabaceae and Chenopodiaceae, have been described as the downy mildew pathogens infecting Caryophyllaceae [4]. In Korea, however, only two species, P. campestris (ex Arenaria serpyllifolia) and P. conferta (ex Cerastium holostoides), have been reported hitherto [5].

During a survey of the diversity of Peronosporaceae in Korea, numerous samples associated with Peronospora have been collected on plants of two genera of Caryophyllaceae, Stellaria and Pseudostellaria. The Stellaria is a cosmopolitan group with about 90–120 species, but the Pseudostellaria is mainly distributed in Asia, with up to 20 species. So far, six species of Peronospora have been described on different species of Stellaria; P. alsinearum (ex S. media) [6], P. media (ex S. media, S. nemorum), P. parva (ex S. holostea) [7], P. stellariae-aquatica (ex S. aquatica = Myosoton aquaticum),...
Oomycete samples. Plants of Stellaria and Pseudostellaria with downy mildew infections 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.

Morphological analysis. Conidiophores, conidia, and resting organs formed from the infected leaves were transferred to a drop of lactic acid on a slide glass, covered with a cover slip, and briefly heated using an alcohol lamp. The microscope preparations were examined under brightfield- and DIC-light microscopes, and photographed using a model BX53F microscope (Olympus, Tokyo, Japan) equipped with a DigiRetina 16M digital camera (Tuscn, Fuzhou, China). Measurements were performed at ×400 for conidia and ×100–1,000 for other organs, and reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of the number of measurements given in parentheses.

DNA extraction, PCR, and sequencing. Genomic DNA was extracted from the infected host tissue using the MagListo 5M Plant Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). Two barcode markers for oomycetes, the ribosomal internal transcribed spacer (ITS) region and the mitochondrial cytochrome c oxidase subunit II (cox2) gene [11], were amplified with primers ITS1-O [12] and LR0 [13] and primers cox2-F [14] and cox2-RC4 [11], respectively. PCR conditions for ITS and cox2 amplifications were identical as outlined in Choi et al. [15]. The PCR products were purified and sequenced by a DNA sequencing service (Macrogen Inc., Seoul, Korea), with the same primers used for amplification.

Phylogenetic analysis. Sequences of the ITS rDNA and the cox2 mtDNA were edited with the DNASTAR software package ver. 5.05 (DNASTar Inc., Madison, WI, USA). Alignments of each locus were performed using the Q-INS-i algorithm [16] in MAFFT 7 [17], in addition to the reference sequences of Peronospora parasitic to Stellaria species, available in GenBank. Maximum likelihood (ML) and minimum evolution (ME) methods were used to infer the phylogenetic trees. For ML analysis, 1,000 rounds of

Table 1. Information of Peronospora specimens parasitic to Stellaria and Pseudostellaria in Korea

