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, $9\sim11 \times 6\sim8 \mu\text{m}$, with prominent snout-like germ pores. Cheilocystidia abundant, $57\sim68 \times 19\sim25 \mu\text{m}$, and narrowly elongated clavate, often clustered in threes or fours. Pleurocystidia rarely present, $45\sim47.5 \times 12\sim13 \mu\text{m}$, 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 Coprinaceae is cellular with few exceptions, i.e., *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 microscopical 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

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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 (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) [13, 14]. The PCR amplification reaction mixture (25 μ L) contained 50 μ M of each primer, Smart Taq Pre-Mix (Solgent, Seoul, Korea) and 1 μ L 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

Table 1. Internal transcribed spacer sequence list for the phylogenetic analysis

Species	Collection and herbarium	Locality	GenBank accession No.
<i>Lacrymaria velutina</i>	GNU100529-2	Jinju-city, Korea	HQ455789
<i>L. velutina</i>	GeneBank, KACC500079	Korea	AF345811
<i>L. velutina</i>	GeneBank, PBM 2439	USA	DQ490639
<i>Lacrymaria lacrymabunda</i>	GeneBank, EL7-03	Sweden	DQ389724
<i>Psathyrella spadicea</i>	GeneBank, 981111Eptype	Sweden	DQ389729
<i>Psathyrella variata</i>	GeneBank, BRNM:705636	Austria	AM712289
<i>Psathyrella cernua</i>	GeneBank, BRNM:705615	Czech	AM712286
<i>Psathyrella avellaneifolia</i>	GeneBank, K 74418	USA	AM712285
<i>Psathyrella cf. longicauda</i>	GeneBank, LO382-89	Sweden	DQ389667
<i>Psathyrella pratensis</i>	GeneBank, LO23-94	Sweden	DQ389678
<i>Psathyrella bipellis</i>	GeneBank, LO207-96	Sweden	DQ389679
<i>Psathyrella candolleana</i>	GeneBank, TNS-F-12192	Japan	AB306311
<i>P. candolleana</i>	GeneBank, GBDS2191	Korea	AF060082
<i>Psathyrella stercoraria</i>	GeneBank, LO460-05	Sweden	DQ389669
<i>Psathyrella atomata</i>	GeneBank, LO127-01	Sweden	DQ389665
<i>Psathyrella hydrophila</i>	GeneBank, JMT22479	USA	FJ899615
<i>Coprinopsis cinerea</i>	GeneBank, SFSU DEH2065	USA	AY461825
<i>Coprinopsis marcescibilis</i>	GeneBank, LO31-03	Sweden	DQ389728
<i>Coprinopsis atramentaria</i>	GeneBank, PBM 992	USA	DQ486694
<i>Coprinus comatus</i>	GeneBank, 3-XII-2001	USA	AY176346
<i>C. comatus</i>	GeneBank, GBDS536	Korea	AF059584
<i>Parasola conopsea</i>	GeneBank, OSC50296	USA	FJ899613
<i>Parasola plicatilis</i>	GeneBank, SZMC:NL:0295	Hungary	FM163216
<i>Panaeolus uliginosus</i>	GeneBank, DAOM 176594	Canada	AY129363
<i>Panaeolus cyanescens</i> var. <i>bisporus</i>	GeneBank, n. 6576 AQU1	Italy	EU834287
<i>Coprinellus aureogranulatus</i>	GeneBank, CBS973.95	Netherlands	GQ249274
<i>Coprinellus micaceus</i>	GeneBank, UBC F19669	Canada	HM240519
<i>Agrocybe pediades</i>	GeneBank, MSC 378490	USA	AY194536
<i>Agrocybe praecox</i>	GeneBank, MSC 378486	USA	AY194531

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, Rowd gills, adnexed, dark black at maturity.

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; veil, obscure superior hairy ring zone; volva, absent (Fig. 1).

Fig. 2. Microscopic characters of the pileipellis and gill edge of *Lacrymaria velutina* (GNU100529-2) Cap cuticle. A, Cells under light microscope ($\times 400$); B, Gill edge, appearing white and beaded under stereomicroscope ($\times 100$).

