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The genus *Cookeina*

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Abstract

Cookeina (Sarcoscyphaceae) is characterized by large, brightly coloured, stipitate apothecia and eccentrically, operculate asci. This paper provides detailed morphological descriptions of the sexual morphs of all the recorded species within the genus, including a new species, *Cookeina garethjonesii*. Phylogenetic analyses inferred from ITS sequence data strongly support species lineages with corresponding morphological differences.

Key words – apothecia – discomycetes – operculate – phylogeny – taxonomy

Introduction

The genus *Cookeina* Kuntze is an operculate discomycete genus of Pezizomycetes, typified by *Cookeina tricholoma* (Mont.) Kuntze. Several species been added to the genus (Seaver 1913, Denison 1967, Rifai 1968, Pfister & Kaushal 1984). The salient characteristics of this genus are the large, brightly coloured, stipitate apothecia and fasciculate excipular hairs. Paraphyses are branched and anastomose freely at the apex to develop excipulum, asci are cylindrical with tapering bases and an eccentrically placed operculum at the apex, and ascospores are ellipsoid to fusiform and hyaline to subhyaline with longitudinal ribs (Bi et al. 1993, Denison 1967, Iturriaga & Pfister 2006). Species of *Cookeina* are widespread, especially in tropical and subtropical regions (Hanlin 1998, Weinstein el al. 2002, Douanla-Meli & Langer 2005, Iturriaga & Pfister 2006). They are saprobes on dead plant material or on soil (Douanla-Meli & Langer 2005; Iturriaga & Pfister 2006).

The most recent comprehensive account of this genus is by Iturriaga and Pfister (2006). Since this study, several new species have been introduced and others have been synonymized. Although Index Fungorum (2016) lists 25 epithets, only 12 species, plus the new species introduced in this paper should be accepted (Weinstein et al. 2002, Iturriaga & Pfister 2006). In the present study, the morphological characteristics and phylogenetic placements of all accepted *Cookeina* species based on ITS sequence data are given. A new species is described herein as *Cookeina garethjonesii* and compared with allied taxa to support its taxonomic placement.

Materials & Methods

Sample collection specimen examination and deposition

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Four specimens of *Cookeina* were collected from Yunnan Province, southern China and southern Thailand in 2014 to 2015. Macroscopic and microscopic characters of the specimens were recorded. A Motic SMZ-168 stereo microscope was used to observe the structures of the apothecia. Sections of apothecia were made with a razor blade and mounted in water. A Nikon ECLIPSE 80i compound microscope was used to observe microscopic characters. Photomicrography was carried out with a Canon 450D digital camera fitted to the microscope. Measurements of paraphyses, asci and ascospores were made from materials mounted in water and the mean values were used in the descriptions. Measurements were made with the Taro soft (R) Image Frame Work v. 0.9.7 program and images used for figures were processed with Adobe Photoshop CS6 software (Adobe Systems Inc.). The type specimens are deposited in the Mae Fah Luang University Herbarium (MFLU), Chiang Rai, Thailand and in the Herbarium of Cryptogams of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Facesoffungi and Index Fungorum numbers were registered as described in Jayasiri et al. (2015) and Index Fungorum (2016).

DNA extraction, PCR and sequencing

Genomic DNA was extracted directly from the apothecia using a Plant DNA Rapid Extraction Kit (Bio Teke corperation, Beijing, China). Polymerase chain reactions (PCR) for this study were carried out for the internal transcribed spacer (ITS), using ITS4 and ITS5 (White et al. 1990) primers. The PCR mixtures (25 μL) contained ddH_2O (11 μL), PCR Master Mix (QinKe Co., China) (11 μL; 2×), DNA template (1 μL), each primer (1 μL; 10 μM). PCR amplification conditions were consisted an initial denaturation step of 5 min at 94 °C, 35 cycles consisted of denaturation at 94 °C for 1 minute, annealing at 53 °C for 50 seconds and elongation at 72 °C for 3 minute and final extension step of 7 minutes at 72 °C. The PCR products were viewed on 1 % agarose electrophoresis gels, stained with ethidium bromide. PCR products were sent to a commercial sequencing provider, Qinke in Kunming, China.

Sequence alignment and phylogenetic analysis

Newly generated sequences in this study were subjected to a standard BLAST search of GenBank for rough identification. Eighteen sequences belonging to ITS gene regions from representative *Cookeina* species and the out-group taxon *Microstoma floccosum* (Schwein.) Raitv., were downloaded from GenBank (Table 1). The newly generated sequences are deposited in GenBank (Table 1) and alignment in Treebase under number S20159 (http://purl.org/phylo/treebase/phylows/study/TB2:S20159). The consensus sequences for each gene were aligned using MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html). The alignment was improved manually where necessary using Bioedit (Hall 1999). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Maximum likelihood phylogenetic analyses were performed in the CIPRES web portal (Miller et al. 2009) using RAxML-HPC2 Workflow on XSEDE (8.2.9) tool (Stamatakis 2006). The bootstrap analysis for each ML tree was performed with 1000 thorough bootstrap replicates with the same parameter settings using the GTR+ G substitution model selected by MrModel Test. Maximum parsimony analysis (MP) was performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2003) for the ITS gene region. Ambiguously aligned regions were excluded from the analyses; gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Branches of zero length were collapsed and all equally most parsimonious trees were saved. Descriptive tree statistics such as tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC], and homoplasy index [HI], were calculated. The resultant trees were viewed with FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/). Maximum likelihood bootstrap values equal or greater than 60% are given as the first set of numbers above the nodes and Maximum-parsimony bootstrap values equal or greater than 60% are given as the second set of numbers above the nodes (Fig. 1).

Results

Phylogenetic analyses

The ITS, dataset of representative species of *Cookeina* 719 characters (including alignment gaps) for 23 ingroup and one outgroup taxa. Of the 719 characters 358 were constant, 89 were variable and parsimony uninformative. Maximum parsimony analysis of the remaining 272 parsimony-informative characters resulted in 750 most parsimonious trees (TL = 750; CI = 0.717, RI = 0.833, RC = 0.597, HI = 0.283) and the best tree is shown in Fig 1. Resultant tree shows four main clades (Fig. 1). The first clade includes our new species and that is phylogenetically close to *Cookeina sulcipes*.

Table 1 Taxa used in the phylogenetic analyses and ITS GenBank accession numbers.

