Cookeina korfii, a new species hidden in *Cookeina tricholoma*

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Summary: *Cookeina korfii* (Ascomycetes, *Pezizales*, *Sarcoscyphaceae*) is described from the Philippines. Ascospores are smooth, smaller than *Cookeina tricholoma* (Mont.) Kuntze, and hairs are more flexuous than in *C. tricholoma*. Analyses of the ITS region shows it to be close to *Cookeina tricholoma* and *Cookeina sinensis* Z. Wang.
Keywords: *Pezizomycetes*, phylogenetic placement, *Sarcoscyphaceae*, ITS.

Introduction

In our studies of the tropical genera of the *Sarcoscyphaceae*, we have re-evaluated morphological characters of species identified as members of the genus *Cookeina* Kuntze from around the tropical world. There are eight recognized species (Iturriaga & Pfister, 2006). Their relatively large ascomata and bright colored hymenium make it one of the most commonly collected and photographed genera in the *Sarcoscyphaceae*. It occurs in tropical and subtropical regions around the world. In an earlier analysis (Weinstein *et al*., 2002) *C. tricholoma* was shown to be distinct from *C. sinensis* and it was suggested that a single species, *C. tricholoma* was cosmopolitan. In the present study we revisited *C. tricholoma* and bring to light a taxon previously hidden among collections referred to *C. tricholoma*. In this study we evaluated select collections from South Asia that have been identified as *C. tricholoma* and *C. sinensis*.

Material and methods

Collections

Specimens collected from several areas in South Asia that were identified as *C. tricholoma* and *C. sinensis* were examined. Morphological analyses used standard mycological methods (Iturriaga & Pfister, 2006). Measurements of structures and photographs were made with an Olympus SZX-1LLK100 Dissecting Microscope (DM); an Olympus BX40F4 Light Microscope (LM); with an Olympus digital camera (plus Microsuite Special Edition software 3.1. Dried herbarium specimens were rehydrated in distilled water for 1–2 hours and sections of ascomata were made with a freezing microtome. These sections were then mounted in water or Congo red (CR) in ammonia (Vellinga, 2001).

Specimens are deposited as indicated. Slide preparations are deposited with the specimens.

Genomic DNA isolation, PCR and sequencing

Small pieces of apothecia were excised from specimens and ground in Eppendorf tubes using a FastprepTM FP120 Cell Disruptor. Genomic DNA was isolated using DNeasy Plant Mini kit according to the modified protocol of Costa & Roberts (2014). Optimal dilutions of the genomic DNA were used for PCR amplification of the ITS rDNA region. Primers ITS1F (Gardes & Bruns, 1993), ITS4CR (TCCGCCAGATDGCTTYG) or ITS5CR (GGGTATCCCTACCTGATC) were used. All PCR reactions were done in a Peltier Thermal cycler PTC-200 using Econo Taq DNA polymerase. Molecular technique for PCR, purification and sequencing followed Hansen *et al*. (2005). Sequencher 4.6 was used to edit and assemble the DNA sequences. ITS sequences obtained from CUP-SA-2454 and CUP-SA-1797 were deposited in GenBank under the accession numbers KT893781 and KT893782 respectively.

Sequence Analysis

ITS sequences of *C. tricholoma*, *C. sinensis* and *C. speciosa* were retrieved from GenBank. These were those used by Weinstein *et al*. (2002). Alignment of the sequences was done using MAFFT webserver (http://mafft.cbrc.jp/alignment/server, Katoh & Standley, 2013) and then manually optimized in MEGA v6.0 (Tamura *et al*., 2013). The full alignment is available from TreeBASE under accession no. 18354. Maximum parsimony (MP) analysis was applied to the dataset sequences and the tree construction procedure was performed in PAUP* version 4.0b10 (Swofford, 2002). Maximum-Likelihood (ML) analysis was conducted with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org; Miller *et al*., 2009). Bayesian inference (BI) was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck, 2003). Branches that received bootstrap support for ML and MP, Bayesian posterior probabilities (BPP) greater than or equal to 0.95 of BPP, 70% of ML-BP, and 50 % of MP-BP, were considered as significantly supported, respectively.

Results

Morphological analyses

Morphological examination revealed two collections from the Philippines labeled as *C. tricholoma* (Fig. 1), are conspecific and distinct from *C. tricholoma sensu stricto*. Diagnostic characters for this new species are smooth-walled ascospores with a size range of (18–) 22–25 × 9.0–11.5 µm; broad elliptic-fusoid to narrow lemon-shaped spores pointed at both poles, and irregular to rounded apiculi frequently present on one or both poles (Figs. 2 and 3). The relevance of these characters is supported by our phylogenetic results (Fig. 4).

