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A new species of *Cudonia* based on morphological and molecular data

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Abstract: A discomycete collected in western Sichuan, China, is morphologically intermediate between *Cudonia* and *Spathularia*. The fungus has a bright yellow capitate ascigerous head, a white, ridged stalk, and a well-developed membrane covering the whole ascoma. The asci, ascospores, and paraphyses are similar to those of *Cudonia* and *Spathularia*. Based on morphology and DNA sequence analysis, a new species, *Cudonia sichuanensis*, is reported. *Cudonia* and *Spathularia* are closely related to members of Rhytismataceae, as has been suggested previously. The similarity of ascoma and ascospore development between these two genera and *Lophodermium* (Rhytismataceae) is discussed.

Key Words: Chinese fungi, Geoglossaceae, Helotiales, ITS, Leotiaceae, nuc-lsu rDNA, *Spathulariopsis*

INTRODUCTION

The Geoglossaceae (Helotiales) is traditionally composed of taxa such as *Geoglossum* Pers., *Trichoglossum* Boud. and *Microglossum* Gillet, which are commonly known as earth tongues, and other genera, including *Mitrula* Fr., *Nothomitra* Maas Geest., *Cudonia* Fr.:Fr., *Spathularia* Pers., and *Spathulariopsis* Maas Geest. These taxa often have large ascocarps, so they have received a lot of attention (Durand 1908, Corner 1929, 1930, Imai 1941, Maas Geesteranus 1964, Mains 1940, 1955, Korf 1973). Based on the color of the ascospores and the reaction of the ascus pore in Melzer's reagent, the family can be separated into two groups: 1) *Cudonia*, *Spathularia*, and *Spathulariopsis* with hyaline ascospores and a negative reaction of the ascus pore in Melzer's reagent; 2) *Geoglossum* and *Trichoglossum* with dark ascospores and a positive reaction (bluing) of the ascus pore in Melzer's reagent. *Mitrula*, *Nothomitra* and *Microglossum* do not fit in either of these groups (Korf 1973, Spooner 1987, Verkley 1994).

Macromorphology of the ascocarps has been emphasized at the family or subfamily level in Helotiales (Imai 1941, Mains 1956, Korf 1973, Spooner 1987). Most mycologists have been inclined to group the genera with clavate or spathulate ascocarps, such as *Geoglossum*, *Microglossum*, and *Spathularia*, together in the family Geoglossaceae, but opinions have differed regarding the position of the genera with pileate ascocarps, such as *Cudonia*, *Vibrissea* Fr., and *Leotia* Pers. *Cudonia* was put in the Leotiaceae, and *Spathularia* and *Spathulariopsis* in the Geoglossaceae by Korf (1973) based on macromorphology.

The well-developed gelatinized layer of *textura intricata* in the ectal excipulum, as in the genus *Leotia*, was emphasized at family level by Lizon et al (1998). The concept of Leotiaceae in the sense of Korf (1973) was restricted to a narrow sense, and *Cudonia* was excluded (Lizon et al 1998). Based on ultrastructure of the ascus apices, Verkley (1994) suggested that there are two different lineages in the family Geoglossaceae, and that *Geoglossum*, *Trichoglossum* and *Microglossum* are different from *Spathularia*.

Except for the difference in the macromorphology of the ascomata, *Cudonia* and *Spathularia* share many important characters, such as gelatinously sheathed ascospores, curved paraphyses, club-shaped asci with non-bluing pores in Melzer's reagent, and veil structures that are present at least at an early stage of ascoma development. The relationship between *Cudonia* and *Spathularia* has been supported by some molecular studies (Gargas et al 1995, Landvik 1996, Suh and Blackwell 1999, Bhattacharya et al 2000, Platt and Spatafora 2000, Gernandt et al 2001).

The current concept of the Geoglossaceae, in which all stipitate, capitate, or clavulate fungi including *Geoglossum*, *Trichoglossum*, *Microglossum*, *Mitrula*, *Spathularia*, *Cudonia* and other taxa are included, is controversial (R. P. Korf pers comm). More and more data based on morphological and molecular research call into question the position of *Cudonia* and *Spathularia* either in Leotiaceae or in Geoglossaceae (Landvik 1996, Pfister and Kimbrough 2001, Gernandt et al 2001).

Nannfeldt (1942) suggested that *Cudonia* and *Spa-*

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thularia share characters with the members of Phacidiaceae (including *Rhytisma* Fr., which now is a member of Rhytismataceae, Rhytismatales), such as filiform, branched, and circinate paraphyses, and a stromatic layer that covers the hymenium in the early stage of ascoma development. This relationship was supported by parsimony analyses using nuc-ssu rDNA, nuc-lsu rDNA, and RPB2 (Landvik 1996, Platt 1999, Gernandt et al 2001, Lutzoni et al 2001). Three groups of Helotiales were presented by Pfister and Kimbrough (2001), and one of these three groups included *Cudonia*, *Spathularia*, and probably several other taxa of the Rhytismatales (Pfister and Kimbrough 2001).

Nannfeldt's suggestion about the close relationship between *Spathularia* and the Rhytismataceae was partially based on the stromatic layer of *Spathularia velutipes* Cooke & Farlow. Maas Geesteranus (1972) compared both *Spathularia velutipes* and *S. flavida* Pers. (the type species of *Spathularia*), and did not observe a stromatic layer (he called it a veil) in his material of *S. flavida*. He created the monotypic genus *Spathulariopsis* for *S. velutipes* and pointed out that the developmental type in *Spathulariopsis* is hemiangiocarpous, in contrast to the gymnocarpous type he found in *Spathularia flavida* (Maas Geesteranus 1972).

