Identification of *Lacrymaria velutina* (Pers. Ex Fr.) Konrad & Maubl. from Micheon-myeon, Jinju-city, Korea

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We identified *Lacrymaria velutina* of the Coprinaceae in Korea. The unusually large and sturdy fruiting body, fibrillose to fibrillose-scaly cap and stalk without a volva with an obscure superior hairy ring zone or hairy annulus, and blackish brown, warted spores distinguished this species from closely related *Psathyrella* species. An illustrated account of the microscopic traits is presented. Fruiting bodies with obtusely hemispherical caps, 2.5–6 cm, becoming convex with age; surface dry, densely fibrillose-scaly with split margin; stipe, 4.5–6 cm, equal, hollow, fibrillose, dry, whitish above the superior ring zone, light brown below; crowded gills, adnexed, dark black at maturity. Pileipellis typically cellular with the gill edge appearing white and beaded. Blackish brown basidiospores that discolor in concentrated sulfuric acid. Spores elliptical, warted, 9–11 × 6–8 µm, with prominent snout-like germ pores. Cheilocystidia abundant, 57–68 × 19–25 µm, and narrowly elongated clavate, often clustered in threes or fours. Pleurocystidia rarely present, 45–47.5 × 12–13 µm, and clavate to utriform. This trait distinguishes our sample as *L. velutina* from other *Psathyrella* spp. of the Coprinaceae, which have smooth spores. This taxon was clarified by the observation that *Psathyrella* spores fade in concentrated sulfuric acid. A molecular phylogenetic study revealed that our specimen was *Lacrymaria velutipes*, which is closely related to *Lacrymaria lacrymabunda*. Moreover, those two species are clearly distinguishable from other *Psathyrella* species, which agreed with the morphologically distinctive traits described above. We believe that this is the first report of this taxon, which has not been described in Korea.

KEYWORDS: Internal transcribed spacer, *Lacrymaria velutipes*, Morphology, Neighbor-Joining, Phylogenetic analysis

We found mushrooms in clusters in Jinju area grassy habitats. Young fruiting bodies had obtusely hemispherical caps that became convex with age. Their surface was dry, densely fibrillose-scaly with a split margin with age. The stipe was equal or appeared swollen due to the aggregated soil without a volva at the base and was fibrillose, dry, whitish above the ring zone, with a light brown stalk below.

According to Miller and Miller [1], the pileipellis of *Coprinus comatus*, the type species of the family, in which the pileipellis are filamentous. This character distinguishes this specimen from other dark-spored families, such as *Strophariaceae*, *Cortinariaceae*, and *Agaricaceae*, which have a filamentous cap cuticle. However, the *Bolbitiaceae* have clavate or rounded cells in the pileipellis, which is similar to the *Coprinaceae*, but have yellow brown cinnamon brown to earth brown smooth spores, which are thick walled with an apical pore. Of the genera in this family, *Conocybe* and *Bolbitius* are distinguished from our samples by a conical cap shape, but *Agrocybe* spp. is rather similar with convex to flat type caps and brown spores.

Cho and Lee [2] reported *Psathyrella candolleana* in Korea. They also described *Psathyrella hydrophila* [3] and *Psathyrella gracillis* from Mt. Mudeong, Kwangju [4]. Park et al. [5, 6] attempted to define the morphological features of coprinoid mushrooms using molecular approaches based on internal transcribed spacer (ITS) II and both ITS I and ITS II sequences. We investigated the macroscopical and microscopic features as well as phylogenetic approaches to identify *Lacrymaria velutina* to the species level.

Materials and Methods

Mushroom samples (GNU100529-2), collected at Micheon-myeon, Jinju, were identified by morphological examination and a phylogenetic approach.

Macroscopic and microscopic characterization. The macroscopic features were observed carefully and recorded according to Smith et al. [7], Largent [8], and Largent and Thiers [9]. The microscopic features were characterized according to Kuo [10], McAdam [11], and Largent et al. [12].

DNA extraction, PCR, and sequencing. Sequences were obtained from fresh material and from herbarium specimens. Total DNA was isolated using the Exgene
Plant-Fungal SV Mini kit (Geneall Biotechnology Co., Seoul, Korea), following the manufacturer’s recommendations. PCR reactions were conducted using a Thermal Cycler TC-512 instrument (Techne, Burlington, NJ, USA). The primers used to amplify the complete ITS region were ITS1F (CTTGGTCAATTAGGAGGAAGTAA) and ITS4 (TCCTCCGCTATTGATATGC) [13, 14]. The PCR amplification reaction mixture (25 µL) contained 50 µM of each primer, Smart Taq Pre-Mix (Solgent, Seoul, Korea) and 1 µL DNA extract. PCR products were isolated using gel electrophoresis on 1.2% (w/v) agarose gels containing ethidium bromide. Bands were visualized using UV light, extracted from the gel, and purified using a Gel Extraction kit (Geneall Biotechnology). Purified PCR products were sequenced on an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). Sequencing was performed using a Bigdye Terminator Cycle Sequencing kit (Applied Biosystems), following the manufacturer’s protocol.

