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A phylogenetic study of the genus *Cookeina*

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Abstract: *Cookeina*, with seven recognized species, is one of the commonly encountered genera of the Sarcoscyphaceae (Pezizales) in tropical and subtropical areas around the world. Morphologically the species are distinguished by combinations of several features including ascospore shape and surface relief, presence and origin of apothecial hairs and presence or absence of gelatinous material within the cortical layer of the excipular tissue. Color of the hymenium, attributed to carotenoid pigments, is particularly variable in some collections especially those referred to as *C. speciosa*. In this study phylogenetic analyses were carried out using rDNA ITS and rDNA LSU sequences. Forty-four collections were studied which included a broad sampling of color variants of *C. speciosa* from a field site in Venezuela. The genus was shown to be monophyletic with several well-supported lineages. These analyses generally support the established, morphologically distinguished taxa within a monophyletic genus *Cookeina*. Collections referred to as *C. speciosa* segregate within a clade in which hymenial color differences are associated with groups within the clade. *Cookeina sinensis* is sister to *C. tricholoma* but is distinct from it; *C. indica* fails to resolve with any of the major clades. The placement of *C. insititia* is ambiguous but it falls within *Cookeina* and thus is considered in the genus *Cookeina* rather than in a separate genus, *Boedijnopeziza*.

Key Words: biogeography, ITS sequences, Pezizales, Sarcoscyphaceae

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INTRODUCTION

Of the tropical and subtropical members of the order Pezizales few are as commonly encountered and collected as are species belonging to the genus *Cookeina* Kuntze (1891). Their brightly colored and relatively large apothecia assure that even the most casual observer will see them. The seven recognized morphospecies are defined on a series of distinctive and unambiguous macroscopic and microscopic characters related to occurrence and distribution of the hairs on the surface of the apothecium, spore shape and surface ornamentation, and presence or absence of gelatinous material in the tissue of excipulum. Species occur on fallen angiosperm branches, trunks and, occasionally, on durable fruits. Some taxa are widespread, such as *C. colensoi* (Berk.) Seaver, *C. tricholoma* (Mont.) Kuntze and *C. speciosa* (Fr. : Fr.) Dennis, but others are more restricted in distribution, such as, *C. sinensis* Z. Wang, *C. venezuelae* (Berk. & M. A. Curtis) Le Gal, *C. insititia* (Berk. & M. A. Curtis) Kuntze, and *C. indica* Pfister & R. Kaushal. Hymenial colors range from white to beige, yellow, orange, scarlet, and even to chocolate brown. Color variation is particularly pronounced among collections of *C. speciosa*. Arpin (1969) analyzed the pigments and reported carotenoids in two species, *C. tricholoma* and *C. sulcipes* (Berk.) Kuntze (=*C. speciosa*).

We initiated this study because we observed both distinct distributional patterns of species in this genus and variation in hymenial colors. The study focuses on the phylogenetic relationships among the taxa and their color variants. We obtained material of all taxa and we sampled broadly across the geographical range of taxa where we had appropriate material. A companion study will treat the genus monographically.

The family Sarcoscyphaceae, in which *Cookeina* has a central position, has a convoluted history, summaries of which may be found in Eckblad (1968), Korf (1970), Rifai (1968) and Harrington et al (1999). The Sarcoscyphaceae has been referred to the suborder Sarcoscyphineae (Rifai 1968). According to an analysis of the Sarcoscyphineae by Harrington et al (1999) it is a paraphyletic assemblage, but a clade containing *Sarcoscypha* (Fr.) Boud., *Phillipsia* Berk., *Cookeina* and related taxa is well supported and represents the Sarcoscyphaceae of most recent authors.

In that study (Harrington et al 1999) *Cookeina* was shown to be sister to *Microstoma* Bernstein, a position that confirms Korf's (1972, 1973) view that these genera are closely related. Korf (1972) proposed a tribe, the *Boedijnopezizeae*, for *Cookeina*, *Microstoma*, and *Boedijnopeziza* S. Ito & S. Imai. These genera share features such as simultaneous maturation of ascospores within apothecia, a particular ascus construction in which the base of the ascus abruptly constricts to a thin supporting hypha and a dense network of anastomosing paraphyses. *Microstoma* is temperate in distribution. *Boedijnopeziza*, based on *B. insititia*, is known from Asia and is recognized as a distinct taxon (Rifai 1968, Korf 1972, 1973) or treated as a synonym of *Cookeina* (Le Gal 1953, Denison 1967, Pfister 1973, Pfister and Kaushal 1984). Korf (1983) suggested a relationship between the Boedijnopezizeae and *Cyttaria* Berk. Subsequent analysis of sequence data indicates that *Cyttaria* is only distantly related to the Pezizales (Landvik 1996).

Our goal in this study was to test the monophyly of the genus *Cookeina* and to evaluate the morphospecies as currently recognized, particularly with regard to biogeography and color variation. To accomplish our goal we used phylogenetic analyses of characters derived from nuclear encoded ribosomal DNA (rDNA).

MATERIALS AND METHODS

Material studied.—Specimens of *Cookeina* used for DNA analysis were collected by the authors or obtained from collectors or herbaria (TABLE I). To distinguish specimens and avoid confusion with herbarium and field numbers, voucher numbers were assigned to collections. The ingroup consisted of 44 specimens of *Cookeina*, including 16 specimens of *C. speciosa* displaying distinct color variants. The outgroup was *Microstoma floccosum*.