Eight species in *Cudonia*, two species in *Spathularia*, and one species in *Spathulariopsis* are estimated (Hawksworth et al 1995). *Cudonia circinans* (Pers.) Fr., *C. confusa* Bres., *C. helvelloides* S. Ito & S. Imai, *C. lutea* (Peck) Sacc., *Spathularia flavida* and *Spathulariopsis velutipes* were reported from China (Zhuang 1998). Two field trips to Sichuan Province, China were made in 1997 and 1998, and about 40 specimens of *Spathularia*, *Cudonia*, and other members of Geoglossaceae were collected. Among them, one capitate fungus is morphologically intermediate between *Cudonia* and *Spathularia*. Morphological and molecular studies suggest that this is a new species in *Cudonia*.

MATERIALS AND METHODS

Specimens and morphological studies.—Specimens were collected in Sichuan Province, China, in 1997 and 1998, and are deposited in the Herbarium of the Institute of Microbiology, the Chinese Academy of Sciences (HMAS) and the Farlow Herbarium (FH) of Harvard University. Morphological descriptions are based on observations of dried or of rehydrated specimens. Microscopic studies were based on squashed tissues and sections cut with a freezing microtome at 20–25 μm thickness. Measurements, illustrations, and photographs were usually made under cotton-blue-lactic-acid using bright field and phase contrast optics (Nikon E600). Ascus pore iodine reactions were examined using Melzer's reagent. Anatomical terms follow Korf (1973).

Molecular techniques.—DNA was isolated from dried fruiting bodies. Approximately 20–30 mg of tissue was ground in liquid nitrogen and extracted in 600 μL of extraction buffer (1% SDS, 0.15 M NaCl, 50 mM EDTA) at 75 C for 1 h, purified with phenol-chloroform-isoamyl alcohol (25:24:1), and precipitated with 95% ethanol and 3 M NaCl overnight. Crude DNA extracts showed strong pigmentation and were diluted with distilled water up to one-thousand-fold for use as PCR templates after an additional purification step with GeneClean (Bio 101, La Jolla, California).

Partial nuclear large subunit rDNA (nuc-lsu-rDNA) was amplified with primers LR0R and LR5 (Vilgalys and Hester 1990) in 11 isolates, representing 4 species of *Cudonia* and *Spathularia* (TABLE I). Internal transcribed spacers 1 and 2 and the 5.8S rDNA were amplified with primers ITS4 and ITS5 (White et al 1990) in 9 isolates, representing 4 species of *Cudonia* and *Spathularia* (TABLE I). PCR reaction mixes (Promega Corp., Madison, Wisconsin) contained 2.5 μL 10x PCR buffer, 5 μM dNTP, 12.5 pM of each PCR primer and 5 μL DNA in 15 μL. The amplification program included 40 cycles of 94 C for 30 s, 45 C for 30 s, and 72 C for 1 min.

PCR products were purified using GeneClean (Bio 101) and sequenced using the ABI Prism Bigdye-terminator cycle sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's protocols. Primers used for sequencing were LR0R, LR3, LR3R, LR5, ITS4, and ITS5. Sequencing reactions were purified using Pellet Paint (Novagen, Madison, Wisconsin) and were run on an Applied Biosystems 377XL automated DNA sequencer. Sequences were edited with Sequencher version 3.1 (GeneCodes Corporation, Ann Arbor, Michigan). Sequences generated in this study were submitted to GenBank (accession numbers AF433136-AF433155; TABLE I).

Phylogenetic analyses.—Sequences were aligned by eye in the data editor of PAUP* 4.0b (Swofford 1999). Two datasets were constructed: 1) a dataset of nuc-lsu rDNA sequences, including the 11 sequences generated for this study and 12 sequences of *Cudonia*, *Spathularia*, *Geoglossum*, *Leotia*, *Lophodermium* Chevall., *Sclerotinia* Fuckel, and *Stictis* Pers., which were downloaded from GenBank (TABLE I); 2) a dataset of nuc-lsu rDNA and ITS sequences from 9 isolates of *Cudonia*, *Spathularia*, and *Lophodermium* (TABLE I). Both datasets were analyzed in PAUP* using equally weighted parsimony, with gaps treated as missing data, and all positions included.

The nuc-lsu rDNA dataset was rooted using *Stictis radiata* (L.) Pers. A heuristic search was performed with one thousand replicate searches, each with a random taxon addition sequence, MAXTREES set to autoincrease, and TBR branch swapping. A bootstrap analysis was performed with one thousand replicates, each with ten random taxon addition sequences, MAXTREES set to 100, and TBR branch swapping.

The nuc-lsu/ITS dataset was rooted using *Lophodermium pinastri* (Schrad.) Chevall. A branch-and-bound analysis was performed with MAXTREES set to autoincrease. A boot-

TABLE I. Specimens used in molecular studies

Species	Collection No.	GenBank accession No.	
		ITS1-5.8S-ITS2 rDNA	28S rDNA
Cudonia circinans[G]	JP 232 (DNA)		AF279379
C. circinans[G]			AF107553
C. lutea	wz 139	AF433151	AF433139
C. lutea	wz 164	AF433149	AF433138
C. lutea	wz 225	AF433150	AF433140
C. sichuanensis	wz 96	AF433148	AF433136
C. sichuanensis	wz 187	AF433147	AF433137
Spathularia cf. *flavida*	wz 214	AF433152	AF433141
S. cf. *flavida*	wz 138	AF433153	AF433142
S. flavida	wz 93		AF433143
S. flavida	wz 95	AF433155	AF433144
S. flavida	wz 135		AF433146
S. flavida	wz 137	AF433154	AF433145
S. velutipes[G]			AF107554
S. velutipes[G]	JP 173(DNA)		AF279411
S. velutipes[G]			AF113734
S. velutipes[G]			AF113735
S. velutipes[G]			AF113736
Geoglossum glabrum[G]			AF113738
Leotia viscosa[G]			AF113737
Lophodermium pinastri[G]		AF013224	AY004334
Sclerotinia veratri[G]			AF113739
Stictis radiata[G]			AF113746

[G] = data obtained from GenBank.