Alignment and phylogenetic analyses. Our DNA sequences, together with relevant sequences from Genebank, are shown in Table 1. Gaps were treated as missing data. The evolutionary history was inferred using the neighbor-joining (NJ) method [15]. A bootstrap consensus tree, inferred from 1000 replicates, represents the evolutionary history of the taxa analyzed [16]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown above the branches [16]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura two-parameter method [17] and are in the units of the number of base substitutions per site. Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). A total of 721 positions were included in the final dataset. Phylogenetic analyses were conducted in MEGA4 [18]. The ITS1-5.8s-ITS2 rDNA sequence was submitted to GenBank for an accession number.

Results

Macroscopic characters. Fruiting bodies were found in groups or clusters in grassy habitats: cap, obtusely

| Species                     | Collection and herbarium | Locality       | GenBank accession No. |
|-----------------------------|--------------------------|----------------|------------------------|
| *Lacrymaria velutina*       | GNU100529-2              | Jinju-city, Korea | HQ455789               |
| L. velutina                 | GeneBank, KACC500079     | Korea          | AF1345811              |
| L. velutina                 | GeneBank, PBM 2439       | USA            | DQ490639               |
| *Lacrymaria lacrymahunda*   | GeneBank, EL7-03         | Sweden         | DQ389724               |
| *Psathyrella spadicea*      | GeneBank, 981111Epitype  | Sweden         | DQ389729               |
| *Psathyrella variata*       | GeneBank, BRNM:705636    | Austria        | AM712289               |
| *Psathyrella cernua*        | GeneBank, BRNM:705615    | Czech          | AM712286               |
| *Psathyrella avellaneifolia*| GeneBank, K 74418        | USA            | AM712285               |
| *Psathyrella cf. longicauda*| GeneBank, LO382-89       | Sweden         | DQ389667               |
| *Psathyrella pratensis*     | GeneBank, LO23-94        | Sweden         | DQ389678               |
| *Psathyrella bipellis*      | GeneBank, LO207-96       | Sweden         | DQ389679               |
| *Psathyrella candolleana*   | GeneBank, TNS-F-12192    | Japan          | AB306311               |
| *P. candolleana*            | GeneBank, GBDS2191       | Korea          | AF606082               |
| *Psathyrella stercorearia*  | GeneBank, LO460-05       | Sweden         | DQ389669               |
| *Psathyrella atomata*       | GeneBank, LO127-01       | Sweden         | DQ389665               |
| *Psathyrella hydrophila*    | GeneBank, JMT22479       | USA            | FJ899615               |
| *Coprinopsis cinerea*       | GeneBank, SFSU DEH2065   | USA            | FJ461825               |
| *Coprinopsis marcescillator*| GeneBank, LO31-03        | Sweden         | DQ389728               |
| *Coprinopsis atramentaria*  | GeneBank, PBM 992        | USA            | DQ486694               |
| *Coprinus comatus*          | GeneBank, 3-XII-2001     | USA            | AY176346               |
| C. comatus                  | GeneBank, GBDS536        | Korea          | AF059584               |
| *Parasola conopila*         | GeneBank, OSC50296       | USA            | FJ899613               |
| *Parasola placitella*       | GeneBank, SZMC:NL:0295   | Hungary        | FM163216               |
| *Panaeolus uliginosus*      | GeneBank, DAOM 176594    | Canada         | AY129363               |
| *Panaeolus cyanescens var. hisporus* | GeneBank, n. 6576 AQUI | Italy          | EU834287               |
| *Cortinarius aureogranulatus*| GeneBank, CBS973.95      | Netherlands    | GQ249274               |
| *Cortinarius micaceus*      | GeneBank, UBC F19669     | Canada         | HM240519               |
| *Agrocybe pediades*         | GeneBank, MSC 378490     | USA            | AY194536               |
| *Agrocybe praecoax*         | GeneBank, MSC 378486     | USA            | AY194531               |
Purple-black, Warted Spores with Distinct Snout-like Germ Pore

umbonate, convex cap with age (2.5–6 cm), surface dry, densely fibrillose-scaly; dull light brown to tawny; margin slightly paler; gills finally deep brown; stipe 4.5–6 cm; equal, fibrillose, dry, whitish above the ring zone, light brown below stalk; veil, obscure superior hairy ring zone; volva, absent (Fig. 1).

