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*Volume 94, pp. 1–6* *October–December 2005*

# *Tuber umbilicatum*, a new species from China, with a key to the spinose-reticulate spored *Tuber* species

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**Abstract**—*Tuber umbilicatum*, a truffle associated with Pinus yunnanensis in southwestern China, is described and illustrated as a new species. It is characterized by inconspicuously papillate ascomata with an umbilicate basal cavity and ellipsoid ascospores ornamented with spines connected by low ridges to form an alveolate reticulum 6-8 meshes across the spore width. A key to seven *Tuber* species bearing spinose-reticulate spores is presented.

**Key words**—*Tuberaceae*, truffle, spore ornamentation, hypogeous fungus

## Introduction

Since Liu (1985) reported the genus *Tuber* from Shanxi province, China, several additional papers on Chinese truffles have been published. The export of Chinese *Tuber* spp. to Europe has stimulated further interest in members of this genus in China. Currently, up to 20 species, including 10 new species, have been reported in this country (Liu 1985, Tao & Liu 1989, Wang & Li 1991, Hu 1992, Wang et al. 1998, Zhuang 1998, Xu 1999, Wang & He 2002, He et al. 2004, Zhang et al. 2005, Song et al. 2005). During study of truffles associated with *Pinus yunnanensis*, a dominant tree in southwestern China, we found a new truffle, characterized by inconspicuously papillate ascomata with an umbilicate basal cavity and ellipsoid, spinose-reticulate ascospores. To distinguish this new species from others with spinose-reticulate spores, a world-wide key to the seven *Tuber* species with such spores is provided.

## Materials and Methods

Macroscopic characters are described from fresh specimen. Microscopic methods of Yang & Zhang (2003) were followed. For scanning electron microscopy (SEM), spores were scraped from the dried gleba onto doubled-sided tape, which mounted directly

*corresponding author

on an SEM stub, coated with gold-palladium, and examined and photographed with a JEOL JMS-5600LV SEM. Herbaria that provided specimens are abbreviated and cited according to Holmgren et al. (1990), except HKAS (Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences) and IFS (Herbarium of Institute of Applied Ecology, Chinese Academy of Sciences), two not yet listed in the *Index Herbariorum*.

## Taxonomy

***Tuber umbilicatum*** Juan Chen & P. G. Liu, **sp. nov.** **Figs. 1-5**

*Ascomata ochracea, glabra vel subglabrabasi umbilicata. Peridium bistratum, 320-500 μm crassum: stratum exterius pseudoparenchymaticum, stratum interius hyphi intertextis. Gleba solida, purpureobruneola vel griseobruneola, venis albis. Asci sporis 1-4 (6). Ascosporae ellipsoideae, ochraceae, 21-40 × 14-32 μm, spinulis 3-5 μm altis, reticulato alveolato < 1μm alto connexis ornatae. Holotypus hic designatus: HKAS 44316.*

*Etymology:* Latin, *umbilicatum*, in reference to the umbilicate depression of the ascomata.

**Ascomata** (Fig. 1) 1.2-1.9 cm broad, globose, with an umbilicate depression at the base, surface smooth or with minute papillae up to 50 μm high, pale yellow, becoming yellow-brown or brown when dried. **Peridium** (Fig. 2) brittle, peeling easily from the gleba, mostly 320-500 μm thick, composed of two layers: outer layer 90-250 μm thick, pseudoparenchymatous, composed of subglobose to subangular, yellow-brown cells 7-16 × 5-11 μm, the walls 1-3 μm thick; inner layer 150-400 μm thick, of intricately interwoven, hyaline hyphae 2-5 μm in diam, the walls thin to somewhat thickened. **Gleba** purple-brown or grey-brown with pink tint at maturity, marbled with numerous, narrow, branching, white veins radiating from the basal cavity.

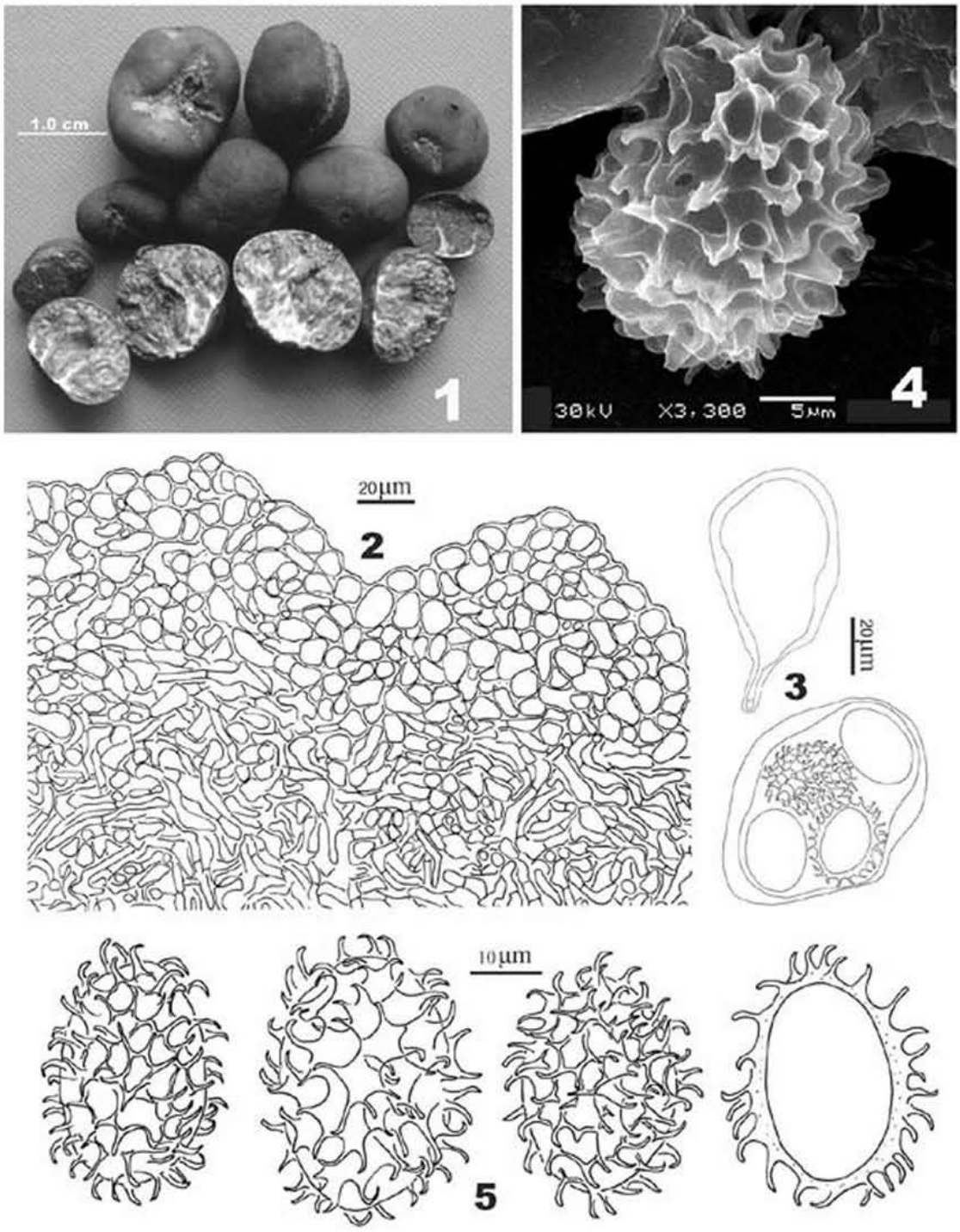
**Asci** (Fig. 3) 50-83 × 37-67 μm excluding the stalk, globose to subglobose, ellipsoid or irregular, sessile or sometimes with a short stalk, 1-4 (-6) spored. **Ascospores** (Fig. 4, 5) ellipsoid, yellow-brown at maturity, the walls up to 2 μm thick, in 1-spored asci (28-) 33-40 × 20-32 μm excluding ornamentation, 2-spored asci (23-) 25-37 × 17-26 μm, 3-spored asci (21-) 23-33 (-36) × 17-23 μm, 4-spored asci 21-30 (-32) × 15-22 (-25) μm, 5-spored asci 21-26 × 14-20 μm; Q = (1-) 1.2-1.6 (-1.8), Q = 1.4 ± 0.13; ornamentation of spines 3-5 (-6) μm tall connected by an alveolate reticulum < 1 μm tall, the alveolae 3-6 (-7) × 2.5-5 μm, 6-8 across the spore width and (6-) 7-10 along the spore length.

**Habitat:** Hypogeous under *Pinus yunnanensis*.

**Specimen examined**—CHINA: **Yunnan province**, Chengjiang county, Tigu Village, elev. 1900-2000 m, 31 Oct. 2003, Juan Chen 145 (HKAS 44316 – holotype).

**Discussion**—The surface of the ascomata of *T. umbilicatum* is glabrous to subglabrous to the naked eye, but very fine papillae can be seen with a stereomicroscope. These papillae give the peridium a wavy outer edge under the compound microscope. The combination of umbilicate, nearly smooth ascomata and spinose-reticulate spores is distinctive.

Species of *Tuber* can be divided on the basis of spore ornamentation into three groups: 1) spiny (representative species: *T. melanosporum* Vittad.), 2) reticulate (representative species: *T. borchii* Vittad.) and 3) spinose-reticulate (representative species:




Figs. 1-5 *Tuber umbilicatum* (HKAS 44316, Holotype)
1. Fresh ascomata; 2. Vertical section of the peridium; 3. Asci;
4. SEM of spore, showing the detail of ornamentation; 5. Ascospores.

*T. spinoreticulatum* Uecker & Burds.). *T. umbilicatum* belongs to the *T. spinoreticulatum* group, which includes *T. taiyuanense* B. Liu, *T. pseudoexcavatum* Y. Wang et al. and *T. huidongense* Y. Wang, known only from China; *T. lyonii* Butters (=*T. texense* Heimsch) and *T. spinoreticulatum* from North America; and *T. malacodermum* E. Fisch. from

Germany and Switzerland (Fischer 1923, Heimsch 1958, Uecker & Burdsall 1977, Liu 1985, Wang et al. 1998, Wang & He 2002, Trappe et al. 1996). These species can be difficult to distinguish because of their morphological similarity. Accordingly, to clarify differences among these species we re-examined the holotypes of *T. huidongense* (IFS 89923) and *T. texense* (OSC 42353), isotype of *T. spinoreticulatum* (OSC 38860), a neotype of *T. taiyuanense* (HMAS 75888) and two specimens of *T. pseudoexcavatum* (HKAS 47617, 41313). Ascomata and SEM photomicrographs of spores are illustrated in Figs. 6-11. Although we did not obtain specimens of *T. malacodermum*, Fischer's (1923) description as confirmed by study of the type collection by J. M. Trappe (personal communication) showed it to have a pseudoparenchymatous peridium composed of cells inflated up to 20-70 µm broad. A key to the spinose-reticulate species is presented here, based on these studies.

## Key to species with spinose-reticulate spores

1. Peridium with rounded cells inflated to 20-70 µm . . . . . ***T. malacodermum***
1. Peridium of interwoven hyphae or with rounded cells mostly < 20 µm broad . . 2

2. Ascomata with a basal cavity . . . . . 3
2. Ascomata without a basal cavity . . . . . 5

3. Peridium smooth or with inconspicuous papillae 10-50 µm high; spores ellipsoid, Q > 1.3 . . . . . ***T. umbilicatum***
3. Peridium with conspicuous warts 100-150 µm high; spores mostly broadly ellipsoid, Q ≤ 1.3 . . . . . 4

4. Cavity conspicuous; asci 1-8 spored, sessile. . . . . ***T. pseudoexcavatum***
4. Cavity inconspicuous; asci 1-4 spored, stipitate . . . . . ***T. spinoreticulatum***

5. Ascomata smooth; ascospores broadly ellipsoid, Q < 1.3, mostly 1-1.25. . . . . ***T. taiyuanense***
5. Ascomata with minute papillae up to 55 µm high; ascospores ellipsoid, Q > 1.3, mostly 1.3-1.6. . . . . 6

6. Spores with longer spines mostly > 3 µm long, reticulum complete and regular, meshes size bigger 4-8 × 4-6 µm . . . . . ***T. huidongense***
6. Spores with shorter spines 2-3 µm long, reticulum partial to complete, meshes size smaller 3-6 × 2-4 µm. . . . . ***T. lyonii*** (=*T. texense*)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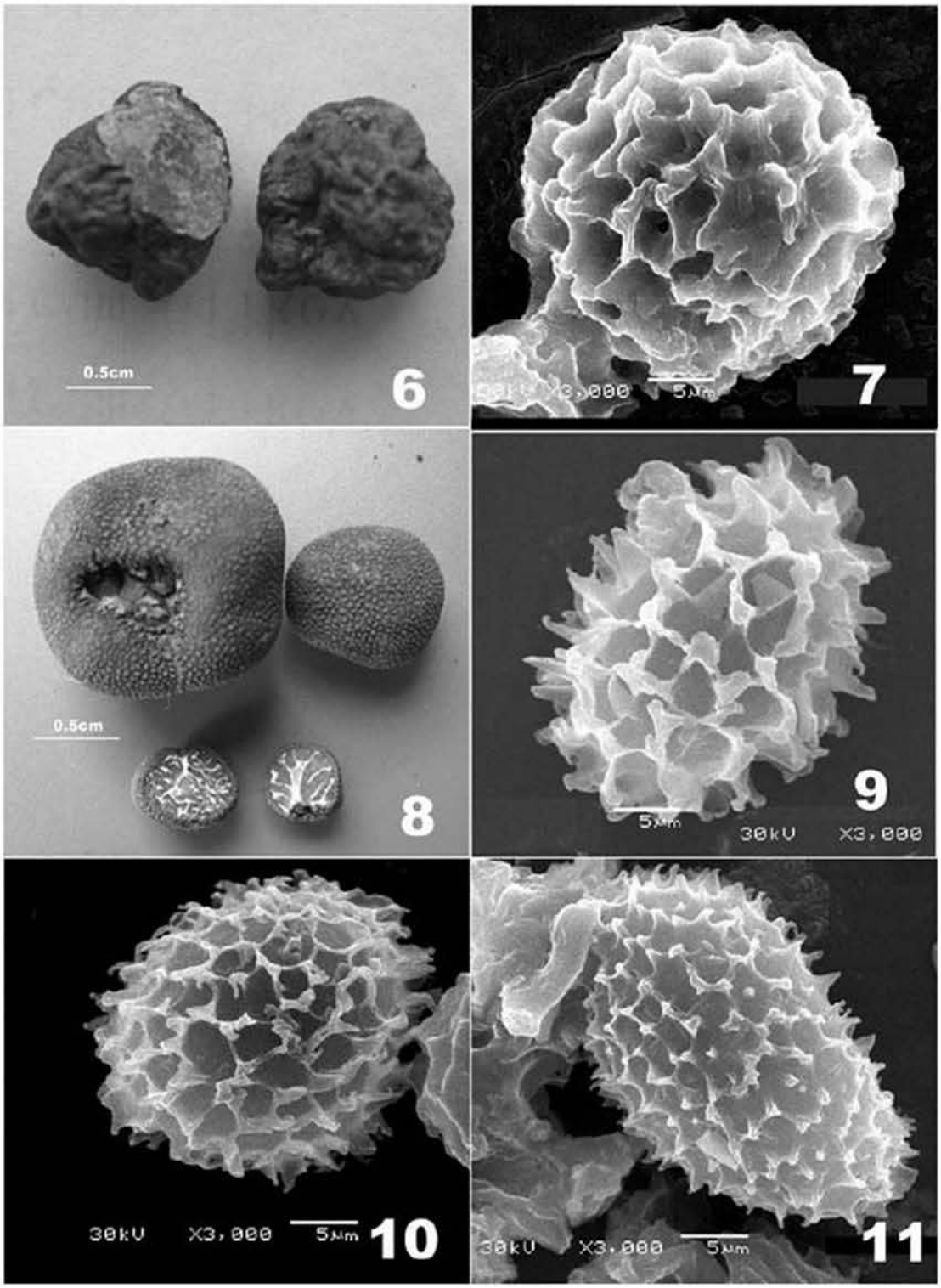
Prof. James Trappe and Dr. Michael Castellano reviewed the manuscript and offered invaluable comments and suggestions. Specimens were generously lent to us by the herbaria of the Department of Botany & Plant Pathology, Oregon State University, Royal Botanic Gardens, Kew, and Institute of Microbiology, Chinese Academy of Sciences. Sincere gratitude is offered to Prof. M. Zang, Dr. Zhu L. Yang and Mrs. X. H. Wang, Kunming Institute of Botany, for their valuable suggestions on earlier version of the manuscript. Many thanks to my colleagues, F. Q. Yu, H. D. Zheng and J. Y. Chen for providing morphological photos. The study was supported in part by National Science Foundation of China (No. 30470011), the Knowledge Innovation Program of Chinese Academy of Sciences (No. KSCXZ-1-09-06 & KSCXZ-SW-101C) and the Nature Science Foundation of Yunnan province (No. 2004C0050M).




Figs. 6-11. *T. taiyuanense* (HMAS 75888, neotype): 6. Ascomata; 7. SEM micrograph of ascospore. *T. pseudoexcavatum* (HKAS 47617): 8. Ascomata. *T. huidongense* (IFS 89923, holotype): 9. Ascospore. *T. spinoreticulatum* (OSC 38860, isotype): 10. Ascospore. *T. texense* (OSC 42353, holotype): 11. Ascospore.

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*Volume 94, pp. 7–14* *October–December 2005*

# *Blumenavia toribiotalpaensis*: a new species of *Clathraceae* from Jalisco, Mexico

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**Abstract**—A new stink horn species, *Blumenavia toribiotalpaensis* sp. nov. *(Clathraceae)* from Mexico, is described and illustrated. *Blumenavia toribiotalpaensis* differs from previously described species of the genus by its larger receptacle and basidiospores, distribution of the gleba over the upper half of the columns and presence of no more than four columns.

**Key words**—Basidiomycota, phalloid, systematics, Talpa de Allende

## Introduction

During the course of studies of the distribution and demography of a recently discovered *Acer saccharum* subsp. *skutchii* (cloud forest sugar maple) population in Jalisco, Mexico (Vargas-Rodriguez 2005), we collected fungal specimens with novel features. A survey of the literature revealed that the specimens are an undescribed species of *Blumenavia* (*Clathraceae*).

The genus *Blumenavia* was established to accommodate *B. rhacodes* Möller (Möller 1895). Currently, two species, *B. rhacodes* and *B. angolensis* (Welw. & Curr.) Dring, are included in the genus (Dring 1980). The genus is characterized primarily by the small number of columns that lack transverse arms. In addition, the columns are free at the base and united at their apices with glebifers consisting of membranes attached by one side to each of the two inner angles of the column (Dring 1980, Sáenz 1980).

The new *Blumenavia* species occurs in a pine-cloud forest transition considered a Tertiary refuge (Vargas-Rodriguez 2005). The subtropical montane cloud forest is unique in having high plant species richness and includes a number of endangered and relict plants, comparable only to certain Asian forests (Graham 1999, Vargas-Rodriguez 2005). The forest contains temperate disjunct tree genera with East Asia and East North America, such as *Acer*, *Magnolia*, *Carpinus*, *Cornus*, *Fraxinus*, *Juglans*, *Tilia*, and *Ostrya* (Graham 1999, Vázquez-García et al. 2000). This exceptional community is proposed for protection as a biosphere reserve with 3,000 inhabitants supporting the movement

(Vargas-Rodriguez 2005). The discovery of the novel *Blumenavia* species increases our knowledge and relevance of the biota of this unique region.

## Materials and Methods

We collected fruiting bodies at different stages of development in a pine-montane cloud forest transition (1,800 m a.s.l.), near Talpa de Allende, Jalisco, Mexico, among fallen leaves under the canopy of adult *Pinus* spp. and *Carpinus caroliniana* trees. A second collection was made a year later in the same area and a third one in 2005. Five fruiting bodies were fixed in FAA solution (five parts 40% formaldehyde: five parts glacial acetic acid: 90 parts 95% ethyl alcohol) and seven were dried. A free hand cross section was made to one egg. Spores were mounted on slides in lactophenol and in 3% KOH and examined with a NIKON Microphot compound microscope using differential interference contrast and bright field optical systems. Hand-cut sections of the columns were made from a sample that had been fixed in FAA for nine months. Sections were dehydrated with ethanol, critical point dried, mounted, and coated with gold:palladium 60:40 in an Edwards S-150 sputter coater. We used a Cambridge S-260 scanning electron microscopy (SEM) for observation.

## Taxonomic Description

***Blumenavia toribiotalpaensis*** **Vargas-Rodriguez sp. nov.** FIGS. 1-14

*Ovum fulvum, superficies interdum findens aquamis fulvis angularibus, elipsoidale 2.2-3.8 cm longum x 2.1-3.9 cm altum; adhaesae albae rhizomorphae 1-1.5 cm diametro. Receptaculum expansum 12.1-15.3 cm altum, 3.8-5.6 cm longum, colore vario ab albulo ad dilutum bombax, cylindrale (or cylindratum) ex 3 vel 4 columnis robustis constans; columnis 0.9-1.1 cm diametro ex parte gracillima, 1.9-2.1 cm ex parte latissima, conjunctis superne, inferne liberis, cum sulco in superfacie; semicirculares sectione, constantes ex 9 tubis compositis in 3 ordinibus ab canali abaxiali; proximi canali sunt 5 tubuli circulares sectione in material nova; medius ordo constat ex 3 latioribus tubis, polygonalibus sectione; atque ex singulari magna polygonali tuba constat ordo extremus ab canali abaxiali. Columnae tela glebifera incrassata in facie interna informante cristam per anterior-laterales angulos columnae, glebifera incrassata marginibus unita, crista blebam ferente. Gleba coercita intra laceratam glebiferam cristam, sita in superior parte columnarum, atro-brunnescenti-olivacea, aroma simili piscibus mortuis et nauseosa. Basidiosporae 3.8-4.2 x 1.7-1.9 µm. Associati basidiocarpis siti Scarabaei (Staphilinidae et Leiodidae) intra brachia et muscae (Tephritidae) in gleba maturorum basidiocarporum.*

Egg pale brown, outer surface sometimes cracking into angular brown scales, ellipsoidal, 2.2-3.8 cm wide, 2.1-3.9 cm high, gelatinous layer 5-7 mm thick, traversed by peridial sutures corresponding to each of the four columns, immature glebal mass about 4 mm diameter among columns, glebiferous tissue separated by a cavity opened in one extreme and joined to the column only by the opposite extreme, medullar zone rectangular-like in shape; attached white rhizomorphs 1-1.5 mm in diameter. Expanded receptacle 12.1-15.3 cm high and 3.8-5.6 cm wide, whitish to pale beige, cylindrical, with 3 to 4 robust columns; columns 0.9-1.1 cm diameter at the thinnest part to 1.9-2.1 cm at the widest part, united above, free below with a groove in the outer surface, semicircular in section,

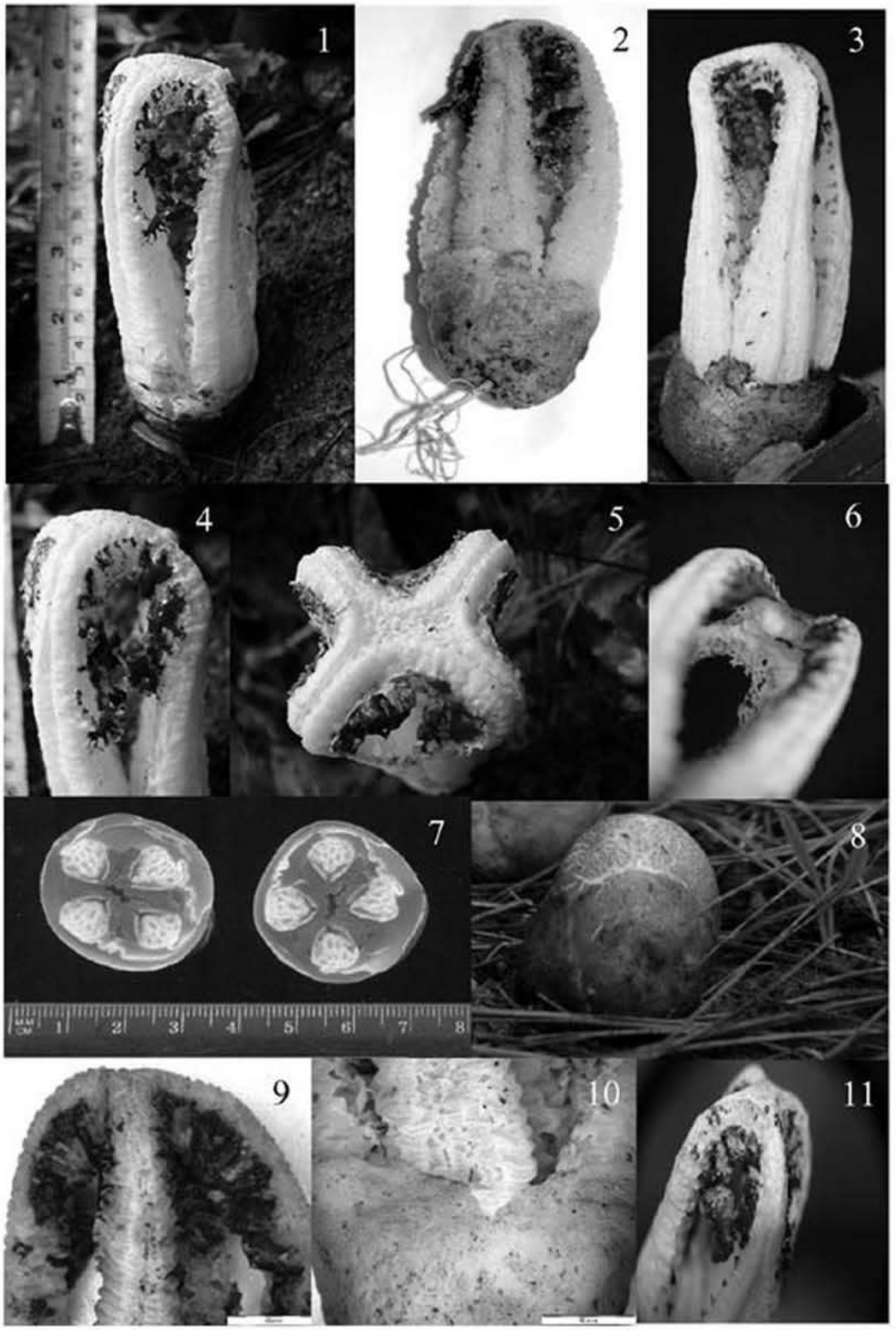


**Figure 1-11.** *Blumenavia toribiotalpaensis*, IBUG 422a. **1,** Habitat photograph showing receptacle and volva. **2,** Receptacle, volva, and rhizomorphs from specimen preserved in FAA. IBUG 456, **3,** Receptacle and volva. IBUG 422a. **4, 5, 9, 10,** Detail of gleba, showing its distribution over the upper half of the receptacle. IBUG 456, **6 & 11,** Detail of gleba. Yalma L. Vargas-Rodriguez 455, **7 & 8.** Freehand cross section of an egg. IBUG 422a, **9,** Detail of the volva from specimen preserved in FAA.

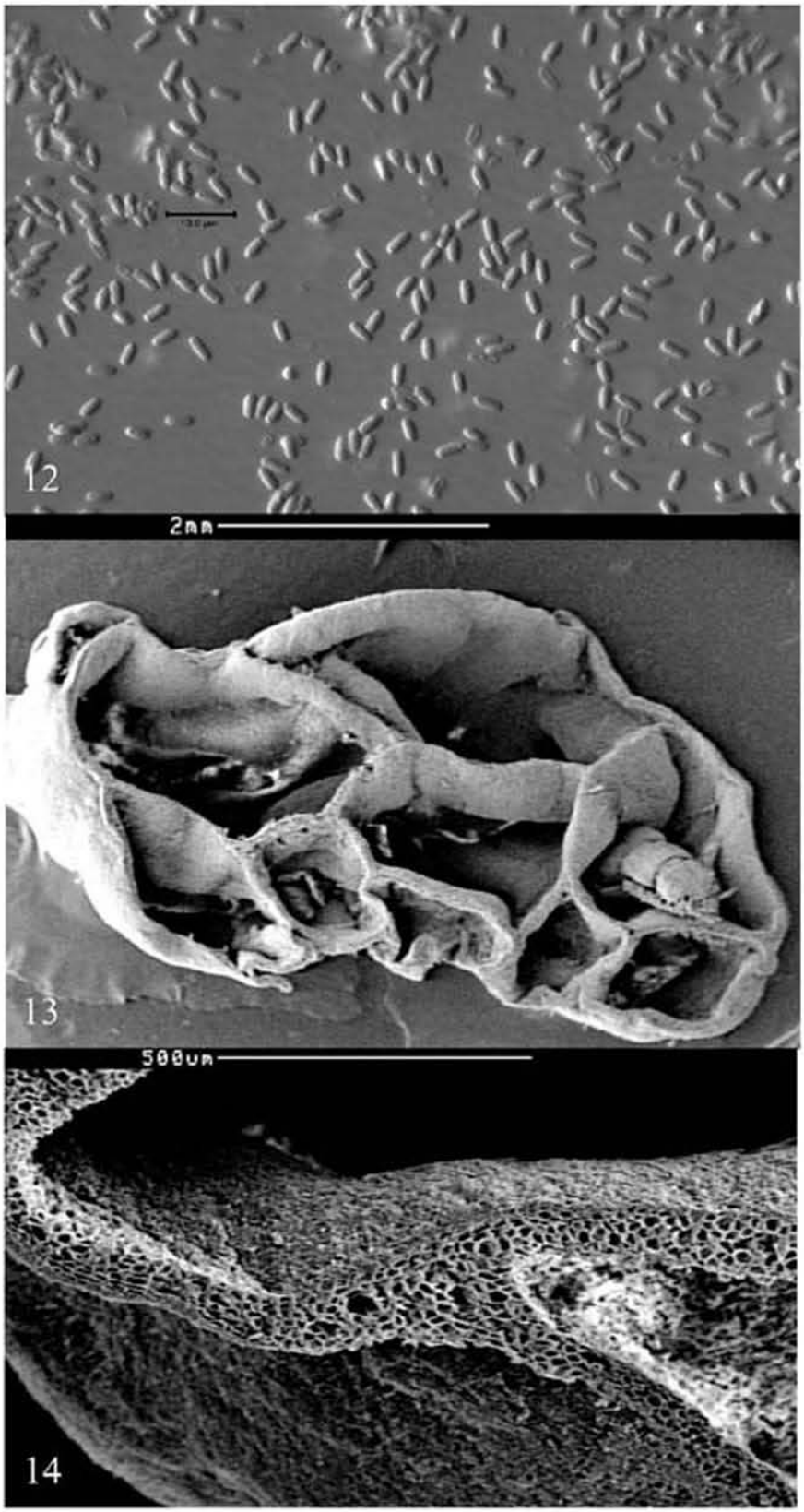


**Figure 12-14.** *Blumenavia toribiotalpaensis*, LSUM 422b. **12,** Basidiospores. **13,** Sections of the arm showing number and arrangement of tubes and associated staphylinid beetle. **14,** Inner (upper side) and outer (bottom) arm surfaces.

comprised of 9 tubes arranged in three ranks from the abaxial groove; nearest to the groove are five small tubes, circular in section in fresh material; the middle rank consists of three wider tubes, polygonal in section; and a single, large polygonal tube comprises the outermost rank from the abaxial groove (Fig. 13). Columns with a thickened glebiferous tissue on the inner surface forming a crest along the anterior-lateral angles of the column, glebiferous tissue united to the arms by the edges, crest bearing the gleba. Gleba restricted to the lacerate glebiferous crest, situated on the upper half of the columns, dark olive-brownish, odor of dead fish, nauseous. Basidiospores 3.8-4.2 x 1.7-1.9 µm, cylindrical, hyaline in KOH. Beetles [Staphilinidae (Fig. 13), Leiodidae] and flies (Tephritidae) associated with basidiocarps, beetles located inside the arms reach the interior through holes in basidiocarps, holes appear in decaying basidiocarps; flies located in gleba of mature basidiocarps.