Species name	Strain number	ITS
Cookeina colensoi	C.col118	AF394532
Cookeina colensoi	C.col121	AF394531
Cookeina cremeirosea	AS142	KU306963
Cookeina cremeirosea	AS210	KU306964
Cookeina garethjonesii	HKAS90509	**KY094617**
Cookeina garethjonesii	HKAS90513	**KY094622**
Cookeina indica	C.ind119	AF394029
Cookeina indica	MFLU 16-0610	**KY094621**
Cookeina insititia	C.ins126	AF394033
Cookeina insititia	C.ins125	AF394032
Cookeina korfii	CUP SA-2454	NR_136141
Cookeina korfii	CUP-SA-1797	KT893782
Cookeina sinensis	C.sin111	AF394028
Cookeina sinensis	C.sin48	AF394027
Cookeina speciosa	C.spe6035	AF394018
Cookeina speciosa	C.spe31	AF394017
Cookeina sulcipes	isolate 2	AF529292
Cookeina sulcipes	isolate 1	AF529291
Cookeina sulcipes	MFLU 15-2358	**KY094620**
Cookeina tricholoma	MFLU 15-2359	**KY094618**
Cookeina tricholoma	HKAS87041	**KY094619**
Cookeina venezuelae	C.ven43	AF394044
Cookeina venezuelae	C.ven42	AF394043
Microstoma floccosum	Micro45	AF394046

Newly generated sequences are in bold

Taxonomy

Cookeina colensoiopsis Iturr. & Pfister, Mycotaxon 95: 151 (2006)
Facesoffungi number: FoF 02666

Saprobic on dead stems. Sexual morph: *Apothecia* 20 × 2–15 mm when fresh, and 14–15 × 1–8 mm when dry, scattered, centrally stipitate, deeply cupulate, bright yellow to orange when fresh, furfuraceous with white hair-like projections. *Stipe* 3–13 × 1–3 mm cream-colored, subcylindrical, wider at the base. *Receptacle* concave, bright yellow when fresh. *Margins* concolorous to receptacle. *Disc* darker than receptacle, bright yellow to orange when fresh, ochre when dry and orange when rehydrated. *Ectal excipulum* 30–90 µm composed of cells of *textura globulosa* to *prismatica* (8–14 µm diam.), 4–5 cell layers present. *Medullary excipulum* has two layers, outer layer 50–100 µm wide, composed of thin-walled cells of *textura oblita* (2–1 µm diam.), hypha immersed in a gel and oriented outwards, perpendicular to the receptacle surface, inner layer 60—600 µm composed of long cells of *textura porrecta* (4–6 µm). *Subhymenium* composed of gelatinized cells of *textura intricata*. *Hymenium* 380–450 µm, hymenial setae present, 430–440 × 2 µm, intermixed with asci and paraphyses, brownish with lanceolate terminal cell, exceeding hymenial elements by 10–20 µm. *Paraphyses* 2–4 µm wide, numerous, filiform, septate, highly branched and slightly swollen to clavate at the apex forming the epithecium. *Asci* 340–420 ×

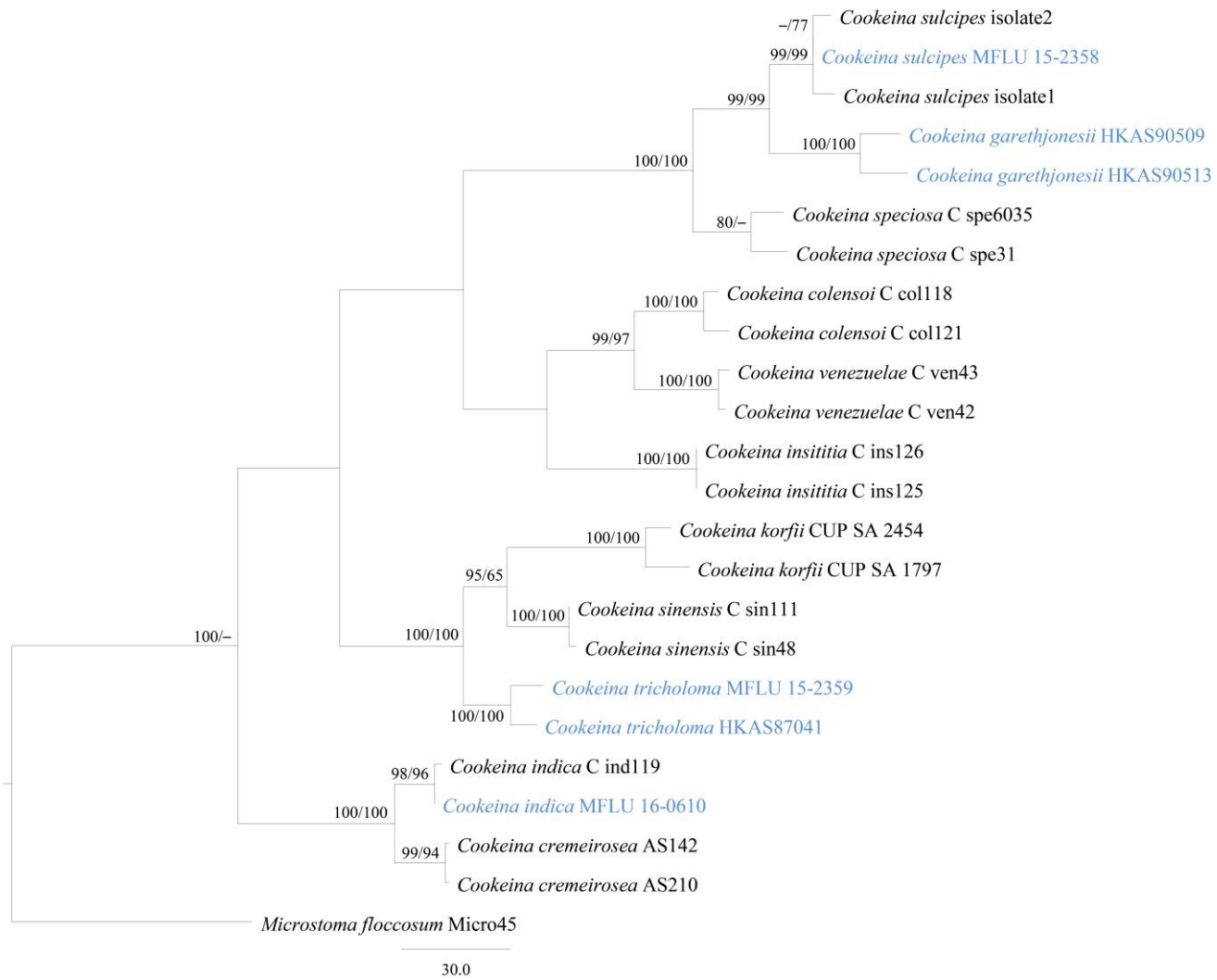

Fig. 1 – Phylogram generated from maximum parsimony analysis of sequences of *Cookeina* based on ITS sequence data. Maximum likelihood bootstrap values ≥60% are given as the first set of numbers and Maximum-parsimony bootstrap values ≥60% are given as the second set of numbers, above the nodes. Strain/culture numbers are given after the taxon names. The newly generated sequences are in blue. The tree was rooted with *Microstoma floccosum* (Micro45).

10–20 µm, long, cylindrical with rounded base, ending in an abrupt manner to connect with hyphal-like basal appendage, a few times tapering slightly at base, basal appendage 4–28 × 4 µm. *Ascospores* 30–34 × 12–14 µm, obliquely uniseriate, broad elliptic, unequally sided, narrowing slightly at the ends, with a short apiculum present at both ends, mostly with two large central oil guttules with two smaller guttules at the ends, hyaline, smooth-walled. Asexual morph: Undetermined (description modified from Iturriaga & Pfister 2006).

Notes – *Cookeina colensoiopsis* is similar to *C. colensoi* in the presence of hair-like appendages and smooth-walled ascospores, but differs in having long stipes and hymenial setae. A similar type of setae also has been observed only in *C. speciosa* (Iturriaga & Pfister 2006).