Fig. 1. Macroscopic characters of *Lacrymaria velutina* (GNU100529-2). A, Young fruiting bodies with obtusely hemispherical pileus; B, Becoming convex with age. 2.5–6 cm, surface dry, densely fibrillose-scaly with split margins with age; C, Stipe, 4.5–6 cm, equal hollow or swollen at base, fibrillose, dry, whitish above the ring zone (under light), light brown below stalk; D, Rowed gills, adnexe, dark black at maturity.

Fig. 2. Microscopic characters of the pileipellis and gill edge of *Lacrymaria velutina* (GNU100529-2) Cap cuticle. A, Cells under light microscope (×400); B, Gill edge, appearing white and beaded under stereomicroscope (×100).
Microscopic characters. Pileipellis typically cellular with a white gill edge that was beaded in a dried specimen (Fig. 2). Spores blackish brown, elliptical, warty, 9–11 × 6–8 µm, with prominent snout-like germ pore; which discolors in sulfuric acid mount (inset); C, Cheilocystidia 57–68 × 19–25 µm, narrowly elongated clavate, often clustered in threes or fours. Abundant; D, Pleurocystidia clavate to utriform, rarely present, 45–47.5 × 12–13 µm.

Fig. 3. Basidia morphology. A, Basidiospore and cystidia of *Lacrymaria velutina* (GNU100529-2) Basidium 29–35 × 12–17 µm; A, B, Spores blackish brown, elliptical, warty, 9–11 × 6–8 µm, with prominent snout-like germ pore; which discolors in sulfuric acid mount (inset); C, Cheilocystidia 57–68 × 19–25 µm, narrowly elongated clavate, often clustered in threes or fours. Abundant; D, Pleurocystidia clavate to utriform, rarely present, 45–47.5 × 12–13 µm.

Fig. 4. Microscopic features of *Lacrymaria velutina* (GNU100529-2). A, Pores, elliptical, finely warty, 9–11 × 6–8 µm, with typical snout-like germ pore; B, Basidia, 29–35 × 12–17 µm; C, Cheilocystidia, 57–68 × 19–25 µm, narrowly elongated clavate, often clustered in threes or fours; D, Pleurocystidia, rarely present 45–47.5 × 12–13 µm; clavate to utriform.

rDNA sequence and phylogeny. The ITS1-5.8s-ITS2 rDNA sequence is available at GenBank as accession number HQ455789 (Table 1). A molecular phylogenetic study revealed that our specimen was identified as *Lacrymaria velutipes* (Fig. 5) with a high bootstrap consensus, which agrees with the morphologically based taxonomy,
and that it is closely related to *Lacrymaria lacrymabunda*. These two species were clearly distinguished from other *Psathyrella* species. Coprinoid taxa were rooted with *Agrocybe* species of *Bolbitiaceae* and were clustered together.

*Parasola*, *Coprinopsis*, and *Coprinellus*, formerly included in *Coprinus*, were distinguishable from *Psathyrella* spp. with the exception of *Psathyrella condolleana* and *P. hydrophila*. *Parasola* conopila and *Parasola* plicatus were clustered together, but long branches were suggestive of a dissimilarity. *Coprinopsis* spp. were rather distinctive in one cluster.

Interestingly, *P. hydrophila* was separated from the other *Psathyrella* species and positioned in *Coprinus* and *Panaeolus* subclusters but with a very long branch. This indicates greater genetic dissimilarity not only between *P. hydrophila* and *Coprinus* or *Panaeolus* but also between *Coprinus* or *Panaeolus* species. *Coprinellus* spp. clustered together in one group but appeared closer to *Psathyrella* taxa.

**Discussion**

Of the six common *Coprinaceae* genera, *Coprinus* and *Coprinopsis* spp. have gills that deliquesce, but the pileipellis of *Coprinus* is microscopically composed of radially arranged filamentous cells, whereas they are elongate in *Coprinopsis*. *Parasola* is a fungus that deliquesces slowly.
and has clavate hymeniform cells in the cap cuticle. The gills of *Coprinellis* are not deliquescent, and round cells are present in the cap cuticle, which looks similar, but has a conic cap with a striate margin. Finally, *Panaeolus* and *Psathyrella* gills do not deliquesce and are closely related with each other, according to Smith et al. [7]. Both have cellular or round cells in the cap cuticle and share common habitats; the stipe is tall and thin with a thin, conic pileus in *Panaeolus*, whereas it is variable in size and shape in *Psathyrella*, with a thick, fleshy, convex pileus. Such confusion is further clarified by the observation that the spores of *Psathyrella* discolor in concentrated sulfuric acid, whereas those of *Panaeolus* retain their original color [1, 20].

