Diversity, Phylogeny, and Host-Specialization of Hyaloperonospora Species in Korea

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Abstract The genus Hyaloperonospora (Peronosporaceae; Oomycota) is an obligate biotrophic group that causes downy mildew disease on the Brassicaceae and allied families of Brassicales, including many economically relevant crops, such as broccoli, cabbage, radish, rape, and wasabi. To investigate the diversity of Hyaloperonospora species in northeast Asia, we performed a morphological analysis for the dried herbarium specimens collected in Korea, along with molecular phylogenetic inferences based on internal transcribed spacer rDNA and cox2 mtDNA sequences. It was confirmed that 14 species of Hyaloperonospora exist in Korea. Of these, three species, previously classified under the genus Peronospora, were combined to Hyaloperonospora: H. arabidis-glabrae comb. nov. (ex Arabis glabra), H. nasturtii-montani comb. nov. (ex Rorippa indica), and H. nasturtii-palustris comb. nov. (ex Rorippa palustris). In addition, finding two potentially new species specific to northeast Asian plants is noteworthy in support of the view that the species abundance of Hyaloperonospora has been underestimated hitherto.

Keywords Barcoding, Brassicaceae, cox2, Downy mildew, Emerging disease, Peronospora

The Peronosporaceae is the largest obligate biotrophic family of the phylum Oomycota. They cause downy mildew disease on a wide range of mono-and dicotyledonous plants [1], among which the Brassicaceae and allied families of Brassicales are the most common host plants. A total of 140 names have been introduced to accommodate the Peronospora species infecting the brassicaceous plants [2], more than half of which have been described by Gäumann [3, 4], who discriminated them mainly using subtle morphological difference and host specialization. In addition to the establishment of the new genus Hyaloperonospora for the brassicolous downy mildews (BDMs) [5], recent phylogenetic studies revealed a high level of species diversity and host-specialization of this genus [6-12]. The collective results support the view that the narrow species circumscription [3, 4] better reflects the natural taxonomic system for BDMs. The results refute the broad species concept of Yerkes and Shaw [13], who attributed the disease to a single species, H. parasitica, although for the last 50 years this concept has been more widely applied to plant pathology and related fields.

Downy mildew is an important disease for many economically relevant brassicaceous crops, particularly species or varieties of Brassica, e.g., the flowerhead types (cauliflower, broccoli), the leaf brassicas (cabbage, Brussels sprouts), the root brassicas (turnips, swedes), oil brassicas (rape), and the horticultural variety (B. oleracea var. acephala). In Korea, commercial crops, including brassicas and radishes, have experienced ongoing severe economic losses [14]. Furthermore, the active importation and cultivation of various overseas crops have led to the emergence of new diseases affecting broccoli [15], tatsoi [16], and spider flower [17]. Previously, Choi et al. [18] described a high phylogenetic diversity for Hyaloperonospora specimens collected in Korea, but the discovered lineages could not be assigned at the species level because of the lack of morphological data and the broad species concept, which prevailed at that time in the taxonomy of downy mildews. The concept has continued to the first monographic studies for the Peronosporaceae in Korea [19]. However, in keeping with the aforementioned taxonomic system for downy...
mildews, recent studies have certainly reflected the narrow species concept for *Hyaloperonospora* species parasitic to *Cardamine* [12] and *Draba* [11] in Korea (Table 1).

This study evaluated the species diversity of BDMs in Eastern Asia using the herbarium specimens collected in Korea, for which mycological characteristics, phylogeny, and host-specialization of *Hyaloperonospora* species were investigated. We report the presence of eight interesting species, including three new combinations, two new species candidates, and three species that have been previously unrecorded in Korea (Table 1).

**MATERIALS AND METHODS**

**Oomycete samples.** Brassicaceous plants with downy mildew infections were collected from different sites of Korea for the past 15 years. Information on the dried herbarium samples selected for morphological and molecular phylogenetic analyses is provided in Table 2.