Molecular techniques.—DNA was isolated from approximately 50 mg of dried apothecial tissue of each sample, which was first cleaned of extraneous material using a small paintbrush followed by short blasts of compressed air. Dried apothecia were ground to a fine powder in liquid nitrogen. Powdered samples were extracted in 800 μL SDS lysis buffer (1% SDS; 200 mM Tris, pH 7.5; 250 mM NaCl; 25 mM EDTA; 1% polyvinylpyrolidone, adjusted to pH 6.8) and incubated, with occasional mixing, in a water bath for 1 h at 65 C. The supernatant was extracted twice with an equal volume of chloroform-isoamyl alcohol (24:1) and transferred to a 2.0 mL microcentrifuge tube. DNA was purified with a QIAGEN Blood and Tissue kit (Valencia, California) by incubating at 70 C for 10 min with an equal volume of buffer AL (1:1 supernatant: buffer) followed by the addition of 100% ethanol equal in volume to the original supernatant. DNA was purified using QIAamp spin columns; bound DNA was washed twice with applications of 500 μL wash buffer (buffer AW, Qiagen) and then eluted from the spin column with 100–200 μL ddH_2O warmed to 70 C. Eluate was passed through the column a second time to increase yield.

DNA extract was used for polymerase chain reaction (PCR) of the internal transcribed spacers (ITS 1 and ITS 2) and the 5.8S region of the nuclear ribosomal DNA using the fungus-specific oligonucleotide primers ITS 4 and ITS 5 (White et al 1990). Four μL of PCR product was quantified on an ethidium bromide-stained 1.0% agarose gel, and the remaining PCR product was purified using Microcon 100 microconcentrator columns (Amicon Inc., Beverly, Massachusetts).

For dye-terminator cycle-sequencing involving the ITS region, ITS primers 2, 3, 4 and 5 were used; for the LSU region primers LR0R, LR3R, LR3 and LR5 were used. Sequencing reactions were run on an Applied Biosystems 377 automated DNA sequencer.

Analytical techniques.—Sequences were edited and assembled using Sequencher 3.0 (GeneCodes, Ann Arbor, Michigan) and aligned manually in the data editor of PAUP 4.0d64 (Swofford 1991) with alignment gaps inserted to maximize aligned sites. Sequences have been deposited in GenBank (TABLE I) and the data matrix is available from TreeBASE as accession number SN1105.

Phylogenetic analyses were performed in PAUP 4.0d64 (Swofford 1991). After coding gaps in various fashions and finding basically similar results, we determined to treat gaps as missing data (gaps = missing coding); ambiguous regions in the alignment (characters 67–76, 79–93, 123–145, 160–182, 238–263, 266–275, 286–312, 530–545, 545–562, 687–716) were recoded as single characters (characters 782–791, respectively) using the methods described by LaGreca (1999), with each newly designated character given a weight equal to the number of characters from the corresponding ambiguous region. Using this system, the number of informative characters was increased from 246 to 256. Due to the size of the data set, we were limited to heuristic searches, which were performed in two parts. First, 100 heuristic searches were performed with random taxon addition and TBR branch swapping, with MAXTREES set to 200, keeping up to 2 trees per replicate. Second, all the shortest trees from the first part of the analysis were used as starting trees for complete TBR branch-swapping with MAXTREES set to 15 000. Relative robustness of individual clades was assessed by the bootstrap (Felsenstein 1985) using 100 heuristic searches, simple taxon addition sequences, TBR branch swapping and MAXTREES set to 1000.

Scanning electron microscopy (SEM), light microscopy (LM) and morphological studies.—Ascospores of *Cookeina* collections were gathered by rehydrating a small piece of apothecium in several drops of water and then carefully separating the hymenial tissue from the excipular tissue. The hymenial tissue was macerated using a scalpel blade and further squashed with a pipette tip. An aliquot of this macerate was examined under LM. When free floating spores were observed, a drop of this spore suspension was pipetted onto a cover slip, dried, placed on a stub and sputter-coated with a gold-palladium alloy. Observations were made on an AMRAY model 1000 SEM.

TABLE I. List of collections and sequences of *Cookeina* and *Microstoma* species used for phylogenetic analysis