Fig. 3. Basidia morphology. A, Basidiospore and cystidia of *Lacrymaria velutina* (GNU100529-2) Basidium $29\sim35 \times 12\sim17 \mu\text{m}$; A, B, Spores blackish brown, elliptical, warted, $9\sim11 \times 6\sim8 \mu\text{m}$, with prominent snout-like germ pore; which discolors in sulfuric acid mount (inset); C, Cheilocystidia $57\sim68 \times 19\sim25 \mu\text{m}$, narrowly elongated clavate, often clustered in threes or fours. Abundant; D, Pleurocystidia clavate to utriform, rarely present, $45\sim47.5 \times 12\sim13 \mu\text{m}$.

Microscopic characters. Pileipellis typically cellular with a white gill edge that was beaded in a dried specimen

Fig. 4. Microscopic features of *Lacrymaria velutina* (GNU100529-2). A, Pores, elliptical, finely warty, $9\sim11 \times 6\sim8 \mu\text{m}$, with typical snout-like germ pore; B, Basidia, $29\sim35 \times 12\sim17 \mu\text{m}$; C, Cheilocystidia, $57\sim68 \times 19\sim25 \mu\text{m}$, narrowly elongated clavate, often clustered in threes or fours; D, Pleurocystidia, rarely present $45\sim47.5 \times 12\sim13 \mu\text{m}$; clavate to utriform.

(Fig. 2). Spores blackish brown, elliptical, warted, $9\sim11 \times 6\sim8 \mu\text{m}$, with prominent snout-like germ pores (Figs. 3A, 3B and 4B) that discolored or faded in concentrated sulfuric acid mounts (Fig. 3B inset). Cheilocystidia, $57\sim68 \times 19\sim25 \mu\text{m}$, narrowly elongated clavate, often clustered in threes or fours; abundant, (Figs. 3C and 4C). Pleurocystidia clavate to utriform, rarely present, $45\sim47.5 \times 12\sim13 \mu\text{m}$ (Figs. 3D and 4D). This character distinguishes our sample as *Psathyrella velutina* from other *Psathyrella* spp. of the *Coprinaceae*, which have smooth spores [19]. This taxon was clarified by the observation that the spores of *Psathyrella* fade in concentrated sulfuric acid (Fig. 3B inset), whereas *Paneolus* spores do not. *L. velutina* has larger spores, $9\sim12 \times 6\sim7 \mu\text{m}$, compared to *P. lacrymabunda*, which has smoother smaller spores, $6\sim7.5 \times 3.2\sim4 \mu\text{m}$ [7]. The macroscopic and/or microscopic morphological traits are summarized in Table 2.

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,

Table 2. *Lacrymaria velutina* (GNU100529-2) characters

Character	<i>Lacrymaria velutina</i> (GNU100529-2)
Location	Micheon-myeon, Jinju city
Date sampled	2010. 05. 29
Pileus, Pileipellis	Umbonate hemispherical, convex in age (2.5~6 cm), cellular
Flesh	Thick, brownish to ochre
Stipe	4.5~6 cm; equal or swollen at base, fibrillose, dry, whitish above the ring zone, light brown below
Veil	Obscure superior hairy ring zone
Volva	Absent
Gills	Crowded, adnexed, pale yellow to light brown, finally deep brown; edge white
Spore	Blackish brown, elliptical, warted, 9~11 × 6~8 µm, with prominent snout-like germ pore
Basidium	29~35 × 12~17 µm
Pleurocystidia	45~47.5 × 12~13 µm; present rarely, clavate to utriform
Cheilocystidia	57~68 × 19~25 µm; abundant, narrowly elongated clavate, often clustered in threes or fours

Fig. 5. Phylogenetic tree for the ribosomal internal transcribed spacer (ITS)1-5.8s-ITS2 dataset based on the neighbor-joining method. Bootstrap values > 50% are given above the branch. The scale at the bottom of the figure shows the number of substitutions per site represented by a line of particular length.

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 conopsea* 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, warted 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, $9\text{--}12 \times 6\text{--}7\text{ }\mu\text{m}$, compared to *P. lacrymabunda* which has smooth, smaller spores, which are $6\text{--}7.5 \times 3.2\text{--}4\text{ }\mu\text{m}$ [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*. *Lacrymaria 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 $6\text{--}7.5 \times 3.2\text{--}4\text{ }\mu\text{m}$. 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.

Lacrymaria 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 monophyly of the large genus *Psathyrella*. *L. velutina* is closely related to *Psathyrella* species and belongs in *Lacrymaria* 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. velutipes* 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 conopsea* 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 phylogeneticists over the last decade. Such a molecular phylogeny was also supported by Walther *et al.* [30], who provided relevant anamorphic conidigenous 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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