| Oomycete species          | Host plant                   | Geographic origin       | Herb. No.          | GenBank accession No. |
|---------------------------|------------------------------|-------------------------|--------------------|-----------------------|
| **P. stellariae-uliginosae** | **Stellaria alpine**         | Korea, Chuncheon        | KUS-F18827         | MF784738              |
| **P. stellariae-uliginosae** | **S. aline**                 | Korea, Chuncheon        | KUS-F15790         | MF784726              |
| **P. stellariae-uliginosae** | **S. aline**                 | Korea, Yangpyeong       | KUS-F21734         | MF784739              |
| **P. stellariae-aquaticae** | **S. aquatic**               | Korea, Chuncheon        | KUS-F17269         | MF784740              |
| **P. stellariae-aquaticae** | **S. aquatic**               | Korea, Hongcheon        | KUS-F17326         | MF784741              |
| **P. stellariae-aquaticae** | **S. aquatic**               | Korea, Chuncheon        | KUS-F29741         | MF784742              |
| **P. parva**               | **S. holostea**              | Austria, Burgenland     | WU22915            | MF784729              |
| **P. parva**               | **S. holostea**              | Germany, Sachsen-Anhalt | GLM65627           | MF784730              |
| **P. ailsinicum**          | **S. media**                 | Korea, Jeju             | KUS-F21708         | MF784730              |
| **P. aisincum**            | **S. media**                 | Korea, Jeju             | KNUH88             | MF784731              |
| **P. ailsinicum**          | **S. media**                 | Austria, Vienna         | WU22876            | MF784731              |
| **P. ailsinicum**          | **S. media**                 | Germany, Sachsen        | GLM78867           | MF784732              |
| **P. pseudostellariae**    | **Pseudostellaria palibiniana** | Korea, Hongcheon     | KUS-F18840         | MF784745              |
| **P. pseudostellariae**    | **Ps. palibiniana**          | Korea, Chuncheon        | KUS-F26706         | MF784746              |
| **P. pseudostellariae**    | **Ps. palibiniana**          | Korea, Hongcheon        | KUS-F24933         | MF784747              |
| **P. pseudostellariae**    | **Ps. palibiniana**          | Korea, Hongcheon        | KUS-F25730         | MF784748              |
| **P. pseudostellariae**    | **Ps. palibiniana**          | Korea, Hongcheon        | KUS-F25743         | MF784749              |
| **Peronospora sp.**        | **Ps. davidii**              | Korea, Hongcheon        | KUS-F18847         | MF784730              |
| **Peronospora sp.**        | **Ps. davidii**              | Korea, Chuncheon        | KUS-F25822         | MF784731              |
random addition of sequences as well as 10,000 fast bootstrap replicates were performed with RAxML 7.0.3 [18] using the GTRCAT model. ME analysis was done using MEGA 7.0 [19] 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 10,000 bootstrap replicates.

RESULTS AND DISCUSSION

The phylogenetic relationships of Peronospora species parasitic on Stellaria and Pseudostellaria species were inferred using ML and ME analyses of the ITS rDNA and cox2 mtDNA sequences. As the tree topologies generated from ML and ME inferences were fully compatible (data not shown), except for minor differences in bootstrapping support values, only the ML tree is shown for each locus (Fig. 1A for ITS and 1B for cox2), with ML and ME bootstrap values higher than 70% at first and second position above the branches.

In both ITS and cox2 trees, the accessions of Peronospora originated from three species of Stellaria and two species of Pseudostellaria formed four highly supported clades; the first monophyletic clade consisting of two Peronospora species, P. pseudostellariae parasitic on Ps. palibiniana and P. stellariae-uliginosae on Stellaria alsine, the second of P. stellariae-aquaticae on Stellaria aquatica, the third of P. alsinearum on S. media, and the fourth of an undetermined species of Peronospora on Ps. davidii. Interestingly, in the first clade, P. pseudostellariae and P. stellariae-uliginosae, were indistinguishable in both gene trees, although a specimen (KUS-F21734) of P. stellariae-uliginosae revealed only one substitution in the ITS sequence, providing evidence of their close genetic affinity. However, we postpone a synonymization of the epithet P. pseudostellariae under P. stellariae-uliginosae to a further study using additional

Fig. 1. Maximum likelihood trees based on the complete internal transcribed spacer (ITS) (ITS1, 5.8S rDNA, and ITS2) sequences (A) and the cox2 mitochondrial DNA sequences (B), with support values in minimum evolution inference. Bootstrapping support values (maximum likelihood/minimum evolution) higher than 70% are given above the branches. The scale bar equals the number of nucleotide substitutions per site.
samples and more variable gene markers. In the third clade of *P. alsinearum*, despite all specimens originating from the same host plant species, *S. media*, a significant level of genetic divergences were found, which was most likely due to the distance in the geographic origin: two samples were obtained from Kores, but the remaining from Austria (ITS: AY198279) and Germany (cox2: KJ654256).