**Morphological analysis.** Conidiophores and conidia formed from the underneath of infected leaves or stems were transferred to a drop of lactic acid on a slide glass, covered with a cover slip, and briefly heated using an alcohol lamp. Slides were examined and photographed using a model BX53F microscope (Olympus, Tokyo, Japan) equipped with a DigiRetina 16M digital camera (Tucsen, Fuzhou, China). Measurements are reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of a number of measurements given in parentheses.

**DNA extraction, PCR, and sequencing.** Genomic DNA was extracted from the infected dry host tissue using the DNeasy Plant Mini kit (Qiagen, Hilden, Germany). The ribosomal internal transcribed spacer (ITS) regions were amplified using primers ITS1-O [24] and LR0 [25]. For the mitochondrial cytochrome c oxidase subunit II (cox2) sequences, primers cox2-F [26] and cox2-RC4 [27] were used for amplification and sequencing. PCR conditions for ITS and cox2 amplifications were identical as outlined in Choi et al. [28]. 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.** The ITS rDNA and the cox2 mtDNA sequences were edited using the DNAStar software package ver. 5.05 (DNAStar, Inc., Madison, WI, USA). Alignments of each locus were performed using MAFFT 7 [29] and the Q-INS-i algorithm [30], in addition to the previously published reference sequences of *Hyaloperonospora* and an outgroup taxon, *Perofascia lepidii*. Minimum evolution (ME) and maximum likelihood (ML) methods were used to construct two different trees. ME analysis was done using MEGA 7.0 [31] with the default settings of the program, except for replacement with the Tamura-Nei model. ML analysis, 1,000 rounds of random addition of sequences as well as 500 fast bootstrap replicates were performed with RAxML 7.0.3 [32] using the GTRCAT model.