Cookeina colensoi (Berk.) Seaver, Mycologia 5(4): 191 (1913)
Facesoffungi number: FoF 02667

Saprobic on dead wood. Sexual morph: *Apothecia* 1–1.5 × 0.5 cm arising singly, substipitate to stipitate, deep or shallow cupulate, brownish yellow to brownish orange when fresh, pale-yellow when dry, glabrous, gelatinous layer present, wrinkled when dry especially near the base of the cup. *Stipe* <1mm. *Receptacle* concave, margins are covered with rudimentary hairs. *Ectal excipulum* 40 µm (10–60 µm) outer cells composed of cells of *textura globulosa*, inner cells

composed of gelatinized cells of *textura angularis*. *Medullary excipulum* composed of hyaline cells of *textura intricata*. *Paraphyses* numerous, filiform, highly branched and slightly enlarged at the apex producing epithecium. *Asci* 310–475 × 18–20 µm unitunicate, operculate, cylindrical, gradually tapering below. *Ascospore* 28–40 × 11–15 µm, uniseriate or with the ends slightly overlapping, narrowly ellipsoid or fusoid with the ends quite strongly narrowed, smooth, thin-walled, with one or two unequal large oil droplets and granular within, striations consisting of several broad, longitudinal bands extending the length of the spore. Asexual morph: Undetermined (description modified from Iturriaga & Pfister 2006).

Notes – Both *C. colensoi* and *C. venezuelae* have gelatinized ectal excipular layer, but they are differ from spore ornamentation. *Cookeina colensoi* differ from *C. insititia* by ascospore shape (Douanla-Meli & Langer 2005).

Cookeina cremeirosea Kropp Mycoscience (in press) (2016)
Facesoffungi number: FoF 02668

Saprobic on dead stems. Sexual morph: *Apothecia* 1–7 × 8 mm arising singly, centrally-stipitate, deeply cupulate, pink when fresh, reddish yellow when dry. *Receptacle* concave, disc and margins are pink when fresh, minutely granular surface. *Stipe* 1 mm wide, central with a narrow attachment, somewhat compressed and fluted, concolorous with the receptacle but somewhat lighter towards the base. *Hairs* 60–100 µm long. *Hymenium* pinkish orange, fading to pale-yellow in dried specimens. *Ectal excipulum* 35–70 µm composed of nongelatinized cells of textura angularis to *textura globulosa*. *Medullary excipulum* 120–228 µm composed of nongelatinized cells of *textura porrecta*. *Paraphyses* 2–4 µm wide, hyaline, septate, highly branched at the apex producing epithecium. *Asci* 324–336 × 14–17 µm unitunicate, rounded at the base, cylindrical, non-amyloid. *Ascospore* 8–12 × 23–33 µm, uniseriate, amerosporae, ovoid, ends slightly narrowed and subpapillate, hyaline, guttules variable, aseptate, smooth, thin walled. Asexual morph: Undetermined (description modified from Kropp 2016).

Notes – *Cookeina cremeirosea* differs from *C. indica* in having smooth-walled ascospores and pinkish apothecia (Kropp 2016).

Cookeina garethjonesii Ekanayaka, Q. Zhao & K.D. Hyde, sp. nov. Fig. 2
Index Fungorum number: IF552534
Facesoffungi number: FoF 02669

Etymology – The specific epithet *garethjonesii* refers to the significant contribution of Professor E.B. Gareth Jones made to mycology.

Holotype – HKAS90509

Saprobic on dead stems. Sexual morph: *Apothecia* 1–3 × 2–5 cm when fresh, arising singly, stipitate, deep cupulate. *Hymenium* glabrous, bright yellow to orange when fresh. *Receptacle* concave, margins slightly curved, disc and margins are yellow to orange when fresh. *Stipe* 1–1.5 × 0.1–0.3 cm when fresh, slender, hollow, forming a disc-like holdfast at the base, paler than the receptacle. *Hairs* 53–70 × 10–16 µm ($\bar{x}$ = 58.8 × 13.8 µm, n = 30) length and the width at the apex, arranged around the margin as a single row, apical cell slightly swollen, straight, thin-walled, septate, hyaline to yellowish. *Hymenium* hyaline. *Ectal excipulum* 97–132 µm ($\bar{x}$ = 109.8 µm, n = 10) two layered, outer ectal exipulum composed of large, thick-walled, hyaline cells of *textura globulosa*, inner ectal exipulum composed of hyaline thin-walled cells of *textura prismatica*, hyphae in ectal excipulum arranged perpendicular to the receptacle surface. *Medullary excipulum* 112–250 µm ($\bar{x}$ = 177 µm, n = 10) composed of hyaline cells of *textura intricata*, hyphae arranged parallel to receptacle surface. *Paraphyses* 2.2–3.6 µm wide ($\bar{x}$ = 3.3 µm, n = 20), numerous, filiform, septate, highly branched and slightly swollen at the apex, forming the epithecium. *Asci* 280–310 × 18–23 µm ($\bar{x}$ = 300 × 21.3 µm, n = 30), unitunicate, sub-operculate, cylindrical, short pedicellate, obtuse at the base, non-amyloid. *Ascospores* 26–30 × 15–17 µm ($\bar{x}$ = 28.7 × 16.3 µm, n = 40), uniseriate, 1-celled, ovoid, ends obtuse, hyaline or subhyaline, with one or two large oil-drops, aseptate, smooth-walled, thin-walled. Asexual morph: Undetermined.

Material examined – CHINA, XSBN Tropical Botany Garden, Menla County, Yunnan Province, 540m alt., on soil near the vegetation of *Quercus* sp. 5 August 2015, Qi Zhao- 2635 (HKAS90509, holotype), same location, 5 August 2015, Qi Zhao2639 (HKAS90513, isotype).

Notes – *Cookeina garethjonesii* is similar to *C. sulcipes* in its deep cupulate apothecia and straight, thin-walled, short hairs. However, *C. sulcipes* have pinkish apothecia and ascospores with thick gelatinous sheath, while ascospores of *C. garethjonesii* lack a gelatinous sheath and produce yellowish apothecia. *Cookeina garethjonesii* differ from *C. speciosa*, *C. colensoiopsis* and *C. globosa* by not having hairs or setae in the hymenium (Iturriaga & Pfister 2006).