The specimens from *Ps. davidii* were placed as an independent group from Peronospora species on *Stellaria*, as well as *P. pseudostellariae*, and the phylogenetic distances were considerably greater. Their genetic distance from *P. pseudostellariae* was approximately 5.36% (43 out of 802 characters were different) in the ITS sequences and 7.97% (43 out of 539 characters) in the cox2 sequences. Morphological characteristics of Peronospora on *Ps. davidii* markedly differed from those of *P. pseudostellariae*. In the specimens assumed to belong to the new species, the conidia were ellipsoidal, which noticeably distinguished them from the conidia of *P. pseudostellariae* (Fig. 3); a ratio of length to width of Peronospora sp. was (1.21–)1.31–1.54–(1.64) (av. 1.43), while the latter was (1.12–)1.20–1.42(–1.50) (av. 1.29). The conidial size of the former species measured av. 26.64 × 18.72 µm, which are much larger than the latter (av. 18.24 × 14.12 µm). Concerning the length of conidiophores, the former (180–)210–300(–330) µm were shorter than the latter (110–)170–380(–440) µm.

The range of natural hosts of two *Peronospora* species on *Pseudostellaria* hints at an intimate oomycete/plant association. The genus *Pseudostellaria* was subdivided into two series, Glochidiatae and Mamillatae, among which the latter further split into two subseries, Verticillate and Distantes. *Pseudostellaria heterophylla* and *Ps. palibiniana*, the host plants of *P. pseudostellariae*, both belong to the subseries Verticillate, while *Ps. davidii*, the host of *Peronospora* sp., belongs to Distantes [20, 21]. Nonetheless, this correspondence may have resulted from host-shift driven speciation (pseudo-cospeciation), rather than long-term coevolution, as well observed in other downy mildews [22].

**Taxonomy.** Based on the molecular phylogenetic and morphological data, we reported four previously unknown species in Korea, namely *P. alsinearum*, *P. stellariae-aquaticae*, *P. stellariae-uliginosae*, *P. pseudostellariae*, and *P. casparyi* sp. nov.

**Peronospora alsinearum** Casp. (Fig. 2)

**Basionym:** Peronospora alsinearum Casp., Monatsbericht. König. Akad. Wiss. Berlin 1855: 330 (1855) [MB#223417].

**Description:** Down hypophyllous, yellowish to brownish, consisting of scattered patches of conidiophores, rarely of a more or less dense structure. Conidiophores hyaline, slender, (200–)240–350(–390) µm long; trunk straight to slightly curved, (60–)100–220(–260) µm long (n = 50), of more or less uniform width, (6.3–)7.3–10(–11) µm wide at the middle, basal end not differentiated, rarely slightly bulbous, callose plugs absent; branching elaborate, sub- to dichotomous, in (5–)6–7 orders, branches slightly curved; ultimate branchlets mostly in pairs or rarely single, sub-straight to slightly curved to sigmoid, (3–)5–12(–14) µm long, (1.2–)1.5–2.3(–2.6) µm wide at the base (n = 30), apex subtruncate or obtuse. Conidia bright yellowish, broadly ellipsoidal to

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**Fig. 2.** Morphological characteristics of three *Peronospora* species parasitic on *Stellaria* species, *P. alsinearum* ex *Stellaria media* (A–F), *P. stellariae-aquaticae* ex *Stellaria aquatica* (G–O), *P. stellariae-uliginosae* ex *Stellaria alsine* (P–U). A, B, G, H, P–Q, Conidiophores; C, D, I, J, R, S, Ultimate branchlets; E, F, K, L, T, U, Conidia; M–O, Resting organs (scale bars: 100 µm for conidiophores, 20 µm for ultimate branchlets and conidia, and 40 µm for resting organs).
ellipsoidal, (23–)24–29 (–30) µm long (av. 26.97), (18–)19–22 (–23) µm wide (av. 20.76), with a ratio of length to width of (1.13–) 1.20–1.36 (–1.43) (av. 1.30, n = 50), greatest width median, rarely supra-median, tip rounded, base rounded to slightly protruding; germination at the side of conidia, producing germ tubes, up to 100 µm long. Resting organs not seen.

