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Short communication

A new Amazonian species of *Calocera* with dendroid and multi-headed basidiocarp

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ABSTRACT

During a biodiversity survey of Amazonian jelly fungi, we collected a unique dacrymycetous fungus. This fungus is characterized by stipitate, branched and multi-headed basidiocarps with fascicled marginal hyphae on the sterile parts of the basidiocarps and narrow cylindrical to navicular basidiospores. No dacrymycetous species with these morphological characteristics has been reported. Based on phylogenetic analysis of 28S rDNA sequences, the new specimens belong into the Dacrymycetaceae lineage. As an appropriate genus cannot be inferred from the phylogeny, we describe this fungus as a new species, *Calocera arborea*, based on its morphological characters.

Species diversity among Amazonian jelly fungi has been researched by Bernard Lowy, who has described many species within the Tremellales, Auriculariales, and Dacrymycetales (Lowy 1959, 1971, 1981, 1985, 1987), but there has been no more recent research. To reveal the undiscovered species diversity of Amazonian jelly fungi, we investigated the tropical rainforests in Manaus (Amazonas, Brazil). During these surveys, we collected fruit-bodies of a dacrymycetous fungus with unique dendroid basidiocarps from dead branches and trunks of unknown broad-leaved trees on the forest floor. In this short report, the taxonomic position of this dacrymycetous species is discussed based on morphological and molecular data. It is described as a new species of *Calocera* (Fr.) Fr.

Three sites in Manaus were investigated in August 2011: the Reserve Adolpho Ducke, the Reserve Biológica de Campina, and the Reserva Experimental de Silvicultura Tropical. In each site, basidiocarps of jelly fungi were collected from woody debris on the forest floor. Basidiocarps were taken to a laboratory at the Instituto Nacional de Pesquisas da Amazônia (INPA), where macro- and microscopic observations were made. Specimens were air-dried and deposited in the INPA Herbarium (specimen nos.: INPA 241455, 241457 and 241458).

Detailed morphological observations were made in the laboratory of the Fungus/Mushroom Resource and Research Center, Tottori University, Tottori, Japan. The dried

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basidiocarps were rehydrated and sliced using a freezing microtome (REM-710, MC-802A; Yamato Kohki, Saitama, Japan) to make preparations for light microscopic observations. Sections were mounted in lactic acid on microscopic slides. A binocular dissecting microscope (SMZ1500; Nikon) and a light microscope (Eclipse 80i; Nikon, Tokyo, Japan) were used for observations and line drawings.

Genomic DNA was extracted from the basidiocarps following the modified CTAB method described by Matsuda and Hijii (1999). Polymerase chain reactions (PCR) were performed using a Quick Taq HS DyeMix (Toyobo, Osaka, Japan). Each PCR reaction contained a 50-μl mixture (21 μl distilled water, 25 μl master mix, 3 μl ca. 0.5 ng/μl template DNA, and 0.5 μl of each primer [final, 0.25 μM]). The primer pairs ITS1f (Gardes and Bruns 1993)/LR3 (Vilgalys and Hester 1990), or LR0R (Moncalvo et al. 1995)/LR3 were used to obtain the D1/D2 domain of the 28S rDNA. Each DNA fragment was amplified using a PCR thermal cycler (DNA Engine; Bio-Rad, Hercules, CA, USA) with the following thermal cycling schedule: the first cycle consisted of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 50 (ITS1f/LR3) or 54 °C (LR0R/LR3) for annealing, 1 min at 68 °C, and a final cycle of 10 min at 68 °C. The reaction mixture was then cooled at 4 °C for 5 min. PCR products were purified using a QiAquick PCR Purification Kit (Qiagen, Mississauga, Canada) following the manufacturers' instructions. Purified PCR products were sequenced by FASMAC Co., Ltd. (Kanagawa, Japan).