**Specimen examined** — HOLOTYPE **here designated. MEXICO, JALISCO: Talpa de Allende municipality**, pine-cloud forest (*Acer-Podocarpus-Abies*) transition, "Ojo de Agua del Cuervo" ("Crow spring") locality, west of Cumbre de Los Arrastrados (20°11"N; 105°16"W), 1800 m a.s.l., on *Pinus* spp. fallen trunk and debris, 10 Sep 2002, *Yalma L. Vargas-Rodriguez 240*, with *Javier Curiel, J. Antonio Vázquez-García* and *Toribio Quintero Moro*, dry specimen (BPI); 10 Sep 2002, *Yalma L. Vargas-Rodriguez 240a*, with *Javier Curiel, J. Antonio Vázquez-García* and *Toribio Quintero Moro*, dry specimen (IBUG) (Holmgren et al. 1990).

PARATYPE **here designated. MEXICO, JALISCO: Talpa de Allende municipality**, pine-cloud forest (*Acer-Podocarpus-Abies*) transition, "Ojo de Agua del Cuervo" ("Crow spring") locality, west of Cumbre de Los Arrastrados (20°11"N; 105°16"W), 1800 m a.s.l., on fallen leaves of *Pinus* spp. and *Carpinus caroliniana*, 14 Sep 2003, *Yalma L. Vargas-Rodriguez 422b, 423a, 423b, 424a* and *J. Antonio Vázquez-García*, FAA preserved material (LSUM); 14 Sep 2003, *Yalma L. Vargas-Rodriguez 422a* and *J. Antonio Vázquez-García*, FAA preserved material (IBUG); 13 Sep 2005, *Yalma L. Vargas-Rodriguez 454, 456, 462* and *J. Antonio Vázquez-García*, dry specimen (IBUG); 13 Sep 2005, *Yalma L. Vargas-Rodriguez 459, 463* and *J. Antonio Vázquez-García*, dry specimen (LSUM).

**Etymology**—From the Latin *talpaensis*, referring to the municipality where the fungus was collected and *toribio*, referring to Toribio Quintero Moro, a remarkable forest conservationist. He has promoted the protection of the Talpa de Allende forests by collecting 3,000 signatures from Talpa de Allende habitants and petitioning state and federal Mexican authorities for the creation of a new biosphere reserve in the area.

**Known distribution**—Jalisco: Only known from the type locality. The species is not common in the area; individuals are patchy distributed along 100 m. The species was not previously known by local people. Only three other species (*Clathrus crispus, C. cancellatus*, and *C. ruber*) of the *Clathraceae* family are known for the Jalisco state and these do not co-occur with *Blumenavia toribiotalpaensis*. This is the first record of the genus for western Mexico and the second one for the country.

**Habit and habitat**—Occasionally gregarious. Among plant leaves and debris, under *Pinus* spp., *Carpinus caroliniana* and *Acer saccharum* subsp. *skutchii* canopy, in transitional pine forest to montane cloud forest.

## Discussion

*Blumenavia* differs from similar stink horn genera in the glebifer form. Especially notable is the unique gleba borne on lateral flaps of tissue that is lacking in *Clathrus*, *Laternea*, and *Ligiella*.

The differences between *Blumenavia toribiotalpaensis* and the two previously described species include the distribution of the gleba and the size of the receptacle and basidiospores (Table 1). The gleba is distributed along half the length of the column in *B. toribiotalpaensis*; in *B. rhacodes* the gleba is present over the entire length of the column and in *B. angolensis* restricted to the upper quarter or one third of the receptacle. The receptacle and basidiospores are larger in *B. toribiotalpaensis* than in the other two species (Table 1).

Table 1. Differences among species of *Blumenavia*

| | *Blumenavia rhacodes* | *Blumenavia angolensis* | *Blumenavia toribiotalpaensis* |
|---|---|---|---|
| Expanded receptacle | 8.5-13x8 cm | 10x3 cm | 12.1-15.3x3.8-5.6 cm |
| Receptacle color | Clear orange to yellow | White | Whitish to pale beige |
| Sections of columns; number of tubes | Triangular and trapezoidal; about 10 tubes | Subtriangular or quadrangular; about 6 tubes | Semicircular; 9 tubes |
| Number of columns | 3-6 | 3-5 | 3-4 |
| Groove along outer side of column | Present | Lacking | Present |
| Basidiospores | 3-4x1-1.5 µm | 3-3.5x1.5 µm | 3.8-4.2 x 1.7-1.9 µm |
| Gleba distribution | Entire length of the column | Upper quarter or one third of the receptacle | Upper half |
| Habitat and distribution | In coffee plantations in cloud forest in Mexico. Mexico: Veracruz; The Caribbean: Trinidad; South America: Brazil. | Habitat not noted. Africa: Angola, Tanzania, South Africa; US: Texas; The Caribbean: Puerto Rico; South America: Brazil. | Pine-montane cloud forest transition. Mexico: Jalisco. |

Mature specimens of *B. toribiotalpaensis* usually have four columns but one immature specimen has only three. The white columns in fresh specimens separate *B. toribiotalpaensis* from *B. rhacodes* with orange-yellow columns; *B. angolensis* however, also has white columns. The spongy texture of the columns of *B. toribiotalpaensis* distinguishes this species from the more rigid, less spongy texture of *B. angolensis*. Number, shape and arrangement of tubes in transverse sections of columns differ, being more numerous in *B. toribiotalpaensis* than in *B. angolensis*, with a semicircular shape, and arranged differently from *B. rhacodes* (Table 1).

Habitat and known geographical distribution differ among the three *Blumenavia* species. Although both *B. toribiotalpaensis* and *B. rhacodes* are known from Mexico, *B. rhacodes* has been found in coffee plantations established under the canopy of cloud forest trees at 1300 m a.s.l. in Teocelo, Veracruz, Mexico (López et al. 1981), while *B. toribiotalpaensis* occurs at higher elevations (1800 m a.s.l.) in the transition of pine and cloud forest. In Mexico, *B. rhacodes* is also known from Xalapa, Veracruz (Calonge et al. 2004). This is the first record of the genus for western Mexico and is the second for the country, which was previously found in eastern Mexico (Veracruz state). Individuals of *Blumenavia* species have low density in Mexico. *Blumenavia angolensis* is known from Angola, Tanzania, South Africa and Brazil, although the habitats were not reported (Dring 1980) (Table 1).

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# Studies on Basidiomycetes in Greece 1: The genus *Crepidotus*

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**Abstract**—The diversity of *Crepidotus* in the Eastern Mediterranean region is poorly known, and data from Greece are scarce. The present work aims at the record and study of the diversity of the genus in Greece and at the contribution to the knowledge of the distribution of the genus in Europe. Forty-four collections have been examined and ten taxa have been identified. *Crepidotus autochthonus, C. lundellii, C luteolus, C. subverrucisporus* and *C. applanatus* var. *subglobiger* are newly recorded from Greece and most taxa are recorded on new substrates for both Greece and Europe. Detailed descriptions, ecological notes and taxonomical comments on all studied taxa are given.

**Key words**—lignicolous fungi, mycodiversity, biodiversity, taxonomy, *Crepidotaceae*

## Introduction

*Crepidotus* is a distinct and well-defined genus, and although most species remain not yet completely documented and clarified, several monographic works, as well as critical revisions and regional studies based mainly on morphological studies, provide comprehensive systematic treatment of many species (Singer 1947; Pilát 1948; Hesler & Smith 1965; Singer 1973; Watling & Gregory 1989; Nordstein 1990; Stangl et al. 1991; Senn-Irlet 1995; Senn-Irlet & De Meijer 1998; Aime 2001; Krisai-Greilhuber et al. 2002; Bandala & Montoya 2002a, 2002b, 2004). The systematic position of *Crepidotus* was until very recently debatable, as it was placed either in family *Crepidotaceae* (Moser 1978; Jülich 1981; Singer 1986; Hawksworth et al. 1995), *Strophariaceae* (Kühner 1980) or *Cortinariaceae* (Bas 1988; Kirk et al. 2001). Recent phylogenetic analyses (Aime 1999; Moncalvo et al. 2002; Aime et al. 2005) allow to redefine the *Crepidotaceae* within a broader phylogenetic framework of the agarics, and the genera *Crepidotus* and *Simocybe* are better supported in family *Crepidotaceae* s.s., which represents a separate lineage of dark-spored euagarics.

In the Mediterranean region some studies on *Crepidotus* exist, mainly concerning N. Africa (Malençon & Bertault 1975), Spain (Ortega & Buendia 1989) and Italy (Lonati 2000). However, the knowledge of the diversity of *Crepidotus* in the Eastern Mediterranean region is poor, as few papers have been published and most of these are not easily accessible. The data from Greece are scarce. To date, only seven taxa were

recorded from Greece, usually without descriptions, and most collected very few times (Diapoulis 1939; Maire & Politis 1940; Avtzis & Diamandis 1988; Minter 1988; Diamandis & Perlerou 1990; Pantidou 1991; Diamandis 1992; Zervakis et al. 1998; Dimou et al. 2002a; Dimou et al. 2002b; Konstantinidis 2002; Polemis et al. 2002). Few papers from neighboring countries have been recently published, such as Croatia (Tkalčec & Mešić 2003) and Turkey (Öztürk et al. 2003; Ersel & Solak 2004; Sesli & Denchev 2005). Most of the above mentioned papers are floristic studies, lacking detailed descriptions or exsiccata.

The aim of the present study is to provide a better understanding of the biodiversity of the genus in Greece, concerning its morphology (including the range and importance of its variability), ecology and chorology. Collections have been made from various regions of central and southern continental Greece, mainly from coniferous and deciduous forests, as well as riparian and maquis vegetation.

The following ten taxa are identified and described in this work: *C. applanatus* var. *subglobiger*, *C. autochthonus*, *C. calolepis*, *C. cesatii* var. *cesatii*, *C. epibryus*, *C. lundellii*, *C. luteolus*, *C. mollis*, *C. subverrucisporus* and *C. variabilis*.

## Materials and methods

Microscopical observations were made in bright field or phase contrast using a standard light transmission microscope. Sections of dried material were mounted in 3% KOH, with or without the addition of Phloxine. All measurements were made under 1000× magnification. At least 20 spores and 10 basidia and cheilocystidia were measured per specimen. The spores were measured from the surface of the pilei or from a spore deposit (when available). The spore sizes are given in approximation to 0.5 μm, with extreme values given in parentheses, followed by the length-width ratio of the spores (Q). Habitat references in the descriptions refer exclusively to the collected material. Greek localities are transcribed into latin according to ISO 843: 1997 (E). Authorities' abbreviations are in accordance to *Authors of Fungal Names* by Kirk & Ansell (1992). We have adopted the infrageneric classification proposed by Senn-Irlet (1995).

Material from other collectors or researchers (published or unpublished), was examined when available. The specimens collected from the authors are deposited in the Mycological Herbarium of the University of Athens (ATHU–M).

## Taxonomic descriptions

### *Crepidotus* (Fr.: Fr.) Staude 1857

### *Crepidotus* subgenus *Crepidotus*

***Crepidotus calolepis*** (Fr.) P. Karst. **Figs 1a–b; 9b, d; 11g**

*Crepidotus calolepis* (Fr.) P. Karst., Bidr. Känn. Finl. Nat. Folk 32: 414 (1879); *Crepidotus mollis* var. *calolepis* (Fr.) Pilát, Ann. Hist. –Nat. Mus. Natl. Hung., n.s. 2B: 74 (1940); *Crepidotus mollis* ssp. *calolepis* (Fr.) Nordstein, Synopsis Fungorum (Oslo) 2: 67 (1990).

**Pileus** 10–70 mm, semicircular to flabelliform, convex to plano-convex, laterally or almost laterally attached to the substrate, with incurved and later even margin, surface viscid-sticky to dry, densely minutely tomentose-scaly with yellowish brown to brown

fibrillose scales on a dirty whitish, cream to ochre-yellowish background, consistency tough, elastic. **Lamellae** whitish in young specimens, then spotted brownish and finally uniformly ochre-brown to cinnamon, moderately crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary. **Spore print** yellowish brown.

**Basidiospores** 7.5–11.0 × 5.5–7.0 µm, Q = 1.21–1.69, ellipsoid, amygdaliform in side view, smooth, yellowish brown in KOH, thick-walled, apex obtuse, depressed or occasionally mucronate; in some spores inner wall curving inwards at apex, resembling a callus or an indistinct germ pore when accompanied by a small apical depression (Figs 1a, 9b). **Basidia** 25–32 × 7–9 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 25–65 × 4–14 µm, clavate, cylindrical, irregularly cylindrical, somewhat fusiform to narrowly lageniform, sometimes branched, apex obtuse, often subcapitate (Fig. 1b), frequently embedded in gelatinous material entirely covering the lamellar edge. **Pleurocystidia** absent but pleurocystidioid-like bodies present in some specimens, clavate, with a short, apical or rarely lateral, finger-like protuberance (Fig. 11g). **Lamellar trama** often gelatinized. **Pileipellis** with an underlying layer of parallel hyphae, 3–5 µm wide, hyaline, not encrusted, and an upper layer of parallel to somewhat ascending hyphae, 4–12 µm wide, hyaline to pale ochraceous, with encrusting zebra-like pigment, amongst which ascending scale-forming hyphae, 4–15 µm wide, brown to dark brown, thin- to somewhat thick-walled, short-celled, with strongly encrusting, zebra-like pigment (Fig. 9d), in some specimens with markedly large, plate-like encrustations. **Pileal trama** partly gelatinized, gelatinous layer underlying the pileipellis usually distinct, up to 200 µm thick, but occasionally thin, rudimentary, and hence difficultly observed. **Secretory hyphae** occasionally present in pileipellis, pileal trama and lamellar trama, hyaline to golden yellow in KOH. **Clamp connections** absent in all tissues.

**Habitat:** Solitary to gregarious on standing or fallen trunks and branches of coniferous or deciduous trees.

**Specimens examined** — Mt. Taygetos, Messinia, on wood of *Platanus orientalis*, 29 Nov. 1968, Pantidou, ATHU–M 1071 (as *C. mollis*); Mt. Kandilio, Pagontas-Prokopi, Evvoia, forest of *Pinus* sp., on fallen branches of *Pinus* sp., 13 Dec. 1986, Gonou, ATHU–M 3781; Mt. Taygetos, Messinia, forest of *Pinus nigra* & *Abies cephalonica*, on fallen branches, 17 Nov. 1997, Gonou, ATHU–M 3782; Mt. Aroania, Zarouchla, Achaïa, forest of *A. cephalonica*, on stump of *A. cephalonica*, 27 Nov. 1997, Delivorias, ATHU–M 3970; Mt. Parnitha, Attiki, forest of *A. cephalonica*, on fallen branches of *A. cephalonica*, 4 Dec. 1997, Gonou, ATHU–M 3783; Mt. Vardousia, Artotina, Fokida, forest of *P. nigra* & *Abies* sp., on fallen branches, 26 Sep. 1999, Gonou, ATHU–M 5107; Mt. Parnonas, Agios Petros, Arkadia, forest of *Castanea sativa* & *Quercus* sp., on fallen branches, 21 Nov. 1999, Gonou, ATHU–M 5105; river Agrafiotis, Epiniana, Evrytania, riparian vegetation, on living trunk and branches of *Pl. orientalis*, 15 Oct. 2000, Delivorias, ATHU–M 5122; Gardiki, Fthiotida, forest of *Alnus glutinosa*, 15 Oct. 2000, Dimou, 731; Ano Chora, Nafpaktia, Aitoloakarnania, dead trunk of *Pl. orientalis*, 22 Nov. 2001, Dimou, 988; Mt. Tymfristos, Agios Nikolaos, Evrytania, forest of *Pl. orientalis*, *Quercus* sp. & *C. sativa*, on trunk base of *Pl. orientalis*, 8 Nov. 2003, Gonou, ATHU–M 5108; Aetos, Messinia, on branches of *Pl. orientalis*, 6 Dec. 2003, Kapsanaki, ATHU–M 5111; Mt. Liakoura, Granitsa, Evrytania, forest of *Abies borisii-regis*, on fallen twigs of *A. borisii-regis*, 27 Sep. 2004, Delivorias, ATHU–M 5161; Mt. Liakoura, Limeri, Evrytania, forest of *P. nigra*, on

fallen branches of *P. nigra*, 13 Oct. 2004, Delivorias, ATHU–M 5174; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *Pl. orientalis, Quercus frainetto, C. sativa* and *A. borisii-regis*, on branches of *Pl. orientalis*, 23 Oct. 2004, Gonou, ATHU–M 5169; Mt. Liakoura, Granitsa, Evrytania, forest road, on living branches of *Pl. orientalis*, 11 Nov. 2004, Delivorias, ATHU–M 5175; Mt. Liakoura, Granitsa, Evrytania, forest of *A. borisii-regis* and *Pl. orientalis*, on fallen trunk of *Pl. orientalis*, 11 Nov. 2004, Delivorias, ATHU–M 5178.

**Remarks:** *C. calolepis* is very closely related to *C. mollis* and is considered by some authors as a variety or subspecies of the latter (Pilát 1948; Nordstein 1990). Others (Singer 1973; Watling & Gregory 1989; Senn-Irlet 1995, Bandala & Montoya 2004) consider the two taxa distinct at a specific level. Both species are characterized by the presence of a gelatinous layer in the pileal trama, considered to be more developed in *C. mollis* and less developed or absent in *C. calolepis*. This, however, has not been considered a reliable distinguishing feature (Nordstein 1990, Senn-Irlet 1995). We have accepted the species-concept of *C. calolepis* as portrayed by Senn-Irlet (1995), who has performed the most detailed work on the European species of the genus. According to this concept, *C. calolepis* is distinguished from *C. mollis* by the somewhat broader basidiospores and the presence of yellowish brown, fibrillose scales on the pileal surface formed by brownish hyphae with encrusting pigment. The pileal surface of *C. mollis* is glabrous or with scattered innate fibrils that may form indistinct pale scales, but the hyphae of the pileipellis are not pigmented and never heavily encrusted.

Our collections include specimens with strongly fibrillose-scaly pilei and specimens with almost or completely glabrous pilei, as well as many transitional forms. We cross-examined the morphology of the pileal surface, the structure of the pileipellis and the spore dimensions and concluded that two distinct forms exist amongst our collections. The first form is characterized by whitish pilei, glabrous throughout or with few fibrillose scales at the centre, in which the pileipellis consists of hyaline to pale yellowish hyphae with minute encrustations and the spores are consistently narrower (only exceptionally exceeding 6 µm in width and never more than 6.5 µm). The second form is characterized by yellowish to yellowish-brown, minutely to strongly fibrillose-scaly pilei, often throughout, in which the scale-forming hyphae of the pileipellis are consistently more or less strongly pigmented (yellowish brown to dark reddish brown in KOH) and in all cases heavily encrusted, and the spores are broader (most exceeding 6 µm and frequently reaching 7 µm in width). We identified the former as *C. mollis* and the latter as *C. calolepis*.

The Mediterranean variety *C. calolepis* var. *squamulosus* (Cout.) Senn-Irlet is not clear to us. It is reported by Senn-Irlet (1995) to have slightly larger basidiospores than var. *calolepis* (8.5–12 × 6–7.5 µm versus 7.5–10 × 5–7 µm) and broader scale-forming hyphae (up to 22 µm wide, instead of 14 µm wide). The spore-size in our specimens holds an intermediate position between the two varieties, as, in most collections, a significant portion of the spores exceed 10 µm in length, but we have not measured any spores larger than 11 µm in length or 7 µm in width. Also, we have not encountered any scale-forming hyphae broader than 15 µm. Although the spores in our specimens are slightly larger than those reported by Senn-Irlet, this has been reported by other authors as well (Bandala & Montoya 2004) and cannot be considered a significant enough deviance to justify a distinction at a variety level. We have therefore concluded

that all of our specimens must belong to a single taxon, i.e. var. *calolepis*. Lonati (1993) reports *C. mollis* var. *squamulosus* Cout. with a spore size of 7–10 × 5–6 (–6.5) µm and scale-forming hyphae 3–8 µm wide. Judging on his description, Lonati's *C. mollis* var. *squamulosus* must in fact be *C. calolepis* var. *calolepis*. Malençon & Bertault (1975) also report having found *C. mollis* var. *squamulosus*, but they distinguish it from *C. calolepis* on the grounds that the latter represents a small-sized species with a non-gelatinized or little-gelatinized pileipellis, features that are not considered diagnostic (Nordstein 1990). They make no reference to the spore-size or the structure of the pileipellis. It is unclear to us whether their *C. mollis* var. *squamulosus* represents *C. calolepis* var. *calolepis* or var. *squamulosus*.

An interesting deviant feature in our examined collections is that many basidiospores present a curving of the inner wall at the spore apex, with the outer wall either remaining obtuse and thus giving the impression of a callus-like formation, or having a small depression, resembling an indistinct germ pore (Figs 1a, c; 9a–b). This was constantly observed in all examined specimens of both *C. mollis* and *C. calolepis* and should be considered characteristic for the two species. Singer (1973) also reports similar characteristics. Senn-Irlet (1995) refers that in SEM analyses, spores of *C. calolepis* and *C. mollis* reveal a small apical depression, which may be interpreted as an apical thinning of the wall, yet neither a truncate spore apex or an apical thinning was visible under the light microscope. When describing in general the spores of the genus, Singer (1986) reports that the spores may occasionally have an indistinct callus or rarely an indistinct germ pore.

In some specimens of *C. calolepis*, as well as *C. mollis*, the lamellar trama and margin are distinctly gelatinous, the gelatinous material often covering the lamellar edge entirely. The presence of this material is possibly related to environmental humidity.

Differentiatied pleurocystidioid-like bodies were observed in the hymenium of some *C. calolepis* specimens, being more frequent near the margin. Singer (1986) reports that cystidioles may often be present on the sides of the lamellae in *Crepidotus* species and Hesler & Smith (1965) go as far as to acknowledge these elements as pleurocystidia. Senn-Irlet (1995) observed such bodies in *C. cesatii* and interpreted them as abnormalities induced by drought and therefore of no taxonomic significance. Apart from *C. calolepis*, we have also encountered such bodies in specimens of *C. subverrucisporus*. It is doubtful that these elements are of taxonomical significance in either case, as their presence is not constant and could not be correlated with any other deviant feature.

*C. calolepis* seems to be by far the most common representative of the genus in Greece, as we have collected it on several substrates from various locations. However, it was formerly recorded only by Maire & Politis (1940) on a stump of *Pinus halepensis* and dead trunks of *Platanus* sp., collections dating back to 1904 and 1906. On the other hand, the closely related *C. mollis* is recorded a number of times in the literature (Diapoulis 1939; Maire & Politis 1940; Pantidou 1991; Zervakis et al. 1998; Dimou et al. 2002a). We examined a collection of Pantidou (ATHU–M 1071), identified as *C. mollis*. It consists of a single, well-preserved specimen. The pileus is covered almost throughout with minute fibrillose scales and the scale-forming hyphae are yellowish brown to brown with strongly encrusting pigment. The spore dimensions fit accurately to our measurements of other specimens of *C. calolepis*. We have therefore concluded that this specimen formerly attributed to *C. mollis* in fact belongs to *C. calolepis* as here

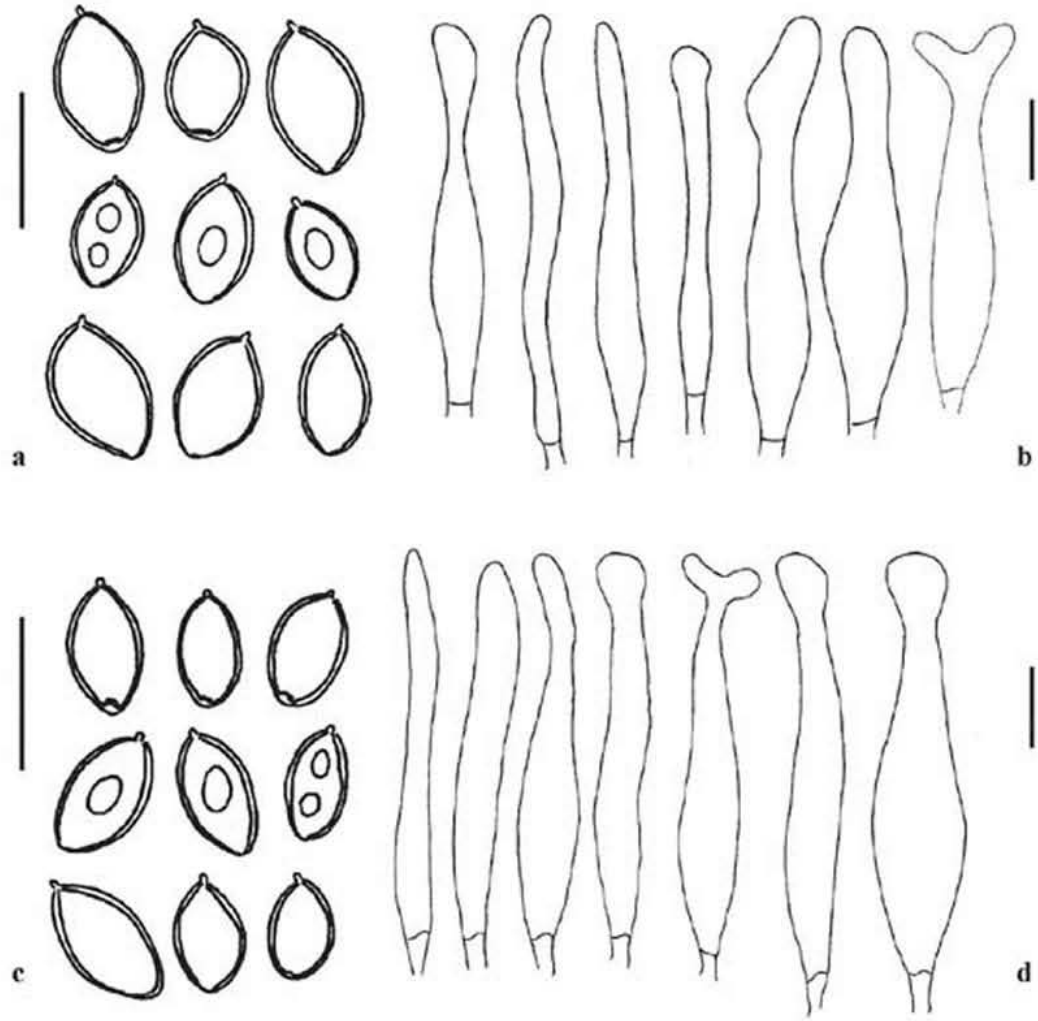


**Fig. 1. a–b.** *C. calolepis:* **a.** basidiospores, **b.** cheilocystidia, **c–d.** *C. mollis:* **c.** basidiospores, **d.** cheilocystidia. Scale bars = 10 µm.

interpreted. We also examined three specimens collected by Dimou (pers. com.), two of which he had identified as *C. mollis* var. *calolepis* (731, 988, unpubl.), and one as *C. mollis* var. *mollis* (961, unpubl.), and we concur with his judgement. Typical forms of *C. mollis* and *C. calolepis* are easily distinguished from one another in the field, but we have encountered many non-typical specimens, transitional in appearance, that could easily be misidentified if not carefully examined under the microscope. The presence of fibrillose scales on the pileal surface can be variable in abundance, and specimens collected in wet weather often have apparently glabrous pilei, to the naked eye, as stressed by Bandala & Montoya (2004). It is our conviction that *C. calolepis* may have occasionally been mistaken for *C. mollis* in the past and is in fact much more common in Greece than the latter. Pilát (1948) states that *C. calolepis* is more common in dry areas or drier inland climates. This may explain the frequent occurrence of *C. calolepis* in Greece, a country with a drier climate in comparison to most European countries.

Most authors (Malençon & Bertault 1975, Ortega & Buendia 1989, Watling & Gregory 1989, Nordstein 1990, Senn-Irlet 1995, Breitenbach & Kränzlin 2000, Krisai-Greilhuber et al. 2002) report either or both *C. mollis* and *C. calolepis* solely on wood of broad-leaved trees. Scarce reports exist from wood of coniferous trees (Maire & Politis

1940, Bandala & Montoya 2004). In Greece, *C. calolepis* has been reported on a stump of *Pinus halepensis* (Maire & Politis 1940), now it is newly recorded on branches of *Abies cephalonica, Abies borisii-regis* and *Pinus nigra.* Of the material collected in this work, seven collections of *C. calolepis,* as well as two collections of *C. mollis,* were found on wood of coniferous trees (*Abies* and *Pinus*), which have not been recorded as substrates of either species in Europe. It has also been recently collected on branches of *Alnus glutinosa* (Dimou, pers. com.). Furthermore, eight collections of *C. calolepis* were found on wood of *Platanus orientalis,* a host also not included in the substrates of this species for Europe (Senn-Irlet 1995).

***Crepidotus mollis*** (Schaeff.: Fr.) Staude **Figs 1c–d; 9a, c; 11b, d, k**

*Crepidotus mollis* (Schaeff.: Fr.) Staude, Schwämme Mitteldeutschl. 25: 71 (1857)

**Pileus** 10–50 mm, semicircular to flabelliform, convex to plano-convex, laterally or almost laterally attached to the substrate, with incurved and later even margin, surface viscid-sticky to dry, white to cream, glabrous to minutely fibrillose, forming scattered, indistinct fibrillose scales, more evident in dried specimens, consistency tough, elastic. **Lamellae** whitish in young specimens, then spotted brownish and finally uniformly ochre-brown to cinnamon, moderately crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary.

**Basidiospores** 7.0–10.0 × 5.0–6.0 (–6.5) µm, Q = 1.42–1.82, ellipsoid, amygdaliform in side view, smooth, yellowish brown in KOH, thick-walled, usually with one and less often two large oil drops as well as few small ones, apex often mucronate and thin-walled, in some spores inner wall folding inwards resembling a callus or an indistinct germ pore (Figs 1c, 9a). **Basidia** 12–30 × 6–9 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 32–55 × 6–10 µm, irregularly cylindrical, lageniform to slightly fusiform, apex obtuse, sometimes subcapitate, rarely branched or septate, frequently embedded in gelatinous material entirely covering the lamellar edge (Fig. 1d, 11b). Basidioles, basidia and cheilocystidia rarely with golden-yellow, smooth content (Fig. 11d). **Lamellar trama** seldom gelatinized. **Pileipellis** with an underlying layer of parallel hyphae, 3–5 µm wide, hyaline, not encrusted, and an upper layer of hyaline to pale yellowish hyphae, 4–12 µm wide, with granular or minutely encrusting pigment but never heavily encrusted or strongly pigmented (Fig. 9c). **Pileal trama** partly gelatinized, gelatinous layer underlying the pileipellis, usually well-developed. **Secretory hyphae** occasionally present in pileipellis, pileal trama and lamellar trama (Fig. 11k), scarce to abundant, hyaline to golden yellow in KOH. **Clamp connections** absent in all tissues.