Habitat: Infested leaves of Stellaria media.

Specimen examined: Korea, Jeju-do, Jeju-si, Hallasan National Park, 33°28′11″ N, 126°29′37″ E, 3 Dec 2004, H. D. Shin and Y. J. Choi, KZITFG0000000013 (KUS-F21011); also see Table 1.

Note: Two species of Peronospora, P. alsinicirum [6] and P. media [7], have been described on Stellaria media, but Gustavsson [23] thought that the latter species is conspecific with P. alsinicirum. All morphological characteristics of the Korean samples are consistent with the descriptions for P. alsinicirum [7, 23]. This is the first record of P. alsinicirum in Korea.

**Peronospora stellariae-aquaticae** Sawada (Fig. 2)

**Basionym:** *Peronospora stellariae-aquaticae* Sawada, sec. Sawada 1927, Descript. Cat. Formosa Fungi: 58 (1925) [MB#278783].

**Description:** Down hypophyllous, whitish to greyish, consisting of dense patches of conidiophores, Conidiophores hyaline, slender, (210–)280–470 (–550) µm long; trunk straight to slightly curved, (90–)150–360 (–420) µm long (n = 50), of more or less uniform width, (6–)8–11 (–13) µm, basal end not differentiated, rarely slightly bulbous, callose plugs absent; branching elaborate, sub- to dichotomous, in (5–)6–7 orders, branches slightly curved; ultimate branchlets mostly in pairs, rarely single, straight to slightly curved, (4–)6–8 (–10) µm long, (1–)1.3–1.5 (–1.8) µm wide at the base (n = 30), apex subtruncate or obtuse. Conidia brightly yellowish, ellipsoidal, (14–)15–19 (–20) µm long (av. 17.40), (12–)13–15 (–16) µm wide (av. 14.24), with a ratio of length to width of (0.6–)1.11–1.31 (–1.36) (av. 1.22, n = 50), greatest width median, rarely supra-median, tip rounded, base rounded or narrowing, pedicel absent or hardly visible as a scar to slightly protruding; germination at the side of conidia, producing germ tubes, often various swellings and whirling. Resting organs not seen.

Habitat: Infested leaves and stems of Stellaria alsine.

Specimen examined: Korea, Gangwon-do, Chuncheon-si, Bongmyeong-ri, 37°50′20″ N, 127°47′00″ E, 4 Nov 2004, H. D. Shin & Y. J. Choi, KZITFG0000000016 (KUS-F20953); also see Table 1.

Note: *Peronospora stellariae-uliginosae* has been described on Stellaria uliginosa (now, a synonym of S. alsine). The conidial size of the Korean samples is somewhat smaller than ones of the original description [24]. This is the first record of *P. stellariae-uliginosae* in Korea.

**Peronospora stellariae-uliginosae** Sawada (Fig. 2)

**Basionym:** *Peronospora stellariae-uliginosae* Sawada, see Sawada 1927, Descript. Cat. Formosa Fungi: 59 (1925) [MB#278785].

**Description:** Down on to lower surface of leaves but rarely on stems, greyish to yellowish, consisting of dense patches of conidiophores. Haustoria hyphal, branched, filling the host cell almost completely. Conidiophores hyaline, slender, (170–)280–520 (–600) µm long; trunk straight to slightly curved, (50–)140–310 (–400) µm long (n = 50), of more or less uniform width, (6–)7–10 (–11) µm wide at the middle, callose plugs absent; branching elaborate, sub- to dichotomous, in (5–)6–7 orders, branches slightly curved; ultimate branchlets mostly in pairs, rarely single, sub-straight to slightly curved, (4–)6–8 (–10) µm long, (1–)1.3–1.5 (–1.8) µm wide at the base (n = 30), apex subtruncate or obtuse. Conidia brightly yellowish, ellipsoidal, (14–)15–19 (–20) µm long (av. 17.40), (12–)13–15 (–16) µm wide (av. 14.24), with a ratio of length to width of (0.6–)1.11–1.31 (–1.36) (av. 1.22, n = 50), greatest width median, rarely supra-median, tip rounded, base rounded or narrowing, pedicel absent or hardly visible as a scar to slightly protruding; germination at the side of conidia, producing germ tubes, often various swellings and whirling. Resting organs not seen.