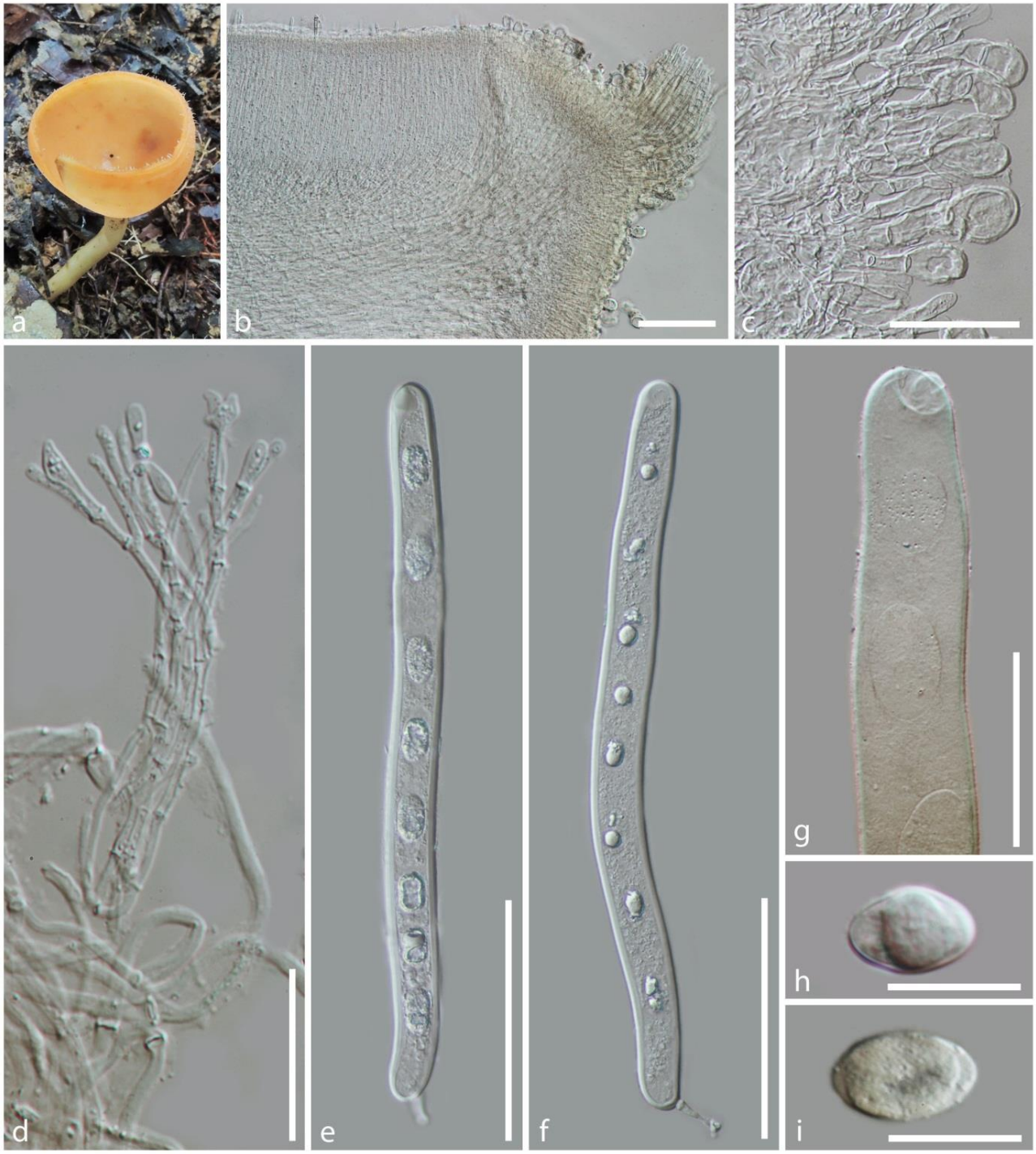

Fig. 2 – *Cookeina garethjonesii* (HKAS90509). a Apothecia on wood, b Cross section of an apothecium, c Cylindric, short hairs, d Septate, branched paraphyses, e–f Cylindrical asci, g Sub operculate apical apex (open state), h–i Ovoid ascospores. – Scale bars b = 100 µm, c = 50 µm, d = 35 µm, e–f = 100 µm, g = 50 µm, h–i= 25 µm.

Cookeina globosa Douanla-Meli, Mycotaxon 92: 225 (2005)
Facesoffungi number: FoF 02670

Saprobic on dead stems. Sexual morph: *Apothecia* 8–20 × 7–17 mm in diameter, gregarious, subsessile to short stipitate, deeply cupulate, pink to pale lead when fresh and brownish-yellow when dry. *Receptacle* 4–12 mm deep, concave, fertile surface smooth, shiny, lower sterile surface glabrous to finely velvet. *Margin* entire, erect but slightly incurved when dry. *Stipe* 3–5 mm long, cylindrical, often curved, composed of pale orange cells of *textura porrecta* (up to 5 µm diam), hyaline, simple septate, nearly thick-walled. *Hairs* 1 mm long on flanks and margins, cylindrical, straight, white. *Ectal excipulum* 60–100 µm thick, composed of cells of *textura angularis* to *globulosa*, few layers of polygonal cells in gelatinous tissues, the outer cells globose, larger with thickened yellowish-brown walls, inner cells hyaline. *Medullary excipulum* composed of hyaline cells of *textura intricata* to *porrecta*, loosely interwoven hyphae, up to 6 µm diameter. *Subhymenium* 10–25 µm thick, composed of cells of *textura intricata*. *Hymenium* pink, hymenial hairs present. *Paraphyses* 1–4 µm wide, numerous, filiform, highly branched and slightly swelled at the apex producing epithecium, protruding up to 45 µm above the asci level. *Asci* 300–370 × 13–20 µm unitunicate, thick-walled, cylindrical, slightly tapering at the base and usually contracted into a short narrow stalk, 8-spored, suboperculate with operculum arranged obliquely on ascus apex, non-amyloid. *Ascospores* 11–16 × 9–12 µm, uniseriate, subglobose to globose, thin-walled, smooth, usually fined with two large and several small oil droplets. Asexual morph: Undetermined (description from Douanla-Meli & Langer 2005).

Notes – *Cookeina globosa* is similar to *C. colensoi* in having glabrous, subsessile apothecia, but the *C. colensoi* differs in spore ornamentation and not having a gelatinized ectal excipular layer (Douanla-Meli & Langer 2005).

Cookeina indica Pfister & R. Kaushal, Mycotaxon 20(1): 117 (1984) Fig. 3
Facesoffungi number: FoF 02671

Saprobic on dead stems. Sexual morph: *Apothecia* 1–3 × 4–8 cm arising singly or gregarious, stipitate, deep or more shallow cupulate, yellow to orange when fresh, stalk 220 × 1.5 mm. *Receptacle* concave. *Disc* yellow to orange when fresh, nearly smooth except at the margin where it is minutely furfuraceous. *Margins* entire, lighter in colour than disc. *Hymenium* yellow, smooth. *Ectal excipulum* 70–100 µm ($\bar{x}$ = 79.8 µm, n = 10) composed of large, thin-walled, hyaline cells of *textura prismatica* to *globulosa*. *Medullary excipulum* 100–140 µm ($\bar{x}$ = 117.9 µm, n = 10), composed of hyaline cells of *textura intricata*. *Paraphyses* 2.5–4.5 µm wide ($\bar{x}$ = 3.3 µm, n = 20), numerous, filiform, septate, highly branched and slightly swollen at the apex forming the epithecium. *Asci* 420–520 × 24–30 µm ($\bar{x}$ = 487.2 × 27.1 µm, n = 30), unitunicate, operculate, cylindrical, base narrow-hyphoid, short pedicellate, thick-walled, apices obtuse, non-amyloid. *Ascospores* 31–37 × 14–16 µm ($\bar{x}$ = 33.6 × 14.9 µm, n = 40), uniseriate, 1-celled, ovoid, ends slightly narrow, often inequilateral, hyaline with 3 guttules or multiguttulate, aseptate, ornamentation of fine longitudinal, parallel ridges. Asexual morph: Undetermined (description modified from Iturriaga & Pfister 2006).