**RESULTS**

**Phylogenetic analyses.** After the exclusion of excessive gap regions and large insertions present in the ITS rDNA regions of some *Hyaloperonospora* species, e.g., *H. parasitica* s.s. ex *Capsella bursa-pastoris*, 1,222 characters were included in analyses, of which 379 were parsimony informative. The cox2 matrix contained 509 characters, from which 150...
| Oomycete species | Host plant | Geographic origin | Herb. No. | ITS | cox2 |
|------------------|------------|-------------------|-----------|-----|------|
| H. arabidis-alpinae | Arabis alpina | Austria, Niederösterreich | HV408-409 (WU) | AY531466 | - |
| H. arabidis-glabra | Arabis glabra | Korea, Hoengseong | KUS-F21788 | MF784703 | MF784663 |
| H. arabidis-glabra | Arabis glabra | Korea, Yangpyeong | KUS-F25778 | MF784704 | MF784664 |
| H. parasitica s. lat. | Arabis soweri | Austria, Kärnten, Flattach | HV508-510 (WU) | AY531392 | - |
| H. arabidis-turritae | Arabis turrita | Austria, Niederösterreich, Giefhühl | HV2121 (WU) | EU049223 | - |
| H. brassicae | Armoracia rusticana | Austria, Oberösterreich, Raab | HV1006-1008 (WU) | AY531406 | - |
| H. brassicae | Brassica campestris | Korea, Gangneung | KUS-F13731 | AY210985 | MF784665 |
| H. brassicae | Brassica campestris | Korea, Seoul | KUS-F16040 | AY210986 | MF784666 |
| H. brassicae | Brassica campestris | Korea, Sacheon | KUS-F19538 | MF784705 | MF784667 |
| H. brassicae | Brassica napus | Germany, Sachsen-Anhalt, Libbesdorf | J1106/01 (TUB12465) | EU049254 | - |
| H. brassicae | Brassica napus | Germany, Sachsen-Anhalt, Unterrissdorf | J904/01 (TUB12441) | EU049216 | - |
| H. brassicae | Brassica napus | Korea, Pyeongtaek | KUS-F26445 | KX01055 | - |
| H. brassicae | Brassica oleracea var. italica | Korea, Jeju | KUS-F22524 | EU137726 | - |
| H. parasitica | Capsella bursa-pastoris | Korea, Seoul | KUS-F15691 | AY210987 | MF784668 |
| H. parasitica | Capsella bursa-pastoris | Korea, Hongcheon | KUS-F18835 | AY210988 | MF784669 |
| H. parasitica | Capsella bursa-pastoris | China, Yantai, Maoping | KUS-F22302 | MF784706 | MF784670 |
| H. parasitica | Capsella bursa-pastoris | Korea, Gunsan | KNUH17 | MF784707 | MF784671 |
| H. dentariae | Cardamine bulbifera | Austria, Niederösterreich, Giefhühl | HV2029 (WU) | KC49997 | KC499957 |
| H. cardamines-laciniatae | Cardamine diphylla | USA, Tennessee, Knoxville | HV-PA10 (WU) | KC49994 | KC499953 |
| H. cardamines-enneaphyllos | Cardamine enneaphyllos | Austria, Niederösterreich, Mannersdorf/Leithaged | HV2099 (WU) | KC4995018 | KC499978 |
| H. cardamines-enneaphyllos | Cardamine enneaphyllos | Austria, Niederösterreich, Giefhühl | HV2105 (WU) | KC4995019 | KC499979 |
| H. malyi | Cardamine graeca | Greece, Korfos, Episkopi | HV2895 (WU) | KC4995022 | KC499982 |
| H. dentariae | Cardamine heptaphylla | Italy, Lombardia, Lecco, Canzo, Gajum | HV2935 (WU) | KC4995005 | KC4994956 |
| H. nasturtii-aquatici | Cardamine hirsuta | Austria, Wien, Landstrasse, Botanischer Garten | HV2030 (WU) | KC4995026 | KC499486 |
| H. dentariae | Cardamine impatiens | Korea, Hongcheon | KUS-F20166 | MF784708 | MF784672 |
| H. dentariae | Cardamine impatiens | Korea, Hoengseong | KUS-F21782 | MF784709 | MF784673 |
| H. dentariae | Cardamine impatiens | Korea, Hongcheon | KUS-F21377 | MF784710 | MF784674 |
| H. dentariae | Cardamine impatiens | Korea, Hoengseong | KUS-F21908 | MF784711 | MF784675 |
| H. dentariae | Cardamine impatiens | Korea, Pyeongchang | KUS-F20305 | MF784712 | - |
| H. dentariae | Cardamine impatiens | Korea, Cheorwon | KUS-F23320 | MF784713 | MF784676 |
| H. dentariae | Cardamine impatiens | Austria, Niederösterreich, Giefhühl | HV2289 (WU) | KC4995009 | KC4994970 |
| H. cardamines-laciniatae | Cardamine lacinia | USA, Maryland, Beltsville | HV2949 (WU) | KC499496 | KC4994955 |
| H. dentariae-macrophyllae | Cardamine leucantha | Korea, Chuncheon | KUS-F17273 | AY210990 | KC4994974 |