**Habitat:** Gregarious on living or dead trunks and branches of *Abies borisii-regis* and *Platanus orientalis.*

**Specimens examined** — Mt. Zygourolivado, Pefkofyto, Karditsa, forest of *Abies borisii-regis,* on a fallen trunk of *A. borisii-regis,* 19 Sep. 1999, Delivorias, ATHU–M 5117; Mt. Zygourolivado, Pefkofyto, Karditsa, forest of *A. borisii-regis,* on a fallen trunk of *A. borisii-regis,* 17 Nov. 2001, Delivorias, ATHU–M 5123; Tatoi, Attiki, on fallen twigs of a deciduous tree, Dimou, 961; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *Platanus orientalis, Quercus frainetto, Castanea sativa* and *A. borisii-regis,* on branches of *P. orientalis,* 23 Oct. 2004, Gonou, ATHU–M 5170; Mt. Liakoura, Granitsa, Evrytania, forest of *A. borisii-regis* and *P. orientalis,* on living trunk and branches of *P. orientalis,* 11 Nov. 2004, Delivorias, ATHU–M 5177; Mt. Liakoura, Granitsa, Evrytania, forest of *A.*

*borisii-regis* and *P. orientalis*, on dead branches of *P. orientalis*, 11 Nov. 2004, Delivorias, ATHU–M 5180.

**Remarks**: The aforementioned collections are those we have encountered to fit *C. mollis*. For further notes on *C. mollis* and comparison with *C. calolepis* see remarks under *C. calolepis*.

Secretory hyphae were observed in abundance in the lamellar trama of basidiocarps from collection ATHU–M 5123. However, in basidiocarps of ATHU–M 5117, collected two years earlier from the same trunk, these hyphae were scarce, and in specimen 961 collected by Dimou, no secretory hyphae were found. The presence of these hyphae is most likely due to environmental conditions or the stage of maturity of the basidiocarps, and is of little or no taxonomical merit. Similar secretory hyphae were also observed in some specimens of *C. calolepis*.

*C. mollis* is newly recorded for Greece on *Abies borisii-regis*. According to the substrate's list of the species in Europe (Senn-Irlet 1995), there is only one reference of the species from *Platanus* and none from conifers.

## *Crepidotus* subgenus *Dochmiopus* (Pat.) Pilát, 1948

### Section *Dochmiopus*

***Crepidotus applanatus* var. *subglobiger*** Singer — Figs 2a–d; 10h; 11h-j

*Crepidotus applanatus* var. *subglobiger* Singer, Nova Hedwigia Beih. 44: 478 (1973)

**Pileus** 5–30 mm, semicircular, flabelliform to petaloid, convex to plano-convex, laterally attached to the substrate, margin incurved to even, hygrophanous, translucently striate at margin when wet, surface smooth, somewhat felty at point of attachment, pure white to pale cream, becoming yellowish brown in dried specimens. **Lamellae** whitish in young specimens, then buff to snuff brown, moderately distant, adnate to decurrent, margin minutely fimbriate, remaining whitish (observed under lens). **Stipe** rudimentary, with white tomentum on the substrate.

**Basidiospores** (5.0–) 5.5–6.5 (–7.0) × (5.0–) 5.5–6.0 (7.0) µm, Q = 1.00–1.20, globose to subglobose, yellowish brown in KOH, with pinkish content, finely verrucose, with a perispore (Figs 2a, 10h). **Basidia** 18–26 × 6–8 µm, cylindrical-clavate, 4-spored, with basal clamp, content with numerous small oil drops. **Cheilocystidia** 45–110 × 5–12 µm, variable, typically cylindrical to narrowly lageniform, rather often subcapitate or curved at apex, some branched, some septate, in some cases with two septa on the same cystidium, hyaline, thin-walled but rather often thick-walled at the medial part, in some cystidia markedly so, up to 3 µm thick (Fig. 2b, d; 11h-j). **Pleurocystidia** absent but abundant pleurocystidioid-like bodies present, 14–22 × 5–6 µm, irregularly cylindrical to clavate, often curved, twisted or constricted, frequently with an apical to lateral, finger-like protuberance (Fig. 2c). **Pileipellis** a cutis, hyphae 4–8 µm wide, hyaline or with pale yellowish, diffuse to somewhat granular intracellular pigment, occasional hyphal ends exerting as pileocystidia, 30–70 × 6–9 µm, narrowly lageniform, cylindrical to subcapitate, hyaline. Secretory hyphae present in pileipellis, pileal trama and lamellar trama. **Clamp connections** present in all tissues.

**Habitat:** Scattered on a rotten fallen trunk of *Picea abies*.

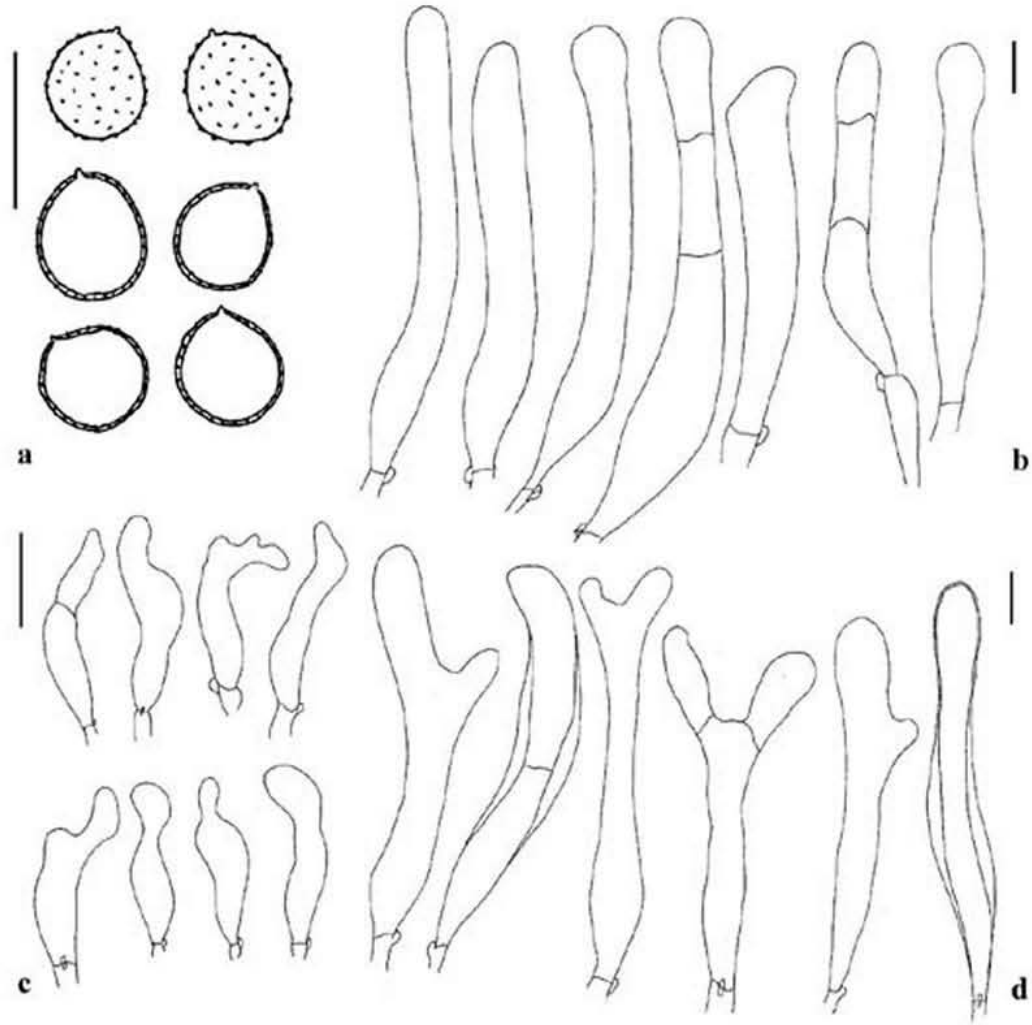


**Fig. 2.** *C. applanatus* var. *subglobiger:* **a.** basidiopores, **b, d.** cheilocystidia, **c.** pleurocystidoid bodies. Scale bars = 10 µm.

**Specimens examined** — W. Rodopi Mts., Elatia, Drama, alt. 1550 m, on a fallen trunk of *Picea abies*, 5 Oct. 2005, Gonou & Floudas, ATHU–M 5332.

**Remarks:** *C. applanatus* var. *subglobiger* is distinguished from the typical variety by the shape of the cheilocystidia which are longer, narrowly lageniform to cylindrical instead of clavate to capitate. Furthermore, var. *applanatus* prefers hardwoods, whereas var. *subglobiger* seems to be restricted to coniferous wood.

We have collected this taxon only once, from a *Picea* forest in Northern Greece. Microscopic examination revealed a few deviant features, such as the presence of septa on many cystidia, and even, in many occasions, two septa on the same cystidium. This was not a constant feature however, as showed by examination of different lamellar margins from the same basidiocarp. In one lamella the cheilocystidia were almost predominately septate, whereas in a nearby lamella the septate cystidia were scarce to almost absent. Also, many cystidia with a markedly thick-walled medial part were encountered, with the remaining cystidium being thin- to slightly thick-walled. Finally, we observed many branched cystidia at the apices, either forked or laterally branched, in most cases with

two, rarely three, branches. It was suprisingly difficult to find basidia, although most specimens were fully mature, with abundant basidiospores in all preparations. We encountered instead many basidioles and pleurocystidioid bodies, these most probably being abnormally developed basidia. All the above mentioned abnormalities, if they be such, might be induced by environmental conditions.

Hesler & Smith (1965) described two varieties of *C. applanatus* based on the morphology of the cheilocystidia: var. *phragmocystidiosus* with septate cystidia and var. *diversus* with branched or knobbed cystidia. These varieties are considered conspecific by Aime (2001) with *C. applanatus* s. Joss. She concludes, after detailed examination, that cheilocystidia in this taxon are, under the influence of environmental conditions, capable of secondary growth that can alter their shape and size as well as the number of septa per cystidium. This, however, does not seem to be the case in our specimens, as the septate and non-septate cystidia are morphologically similar. We agree, nevertheless, with Aime's deduction that taxonomic delineation in *Crepidotus* cannot be based on cystidial morphology alone, as the form of the cheilocystidia may vary greatly within a single taxon and, as observed in this case, even within individual collections.

*C. applanatus* has been reported twice from Greece, from a fallen trunk of *Abies borisii-regis* (Diamandis & Perlerou 1990) and from dead branches of *Fagus* (Diamandis 1992). The latter collection is accompanied by a description, but with no reference to the morphology of the cheilocystidia. Judging by the habitat, the first collection might be var. *subglobiger* and the second var. *applanatus* but the authors make no such distinction.

*C. applanatus* var. *subglobiger* is newly recorded for Greece.

***Crepidotus cesatii* var. *cesatii*** (Rabenh.) Sacc. Figs 3a–b; 9e

*Crepidotus cesatii* (Rabenh.) Sacc., Michelia 1: 2 (1877); *Dochmiopus sphaerosporus* (Pat.) Pat., Hyménomyc. Eur.: 113 (1887); *Crepidotus sphaerosporus* (Pat.) J.E. Lange, Dansk Bot. Ark. 9 (6): 52 (1938); *Crepidotus cesatii* var. *sphaerosporus* (Pat.) A. Ortega & Buendia, Int. J. Myc. Lich. 4 (1–2): 96 (1989)

**Pileus** 3–22 mm, circular to semicircular or roundedly flabelliform, rarely somewhat lobed, convex to plano-convex, centrally to eccentrically or almost laterally attached to the substrate, margin incurved, becoming even only in fully mature specimens, surface dry, felty, pure white, remaining so in dried specimens or becoming pale cream. **Lamellae** whitish in young specimens, often with a pinkish tint, later cream, pinkish buff to pale cinnamon, never significantly darker, distant to subdistant, adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary.

**Basidiospores** (5.5–) 6.5–8.0 (–9.0) × 4.5–7.0 µm, Q = (1.00–) 1.10–1.33 (–1.46), globose, subglobose to broadly ellipsoid, pale yellowish in KOH, finely echinulate (Figs 3a, 9e). **Basidia** 20–37 × 6–9 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 28–80 × 4–11 µm, diverticulate, clavate, cylindrical, irregularly cylindrical, fusiform, lageniform, usually branched, frequently multiply so, apices obtuse, hyaline, thin-walled (Fig. 3b). **Pileipellis** a trichodermium of loosely interwoven hyphae with transitions to a loose cutis, hyphae often coiled, 3–6 µm wide, hyaline, thin-walled. **Clamp connections** present in all tissues.

**Habitat:** Solitary or in small groups on dead or living branches of *Platanus orientalis* and, in one case, *Pinus nigra*.

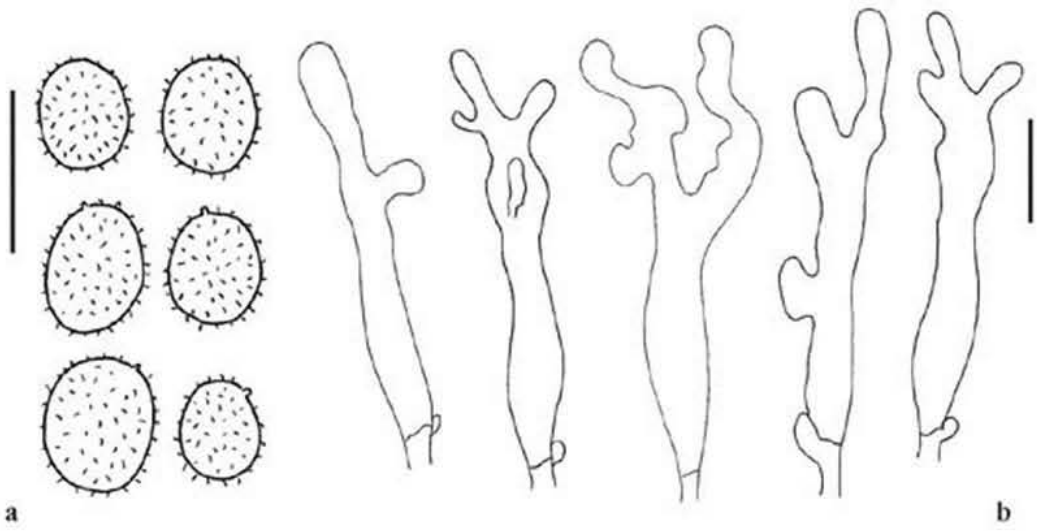


Fig. 3. *C. cesatii* var. *cesatii:* **a.** basidiopores, **b.** cheilocystidia. Scale bars = 10 μm.

**Specimens examined** — Mt. Zygourolivado, Anthochori, Karditsa, riparian vegetation, on a living branch of *Platanus orientalis,* 19 Sep. 1999, Delivorias, ATHU–M 5116; Mt. Katachloro, Kedros, Karditsa, riparian vegetation, on branches of *Pl. orientalis,* 13 Nov. 1999, Delivorias, ATHU–M 5118; Mt. Tymfristos, Raches Tymfristou, Evrytania, forest of *Pinus nigra* and *Abies borisii-regis,* on branches of *P. nigra,* 25 Oct. 2003, Delivorias, ATHU–M 5125; Mt. Tymfristos, Agios Nikolaos, Evrytania, forest of *Pl. orientalis, Quercus* sp. & *Castanea sativa,* on fallen twigs and branches of *Pl. orientalis,* 8 Nov. 2003, Gonou, ATHU–M 5109; Mt. Liakoura, Granitsa, Evrytania, forest of *A. borisii-regis* and *Pl. orientalis,* on dead twigs and branches of *Pl. orientalis,* 27 Sep. 2004, Delivorias, ATHU–M 5162; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *Pl. orientalis, Quercus frainetto, C. sativa* and *A. borisii-regis,* on branches of *Pl. orientalis,* 23 Oct. 2004, Gonou, ATHU–M 5173; Mt. Liakoura, Granitsa, Evrytania, forest road, on living branches of *Pl. orientalis,* 11 Nov. 2004, Delivorias, ATHU–M 5176; Mt. Liakoura, Granitsa, Evrytania, forest of *A. borisii-regis* and *Pl. orientalis,* on dead branches of *Pl. orientalis,* 11 Nov. 2004, Delivorias, ATHU–M 5179.

**Remarks:** The typical variety of *C. cesatii* is characterized by the distant lamellae, the globose to subglobose, finely echinulate basidiospores, the diverticulate cheilocystidia and the coiled hyphae of the pileipellis. The only other variety recognized by Senn-Irlet (1995), *C. cesatii* var. *subsphaerosporus* (J.E. Lange) Senn-Irlet, is reported to have broadly ellipsoid basidiospores, mostly straight hyphae on the pileipellis and to grow on branches of coniferous trees. Also, Watling & Gregory (1989), report that the latter variety lacks the characteristic pink tinge on the lamellae and has a darker spore print, although Senn-Irlet does not make such a reference. In all specimens examined in this work, at least a portion of the basidiospores were found to be broadly ellipsoid, and, in some specimens, these spores predominate. However, in all specimens the hyphae of the pileipellis were clearly coiled and the only collection made from coniferous trees does not seem to deviate microscopically from the remaining collections. As we have been unable to determine a correlation between the variation in shape of the basidiospores and either the structure of the pileipellis, the colour of the lamellae or the habitat, we have concluded that our findings consist of a single taxon, *C. cesatii* var. *cesatii,* in which

the basidiospores may range from perfectly globose to broadly ellipsoid in their extreme variation, with a Q ratio reaching up to 1.46.

*C. cesatii* var. *cesatii* is newly recorded for Greece on twigs and branches of *Pinus nigra* and *Platanus orientalis*, the latter seeming a rather common substrate for the species in Greece, in contrast to the references for the distribution of the species in Europe (Senn-Irlet 1995).

***Crepidotus variabilis*** (Pers.: Fr.) P. Kumm. Figs 4a–b, 10g

*Crepidotus variabilis* (Pers.: Fr.) P. Kumm., Führ. Pilzk.: 74 (1871)

**Pileus** 5–15 mm, circular to semicircular or roundedly flabelliform, often lobed, convex to plano-convex, centrally to eccentrically or almost laterally attached to the substrate, with incurved and later even margin, surface dry, felty to smooth, pure white to dirty white, remaining so in dried specimens. **Lamellae** whitish in young specimens, often with a pale pinkish tint, later clay buff and finally cinnamon brown, moderately crowded to crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary.

**Basidiospores** (5.0–) 5.5–7.0 (–7.5) × (2.5–) 3.0–3.5 (–4.0) µm, Q = 1.57–2.17, short cylindric to elongate, oblong, pale yellowish in KOH, minutely but distinctly punctate-warty (Figs 4a, 10g). **Basidia** 20–25 × 5–7 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 22–53 × 6–11 µm, diverticulate, clavate, cylindrical, irregularly cylindrical, fusiform, mostly branched, often multiply branched, hyaline, thin-walled (Fig. 4b). **Pileipellis** a trichodermium of loosely interwoven hyphae, 2–5 µm wide, hyaline, thin-walled. **Clamp connections** present in all tissues.

**Habitat:** Solitary to gregarious on branches of *Quercus frainetto*, *Quercus coccifera* and *Cistus* sp.

**Specimens examined** — lake Plastira, Agios Athanasios, Karditsa, forest of *Quercus frainetto*, on fallen branches of *Q. frainetto*, 1 Nov. 1998, Delivorias, ATHU–M 5112; lake Plastira, Agios Athanasios, Karditsa, forest of *Q. frainetto*, on fallen branches of *Q. frainetto*, 1 Nov. 1998, Delivorias, ATHU–M 5113; lake Plastira, Agios Athanasios, Karditsa, forest of *Q. frainetto*, on fallen branches of *Q. frainetto*, 18 Sep. 1999, Delivorias, ATHU–M 5114, Domokos, Fthiotida, maquis vegetation, on branches of *Quercus coccifera*, 13 Nov. 1999, Delivorias, ATHU–M 5115; Mt. Ymittos, Attiki, maquis vegetation, on twigs of *Cistus* sp., 7 Dec. 2002, Dimitriadis, ATHU–M 4648.

**Remarks:** *C. variabilis* is characterized by the lobed pileal margin (not always distinct, however), the small-sized, cylindrical, punctate-warty basidiospores and the diverticulate cheilocystidia. The lobed pileal margin may be a good distinctive feature for macroscopical identification when clearly formed, but, as in all white species of *Crepidotus*, careful microscopical examination is essential for identification. The characteristic small-sized, cylindrical basidiospores provide a reliable distinguishing feature. *C. variabilis* var. *trichocystis* Hesler & A.H. Sm. is reported to have larger basidiospores and longer, narrowly cylindrical to narrowly lageniform cheilocystidia (Senn-Irlet 1995).

*C. variabilis* seems to be common in Greece, as it is reported a number of times in the literature (Maire & Politis 1940, as *Dochmiopus variabilis*; Minter 1988; Avtzis & Diamandis 1988; Konstantinidis 2002) and we have collected it a few times ourselves.

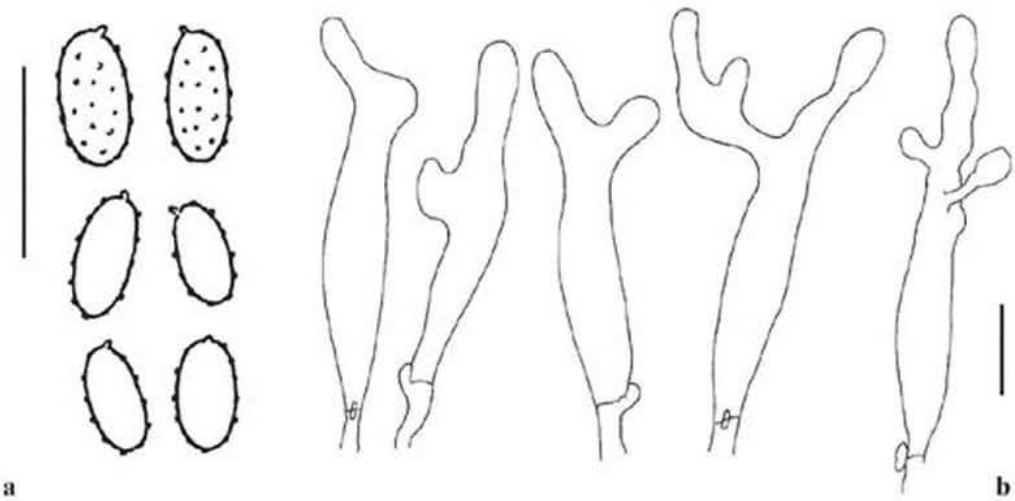


**Fig. 4.** *C. variabilis:* **a.** basidiospores, **b.** cheilocystidia. Scale bars = 10 µm.

It has also been recently collected on branches of *Alnus glutinosa* and *Quercus coccifera* (Dimou pers. com.). It is newly recorded for Greece on branches of *Quercus frainetto, Q. coccifera* and twigs of *Cistus* sp., the last two being representative plants of the maquis vegetation.

## Section *Crepidotellae* Hesler & A.H. Sm., 1965

## Subsection *Autochthoni* Senn-Irlet, 1995

***Crepidotus autochthonus*** J.E. Lange — Figs 5a–b, 9f, 11c, e

*Crepidotus autochthonus* J.E. Lange, Dansk bot. Ark. 4 (6): 51 (1938)

**Pileus** 10–40 mm, semicircular to flabelliform, convex to plano-convex, laterally or almost laterally attached to the substrate, with incurved, later even to undulating margin, surface dry, glabrous to minutely fibrillose-tomentose, dirty whitish, cream to yellowish buff. **Lamellae** whitish in young specimens, then spotted brownish and finally uniformly cinnamon brown to fulvous, crowded, emarginately adnate, margin even. **Stipe** absent or rudimentary. **Spore print** yellowish brown to umber.

**Basidiospores** 7.0–9.0 × 5.0–6.0 µm, Q = 1.27–1.64, ellipsoid, amygdaliform or lemoniform in side view, with a more or less acute apex, smooth, thick-walled, occasionally wall thinning at acute apex, yellowish to yellowish brown in KOH, usually with a large oil drop (Figs 5a, 9f). **Basidia** 25–30 × 7–9 µm, cylindrical-clavate, with 4 sterigmata. **Cheilocystidia** 17–32 × 7–13 µm, cylindrical, clavate, some subcapitate, short lageniform, not branched, rarely septate, thin to thick-walled (Figs 5b, 11c). Basidioles and cheilocystidia sometimes with yellow-golden, smooth content (Fig. 11e). **Pileipellis** a cutis of hyaline hyphae, 3–5 µm wide, some ascending. **Pileal trama** without gelatinous layer. **Lamellar trama** with few secretory hyphae, usually hyaline, seldom golden-yellow. **Clamp connections** present in all tissues.

**Habitat:** Gregarious or in small groups on ground, in forest of *Quercus frainetto* or mixed *Q. frainetto* and *Abies borisii-regis*.

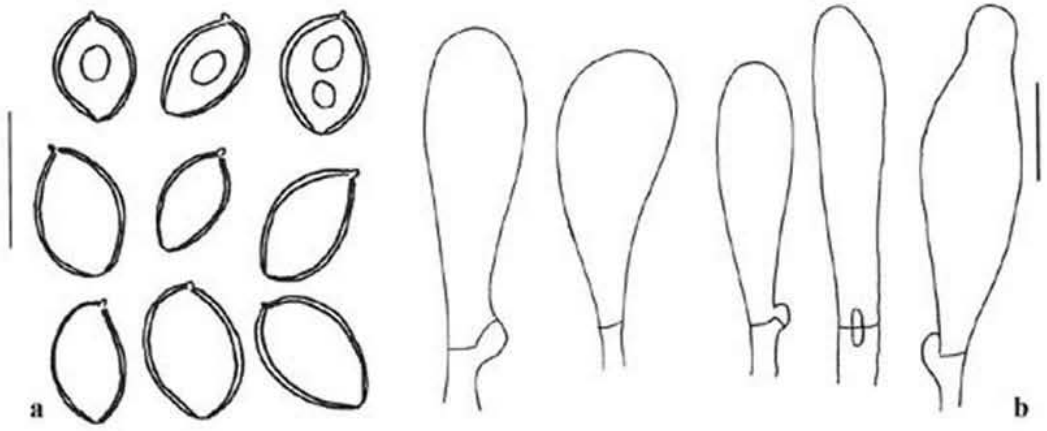


Fig. 5. *C. autochthonus:* a. basidiospores, b. cheilocystidia. Scale bars = 10 μm.

**Specimens examined** — Lake Plastira, Kryoneri, Karditsa, clearing of forest of *Quercus frainetto*, on ground, 14 Oct. 2000, Delivorias, ATHU–M 5121; lake Plastira, Kastania, Karditsa, mixed forest of *Q. frainetto* and *Abies borisii-regis*, on ground, 7 Sep. 2002, Delivorias, ATHU–M 5124.

**Remarks:** *C. autochthonus* is rather similar-looking macroscopically to *C. mollis* and has practically identical basidiospores, which nevertheless can be distinguished by their acute apex, without the characteristic wall curving or apical depression of *C. mollis* spores. It is also easily identifiable by the terrestrial habit, the lack of a gelatinous layer in the pileal trama, the shape of the cheilocystidia and the presence of clamp-connections. According to Senn-Irlet (1995), it is the only terrestrial species of *Crepidotus* in Europe.

*C. autochthonus* is newly recorded from Greece and is reported for the first time in Europe in forests of *Quercus* spp.

## Subsection *Pleurotellus* (Fayod) Senn-Irlet, 1995

***Crepidotus epibryus*** (Fr.: Fr.) Quél. Figs 6a–b; 10i; 11f

*Crepidotus epibryus* (Fr.: Fr.) Quél., Mém. Soc. Emul. Montbéliard, sér. 2, 5: 138 (1872); misdet.: *Crepidotus perpusillus* (Lumn.: Fr.) Maire, Fungi Catal. II: 102 (1937)

**Pileus** up to 10 mm, rounded flabelliform or campanulate when young, circular with age, spreading out on the substrate, becoming almost resupinate, with white tomentum around the margin where attached, sessile, surface tomentose, white, even when dried. **Lamellae** rather distant to moderately crowded, adnexed, whitish to pale ochraceous in fresh specimens, remaining so or darkening to fulvous in dried ones, margin concolorous, even to slightly uneven, often browning at places, especially when dried. **Flesh** very thin, white. **Stipe** absent.

**Basidiospores** 6.0–9.0 × 2.5–3.0 μm, cylindrical, somewhat fusoid to narrowly amygdaliform or pip-shaped, some slightly curved, smooth, hyaline or pale yellow in KOH, yellow in the commonly formed masses (of two, four or more) (Figs 6a, 10i). **Basidia** 15–20 × 5–6 μm, clavate, 4-spored, usually hyaline; some disintegrating basidioles and

basidia with granular, golden brown content, larger in size (Fig. 11f). **Cheilocystidia** up to 50 × 7 µm, cylindrical to narrowly lageniform, flexuous, sometimes with strongly curved or whirled apex, rarely branched, often difficult to be observed (Fig. 6b). Parts of the hymenium covered with golden-brown granular material. **Pileipellis** a transition between a cutis of interwoven hyphae and a trichodermium of erect, straight, filiform, hyaline hyphae. **Clamp connections** absent in all tissues.

**Habitat:** Solitary on stalks and laminas of fallen leaves of *Castanea sativa*.

**Specimens examined:** Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *Castanea sativa, Abies borisii-regis, Quercus* sp. and *Corylus avelana*, on fallen leaves of *C. sativa*, 14 Nov. 1998, Gonou, ATHU–M 5106.

**Remarks:** Distinctive microscopical characters of *C. epibryus* are the size and shape of the basidiospores as well as the narrowly lageniform, flexuous and/or curled at the apex cheilocystidia. The cheilocystidia were scarce and difficult to find, possibly because the lamellar edge had been injured. The golden-brown material covering parts of the hymenium is probably the result of the excretion of necropigments from the concolorous disintegrating basidia and basidioles and corresponds macroscopically to the browning spots of the lamellae. Similar pigmented and amorphous aggregations on the hymenium are reported from species of *Lyophyllum* as well as pigmented basidia from species of *Inocybe, Cortinarius* and *Pholiota* (Clémençon 1997).

*C. epibryus* has been twice reported from Greece, on leaves of *Fagus sylvatica* (as *Crepidotus perpusillus*, Maire & Politis 1940) and on leaves of *Quercus cerris* (Polemis et al. 2002). Our collection is the first recording of *C. epibryus* on leaves of *Castanea sativa* in Greece and one of the very few in Europe (Senn-Irlet 1995).