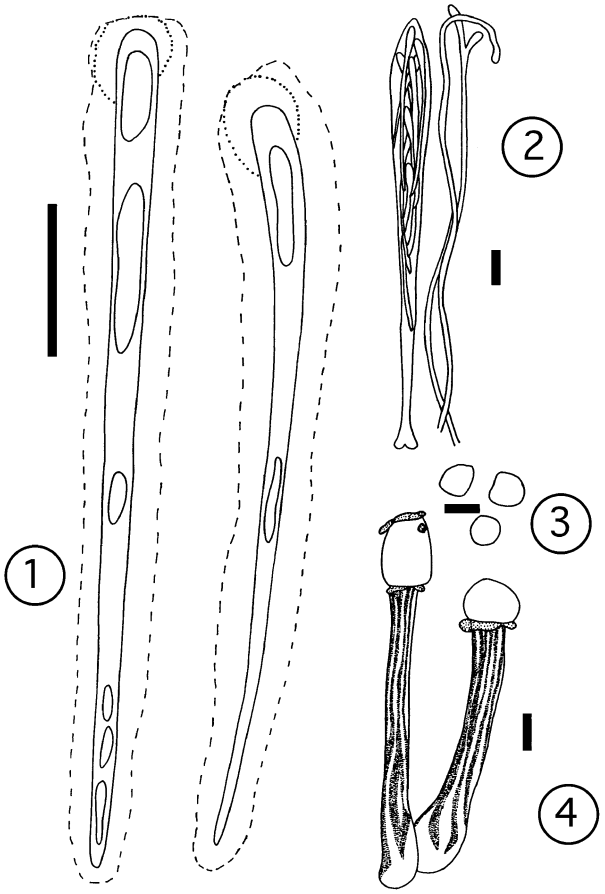
strap analysis was performed with one thousand branch-and-bound replicates with MAXTREES set to autoincrease. Alignments are available at TreeBASE (study accession number S702).

RESULTS

Morphology.—Twenty-two specimens were examined, including four collections of the new species, *Cudonia sichuanensis* (FIGS. 1–10). The ascomata of *C. sichuanensis* are distinctly capitate, and the broken membrane forms a collar-like structure connected with the stalk along the margin of the ascogenous head (FIGS. 4, 5, 8). The membrane is about 50 μm thick and is composed of several layers of angular cells. The outermost layer is composed of smaller cells (3–5 μm in diam) and is covered with duff or soil debris (FIGS. 9–10). Such a well-developed membrane is not found in the other species of *Cudonia* and *Spathularia* from the Chinese collections. However, sometimes a kind of cortex remains on the surface of the stalk of Chinese *Cudonia* and *Spathularia* collections, which is composed of hyaline to brownish and angular to globose cells, coated with some plant debris and sand. Remnants of veils are observed at the juncture between the stipe and the hymenium from the North American species of *Cudonia* and *Spathularia* (D. Pfister pers comm). A gelatinous sheath and cap-like structure were observed on the ascospores of all the collections in rehydrated specimens (FIGS. 6–7). Similar structures were observed by Johnson (1994) in *Lophodermium pinastri*. The size of the ascospores in *C. sichuanensis* ranges from 46–66 × 2.0–2.2 μm. Globose ascoconidia are produced by the ascospores in asci from all the collections of *Cudonia* and *Spathularia*, and the paraphyses show no morphological difference among those collections.

Molecular data.—PCR products of nuc-lsu-rDNA ranged from 898 bp in *Cudonia sichuanensis* (WZ187) to 1206–1210 bp in *Spathularia flavida* (WZ95, WZ135, and WZ137). The difference in size results from the presence of 283–290 bp introns in *S. flavida* (WZ95, WZ135, and WZ137). Similar introns were observed in the sequence of *Lophodermium pinastri* AY004334 (which was downloaded from GenBank). The intron sequences were excluded from the phylogenetic analysis. PCR products of ITS 1 and 2 and 5.8S rDNA ranged from 523 bp in *C. sichuanensis* to 528–540 bp in *C. lutea*, and *S. flavida*.

The nuc-lsu rDNA dataset included 962 aligned positions, with 189 variable positions and 95 parsimony-



FIGS. 1–4. *Cudonia sichuanensis* HMAS 75140. 1. Ascospores. 2. Ascus and paraphyses. 3. Ascoconidia. 4. Ascocarps. Scale bars: 1–2 = 10 μm, 3 = 2 μm, 4 = 2 mm.