| H. dentariae-macrophyllae | Cardamine leucantha | Korea, Hongcheon | KUS-F17298 | AY210992 | KC4994976 |
| H. dentariae | Cardamine pentaphylla | Austria, Kärnten, Flattach, Tschepasschlucht | HV2334 (WU) | KC4995010 | KC499971 |
| H. nasturtii-aquatici | Cardamine pratensis | Austria, Niederösterreich, Mariensee | HV2400 (WU) | KC4995031 | KC4994992 |
| Hyaloperonospora sp. | Cardamine scutata | Korea, Chuncheon | KUS-F18833 | AY210994 | D099=D420 |
| Hyaloperonospora sp. | Cardamine scutata | Korea, Pyeongchang | KUS-F19563 | MF784714 | MF784678 |
| Hyaloperonospora sp. | Catolobus cf. pendulifolius | Korea, Samcheok | KUS-F18194 | AY210108 | MF784679 |
| Oomycete species | Host plant | Geographic origin | Herb. No. | ITS   | cox2   |
|------------------|------------|-------------------|-----------|-------|--------|
| **Hyaloperonospora sp.** | *Catolobus cf. pendulus* (= *Arabis pendula*) | Korea, Samcheok | KUS-F18892 | MF784715 | MF784680 |
| **H. drabae** | *Draba nemorosa* | Korea, Chuncheon | KUS-F15718 | GU189409 | MF784681 |
| **H. norvegica** | *Draba nemorosa* | Korea, Muju, Mt. Dugyu | KUS-F21277 | GU189411 | MF784683 |
| **H. norvegica** | *Draba nemorosa* | Korea, Muju, Mt. Dugyu | KUS-F21275 | GU189412 | MF784684 |
| **Hyaloperonospora sp.** | *Eruca sativa* | Korea, Icheon | KUS-F24009 | GQ919015 | MF784685 |
| **H. thlaspeos-perfoliati** | *Microthalmapi perfoliatum* | Germany, Baden-Württemberg, Öschingen | MG1879 (TUB12275) | AY531432 | - |
| **H. thlaspeos-perfoliati** | *Microthalmapi perfoliatum* | Austria, Niederösterreich | HV2079 (WU) | EU049219 | - |
| **H. brassicaceae** | *Raphanus sativus* | Spain, Avila, nearby Navalosa | GG253 (MA-Fungi72986) | EU049277 | - |
| **H. narcissi-montani** | *Rorippa indica* | Korea, Busan | KUS-F23202 | MF784718 | MF784690 |
| **H. narcissi-palustris** | *Rorippa palustris* | Korea, Gangneung | KUS-F11409 | MF784692 | - |
| **H. narcissi-palustris** | *Rorippa palustris* | Korea, Namyangju, Deokso | KUS-F18713 | MF784693 | - |
| **H. nassurtii-montani** | *Rorippa indica* | Korea, Chuncheon | KUS-F18834 | MF784694 | - |
| **H. nassurtii-montani** | *Rorippa indica* | Korea, Namyangju, Deokso | KUS-F18859 | MF784695 | - |
| **H. nassurtii-palustris** | *Rorippa indica* | Korea, Gangneung | KUS-F23202 | MF784718 | MF784690 |
| **H. nassurtii-palustris** | *Rorippa palustris* | Korea, Namyangju, Deokso | KUS-F23409 | MF784720 | MF784696 |
| **H. rorippae-palustris** | *Rorippa palustris* | Austria, Tiral, Steeg | GLM46904 | EU049235 | - |
| **H. rorippae-palustris** | *Rorippa palustris* | Austria, Tiral, Steeg | GLM46879 | EU049234 | - |
| **H. brassicaceae** | *Sinapis alba* | Germany, Rheinland-Pfalz, Kastellaun | J2247/01 (TUB12435) | EU049211 | - |
| **H. brassicaceae** | *Sinapis alba* | Germany, Baden-Württemberg, Tübingen | MG1866 | AY531403 | - |
| **H. brassicaceae** | *Sinapis arvensis* | Germany, Sachsen, Wollenstein/Streckenwalde | D19/7/97 | EU049218 | - |
| **Hyaloperonospora sp.** | *Tarenaya hassleriana* (= *Cleome spinosa*) | Korea, Ganghwa | KUS-F25462 | JQ301468 | MF784697 |
| **Hyaloperonospora sp.** | *Tarenaya hassleriana* (= *Cleome spinosa*) | Korea, Ganghwa | KUS-F25462 | MF784721 | MF784697 |
| **H. thlaspeos-avensis** | *Thlaspi arvense* | Korea, Chuncheon | KUS-F17271 | AY211009 | MF784698 |
| **H. thlaspeos-avensis** | *Thlaspi arvense* | Korea, Chuncheon | KUS-F18832 | AY211010 | MF784699 |
| **H. thlaspeos-avensis** | *Thlaspi arvense* | Korea, Muju, Mt. Dugyu | KUS-F21272 | MF784722 | MF784700 |
| **H. thlaspeos-avensis** | *Thlaspi arvense* | Austria, Oberösterreich, St. Willibald | HV762/HV764 (WU) | AY531445 | - |
| **Peroferasia lepidii** | *Lepidium virginicum* | Korea, Gangneung | KUS-F17311 | MF784723 | - |
| **Pf. lepidii** | *Lepidium virginicum* | Korea, Changwon | KNUH33 | MF784724 | MF784702 |
| **Pf. lepidii** | *Lepidium virginicum* | Korea, Seoul | KUS-F17250 | AY211013 | MF784701 |

