Bremia itoana (Oomycota, Peronosporales), a Specialized Downy Mildew Pathogen on an East Asian Plant, Crepidiastrum sonchifolium (Asteraceae)

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ABSTRACT

Crepidiastrum sonchifolium, a flowering plant in the daisy family (Asteraceae), is native to East Asia. In Korea, this plant is a locally cultivated vegetable, and its market size is gradually growing. Since the plants with downy mildew infection were initially found at a private farm of Chuncheon city, the occurrences have continued in commercial farms of other regions, highlighting that this disease is spreading throughout Korea. The pathogen was attributed to a member of the genus Bremia that contains many specialized species, each of which displays a narrow host spectrum on Asteraceae. Based on morphological and molecular phylogenetic analyses, along with the high host specificity recently proven for Bremia sp. species, the identity of the causal agent was confirmed as a so far undescribed species of Bremia. Here, we introduce Bremia itoana sp. nov., specific to C. sonchifolium.

1. Introduction

Crepidiastrum sonchifolium (Maxim.) Pak & Kawano, called “sonchus-leaf crepidiastrum”, is native to East Asia. In Korea, it is an economically important vegetable, which is consumed to make kimchi, and its cultivated area is gradually expanding throughout the country [1–4]. In addition to its culinary value, this plant has been traditionally used as a folk medicine because of its digestive, diuretic, and anti-inflammatory activities [5].

Since downy mildew disease caused by the genus Bremia (Oomycota, Peronosporales) has been initially found on C. sonchifolium at a private farm in Chuncheon city, Korea in 1998, this disease has been continuously spreading to other regions, although it does not seem to have reached outside of Korea. On a large number of genera or species of the family Asteraceae, including this crop, downy mildew is a notorious disease, among which Bremia lactucae is one of the most well-known species, which causes devastating damage in the cultivation of lettuce (Lactuca sativa) [6]. According to the broad species concept that a downy mildew species is responsible for all infections occurring on a host family [7], the causal pathogen affecting C. sonchifolium has been presumed to be B. lactucae. However, recent phylogenetic studies with multigene sequences have found several well-supported groups in Bremia but also a better resolution power for discriminating them at the species level [8–13]. As a result, it revealed that Bremia is not monotypic, which consists of a dozen of highly host-specific species, and in addition, Choi et al. [10] found several previously overlooked lineages of Bremia, including Bremia sp. affecting C. sonchifolium, which was distant from other East Asian species of Bremia, parasitic to Crepidiastrum and two allied genera, Ixeris and Youngia. In the present study, we aimed to clarify the identity of the downy mildew pathogen, parasitic to C. sonchifolium, using both morphological and molecular phylogenetic approaches.

2. Materials and methods

2.1. Morphological analysis

C. sonchifolium plants with downy mildew symptoms were collected from various regions of Korea. For morphological investigation, conidiophores and conidia formed from the lower surface of the infected leaves were transferred onto a drop of lactic acid on a slide glass, covered with a coverslip, and gently warmed up using an alcohol lamp. A detailed microscopic examination was performed using an...
Olympus BX53F microscope (Olympus, Tokyo, Japan) equipped with a DigiRetina 16 M digital camera (Tucsen, Fuzhou, China). The following morphological characteristics were observed at 100–200× for conidiophores and at 400× for conidia and ultimate branchlets. Measurements were reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of the number of measurements given in parentheses. Among the specimens examined morphologically, three specimens were selected for phylogenetic analyses (Table 1), and deposited at the Korea University Herbarium (KUS-F, Seoul, Korea) and the National Institute of Biological Resources (NIBR, Incheon, Korea).

2.2. Molecular phylogenetic analysis

About 10–20 mg of infected leaves from herbarium specimens were disrupted with homogenizing pestles in 1.5-mL tube. Genomic DNA extraction was performed using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Three nuclear (ITS1, LSU D1-3 rDNA, BrRxLR11) and three mitochondrial (cox1, cox2, cox2-1 spacer mtDNA) markers were amplified by PCR as outlined previously [10–12]. The PCR products were purified and sequenced by a DNA sequencing service (Macrogen Inc., Seoul, Korea). For phylogenetic analysis, multigene sequences of Bremia species used in the previous studies [10–12] were retrieved from GenBank. Alignments for each of multigene datasets were done using the MAFFT 7 [14], by choosing the Q-INS-i algorithm [15]. After ensuring no strongly supported conflicting topologies among the trees inferred from individual loci, the alignments were concatenated using SequenceMatrix [16]. Two different phylogenetic inference methods were performed on the concatenated alignment. Minimum Evolution (ME) was done in MEGA6.0 [17] using the Tamura-Nei substitution model, and the robustness of internal branches was evaluated by performing 10,000 bootstrap replicates. Maximum Likelihood (ML) inference was computed using RAxML 7.0.3 [18], with default settings on the RAxML BlackBox web server [19].