Phylogenetic Analyses

Parsimony analyses based on the ITS gene sequences showed that there were 121 parsimony-informative characters out of 649 total characters, yielding one parsimonious trees by 162,098 steps, in which the monophyletic placement of these collections was significantly supported by 1 of BPP, 100% of ML-BP and 100% of MP-BP (Fig. 4).

Taxonomy

Cookeina korfii Iturr., F. Xu & Pfister, *sp. nov.* — Mycobank 814823

Apothecia solitary or scattered, cupulate, with an eccentric stipe, up to 18 mm high and 35 mm diam when dry (Fig. 1). No record of color when fresh. Orange pigments exuded into water in some dried collections. **Receptacle** concolorous or paler than the disc, light yel-

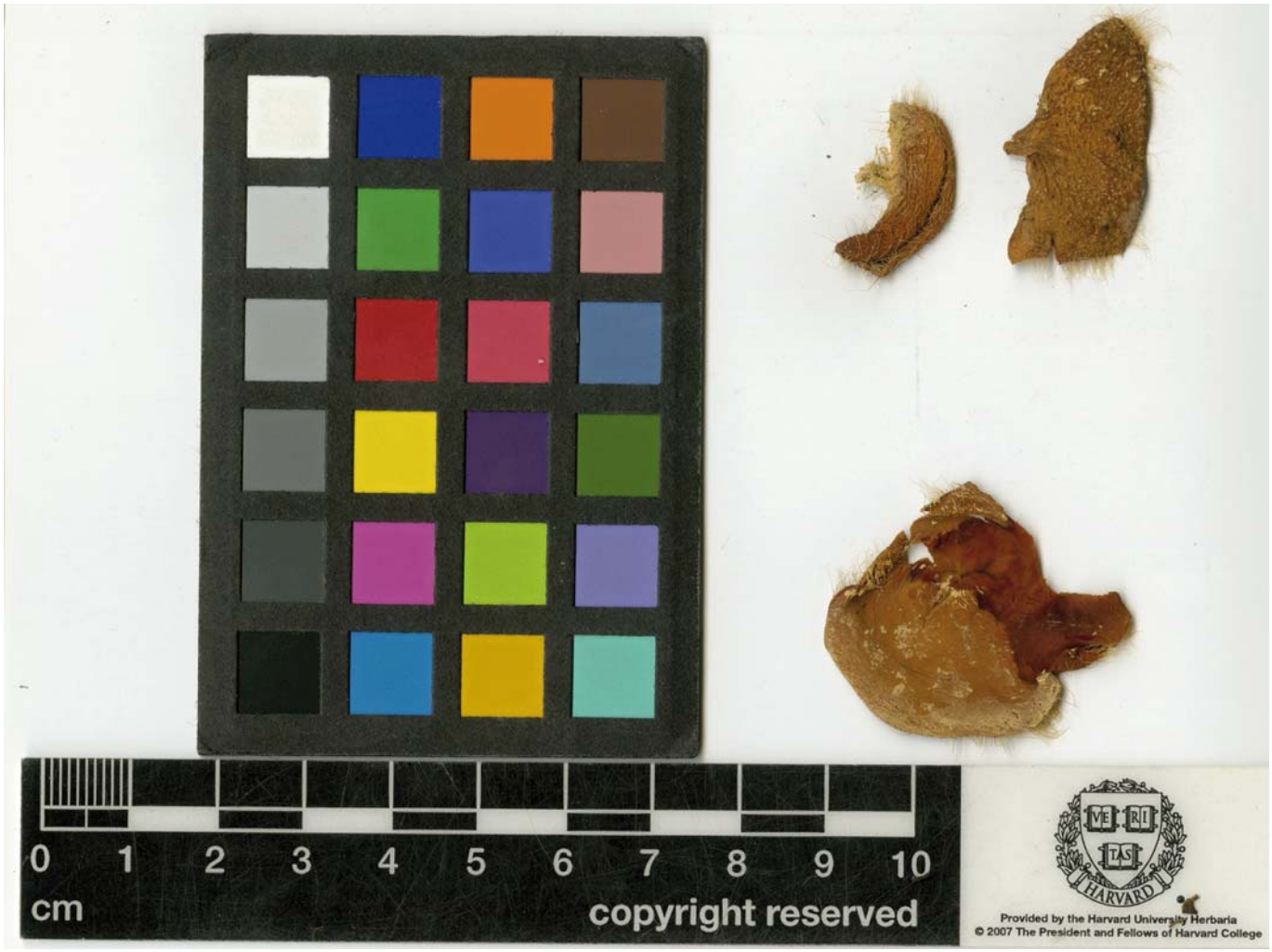


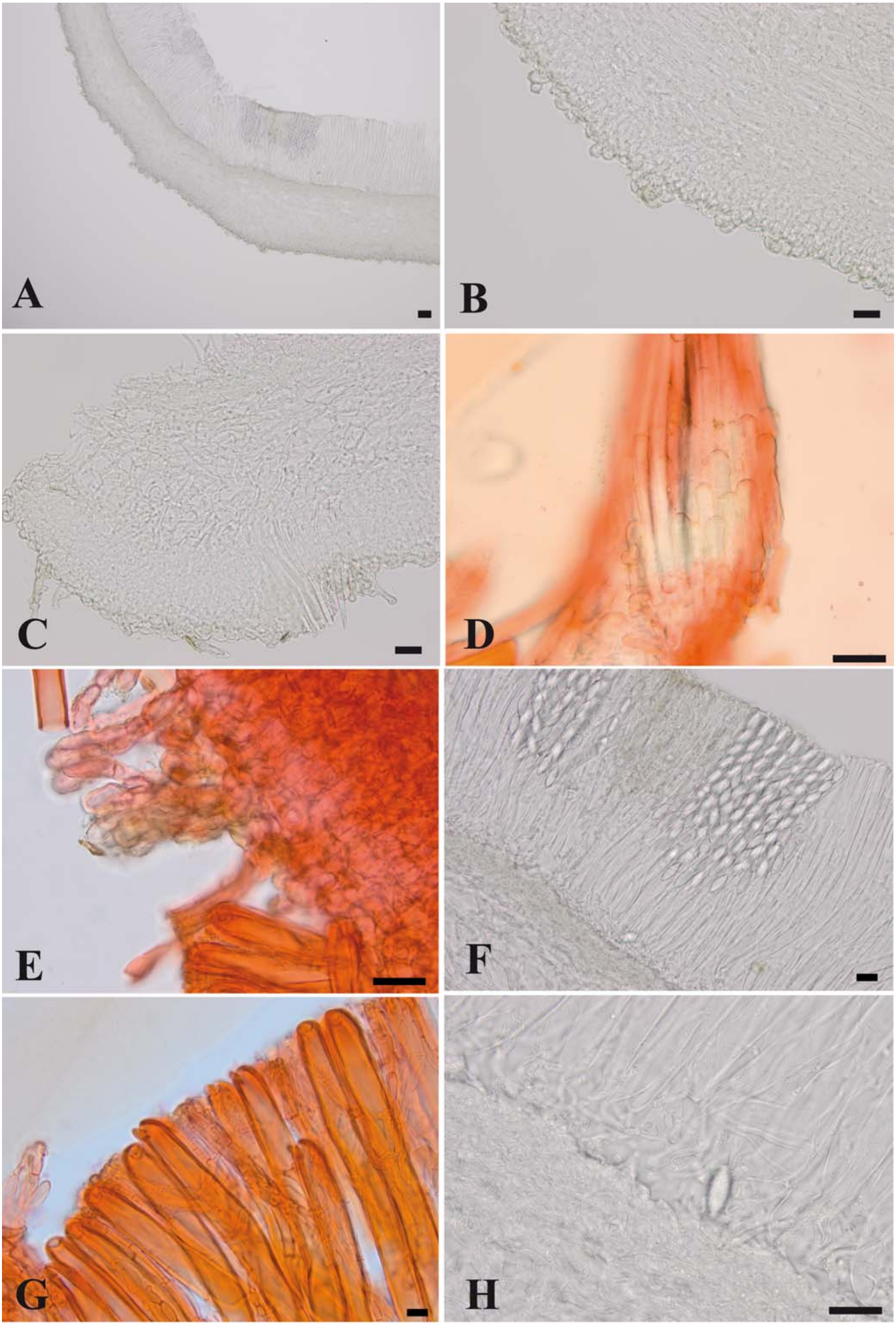
Fig. 1 – Apothecia of *Cookeina korfii* CUP-SA-2454 specimen