Species	Geographic origin	Specimen[a]	Color[b]	Code	GenBank accession
C. colensoi	India	**FH, PAN** 18557	Not recorded	118	AF394532
C. colensoi	Mexico	**CUP** 62500	Not recorded	60	AF394040
C. colensoi	New Zealand	**PDD** 68535	Not recorded	112	AF394034
C. colensoi	New Zealand	**PDD** 66040	Not recorded	113	AF394035
C. colensoi	New Zealand	**PDD** 68628	Not recorded	114	AF394036
C. colensoi	New Zealand	**PDD** 55306	Not recorded	115	AF394037
C. colensoi	Australia	**DAR** 63642	Orange	116	AF394038
C. colensoi	Australia	**DAR** 63646	Orange-saffron	117	AF394039
C. colensoi	Guizhou, China	**HMAS** 59537	Not recorded	121	AF394532
C. indica	Yunnan, China	**HMAS**	Not recorded	119	AF394531
C. insititia	Yunnan, China	**HMAS** 70078	Not recorded	123	AF394030
C. insititia	Guizhou, China	**HMAS** 71942	Not recorded	124	AF394031
C. insititia	Yunnan, China	**FH** Wang sp 1	Not recorded	125	AF394032
C. insititia	Yunnan, China	**FH** Wang sp 2	Not recorded	126	AF394033
C. sinensis	Yunnan, China	**HKAS** 14679	Not recorded	111	AF394028
C. sinensis	Yunnan, China	**HMAS** 70088	Not recorded	48	AF394027
C. speciosa	Venezuela	**FH** Iturriaga 10E-D5	Light brown (9C5-9C6)	22	AF394015
C. speciosa	Venezuela	**FH** Iturriaga 2610	Pale yellow (4A3-4A4)	25	AF394005
C. speciosa	Venezuela	**FH** Iturriaga 7A-D4	Light coral (10A5)	27	AF394006
C. speciosa	Venezuela	**FH** Iturriaga D2-5A	Coral (10A7)	28	AF394007
C. speciosa	Venezuela	**FH** Iturriaga D2-4A	Coral (10A7)	29	AF394012
C. speciosa	Venezuela	**FH** Iturriaga 1D-D6	Mauve (10C4-10C6)	30	AF394016
C. speciosa	Venezuela	**FH** Iturriaga 2D-D4	Mauve (10C4-10C6)	31	AF394017
C. speciosa	Venezuela	**FH** Iturriaga 4D-D4	Mauve (10C4-10C6)	32	AF394004
C. speciosa	Venezuela	**FH** Iturriaga D2-2A	Orange (7A8)	33	AF394008
C. speciosa	Venezuela	**FH** Iturriaga 1C-D4	Orange (7A8)	34	AF394011
C. speciosa	Venezuela	**FH** Iturriaga 4A-D4	Deep coral (11A8)	36	AF394014
C. speciosa	Venezuela	**FH** Iturriaga 1E-D5	Deep coral (11A8)	37	AF394003
C. speciosa	Thailand	**FH** Pfister 7131	Not recorded	7131	AF394009
C. speciosa	Thailand	**FH** Pfister 7143	Not recorded	7143	AF394010
C. speciosa	Borneo, Malaysia	**C** TL 6035	Not recorded	6035	AF394018
C. speciosa	Colombia	**FH** Muneton 296	White	110	AF394013
C. tricholoma	Yunnan, China	**HMAS** 23238	Not recorded	120	AF394025
C. tricholoma	Yunnan, China	**HMAS**	Not recorded	122	AF394026
C. tricholoma	Venezuela	**FH** Iturriaga 1D-D5	Orange (7A8)	38	AF394021
C. tricholoma	Venezuela	**FH** Iturriaga 1B-D5	Orange (7A8)	39	AF394022
C. tricholoma	Venezuela	**FH** Iturriaga 2705	Not recorded	40	AF394023
C. tricholoma	Puerto Rico	**FH** Pfister 38	Not recorded	15	AF394024
C. tricholoma	Thailand	**FH** Pfister 7170	Not recorded	7170	AF394020
C. tricholoma	Borneo, Malaysia	**C** TL 6101	Not recorded	6101	AF394019
C. venezuelae	Guadeloupe	**FH** Pfister 1161	Not recorded	19	AF394042
C. venezuelae	Puerto Rico	**FH** Cantrell 3381	Not recorded	20	AF394041
C. venezuelae	Venezuela	**FH** Iturriaga 6066	Salmon (10A5)	42	AF394043
C. venezuelae	Venezuela	**FH** Iturriaga 6065	Salmon (10A5)	43	AF394044
M. floccosum	Mexico	**FH** K. Griffith	Not recorded	45	AF394045
M. floccosum	Mexico	**FH** K. Griffith	Not recorded	46	AF394046

[a] Voucher specimens are deposited in the herbaria indicated in boldface type as follows: **CUP**, Plant Pathology Herbarium, Cornell University; **DAR**, Plant Pathology Branch Herbarium, Biological and Chemical Research Institute, New South Wales; **HMAS**, Mycological herbarium, Systematic Mycology and Lichenology Laboratory, Beijing; **FH**, Farlow Herbarium, Harvard University; **PAN**, Botany Department Punjab University; **PDD**, New Zealand Fungal Herbarium, Landcare Research, Auckland, New Zealand. Code refers to the numbers used in this study. Color from Methuen is noted in parentheses where available.

[b] Numbers and letters in parentheses refer to Methuen (Kornerup and Wanscher 1978) notations.

Field studies.—A detailed field study was undertaken in the Amazonian rainforest in Yutajé, Amazonas State, Venezuela. *Cookeina* species were collected in numbered plots on precisely designated and coded substrates. Thus, single collections could be traced precisely to a particular substrate and its neighbors on that substrate could be identified.

Color nomenclature.—Colors were assigned in the field and correlated with Methuen (Kornerup and Wanscher 1978) color names and numbers. Methuen color notations are given in TABLE I.

RESULTS

Alignment.—ITS1 and ITS2 were aligned along with the intervening 5.8S region and flanking partial sequences of 18S and 25S rDNA. All positions were generally able to be aligned within collections of the same species; alignment across species often required the addition of gaps. Because of the large data set this resulted in ten main areas of ambiguous alignment (as described in the Methods). The 5.8S region was almost identical across all taxa, and the aligned length of all sequences (including inserted gaps) was 781 bp.

Parsimony analyses.—Under gap = missing data there were 256 informative characters yielding 15 000 equally parsimonious trees of 3083 steps (consistency index, CI = 0.960; retention index, RI = 0.980). Phylogenetic analysis identified four well-supported lineages of rDNA as measured by bootstrapping (FIG. 1). The monophyly of the *C. speciosa* and *C. tricholoma* lineages was strongly supported (for each, bootstrap = 100%) and the lineage consisting of *C. colensoi* and *C. venezuelae* was supported by a bootstrap value of 99%. Further bootstrap support of 100% and 93%, respectively, was obtained for both the *C. colensoi* and *C. venezuelae* clades, while the *C. insititia* clade was supported by a bootstrap value of 100%. The only specimen of *C. indica*, although falling within *Cookeina*, failed to clearly resolve with any of the greater clades.