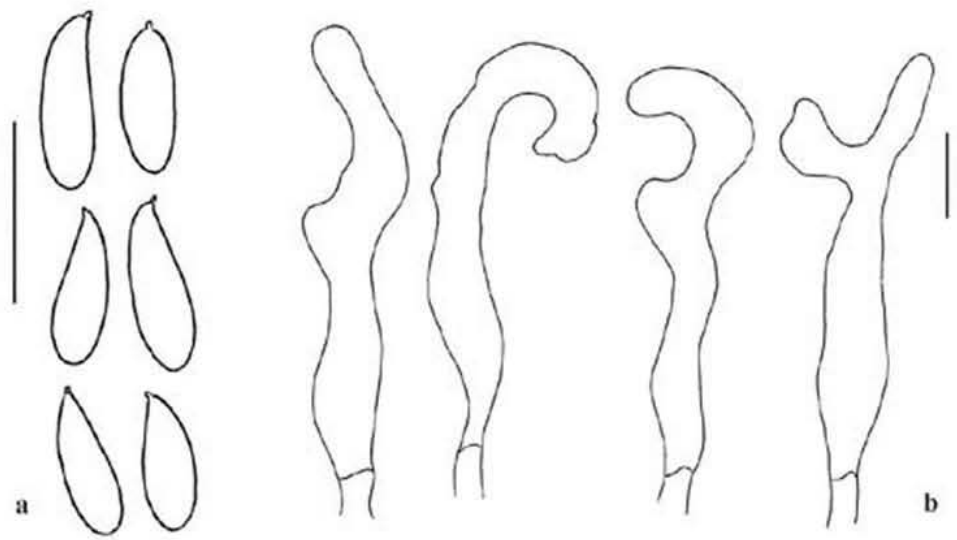


Fig. 6. *C. epibryus:* **a.** basidiospores, **b.** cheilocystidia. Scale bars = 10 µm.

## Subsection *Fibulatini* Singer, 1947

### *Crepidotus lundellii* Pilát

Figs 7a–b; 10a, d

*Crepidotus lundellii* Pilát, Fungi Exsiccati Suecici fasc. V-VI: 10 (1936)

**Pileus** 3–20 mm, circular to semicircular or roundedly flabelliform, convex to planoconvex, eccentrically to almost laterally attached to the substrate, with incurved and later even margin, surface dry, felty, pure white to pale cream. **Lamellae** whitish in young specimens, then clay buff and finally cinnamon brown to fulvous, moderately crowded to crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent or rudimentary.

**Basidiospores** 6.5–8.5 (–9.0) × 4.5–5.5 (–6.0) µm, Q = 1.40–1.80, in frontal view broadly ovoid to ellipsoid, in side view ellipsoid to slightly amygdaliform, yellowish in KOH, wall slightly roughened, almost smooth, frequently seemingly smooth even under oil immersion but never actually completely smooth (Figs 7a, 10a). **Basidia** 21–30 × 6–8 µm, cylindrical-clavate, 4-spored. **Cheilocystidia** 32–56 × 7–12 µm, clavate, cylindrical, fusiform, with an obtuse, sometimes subcapitate apex, rarely branched, hyaline, thin-walled (Figs 7b, 10d). **Pileipellis** a trichodermium of loosely interwoven hyphae 3–5 µm wide, hyaline, thin-walled. **Clamp connections** present in all tissues.

**Habitat:** Gregarious on fallen branches of *Platanus orientalis*.

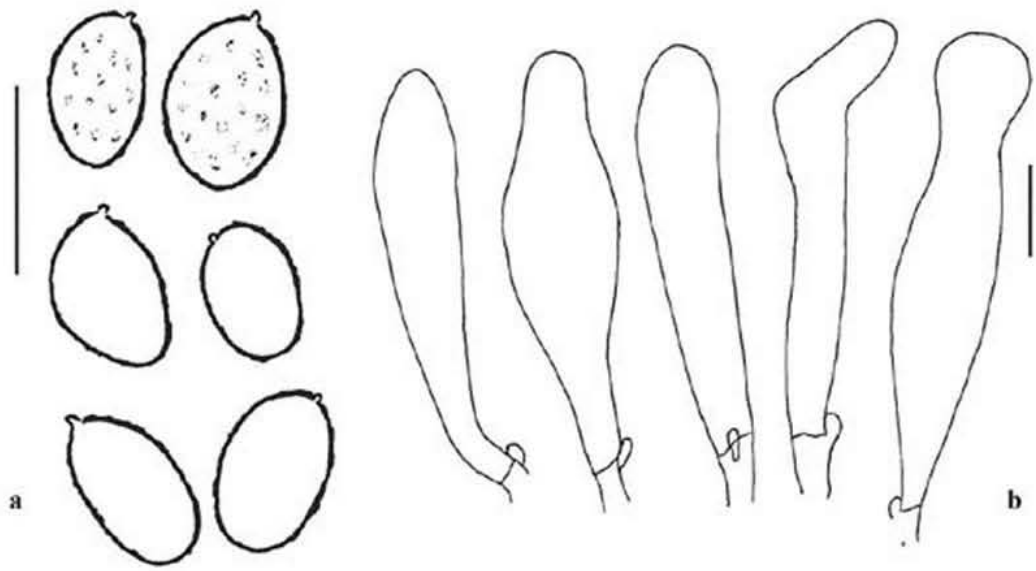


Fig. 7. *C. lundellii*: **a.** basidiospores, **b.** cheilocystidia. Scale bars = 10 µm.

**Specimens examined** — Mt. Katachloro, Kedros, Karditsa, riparian vegetation, on fallen branches of *Platanus orientalis*, 13 Nov. 1999, Delivorias, ATHU-M 5119; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *P. orientalis*, *Quercus frainetto*, *Castanea sativa* and *Abies borisii-regis*, on branches of *P. orientalis*, 23 Oct. 2004, Gonou, ATHU-M 5171.

**Remarks:** *C. lundellii* is characterized by the unique ornamentation of the basidiospores, consisting of very low warts and ridges. The spores seem smooth in low magnification

and the ornamentation is revealed only under oil immersion. The basidiospores are ellipsoid to slightly amygdaliform and faint yellowish in KOH. In the macroscopically similar *C. subverrucisporus*, the spore ornamentation is much more distinct, the spore colour is darker and the cheilocystidia are more consistently narrowly lageniform (apically tapered) (Senn-Irlet 1995; Bandala et al. 1999).

*C. lundellii* is newly recorded for Greece and is reported for the first time on *Platanus* in Europe (Senn-Irlet 1995). It has also been recently collected on branches of *Alnus glutinosa* (Dimou pers. com.).

***Crepidotus luteolus*** (Lambotte) Sacc. Figs 8a–b; 10b, e

*Crepidotus luteolus* (Lambotte) Sacc., Syll. Fung. (Abellini) 5: 888 (1887)

**Pileus** 2-15 mm, sessile, young ungulate, campanulate, later convex to plano-convex, flabelliform, reniform or semicircular when seen from above, laterally or dorsally attached, often spread out over the substrate, almost resupinate, margin even, straight, remaining so or becoming undate, lobate, white-yellowish to yellowish-cream when wet, straw to buff when dried, surface first tomentose-hirsute with a smooth margin, later smooth throughout or hirsute only near the point of attachment, usually with a rich tomentum on the substrate. **Lamellae** adnexed to narrowly adnate, moderately crowded, whitish or pale yellowish at first, later buff to buffish brown, fulvous when dried, margin whitish, fimbriate when young, concolorous and almost smooth when mature. **Flesh** white. **Stipe** absent or rarely observed in very young fruit bodies.

**Basidiospores** 8.0–9.5 (–10.5) × 4.5–5.5 (–6.0) µm, Q = 1.55–2.10, ellipsoid in frontal view, ellipsoid to usually amygdaliform in side view, yellowish brown in KOH, minutely roughened (Figs 8a, 10b). **Basidia** 20–30 × 7–9 µm, clavate, usually strongly granular, 4-spored. **Cheilocystidia** 45–60 × 5–8 µm, cylindrical to narrowly lageniform, strongly flexuous, often branched or rarely angular, hyaline, thin walled (Figs 8b, 10e). **Lamellar edge** in some sections somewhat gelatinous, holding cheilocystidia rather packed. **Pileipellis** either a cutis of interwoven hyphae with transitions to a trichodermium bearing bundles of exerted hyphal ends, or a real trichodermium with erect hyphae, hyphae straight, flexuous or winding, thin to slightly thick walled, hyaline or pale yellow, some of the latter slightly encrusted. **Clamp connections** present in all tissues.

**Habitat:** Solitary or in small groups, rather gregarious, on fallen twigs of *Platanus orientalis*.

**Specimens examined** — Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed deciduous forest of *Platanus orientalis*, *Quercus* sp. and *Castanea sativa*, on fallen twigs of *P. orientalis*, 8 Nov. 2003, Gonou, ATHU-M 5110.

**Remarks:** *C. luteolus* is characterized macroscopically by the yellowing colors of the basidiocarps and microscopically by the faintly ornamented, amygdaliform, relatively long and narrow basidiospores as well as the polymorphic, flexuous, frequently branched cheilocystidia. Our specimens of *C. subverrucisporus* exhibit the same yellowing colors when fresh and could be confused macroscopically with those of *C. luteolus*. The two species can be distinguished microscopically by their basidiospores and cheilocystidia. The basidiospores of *C. subverrucisporus* differ in being more ornamented and ellipsoid (broader) rather than amygdaliform in side view, while the cheilocystidia are less flexuous and branched.

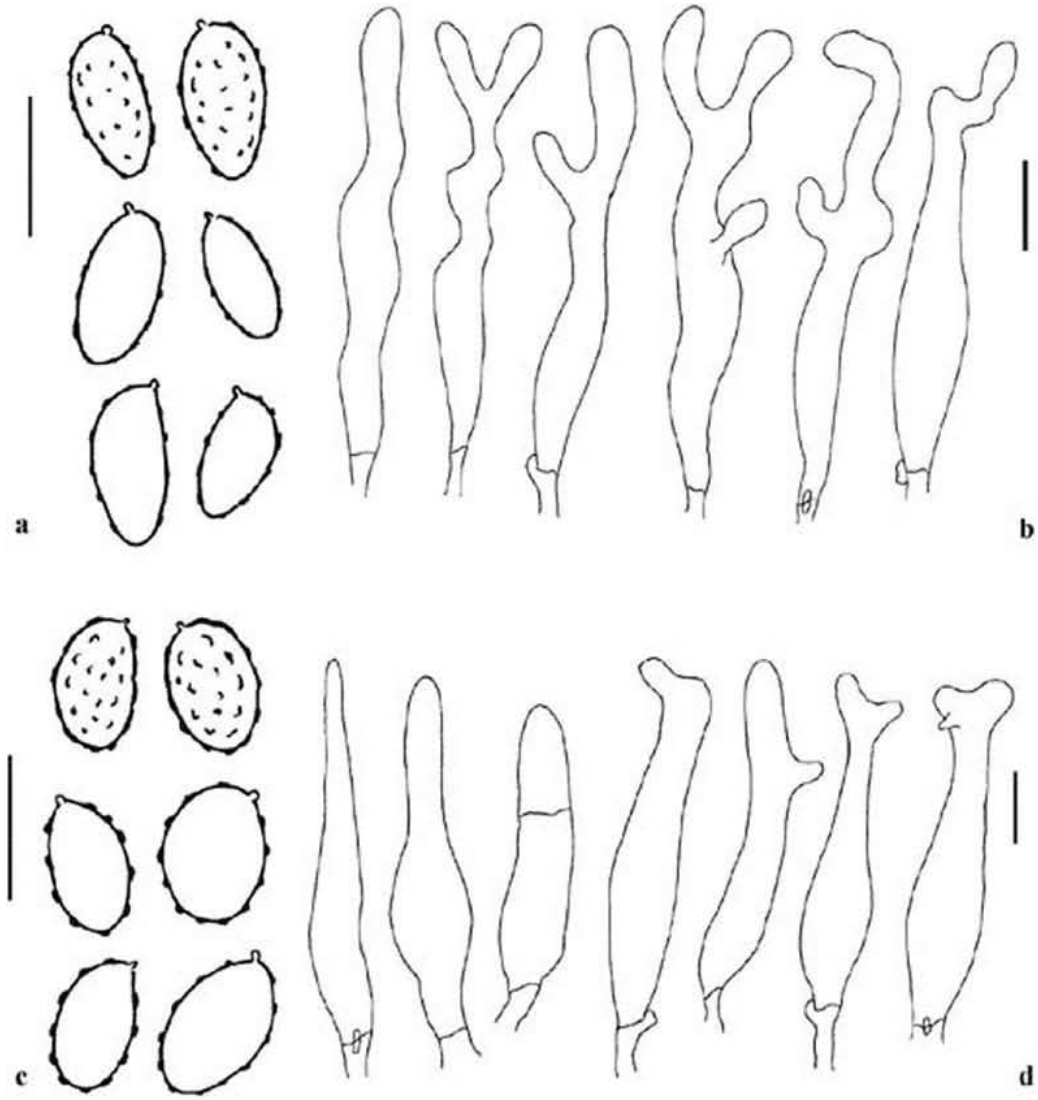


**Fig. 8. a–b.** *C. luteolus:* **a.** basidiospores, **b.** cheilocystidia; **c–d.** *C. subverrucisporus:* **c.** basidiospores, **d.** cheilocystidia. Scale bars = 10 μm.

It is worth mentioning that besides *C. luteolus,* specimens of *C. cesatii* were collected on the same day from the same locality, on nearby fallen twigs of *Platanus orientalis.* Basidiocarps of *C. luteolus* exhibited a pale yellow color on the pileus in contrast to the whitish color of *C. cesatii.*

*C. luteolus* is newly recorded from Greece and is reported for the first time on *Platanus* in Europe. It has also been recently collected on *Nerium oleander* (Dimou pers. com.)

### *Crepidotus subverrucisporus* Pilát — Figs 8c–d; 10c, f; 11a

*Crepidotus subverrucisporus* Pilát, Studia Botanica Čechoslavaca 10: 151 (1949)

**Pileus** 3–10 mm, circular to semicircular or roundedly flabelliform, convex to plano-convex, eccentrically to almost laterally attached to the substrate, where usually with a whitish or yellowish tomentum, margin incurved and later even, surface dry, felty, white

or dirty whitish to pale yellowish cream. **Lamellae** whitish in young specimens, later buff and finally cinnamon brown to fulvous, moderately crowded, emarginately adnate, margin minutely fimbriate, remaining whitish. **Stipe** absent.

**Basidiospores** (7.0–) 7.5–10.0 (–11.0) × (4.5–) 5.0–6.0 (–7.0) µm, Q = 1.45–1.78, ovoid to ellipsoid, slightly amygdaliform in side view, yellowish brown in KOH, minutely but distinctly rugulose (Figs 8c, 10c). **Basidia** 20–25 × 7–8 µm, cylindrical-clavate, 4-spored, some 2-spored. **Cheilocystidia** 28–65 × 4–10 × 3–5 µm, cylindrical, broadly lageniform to narrowly, elongate lageniform, often angular or sometimes branched towards the apex or apex subcapitate, sometimes septate at upper 1/3, hyaline, thin-walled (Figs 8d, 10f, 11a). **Pleurocystidia** absent but seldom lageniform pleurocystidioid bodies present. Basidioles and pleurocystidioid bodies sometimes with golden-yellow content. **Pileipellis** a trichodermium with transitions to a cutis, hyphae loosely interwoven, 3–6 µm wide, hyaline, thin-walled. **Clamp connections** present in all tissues.

**Habitat:** Gregarious on fallen branches of *Platanus orientalis*.

**Specimens examined** — Mt. Katachloro, Kedros, Karditsa, riparian vegetation, on fallen branches of *Platanus orientalis*, 13 Nov. 1999, Delivorias, ATHU–M 5120; Mt. Tymfristos, Agios Nikolaos, Evrytania, mixed forest of *P. orientalis*, *Quercus frainetto*, *Castanea sativa* and *Abies borisii-regis*, on mossy branches of *P. orientalis*, 23 Oct. 2004, Gonou, ATHU–M 5172.

**Remarks:** The fresh fruit bodies from both collections exhibited a pale yellowish color in the pileus which was retained in the exsiccata. *C. subverrucisporus* is characterized by the minutely but distinctly rugulose, slightly amygdaliform, moderately dark coloured basidiospores and the rather simple lageniform cheilocystidia. The cheilocystidia are reported to be generally unbranched (Watling & Gregory 1989; Senn-Irlet 1995; Breitenbach & Kränzlin 2000). Type studies performed by Senn-Irlet (1993) showed that the cheilocystidia in the holotype (found on *Robinia pseudoacacia*) are often septate (also observed by Bandala et al. 1999) and sometimes branched. The same observation was made by Senn-Irlet (1995) on material collected from Italy, also on *Robinia*. We have encountered in our specimens both septate and branched at the tips cystidia.

We have also occasionally observed pleurocystidioid-like bodies, some with a golden-yellow content, but they are probably of no taxonomic significance. Bandala et al. (1999) also report sterile basidiole-like elements rarely present on the sides of the lamellae and consider them probably to be abnormal basidia or basidioles.

*C. subverrucisporus* is newly recorded from Greece and is reported for the first time on *Platanus* in Europe.

## Discussion

With few exceptions, such as *C. calolepis* or *C. cinnabarinus*, the species of *Crepidotus* are more or less macroscopically similar, i.e. whitish, small to medium sized basidiocarps, with whitish to pale brownish lamellae and without a distinctive smell or taste. Hence, careful microscopic examination is absolutely essential for determination at a species level. The most important microscopic features from a taxonomical standpoint are the morphology of basidiospores, cheilocystidia and, to a lesser degree, pileipellis, and the

presence or absence of clamp connections (Nordstein 1990; Senn-Irlet 1995; Bandala & Montoya 2004). In few species other features are also of taxonomical importance, such as the presence of a gelatinous layer in the pileal trama in *C. calolepis* and *C. mollis*, the distant, pinkish lamellae in *C. cesatii*, or some striking habitat preferences, such as the growth on stems, leaves or litter in *C. epibryus*, or directly on soil in *C. autochthonus*. In general, however, habitat preferences are an indicative and not a decisive taxonomical feature. *C. calolepis* and *C. mollis* are considered by most authors to grow exclusively on deciduous trees, but we have collected both species on conifers in more than a few occasions. *C. cesatii* var. *cesatii* is considered to prefer deciduous trees whereas var. *subsphaerosporus* coniferous trees, but the decisive distinctive feature between the two varieties is the shape of the basidiospores and not the habitat. We have collected specimens of *C. cesatii* on both deciduous and coniferous trees, but without any notable microscopic differences between them.

We noted many deviant features in our specimens, such as a callus-like structure at the apex of the basidiospores in *C. calolepis* and *C. mollis*, pleurocystidioid-like bodies in *C. calolepis*, *C. applanatus* var. *subglobiger* and *C. subverrucisporus*, septate cheilocystidia in *C. mollis*, *C. applanatus* var. *subglobiger*, *C. autochthonus* and *C. subverrucisporus*, thick-walled cheilocystidia in *C. applanatus* var. *subglobiger*, basidia, basidioles and cheilocystidia with pigmented content in *C. mollis*, *C. autochthonus* and *C. subverrucisporus*, amorphous material on the hymenium in *C. epibryus*, gelatinized lamellar trama in *C. calolepis* and *C. mollis*, and secretory hyphae in many species. The presence of the callus-like structure in *C. calolepis* and *C. mollis*, although sporadic, is constant and should be considered characteristic for the two species. Singer (1973) also mentioned such features. All the other features have an inconstant appearance and are due most probably to environmental conditions or different stages of maturity, and therefore no taxonomic value can be attributed to them.

The determination of the diversity and distribution of *Crepidotus* species is not an easy task, as they usually form small-sized basidiocarps that may be overlooked. Prior to this work, 7 taxa of *Crepidotus* were reported from Greece: *C. applanatus* (Diamandis & Perlerou 1990; Diamandis 1992), *C. cesatii* (Maire & Politis 1940, as *Dochmiopus sphaerosporus*, Dimou et al. 2002a, as *Crepidotus sphaerosporus*), *C. calolepis* (Maire & Politis 1940), *C. cinnabarinus* (Dimou et al. 2002b), *C. epibryus* (Maire & Politis 1940, as *Crepidotus perpusillus*; Polemis et al. 2002), *C. mollis* (Diapoulis 1939; Maire & Politis 1940; Pantidou 1991; Zervakis et al. 1998; Dimou et al. 2002a) and *C. variabilis* (Maire & Politis 1940; Minter 1988; Avtzis & Diamandis 1988; Diamandis 1992; Konstantinidis 2002). Most of these taxa were reported only once or twice, with the exceptions of the seemingly common *C. variabilis* and *C. mollis*. Concerning the latter, as stated in this work, it is possible that some collections attributed to *C. mollis* may in fact represent collections of *C. calolepis*. Some older collections (Diapoulis 1939; Maire & Politis 1940) cannot be accounted for and probably have not survived to this day. We have examined collection ATHU-M 1071 identified as *C. mollis* (Pantidou 1991) and have attributed it to *C. calolepis*, and have also cross-examined two specimens of *C. calolepis* and one of *C. mollis*, kindly provided to us by Dimou. We are convinced that *C. calolepis* is much more common in Greece than *C. mollis*.

*Crepidotus* species never form the dominant element in any European vegetation unit (Senn-Irlet 1995), and this obviously applies for Greece as well. Most of the gathered collections consist of rather few individuals. Few of the collected species were found to grow gregariously, i.e. *C. calolepis, C. mollis, C. autochthonus* and, to a lesser degree, *C. luteolus* and *C. subverrucisporus*. However, in some occasions we collected more than a few different species from the same location. Seven taxa: *C. calolepis, C. mollis, C. lundellii, C. subverrucisporus, C. cesatii* var. *cesatii, C. luteolus* and *C. epibryus*, were collected from a small area on Mt. Tymfristos (Agios Nikolaos), the first five on the same day. In addition, *C. calolepis* and *C. cesatii* were collected in two successive years from that area. Three of the similar-looking white species, *C. cesatii, C. lundellii* and *C. subverrucisporus*, were collected on the same day from the same location (Mt. Katachloro). In one case, basidiocarps of two species, *C. cesatii* and *C. calolepis*, were found on nearby branches of the same *Platanus orientalis*, a few meters apart from a second *Platanus* colonized by *C. mollis* (Mt. Liakoura). We have not, however, encountered more than one species on the same branch. Senn-Irlet (1995) states that in no case had she encountered basidiocarps of more than one *Crepidotus* species on the same substrate.

Floristic studies in other countries of the Mediterranean region reveal a similar diversity of *Crepidotus* species as in Greece. Senn-Irlet (1995) provides data for most countries surrounding the Mediterranean, such as Bulgaria, Jugoslavia, Turkey, Italy, France and Spain. A recent checklist of *Crepidotus* in Croatia includes eight species in total, all of which have also been found in Greece (Tkalčec & Mešić 2003). The same applies for the seven species of *Crepidotus* reported from Turkey (Selsi & Denchev 2005). The diversity of *Crepidotus* in Spain (Ortega & Buendía 1989) is also similar to that of Greece. Lonati (2000) presented a paper concerning the diversity of *Crepidotus* in the Mediterranean area, in which he reports sixteen species (twelve, if synonymy is considered) collected from various provinces of Italy. Malençon & Bertault (1975) report eight species from Morocco, Algeria and Tunisia.

In conclusion, the biodiversity of *Crepidotus* in Greece includes, to date, 12 taxa in total. A survey of all species recorded in Greece is given in Table 1, where both published and unpublished data are included. Five taxa are newly recorded from Greece: *C. applanatus* var. *subglobiger, C. autochthonus, C. lundellii, C. luteolus* and *C. subverrucisporus*. Other five taxa are recorded on new substrates for Greece: *C. calolepis* on trunks and branches of *Abies cephalonica, Abies borisii-regis* and *Pinus nigra, C. cesatii* var. *cesatii* on twigs and branches of *Platanus orientalis* and *Pinus nigra, C. epibryus* on leaves of *Castanea sativa*, and *C. variabilis* on twigs and branches of *Cistus* sp., *Quercus frainetto* and *Q. coccifera*. Moreover, *C. calolepis* and *C. mollis* are encountered for the first time in Europe on conifers, *C. calolepis, C. lundellii, C. luteolus* and *C. subverrucisporus* on *Platanus, C. autochthonus* in *Quercus* forests and *C. variabilis* on *Cistus*.

## Acknowledgments

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Table 1. A survey of *Crepidotus* species recorded from Greece.
(*) = first record of species and/or its substrate for Greece, (♦) = first record of substrate for Europe.

| Species | Substrate | Habitat | Publication |
|---|---|---|---|
| *C. applanatus* | fallen branches of *Fagus moesiaca* | forest of *F. moesiaca* | Diamandis 1992 |
| | fallen trunks of *Abies borisii-regis* | mixed forest of *A. borisii-regis* and *Pinus nigra* | Diamandis & Perlerou 1990 |
| *C. applanatus* var. *subglobiger* | fallen trunk of *Picea abies* | forest of *Picea abies* | this work (*) |
| *C. autochthonus* | soil | clearing of forest of *Quercus frainetto* (♦) | this work (*) |
| | soil | mixed forest of *Q. frainetto* & *A. borisii-regis* (♦) | this work (*) |
| *C. calolepis* | fallen twigs of *Pinus* sp. | forest of *Pinus* sp. | this work |
| | fallen branches of conifers | mixed forest of *P. nigra* & *Abies cephalonica* | this work |
| | stump of *A. cephalonica* (♦) | mixed forest of *A. cephalonica* & *P. nigra* | this work |
| | fallen twigs of *Platanus orientalis* (♦) | mixed forest of *A. cephalonica* & *Pl. orientalis* (sporadically) | this work |
| | fallen branches of conifers | mixed forest of *P. nigra* & *Abies* sp. | this work |
| | fallen branches of hardwoods | mixed forest of *Castanea sativa* & *Quercus* sp. | this work |
| | living trunk of *Pl. orientalis* (♦) | riparian vegetation | this work |
| | base of trunk of *Pl. orientalis* (♦) | mixed forest of *Pl. orientalis*, *Quercus* sp. & *C. sativa* | this work |
| | fallen twigs of *Pl. orientalis* (♦) | | this work |
| | fallen twigs of *A. borisii-regis* (♦) | mixed forest of *A. borisii-regis* & *Pl. orientalis* (sporadically) | this work |
| | branches of *Pl. orientalis* (♦) | mixed forest of *Pl. orientalis*, *Q. frainetto*, *C. sativa* and *A. borisii-regis* | this work |
| | fallen branches of *P. nigra* (♦) | forest of *P. nigra* | this work |
| | living branches of *Pl. orientalis* (♦) | forest road | this work |
| | fallen trunk of *Pl. orientalis* (♦) | mixed forest of *A. borisii-regis* and *Pl. orientalis* | this work |
| | dead trunk of *Platanus* sp. | | Maire & Politis 1940 |
| | stump of *Pinus halepensis* | | Maire & Politis 1940 |
| | wood of *Pl. orientalis* (♦) | | as *C. mollis*; Pantidou 1991 |
| | wood of *Fagus sylvatica* | | as *C. mollis*; Zervakis et al. 1998; Dimou et al. 2002a |
| | | | as *mollis* var. *calolepis*; pers. com., Konstantinidis |
| | fallen branches of *Alnus glutinosa* | | as *C. mollis*; pers. com., Dimou |
| | dead trunk of *Pl. orientalis* (♦) | | as *C. mollis* var. *calolepis*; pers. com., Dimou |
| *C. cesatii* var. *cesatii* | living trunk of *Pl. orientalis* (*) | riparian vegetation | this work |
| | fallen twigs of *Pl. orientalis* (*) | riparian vegetation | this work |
| | fallen twigs of *Pl. orientalis* (*) | mixed forest of *Pl. orientalis*, *Quercus* sp. & *C. sativa* | this work |
| | twigs of *P. nigra* (*) | mixed forest of *P. nigra* & *A. borisii-regis* | this work |
| | dead twigs and branches of *Pl. orientalis* (*) | mixed forest of *A. borisii-regis* and *Pl. orientalis* | this work |
| | branches of *Pl. orientalis* (*) | mixed forest of *Pl. orientalis*, *Q. frainetto*, *C. sativa* and *A. borisii-regis* | this work |
| | living branches of *Pl. orientalis* (*) | forest road | this work |
| | dead branches of *Pl. orientalis* (*) | mixed forest of *A. borisii-regis* and *Pl. orientalis* | this work |
| | fallen twigs & leaves of *Fagus* sp. | mixed forest of *P. nigra* & *A. borisi-regis* | as *Dochniopus sphaerosporus*; Maire & Politis 1940 |
| | wood of *F. sylvatica* | | as *C. sphaerosporus*; Dimou et al. 2002a |
| | wood of *Pl. orientalis* (*) | | pers. com., Dimou |
| | hardwoods | | pers. com., Dimou |

| Species | Substrate | Habitat | Publication |
|---|---|---|---|
| *C. cinnabarinus* | branches of *Alnus glutinosa* | | Dimou et al. 2002b |
| | wood of *Pl. orientalis* | riparian vegetation | pers. com., Diamandis |
| *C. epibryus* | fallen leaves of *C. sativa* (*) | mixed forest of *Pl. orientalis*, *Quercus* sp. & *C. sativa* | this work |
| | fallen leaves of *Fagus* sp. | | as *C. perpusillus*; Maire & Politis 1940 |
| | | | Polemis et al 2002 |
| *C. lundellii* | twigs of *Pl. orientalis* (♦) | riparian vegetation | this work (*) |
| | branches of *Pl. orientalis* (♦) | mixed forest of *Pl. orientalis*, *Q. frainetto*, *C. sativa* and *A. borisii-regis* | this work (*) |
| | branches of *Alnus glutinosa* | | pers. com., Dimou |
| *C. luteolus* | twigs of *Pl. orientalis* (♦) | mixed forest of *Pl. orientalis*, *Quercus* sp. & *C. sativa* | this work (*) |
| | *Nerium oleander* | | pers. com., Dimou |
| *C. mollis* | fallen trunk of *A. borisii-regis* (♦) | forest of *A. borisii-regis* | this work |
| | branches of *Pl. orientalis* | mixed forest of *Pl. orientalis*, *Q. frainetto*, *C. sativa* and *A. borisii-regis* | this work |
| | living trunk and branches of *Pl. orientalis* | mixed forest of *A. borisii-regis* and *Pl. orientalis* | this work |
| | dead branches of *Pl. orientalis* | mixed forest of *A. borisii-regis* and *Pl. orientalis* | this work |
| | old trunks of *Quercus* sp. | | Diapoulis 1939 |
| | stump of *Platanus* sp | | Maire & Politis 1940 |
| | | | pers. com., Konstantinidis |
| | hardwoods | | pers. com., Dimou |
| *C. subverrucisporus* | fallen trunk of *Pl. orientalis*(♦) | riparian vegetation | this work (*) |
| | mossy branches of *Pl. orientalis* (♦) | mixed forest of *Pl. orientalis*, *Q. frainetto*, *C. sativa* and *A. borisii-regis* | this work (*) |
| *C. variabilis* | twigs of *Q. frainetto* (*) | forest of *Q. frainetto* | this work |
| | twigs of *Quercus coccifera* (*) | maquis vegetation | this work |
| | twigs of *Cistus* sp. (♦) | maquis vegetation | this work |
| | dead twigs of *Abies* sp. | | as *Dochmiopus variabilis*; Maire & Politis 1940 |
| | fallen branches of *Fagus moesiaca* | | Avtzis & Diamandis 1988; Diamandis 1992 |
| | dead twig | | Minter 1988 |
| | wood of deciduous trees & shrubs | | Konstantinidis 2002 |
| | branches of *Alnus glutinosa* | | pers. com., Dimou |
| | branches of *Q. coccifera* | | pers. com., Dimou |
| | wood of *C. sativa* | forest of *C. sativa* | pers. com., Diamandis |
| | wood of *A. borisii-regis* | mixed forest of *A. borisii-regis* and *P. nigra* | pers. com., Diamandis |
| | wood of *C. sativa* | forest of *C. sativa* | pers. com., Diamandis |
| | wood of *Q. frainetto* (*) | forest of *Q. frainetto* | pers. com., Diamandis |
| | wood of *Quercus* sp. | boundaries of plantation of *P. nigra* | pers. com., Diamandis |
| | wood of *A. borisii-regis* | forest of *A. borisii-regis* | pers. com., Diamandis |