Our specimens collected on May 29, 2010 from Michenmyeon, Jinju city revealed a cellular pileipellis and dark purple-black, warty spores with distinct snout-like germ pores (Fig. 4). This observation distinguishes our sample as *P. velutina* from the other *Psathyrella* spp. of the *Coprinaceae*, which have smooth spores [19]. This taxon was confirmed to have spores that fade in concentrated sulfuric acid, whereas those of *Panaeolus* do not. *P. velutina* has larger spores, 9–12 × 6–7 µm, compared to *P. lacrymabunda* which has smooth, smaller spores, which are 6–7.5 × 3.2–4 µm [7].

However, mycologists have recognized the fibrillose cap and the barely noticeable “ring zone” on the upper stipe resulting from the deterioration of the partial veil as unusual or rather un-*Psathyrella*-like over the years and have debated where to place it [21]. Such an opinion was shared by Arora [20] who commented that this species is unusually large and sturdy for a *Psathyrella* and likely to be another genus. The fibrillose to fibrillose-scaly cap and stalk, obscure hairy annulus, and blackish brown spores are distinctive. Arora [20] suggested *L. velutina* as a synonym for *P. velutina*. *Lachrymaria lacrymabunda* has also been suggested as a synonym for *P. velutina* [1], but it has smooth spores [7], which is not acceptable based on the spore shape and spore size of 6–7.5 × 3.2–4 µm. Nevertheless, the literature on the cystidia description of the gill has not been consistent for the presence of either pleurocystidia [7, 21] or cheilocystidia [1, 19, 22]. We found both types of cystidia on our specimen and confirmed that cheilocystidia are abundant and common, whereas pleurocystidia are rarely present.

*Lachrymaria* was recently confirmed as a distinct genus by Hopple and Vilgalys [23]. Moncalvo et al. [24] have also confirmed that the psathyrella clade is not a monophyle of the large genus *Psathyrella*. *L. velutina* is closely related to *Psathyrella* species and belongs in *Lachrymaria* based on phylogenetic analyses of the nLSU rDNA nucleotide sequence. Since then, *L. velutina* has been accepted in the mycological community [21, 22].

Some concerns exist that NJ methods yield slightly different topologies depending on the distance parameters. Adding or omitting taxa significantly changes the topologies and the clade composition [25]. The average pairwise Jukes-Cantor (JC) distance should be < 1.0, which makes data suitable for NJ trees, according to Nei and Kumar [26], authors of MEGA. Therefore, we confirmed that our data had a JC distance of 0.132, and, thus, were suitable for the NJ tree method [27]. A molecular phylogenetic study revealed that our specimen is *L. velutina* with high bootstrap support, based on clustering with Korean strain KACC500079 (Table 1) and US strain PMB 2439 (Fig. 5). The Korean common name is “large teardrop mushroom” [28], and the common name is “weeping widow,” which refers to the water droplet that exudes from the gills of this mushroom [29]. This species was closely related to *L. lacrymabunda*. Moreover, those two species are clearly distinguished from other *Psathyrella* species, which agrees with the morphologically distinctive traits discussed above.

*Parasola, Coprinopsis, and Coprinellus*, were formerly included in *Coprinus* and distinguishable from *Psathyrella* *hydrophila*. *Parasola conopila* and *Parasola plicatus* grouped together but with long branches suggestive of dissimilarity. *Coprinopsis* spp. were rather distinctive in one cluster, although this genus was reported to be closer to *Psathyrella* taxa by Walther et al. [30] and Larsson and Örstadius [31].

*Psathyrella hydrophila* was separated from the other *Psathyrella* species and positioned in *Coprinus* and *Panaeolus* clusters but with a very long branch. This also indicates greater genetic dissimilarity between them. *Psathyrella* appears to be polyphyletic, as evidenced by Vasutová et al. [32].

The family Psathyrellaceae was established from the traditional family Coprinaceae [23, 24] and *C. comatus*, the type species, was transferred to the lepiotoid fungi in *Agaricaceae* based on ITS-LSU data [25]. Nevertheless, this has been a source for significant controversy between mycologists and molecular phylogenists over the last decade. Such a molecular phylogeny was also supported by Walther et al. [30], who provided relevant anamorphic congeniduous hyphae to distinguish *Psathyrella* (chain shortening), *Panaeolus* (coiling), and *Coprinus* (swelling of hyphal ends to the conidia is typical for *Agaricaceae*).

Our method certainly resolved the identity of our specimen as *L. velutina*, although the phylogenetic tree did not support the results of Vellinga [25]. To clarify the phylogenetic relationship of coprinoid taxa, additional *Psathyrella* and coprinoid species must be examined morphologically and in studies that include a method capable of considering very large phylogenies.

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