The *C. speciosa* clade includes color variants from Venezuela, a white specimen from Colombia, two isolates from Thailand and one from Sabah, Malaysia, which together form two sub-clades with good support. One major sub-clade consisting of mostly Venezuelan specimens (bootstrap = 95%) contains two groups: one that includes ''deep coral'' variants (specimen nos. 36 and 37; bootstrap = 100%) and another with ''mauve/light brown'' variants (specimen nos. 22, 30, 31 and 32; bootstrap = 97%). The specimen from Sabah, Malaysia is basal to this subclade. The other major well-supported sub-clade (bootstrap = 99%) also consists of two smaller groups. One of these groups (specimen nos. 25, 27, 28, 29, 33, 34, and 110; bootstrap = 100%) consists mostly of Venezuelan specimens ranging in color from orange, coral, light coral, to yellow. In addition, the white specimen from Colombia falls into this clade. The other group consists of collections from Thailand (bootstrap = 100%).

The *C. tricholoma* clade includes specimens from Sabah, China, Puerto Rico, Thailand and Venezuela. There is very little difference in ITS sequences within this clade despite the geographical spread of the collections (FIG. 2). *Cookeina sinensis* is basal in the *C. tricholoma* clade with 100% bootstrap support.

The clade comprising *C. venezuelae* and *C. colensoi* is strongly supported at the highest order (bootstrap = 99%) and resolution for each sub-clade alone is high (bootstrap of 93% and 100% respectively). Regional variation is apparent in each of these clades: all southern hemisphere (Australia and New Zealand) specimens of *C. colensoi* cluster together with virtually no ITS sequence differences among specimens while the specimens from the northern hemisphere (China, India, and Mexico) resolve basally to the southern hemisphere collections. *Cookeina venezuelae* shows considerable variation within the relatively local Caribbean region, with Venezuelan specimens resolving together (bootstrap = 100%), and specimens from Guadeloupe and Puerto Rico clustering together (bootstrap = 100%).

Cookeina insititia also demonstrates considerable variation within the clade, especially considering that each of the four specimens analyzed were collected in China, three from Yunnan Province (FIG. 2).

SEM spore images.—SEM images of spores from *Cookeina* (FIG. 3) aid and clarify descriptions derived from LM studies. Spores of several species are described as striate in LM studies but SEM reveals that the nature and arrangement of the striation varies. Spore ornamentation in *C. speciosa*, described as striate, consist of irregular anastomozing longitudinal ridges that appear somewhat wrinkled. Spores of *C. tricholoma*, also described as striate under LM, exhibit a more regular pattern of relatively straight and parallel longitudinal ridges. *Cookeina indica*, described as having fine longitudinal markings, has regular parallel longitudinal ridges that are wider than those in *C. tricholoma*. *Cookeina venezuelae*, typically described as having both longitudinal and transverse striations, can be more accurately described as having longitudinal ribs with fine transverse interconnecting ridges. Spores of both *C. colensoi* and *C. insititia* are uniformly smooth. Spores of *C. sinensis* were difficult to obtain because the material was scanty and questionably mature, but those we found were smooth.