The sequences determined in this study, the dacrymycetous D1/D2 sequences analyzed by Shirouzu et al. (2009) and the D1/D2 sequence of *Calocera lutea* (Massee) McNabb (AB712379) were combined into a dataset. A multiple alignment was conducted using MAFFT v6 (Katoh and Toh 2008; mafft.cbrc.jp/alignment/software), and nucleotide sites that contained gaps were excluded from the analysis. The alignment can be viewed at TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S12760). Maximum likelihood (ML) and parsimony (MP) methods were used for phylogenetic analyses. The ML analyses were done using RAxML 7.2.6 (Stamatakis 2006), under a GTRGAMMA model. The bootstrap proportions (MLBP) and trees were obtained by running rapid bootstrap analysis of 1000 pseudo-replicates followed by a search for the tree with the highest likelihood. MP analyses were carried out using PAUP v4.0b10 (Swofford 2003), with the heuristic search option and the tree-bisection-reconnection algorithm with 1000 random sequence additions, to find the global optimum tree. All sites were treated as unordered and unweighted. A bootstrap analysis with 1000 replicates (in each replicate performing one round of heuristic search with random addition of sequences and implementing TBR branch swapping) was used to estimate clade support by bootstrap proportions (MPBP).

1. Taxonomy

Calocera arborea Shirouzu, sp. nov. Figs. 1 and 2.
MycoBank no.: MB 800473.

Calocera arborea is characterized by stipitate, branched and multi-headed basidiocarps. The fascicled marginal hyphae on the sterile parts of basidiocarps and narrow cylindrical to navicular basidiospores are also diagnostic characters of this species.

Holotype: Brazil, Amazonas, Manaus, Reserve Adolpho Duke, on a decorticated dead branch of a broad-leaved tree on the forest floor, 6 Aug 2011, *TSBR005*, leg. D.L. Komura (INPA 241458).

Additional specimens examined: Brazil, Amazonas, Manaus, Reserve Adolpho Duke, on a decorticated dead branch of

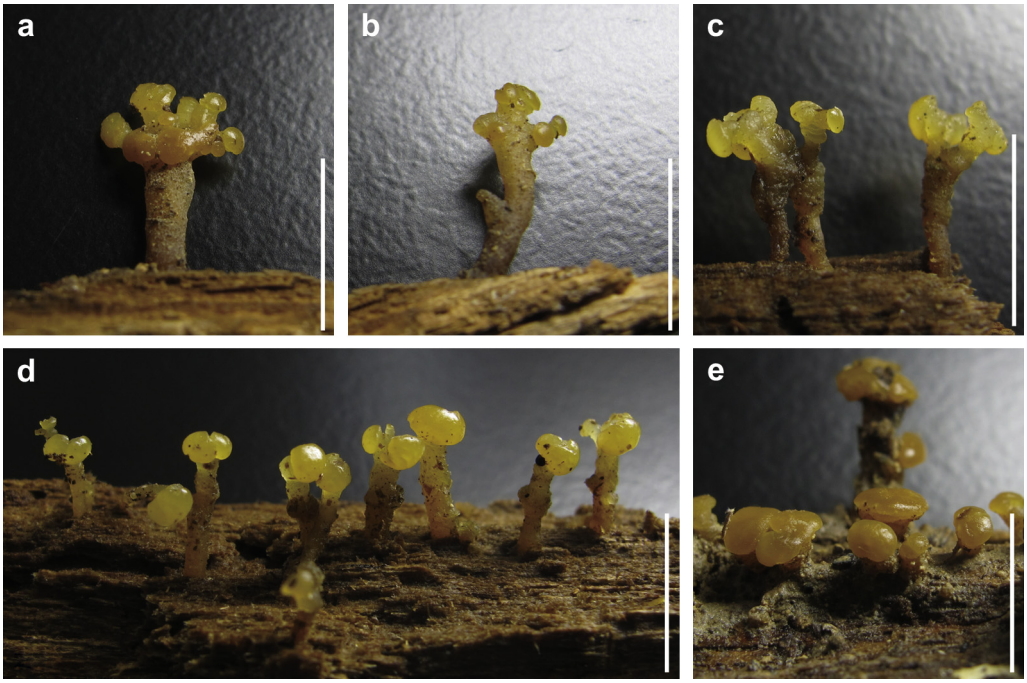

Fig. 1 – Basidiocarps of *Calocera arborea*. a, b: INPA 241457. c, d: INPA 241458 (holotype). e: INPA 241455. *Bars* 10 mm.