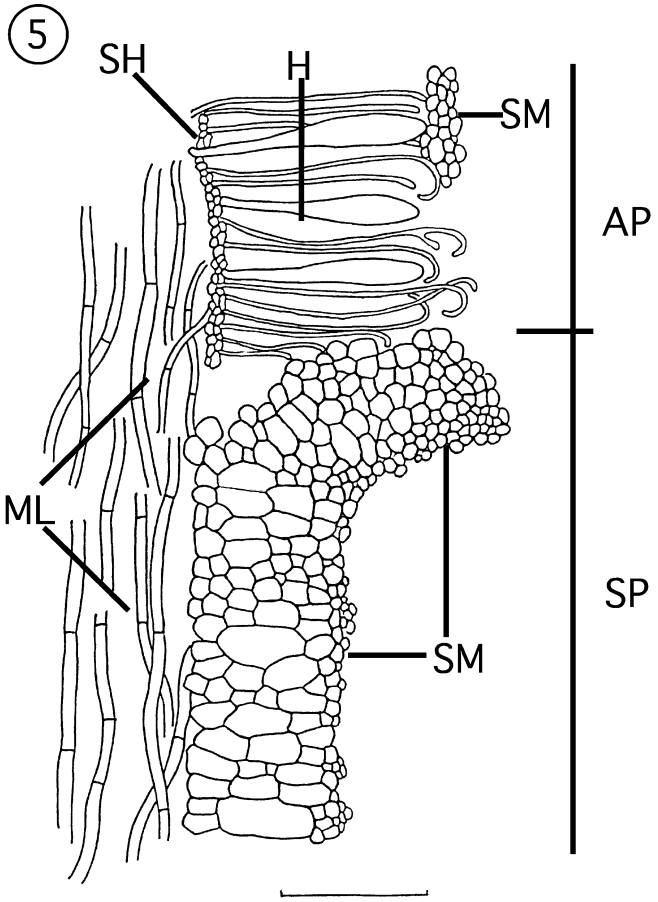


FIG. 5. Longitudinal section through the fruitbody of HMAS 75140 shows ascigerous portion (AP), stalk portion (SP), subhymenium (SH), hymenium (H), stromatic membrane (SM), and Medullar layer in both stalk and ascigerous potion. Scale bar 5 = 50 μm.

informative positions. Parsimony analysis of the nuc-lsu rDNA dataset resulted in twelve equally parsimonious trees of 297 steps (CI = 0.771, RI = 0.724; FIG. 11). *Cudonia* plus *Spathularia* form a strongly supported monophyletic group (bootstrap = 91%; FIG. 11). *Lophodermium pinastri* is strongly supported as the sister group of the *Spathularia-Cudonia* clade (bootstrap = 100%; FIG. 11). Within the *Spathularia-Cudonia* clade there is little resolution or support, except for three groups that include putatively conspecific isolates of *C. circinans*, *C. lutea*, and *S. flavida*. Neither *Spathularia* nor *Cudonia* is resolved as monophyletic (FIG. 11). The two isolates of *C. sichuanensis* were weakly supported as conspecific (bootstrap < 50%; FIG. 11).

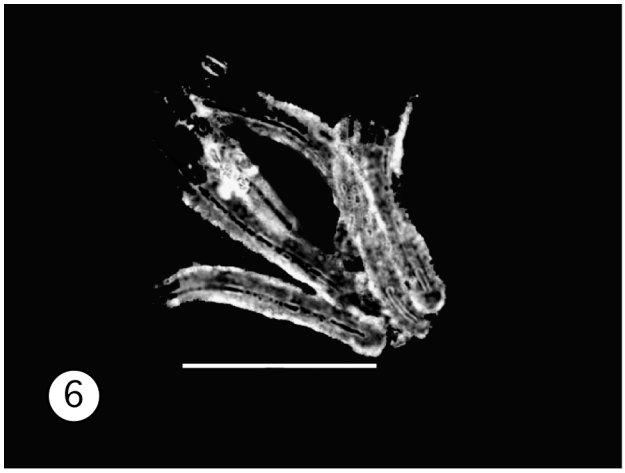
The nuc-lsu/ITS dataset included 1415 aligned positions, with 145 variable positions and 72 parsimony-informative positions. Branch-and-bound analysis of the nuc-lsu/ITS dataset produced a single tree of 203 steps (CI = 0.823, RI = 0.748; FIG. 12). *Spathularia* and *Cudonia* were both weakly supported as monophyletic groups. The two isolates of *C. sichuanensis* were strongly supported as conspecific (bootstrap = 90%; FIG. 12).

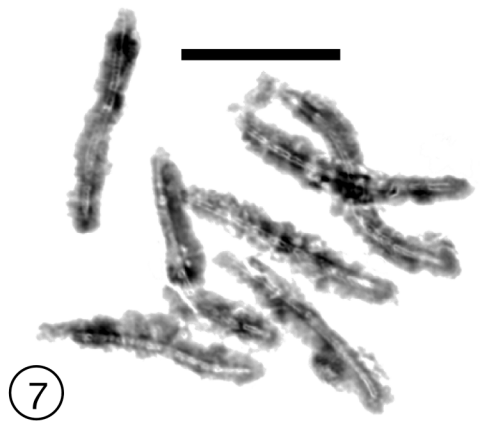
TAXONOMY

A morphological comparison between *Cudonia*, *Spathularia*, *Geoglossum*, *Cryptohymenium*, and *Lophodermium* is shown in TABLE II. (Data were based Mains 1940, Imai 1941, Samuels and Kohn 1986, Spooner 1987, Johnston 1989, and pers obs).