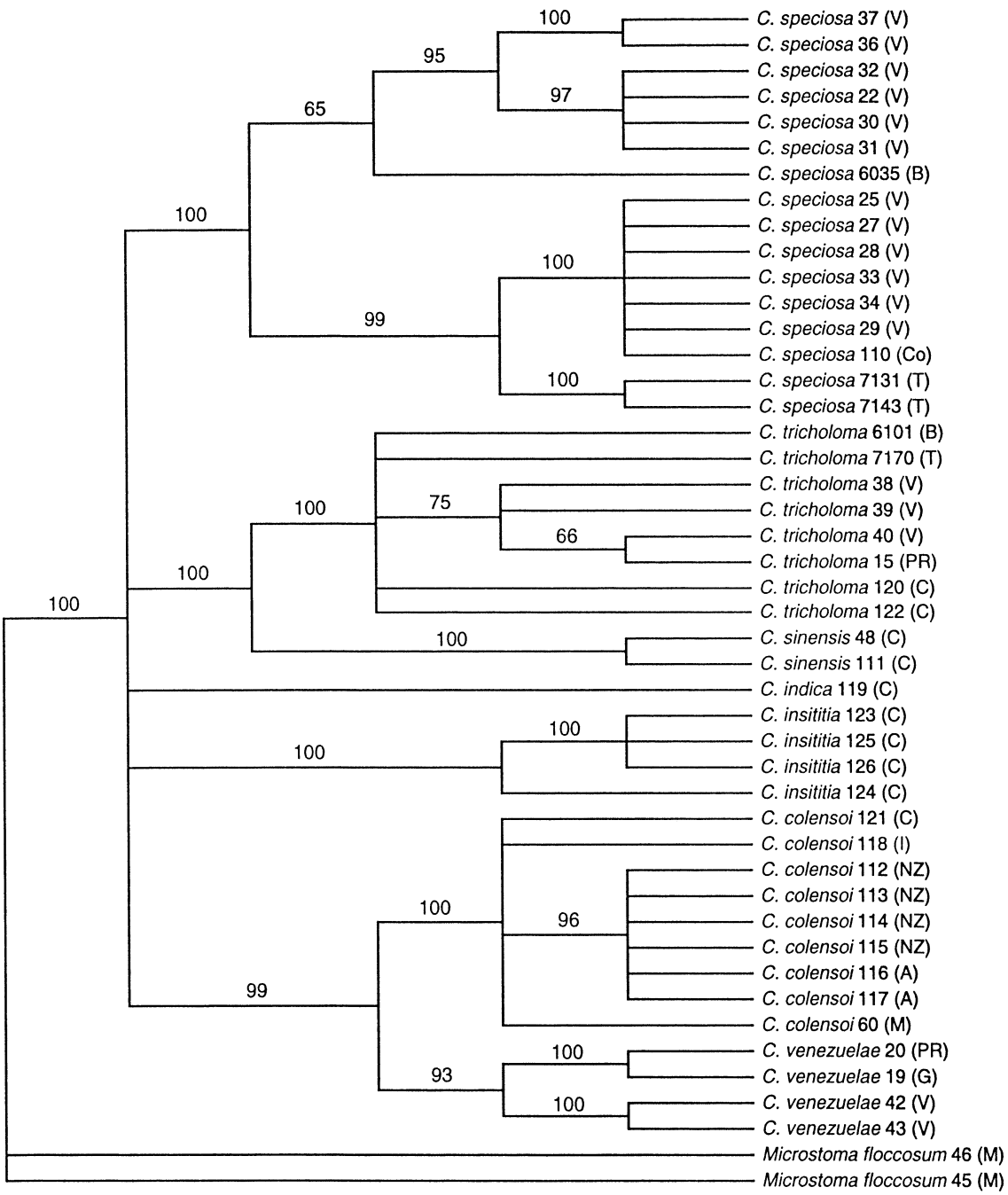

FIG. 1. Strict consensus of 15 000 equally parsimonious trees (3083 steps) generated under gap = missing data. Numbers above the branches represent bootstrap support for 1000 replicates. Location codes are as follows: A—Australia, B—Borneo (Sabah), C—China, Co—Colombia, G—Guadeloupe, I—India, M—Mexico, NZ—New Zealand, PR—Puerto Rico, T—Thailand, V—Venezuela.

Field studies.—The field study focused on *C. speciosa* but also yielded collections of *C. tricholoma.* Twelve collections of *C. speciosa* from the field plots were included in this study. Two collections, nos. 27 and 36, recorded as different in color, were collected on the same substrate in the same plot on the same day. However, these collections occur in different clades of the *C. speciosa* group. This strongly supports the hypothesis that the color variants are distinct. Similarly, two other collection, nos. 22 and 37, were made in the same plot, on the same substrate, on the same day. Both of these collections fall in the same clade.