low to cream to orange to rusty when dry, covered by uniformly distributed long, conspicuous hairs. **Stipe** mostly short, 4–8 (–20) × 2.5–4 mm when dry, concolorous with receptacle, sub-cylindrical, slightly wider at the base, with longitudinal ridges and furrows over its entire length when dry, the furrows, extending to the receptacle. **Disc** deeply concave, paler than the receptacle when dry. **Margin** not enrolled when dry, provided with four distinct concentric ridges concolorous or lighter colored than the receptacle, from which hairs arise. **Hairs** composed of fascicles of parallel hyphae, flexuous when rehydrated, though stiff when dry, white to whitish when rehydrated, to light-brown to brown when dry, 3–6 (–7) mm long, arising from within the medullary excipulum, hyphae of the hairs septate, thick-walled, wider at the base from 62–76 µm, tapering gradually towards the apex to 30–40 µm wide in the middle, 8–9 µm wide at the apex; individual hyphae 6–8 µm diam, walls 1.5–2.5 µm wide; shorter hyphae surrounding the base of the hairs, with rounded apices (Fig. 2C, 2D and 2E). Tomentum absent, or scarcely present, composed of short monilioid hair-like processes. **Outer excipulum** 46–65 4m thick, *textura globulosa*, cells arranged perpendicularly to the surface of the receptacle, 13–19 1m diam, hyaline. In some cases these cells aggregate to form masses of loosely connected cells, which give the receptacle a pruinose surface (Fig. 2A and 2B). **Inner ectal excipulum** 88-125 µm thick layer of dense *textura porrecta*, thin-walled hyphae 4–7 µm diam, no gel present (Fig. 2B and 2C). **Medullary excipulum** 110–150 µm thick, of *textura intricata*; hyphae septate, 7–10 µm wide (Fig. 2B and 2C). **Subhymenium** 30 µm wide, of *textura intricata* (Fig. 2A, 2F and 2H). **Hymenium** 390–430 µm, easily separated from the excipular layer (Fig. 2A and 2F). **Asci** long-cylindrical, 230–255 × 9–11 µm, abruptly constricted and narrow-hyphoid at the base, thick-walled, up to 2–3 µm (Fig. 2F, 2G and 2H), with 8 ascospores. **Ascospores** elliptic-fusoid to narrow lemon-shaped, pointed at both poles, and with irregular to rounded apiculi frequently present at one or both poles; ascospores hyaline to light yellow smooth-walled, 0 (to 1) to 2-guttulate, (18–) 22–25 × 9.0–11.5 µm (Fig. 3A, 3B, 3C and 3D). **Paraphyses** slender, septate, sometimes constricted at the septa, branched and anastomosing in a net pattern, 2–3 µm wide (Fig. 2F and 2G).

Habitat and occurrence: On fallen twigs of unknown angiosperm host, found in the months of October and December.

Distribution: from the Philippines.

Fig. 2 – *(next page)* Micro-morphological characters of *Cookeina korfii* sp. nov.
A: Partial section under water; B: Ectal excipulum and medullary excipulum under water; C: Ectal excipulum, medullary excipulum, and long hair arising from the medullary excipulum under water; D: hair structure at the base under Congo red; E: Section at the margin under Congo Red. More abundantly aggregated hair-like processes in tufts, medullary excipulum showing arrangement as *textura porrecta*; F: Hymenial layer, asci bases, subhymenium under water; G: Asci which have undergone ascospores released, with open suboperculi under Congo red. Paraphyses with net arrangement that can be seen under the layer of asci; H: Asci basal appendages under water. Scale bars=20 µm.


A
B
C
D
E
F
G
H

Notes: Apothecia of CUP-SA-2454 are larger than the other sample examined: Stipe up to 20 mm long. Receptacle considerably wider than the other ascomata examined: 35 mm wide. Receptacle hairs are up to 7 mm long. It is of a darker color, being orange rusty-brown; the other specimen is of a paler color. Tomentum is present, as short monilioid hair-like processes.

Ecology: Both specimens were collected on fallen twigs, in a tropical rainforests, at low altitudes less than 30 m a.s.l., collected in October and December.

Etymology: This new species is dedicated to Richard P. Korf on the occasion of his 90th birthday. His mentorship to D.H. Pfister and to T. Iturriaga provided them with insight, dedication and love of the study of the discomycetes. F. Xu acknowledges his appreciation. He has benefited indirectly from Professor Korf's work throughout his studies.