Material examined – CHINA, Kunming, Yunnan Province, 4 December 2013, S.C. Karunarathna, NB036 (MFLU 16-0610).

Notes – *Cookeina indica* is distinct from other species of the genus in having striate spores with pointed apices, a yellow hymenium and a non-hairy, nearly smooth outer receptacle surface (Iturriaga & Pfister 2006).

Cookeina insititia (Berk. & M.A. Curtis) Kuntze, Revis. gen. pl. (Leipzig) 2: 849 (1891)
Facesoffungi number: FoF 02672

Saprobic on dead stems. Sexual morph: *Apothecia* 4–11 × 9–25 mm tall when dry, arising singly to gregarious, centrally stipitate, deep cupulate, white to pale cream when fresh. *Receptacle* concave, urceolate to turbinate, white to pale cream, when dry beige or yellow-ochre. *Disc* darker than the receptacle. *Margin* concolorous to the receptacle. *Hairs* three types, all originated from

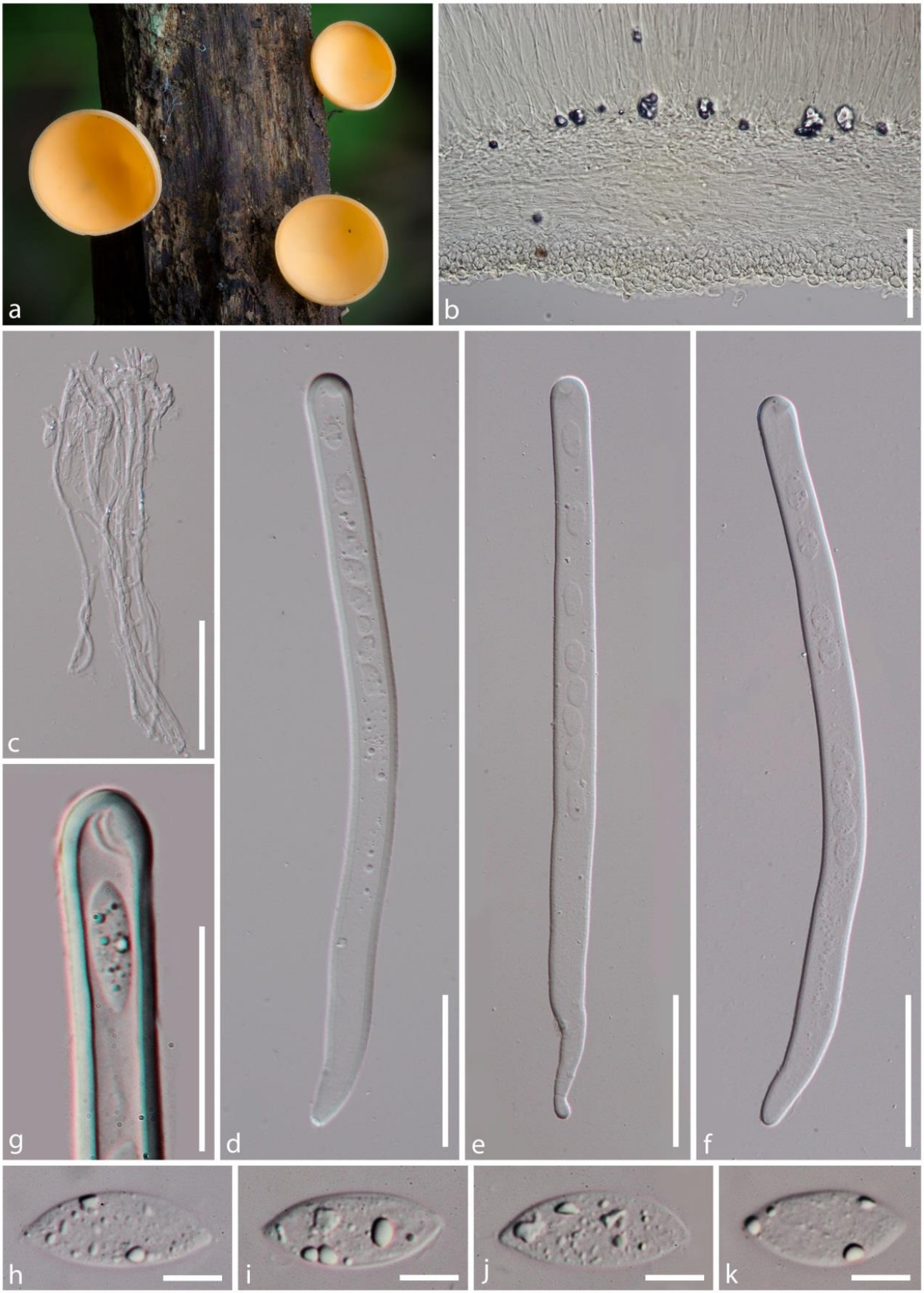

Fig. 3 – *Cookeina indica.* a Apothecia on wood, b Cross section of an apothecium, c Septate, branched paraphyses, d–f Cylindrical asci, g Sub operculate apical apex (closed state), h–k Ovoid ascospores. – Scale bars b = 150 µm, c–f = 100 µm, g = 50 µm, h–k = 10 µm.

outer ectal excipulum 1) marginal twisted (when dry) hairs up to 2 × 0.5 mm, light yellow, triangular and flattened, in 1–2 rows, gradually tapered towards the apex, 2) hairs, straight, in the margin intermixed with the twisted hairs, covering mainly the upper part of the receptacle, half the size of the marginal ones, similar in structure to them, 3) hyphal projections covering receptacle and stipe. Stipe whitish, 3–16 × 1–2 mm when dry, up to 40 mm long when fresh, sometimes with a disc-shaped point of attachment. Ectal excipulum composed of two layers, outer ectal excipulum 30–50 µm, composed of thick and sometimes warted-walled, hyaline cells of textura globulosa to angularis, inner ectal excipulum 20–80 µm, composed of cells textura oblita, loosely interwoven, delicate, septate, branched, thin-walled hyphae immersed in a distinct gelatinous matrix and oriented perpendicular to the receptacle surface. Medullary excipulum composed of septate, typically unbranched cells of textura porrecta to intricata. Subhymenium composed of cells of textura intricata. Paraphyses 1–2 µm wide, filiform, septate, highly branched and slightly swollen at the apex forming an epithecium. Asci 400–453 × 12–16, µm unitunicate, operculate, cylindrical, tapering to a long thin obconical base. Ascospores 36–52 × 8–16 µm, uniseriate, narrow sub-fusoidal to fusoida1, asymmetrical or distinctly curved, with pointed ends, hyaline, smooth-walled, containing numerous guttules. Asexual morph: Undetermined (description modified from Iturriaga & Pfister 2006).