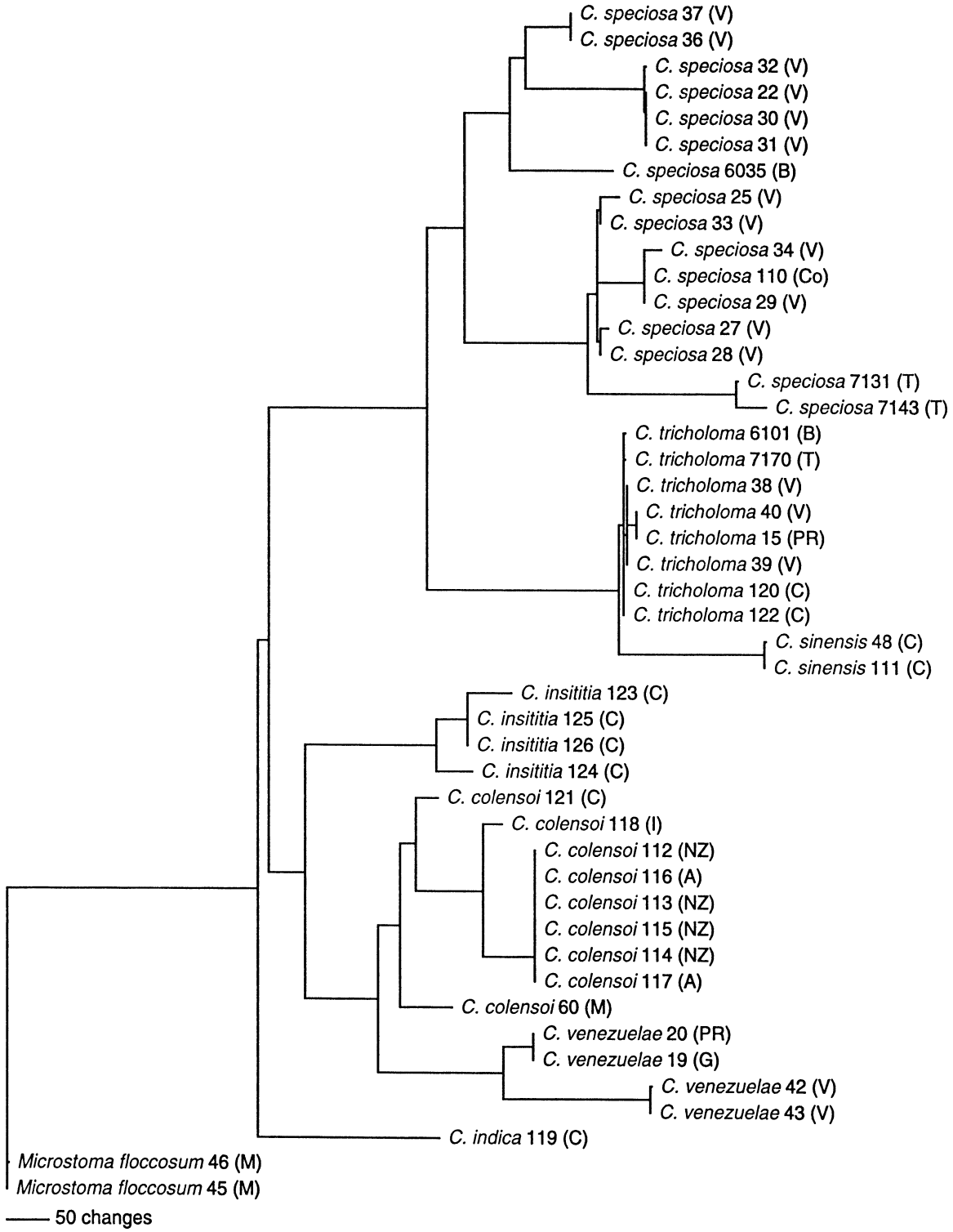

FIG. 2. Phylogram representing one of 1000 equally parsimonious trees for ITS sequences. Terminal taxa are individual collections (see TABLE I); branch lengths are according to scale. Geographical codes are as for FIG. 1.

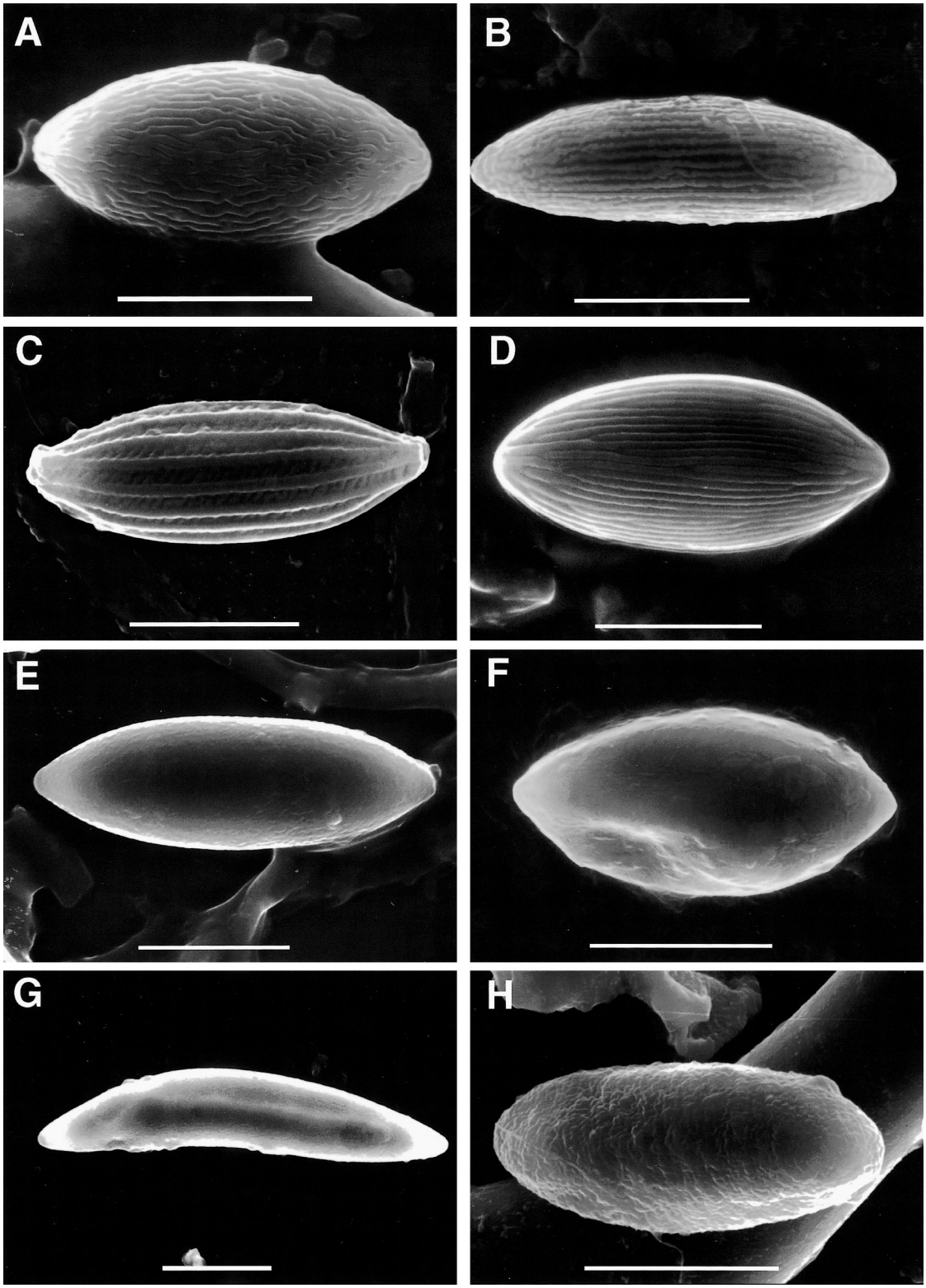

FIG. 3. Ascospore ornamentation as seen by SEM. A. *Cookeina speciosa*. Face view showing anastomosing ridges (FH, Aldava 296). B. *Cookeina indica*. Face view showing 13–15 longitudinal ribbon-like ridges (HMAS, Z. Wang). C. *Cookeina venezuelae*. Profile view showing 6–7 longitudinal ribs with regular, fine transverse connecting ridges (FH 1107). D. *Cookeina tricholoma*. Face view showing 22–24 longitudinal ridges (OSC 67751). E. *Cookeina colensoi*. Face view (PDD 68628). F. *Cookeina sinensis*. Profile view (HMAS 14679). G. *Cookeina insititia*. Profile view, surface smooth (HMAS, Z. Wang). H. *Microstoma floccosum*. (FH, K. Griffith). Scale bar = 10 μm.