Habitat: Infested leaves of Stellaria alsine.

Specimen examined: Korea, Gangwon-do, Hoengeun-gun, Chudong-ri, 37°28′42″ N, 128°01′34″ E, 8 Sep 2011, H. D. Shin & Y. J. Choi, KZITFG0000000015 (KUS-F26123); also see Table 1.

Note: This is the first record of *P. stellariae-aquaticae* in Korea. The morphological features of the present specimen are consistent with the original description by Sawada [24], except that conidia in the Korean sample (av. 19.55 × 16.51 µm) are somewhat smaller than those of the original description (av. 21.1 × 17.6 µm).
record of this species in Korea. So far, the distribution of *P. pseudostellariae* has been restricted to China [10, 27], but the present study suggests that this species may be commonly present in East Asia.

**Peronospora casparyi** Jae S. Lee & Y. J. Choi, sp. nov. (Fig. 3) [MB#823746]

**Etymology:** Named in honour of Robert Caspary, who has first recorded the downy mildew species parasitic on the genus *Stellaria* s. lat. (including *Pseudostellaria*).

**Description:** The attacked tissues show 3–8 mm spots on leaves, covering much larger areas by coalescing, pale green to yellowish, margin diffuse to vein limited; the tissues are finally necrotic and brittle. Down hypophyllous, whitish, consisting of scattered to rarely dense patches of conidiophore. Haustoria hyphal, branched, filling the host cell almost completely. Conidiophores hyaline, slender, (180–)210–300 (–330) μm long; trunk straight to slightly curved, (100–)120–180 (–200) μm long (n = 50), of more or less uniform width, (5–)6–8 (–9) μm wide below the first branch, 7–10 μm at the base, basal end not differentiated, rarely slightly bulbous and then up to 14 μm wide, callose plugs absent; branching elaborate, monopodial, in 5–6 (–7) orders, branches slightly curved; ultimate branchlets in pairs or single, from slightly curved to sigmoid, (5–)8–12 (–15) μm long, (1–)1.5–2.8 (–3.3) μm wide at the base (n = 30), apex subtruncate or obtuse. Conidia pale olivaceous, ellipsoidal, (23–)24–29 (–30) μm long (av. 26.64), (16–)17–21 (–22) μm wide (av. 18.72), with a ratio of length to width of (1.21–)1.31–1.54 (–1.64) (av. 1.43, n = 50), greatest width median, rarely supermedian, tip rounded, base rounded or narrowing, wall ca. 0.5 μm thick; pedicel absent or hardly visible as a scar to slightly protruding; germination at the randomly located sites of the wall, producing germ tubes, often various swellings and whirling. Resting organs not seen.

**Habitat:** Infested leaves of *Pseudostellaria palibiniana*.

**Specimens examined:** Korea, Gangwon-do, Chuncheon-si, Bongmyeong-ri, 37°46’23” N, 127°48’44” E, 5 Jun 2012, H. D. Shin and Y. J. Choi, KZITFG0000000014 (KUS-F26706); also see Table 1.

**Note:** *Peronospora* materials from *Ps. palibiniana* are in agreement with the description of *P. pseudostellariae* [10]. Yin and Yang [10] reported *Pseudostellaria heterophylla* as the original host plant of *P. pseudostellariae*, but the present study added *Ps. palibiniana*, a common wild plant in Korea [25, 26], as a new host of the species. This is the first study added *Ps. pseudostellariae* parasitic on *P. pseudostellariae* the original host plant of *P. pseudostellariae*.

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