3. Results and discussion

Trees based on each alignment of three nuclear (ITS, LSU D1-3 rDNA, BrRxLR11) and three mitochondrial (cox1, cox2, cox2-1 spacer mtDNA) loci showed no strong conflicting support with a phylogeny based on the concatenated alignment of all six loci. Thus, only the phylogeny based on concatenation of all loci was used for phylogenetic reconstruction. The final concatenated alignment displayed 4148 total characters, including 903 variable characters, 800 of which were parsimony-informative for Bremia species. Because the dataset revealed no significant conflicts in the topologies derived from ME and ML analyses, only the tree from the ME inference was shown in Figure 1. The overall topology and major groupings were in line with those of the previous studies [8–12], with unsupported replacements of a few branches. Three specimens affecting C. sonchifolium formed an independent lineage with maximum support and was separated from all previously accepted species of Bremia. Interestingly, this lineage is quite distant from another well-supported group consisting of four East Asian species, B. microspora, B. ovata, B. polycephala, and B. sawadae, despite their host plant affinity within the tribe Cichorieae (subfamily Cichorioideae). Instead, Bremia sp. affecting C. sonchifolium grouped with other Bremia species, parasitic to two subfamilies Asterioideae and Carduoideae, with moderate support of 70% in ME and 78% in ML analyses.

In agreement with the previous studies [8,11,20,21] that all Bremia species, parasitic to Cichorieae, have smaller conidia than other accepted species of Bremia, the present pathogen affecting C. sonchifolium could be characterized by small conidia of av. 15.9 × 14.1 μm, which is similar to B. microspora (av. 15.9 × 13.7 μm) and B. polycephala (av. 15.6 × 14.0 μm), but somewhat larger than B. ovata (av. 13.2 × 11.2 μm) and B. microspora (av. 13.4 × 12.7 μm). However, in line with the present phylogenetic results, this species exhibits several morphological differences. First, the length of conidiophores was markedly shorter as 200–410 (av. 305) μm than other four species (at least more than av. 400 μm). About 20–40% of conidiophores in Bremia sp. are shorter than 250 μm, but in other species, such short ones were <5%. As a related feature, the length of trunks was also shorter; av. 150 μm in Bremia sp. versus at least av. 237 μm in other species. In addition, the position of the first branching in Bremia sp. was somewhat lower as 4/10–6/10, thus often rendering the branching part occupying over half of the conidiophore’s length,

| Herb. No. [KUS-F] | Seq. ID | Collection year | Geographic origin | 18S + ITS1/LSU D1-3/BrRxLR11 nDNA | cox2/cox1/cox2-1 spacer mtDNA |
|-------------------|---------|-----------------|------------------|---------------------------------|--------------------------------|
| 19257             | D216    | 2002            | Korea; Hongcheon | KT249120/ KT249315/ KT249705    | MH665654/MH665655/MH665656     |
| 19490             | D217    | 2003            | Korea; Chunchon  | KT249121/ KT249316/ KT249706    | MH665652/MH665655/MH665653     |
| 23946             | D543    | 2008            | Korea; Yangpyeong| KT249510/ KP684694/ KP684894    | MH665652/MH665651/MH665653     |

Table 1. Herbarium specimens of Bremia sp. parasitic to Crepidiastrum sonchifolium.
while in other species they branched mostly at 3/10. As *Bremia* sp. and *B. sawadae* have the close host affinity [22], both parasitic to the genus *Crepidiastrum*, their morphological characteristics were compared in detail (Table 2). In addition to the differences listed above, the shapes of conidia and ultimate branchlets can discriminate between the two species; oblong to ovoid conidia in *Bremia* sp. versus ovoid conidia in *B. sawadae*, and often confluent vesicles at the end of ultimate branchlets in *Bremia* sp. versus mostly spherical vesicles in *B. sawadae*.

The host range of *Bremia* species is restricted to the plant family Asteraceae, favouring three subfamilies, Asteroideae, Carduoideae, and Cichorioidae [10]. Especially, small conidia-possessing species of *Bremia*, including the present species, all colonize the three East Asian genera of the subtribe

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**Table 2. Comparison of morphological characteristics of *Bremia* species parasitic to *Crepidiastrum* species.**