Five collections (nos. 27, 31, 32, 34, 36) were made in plot D4. Of these nos. 31, 32, and 36 group together in the dark-color clade; nos. 27 and 34 are in the light-color sister clade. Three collections (nos. 28, 29, 33) were made in plot D2. Collection 28 and 29 were from the same substrate, and together with 33 resolve in the light-color clade.

DISCUSSION

General morphology.—Phylogenetic analyses reveal several distinct rDNA lineages with resolution into clades that correlate with the morphological groups that have been recognized as species. The monophyletic group consisting of *C. speciosa*, *C. tricholoma*, and *C. sinensis* are hirsute, stipitate and lack a defined gelatinous layer in the excipulum at maturity. Spores are longitudinally ridged in the two former species and smooth in the latter. *Cookeina venezuelae* and *C. colensoi*, which also constitute a monophyletic group, have a well-defined excipular gel layer, are sessile or short stipitate and glabrous or with low scales or pustules. Spores are ornamented in *C. venezuelae* and smooth in *C. colensoi*. *Cookeina indica*, with ornamented spores, and *C. insititia*, with smooth spores, are intermediate regarding excipular features and did not clearly resolve in relation to the larger groupings.

Spore ornamentation has no clear correlation to any of the major lineages. *Cookeina sinensis*, for example, has smooth spores or slightly wrinkled spores (Wang 2001) yet resolves with taxa having striate spores, while *C. venezuelae*, with a unique spore ornamentation, as dissimilar to striate spores as they are to smooth spores, resolves with the smooth-spored *C. colensoi*. This lack of correlation contrasts with observations by Hansen et al (1999), who found that ITS lineages within *Phillipsia*, also a member of the Sarcoscyphaceae, were all supported by spore morphology.

Cookeina insititia is the only species with stipitate, hirsute apothecia and a gelatinous layer, but its relationship to one or the other of the main groups is uncertain. Likewise, *C. indica*, the only stipitate, glabrous species without a gelatinous layer, also fails to consistently resolve with one or the other of the greater monophyletic clades.

Color and regional variation in C. speciosa.—*Cookeina speciosa* is noteworthy within the Pezizales for having a broad spectrum of apothecial colors. Our studies to date show no consistent anatomical differences among the color forms. On the other hand, rDNA analysis indicates collections in the same color ranges group together into two well-supported clades. Thus, one clade with a 65% bootstrap includes collections with darker colored apothecia, in the brown-mauve-dark coral (purplish) range; the second clade, with a 99% bootstrap, has lighter colored apothecia, in the yellow, orange, light coral, coral and white range. In Arpin's (1969) study he found no evidence for differences in the carotenoid content in two color variants of *C. speciosa* (as *C. sulcipes*) he studied.

In this study the northern region of South America represents the reference point from which variation can be assessed since a range of color forms were documented from there and incorporated in the data set. It is interesting therefore that the collection from Sabah groups with the more darkly pigmented specimens from Venezuela, while the collections from Thailand always group with the clade comprising the more lightly pigmented specimens from Venezuela. The sequence difference between the Sabah and Thailand specimens is considerable, given the geographical proximity of the two locations. This disparity cannot be accounted for by biogeographical features such as the Wallace line, which passes to the east of Borneo. The two regions are part of the same biogeographical zone, although associated with different forest types. Furthermore, field studies in Venezuela demonstrate a range of ITS and apothecial color differences present even within small areas and on the same pieces of downed wood.

Color as a reliable taxonomic character.—Conventions in taxonomic mycology have had an important influence on species concepts within certain groups of fungi. Ascomycete systematists have largely discounted color variations in favor of reliance on microscopic characters. Although color variation was noted and used by some earlier mycologists to distinguish species of *Cookeina*, more recent authors have grouped the various color forms into more or less anatomically uniform morpho-species. Our data suggests that a closer look at these species complexes is required to understand the characters used in classification. We believe there are at least two taxa within the *C. speciosa* complex.

Resolution of the taxonomic status of C. sinensis.—*Cookeina sinensis* was recognized on morphological grounds. It has smooth or slightly wrinkled spores but otherwise is morphologically similar to *C. tricholoma*. We examined both the sterile holotype and a fertile paratype of *C. sinensis*. Our morphological observations are still tentative because of the inadequacy of the available material. Even the paratype material is barely mature and it is possible that the smooth spores of *C. sinensis* could represent immature spores of *C. tricholoma*. Our studies indicate that the striations on ascospores of *C. tricholoma* form relatively

late in the development of the ascospores. On molecular grounds *C. sinensis* falls outside and sister to the *C. tricholoma* group. The *C. tricholoma* collections from such disparate locations as Sabah, China, Puerto Rico, Thailand, and Venezuela, show short internal branch lengths (FIG. 2). While this lack of variation may somehow be unique to the biology of *C. tricholoma*, it also highlights the distinct taxonomic provenance of *C. sinensis*, which always resolves sister to the *C. tricholoma* collections (bootstrap = 100%). Furthermore, both specimens of *C. sinensis* and two specimens of *C. tricholoma* were collected in Yunnan Province of China, further weakening the argument that *C. sinensis* may represent either an immature collection of *C. tricholoma* or a regional variant of it. These phylogenetic results, along with the morphological difference that characterizes *C. sinensis*, provide evidence for its recognition as a distinct species. Wang (2001) reported *C. sinensis* from Taiwan and provided SEM photomicrographs of spores from a type collection and one from Taiwan.