Specimens examined: Holotype: On twig, in vicinity of Mud Springs, Mt. Makiling, Los Baños (Philippines), Luzon, coll. K. P. Dumont, 10.XII.1966, CUP-SA-2454 [as *Cookeina tricholoma*]. Additional specimen: On twigs, 2 km South of Quezon, Palawan (Philippines), coll. K. P. Dumont and J. V. Pancho, 3.X.1966, CUP-SA-1797 [as *Cookeina tricholoma*].

Diagnostic characters: *C. korfii* possesses smooth-walled ascospores of smaller size than *C. tricholoma* or *C. sinensis*, and fusoid-apiculate shape.

Discussion

Our attention focused on specimens from South Asia with morphologies similar to *C. tricholoma* and *C. sinensis*. *Cookeina sinensis* has been recorded from China (Wang, 2001; Weinstein *et al*., 2002; Iturriaga & Pfister, 2006), and India (Patil *et al.*, 2012). Upon examination of specimens from FH, CUP and DUKE, we identified two specimens from the Philippines that were at variance with *C. tricholoma* and *C. sinensis*. These are different from *C. sinensis* (ascospores smooth-walled but much larger, 28–40 × 12–12.5 µm), and from *C. tricholoma* (ascospores with longitudinal ridges on the walls and also larger 25–39 × 11–13.5 µm).

In our phylogenetic analyses, a small dataset was analyzed, using *C. speciosa* as the outgroup. Phylogeny based on ITS rDNA resolved the relationships of *C. korfii* with its morphological closely related species and results show that the two collections of "*C. tricholoma*" from the Philippines are a distinct species in a clade with *C. tricholoma* and *C. sinensis*. It is shown to be closest to *C. sinensis* with high support (Fig. 4). Our resolution of *C. sinensis* and *C. tricholoma* as sister groups agree with those of Weinstein *et al.* (2002). *Cookeina tricholoma* collections studied by Weinstein *et al.* (2002) from South America (Puerto Rico and Venezuela) belong to another clade (supported by 87% ML-BP and 64% MP-BP). With the discovery of this new species, the genus *Cookeina* in South Asia proves to be more diverse than previously recognized.