Table 2. Continued
Hyaloperonospora Species in Korea

were parsimony informative. As tree topologies of ME and ML inferences were fully compatible (data not shown), only each ME tree is shown for each locus (Fig. 1 for ITS, Fig. 2 for cox2), with ME and ML bootstrap values above 60% at first and second position above/below the branches.

In phylogenetic investigations (Figs. 1 and 2), the accessions from different species of Arabis were divided into four
Lee et al. distant lineages: one representing *H. arabidis-alpinae* parasitic on *Arabis alpina*, a second lineage of *H. arabidis-turritae* on *Arabis turrita*, a third lineage on *Arabis soyeri* previously described by Göker et al. [7], and a fourth clade with two Korean accessions on *Arabis glabra*, representing a new combination, *H. arabidis-glabrae*. An unknown lineage of *Hyaloperonospora* originating from *Catolobus pendulus* (= *Arabis pendula*) was supported by high supporting values in both ME/ML analyses of ITS and *cox2*. It further clustered together with *H. arabidis-turritae*, *H. mayli*, and *H. thlaspeos-perfoliati*. There has been no prior report of downy mildew on this plant, but, considering the phylogenetic divergence, it could be an unknown species.

The accessions from *Rorippa* species were contained in two major clades (Fig. 1). The first monophyletic clade represented *H. nasturtii-montani*. The second clade consisted of *H. nasturtii-islandicae* and *H. nasturtii-palustris*. The grouping of the latter two species, which are parasitic to only a single plant *Rorippa palustris*, was highly supported with the values of 91/98%, but each combination harbored only a distinct *Hyaloperonospora* species.

In the ITS tree, the accessions of the *H. brassiccae* complex were contained in four closely related clades, as suggested by Göker et al. [7]: a first highly supported monophyletic clade consisting of the specimens parasitic on *B. campestris*, *B. narinosa*, and *B. oleracea*, a second on *Sinapis alba*, a third on *Sinapis arvense*, and a fourth on *Raphanus sativus* and *R. raphanistrum*. Interestingly, the accessions from

Fig. 2. Minimum evolution trees based on the *cox2* mtDNA sequences, with support values in Bayesian inference. Bootstrapping support values (minimum evolution/maximum likelihood) higher than 60% are given above/below the branches. The specimens collected in Korea are shown in bold, and previously unknown species in Korea were highlighted in the colored boxes. The scale bar equals the number of nucleotide substitutions per site.
spider flower, Tarenaya hasleriana (= Cleome spinosa), were placed within the first clade of H. brassicae (Figs. 1 and 2), with no nucleotide differences in the ITS sequences and only one difference in the cox2 sequences, hinting at their close genetic relationship.

The Korean accessions collected from Cardamine impatiens fell within the H. dentariae clade, with weak supporting values in ITS dataset and the maximum value in cox2 mtDNA. In the cox2 dataset, they further formed their own well-supported subclade with the maximum supporting value in both analyses, as shown by Voglmayr et al. [12], who observed the presence of host-specific structures within the H. dentariae clade. Interestingly, there was a phylogenetic divergence between European and Korean accessions originating from the same plant, C. impatiens. An unknown lineage of Hyaloperonospora originated from Cardamine scutata. It was closest to H. sp. ex Arabis soyeri, but could be a hitherto overlooked species. The Korean accessions collected from Thlaspi arvense grouped with the GenBank sequences of H. thaalspeos-arvensis, with the maximum support in the ITS sequences. This is the first report of H. dentariae and H. thaalspeos-arvensis in Korea.