Notes – *Cookeina insititia* is distinct from other species of the genus in having three types of hairs. Meléndez-Howell et al. (2003) showed that although smooth-walled under the light microscope, ascospores have low convolute markings under the SEM. Moreover the ascus apex of *C. insititia* differs from other species by the absence of the d2 wall layer (Meléndez-Howell et al. 2003). The other unique characteristic of *C. insititia* is the presence of single ascospore wall layer (Boedjin 1933, Pfister 1973).

Cookeina korfii Iturr., F. Xu & Pfister, Ascomycete.org 7(6): 331 (2015)
Facesoffungi number: FoF 02673

Saprobic on dead stems. Sexual morph: *Apothecia* 18 × 35 mm when dry, arising singly or scattered, stipitate, deeply cupulate with an eccentric stipe, orange pigments exuded into water in dry specimens. Stipe 4–20 × 2.5–4 mm when dry, eccentric, 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. *Receptacle* concave, light yellow to orange when dry, covered by uniformly distributed long, conspicuous hairs, sometimes pruinose surface. Disc paler than the receptacle when dry. Margin concolorous or lighter colored than the receptacle. *Spines* 3–7 mm long, wider at the base and tapering gradually towards the apex, composed of fascicles of parallel hyphae, on flanks and margins, thick-walled, septate, flexuous when rehydrated, though stiff when dry, white to whitish when rehydrated, to light-brown to brown when dry. *Hairs* shorter hyphae surrounding the base of the hairs, with rounded apices. *Ectal excipulum* 2 layers, outer ectal excipulum 46–65 µm, composed of hyaline cells of *textura globulosa*, cells arranged perpendicularly to the surface of the receptacle, Inner ectal excipulum 88–125 µm, composed of dense, thin-walled *textura porrecta*. *Medullary excipulum* 110–150 µm composed of hyaline cells of *textura intricata*. *Subhymenium* 30 µm, composed of *textura intricata*. *Paraphyses* 2–3 µm wide, numerous, filiform, septate, sometimes constricted at the septa, highly branched at the apex, forming the epithecium. *Asci* 230–255 × 9–11 µm, unitunicate, operculate, long, cylindrical, abruptly constricted and narrow-hyphoid at the base. *Ascospores* 18–25 × 9–11.5 µm, uniseriate, elliptic-fusoid to narrow, light yellow, aseptate, smooth, thin-walled, pointed at both poles, and with irregular to rounded apiculi frequently present at one or both poles, 0 (to 1) to 2-guttulate. Asexual morph: Undetermined (description modified from Iturriaga et al. 2015).

Notes – *Cookeina korfii* is most similar to *C. tricholoma* and to *C. sinensis*, but differs in having smooth-walled, smaller and fusoid-apiculate ascospores (Iturriaga et al. 2015).

Cookeina sinensis Zheng Wang, Mycotaxon 62: 293 (1997)
Facesoffungi number: FoF 02674

Saprobic on dead stems. Sexual morph: *Apothecia* up to 25 × 50 mm when dry, arising singly and scattered, centrally stipitate, deeply cupulate, pink to orange when fresh. *Stipe* short, subcylindrical. *Receptacle* concave, pinkish orange, covered more or less uniformly with conspicuous long hairs. *Margin* somewhat in-rolled. *Hairs* 3–7 mm long 6–8 µm wide, on flanks and margins, fasciculate, stiff, bristle-like, white to brownish. *Ectal excipulum* about 50 µm, composed of thick-walled, hyaline cells of *textura angularis*. *Medullary excipulum* 230–300 µm, composed of hyaline cells of *textura intricata*. *Paraphyses* 2.5–4 µm wide, moniliform, slender, septate, highly branched at the apex, forming the epithecium. *Asci* 275–350 × 15–20 µm, unitunicate, suboperculate, cylindrical, narrow-hyphoid at base, thick-walled, non-amyloid. *Ascospores* 16 × 34–40 µm, broad ellipsoid or subfusoid, pointed at both ends, pale yellow, smooth-walled, biguttulate. Asexual morph: Undetermined (description modified from Iturriaga & Pfister 2006).

Notes – *Cookeina sinensis* is distinct from other species in having conspicuous hairs arising from medullary excipulum and moniliform paraphyses. *Cookeina sinensis* is similar to *C. colensoi*, *C. sinensis* and *C. tricholoma* in apothecial shape, structure and colour, but differs in apothecial size (up to 50 mm in diameter) and having smooth-walled ascospores. Both *Cookeina sinensis* and *C. colensoi* bear smooth-walled ascospores, but they differ by having a non-gelatinized ectal excipulum (Iturriaga & Pfister 2006).

Cookeina speciosa (Fr.) Dennis, Mycotaxon 51: 239 (1994)
= ***Cookeina amoena***, (Lév.) Kuntze, Revis. gen. pl. (Leipzig) 2: 849 (1891)
= ***Cookeina javanica*** (Nees & Lév.) Kuntze, Revis. gen. pl. (Leipzig) 2: 849 (1891)
= ***Cookeina sumatrana*** Boedijn, J. Indian bot. Soc. 26: 407 (1929)
Facesoffungi number: FoF 02675

Saprobic on dead stems. Sexual morph: *Apothecia* 10–50 × 10–80 mm, arising singly or gregarious, centrally stipitate, deep or more shallow cupulate or goblet-shaped. *Receptacle* concave, whitish. *Stipe* 3–75 × 1–6 mm slender, hollow, slightly attenuate below and often forming a disc-like holdfast at the bottom, concolorous or even paler than the receptacle. *Hairs* up to 2 mm long, arranged around the margin as a single row, composed of bundles of septate and thick-walled hyphae. *Ectal excipulum* of two layers, outer ectal exipulum 28–80 µm, composed of thick-walled, hyaline to light brown cells of *textura globosa* to *angularis* or *prismatica*, lying perpendicular to the surface of the receptacle, inner ectal excipulum 30–300 µm composed of cells of *textura intricata*, layer is gelatinized only in fertile young specimens, disappearing at maturity. *Medullary excipulum* composed of cells of *textura intricata* to *porrecta*. *Subhymenium* 15–30 µm, composed of cells of *textura intricata* to *porrecta*, gelatinized in immature specimens. *Hymenium* surface smooth, yellow orange, grayish brown to pale chocolate-brown, highly hygrophanous, becoming upon drying uniformly concolorous, pastel yellow to brownish-yellow. *Hymenial setae* interspersed with asci and paraphyses, sometimes dark, tortuose, thick-walled, exceeding the hymenium by 22–40 µm. *Paraphyses* 2–4 µm wide, numerous, filiform, highly branched at the apex producing epithecium. *Asci* 300–370 × 17–19 µm, unitunicate, operculate, cylindrical. *Ascospores* 22–33 × 10–16 µm, ellipsoid, thick-walled, surface with longitudinal ridges, biguttulate. Asexual morph: undetermined (description modified from Iturriaga & Pfister 2006).

Notes – *Cookeina speciosa* is distinct in having orange-yellow, orange to chocolate-brown apothecia (when fresh) and ascospores with a sinusoid surface ornamentation (Weinstein et al. 2002; Meléndez-Howell et al. 2003). *Cookeina speciosa* is similar to *C. tricholoma*, but differs in having evenly distributed hairs arising from medullar excipulum (Wang 1997). Both *Cookeina speciosa* and *C. sinensis* have hirsute apothecia, but they differ in ascospore ornamentation.