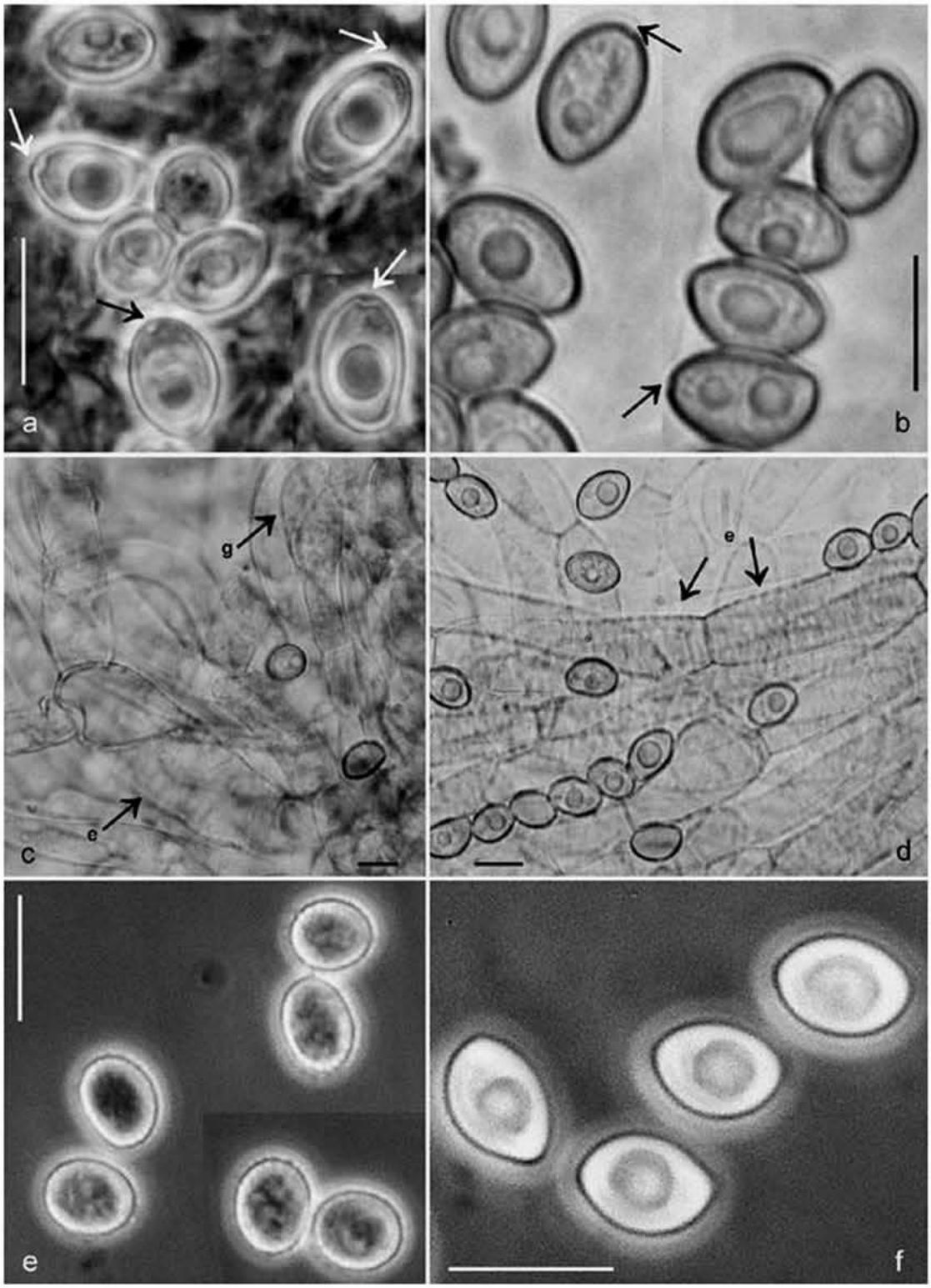


**Fig. 9. a–b.** Thick-walled basidiospores with apical wall curving and small depression (arrows); **c.** pale yellow hyphae of the pileipellis with granular (g) or minutely encrusting (e) pigment; **d.** yellow-brown hyphae of the pileipellis with strongly encrusting zebra-like pigment (e): **a, c.** *C. mollis* (ATHU–M 5123); **b, d.** *C. calolepis* (Dimou 731). **e–f.** Basidiospores: **e.** *C. cesatii* var. *cesatii* (ATHU–M 5109, 5125); **f.** *C. autochthonus* (ATHU–M 5121). Scale bars = 10 μm.

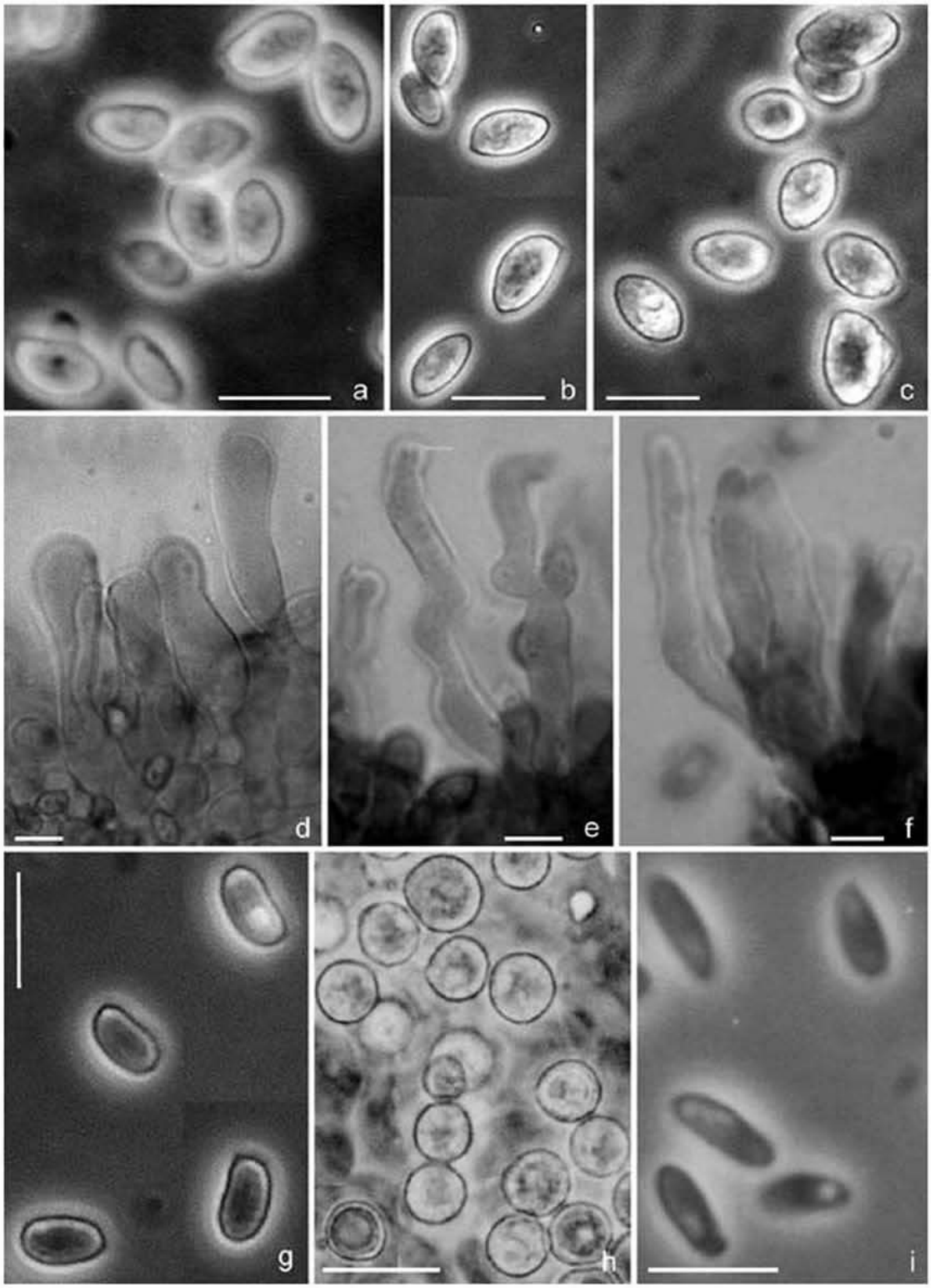


**Fig. 10. a–c.** Basidiospores; **d–f.** Cheilocystidia: **a, d.** *C. lundellii* (ATHU–M 5119); **b, e.** *C. luteolus* (ATHU–M 5110); **c, f.** *C. subverrucisporus* (ATHU–M 5120). **g–h.** Basidiospores: **g.** *C. variabilis* (ATHU–M 5112); **h.** *C. applanatus* var. *subglobiger* (ATHU–M 5332); **i.** *C. epibryus* (ATHU–M 5106). Scale bars = 10 μm.

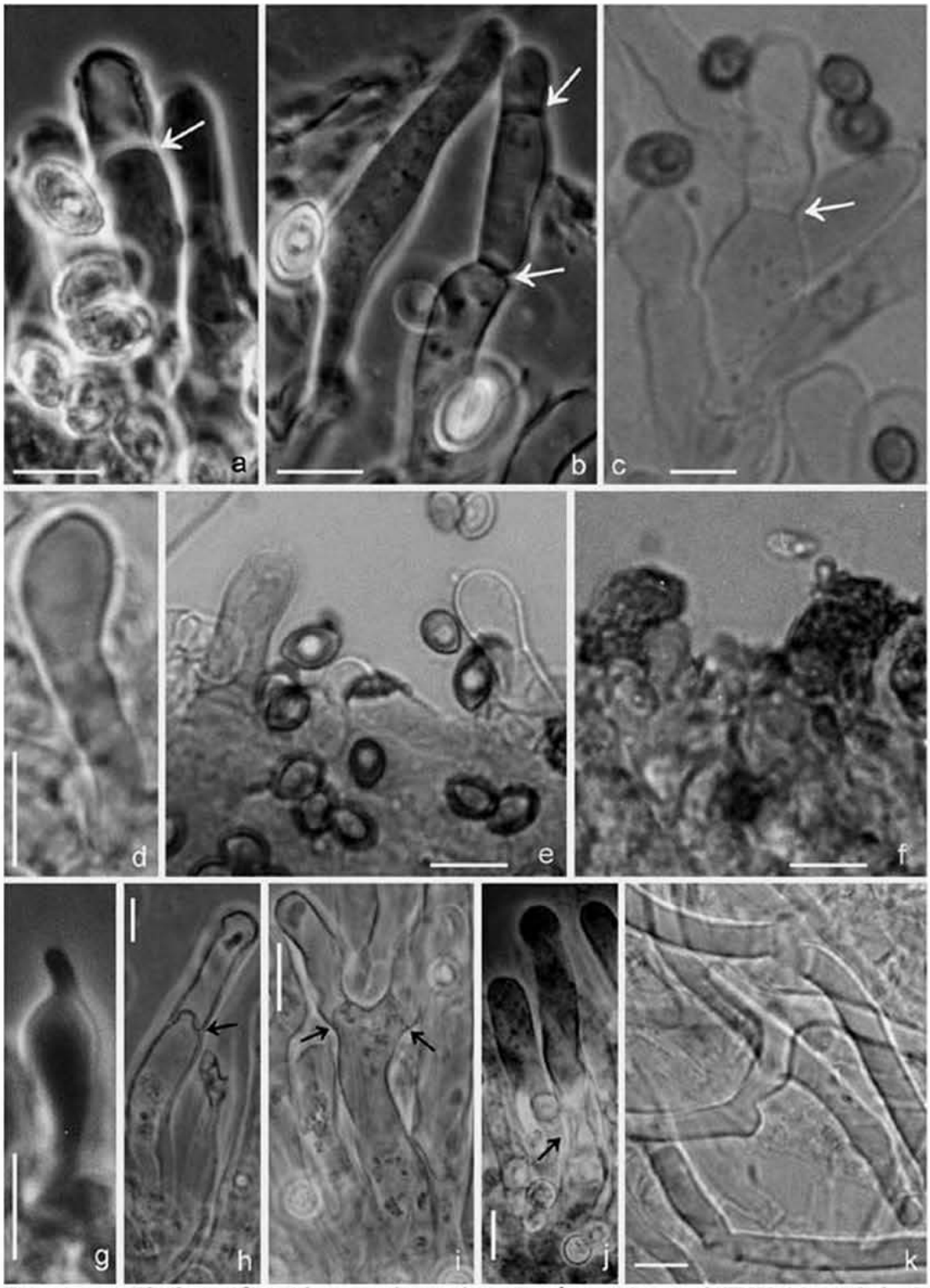


**Fig. 11. a–c.** Cheilocystidia with septae (arrows): **a.** *C. subverrucisporus* (ATHU–M 5120); **b.** *C. mollis* (ATHU–M 5123); **c.** *C. autochthonus* (ATHU–M 5121). **d.** Yellowing basidiole: *C. mollis* (ATHU–M 5123). **e.** Yellowing, thick-walled cheilocystidia: *C. autochthonus* (ATHU–M 5121). **f.** Disintegrating basidiole and basidium with yellow-brown, strongly granular content: *C. epibryus* (ATHU–M 5106). **g.** Pleurocystidioid body: *C. calolepis* (ATHU–M 5105). **h–j.** Septate, branched and thick-walled cheilocystidia: *C. applanatus* var. *subglobiger* (ATHU–M 5332). **k.** Secretory hyphae in the lamellar trama: *C. mollis* (ATHU–M 5123). Scale bars = 10 μm.

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MYCOTAXON

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# The lichen flora of the Termessos National Park in Southwestern Turkey

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**Abstract** – Between March 2002 and 2003, the lichen flora of the Termossos National Park was studied for the first time. In all, 161 taxa (152 species, 4 subspecies, 5 varieties) were determined from 1114 lichen samples, of which 86 were new to Antalya Province and 5 were new to Turkey. The complete checklist can be downloaded as PDF file from www.mycotaxon.com/resources/weblists/html.

**Key words** – lichens, Güllük Mountain, Antalya

## Introduction

The number of studies on the lichen flora of Turkey has increased significantly over the last two decades (e.g., Aslan 2000, Breuss & John 2004, Çiçek & Özdemir Türk 1998, Güvenç et al. 1996, Özdemir Türk & Güner 1998, Öztürk & Güvenç 2003). Although the lichen flora of the Mediterranean phytogeographical region of Turkey has received more attention than the other regions of Turkey (John 1996, 2003; John & Nimis 1998; Nimis & John 1998; John et al. 2000), even this area needs additional research. To determine the lichen flora of the region, floristic studies that focus on small areas with high biodiversity are needed.

Throughout the Mediterranean region of Turkey, there are ruins and cities from the ancient civilizations, but until now no lichen floristic or biodeterioration study has been published from such places. Termessos (Güllük Mountain) National Park, located on the West side of the Taurus Mountains in Antalya province, southwestern Turkey, is such a site. It is famous for its ancient city, Termessos, which is situated on a natural platform at the top of Güllük Mountain, which has been formed by chemical erosion and tectonic movements. It includes a canyon with very steep walls as high as 500-600 m.

## Material and Methods

The research is based on 1114 lichen samples, which were collected from 54 localities (Table 1) in Termessos National Park between March 2002 and September 2003. In every locality coordinates and altitude were measured by GPS (Garmin 12X) and all lichen samples were taken together with their substratum. The samples were brought the laboratory and air-dried under room conditions (25 ± 2 °C, RH 60±10).

For identification, macroscopic and microscopic characters were examined with stereo- and light microscopes and by reference to recent literature (e.g. Wirth 1995, Purvis et al. 1992, Clauzade & Roux 1985, Giordani et al. 2002, Jørgensen 1997, Zeybek et al. 1993, Breuss 1990, Moberg 1977). Following identification, the lichens were deposited in Akdeniz University Herbarium (AKDU).

## Results

A PDF file containing the list of lichen species (including locality numbers and substrata) encountered in Termessos National Park, table of localities, and map of the study area can be downloaded from **http://www.mycotaxon.com/resources/ weblists.html.** The abbreviations of authors are in accordance with Brummitt & Powell (1992). Lichen taxa new to Turkey are indicated by *, those new to Antalya province by #.

## Discussion

This study reports 161 taxa from Termessos National Park, of which *Collema conglomeratum*, *Lecania inundata*, *Leptogium furfuraceum*, *Peltigera monticola* and *Physconia servitii* are new to Turkey and 86 are new to Antalya province. Although the lichen flora of the Mediterranean region of Turkey is reasonably well studied, it is quite remarkable to find still so many new lichen records for the region as well as some new to the country, emphasizing that much more explorative effort should be made on its lichen flora.

In the study area, in addition to common lichen species for the Mediterranean Region, such as *Lecanora bolcana* and *Diploschistes ocellatus*, we determined "manna" lichens, such as *Aspicilia desertorum* and *A. hispida*, which usually grow in steppes, suboceanic species such as *Degelia plumbea* and *Staurolemma omphalarioides*, and oceanic species, such as *Collema furfuraceum* and *C. nigrescens*. Although the study area is relatively small (ca. 6702 ha), the wide variation in its topology, formed by high mountains, valleys and a deep canyon, evidently provides habitats for a rich lichen biodiversity.

Calcareous species are dominant due to the widespread occurrence of calcareous rocks throughout the study area, and because of the frequency of trees with acidic bark, such as *Pinus nigra* and *Quercus coccifera*, most of the epiphytic species are acidophytic.

Of particular note are *Anthracocarpon virescens*, *Caloplaca adriatica*, *Hypocenomyce anthracophila*, *Neocatapyrenium rhizinosum*, *Pertusaria hymenea*, *Placidium pilosellum*, *Solenopsora liparina* and *Staurolemma omphalarioides*, for which there are only one or two records from Turkey (Pisut 1970; Breuss 1998; Nimis & John 1998; John et al. 2000; John 1996, 2003; Breuss & John 2004).

On the ruins of the ancient city of Termessos, not only species with a wide ecological amplitude, such as *Aspicilia calcarea*, *Caloplaca aurantia*, *Lecanora muralis*, *Lobothallia radiosa*, *Placynthium nigrum* and *Xanthoria elegans* were found, but also species mainly found in the Boreal-Mediterranean region and between the south of Central Europe and the Mediterranean Region such as *Aspicilia farinosa*, *Caloplaca chrysodeta*, *C. xantholyta*, *Collema cristatum*, *Diploschistes ocellatus*, *Lepraria nivalis*, *Solenopsora candicans* and

*Solenopsora liparina* (Wirth 1995). Because the ancient city was built from local stones, the species on the ruins are similar to the lichen flora found elsewhere in the study area.

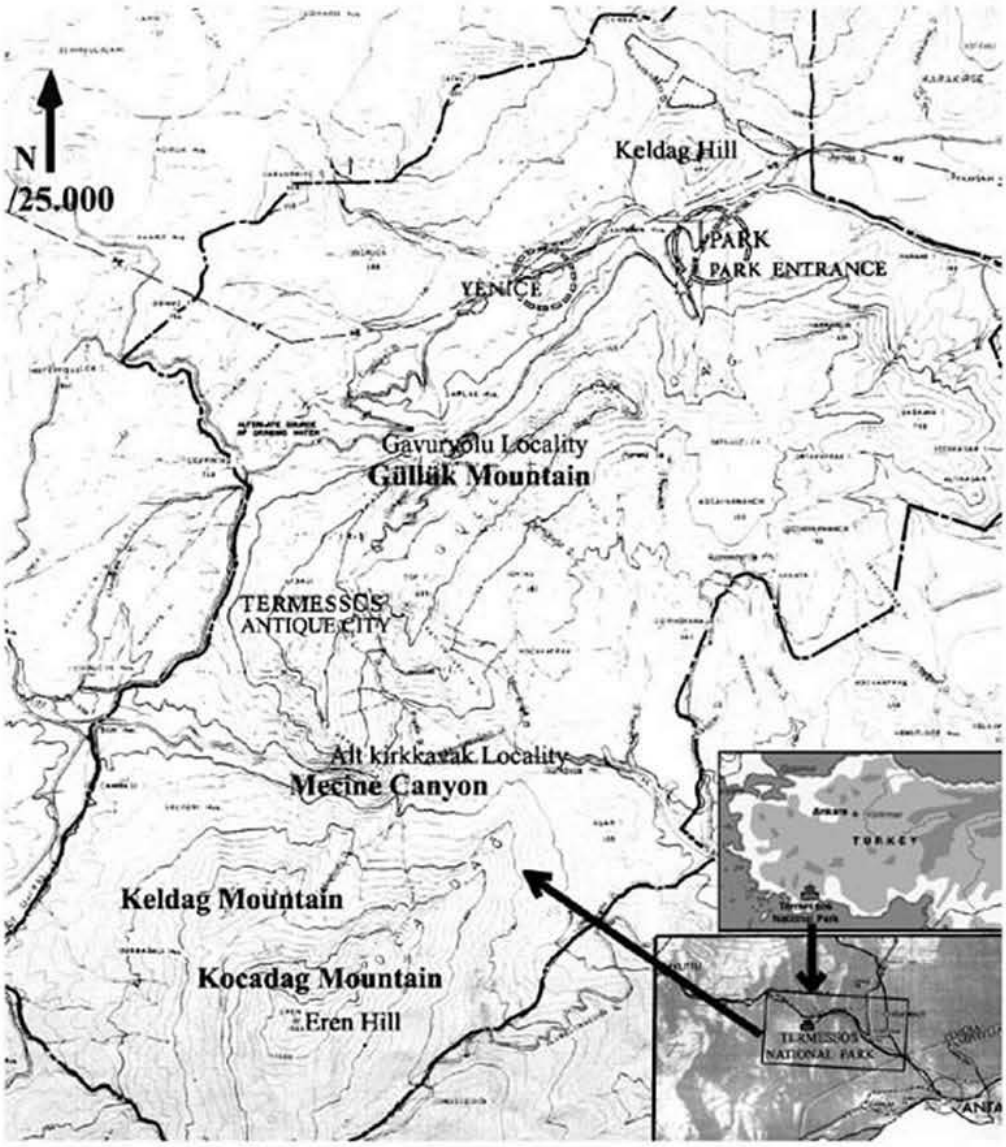


Figure 1: Map of Termessos National Park

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# Two new species of *Anthracoidea* (*Ustilaginales*) from China

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**Abstract**—Two new species, *Anthracoidea setosae* on *Carex setosa* and *A. xizangensis* on *Kobresia duthiei*, are described and compared with *A. misandrae* and related species.

**Key words**—smut fungi, Ustilaginomycetes, taxonomy

A new species of *Anthracoidea* on *Carex setosa* (Subgen. *Carex*, Sec. *Frigidae*) was discovered from our herbarium (HMAS 67908, 34929, 34930). These specimens were wrongly identified by the author (Guo 1994) as *A. misandrae*. The new species of *Anthracoidea* is similar to *A. misandrae* and *A. sempervirentis* Vánky on host plants in the same section of *Carex* in having ustilospores of the same size. It differs mainly from *A. misandrae* by ustilospores with minute warts measuring 0.125-0.3 µm in diam., while *A. misandrae* has ustilospores with larger warts [(0.2-)0.4-0.8(-1) µm in diam. (Kukkonen 1963: 83)]. It differs mainly from *A. sempervirentis* by ustilospores with regularly distributed warts as seen by SEM (scanning electron microscopy), while *A. sempervirentis* has ustilospores with a "surface by SEM partly with sparse, 0.1-0.2 µm high knobs, partly with abundant and dense, up to 0.5 µm high, irregular, often confluent, rounded warts." (Vánky 1979: 226). The new species is described as:

***Anthracoidea setosae*** L. Guo, **sp. nov.** **Figs. 1-2**

*Sori in ovariis, subglobosi vel ovoidei, 1.2-2 mm longi, 1-1.8 mm lati, primum membrana cinerascenti, fungali cooperti, deinde expositi. Massa sporarum nigra, semiagglutinata. Ustilosporae a fronte globosae, ellipsoideae, leviter irregulares vel irregulares, 17.5-25(-27) x 12.5-20 µm, ab acie 10-15 µm latae, flavidobrunneae vel atrobrunneae; pariete aequaliter vel inaequaliter incrassato, 1-2.5(-3) µm crasso, tumores interni desunt, regiones lucem repercutientes desunt, superficie minute et dense verruculoso sub SEM.*

Sori in ovaries, subglobose or ovoid, 1.2-2 mm long and 1-1.8 mm wide, at first covered by a grayish, fungal membrane, later becoming exposed. Spore mass black, semi-agglutinated. Ustilospores in plane view globose, ellipsoidal, slightly irregular or irregular, 17.5-25(-27) x 12.5-20 µm, in side view 10-15 µm wide, yellowish-brown or blackish-brown; wall evenly or unevenly thickened, 1-2.5(-3) µm, no internal swellings, no light reflective areas, surface minutely and densely verruculose as seen by SEM.

On *Carex setosa* Boott (*Cyperaceae*, Subgen. *Carex*, Sect. *Frigidae*), Gansu: Zhouqu, Shatanlinchang, alt. 3050 m, 4 IX 1992, L. Guo 1276, HMAS 67908 (holotypus hic

designatus); Sichuan: Emei Shan, Leidongping, alt. 2500 m, 10 VII 1969, C. M. Wang, Y. X. Han & Q. M. Ma 314, HMAS 34930 (paratypus); Emei Shan, Jindingsi, alt. 3150 m, 9 VII 1969, C. M. Wang, Y. X. Han & Q. M. Ma 296, HMAS 34929 (paratypus).

*Etymology*: Refers to the host plant *Carex setosa*.

*Anthracoidea misandrae* was discovered in the Herbarium of the Institute of Botany, Chinese Academy of Sciences (PE) and collected by Prof. Kuan Kechien from Xinjiang Uygur Autonomous Region in 1957. It is described as:

***Anthracoidea misandrae*** Kukkonen, Ann. Bot. Soc. Zool.-Bot. Fenn. „Vanamo" 34(3): 82, 1963. **Figs. 3-4**

Sori in ovaries, ellipsoidal, 2.5-3.5 mm long and 1.5-2.5 mm wide, at first covered by a grayish, fungal membrane, later becoming exposed. Spore mass black, semi-agglutinated. Ustilospores in plane view subglobose, ellipsoidal or ovoid, 17.5-25(-26) x 15-20(-22) µm, in side view 10-14 µm wide, dark reddish-brown; wall evenly thickened, ca. 1 µm, no internal swellings, no light reflective areas, surface verrucose.

On *Carex stenocarpa* Turcz. ex V. Krecz. (*Cyperaceae*, Subgen. *Carex*, Sect. *Frigidae*), Xinjiang: Nilka Xian, 60 Km N of Wulasitai (in the Borohoro Mountains) 31 VIII 1957, K. C. Kuan 3991, HMAS 132710.

Another new species of *Anthracoidea* on *Kobresia duthiei* (Sec. *Elyna*) was discovered from our herbarium (HMAS 67973) and collected by Prof. Zhuang Jianyun in 1990. The specimen was wrongly identified by the author as *A. filifoliae* L. Guo (1995-1996) on the section *Kobresia*. The new species differs from *A. filifoliae* by minute warts on the surface of the ustilospore as seen by SEM and host plants in different sections of the genus *Kobresia*, while *A. filifoliae* has dense and minute warts between the larger warts on the surface of the ustilospores. Only *A. elynae* (Syd.) Kukkonen (1963: 65) has been recorded previously on the section *Elyna*. The new species differs from *A. elynae* by having warts on the surface of the ustilospores, while the surface of the ustilospores of *A. elynae* is smooth. The new species is described as:

***Anthracoidea xizangensis*** L. Guo, **sp. nov.** **Figs. 5-6**

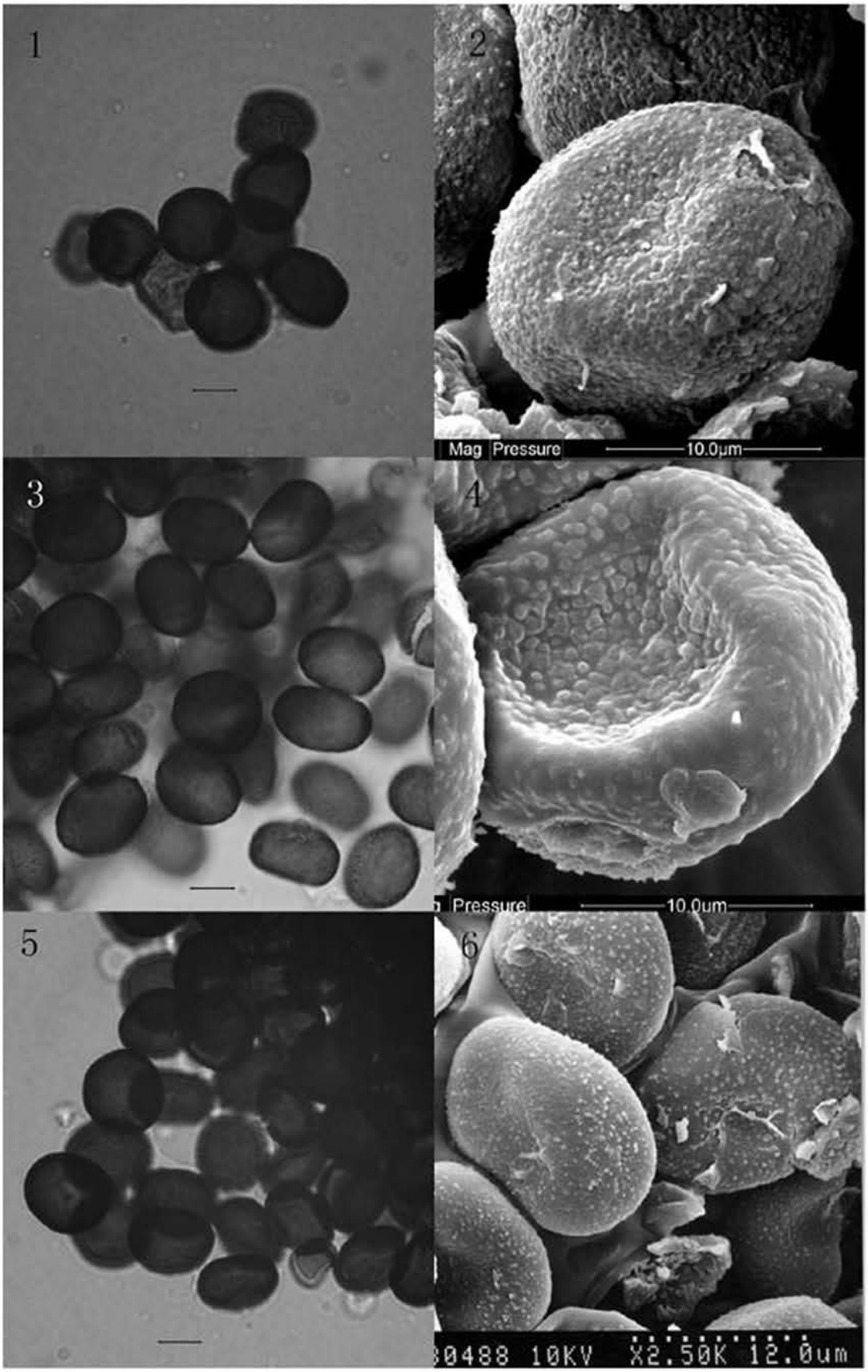
*Sori in ovariis, ellipsoidei vel ovoidei, 1-2 mm longi, 0.7-1 mm lati, primum membrana cinerascenti, fungali cooperti, deinde expositi. Massa sporarum nigra, semiagglutinata. Ustilosporae a fronte globosae, ellipsoideae vel ovoideae 17-22.5 x 15-18 µm, ab acie 10-15 µm latae, atrobrunneae; pariete aequaliter incrassato, 1.5-2 µm crasso, tumores interni desunt, regions lucem repercutientes desunt, superficie minute et dense verruculoso sub SEM.*

Sori in ovaries, ellipsoidal or ovoid, 1-2 mm long and 0.7-1 mm wide, at first covered by a grayish, fungal membrane, later becoming exposed. Spore mass black, semi-

Figs. 1-2. Ustilospores of *Anthracoidea setosae* on *Carex setosa* as seen by LM (light microscopy) and SEM (HMAS 67908, holotypus).