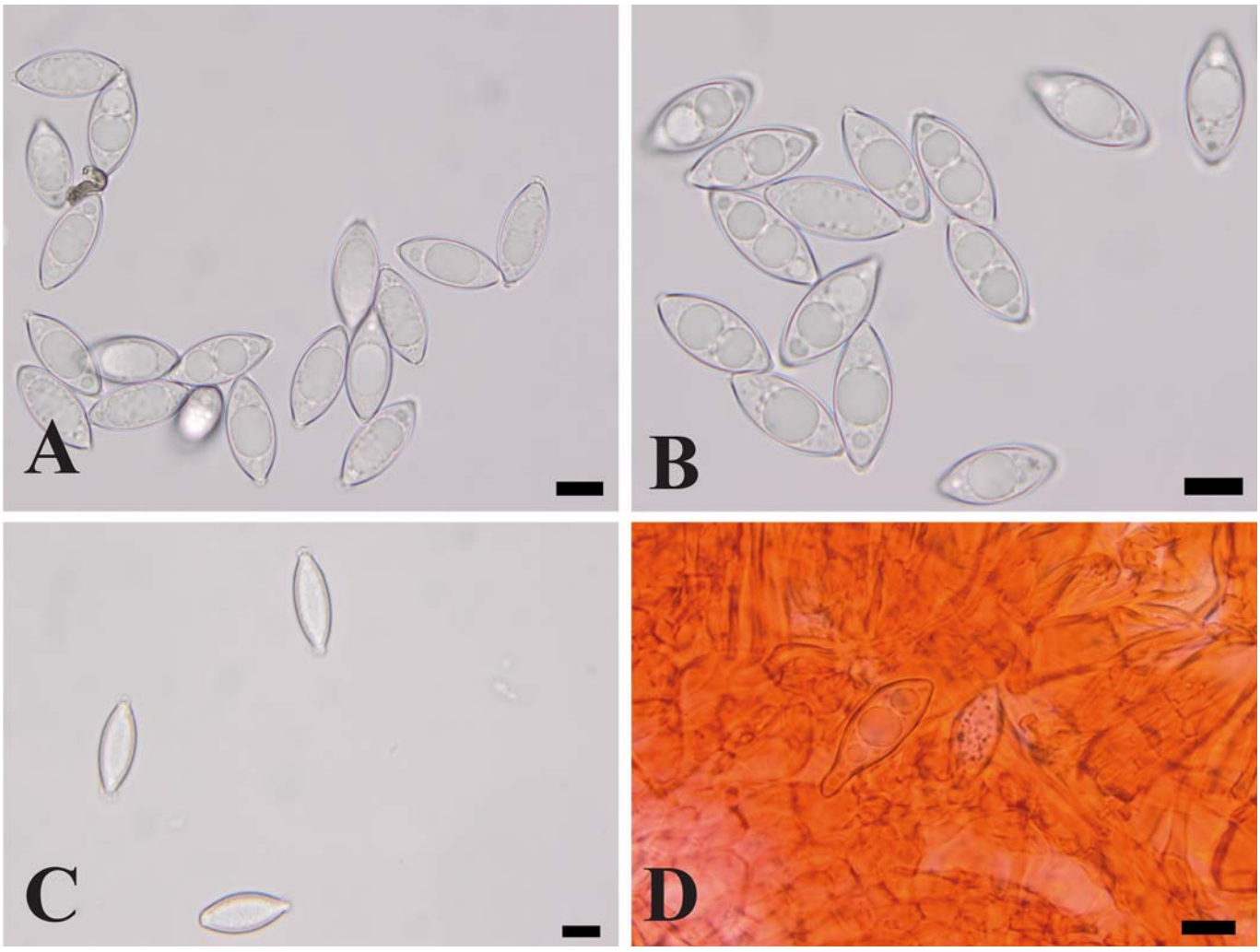


Fig. 3 – Ascospores of *Cookeina korfii* sp. nov.
A and B: Ascospores with polar apiculi under water; C: Smooth wall surface with no striation under water; D: germinating ascospore under Congo red. Scale bars=10 µm.

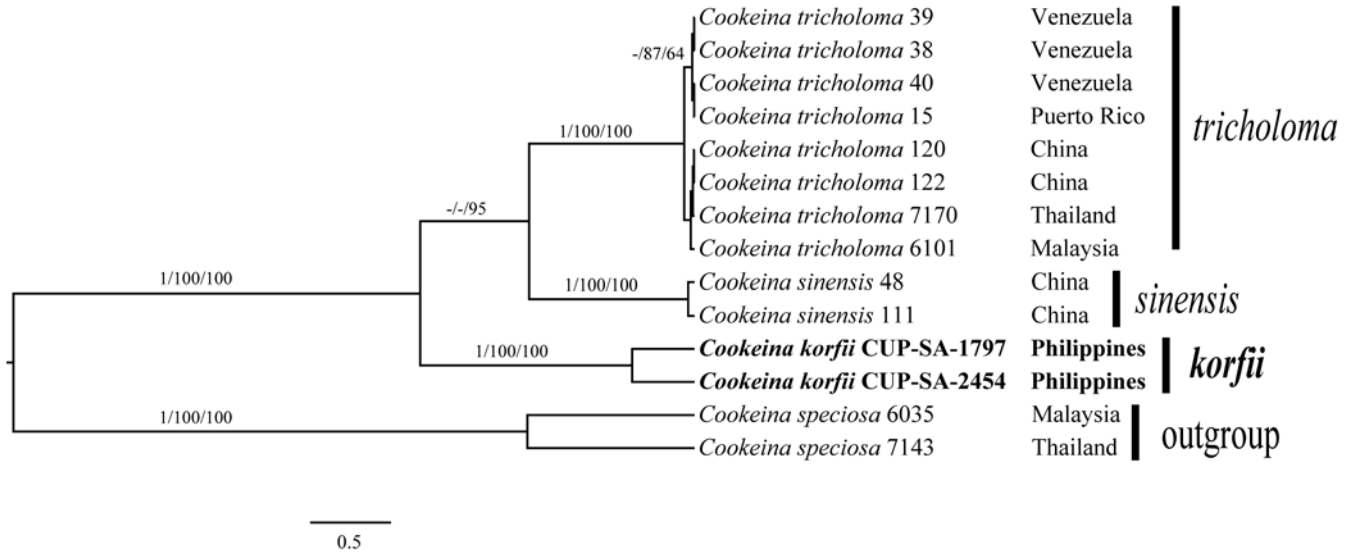


Fig. 4 – Phylogenetic placement of *Cookeina korfii* and its closest related species produced from MrBayes analysis based on ITS. Sequences of *Cookeina speciosa* were used to root the phylogeny. Only branches which were highly supported in the analyses (Bayesian posterior probabilities ≥ 0.95, Maximum likelihood bootstrap ≥ 70% and Maximum parsimony bootstrap ≥ 50%, respectively) are indicated.

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