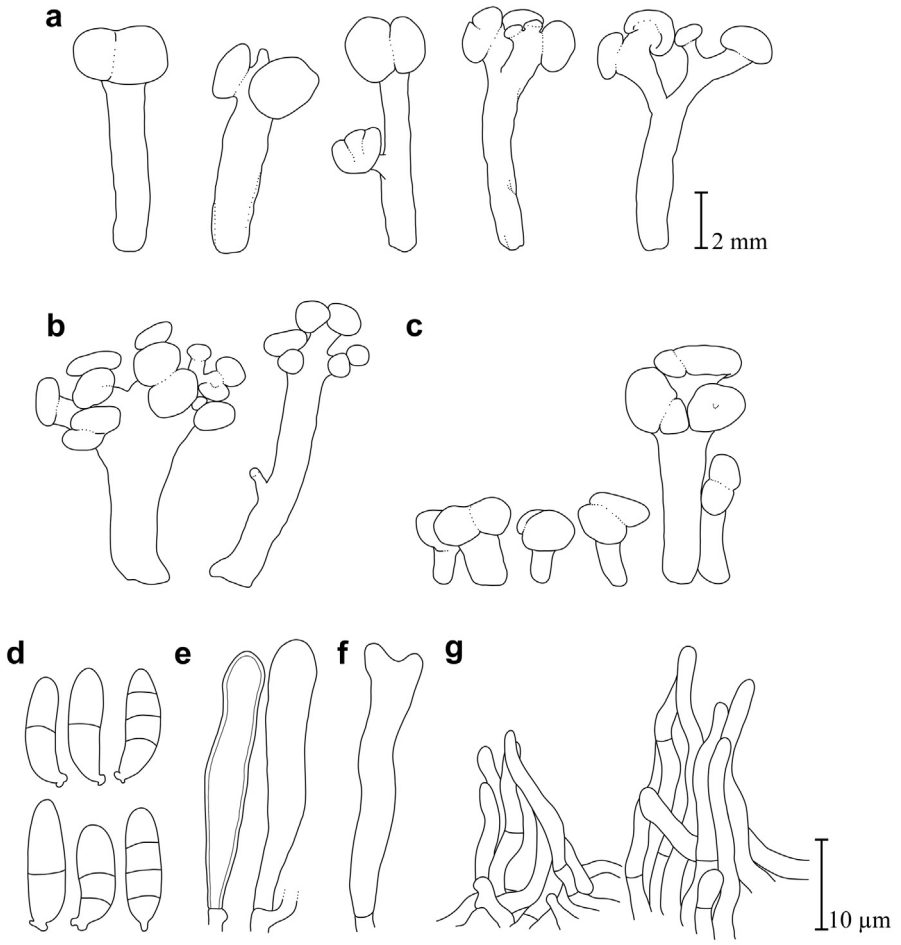

Fig. 2 – *Calocera arborea*. a–c: basidiocarps. d: basidiospores. e: Probasidia. f: developing basidium. g: marginal hyphae. a, d–g: INPA 241458 (holotype). b: INPA 241457. c: INPA 241455.

a broad-leaved tree on the forest floor, 28 Jun 2011, *GLO012*, leg. G.O. Luz (INPA 241472); *GLO013*. leg. G.O. Luz (INPA 241473); 6 Aug 2011, *TSBR002*, leg. N.K. Ishikawa (INPA 241455); on a decorticated dead trunk, *TSBR004*, leg. N.K. Ishikawa (INPA 241457).

28S rDNA sequence: AB723514 (ex holotype, INPA 241458); AB723513 (INPA 241457).

ITS1-5.8S-ITS2 region sequence: AB744230 (ex holotype, INPA 241458).

Etymology: *Arboreus*, from the shape of basidiocarp.

Basidiocarps scattered, cylindrical, simple or branched, dendroid, stipitate, pileate, bearing a subglobose to hemispherical head on the main axis and each branch, yellow to brown, soft-cartilaginous to firm-gelatinous, 5–11 mm high, 1–2 mm in diameter at the stipe, 4–8 mm in diameter at the upper branching part, in transverse section through the pileus showing an organization into three zones: a central core of compact parallel hyphae surrounded by a zone of loosely interwoven hyphae enclosed by the hymenium. Marginal hyphae on sterile surfaces of basidiocarps cylindrical, simple or branched, straight or flexuous, septate, thin-walled, hyaline, forming fascicles of 20–30 × 20 μm. Internal hyphae branched, septate, thin- or thick-walled, hyaline, 2–3 μm in diameter, without clamp connections. Hymenium limited to the surfaces of the heads, usually amphigenous, rarely unilateral. Probasidia cylindrical to clavate, pale yellow, 25–35 × 4–5 μm, without basal clamp connections, becoming bifurcate. Basidiospores cylindrical to navicular, straight or curved, with an apiculum at the base, thin-walled, hyaline, 11–15 × 3.5–4.5 μm, 13 × 4 μm on average ($n = 20$), 1–3 septate, germination via germ tubes.