| Characteristics | *B. sawadae* | *B. itoana* |
|----------------|-------------|-------------|
| **Host plant** | *Crepidiastrum denticulatum* | *Crepidiastrum sonchifolium* |
| **Conidiophores (n = 50)** | | |
| Length | (355–424–536–648(–718)) μm | (150–200–300–410(–500)) μm |
| length of trunk | (224–288–374–460(–514)) μm | (40–100–150–200(–300)) μm |
| Width of trunk | (5.4–16.2–7.1–8.0(–9.4)) μm | (4.9–16.5–7.7–9.3(–10.9)) μm |
| Position of the first branch | 2/10–4/10 | 4/10–6/10 |
| Branching type | Dichotomous | Dichotomous |
| No. of branch orders | 4–6 | 3–5(–6) |
| Callose plugs | Often present in trunk, rarely in branches | Often present in trunk and branches |
| **Ultimate branchlets (n = 50)** | | |
| Shape | Curved | Slightly curved to substraight |
| Length | 8–20(–30) μm | 6–17 μm |
| **Vesicles (n = 50)** | | |
| Shape | Spherical | Spherical or confluent |
| Size (diam.) | 8–10 μm | 8–12 μm |
| No. of extensions | 4–6(–7) | 4–6 |
| Length of extensions | 5–7 μm | 6–8 μm |
| Shape of tips | Obtuse to somewhat swollen | Obtuse to somewhat swollen |
| Width of tips | 1.3–1.8 μm | 1.4–1.9 μm |
| **Conidia (n = 100)** | | |
| Shape | Ovoid | Oblong to ovoid |
| Colour | Hyaline | Hyaline |
| Length (μm) | (11.5–12.5–13.4–14.3(–14.7)) μm | (14.0–15.1–15.9–16.7(–17.7)) μm |
| Width (μm) | (10.6–11.1–11.7–12.4(–14.1)) μm | (13.0–13.4–14.1–14.8(–15.7)) μm |
| l/w ratio | (1.06–1.1–1.1–1.19(–1.26)) μm | (1.05–1.09–1.13–1.17(–1.22)) μm |
| Pedicel | Mostly present | Mostly present |
| Reference | Park et al. [12] | The present study |
Crepidinae of Cichorioideae, Crepidiastrum, Ixeris, and Youngia \[8,10,11\]. However, although they infect the close host plants in the same area, *Bremia* sp. affecting *C. sonchifolium* seems to have evolved independently from other species. It is most likely that long after branching of the main group infecting Crepidinae, *Bremia* sp. has host-jumped from another lineage infecting Cichorioideae onto *C. sonchifolium*. Despite the similar morphology to Cichorioideae-infecting species but the close phylogenetic relationship to Asteroideae- and Carduoideae-infecting species, it is unlikely that this species served as an evolutionary bridge connecting the two groups because the host plant was always limited in East Asia.

The present study provided evidence that the causal agent of downy mildew occurring on the cultivated crop, *Crepidiastrum sonchifolium*, is independent from all previously known species of *Bremia*, for which we introduce a new species here.

**Taxonomy**

*Bremia itoana* Y.J. Choi & H.D. Shin, sp. nov. [MB827100] **Figure 2.**

**Etymology:** named in honour of Seiya Ito for his outstanding studies on East Asian species of *Bremia*.

Lesions commonly causing discolouration of the tissues, pale green or yellow, later becoming dark brown, vein-limited, poly-angular, frequently covering larger areas by coalescing; infected tissues become necrotic. Down present on the under the surface of host leaf, whitish, consisting of scattered conidiophores, only one or two in a fascicle, sparse. Conidiophores emerging through stomata, colourless, straight, \((150–)200–305–410(–500)\) \(\mu\)m; trunk straight, \((60–)100–150–200(–300)\) \(\mu\)m long, \((4.9–)6.5–7.7–9.3(–10.9)\) \(\mu\)m wide below the first branch; basal end not differentiated to slightly bulbous, \((6.4–)8.6–10–11.4(–12.2)\) \(\mu\)m wide; callose plugs often present in trunk and branches; branches dichotomous, 3–5(–6) orders. Ultimate branchlets in pairs in most branchlets, rarely single, slightly curved to straignt, \(6–17\) \(\mu\)m long, obtuse to somewhat swollen; vesicles spherical or confluent, \(8–12\) \(\mu\)m diam., bearing 4–6 extensions with lengths of \(6–8\) \(\mu\)m. Conidia colourless, oblong to ovoid, almost symmetrical at the equatorial plane, \((14.0–)15.1–15.9–16.7(–17.7)\) \(\mu\)m long, \((13.0–)13.4–14.1–14.8(–15.7)\) \(\mu\)m wide, a l/w ratio of \((1.05–)1.09–1.13–1.17(–1.22)\), greatest width median to submedian, tip round, base broadly round; pedicel mostly present, having thin-walled papilla. Germination directly with a germ tube only at the tip.
up to 215 μm long, rarely branched. Resting organs not seen.

**Typus:** KOREA; Chuncheon-si, Dongnaje-myeon, Goeun-ri (37°50′19″N 127°46′58″E), 21 May 2003, Y.J. Choi & H.D. Shin, NIBRFG000051456 (holotypus).

**Habitat:** On living leaves of *Crepidiastrum sonchifolium* (Asteraceae).

**Additional specimens examined for morphological investigation:** KUS-F15622 (Nov. 1 1998, Chang-ri, Nam-myeon, Yanggu, Korea), 20946 (Nov. 4 2004, Goeun-ri, Dongnaje-myeon, Chuncheon, Korea), 21516 (Oct. 17 2005, Jangjeonpyeong-ri, Hongcheon-eup, Hongcheon, Korea), 21684 (Nov. 11 2005, Goeun-ri, Dongnaje-myeon, Chuncheon, Korea), 21688 (Nov. 12 2005, Seosang-ri, Seo-myeon, Chuncheon, Korea).

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