Figs. 3-4. Ustilospores of *Anthracoidea misandrae* on *Carex stenocarpa* as seen by LM and SEM (HMAS 132710).

Figs. 5-6. Ustilospores of *Anthracoidea xizangensis* on *Kobresia duthiei* as seen by LM and SEM (HMAS 67973, holotypus). Bars = 10 µm


1
2
Mag Pressure
10.0µm
3
4
Pressure
10.0um
5
6
0488 10KV X2.50K 12.0um

agglutinated. Ustilospores in plane view globose, ellipsoidal, or ovoid, 17-22.5 x 15-18 µm, in side view 10-15 µm wide, blackish-brown; wall evenly thickened, 1.5-2 µm, no internal swellings, no light reflective areas, surface minutely and densely verruculose as seen by SEM.

On *Kobresia duthiei* C. B. Clarke (*Cyperaceae*, Sect. *Elyna*), Xizang: Dinggye, Yala Shan, alt. 4950 m, 15 VIII 1990, J. Y. Zhuang 2919, HMAS 67973 (holotypus hic designatus).

*Etymology*: Refers to the locality Xizang Autonomous Region (Tibet).

Since 2000 (Guo 2000), eight new species and three new records of the genus *Anthracoidea* have been recorded in China (Guo 2002, 2004, Guo & Wang 2005, Guo & Zhang 2004, Wang & Piepenbring 2002, Zhang & Guo 2004), including *A. setosae* and *A. xizangensis* (in this paper).

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# A new species of *Lepiota* (*Agaricaceae*, Basidiomycetes) from China

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**Abstract**—A new species, *Lepiota catenariocystidiata*, is described and illustrated. It is compared with similar species.

**Key words**—*Agaricales*, taxonomy

## Introduction

During our study of lepiotaceous fungi, we came across an undescribed species of the genus *Lepiota* (Pers.: Fr.) Gray. It is described and illustrated herein. In descriptions of the basidiomata, color designations (e.g., 1A1) are from Kornerup & Wanscher (1981), and color names with first letters capitalized (e.g., Pale Smoke Gray) are from Ridgway (1912). In descriptions of basidiospores, the notation [*n*/*m*/*p*] shall mean *n* basidiospores measured from *m* basidiomata of *p* collections in Melzer's reagent. *Q* is used to mean quotient of length and width of a spore in side view; **Q** means average *Q* of all basidiospores ± sample standard deviation. Herbarium code HKAS = Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences.

## Taxonomy

***Lepiota catenariocystidiata*** Han C. Wang & Zhu L. Yang, **sp. nov.** **Figs. 1-4**

*Pileus 3-5 cm latus, initio subcampanulatus, deinde convexus vel applanatus, albidus vel griseolus, squamulis tomentosis, griseis vel obscure griseis. Lamellae liberae, albidae vel cremeae. Stipes 4-6 × 0.3-0.6 cm, subcylindricus, albidus, annulatus, squamulis confertis,*

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*tomentosis, griseis infra annulum. Annulus superus, membranaceus. Basidiosporae (7.0) 7.5-9.0 (10.0) × (3.5) 4.0-4.5 (5.0) µm, oblongae vel subcylindricae, incoloratae, hyalinae, dextrinoideae. Basidia 20-30 × 7.5-10 µm, subclavata, 4-sporigera, raro 2-sporigera. Pleurocystidia nulla. Cheilocystidia 10-20 × 8-12 µm, subglobosa, ovata vel breviclavata, catenaria, incolorata. Squamulae pilei ex hyphis subcylindricis terminalibus compositae. Fibulae praesentes.*

**Holotype:** CHINA, Yunnan Prov., Mengla County, Menglun, 2. XI. 1989, Z.L. Yang 918 (HKAS 22145).

**Etymology:** Named because of the cheilocystidia often in chains.

*Basidiomata* (Fig. 1) scattered. *Pileus* 3-5 cm in diam, at first subcampanulate, then convex to applanate, with an obtuse umbo or non-umbonate; pileal surface dry, whitish to grayish (1A1-1B1; Pale Smoke Gray) but with pinkish tinge (11A2-11B2; Light Brownish Drab) at center, densely covered with minute, dark gray (11C1 + 11D1 + 11E1; Dark Neutral Gray to Blackish Slate) tomentose squamules over disc, with small, concentrically arranged, grey to dark grey squamules towards the margin, margin often slightly exceeding lamellae. *Lamellae* free, whitish to cream-coloured, moderately crowded, in 2-3 ranks, up to 0.7 cm broad, with white to concolorous eroded edge. *Stipe* 4-6 × 0.3-0.6 cm, central, subcylindrical, hollow, slightly enlarged near base, surface whitish, lower part covered with grey (11C1 + 11D1; Dark Neutral Gray to Hair Brown) tomentose squamules often in belts. *Annulus* superior, membranous, upper surface white and glabrous, lower surface covered with grey tomentose squamules, persistent or fugacious. *Context* whitish, unchanging; odor indistinct; taste slightly hot.

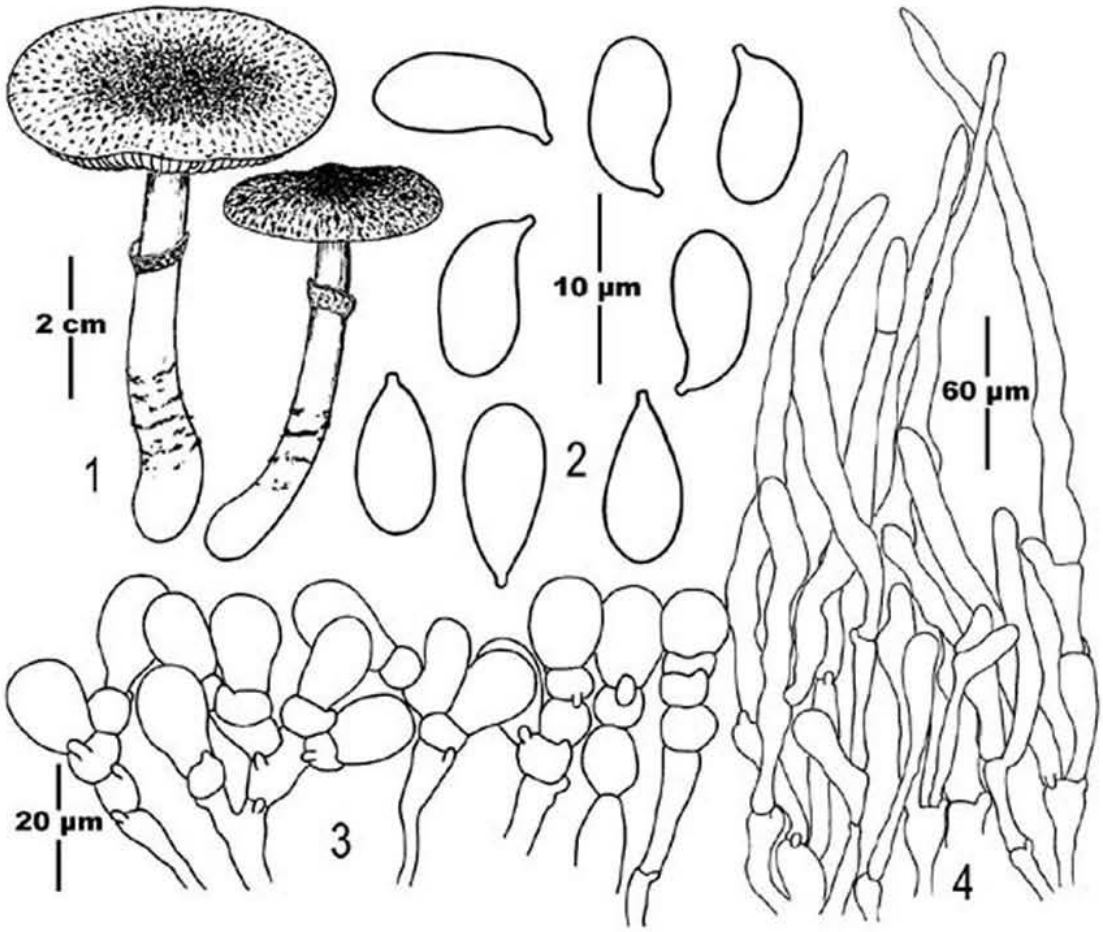
*Basidiospores* (Fig. 2) [42/2/2] (7.0) 7.5-9.0 (10.0) × (3.5) 4.0-4.5 (5.0) µm [Q = (1.67) 1.75-2.25, Q = 2.01 ± 0.18], oblong to subcylindrical, with a distinct suprahilar depression in side view, water-drop-shaped in front view, broader at apical part, attenuate towards the base, slightly thick-walled, colorless, hyaline, dextrinoid, reddish in Congo Red, not metachromatic in Cresyl Blue. *Basidia* 20-30 × 7.5-10 µm, subclavate, hyaline, thin-walled, 4-spored, rarely 2-spored. *Pleurocystidia* absent. *Cheilocystidia* (Fig. 3) abundant, 10-20 × 8-12 µm, subglobose, ovoid to short clavate, often in chains, colorless and hyaline. *Squamules* (Fig. 4) on pileus a disrupted trichodermium consisting of loose fascicles of long, more or less erect, subcylindrical, terminal elements (45-300 × 10-17 µm) with tapering or round apex and pale yellow-brown to dark yellow-brown intracellular pigments; with short clavate cells at the base of these long elements; the repent hyphae at the base of erect elements sometimes with incrusting pigments. Squamules on surface of stipe similar to those on pileus. *Clamp connections* abundant in basidiomata.

**Habitat:** On well-rotten wood with soil in limestone monsoon forests; fruiting in summer and autumn in southwestern China at 600–700 m elev.

**Known distribution:** Known from tropical Yunnan only.

**Additional material examined:** CHINA, Yunnan Prov., Mengla County, Menglun Nature Reserve, 22. X. 1988, Zang 11515 (HKAS 20358).

**Notes:** *Lepiota catenariocystidiata* is well characterized by its whitish to grayish pileus with a pinkish center, gray to dark gray squamules on the pileus made up of a disrupted trichodermium with long subcylindrical terminal elements and short clavate cells at the base of these long elements, water-drop-shaped basidiospores in front view, catenulate cheilocystidia and the common presence of clamp connections in the basidiomata.




Figs. 1-4. *Lepiota catenariocystidiata* (from holotype)
1. Basidiomata. 2. Basidiospores. 3. Cheilocystidia. 4. Squamules on pileus

Due to the trichodermium type of the squamules on the pileus and the oblong to subcylindrical basidiospores, *L. catenariocystidiata* may belong to *Lepiota* sect. *Ovisporae* (J.E. Lange) Kühner. Species with pileal squamules made up of long, erect elements and short, clavate elements in between were put in the subsect. *Felininae* Bon within sect. *Ovisporae* (Vellinga 2001). Because *L. catenariocystidiata* has short, clavate elements at the base of long, erect ones, it should be placed in the subsection. However, according to recent molecular phylogenetic studies, the ITS data set does not support sect. *Ovisporae*, and thus a re-evaluation of this section is needed (Vellinga 2003). The present species may be clustered within the Clade 1 of *Lepiota* s. l. (Vellinga 2003).

*Lepiota catenariocystidiata* may be related to *L. felina* (Pers.) P. Karst., *L. pseudolilacea* Huijsman and *L. pseudohelveola* Kühner ex Hora. However, the latter two species have differently coloured basidiomata, ellipsoid to oblong basidiospores in front view and rarely with a suprahilar depression in side view, and differently shaped cheilocystidia (Kühner 1936; Huijsman 1947; Hora 1960; Enderle & Krieglsteiner 1989; Bon 1981, 1996; Candusso & Lanzoni 1990). According to Vellinga (2001), *L. pseudohelveola* should be regarded as a synonym of *L. pseudolilacea*. *Lepiota felina* differs from *L. catenariocystidiata* in the colour of the basidiomata, the shape of the cheilocystidia and

basidiospores (Kühner 1936; Huijsman 1947; Hora 1960; Bon 1981, 1996; Enderle & Krieglsteiner 1989; Candusso & Lanzoni 1990; Vellinga 2001).

The basidiospores of *L. catenariocystidiata* are very similar to those of *L. plumbicolor* (Berk. & Broome) Sacc., originally described from Sri Lanka. However, the latter has blackish purple squamules on pileus with elongate clavate terminal elements with an obtusely rounded apex and clavate-cylindrical cheilocystidia (Pegler 1972, 1986).

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# ITS sequence analysis and ascomatal development of *Pseudogymnoascus roseus*

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**Abstract**—ITS sequence analysis and ascomatal development of *Pseudogymnoascus roseus* strains isolated from sclerotia of *Cordyceps sinensis* collected from the Tibetan Plateau, China, are reported in this paper. The ITS sequences of three strains from different locations were identical and were compared with sequences obtained from the BLAST search in GenBank. The strains display the same morphology as the reference collection deposited in K, matching the species description of *P. roseus*. Ascomatal development of the *P. roseus* strains is described. Ascomata of *P. roseus* were found to comprise an aggregation of asci from several different ascomatal initials enveloped by a loose, thick-walled hyphal network. In the parsimony analysis, ITS sequences of *P. roseus* and other *Myxotrichaceae* grouped outside the *Onygenales* and clustered with those of discoid fungi. Members of *Myxotrichaceae* were considered closely related to discomycetes, but greatly diverged from onygenalean fungi. *Myxotrichaceae* did not form a monophyletic group in the ITS tree.

**Key words**—DNA, fungal culture, taxonomy

## Introduction

*Pseudogymnoascus* Raillo, a genus of Ascomycetes established with two species in 1929, has been referred to either *Gymnoascaceae* Baran. (e.g. Kuehn 1958, Arx 1971, Alexopoulos & Mims 1979, Orr 1979, Benny & Kimbrough 1980, Eriksson & Hawksworth 1986, 1993) or to *Onygenaceae* Berk. (Arx 1987). However, it was placed by Currah (1985) in *Myxotrichaceae* Locq. ex Currah, based on cellulose degradation capacity, smooth ascospores and rhexolytically dehiscing conidia. The latter taxonomic treatment has been widely accepted (Alexopoulos et al. 1996, Kirk et al. 2001). Species of this genus have yellow or rose, globose to subglobose, discrete or confluent ascomata; ascomatal peridium composed of a network of slightly thick-walled hyphae; appendages simple and not distinct; asci globose to ellipsoid, normally 8-spored; ascospores ellipsoid to fusoid, smooth, hyaline, yellow, orange to pink (Cejp & Milko 1966, Orr 1979, Currah 1985).

In addition to the original two species of *Pseudogymnoascus*, *P. roseus* and *P. vinaceus* Raillo, several more species have since been described, e.g. *P. caucasicus* Cejp & Milko,

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*P. bhattii* Samson, *P. alpinus* E. Müll. & Arx and *P. dendroideus* Locq.-Lin. However, Samson (1972) considered *P. roseus* and *P. vinaceus* identical and lectotypified the genus with *P. roseus*. Orr (1979) discussed the status of the genus and recognised two species, *P. vinaceus* and *P. roseus*, listing *P. bhattii* as a synonym of *P. vinaceus*. In his monographic study of *Onygenales*, Currah (1985) treated both *P. vinaceus* and *P. bhattii* as synonyms of *P. roseus*. Although Samson's (1972) choice of *P. roseus* as the type species has been followed by others (Arx 1974, 1981, 1987, Currah 1985, Sigler et al. 2000), the genus was also typified by *P. vinaceus* early (Kuehn 1958, Orr 1979).

As more species of *Pseudogymnoascus* were described, the range of morphological characters became more diverse. For example, *P. alpinus* was described as having navicular-fusoid ascospores, and ascomata with branched and recurved appendages (Müller & Arx 1982); *P. dendroideus* with ramified ascomatal appendages and striated ascospores (Locquin-Linard 1982); and *P. roseus* var. *ornatus* Udagawa & Uchiy. with irregularly lobate-reticulate ascospores (Udagawa & Uchiyama 1999). Further, a weakly cellulolytic species having fusoid ascospores with a longitudinal sigmoid crest was assigned to the genus as *Pseudogymnoascus* sp. (Lumley et al. 2000).

Anamorphs of *Pseudogymnoascus* have been referred to *Geomyces* Traaen (Orr 1979, Currah 1985). *Geomyces pannorum* var. *vinaceus* (Dal Vesco) Oorschot is considered as the anamorph of *P. roseus* (Sigler & Carmichael 1976, Oorschot 1980).

Systematically, *Myxotrichaceae* was placed in the order *Onygenales*, along with *Arthrodermataceae* Locq. ex Currah, *Gymnoascaceae* and *Onygenaceae*, by Currah (1985). Species of *Myxotrichaceae* are saprobic, cellulose-degrading, and usually inhabit forest soils and decay plant material. Members of *Gymnoascaceae* do not exhibit strong substrate preferences, and are neither keratinolytic nor cellulolytic. The *Arthrodermataceae* and the *Onygenaceae* degrade keratin and usually inhabit soils enriched with keratin or dung. Currah (1994) further restricted the *Onygenales* to keratinolytic genera and suggested that the *Myxotrichaceae* might represent a distinct evolutionary line from typical members of *Onygenales* and derive from the inoperculate discomycetes and that it merited a placement in its own order. The distant relationship between the *Myxotrichaceae* and other members of *Onygenales* has been confirmed by recent studies (Sugiyama et al. 1999, Mori et al. 2000, Kirk et al. 2001).

Recently, molecular approaches have been introduced to the systematic study of *Myxotrichaceae* and related fungi (Bowman & Taylor 1993, LeClerc et al. 1994, Hambleton et al. 1998, Sugiyama et al. 1999, Sugiyama & Mikawa 2001). Some molecular evidence indicates that *Myxotrichaceae* are distantly related to other members of *Onygenales* (Sugiyama et al. 1999, Mori et al. 2000). Sugiyama et al. (1999) examined the molecular systemtatics of onygenalean taxa (including *P. roseus* and members of *Amauroascaceae* Arx, *Arthrodermataceae*, *Gymnoascaceae* and *Onygenaceae*) based on 18S rDNA sequences and suggested that the *Myxotrichaceae* should be placed in an independent position among the *Helotiales* and the *Erysiphales*, on a different lineage from the keratin-degrading fungi. Based on sequences of 18S rDNA and partial sequences of 28S rDNA, Mori et al. (2000) demonstrated that the *Myxotrichaceae* was distantly related to the other onygenalean families, as a sister group to the *Erysiphales* and that the *Erysiphales/Myxotrichaceae* clade was also closely related to some discomycetous fungi, e.g. *Helotiales* and *Thelebolaceae* (Brumm.) Eckblad. *Pseudogymnoascus* has also been used as the outgroup in other molecular systematic studies, e.g. Hambleton et al. (1998)

and Sugiyama & Mikawa (2001). The molecular research has provided more evidence to support the idea that the *Myxotrichaceae* is not closely related to onygenalean fungi.

Ascomatal development of *Pseudogymnoascus* has been described in some taxonomic studies (Samson 1972, Locquin-Linard 1982, Müller & Arx 1982, Tsuneda 1982, Ito & Yokoyama 1987). Samson (1972) described the ascomatal initials as borne on the vegetative hyphae, consisting of coiled ascogonia and producing loose wefts of ascogenous hyphae inside hyphal tufts, and illustrated the ascomatal initials and the ascus formation. When describing the new species *P. alpinus*, Müller & Arx (1982) indicated that ascomatal initials grew as aerial branches of vegetative hyphae, loosely interwoven and sympodially branched once or twice. Locquin-Linard (1982) provided three drawings to describe the different stages of ascomatal develolpment of *P. dendroideus*. Tsuneda (1982) illustrated the development of ascomata in a scanning electron microscopic study of *P. roseus* and Ito & Yokoyama (1987) provided a photograph of the ascomatal initial but without description. The process of ascomatal development in *Pseudogymnoascus* has not yet been described in detail.

*Pseudogymnoascus roseus* is a species of worldwide distribution frequently found in soil, usually from alpine or forest areas (Christensen et al. 1962, Orr 1979, Ito & Yokoyama 1985, 1987, Currah 1985, Yokoyama et al. 1989, Udagawa & Uchiyama 1999), and occasionally on dung (Ellis & Ellis 1988). It has been also reported from tropical regions (Farrow 1954, Siddiqi 1964, as *P. vinaceus*). *Pseudogymnoascus roseus* was also reported to form mycorrhizal associations with *Vaccinium angustifolium* Aiton in the laboratory (Dalpé 1989), and typical ericoid mycorrhiza with salal (*Gaultheria shallon* Pursh), and also to degrade cellulose and to use organic forms of nitrogen (Xiao & Berch 1995).

During fieldwork undertaken on the Tibetan Plateau for investigation of the Chinese Caterpillar Fungus (*Cordyceps sinensis*, a well-known fungus used as a tonic in traditional Chinese medicine), some other fungi were also isolated from sclerotia of the fungus. Among them, three strains displayed different characters from those of *C. sinensis* in culture. Sequences of internal transcribed spacers (ITS) in the nuclear ribosomal DNA (nrDNA) were obtained for molecular systematic analysis and observations on the cultures were made to elucidate the development of ascomata in these strains. The taxonomic position of the strains was determined as *P. roseus* based on both molecular data and morphological observation. The results are reported here to provide further information on the biology of this fungus.

## Materials and Methods

### Fungal cultures

Fungal cultures used in this study were isolated from *C. sinensis* specimens, collected from Xiaojin County, Sichuan Province, and Yulong Snow Mountain, Yunnan Province of China, on the southeast of the Tibetan Plateau. Soil and plant debris on the fresh specimens were removed and the surface of the specimens was sterilised with 70% ethanol before isolation. The exoskeleton of the host larva was peeled off by using a scalpel and small pieces of the inner tissue of sclerotium of *C. sinensis* were transferred to fresh slopes of bran agar-peptone medium (potato dextrose agar (PDA) supplemented with 5 % wheat bran and 0.5 % peptone). A few pieces of the exoskeleton were also used

as inocula for comparison. Pure cultures were obtained by sub-culturing hyphal tips of primary isolates. Living cultures were maintained at the Institute of Microbiology, Chinese Academy of Sciences, and dried voucher specimens are deposited in HAMS (Chinese Academy of Sciences, Beijing, China) and K (Royal Botanic Gardens, Kew, UK). The details of strains used in this study are listed in Table 1. The cultures were kept at 4 °C for 3–16 weeks for morphological observation and molecular experiments.

## DNA extraction

Samples of fresh mycelium were obtained by scraping the culture from the surface of the nutrient slopes after 6 weeks of incubation. DNA extraction was carried out following a modification of Yao et al. (1999). About 0.1 g of fresh mycelium (including some agar) was ground into powder in liquid nitrogen and transferred to a 1.5 ml tube. The lysis buffer of 600 µl 2% CTAB was added, followed by incubation in a water bath at 65 °C for 1h or more. An equal volume of phenol/chloroform/isoamylol (25:24:1) was added and mixed, then centrifuged at 13000 rpm for 10 min. The supernatant was transferred to a fresh 1.5 ml tube, followed by extraction of the chloroform/isoamylol alcohol (24:1). After centrifugation, the supernatant was transferred to a fresh tube and 250 µl isopropanol was added to precipitate the DNA at –20 °C for 4 h or overnight. The precipitate was centrifuged at 13000 rpm for 10 min, then the liquid was drained off and the tube dried at room temperature for more than 2 h. The DNA preparation was resuspended in 40 µl of sterile deionised water. The crude extracts containing unquantified DNA amounts were used as templates for PCR amplification. Dilution of these extracts 2–10 times was sometimes required for successful DNA amplification.

## PCR amplification and sequencing

The entire ITS region of nrDNA, including ITS1, ITS2 and 5.8S gene, was amplified by polymerase chain reaction (PCR) utilizing the ITS5/4 primers (White et al. 1990). The amplification was performed in 25 µl volumes of reaction mixture containing: 10 mM Tris/HCl (pH 8.3), 2.5mM $MgCl_2$, 0.2 mM of each of the four deoxyribonucleotide triphosphates, 0.4 µM of each of the two primers, 24 U $ml^{-1}$ Taq polymerase (Sino-American Biotechnology Co.), 1 µl of DNA template (some of them were diluted from the crude DNA extracts). The PCR was performed with an initial denaturation of 97 °C for 2.5 min and 35 cycles of 97 °C for 30 sec, 50 °C for 1 min, 72 °C for 1.5 min and final 72 °C for 10 min.

Products were purified using Watson's PCR Purification Kit (Watson Ltd). Sequencing was performed by the cyclic reaction termination method using fluorescently labelled dideoxyribonucleotide triphosphates, according to the manufacturer's protocols on the Geneamp PCR System 2400 or 9700 (Perkin-Elmer). The sequencing products were purified by ethanol precipitation according to the sequencing kit protocol (ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit, Original and Version 2.0, ABI). Sequencing was performed on an ABI Prism® 3100 Genetic Analyzer (Applera Corporation) and data collected on a Dell computer with the DNA Sequencing Analysis programme (ABI Prism®DNA Sequencing Analysis Software™, Version 3.7). Each fragment was sequenced in both directions for confirmation and the two strands of sequences were assembled with Seqscape programme (ABI Prism® SeqScape Software™, Version 1.1).

**Table 1.** Test strains of *Pseudogymnoascus roseus* and *Cordyceps sinensis* used in this study.

| Isol. # | Fungus | Location | Elev. | Isolation source | Coll. date | Voucher | GenBank Access. # |
|---|---|---|---|---|---|---|---|
| CS 6-61 | *Pseudogymnoascus roseus* Raillo | Xiaojin County, Sichuan | 3700m | Sclerotium of *Cordyceps sinensis* | 8 June, 2000 | HMAS 79435 | AY608923 |
| CS 20 | *Pseudogymnoascus roseus* | Yulong Snow Mountain, Yunnan | 4060m | Exoskeleton of *C. sinensis* host larva | 31 May, 2001 | HMAS 79436 | AY608924 |
| CS 22 | *Pseudogymnoascus roseus* | Yulong Snow Mountain, Yunnan | 4060m | Sclerotium of *C. sinensis* | 31 May, 2001 | HMAS 79438 K(M) 108601 | AY608922 |
| CS 18 | *Cordyceps sinensis* (Berk.) Sacc. | Yulong Snow Mountain, Yunnan | 4060m | Sclerotium of *C. sinensis* | 31 May, 2001 | HMAS 79439 | AY608925 |

## DNA sequence analysis and molecular identification of the strains

ITS sequences obtained from this study were compared with existing sequences in GenBank by BLAST database search (Altschul et al. 1997). Additional ITS sequences from *Myxotrichaceae* (*Myxotrichum arcticum*) and from families in the *Onygenales* (including *Gymnoascus* Baran. of *Gymnoascaceae*, *Ajellomyces* McDonough & A. L. Lewis of *Onygenaceae*, *Amauroascus* J. Schröt. of *Onygenaceae* and *Arthroderma* Curr. of *Arthrodermataceae*), two representatives of pyrenomycetes, *Neurospora crassa* Shear & B. O. Dodge and *Erysiphe cichoracearum*, and two species of basidiomycetes were also retrieved from GenBank. One of the *C. sinensis* strains among the isolates obtained from this study was also included for ITS sequence analysis. All the sequences from GenBank are listed in Table 2. Sequences were initially aligned with BioEdit 5.0.6 (Hall 1999) and analysed in PAUP 4.0b10 for Macintosh (Swofford 2001). Extra bases of the ITS fragment in several sequences from Genbank were edited. The alignment was further manually adjusted to reduce some obvious mismatch of sequences created by computer alignment.

A total of 29 sequences was included in the analysis and a data matrix containing 761 base pairs of nucleotides was established. A few dozen bases at both ends were excluded from the analysis owing to uncertainty in determining the sequence. Heuristic searches (Swofford & Olsen 1990, Maddison 1991), including TBR (tree bisection-reconnection) swapping for 1000 replicates of random taxon addition using equal weights were used to explore the set of possible trees from many starting points. Ten trees were saved at each replicate. Nucleotide substitutions were treated as unordered and alignment gaps as missing. Relative supports were assessed by bootstrapping (Felsenstein 1985) using equally weighted characters for 1000 replicates. The tree was rooted with the two basidiomycetes, *Agaricus bisporus* and *Ustilago maydis*.

Table 2. ITS sequences from GenBank used for sequence analysis.

| Fungus | Taxonomic position | Accession # |
|---|---|---|
| *Agaricus bisporus* (J. E. Lange) Imbach | *Agaricaceae; Agaricales* | AF465404 |
| *Ajellomyces capsulatus* (Kwon-Chung) McGinnis & Katz | *Onygenaceae; Onygenales* | AF038353 |
| *Amauroascus mutatus* (Quél.) Rammeloo | *Onygenaceae; Onygenales* | AJ271565 |
| *Arthroderma persicolor* (Stockdale) Weitzman et al. | *Arthrodermataceae; Onygenales* | AJ000614 |
| *Bisporella citrina* (Batsch) Korf & S.E. Carp. | *Helotiaceae; Helotiales* | AF335454 |
| *Dermea viburni* J. W. Groves | *Dermateaceae; Helotiales* | AF141163 |
| *Elytroderma deformans* (Weir) Darker | *Rhytismataceae; Rhytismatales* | AF203469 |
| *Erysiphe cichoracearum* DC. | *Erysiphaceae; Erysiphales* | AF011295 |
| *Gelatinipulvinella astraeicola* Hosoya & Y. Otani | *Helotiaceae; Helotiales* | U72611 |
| *Geomyces asperulatus* Sigler & J. W. Carmich. | Anamorph | AJ390390 |
| *Geomyces pannorum* (Link) Sigler & J. W. Carmich. | Anamorph | AJ509872 |
| *Geomyces pannorum* | Anamorph | AF015789 |
| *Geomyces pannorum* (Link) Sigler & J. W. Carmich. var. *pannorum* | Anamorph | AF307760 |
| *Gymnostellatospora japonica* Udagawa et al. | *Myxotrichaceae* | AF062818 |
| *Gymnoascus petalosporus* (G. F. Orr et al.) Arx | *Gymnoascaceae; Onygenales* | AJ315829 |
| *Gymnoascus punctatus* (B. G. Dutta & G. R. Ghosh) Arx | *Gymnoascaceae; Onygenales* | AJ315825 |
| *Myxotrichum arcticum* Udagawa et al. | *Myxotrichaceae* | AF062810 |
| *Neofabraea malicorticis* H. S. Jacks. | *Dermateaceae; Helotiales* | AF141189 |
| *Neurospora tetrasperma* Shear & B. O. Dodge | *Sordariaceae; Sordariales* | AF388929 |
| *Pezicula ocellata* (Pers.) Seaver | *Dermateaceae; Helotiales* | AF141199 |
| *Pseudogymnoascus roseus* | *Myxotrichaceae* | AF062819 |
| *Pseudogymnoascus roseus* | *Myxotrichaceae* | AF081431 |
| *Scleromitrula spiraeicola* (Dennis) T. Schumach. & Holst-Jensen | *Rutstoemiaceae; Helotiales* | Z81448 |
| *Sclerotinia trifoliorum* Erikss. | *Sclerotiniaceae; Helotiales* | Z99676 |
| *Ustilago maydis* (DC.) Corda | *Ustilaginaceae; Ustilaginales* | AF038826 |

## Morphological observation

The cultivated strains were examined frequently over 12 weeks to observe the growth of the colony. When ascomata were visible, daily observation was carried out to distinguish the different stages of ascomatal development. For ascomatal initial stages, minute tufts of hyphae were removed and placed in a drop of water on a slide for microscopic observation. For later ascomatal development stages, the ascomata were removed under the dissecting microscope and sectioned by using a freezing microtome or dissected on the slide to spread the asci and ascospores. Most preparations were mounted in water and observed immediately under the microscope. Some of the slides were stained with cotton blue in lactic acid to preserve the structure for later observation and photographing.