In the molecular phylogeny, *C. arborea* (AB723513 and AB723514) was positioned in the Dacrymycetaceae lineage (Fig. 3). The family Dacrymycetaceae is a morphologically diverse group including pulvinate, turbinate, stipitate spathulate and dendroid species (McNabb and Talbot 1973; Oberwinkler 1993; Shirouzu et al. 2009). *Calocera arborea* clusters with *Dacrymyces minutus* (L.S. Olive) McNabb, but MLBP and MPBP supports are low. The analyses suggest that *C. arborea* is a member of Dacrymycetaceae, but its closest relatives are unclear. Additional analyses based on a dataset including gap sites showed the same results (data not shown).

Deciding on the appropriate genus was difficult. *Calocera arborea* has stipitate, branched basidiocarps like species of *Calocera* (McNabb 1965a). However, it has fascicled marginal hyphae on the sterile surfaces of basidiocarps, and unilateral

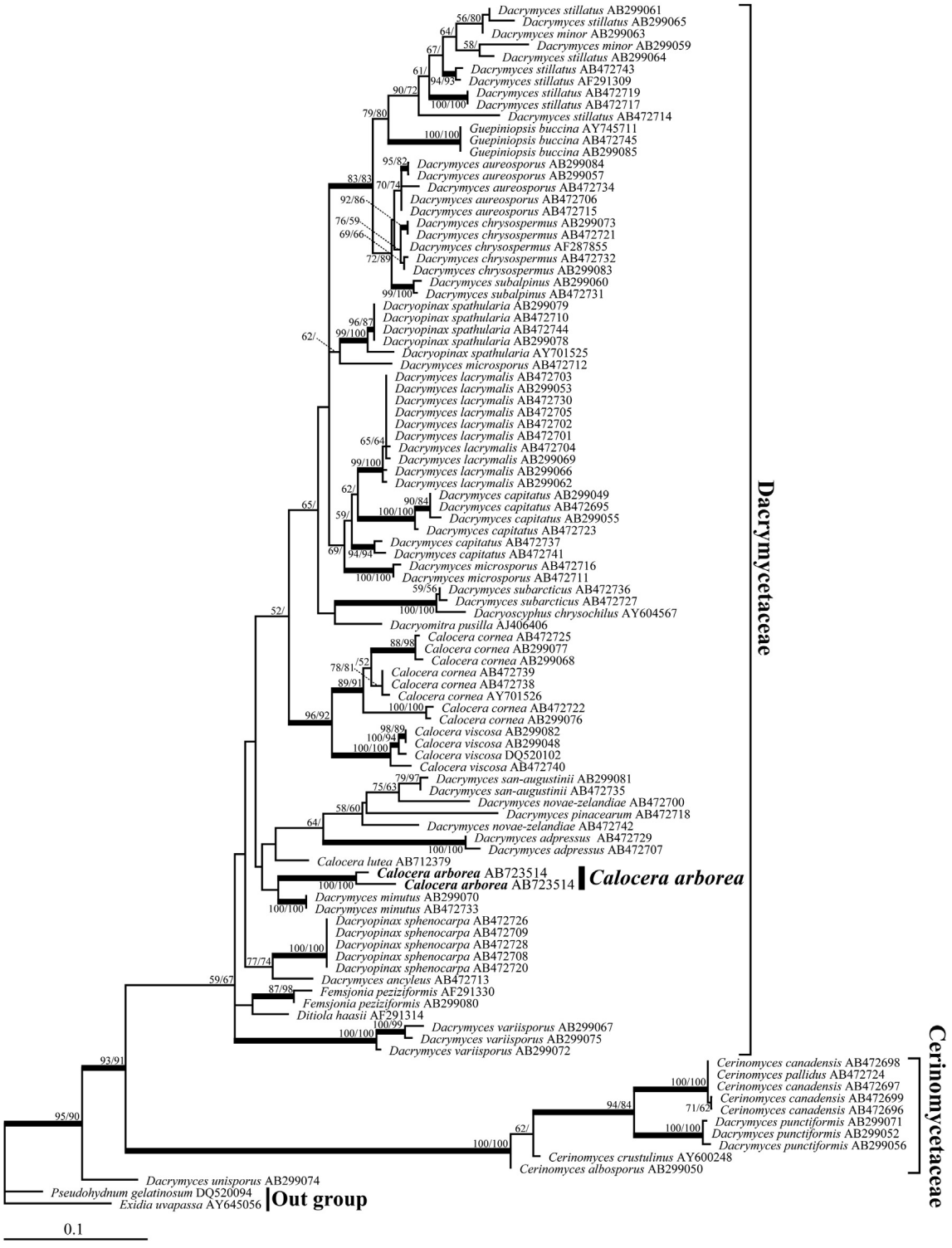

Fig. 3 – Molecular phylogenetic position of *Calocera arborea* within the Dacrymycetes. ML tree based on the dataset of 28S rDNA D1/D2 regions analyzed by Shirouzu et al. (2009). MLBP ≥ 50%/MPBP ≥ 50% are shown near the branches. MLBP ≥ 80%/ MPBP ≥ 80% are indicated by thickened branches.

hymenia were rarely observed. These are diagnostic characters of *Dacryopinax* G.W. Martin (McNabb 1965b). Stipitate and pileate basidiocarps are also found in *Ditiola* Fr. and *Dacrymyces* Nees (McNabb 1966, 1973). In addition, the generic circumscriptions in the Dacrymycetaceae are still unclear based on recent studies showing that *Dacrymyces* and *Dacryopinax* are polyphyletic (Shirouzu et al. 2009). Our phylogenetic tree shows that *C. arborea* may be related with a turbinate species, *D. minutus*, but this hypothesis has low support (Fig. 3). The phylogenetic analysis did not provide support for a particular generic position. We tentatively decided to assign it to a genus based on the morphological

characters, such as the stipitate, branched and dendroid basidiocarps, and three zoned internal structure. These are remarkable characteristics of *Calocera* (McNabb 1965a). Thus, we place this dacrymycetous species in the genus *Calocera*. More research into the phylogenetic relationships and generic circumscriptions within the Dacrymycetaceae is necessary in the future, and will hopefully resolve the current generic uncertainties.