Cudonia sichuanensis Zheng Wang, sp. nov.
(FIGS. 1–10)

Ascomata solitaria vel gregaria, stipitata, capitata 17–35 mm longitudine; caput ascomatis globosum, luteum vividum sed siccatum fulvum, 1.5–3 mm diametro; semper adsunt fragmenta structurae membranaceae in parte superiore stipitis et secundum marginem conjunctam stipiti. In stipite corticis textura angularis, 30–50 μm crassitudine; asci clavati, ad basem certe angustiores; porus iodo non caerulescens, 136–152 × 12–14 μm; octosporae in asco; ascos-





FIGS. 6–7. Ascospores of *Cudonia sichuanensis* HMAS 75140. 6. Spores in black ink show the gelatinous sheaths and caps. 7. The gelatinous sheaths surrounded by the ink debris dissolved quickly in water. Scale bars: 6–7 = 50 μm.

porae clavato-filiformes vel aciculares, hyalinae, non-septatae, vaginatae in gelatin, 46–66 × 2–2.2 μm; paraphyses filiformes, apice curvat vel circinat. Holotypus: HMAS 75140.

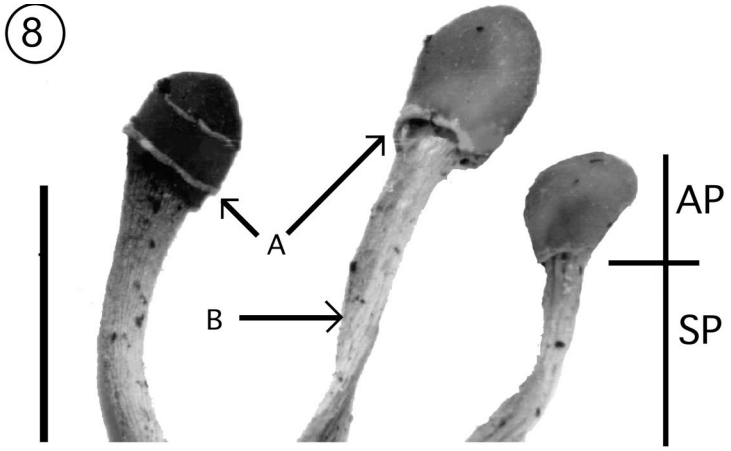
Ascomata scattered to gregarious, capitate, stipitate, 17–35 mm in height. Ascigerous portion capitate, with remains of a membrane-like structure on the upper part and along the margin connecting to the stalk (FIGS. 4, 5, 8), bright yellow when fresh, brownish when dry, 1.5–3 mm in diam, slightly swollen compared with the stalk. Stalk white, slender, smooth or slightly tomentose, striate to ridged, 15–25 mm in length. The interior of the stalk and ascigerous portion of *textura intricata*, loosely interwoven hyphae becoming more compact and parallel toward the outside (FIGS. 5, 9–10). The stromatic membrane of regular *textura angularis*, well-developed, sometimes broken into pieces as the hymenium expands, about 40–50 μm thick over the stalk and 30–40 μm thick over the hymenium, outermost layer composed of smaller cells encrusted with soil and debris, brownish. Cells of the stromatic layer array at a right angle to the axis of the stalk, angular to globose from inner side to outer surface, 3 × 5–15 × 25 μm. Hymenium about 120–150 μm thick. Asci clavate, narrower towards the base, 8-spored, apical pore J-, 136–152 × 12–14 μm. Ascospores hyaline, acicular, rounded above, acuminate below, multiguttulate, nonseptate, 46–66 × 2.0–2.2 μm, the wall with a gelatinous layer swelling in water to 1.5–2 μm thick, prominent gelatinous cap present at the wider end of the ascospore, and sometimes at both spore poles. Ascoconidia subspherical, irregular ellipsoid or obovoid, 1–2 × 1–2 μm, 1-celled, hyaline, sometimes replacing the ascospores and filling the asci. Paraphyses filiform, simple or branched below, not or irregularly branched above, strongly curved to circinate or straight above, hyaline, about 2 μm in diam.

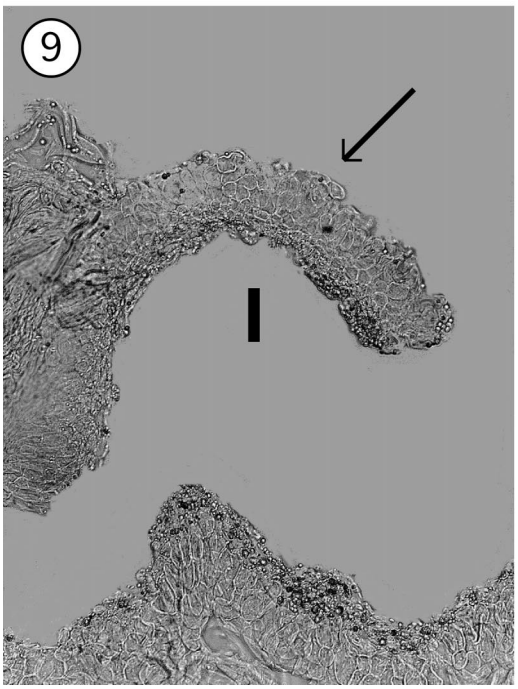
Specimens examined. CHINA. SICHUAN PROVINCE: Xiangchenxian, Daxuesan Mountains, on duff of *Abies* sp., 24 Jul 1998, Zheng Wang WZ0178 (HOLOTYPE HMAS75140, ISOTYPE FH-WZ0178); between Xiangchenxian and Dongwangxian, on duff, 26 Jul 1998, *Zheng Wang* WZ202 HMAS 75139; between Xiangchenxian and Dongwangxian, on duff, 26 Jul 1998, *Zheng Wang WZ187* HMAS 75141; Xiangchenxian, Wumingsan, on duff, 12 Jul 1998, *Zheng Wang WZ96* HMAS 75143.