# Results

## Molecular analysis

The complete sequences of the ITS region of the strains CS20, CS22, CS6-61 and CS18 were 521–543 bp long. The ITS sequences from CS20, CS22 and CS6-61 were identical and different from that of CS18, which was identified as *C. sinensis*. The sequences have been submitted to GenBank with accession numbers from AY608922 to AY608925.

The sequences obtained from the BLAST search could be divided into several groups based on the taxonomic position of the fungi. They were named *Pesudogymnoascus* and its related fungi, including *P. roseus*, *Geomyces*, *Gymnostellatospora* Udagawa et al. and *Chrysosporium* Corda. The majority of the sequences were of discomycetes, including *Helotiales* and *Rhytismatales*, and of other ascomycetes, including *Dothideales* and *Erysiphales*. The sequences selected for analysis represented the major groups found in the BLAST search. To clarify the systematic relationship of CS20, CS22 and CS6-61 with *Onygenales* species, sequences of *Gymnoascus*, *Myxotrichum* Kunze, *Amauroascus*, *Arthroderma* and *Ajellomyces* were included in the analysis.

A total of 675 bp of the ITS regions was used in the analyses. Among the nucleotides, 185 were constant. Of the remaining variable bases, 329 were potentially parsimony informative. Ten most parsimonious trees were obtained with this alignment. One of them is shown in Fig. 1. The sequences formed four groups marked as pseudogymnoascean, discomycetous, pyrenomycetous and onygenalean according to the species within each group. The other nine trees differed from Fig. 1 in the positions of AF081431-*Pseudogymnoascus roseus* and AF062818-*Gymnostellatospora japonica* within the pseudogymnoascean group; the position of AF203469-*Elytroderma deformans* being a sister group to both the pseudogymnoascean group and the major clade of the discomycetous group in some other trees; and the positions of AF335454-*Bisporella citrina* and U72611-*Gelatinipulvinella astraeicola* (as '*astraoeca*' in Genbank) within the discomycetous group.

The pseudogymnoascean group comprised sequences of *P. roseus*, *Geomyces* and *Gymnostellatospora* and was supported by bootstrap analysis at 88% (Fig. 1). The sequences of CS20, CS22, CS6-61 and of AF062819, named *P. roseus* in GenBank, were identical. AF081431-*P. roseus* is an incomplete ITS sequence containing only ITS2 and partial 5.8S gene, almost identical to the same part of ITS sequences of AF062819-*P. roseus*, CS20, CS22 and CS6-61. Although it was shown with no change from others

of these sequences in Fig. 1, it was sometimes placed as a sister group to AF062819-*P. roseus*, CS20, CS22 and CS6-61 in the other parsimony trees. The grouping of these sequences in a terminal clade received 92% bootstrap support. Sequences of AJ509872 and AF015789, both named as *Geomyces pannorum* in Genbank, grouped together with 83% bootstrap support. They formed a clade with the *P. roseus* sequences having bootstrap support of 74%. The other two sequences of *Geomyces*, AF307760-*G. pannorum* and AJ390390-*G. asperulatus*, are almost identical with only two nucleotide substitutions. The two sequences clustered with AF062818-*Gymnostellatospora japonica* having 69% support in Fig. 1, but the latter was grouped with the *P. roseus* and *Geomyces pannorum* clade in some other trees. Several records of named *Geomyces pannorum* in GenBank (accession numbers from AJ509866 to AJ509871) have similar sequences to AJ509872 and AF015789 and were represented by the latter two in this analysis.

The discomycetous group mainly contained *Helotiales* and *Rhytismatales*. *Myxotrichum arcticum* (*Myxotrichaceae*) and *Erysiphe cichoracearum* (*Erysiphaceae*) were also included in this group. The discomycetous group was the sister group to the pseudogymnoascean group, but it was polyphyletic because one of the discomycetes, *Elytroderma deformans*, was placed as an immediate sister group to the pseudogymnoascean group (Fig. 1). In fact, the pseudogymnoascean group is imbedded within the discomycete taxa.

*Neurospora tetrasperma* (AF388929) and *Cordyceps sinensis* (CS18) were clustered together to form the pyrenomycetous group, which was supported by 100% in bootstrap analysis. The pyrenomycetous group is the sister group to the clade containing pseudogymnoascean and discomycetous groups.

Five species from four families of *Onygenales* were clustered together forming the onygenalean group. The support for this group was very strong, reaching 95% in bootstrap analysis although they demonstrated many variations in ITS sequences.

## Morphological descriptions

Morphological characters of the strains, including colony, anamorph and teleomorph, were observed and described from culture. The developmental sequence of ascomata is described and illustrated in detail to demonstrate the formation of a massive ascoma.

**Culture:** Isolates of CS20, CS22 and CS6-61on bran-agar medium formed white colonies with thick aerial mycelium covering the sclerotium tissue. Colonies of the cultures were flocculant with woolly aerial hyphae, white at first but later becoming pinkish brown to purple, with pigmentation varying in the same colony. Reverse of the colony was red-brown to purplish brown. Ascomata appear in 6–8 weeks. Mature ascomata were pale yellowish-brown to pinkish brown and were either scattered upon the surface of the colony or aggregated into dense clusters. Finally the woolly aerial hyphae disappear and the colonies are covered with clay-pink, farinaceous granules.

**Microscopic observation:** Vegetative hyphae were hyaline, 1.0–2.5 μm diam. Ascomata were discrete or confluent, globose to subglobose, 52–320 μm diam., at first white, finally pale pink to pinkish brown under the dissecting microscope. The outer part of the ascoma formed a defined layer of hyphae, which was usually regarded as a peridium. The peridium was composed of pale yellow to yellow-brown, septate, thick-walled hyphae, 2.0–3.0 μm diam., sometimes thickening at the node reaching 3.5 μm diam. Thin-walled hyphae arising from the thick-walled hyphae in the peridium were regarded as appendages, which were simple and up to 40 μm long. Asci were hyaline,

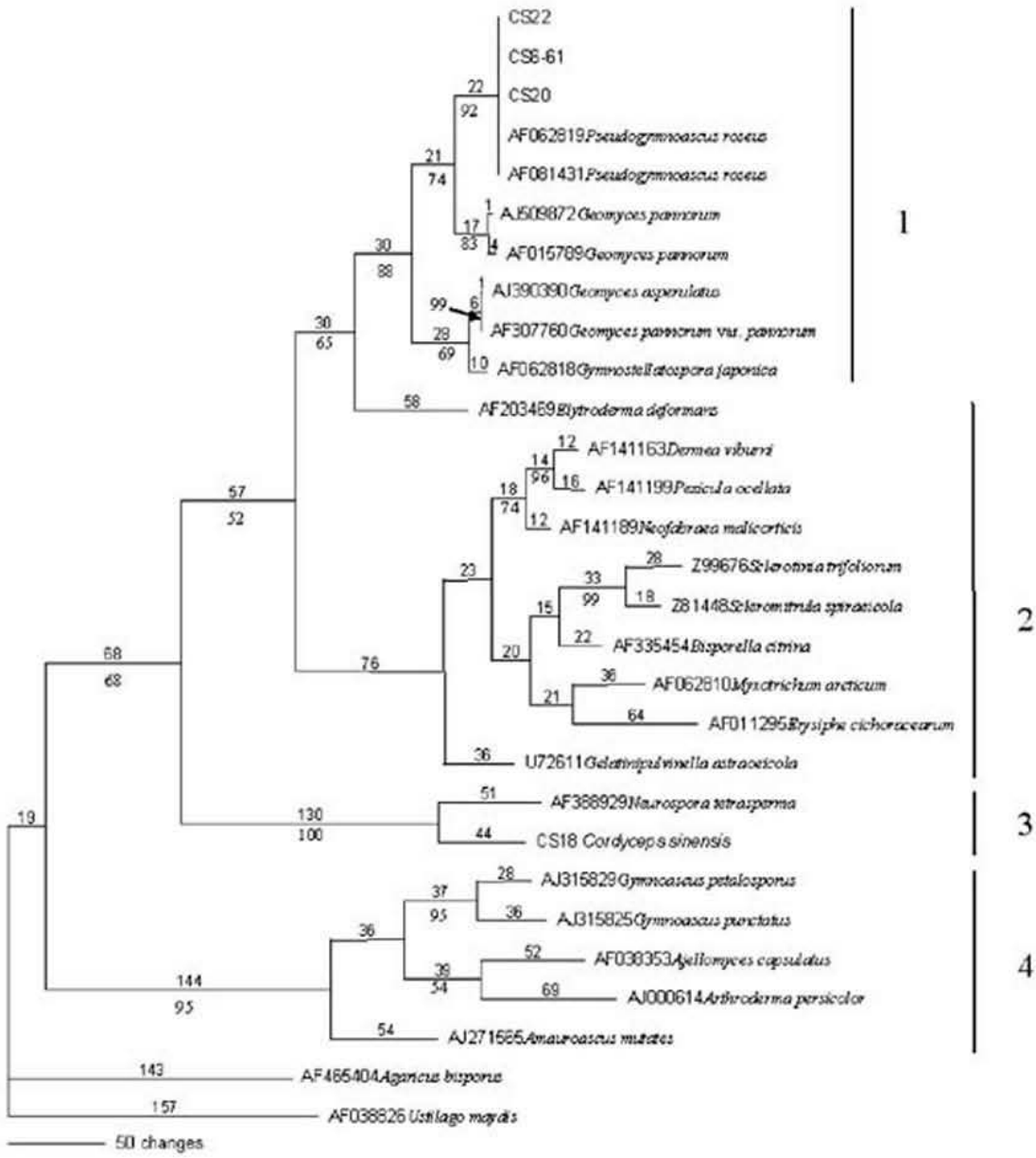
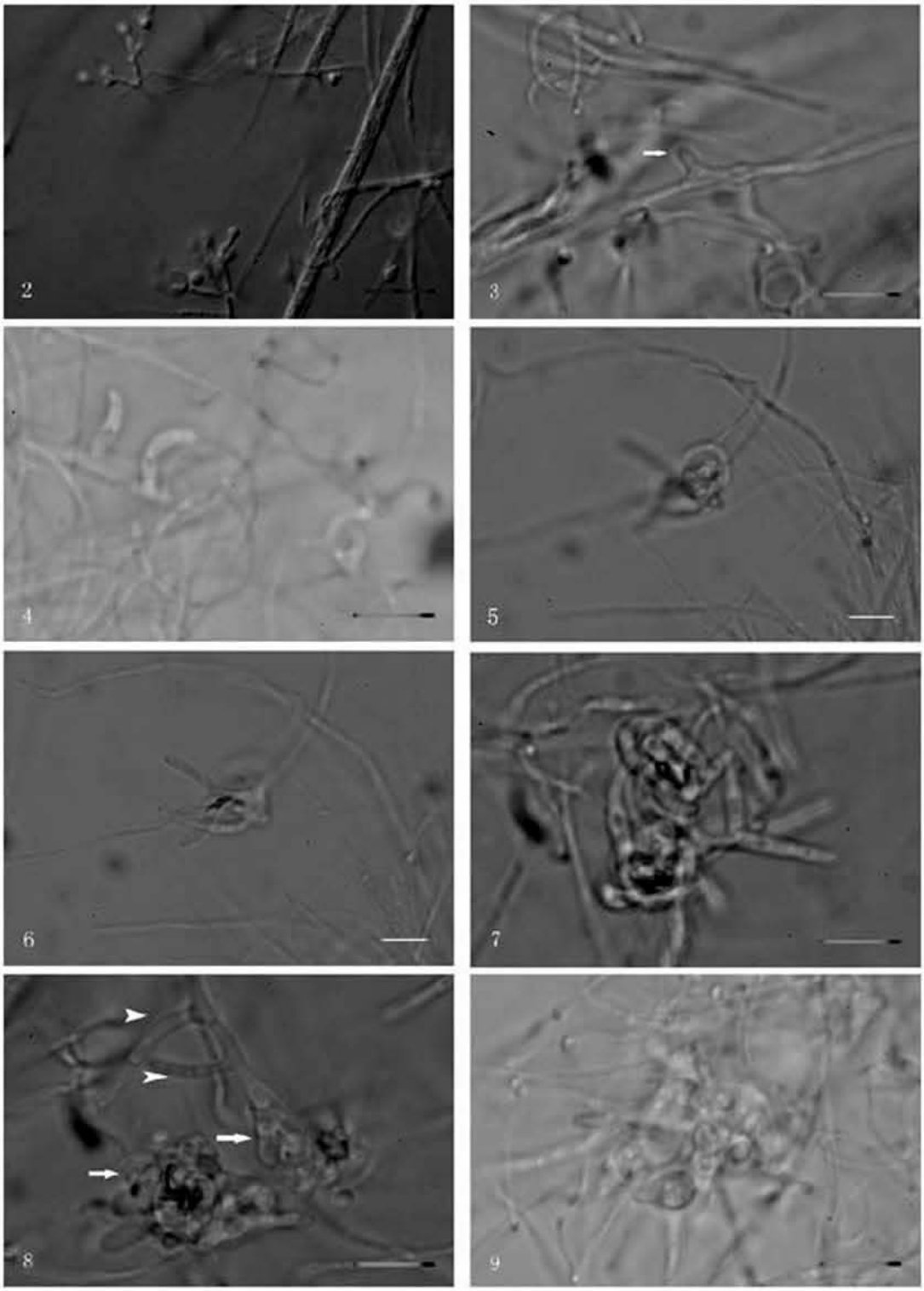



Fig. 1. One of the ten most parsimonious trees obtained from the analysis of nucleotide sequences of ITS regions (nrDNA). The upper and lower numbers on each branch denote the number of estimated substitutions and the percentage of bootstrap replicates respectively. Only bootstraps higher than 50% are shown. The length of the tree is 1607 steps, with consistency index=0.5083 and retention index=0.5401. Group 1=pseudogymnoascean, 2=discomycetous, 3=pyrenomycetous and 4=onygenalean.

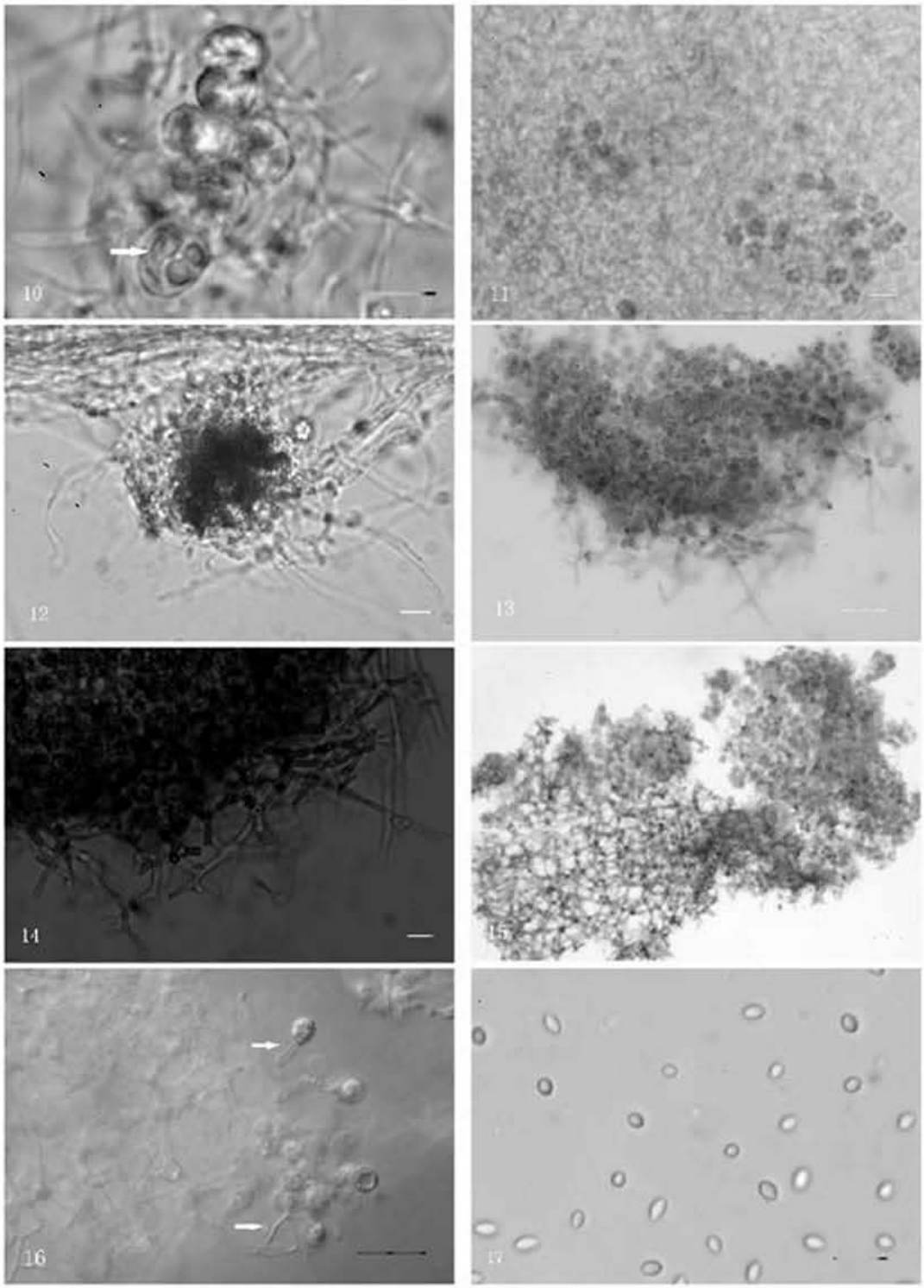
subglobose to oval, stalked, 8-spored, (6.0–) 7.0–9.0 µm diam. Ascospores were hyaline, ellipsoid to fusoid-ellipsoid or fusoid, pale olivaceous to pink, smooth, yellowish brown in mass, 3.5–5.5×1.8–3.0 µm.

**Anamorph:** Hyphae were hyaline, smooth and thin-walled, 0.8–2.5 µm wide. Conidiophores were hyaline and dendroid, or sometimes absent. Conidia formed terminally or were intercalary, thin-walled, smooth, 3.0–6.0×2.0–3.5 µm. The terminal conidia had a truncate base (about 1.8 µm wide) and were cuneiform, ellipsoid or ampullaceous. The intercalary conidia were barrel-shaped. Numerous conidia sometimes




Figs 2–9. *Pseudogymnoascus roseus*. **Fig. 2.** Anamorphic stage. Terminal and intercalary conidia were borne on dendroid conidiophores. **Fig. 3.** Ascoma initial. The ascoma initial arose as a short branch (arrow) from a vegetative hypha. **Fig. 4.** Two pairs of adjacent ascoma initials. The right two initials curved towards each other whilst the left two curved away to the opposite direction. **Fig. 5.** Ascoma initial coil. The other part of the initial is out of focus. **Fig. 6.** The same ascoma initial in Fig. 5 observed at a different focus. Hyphal branches emerge from the coil. **Fig. 7.** Close-up of two ascoma initials. The initials have complex coiling hyphae and branches. **Fig. 8.** Multiple ascoma initials from one site. Two tight ascoma initial coils (arrow heads) and two initial branches (arrows) are visible. **Fig. 9.** Young asci produced in groups. There is no ascospore delimitation at this stage.

Bar=10 μm, except for Fig. 2, where Bar=20 μm.



Figs 10–17. *Pseudogymnoascus roseus*. **Fig. 10.** Young ascospores formed within asci. Ascospores (arrow) are clearly visible at this stage and there is no envelopment of the asci, which were produced from the same ascoma initial. **Fig. 11.** Groups of asci. The groups of asci lack a defined envelope separating them from the surrounding hyphae and may merge together to form an ascus aggregation. Ascospores are visible within asci. **Fig. 12.** A small and immature globose ascoma. There is a released ascus to the upper right of the ascoma. **Fig. 13.** Part of a section of an ascoma. A large number of asci are enwrapped by a network of thick-walled hyphae (peridium). **Fig. 14.** Close-up of peridium in a section of an ascoma. The peridium hyphae are thick-walled with many branches. **Fig. 15.** Dissection of an ascoma. The asci in the ascoma are released at the upper right from the peridium, which is at the lower left. **Fig. 16.** Dissection of an ascoma. Asci are formed on stalks (arrow). **Fig. 17.** Released ascospores. Bar=10 μm in Figs 10–12, 14 and 17; Bar=20 μm in Fig. 16 and Bar=30 μm in Fig. 13.

aggregated together to form a subglobose conglobation. If the strains were kept for long enough, ascomata of *P. roseus* appeared and the colonies became pinkish coloured in 6–8 weeks.

**Development of ascomata:** Ascomatal initials are scattered among, and formed from, vegetative hyphae. Initially, ascomatal initial branches, usually 2.5 μm diam. wide, arise from vegetative hyphae. The short branch with thicker cytoplasm than surrounding vegetative hyphae (Fig. 3) was the earliest stage of ascomatal initial observed. As ascomatal initials grew, they became curved. Adjacent ascomatal initials might sometimes grow toward each other or apart from each other (Fig. 4). Figure 4 shows two pairs of adjacent initials: the right two initials curved towards each other while the left two curved away to the opposite direction. The conjunction of two initials and differentiation of gametangia were not detected. Ascomatal initials continued to grow and formed coils (Figs 5, 6). Figures 5 and 6 show an ascomatal initial coil observed at different focuses. Some thick branch structure emerged from the coil. The initials coiled further to form helical loops (Figs 5–8). Several initials arising nearby were at the same or different developing stages (Figs 7, 8). Figure 8 shows two tightly coiled ascomatal initials (arrow heads) and two ascomatal initial branches (arrows) which arose from the same hypha. Figures 5–8 also show irregular branches stretched out from the coiled initials. These branches can continue to grow and form new coils, or interweave to form an enveloping network in the later stages.

As the ascomatal initial develops, asci are produced. Asci are globose and produced in groups (Fig. 9). Asci begin to enlarge and ascospores are delimitated and form inside the asci (Figs 10, 11). The asci aggregated to form large structures without any defined envelope separating them from the surrounding hyphal components (Figs 10, 11). Figure 11 demonstrates groups of asci can be produced close to each other, and asci from different initials can result in single ascus aggregations. As the number of asci increases, ascomata also increase in size and appear as dark spheres under the microscope owing to the thickness preventing light transmission (Fig. 12). The early ascomata appear as small white points attached to white vegetative hyphae under the dissecting microscope.

As ascus aggregation develops and merges from different ascomatal initials, a network of thick-walled hyphae also envelops the asci. The thick-walled hyphae interweave to form a loose network, which is pale yellow to yellow in colour. Pigmentation of these hyphae increases as the ascomata enlarge. Eventually, a specified layer of the hyphal network, i.e. the peridium, is formed. Figures 13 and 14 are sections of ascomata showing a large number of asci surrounded by the hyphal network (peridium). The peridial network comprises thick-walled hyphae which are richly branched and anastomosed (Figs 14, 15). Some thin-walled, short branches from the hyphal network extend from the globose ascoma. Figure 15 demonstrates that the peridium is composed of loosely interwoven hyphae, which forms a net enveloping the densely packed asci. The whole ascoma appeared dark coloured under the transmission microscope, but as spheres with some shades of pink to pale purple under the dissecting microscope.

Figure 16 shows the attachment of asci to hyphal stalks squeezed out of an ascoma which had a hyphal network (peridium) enveloping the ascus aggregation. When the ascoma matures, a large number of ascospores are released from asci spreading out of the ascoma and among the hyphal network. The ascospores were fusoid to ellipsoid (Fig. 17).

## Discussion

The sequence comparison revealed that the ITS sequences of CS20, CS22 and CS6-61 were identical to AF062819-*Pseudogymnoascus roseus* in GenBank. The DNA sequence analysis showed that these sequences and AF081431-*P. roseus*, the partial sequence of ITS region included in this study, formed a unique terminal group. The strains CS20, CS22 and CS6-61 should be regarded as the same species as AF062819-*P. roseus*, which was submitted by Hambleton et al. in 1998 from strain UAMH 9163, isolated from roots of *Abies lasiocarpa* (Hook.) Nutt. in Alberta, Canada.

The morphological characters of CS20, CS22 and CS6-61 matched those described for *P. roseus* by Cejp & Milko (1966), Orr (1979) and Currah (1985). A reference collection of *P. roseus* deposited at K (K(M) 116466: *Pseudogymnoascus roseus*, isolated from *Eucalyptus*, Australia, G. Johnson, 1975) was also examined for confirmation and they showed the same morphological features. Based on the molecular and morphological data, CS20, CS22 and CS6-61 were identified as *P. roseus*. The strains also exhibit an anamorphic stage with dendroid conidiophores, and cuneiform to ellipsoid and terminal or intercalary conidia. Compared with the morphological characters of *Geomyces pannorum* var. *vinaceus* (Sigler & Carmichael 1976, Oorschot 1980), the anamorphic stage of these strains should be classified in the genus *Geomyces* and might be the same as *G. pannorum* var. *vinaceus*.

The two *G. pannorum* sequences (AF509872 and AF015789) demonstrated only a few variations from that of *P. roseus* and formed a clade with *P. roseus* in the ITS analysis. This result suggested that the two strains of *G. pannorum* were likely to be related to *P. roseus*. The sequence variation between *G. pannorum* and *P. roseus* was nearly the same as within the *G. pannorum* clade.

Four sequences of *Geomyces*, i.e. three *G. pannorum* and one *G. asperulatus*, were separated into two clades. It is possible that more than one taxon has been involved in the strains named as *G. pannorum*. The sequence of AF307760-*G. pannorum* var. *pannorum* is closer to AJ390390-*G. asperulatus* than to the other two *G. pannorum* isolates. *Geomyces asperulatus* and *G. pannorum* are similar in morphology. *Geomyces asperulatus* has hyaline, narrow, sometimes branching conidiophores 0.5–1.0 µm long, and yellow, barrel-shaped (or cuneiform if terminal), long-chained arthroconidia whist *G. pannorum* differs from *G. asperulatus* in forming conidia in shorter chains and in having aleurioconidia formed laterally on the hypha. It is not easy to distinguish the two taxa based on morphology. It appears that molecular systematic analysis based on the DNA sequence data may be a reliable method to avoid misidentification. *Gymnostellatospora japonica* was clustered with two of the four *Geomyces* sequences (AJ390390 and AF307760), and is possibly related to *P. roseus* and *Geomyces* species, but they may not be the same species judged from the sequence variations and from the parsimony analysis. *Gymnostellatospora* was published and placed in *Myxotrichaceae* by Udagawa et al. (1993), based on gross morphological characteristics. Species of *Gymnostellatospora* possess yellowish or reddish brown ascomata; hyphal network of peridium; indistinct 'appendages'; 8-spored, globose or ovoid, evanescent asci; and fusoid, hyaline to pale yellow ascospores (Udagawa et al. 1993). These features are similar to those of *Pseudogymnoascus*. However, the peridial hyphae of *Gymnostellatospora* are incompositoperidium-type (see Currah 1985) at maturity and the ascospore wall has

narrow longitudinal crests and a convex surface, which is clearly different from that of *Pseudogymnoascus*. If the two genera are more widely sampled for investigation of molecular sytematics, it may be possible to find relationships between them.

The systematic position of the genera *Gymnostellatospora*, *Myxotrichum* and *Pseudogymnoascus* were classified in the same family, *Myxotrichaceae*. Members of these genera are similar in morphology, having brown shaded ascomata; hyaline, globose or subglobose asci; and hyaline, ellipsoid or fusoid ascospores. In the present ITS sequence analysis, *Myxotrichum arcticum* was distantly related to *P. roseus* and *Gymnostellatospora japonica*. *Myxotrichum arcticum* was placed in the discomycetous group, and *P. roseus* and *Gymnostellatospora japonica* formed a separate clade with the anamorphic *Geomyces*. Members of *Myxotrichaceae* in this analysis were not in a monophyletic group, as speculated by Currah (1994) based on ascospore morphology. They were separated and embedded among discomycete taxa. Based on 18S rDNA analysis, Sugiyama et al. (1999) described two separate lineages within *Myxotrichaceae*, in which *Myxotrichum* was separated from *Pseudogymnoascus*. Hambleton et al. (1998) also reported that *Myxotricum* and *Byssoascus* Arx diverged significantly from *Gymnostellatospora japonica* and *P. roseus* in ITS sequence analysis. The result of the present study coincides with those reports by Sugiyama et al. (1999) and Hambleton et al. (1998). Although the close relationship between members of *Myxotrichaceae* and discomycetes has been revealed in molecular studies, their ascomatal structure and morphology differ remarkably. Asci of discomycetes are formed from a hymenium within the apothecium, but a hymenium or apothecium are absent in *P. roseus* and other species of *Myxotrichaceae*.

*Pseudogymnoascus* was formerly placed in *Onygenales* (e.g. Currah 1985, 1988). The onygenalean fungi produce evanescent asci in the mycelium or cleistothecial ascomata, and unicellular ascospores. To confirm their molecular relationship, several ITS sequences of onygenalean fungi were included in this analysis. The sequences of onygenalean fungi greatly diverged from those of *P. roseus*, *Geomyces pannorum* and other selected *Myxotrichaceae*. *Pseudogymnoascus roseus* and *Myxotrichum arcticum* are separated from the onygenalean group by the pyrenomycetous group, and closely related to the discomycetes. The results of the present study reveal that the onygenalean group is distantly related to the *Myxotrichaceae* in ITS sequence and shows the independent evolutionary line of *P. roseus* and *Myxotrichum* from other onygenalean taxa as suggested by Currah (1994). Recent molecular systematic studies (Sugiyama et al. 1999, Mori et al. 2000) based on a different region of rDNA also obtained the same results.

The onygenalean fungi were rather isolated from other ascomycetes tested. The discomycete and pyrenomycete species were more closely related to each other than to the onygenalean fungi (Fig. 1). Furthermore, many variations in ITS sequences were also observed among the onygenalean fungi. It seems that there is a complex relationship in *Onygenales* and great divergence may exist within the order.