**Taxonomy.** Based on the molecular phylogenetic and morphological data, we combined three names of *Peronospora* to Hyaloperonospora: *H. arabidis-glabrae* comb. nov., *H. nasturtii-montani* comb. nov., and *H. nasturtii-palustris* comb. nov. Along with H. dentariae, H. thaalspeos-arvensis, and Hyaloperonospora sp. [*Peronospora* *cf*. *cleomes*], they were previously unknown to Korea.

**Hyaloperonospora arabidis-glabrae** (Gäum.) Jae S. Lee & Y. J. Choi, comb. nov. (MB#821809)

**Basionym:** *Peronospora arabidis-glabrae* Gäum., Beih. Bot. Zbl. 35: 520 (1918) (MB#231470).

**Description:** Down hypophyllous, whitish to yellowish, dense, felt-like. Haustoria filling the host cell partly to almost completely, lobate. Conidiophores emerging through stomata, 20–30 in a fascicle, hyaline, somewhat stout, straight, (250–)330–500 (µm) long, (8–)10–16 (µm) wide (n = 30). Ultimate branchlets mostly in pairs, curved to sigmoid, (8–)10–16 (µm) long, (1.1–)1.2–1.7 (µm) wide at the base; tip obtuse or subtruncate (n = 30). Conidia hyaline, subglobose, (19–)24.0–30.0 (µm) long (av. 24), a length to width ratio of (1.05–)1.09–1.19 (µm) (av. 1.14, n = 30), the greatest width at median, with a rounded tip and base; pedicel mostly absent, but rarely present as a scar. Resting organs not seen.

**Habitat:** On living leaves of *Rorippa indica* (= *Nasturtium montanum*) (Brassicaceae).

**Specimen examined:** KZITFG0000000024 (KUS-F22396), Korea, Pyeongchang, Jinbu-myeon (35°08′9″ N, 128°57′19″ E), 28 Oct 2006, H. D. Shin & Y. J. Choi.

**Hyaloperonospora nasturtii-montani** (Gäum.) Jae S. Lee & Y. J. Choi, comb. nov. (MB#821810)

**Basionym:** *Peronospora nasturtii-montani* Gäum., Beih. Bot. Zbl. 35: 528 (1918) (MB#150102).

**Description:** Down hypophyllous, whitish, dense, felt-like. Haustoria filling the host cell partly to almost completely, lobate. Conidiophores emerging through stomata, 20–30 in a fascicle, hyaline, somewhat stout, straight, (250–)330–500 (µm) long; trunk straight, (200–)250–350 (µm) long, (8–)10–16 (µm) wide at the base; pedicel mostly present as a scar. Resting organs not seen.

**Habitat:** On living leaves and stems of *Arabis glabra* (Brassicaceae).

**Specimen examined:** KZITFG0000000009 (KUS-F25778), Korea, Yangpyeong, Jungmisan Recreational Forest (37°34′53″ N, 127°27′29″ E), 10 Jun 2011, H. D. Shin & Y. J. Choi.

**Note:** On *Arabis glabra*, two species of *Peronospora*, *Peronospora arabidis-glabrae* and *P. arabidis-turritae* (now under *Hyaloperonospora*), were introduced by Gäumann [3], who discriminated between them by the height of conidiophores; 300–400 µm in the former species and 200–250 µm in the latter. The Korean sample was mostly more than 300 µm, well consistent with *P. arabidis-glabrae*. The conidia of the Korean specimen were slightly smaller than *P. arabidis-glabrae* [3] (av. 21.14 × 19.14 µm vs. 23.8 × 21.9 µm), but still larger than *P. arabidis-turritae* (av. 18.9 × 16.4 µm). In addition, these two species were phylogenetically distant (Fig. 1).
**Habitat:** On living leaves and stems of *Rorippa palustris* (Brassicaceae).