DISCUSSION

Cudonia sichuanensis is distinct from other species in the genus in its capitate ascomata (FIGS. 8, 12) and well-developed membrane covering the whole fruitbody. Subglobose or even globose pilei have been reported in *Cudonia*, but generally the ascigerous portions expand widely and the margins of the hymenia are free from the stalk in these species (FIG. 12). The form of the ascigerous areas was a character used to separate *Cudonia* from *Spathularia*. Several ascomata of *C. sichuanensis* also show expansion along the margin of ascogenous head, and the membrane can be seen stretched between the hymenium and the stalk.

The color of the hymenium and the size of the ascospores are key characters in the delimitation of species in *Cudonia*. The bright yellow hymenium and ascospores of 46–66 × 2.0–2.2 μm of the new species are similar to those of *Cudonia helvelloides*, which has large (25 to 70 mm) pileate ascomata (Mains 1940, Imai 1941). *Cudonia convoluta* Lloyd is the only species in the genus so far reported to have capitate ascomata, and was considered as an intermediate between *Cudonia* and *Mitrula* by Lloyd (Lloyd 1916). However, Mains (1940) examined the type specimen of *C. convoluta* and found the species was distinctly





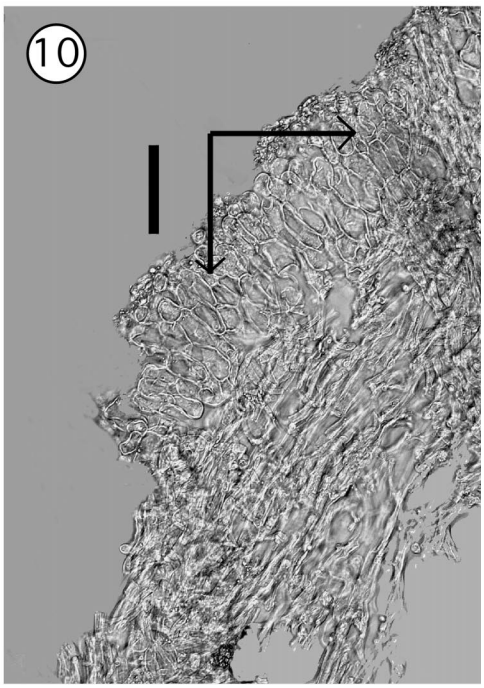


FIG. 8. Dry apothecia of *Cudonia sichuanensis*, HMAS 75140, shows ascigerous portion (AP), stalk portion (SP), and the broken stromatic layer over the hymenium and along the margin of the hymenium. A and B indicate the parts which are shown in FIGS. 8. Scale bar = 20 mm.

FIGS. 9–10. Stromatic layer at different parts of the ascomata of *Cudonia sichuanensis* HMAS 75140. 9. The collar like structure along the margin of the hymenium (arrow). 10. Part of the stromatic layer of *textura angularis* along the stalk (arrows). Scale bars: 9–10 = 50 μm.