Cookeina sulcipes (Berk.) Kuntze, Revis. gen. pl. (Leipzig) 2: 849 (1891) Fig. 4
= ***Cookeina hindsii*** (Berk.) Kuntze, Revis. gen. pl. (Leipzig) 2: 849 (1891)
Facesoffungi number: FoF 02676

Fig. 4 – *Cookeina sulcipes.* a Apothecia on wood, b Apothecia, c Cross section of an apothecium, d Close up of excipulum at margins, e Cylindric, short hairs, f Septate, branched paraphyses, g–j long pedicellate asci, k Sub operculate apical apex (closed state in left and open state in right), l–o Ovoid ascospores. – Scale bars c = 1000 µm, d = 100 µm, e =25 µm, f = 70 µm, g–j = 100 µm, k = 70 µm, l–o = 30 µm.

Saprobic on dead stems. Sexual morph: *Apothecia* 1–3 × 4–8 cm ($\bar{x}$ = 2.2 × 4.8 µm, n = 10) arising singly, stipitate, deep or more shallow cupulate, pink to orange when fresh. *Receptacle* concave, disc and margins are pink to orange when fresh. *Hairs* 13–15 × 2–3 µm ($\bar{x}$ = 14.1 × 2.7 µm, n = 30) on flanks and margins, cylindrical, straight, thin-walled, aseptate, hyaline undifferentiated hyphal tip. *Stipe* 0.5–3 mm long, lighter than the receptacle. *Excipulum* 120–200 µm ($\bar{x}$ = 166.2 µm, n = 10). *Ectal excipulum* composed of large, thin-walled, hyaline to pinkish cells of *textura prismatica* and *medullary excipulum* composed of hyaline to pinkish cells of *textura intricata*. *Hymenium* pinkish orange, fading to pale-yellow in dried specimens. *Paraphyses* 2.2–3.6 µm wide ($\bar{x}$ = 3.3 µm, n = 20), numerous, filiform, septate, highly branched and slightly swollen at the apex forming the epithecium. *Asci* 280–380 × 15–22 µm ($\bar{x}$ = 339.2 × 19.6 µm, n = 30), unitunicate, operculate, cylindrical, short pedicellate, non-amyloid. *Ascospores* 21–30 × 11–18 µm ($\bar{x}$ = 24.8 × 12.5 µm, n = 40), uniseriate, 1-celled, ovoid, ends slightly narrowed, hyaline or subhyaline with one or two large oil-drops, aseptate, smooth, thin-walled, with thick gelatinous sheath. Asexual morph: Undetermined (description modified from Seaver 1913; Patil et al. 2012).

Material examined – THAILAND, Prachuapkhirikhan Province, Pong Prasat, Bang Saphan District, July 30, 2015, A.R. Bandara (MFLU 15-2358).

Notes – *Cookeina sulcipes* differs from all the other species in the genus in having ascospores with thick gelatinous sheath (Iturriaga & Pfister 2006, Patil et al. 2012).

Cookeina tricholoma (Mont.) Kuntze, Revis. gen. pl. (Leipzig) 2: 849 (1891) Fig. 5
Facesoffungi number: FoF 02677

Saprobic on dead stems. Sexual morph: *Apothecia* 1–3 × 4–8 cm ($\bar{x}$ = 2 × 5.2 µm, n = 10), arising singly, stipitate, deeply cupulate. *Receptacle* concave, glabrous, disc orange when fresh. Stipe 1-1.5cm long, 0.2-0.4cm broad, glabrous, lighter than the receptacle surface. *Margin* enrolled, concolorous to receptacle surface. *Spines* 2–7 × 0.5–1 mm ($\bar{x}$ = 3 × 0.8 µm, n = 30) cylindrical, narrowed tips, aseptate. *Hairs* 70–80 × 8–12 µm ($\bar{x}$ = 73.5 × 10.1 µm, n = 30) on flanks and margins, cylindrical, straight, thin-walled, septate, hyaline. *Hymenium* orange to hyaline when fresh. *Ectal excipulum* two layers, outer ectal excipulum 10–100 µm, composed of large, thin-walled, hyaline cells of *textura globulosa*, inner ectal excipulum 30–60 µm, composed of thin-walled hyaline cells of *textura intricata*, hyphae oriented perpendicular to the receptacle surface. *Medullary excipulum* 160–200 µm, composed of hyaline cells of *textura intricata*. *Subhymenium* 40–60 µm composed of loosely arranged hyaline cells of *textura intricata*. *Paraphyses* 2–4 µm wide ($\bar{x}$ = 3.5 µm, n = 20), numerous, filiform, septate, highly branched and slightly swollen at the apex, forming the epithecium. *Asci* 220–320 × 8–22 µm ($\bar{x}$ = 264.4 × 13.5 µm, n = 30) unitunicate, operculate, cylindrical, short pedicellate, non-amyloid. *Ascospores* 10–25 × 7–12 µm ($\bar{x}$ = 21.5 × 9.5 µm, n = 40), uniseriate, 1-celled, ovoid, hyaline to pinkish, aseptate, with 1-2 guttules. Asexual morph: Undetermined (description modified from Iturriaga & Pfister 2006).

Material examined –CHINA, Sky tree, Menla County, Yunnan Province, 400m alt., on soil near the vegetation of *Dipterocarpaceae* 7 July 2014, Xiaobin Liu 394 (HKAS87041).THAILAND, Prachuapkhirikhan Province, Pong Prasat, Bang Saphan District, July 30 2015, A. R. Bandara (MFLU 15-2359).

Notes – *Cookeina tricholoma* and *C. korfii* are distinct from other species in the genus in having both spines and hairs, and hyaline to pink ascospores. However they differ from ascospore ornamentation. Boedijn (1933) and Meléndez-Howell et al. (2003) indicates that the ascospore of *C. tricholoma*, bear parallel longitudinal, low ridges that form the ascospore ornamentation and slightly spinose surface. However the spore ornamentation is only seen in mature spores (Iturriaga & Pfister 2006).