**Specimen examined:** KZITFG0000000006 (KUS-F23085), Korea, Namyangju, Deokso Experiment Farm of Korea University (37°35'03" N, 127°14'11" E), 22 Oct 2007, H. D. Shin & Y. J. Choi.

**Note:** *Peronospora rorippae-islandicae* (now, under *Hyaloperonospora*) has been introduced to accommodate the downy mildew pathogen on *Rorippa palustris* [3]. From the same host plant, the second species, *P. nasturtii-palustris*, was described by Ito and Tomakage [33]. As these two species slightly differed by the size of conidia (av. 16.62 × 13.79 µm vs. av. 18.05 × 14.71 µm), Gustavsson [34] synonymized the latter species under the former species. The Korean accession (av. 18.82 × 14.07 µm), with a typical morphology of *Hyaloperonospora*, is closer to *P. nasturtii-palustris*.

**Hyaloperonospora sp. [Peronospora cf. cleomes]**

**Reference:** *Peronospora cleomes* Hansf., E. African Agric. J. Kenya 8: 250 (1943) (Nom. inval. Article 39).

**Description:** Down hypophyllous, whitish to yellowish, dense, felt-like. Haustoria filling the host cell partly to almost completely, lobate. Conidiophores emerging through stomata, up to 30 in a fascicle, hyaline, somewhat stout, straight, (250–)300–450–450(–590) µm; trunk straight, (100–)200–350–400(–400) µm long, (9–)10–13–17(–17) µm wide (n = 30). Ultimate branchlets mostly in pairs, curved to sigmoid, (5–)17–13–17 µm long, (1.2–)1.5–2.2(–2.5) µm wide at the base; tip obtuse or subtruncate (n = 30). Conidia hyaline, broadly ellipsoidal to subglobose, (12.5–)14.0–18.0(–19) µm long (av. 16.0), (10–)11.3–15.5–17 µm wide (av. 12.4), a length to width ratio of (1.05–)1.11–1.29(–1.35) (av. 1.2, n = 30), the greatest width median, rarely sub- or supra-median, tip and base rounded; pedicel mostly present as a scar. Resting organs rarely present and visible as yellow to brown spot on leaves. Oogonia irregular in shape, 36–57 µm diameter (n = 30); wall wrinkled, yellowish, 2–5(–7) µm thick. Oospores plerotic to aplerotic, globose, (22–25–30) µm diameter, yellowish, 2–3.5 µm thick wall (n = 30).

**Habitat:** On living leaves and stems of *Cardamine impatiens* (Brassicaceae).

**Specimen examined:** KZITFG0000000011 (KUS-F23320), Korea, Cheorwon, Bokjusan Natural Recreation Forest (38°08’40” N, 127°28’23” E), 16 May 2008, H. D. Shin & Y. J. Choi.

**Note:** This cosmopolitan species is parasitic to four different species of *Cardamine*, including *C. impatiens*. The morphotype fits well with the description of *H. dentariae* collected from *C. heptaphylla* [3, 12], except for minor differences in the conidial size. In the *cox2* tree, as suggested by Voglmayr et al. [12], there were a few subclades within "*H. dentariae complex", among which the Korean samples formed a well-supported group (Fig. 2), somewhat apart from other isolates of the complex. Further study is necessary to determine whether it may actually represent taxonomically separable entity at the species or the subspecific level.

**Hyaloperonospora thlaspeos-arvensis** (Gäum.) Göker, Riethm., Voglmayr, M. Weiss & Oberw., Mycol. Prog. 3: 89 (2004) (MB#371143)