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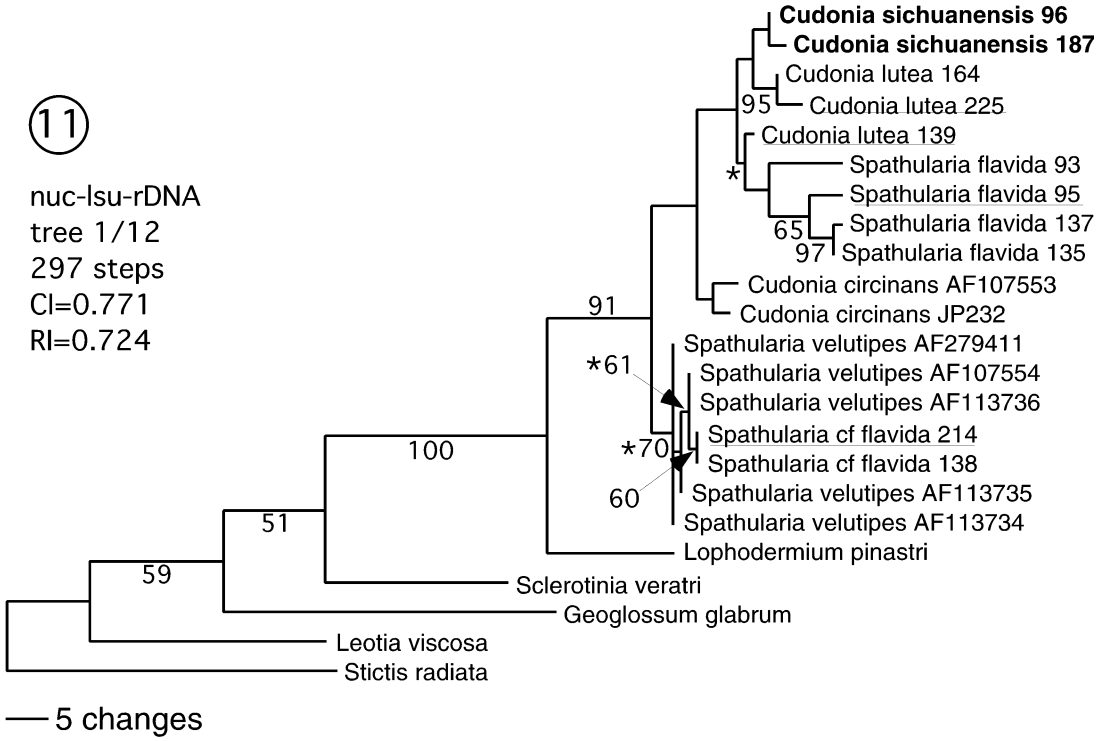
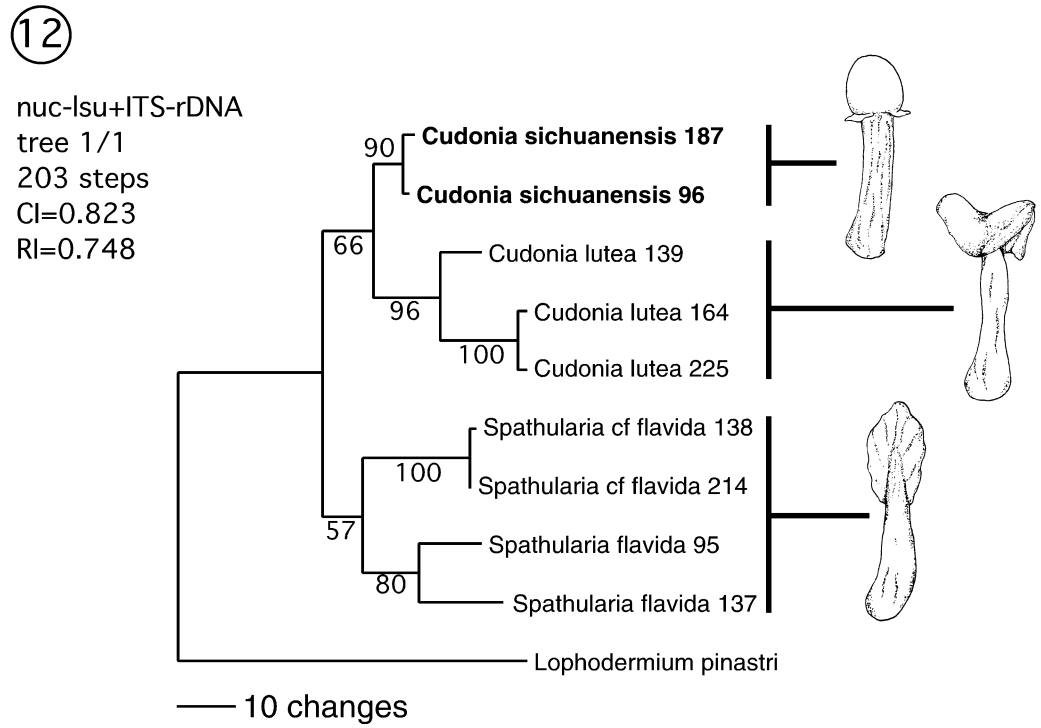
FIGS. 11–12. Phylogenetic relationships of *Cudonia* and *Spathularia* inferred from molecular sequences. 11. Relationships inferred from nuc-lsu-rDNA. One of twelve equally parsimonious trees (297 steps, CI = 0.771, RI = 0.724). Nodes that collapse in the strict consensus tree are marked with an asterisk above the branch. Bootstrap values greater than 50% are





indicated along nodes. Isolates used in ITS-nuc-lsu-rDNA analyses (FIG. 12) are underlined. 12. Relationships inferred from combined nuc-lsu-rDNA and ITS sequences. Single most parsimonious tree (203 steps, CI = 0.823, RI = 0.748). Bootstrap values greater than 50% are indicated along nodes. Habit illustrations of *Cudonia sichuanensis*, *C. lutea*, and *Spathularia flavida* are shown at the corresponding clades.

TABLE II. Morphological comparison of *Cudonia, Spathularia, Geoglossum,* and *Lophodermium.* (Data were based Mains 1940, Imai 1941, Samuels and Kohn 1986, Spooner 1987, Johnston 1989, and personal observation)

	Geoglossum (Geoglossaceae)	*Cudonia, Spathularia*	*Cryptohymenium*	*Lophodermium* (Rhytismataceae)
Habitat	On soil or humus.	On soil or humus.	On soil and decaying herbaceous debris.	Plant parasite, immersed partly in host tissue.
Morphology of ascoma	Capitate to spathulate.	Capitate to pileate.	Capitate.	Discoid.
Stroma and stromatic membrane	Absent.	Present (at least in the early stage of ascoma development).	An epithcium was found to be continuous with cortex of stipe.	Present.
Ascus pore reaction in Melzer's reagent	Positive.	Negative.	Positive.	Negative.
Color of ascospores	Brown to dark brown, rarely hyaline.	Hyaline.	Hyaline.	Hyaline.
Shape of ascospores	Cylindrical, mostly wider than 3 μm wide, distinctly multiseptate.	Filiform, commonly less than 2 μm wide, nonseptate to multiseptate.	Fusoid to subfusoid.	Filiform, commonly less than 2 μm wide, 0–1 septate.
Gelatinous sheath of ascospores	Absent.	Present.	Absent.	Present.
Paraphyses	Mostly wider than 3 μm, straight or curved, brown.	Mostly less than 2 μm wide, curved, hyaline.	2–3 μm wide, straight. Tips subglosose, encrusted in amorphous, brown material.	Mostly less than 2 μm wide, curved, hyaline.

pileate, and could be treated as a synonym of *C. orientalis* Yasuda.

Stunted and degenerate growth forms are occasionally seen in *Spathularia flavida. Spathularia pilatii* Velen., reported as having a globose ascogenous head, turned out to be a misshapen form of *S. flavida* (Mass Geesteranus 1972). *Nothomitra* species have capitate ascocarps and are placed in the family Geoglossaceae. *Nothomitra*, unlike *Cudonia sichuanensis*, has ascospores lacking gelatinous sheaths and amyloid ascus pores (Mass Geesteranus 1964).