Cookeina venezuelae (Berk. & M.A. Curtis) Le Gal, Discomyc. Madagascar (Paris): 239 (1953)
Facesoffungi number: FoF 02678

Saprobic on dead stems. Sexual morph: *Apothecia* up to 30 mm diam. when fresh, up to 20 mm diam. when dry, arising singly to gregarious, sessile to subsessile, deep or shallowly cupulate,

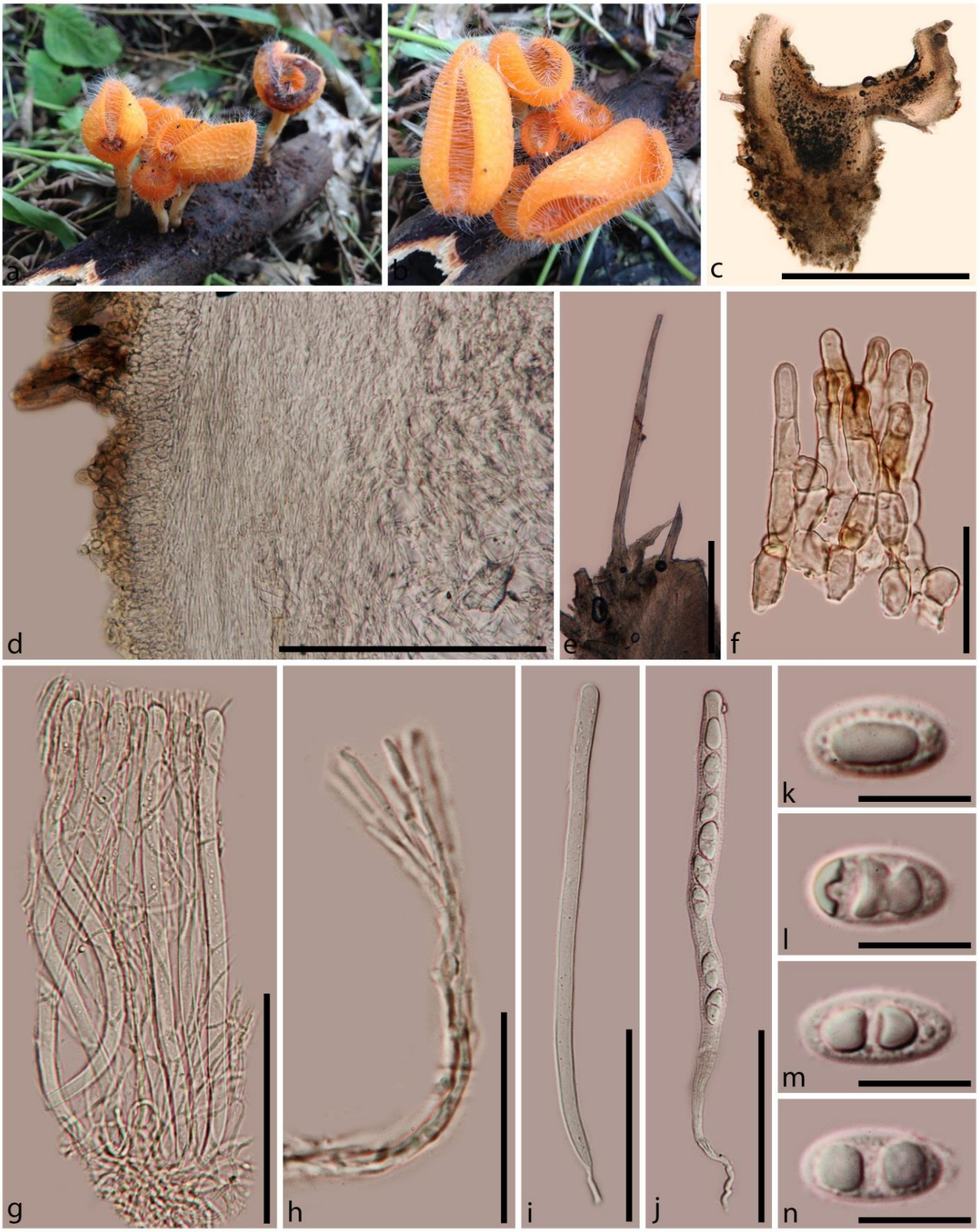

Fig. 5 – *Cookeina tricholoma*. a Apothecia on wood, b Apothecia, c Cross section of an apothecium, d Close up of excipulum, e Cylindric, pointed spines, f Cylindric, short hairs, g Asci and paraphyses in hymenium layer, h Septate paraphyses, i, j long pedicellate asci, k–n Ovoid ascospores. – Scale bars c = 2 cm, d = 200 µm, e = 600 µm, f = 40 µm, g = 100 µm, h = 40 µm, i, j = 80 µm, k–n= 10 µm.

salmon to rose pink when fresh. Receptacle concolorous or slightly lighter than the disc. Disc glabrous, salmon to rose pinkish when fresh, cream colored to light brown when dry. Hairs on flanks and margins, cylindrical, aseptate, undifferentiated hyphal tip. Stipe 1–3 × 2 mm present or

lacking, when present central or slightly eccentric, obconic, concolorous to receptacle. Ectal excipulum 66–88 µm, two layers, outer ectal excipulum composed of large, thick-walled cells of textura prismatica to globulosa and inner ectal excipulum composed of gelatinized thick hyphae (1.5–4 µm in diam.), parallel to one another, perpendicular to the receptacle. Medullary excipulum 80–90 µm, composed of hyaline cells of textura porrecta, hyphae parallel to one another and to the receptacle. Subhymenium composed of textura intricata to porrecta. Paraphyses 2–5 µm wide, numerous, filiform, septate, highly branched and slightly swollen at the apex (up to 5 µm diam.), forming the epithecium. Asci 275–550 × 10–30 µm, unitunicate, operculate, cylindrical, round to slightly tapered at the base. Ascospores 24–43 × 9–18 µm, uniseriate, elliptic, fusoid, pale yellow with two large central guttules, with few longitudinal ribs and many transverse interconnected ribs on ascospore surface, aseptate. Asexual morph: Undetermined (description modified from Iturriaga & Pfister 2006).

Notes – *Cookeina venezuelae* similar to *C. colensoi* in having gelatinous material in the inner ectal excipulum and hairless apothecia with stipes. However the ascospores of *C. colensoi* are smooth and apiculate, whereas those in *C. venezuelae* are non-apiculate and distinctively marked with longitudinal ribs (Le Gal 1953).

Discussion

In the present study we discuss the taxonomy and phylogeny of the genus *Cookeina*, and introduce a new species *Cookeina garethjonesii*. We illustrate collections of *C. indica*, *C. sulcipes* and C. *tricholoma* from Thailand and China. Descriptions of all *Cookeina* species are provided from the literature and an updated phylogenetic tree is shown to illustrate the placement of taxa. In the phylogenetic tree based on ITS sequence data (Fig. 1), there are four major clades in the genus. Clade 1 includes *C. sulcipes*, *C. garethjonesii* and *C. speciosa*. All species in Clade 1 have long, centrally stipitate apothecia with short hair like projections (rudimentary hairs). Clade 2 comprises *C. colensoi*, *C. venezuelae* and *C. insititia* that have a gelatinized ectal excipulum in both mature and immature specimens. *Cookeina speciosa* also have gelatinized ectal excipular layer, but this is apparent in some young fertile specimens (Iturriaga & Pfister 2006). All species in Clade 3 (*C. korfii*, *C. sinensis*, *C. tricholoma*) have both spines and hairs on their apothecia. *Cookeina indica* and *C. cremeirosea* make up Clade 4 and they are characterized by substipitate to sessile apothecia and lack hairs on the receptacle. These results are similar to those of Weinstein et al. (2002), Iturriaga & Pfister (2006), Iturriaga et al. (2015) and Kropp (2016).

Molecular data is not available for *C. globosa* and *C. colensoiopsis*. However, their morphologies are similar to *C. speciosa* in having hymenial setae or hairs, long stipitate apothecia with hair-like projections (Iturriaga & Pfister 2006). Hence phylogenetically *C. globosa* and *C. colensoiopsis* should be members of Clade 1 close to *C. speciosa*.

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