**Description:** Down hypophyllous, whitish, dense, felt-like. Haustoria filling the host cell partly to almost completely, mostly only a haustorium but rarely two in a host cell, vesicle-like or lobate, variable in size (up to 30 µm), with stalk surrounded by thick sheath at the part of entry into the host cell. Conidiophores emerging through stomata, 10–30 in a fascicle, hyaline, somewhat stout, straight, (250–)300–450–550(–590) µm; trunk straight, (180–)200–350–450(–490) µm long, (10–)13–16–18(–18) µm wide (n = 30). Ultimate branchlets mostly in pairs, curved to sigmoid, (12–)18–28(–33) µm long, (1.5–)1.8–2.4(–2.6) µm wide at the base; tip obtuse or subtruncate (n = 30). Conidia hyaline, broadly ellipsoidal to subglobose, (25–)26.5–30.5(–33) µm long (av. 28.04), (18–)20.5–24.0(–25) µm wide (av. 22.1), a length to
width ratio of (1.1–)1.28–1.35 (–1.5) (av. 1.31, n = 30), the greatest width median, rarely sub- or supra-median, tip and base rounded; pedicel mostly present as a scar. Resting organs not seen.

**Habitat:** On living leaves and stems of *Thlaspi arvense* (Brassicaceae).

**Specimen examined:** KZITFG0000000009 (KUS-F25778) for *H. arabidis-glabrae*, KZITFG0000000024 (KUS-F22396) for *H. nasturtii-montani*, KZITFG0000000006 (KUS-F23085) for *H. nasturtii-palustris*, and KZITFG0000000010 (KUS-F25462) for *H. sp.* [Peronospora cf. cleomes] (scale bars: A–D = 100 µm, E–P = 10 µm).

**Fig. 3.** Morphological characteristics of four *Hyaloperonospora* species; *H. arabidis-glabrae* ex *Arabis glabra* (in the first column or the left), *H. nasturtii-montani* ex *Rorippa indica* (second), *H. nasturtii-palustris* ex *Rorippa palustris* (third), and *Hyaloperonospora* sp. [Peronospora cf. cleomes] ex *Tarenaya hassleriana* (fourth). A‒D, Conidiophores; E–H, Ultimate branchlets; I–P, Conidia. Sources: KZITFG0000000009 (KUS-F25778) for *H. arabidis-glabrae*, KZITFG0000000024 (KUS-F22396) for *H. nasturtii-montani*, KZITFG0000000006 (KUS-F23085) for *H. nasturtii-palustris*, and KZITFG0000000010 (KUS-F25462) for *H. sp.* [Peronospora cf. cleomes] (scale bars: A–D = 100 µm, E–P = 10 µm).

**DISCUSSION**

The species abundance of the genus *Hyaloperonospora* remains underestimated in northeast Asia. Two monographs have been published for the family Peronosporaceae in China [37] and Korea [19]. Both studies regarded BDMs as only a species, *H. parasitica* (= *Peronospora parasitica*), or a few species, undoubtedly according to the “one host family-one pathogen” concept of Yerkes and Shaw [13]. The recent phylogenetic investigations, however, clearly favor the narrow species circumscription of Gäumann [3, 4] that a species of *Hyaloperonospora* parasitize a particular genus or species of plants of Brassicaceae [6-12, 38]. The studies highlight the necessity of re-evaluating the species diversity of *Hyaloperonospora* in this region. In the present study, we confirm the presence of 14 species of this genus, of which six species have been unknown in Korea: *H. arabidis-glabrae*, *H. dentariae*, *H. nasturtii-montani*, *H. nasturtii-palustris*, *H. thlaspeos-arvensis*, and *H. sp.*
[Peronospora cf. cleomes]. Although the number of species still seems lower, compared with other European countries, interestingly we discovered two unknown lineages of Hyaloperonospora, specific to two Asian indigenous plants, Cardamine scutata Thunb. and Catolobus pendulus L. (= Arabis pendula). Cardamine scutata is a common herb distributed in northeast Asia, including China [39], Japan [40], Korea [41], and Far Eastern Russia [42]. Similarly, C. pendulus is mainly distributed in northeast Asia, but has also been rarely reported in Europe [43, 44]. To date, there has been no report of downy mildew on these two plants worldwide. However, considering the phylogenetic divergence and the narrow host specialization previously known for Hyaloperonospora species, they are considered two new species. Finding two potentially new species on northeast Asian plants is noteworthy in support of our argument that the diversity of Hyaloperonospora species present in Asia has been underestimated.

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