Phylogenetic analysis suggests that *Cudonia sichuanensis* is a unique species (FIGS. 11, 12). However, sequences of several other species of *Cudonia* were not available in this study, including *C. confusa* and *C. helvelloides*, which have both been found in China (Zhuang 1998).

Some recent molecular studies using 18S nuc-ssu rDNA, 25S nuc-lsu rDNA or RPB2 supported the close relationship among *Cudonia*, *Spathularia*, and the Rhytismataceae (Landvik 1996, Platt 1999, Gernandt 2001, Lutzoni et al 2001). In the present study, data from nuc-lsu rDNA also strongly support the clade including *Cudonia*, *Spathularia*, and *Lophodermium* (FIG. 11). In addition, the intron of about 300 bp in nuc-lsu rDNA of Chinese collections of *S. flavida* shows nearly 70% similarity to that of *Lophodermium pinastri.*

Cudonia sichuanensis represents an intermediate between the pileate species of *Cudonia* and the spathulate species of *Spathularia.* In young ascomata of *Spathularia* the hymenium is covered by an ephemeral stromatic membrane that appears before the stalk develops, and is destroyed as the stalk expands (Z. Wang unpubl). In *S. flavida* the membrane disappears early in the fruitbody development, but in *C. sichuanensis* the membrane is persistent and forms a distinctive cortex structure (FIGS. 5, 9–10). Thus, the ascomata of *Cudonia* and *Spathularia* are hemiangiocarpous, as suggested by Nannfeldt (1932). Johnston (1988) described a mode of ascocarp development in the non-coniferous species of *Lophodermium*, in which a layer of vertically oriented cells between the ascocarp wall and the developing hymenium present at all stages of a *Lophodermium* ascocarp maturity. The layer described by Johnston is similar to the

membrane structure in *Cudonia sichuanensis*. We suggest that the term ''stromatic layer'' introduced by Nannfeldt (1942) be used to refer to the membrane covering the whole ascomata of *Cudonia* and *Spathularia*. The stromatic layer is a developmental character that unites *Cudonia*, *Spathularia*, and members of Rhytismataceae, as suggested by Nannfeldt (1942). Maas Geesteranus described in detail an outermost tissue on the stalk of *Spathularia velutipes* as ''The outer cells of the textura globulosa 8–15 × 6–12 μm, angular, globose, obovoid, with thick brown cell-walls, becoming largely detached one from another and forming short chains at right angels to the axes of the stipe''(Maas Geesteranus 1972). He thought that the outermost tissue is continuous with a veil, which encloses the whole fruitbody in the very beginning of the ascomata, and this presents a type of development called hemiangiocarpous by Nannfeldt (1942). Maas Geesteranus did not see any similar structure on the stalk of other *Spathularia* species, the development type of which he thought to be gymnocarpous, and he also thought there is difference between *Spathularia velutipes* and *Spathularia flavida* on the hyphal construction of the medulla layer in the stalk (Maas Geesteranus 1972). These are the two main reasons Maas Geesteranus erected a new genus *Spathulariopsis* based on *Spathularia velutipes*.

Our observation of several specimens of *Spathularia flavida* and *S. velutipes* (type materials deposited in FH) did not reveal any striking difference in the hyphal construction of the medulla layer in the stalks between these two species. We interpret the membrane found in *S.velutipes* as homologous with that of *Cudonia sichuanensis*. We accept *Spathularia velutipes* rather than *Spathulariopsis velutipes* because the lack of a distinct cortex in the mature ascomata of *Spathularia* cannot be regarded as a different development type to that of *Spathulariopsis*. In fact, membrane-like structures were found frequently in the ascomata in both *Spathularia* and *Cudonia* (Sever 1951, Mains 1955, 1956), and usually on the surface of the hymenia and at the juncture between the stipe and the hymenium.

Samuels and Kohn (1986) described an unusual, capitate discomycete, *Cryptohymenium pycnidiophorum* from New Zealand, in which the hymenial portion of the ascoma is produced below a multiloculate pycnidium. The hymenium of *C. pycnidiophorum* is covered by a cellular epithecium, and in this regard it resembles *C. sichuanensis* and other Rhytismatales (R. P. Korf, pers comm). However, it differs from *Cudonia*, *Spathularia*, and *Rhytisma* in having fusoid to subfusoid ascospores and a positive reaction of the ascus pore in Melzer's reagent, as well as the presence of pycnidia above the hymenium and other characters (Samuels and Kohn 1986; TABLE II).

The presence of ascospores surrounded by a gelatinous sheath is one of the family characters of the Rhytismataceae (Johnston 1994, Hawksworth et al 1995). Ascospore sheath structure was thought to be potentially informative at higher taxonomic level in the family Rhytismataceae, and the lack of an ascospore sheath is always correlated with characters of ascomatal development and structure (Johnston 1994). The ascospore sheaths and caps in species of *Cudonia* (FIGS. 6–7) and *Spathularia* are similar to those of *Lophodermium pinastri* (Johnston 1994) in the Rhytismataceae. This character was overlooked by most mycologists working on *Cudonia* and *Spathularia*. Gernandt et al (2001) concluded that loss of plant parasitism may have occurred within the group of *Cudonia*, *Spathularia* and the members of Rhytismatales, if *Cudonia* and *Spathularia* have common ancestry with Rhytismataceae. In the future, research on the structure and function of the gelatinous sheath of the ascospores and the life history of *Cudonia* and *Spathularia* may provide more information about their relationships with members of Rhytismataceae.

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