The genus *Calocera* includes a similar species, *C. lutea* (= *Dacryomitra lutea* (Massee) Lloyd), with stipitate, pileate, branched and multi-headed basidiocarps (McNabb 1965a). However, this species and *C. arborea* are morphologically distinguishable because *C. lutea* has morchelloid pilei and broader basidiospores (11.5–13.5 × 4.5–5.5 μm). *Calocera lutea* is phylogenetically separate from our new species (Fig. 3). *Dacryopinax maxidorii* Lowy and *D. foliacea* B. Liu & L. Fan are also similar with stipitate, pileate, and multi-headed basidiocarps (Lowy 1981; Liu et al. 1988); however, *D. maxidorii* has flabelliform pilei and *D. foliacea* has disciform to cerebriform pilei differing from those of *C. arborea* as the latter have subglobose to hemispherical heads. In addition, the basidiospores of *D. maxidorii* are uniseptate and shorter (8–10 × 4.5–5.0 μm; Lowy 1981) and those of *D. foliacea* are multi-septate (3–7 septa) and larger (15–22.5 × 4.5–6.5 μm; Liu et al. 1988). The 28S rDNA D1/D2 sequences for *D. maxidorii* and *D. foliacea* are not available. *Dacrymyces microsporus* P. Karst. is also a similar species having stipitate and pileate basidiocarps (McNabb 1973). This species differs from *C. arborea* in having unbranched and single-headed basidiocarps and smaller basidiospores (7.5–11 × 3–4 μm; McNabb 1973); and it is clearly phylogenetically separate from our new species (Fig. 3). *Calocera morchelloides* B. Liu & L. Fan has stipitate and pileate basidiocarps, but it differs from *C. arborea* in having morchelloid pilei and smaller uniseptate basidiospores (7.8–10.4 × 2.86–4.68 μm; Liu and Fan 1990). In addition to these taxonomic comparisons, we conducted a literature search of more than 100 described species of Dacrymycetes. However, no species similar to *C. arborea* could be found. This fungus is a unique dacrymycetous species with branched and multi-headed basidiocarps with subglobose to hemispherical heads. The phylogenetic position of *C. arborea* may aid our future understanding of the diversification of basidiocarp morphologies in the Dacrymycetaceae.

Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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