Resolution of the taxonomic status of C. insititia.—There has been debate over the taxonomic status of *C. insititia*. The spore shape, presence of a gel layer (known also in *C. colensoi* and *C. venezuelae*) and the form and origin of the hairs seem distinct in *C. insititia*. These features led Ito and Imai (1937) to erect a new genus, *Boedijnopeziza*. The genus was supported by some (Rifai 1968, Korf 1972, 1973) and disputed by others (Denison 1967, Eckblad 1968, Le Gal 1953, Pfister 1973, Pfister and Kaushal 1984). Molecular data supports the position of this species within the genus *Cookeina*, but because of lack of resolution in the strict consensus tree, its position in relationship to the other taxa remains problematic. Korf (1971) included *C. colensoi* in *Boedijnopeziza* based on the presence of gel in the excipular tissue of that species. Pfister (1973) recognized that *C. colensoi* and *C. venezuelae* shared this character. One could recognize *Boedijnopeziza* for *C. insititia*, *C. colensoi*, and *C. venezuelae* since the molecular data do not rule out such a position, but there remains little other than the somewhat ambiguous character of gel to unite them morphologically. In our opinion the choice to recognize *Boedijnopeziza* obscures the continuity of characters in this monophyletic group.

Distribution of C. colensoi.—Denison (1967) reported that *C. colensoi* occurred chiefly south of the equator, with one collection from Venezuela and none from Central America. In our monographic studies we have determined that the Venezuelan collection represents a heretofore undescribed species. However, collections from Mexico, India, and China have come to light and have been included in this analysis. The species is best considered sub-tropical, with principle distribution in the southern hemisphere.

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LITERATURE CITED

Arpin N. 1969. Les caroténoïdes des discomycètes: Essai chimiotaxinomique. Bull Mens Soc Linn Soc Bot Lyon 38(suppl):1–169.

Denison WC. 1967. Central American Pezizales. II. The genus *Cookeina*. Mycologia 59:306–317.

Eckblad F-E. 1968. The genera of operculate discomycetes. A re-evaluation of their taxonomy, phylogeny and nomenclature. Nytt Mag Bot 15:1–191.

Felsenstein J. 1985. Confidence limits on phylogenetics: an approach using the bootstrap. Evolution 39:783–791.

Harrington FA, Pfister DH, Potter D, Donoghue MJ. 1999. Phylogenetic studies within the Pezizales. I. 18S rDNA sequence data and classification. Mycologia 91:41–50.

Hansen K, Pfister DH, Hibbett DS. 1999. Phylogenetic relationship among species of *Phillipsia* inferred from molecular and morphological data. Mycologia 91:299–314.

Ito S, Imai S. 1937. Fungi of the Bonin Islands II. Trans Sapporo Nat Hist Soc 15:52–59.

Korf RP. 1970. Nomenclatural notes. VII. Family and tribe names in the Sarcoscyphineae (Discomycetes) and a new taxonomic disposition of the genera. Taxon 19: 782–786.

———. 1971. Some new discomycete names. Phytologia 21: 201–207.

———. 1972. A synoptic key to the genera of the Pezizales. Mycologia 64:937–994.

———. 1973. Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS, eds. The fungi: an advanced treatise. Vol. 4A. New York: Academic Press. p 249–319.

———. 1983. *Cyttaria* (Cyttariales): coevolution with *Nothofagus*, and evolutionary relationship to the Boedijnopezizeae (Pezizales, Sarcoscyphaceae). Austral J Bot, Suppl Ser 10:77–87.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. Ed. 3. London: Eyre Methuen. 252 p.

Kuntze O. 1891. Revisio generum plantarum vascularium omnium atque cellularium multarum secundum leges nomeclaturae internationales cum enumeratione plantarum exoticarum in itinere mundi collectarum. Vol. 2. Leipzig: A. Felix. 1011 p.

LaGreca S. 1999. A phylogenetic evaluation of the *Ramalina americana* chemotype complex (lichenized Ascomycota, Ramalinaceae) based on rDNA ITS sequence data. Bryologist 102:602–608.

Landvik S. 1996. Phylogenetic rDNA studies of discomycetes (Ascomycota). Umea, Sweden: Umea Univ. 42 p.

Le Gal M. 1953. Le discomycètes de Madagascar. Prodr Flore Mycol Madagascar 4:1–465.

Pfister DH. 1973. Notes on Caribbean discomycetes IV. *Cookeina venezuelae*, *C. colensoi* and the genus *Boedijnopeziza*. Phytologia 27:55–62.

———, Kaushal R. 1984. *Cookeina indica*, a new species from India with a key to the species of *Cookeina*. Mycotaxon 20:117–121.

Rifai MA. 1968. The Australasian Pezizales in the herbarium of the Royal Botanic Gardens, Kew. Verh Kon Ned Akad Wetensch, Afd Natuurk, Tweede Sect 57:1–295.

Swofford DL. 1991. PAUP-phylogenetic analysis using parsimony, ver. 4.064d. Champaign, Illinois: Illinois Natural History Survey.

Wang YZ. 2001. Discomycetes of the Sarcoscyphaceae in Taiwan. Mycotaxon 79:329–336.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White T, eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press. p 315–322.