Morphologically, *Pseudogymnoascus* is similar to members of *Onygenales* in many aspects. The ascomatal stucture and brown colour of the reticulate hyphal network (peridium) in *Pseudogymnoascus* is very close to that of *Gymnoascus*. The characters of the peridium were very much emphasised in previous classifications and *Pseudogymnoascus* was considered to be related to *Gymnoascus*, which possesses oblate ascospores with an acute rim. *Pseudogymnoascus* differs from *Gymnoascus* only in the

absence of peridial appendages and ascospore morphology. Currah (1985) noted that characters of ascospore morphology, enzymatic capacities, and nature and the occurrence of anamorphs provide significant information for classification. Based on ascospore morphology and cellulolytic capacities, Currah (1985) placed *Pseudogymnoascus* in *Myxotrichaceae* and *Gymnoascus* in *Gymnoascaceae*, thus separating these two genera in different families. Molecular investigations (Sugiyama et al. 1999, Mori et al. 2000, and this study) also consider that *Pseudogymnoascus* is widely separated from members of *Gymnoascus* and other onygenalean fungi.

Different peridial types evolved within the prototunicate ascomycetes in response to selective pressures affecting spore dispersal from enclosed areas (Currah, 1994). The loose hyphal network of the peridium exposes the asci and ascospores. Along with the evanescent asci, this may contribute to the release and dispersal of ascospores and increase the chance of propagation. The similar peridium occurring in quite different lineages of genera may be the result of convergent evolution.

The close relationship of *P. roseus* with the discomycetes has been suggested by molecular investigations (Sugiyama et al. 1999, Mori et al. 2000, and this study). *Pseudogymnoascus roseus* and other members of *Myxotrichaceae* share some characters in common: unicellular ascospores; scattered (without a hymenium), thin-walled and evanescent asci; cleistothecial ascomata; and ascomatal peridium of a thin hyphal weft. These characters are very different from those of typical discomycetes. Mori et al. (2000) considered that the close relationship between *Erysiphales*, *Myxotrichaceae* and some discomycetous fungi (mainly *Helotiales* and *Thelebolaceae*) suggested a novel evolutionary pathway from cleistothecial discomycetous fungi to *Erysiphales* and *Myxotrichaceae*. The present study also revealed a close relationship between *P. roseus*, other members of *Myxotrichaceae* and discomycete fungi.

*Pseudogymnoascus roseus* shares a similar structure of ascoma and peridium with members of *Onygenales*. The ascomatal structure and loose hyphal network of the peridium found in *P. roseus* and other species of *Myxotrichaceae* may have been reversed from the advanced apothecium to primitive ascoma in *Onygenales*, if the latter is considered to have diverged earlier in the ascomycetes. This reversion may also have occurred more then once (at least in the pseudogymnoascean group and *Myxotrichum arcticum* (Fig. 1) in the course of evolution.

Based on the observation of ascomatal development in this study, the formation of ascoma in *P. roseus* was initiated from short branches of vegetative hyphae. Currah (1985) generalised that the fertile ascomata of *Onygenales* were initiated by the formation of paired gametangial hyphae. Ascomatal development in *P. roseus* may be similar to that in *Onygenales*, but the microscopic observation made in this study did not detect the contact of two gametangia and the migration of nuclei or cytoplasm. The structure of hyphae in the ascomatal initial coils was complex and it proved difficult to distinguish their constituents under the light microscope.

Several ascomatal initials often appeared very close to each other from the same site in the colony of *P. roseus*. Asci from one or several initials might grow together without a defined envelope at early stages. Many asci grouped to form a large globose aggregation. A loose weft of hyphae (peridium) developed later, enveloping the aggregation. From the observation in this study, a large ascoma should be regarded as the ascus aggregation

from several different ascomatal initials because it occupied a large space covering the area where different initials were formed. The peridium is only a loose network of thick-walled hyphae, and was termed a 'reticuloperidium' by Currah (1985). Like the ascoma, the peridium may also come from several different ascomatal initials. Although initial stages of ascomatal development and the structure of mature ascomata have been described (Cejp & Milko 1966, Orr 1979, Currah 1985), detailed observation of ascomatal formation has not been reported elsewhere. It is revealed for the first time that the ascomata in *Pseudogymnoascus* are formed by the aggregation of asci.

The morphology of ascospores and of peridial appendages are important taxonomic characters in recognising species of *Pseudogymnoascus*. The ascospores are generally considered smooth and the appendages not distinct in *Pseudogymnoascus*. Two species of *Pseudogymnoascus* have rather different features from *P. roseus*: *P. dendroideus* has ramified appendages and striated ascospores with slight thickening at the equator (Locquin-Linard 1982) and *P. alpinus* has navicular-fusoid ascospores, and branched and recurved appendages (Müller & Arx 1982). Either the taxonomic position of these two species is in need of reconsideration or the generic concept of *Pseudogymnoascus* must be revised. The species that Lumley et al. (2000) described as '*Pseudogymnoascus* sp.' also requires further investigation because the longitudinal sigmoid crest on the ascospores is not characteristic of *Pseudogymnoascus*. Molecular research may elucidate the relationships of these morphologically different fungi.

*Pseudogymnoascus roseus* is distributed worldwide and has often been isolated from alpine regions. It is interesting that the fungus was also isolated from sclerotia of *C. sinensis* in this study. Because of its medicinal value, *C. sinensis* has been the target of efforts to obtain pure isolates for massive production in industrial fermentation. Various fungi, about 22 names in 13 different genera, have been related in the literature to the anamorph of *C. sinensis*, either for isolates from material of the fungus or for postulated asexual states (Jiang & Yao 2002). Although many of the isolates are not true *C. sinensis*, they may have similar chemical properties and medicinal effects, e.g. *Paecilomyces sinensis* Q. T. Chen et al. and *Tolypocladium sinense* C. Lan Li (Gui & Chen 1983, Li 1988, Liu et al. 1991). Some of those isolates are even widely used in manufacture referring to the products of *C. sinensis* (Jiang & Yao 2002, 2003). It may be ascribed to the interaction of fungi or selective pressure in the same ecological environment that different fungi produce similar medicinal properties. Probably the strains of *Pseudogymnoascus roseus* were present in the alpine soil of the Tibetan Plateau and contaminated the *Cordyceps* isolates. As another micro-organism living in the same niche with *C. sinensis*, whether *P. roseus* has similar chemical properties that can be used for medicinal purpose may deserve further investigation. Currah (1985) suggested that the pale rose reticuloperidium and ascospores of *P. roseus* probably indicated a reliance on burrowing animals for dispersal. The host of *C. sinensis*, the caterpillar larvae of *Hepialus armoricanus* Obertheir, living in the same microcosm may also be one of the animals helping the dispersal of *P. roseus* ascospores.

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# Morphological and molecular characterization of the mycorrhizas of *Inocybe rufuloides* and *I. splendens*

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**Abstract**—The mycorrhizas of *Inocybe rufuloides* on *Pinus pinea* and of *Inocybe splendens* on *Ostrya carpinifolia* were characterized by morphological and molecular methods. Molecular identification was performed by comparing the rDNA ITS sequences obtained from *Inocybe* fruiting bodies with those of the mycorrhizas collected from the same area. Morphological characterization was carried out by a simplified Agerer protocol. This study provides sets of characters which can be used in further studies for the identification of these two *Inocybe* species found contaminating *Tuber* infected plants in experimental truffières.

**Key words**—ectomycorrhizas, ribosomal DNA sequences, morphological description, truffle cultivation

## Introduction

Ectomycorrhizas are dominant in forests of temperate and boreal regions of the Northern Hemisphere (Brundrett et al. 1996). It has been estimated that 6000 or more species of fungi form ectomycorrhizal associations with approximately 10% of the Angiosperms and many Gymnosperms (Trappe 1987). The ectomycorrhizal communities are dynamic and changes in their population structure occur depending on the age of the host plants, season and environmental factors (Molina et al. 1992, Deacon & Fleming 1992). In the past research into ectomycorrhizal fungal diversity were almost exclusively carried out by identifying and counting fruiting bodies that appeared above the ground or by inspecting the roots of trees and grouping their ectomycorrhizas into morphotypes (Selosse 2001). While the first method is relatively quick and easy the number of species of ectomycorrhizal fungi that fruit above the ground is generally considered to be a gross underestimate of the number of ectomycorrhizal fungi present in an environment (Yamada & Katsuya 2001, Selosse 2001). For example, ectomycorrhizal fungi such as *Cenococcum geophilum* and many members of *Telephoraceae*, which are some of the most common mycorrhizas (Richard et al. 2005, Horton & Bruns 2001), do not produce fruiting bodies or form cryptic fruiting structures. In contrast, although morphotyping of ectomycorrhizas give a better picture of fungal diversity and subtle changes that can occur during competition in ectomycorrhizal communities, it is often impossible to relate the morphotypes to a known fungal species (Horton & Bruns 2001).

The recent application of molecular methods has revolutionised our ability to identify ectomycorrhizal fungi. They are useful either for the certification of plants that have been inoculated with commercially valuable species such as *Tuber* and also allow us to carry out more ambitious studies on ectomycorrhizal community structures and competition within them (Amicucci et al. 2002, Dahlberg, 2001). A comparison of ITS sequences obtained, for example, from traditionally identified fruiting bodies with those obtained from mycorrhizas can provide a means for identifying the fungi that form mycorrhizas (Horton & Bruns 2001). However ITS sequences for many ectomycorrhizal fungi have yet to be determined and so their identification remains impossible.

The aim of the work presented here was to provide a set of molecular and morphological characters for the identification of *Inocybe rufuloides* and *Inocybe splendens* by ITS sequencing of the mycorrhizas and of their fruiting bodies and the morphological description of their mycorrhizas which were found as contaminants in cultivated truffières.

## Materials and methods

### Sampling

The fruiting bodies of the two *Inocybe* species used in this study were collected from two different experimental truffières of Emilia Romagna, Italy. *I. rufuloides* was found in October 2003 in a *Pinus pinea - Tuber borchii* productive truffière established in 1990. The plantation is located in Marina di Ravenna on the Adriatic coast (49° 30' 24" N, 17° 60' 70" E) on littoral sandy soil (Zambonelli et al., 2000) while *I. splendens* was collected in March 2004 in a *Tuber aestivum* truffière established in 1997 with *Corylus avellana, Ostrya carpinifolia* and *Quercus pubescens* infected seedlings in the park of S. Giulia, Palagano, Modena (44° 23' 50"N, 10° 39' 40"E, elevation 932 m) (Zambonelli et al. 2005). *I. rufuloides* was found near *P. pinea* in the *T. borchii* truffière and *I. splendens* close to the trunks of the *O. carpinifolia* seedlings in the *T. aestivum* truffière. Dried specimens of each species were deposited in the herbarium of the "Centro di Micologia" of Bologna (*I. rufuloides* n.1994, *I. splendens* n. 5033).

Root samples were also collected from the truffières under fruiting bodies by removing 6 cm diameter soil cores between 0-30 cm. The soil cores were stored overnight in refrigerator at 5 °C and the roots then removed by carefully washing over an 0.8 mm mesh sieve.

### Morphological characterisation

The *I. rufuloides* and *I. splendens* basidiomes were identified on the basis of their macro- and microscopic morphological characters (Heim 1931; Kuyper 1986; Stangl 1989; Moser et al. 1994, 1996; Bon 1997; Franchi et al. 2001). The microscopic features of the basidiomes were examined in both fresh and dried material previous rehydrated in 10% KOH and stained with Congo red.

Samples of mycorrhizas putatively formed by *Inocybe* sp. were selected from the root samples of *Pinus pinea* and *Ostrya carpinifolia* on the basis of their morphological features following Agerer's descriptions of *Inocybe* mycorrhizas (Agerer 1987-2002). Their morphological features were then described using sets of characters suggested

by Agerer (1987-2002, 1991). The colour of the fresh mycorrhizas was recorded under a stereomicroscope and compared with the Royal Botanic Garden colour identification chart (RBG chart) (1969). Mycorrhizal tips were then fixed in FAA bleached by heating to 90 °C in 10% w/v KOH and treated with a few drops of $H_2O_2$ for 20-30 seconds in order to better examine the anatomy of the mantle under the microscope (Giomaro et al. 2000). Ectomycorrhizal tips were fixed in glutaraldehyde (25%), embedded in Tissue Tek OCT (Sakura, Zoeterwounde, Netherlands) compound and then cut with a rotary cryomicrotome (Tissue Tek® II, Miles, Elkhart, IN, USA) (8-10 µm thickness). Serial sections were stained with cotton blue, mounted in lactic acid and observed under a ECLIPSE TE 2000-E microscope (Nikon, Tokyo, Japan).

Mean dimensions of fruiting body and mycorrhiza characters were determined using Axio Vision 2.05 software (Carl Zeiss Vision GmbH, Hallbergmoos, Germany) from images captured with a DXM1200F digital camera (Nikon, Tokyo, Japan) and standard deviations then calculated. Each biometrical character was the mean of at least 50 measurements.

### Molecular characterisation

Molecular identification of the mycorrhizas and fruiting bodies was performed using sequence data of the ITS regions of the ribosomal DNA. Total genomic DNA was isolated by DNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and then eluted in 50 µl of sterile water. Ten mycorrhizas of each morphotype were pooled and 100 µg of fruiting body tissue were used. ITS-1, 5.8S and ITS-2 regions were amplified in a 50 µl volume reaction containing 1-10 ng of genomic DNA, using the primers pair ITS1 and ITS4 (White et al., 1990) in a T gradient Thermal Cycler (BIOMETRA, Göttingen, Germany) according to Amicucci et al. (1996). PCRs were performed using 1.5 units of *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania).

The amplified products were first purified by Gene Clean II kit (BIO 101, Vista, CA, USA) and then sequenced using both the primers mentioned above. Sequence reaction was performed using the ABI PRISM 3700 DNA Analyzer (Applied Biosystem, Foster City, CA, USA). The obtained ITS sequences of fruiting bodies and mycorrhizas were compared each other and with those on the GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) and UNITE (http://unite.zbi.ee/analysis.php3) databases using BLASTN search (Altschul et al., 1997). The ITS sequences of the fruiting bodies obtained in this study have been deposited in GenBank with the following accession numbers: *I. rufuloides* (DQ067579) and *I. splendens* (DQ067580).

## Results

### Morphological characterization

#### *Inocybe rufuloides* Bon

The *I. rufuloides* basidiomes had pileus 15-35 mm broad, campanulate, conico-convex, then plano-convex, at maturity applanate, with or without umbo, with margin inflexed when young, dark brown, orange-brown, tomentose around disc, outwards fibrillose, with fibrils diverging and sometimes squamulose-subsquarrose. Lamellae were adnexed

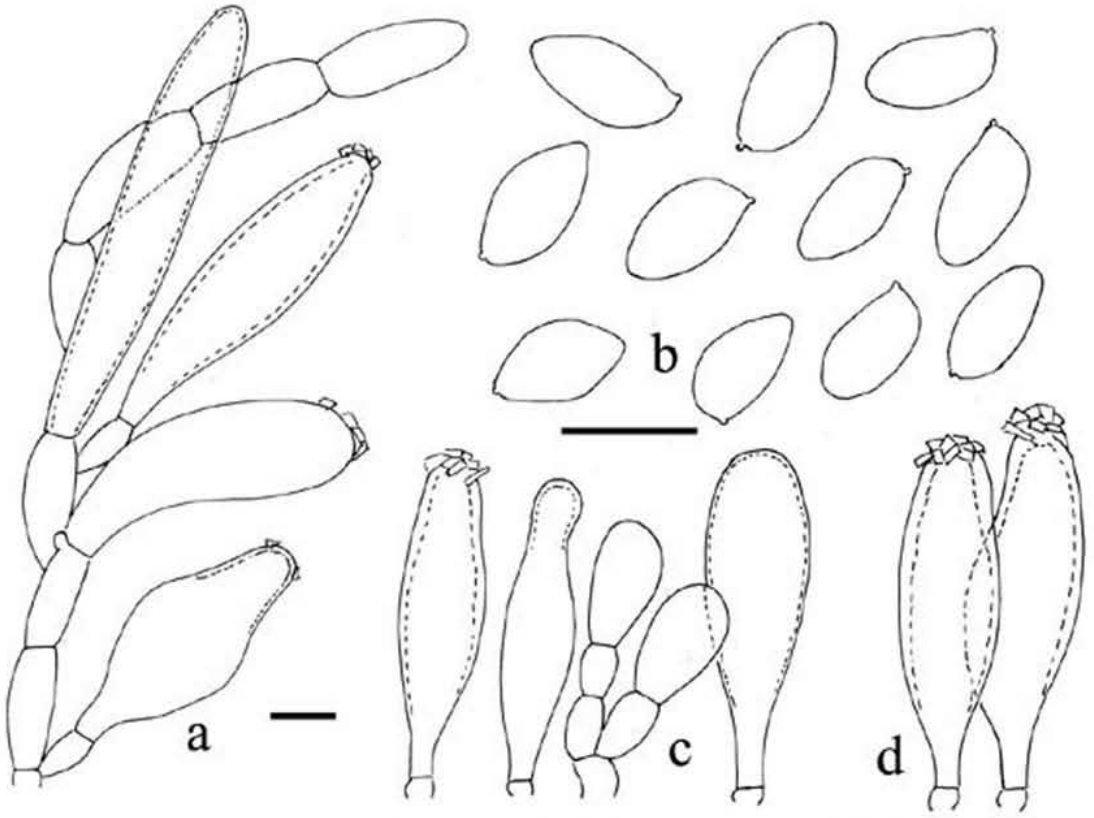


Fig. 1 – Caulocystidia (a), spores (b), cheilocystidia (c) and pleurocystidia (d) of *I. rufuloides* (Bars = 10 µm).

to adnate, L = 28-45, l = 1-3, moderately crowded, ventricose, 4-8 mm broad, pale greyish buff, then brown, dark brown, edge fimbriate, whitish. The stipe was 25-50 x 3-5 (6) mm, equal to clavate, subbulbous, brown to orange brown, extreme apex hairy-pruinose, near base whitish, downwards longitudinally white-fibrillose. The context was white in pileus, red-brown in stipe; smell and taste spermatic. The spores were (8) 10.5 ± 1.4 (13) x (4.7) 5.9 ± 0.7 (7) µm, Q = (1.5) 1.8 ± 0.2 (2.1), smooth, ovoid to amygdaliform, with subobtuse to indistinctly conical apex (Fig. 1b). The basidia were 28-38 x 9-12 µm, 2-4-spored. The hymenial cystidia were 50-70 x 10-20 µm, cylindrico-clavate, clavate, fusiform, lageniform, sometimes subcapitate, with a thick wall 1.5-2.5 µm, slightly yellow with ammoniac (Figs 1c & 1d). Caulocystidia were absent or present only at stipe apex (to 1/10$^{th}$), similar to hymenial cystidia and mixed with cauloparacystidia (Fig. 1a), downwards are present caulocystidioid hairs.

*I. rufuloides* mycorrhizas were simple, ramified or with limited dichotomous branching involving a few lateral root tips, rarely coralloid. The unramified ends were short, straight or slightly twisted, 594.4 ± 167.3 µm long and 419.1 ± 79.0 µm in diameter. The structure of the surface was woolly with whitish emanating hyphae. The mycorrhizal tips were white (RBG chart n. 7) or greyish-cream (RBG chart n. 1) and orange-cream (RBG chart n. 44) at the base. When viewed with a microscope the mantle was plectenchymatous and had three distinct layers. The outermost was composed of partially bundled, loosely woven hyphae which were without a gelatinous matrix (Fig. 3a) whereas the middle and inner layers were made of tightly appressed hyphae (Fig. 3b & 3c). The mantle was 68.5

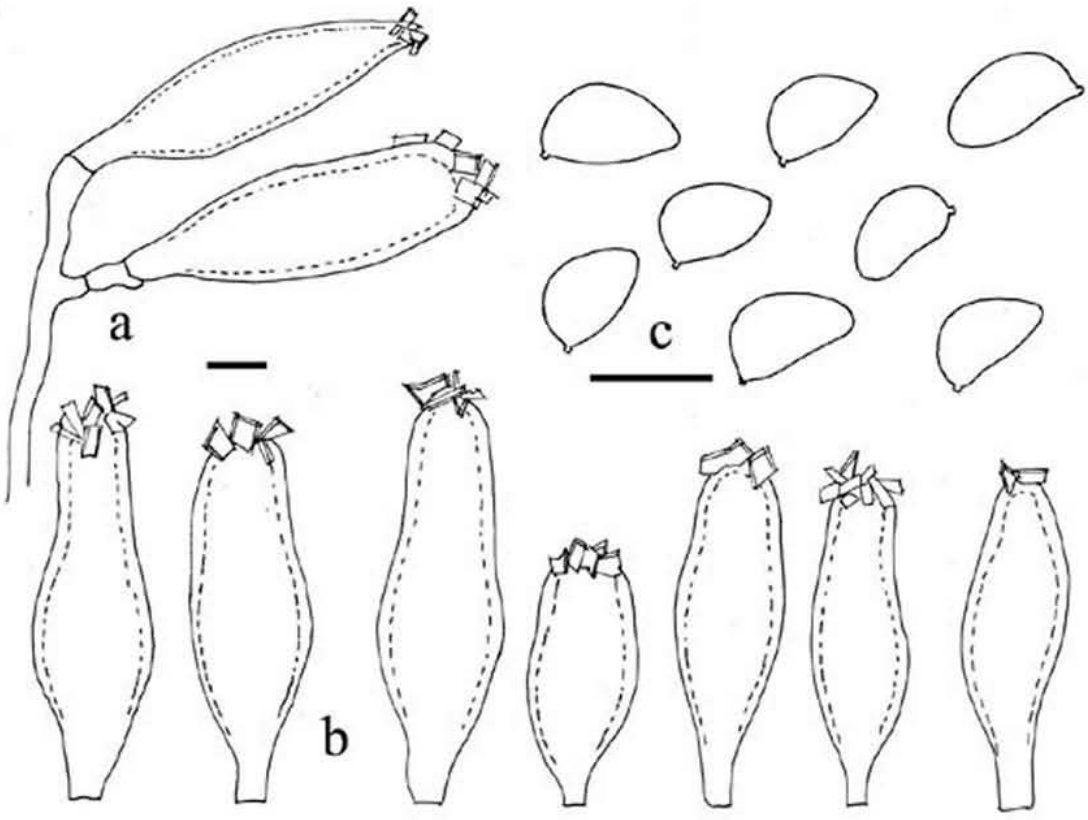


Fig. 2 - Caulocystidia (a), pleurocystidia (b) and spores (c) of *I. spendens* (Bars = 10 µm).

± 13.1 µm thick and the Hartig net was one, rarely two cells deep, 3.5 ± 1.1 µm thick and composed of a single hyphal row. In cross sections the mantle was composed of loosely arranged cells 4.7 ± 0.8 µm x 2.6 ± 0.5 µm in the external layers and 4.4 ± 0.9 µm x 2.8 ± 0.5 µm cells in the inner layer. The root cortical cells were tangentially oval 43.0 ± 9.1 µm x 19.6 ± 4.4 µm. The root cortical cells with Hartig net were radially oval 33.0 ± 7.4 µm x 18.0 ± 4.1 µm. In longitudinal section the mantle was composed of loosely arranged hyphal cells 5.4 ± 1.2 µm x 3.4 ± 0.9 µm in the external layer and of 5.0 ± 1.2 µm x 3.0 ± 0.6 µm in the inner layer. The shape of the root cortical cells was oval 58.3 ± 15.7 µm (tangentially) x 24.7 ± 5.5 µm. The root cortical cells adjacent to the Hartig net were 38.9 ± 9.8 µm (tangentially) x 19.7 ± 4.6 µm. The emanating hyphae (2.1 ± 0.4 µm thick) possessed clamp connections with a distinct hole and had a characteristically constricted fusion point between the arched part of the clamp and the parental hypha (Fig. 3d). Rhizomorphs and cystidia were absent.

### *Inocybe splendens* R. Heim

*I. splendens* basidiomes had pileus 30-50 (70) mm broad, convex, soon plano-convex, finally applanate, with inflexed margin, later straight, often with conspicuous but low broad umbo, brownish-ochraceous to dark brown, sericeous-fibrillose, with fibrils not or diverging, sometimes radially rimulose. Lamellae were adnate to almost free, L=45-65, l=1-3, crowded, ventricose, 3-9 mm broad, whitish then greyish-yellow, finally yellow-brown, sometimes with olivaceous tinge, edge whitish, fimbriate. The stipe was 25-90

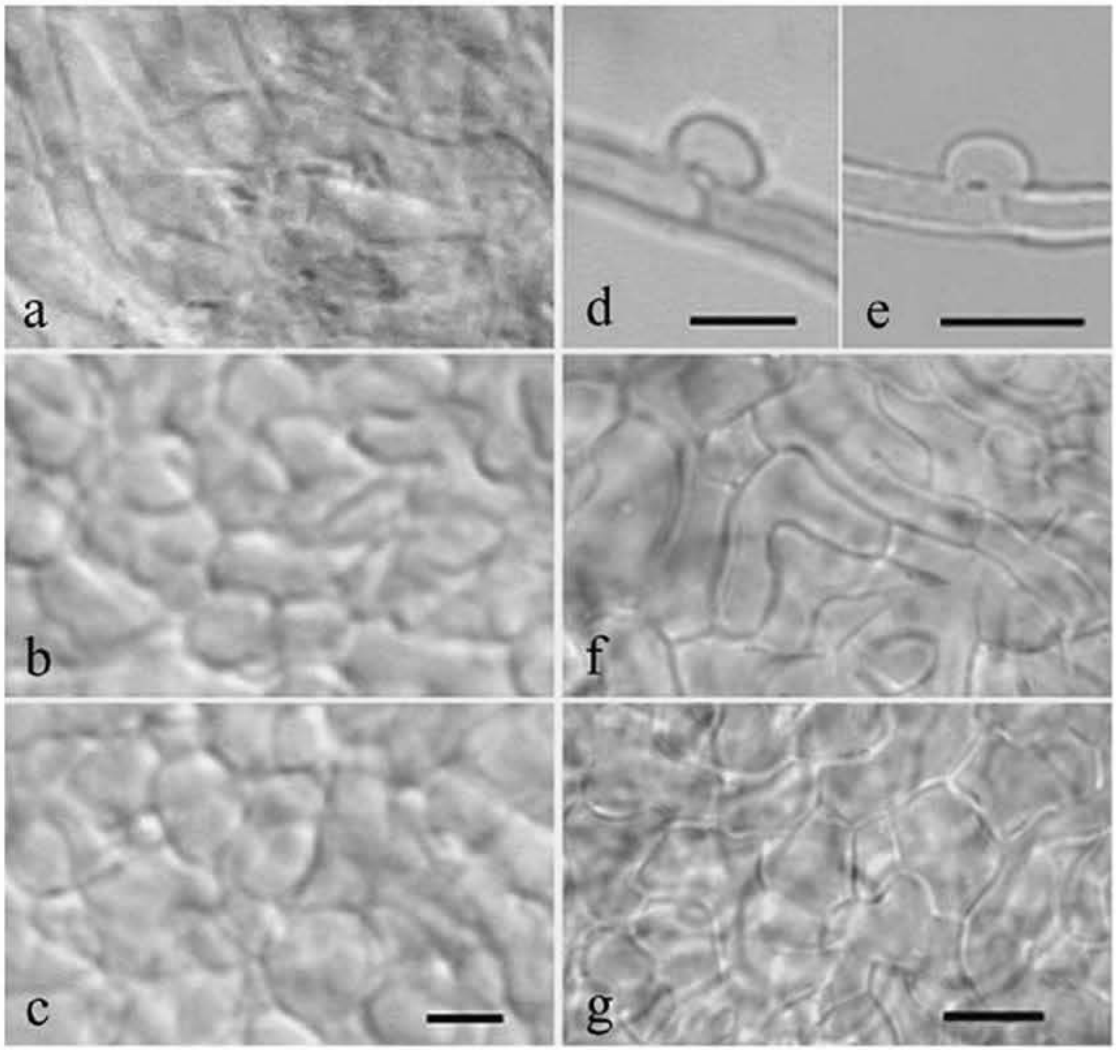


Fig. 3 – Mantle of *I. rufuloides* mycorrhizas (a external, b middle, c internal layers) and of *I. splendens* mycorrhizas (f external, g internal layers) under a light microscope. Clamp connections of *I. rufuloides* (d) and of *I. splendens* (e) (Bars = 5 µm).

x 6-15 mm, sometimes equal, but often marginately bulbous or subbulbous, whitish, finally ochraceous-yellow to pale brownish, pruinose all over. The context was white in pileus, ochraceous-yellow in stipe; smell subspermatic or as *Amanita phalloides*, taste not distinct. The spores were (8) 10.7 ± 1.5 (13) x (4.8) 5.9 ± 0.9 (7) µm, Q = 1.7 ± 0.2 (20), smooth, amygdaliform, with suprahilar depression, with subconical apex (Fig. 2c). The basidia were 26-40 x 8-12 µm, 4-spored. The hymenial cystidia were (45) 50-80 x 14-20 (27) µm, clavate, fusiform to utriform, sometimes sublageniform, with a thick wall 1.5-3.0 µm, colourless or slightly yellow with ammoniac, with crystalliferous at apex (Fig. 2b); paracystidia pyriform, thin-walled, colourless. The caulocystidia were similar to hymenial cystidia, descending almost to base of stipe, mixed with cauloparacystidia throughout (Fig. 2a).

*I. splendens* mycorrhizas were simple or with monopodial ramification with few lateral root tips. The unramified ends were straight or slightly twisted 965.2 ± 353.2 mm long and 222.4 ± 29.4 mm in diameter. The structure of the surface was woolly with whitish

emanating hyphae. The colour of tips was white (RBG chart n. 7) or greyish-cream (RBG chart n. 1) and orange-cream at the base (RBG chart n. 44). Under a microscope the mantle was plectenchymatous, with two distinct layers, formed of closely appressed hyphae and without a gelatinous matrix (Fig. 3f & 3g). The thickness of the mantle was 27.2 ± 5.3 µm. The Hartig net was one, rarely two cells deep, 2.4 ± 0.5 µm thick, and one cell wide. In cross section the mantle was composed of appressed hyphal cells 4.2 ± 1.3 µm x 2.0 ± 0.6 µm in the external layer and of 4.1 ± 1.3 µm x 2.0 ± 0.7 µm in the inner layer. The root cortical cells were approximately tangentially oval 31.7 ± 6.1 µm x 20.9 ± 5.0 µm. The root cortical cells adjacent to the Hartig net were radially oval 27.7 ± 4.5 µm x 14.0 ± 2.7 µm. In longitudinal section the mantle was composed of appressed hyphal cells 4.4 ± 0.8 µm x 2.5 ± 0.6 µm in the external layer and 4.3 ± 1.0 µm x 2.5 ± 0.6 µm in the inner layer. The shape of the root cortical cells was oval 40.0 ± 7.5 µm (tangentially) x 19.9 ± 4.5 µm. The root cortical cells with Hartig net were 37.9 ± 7.4 µm (tangentially) x 13.1 ± 3.5 µm. The emanating hyphae (2.0 ± 0.3 µm thick) possessed clamp connections with a small hole and with a characteristically constricted fusion point where the arched part of the clamp met the parental hypha (Fig. 3e). Rhizomorphs and cystidia were lacking.

### Molecular characterisation

The amplicons of *I. rufuloides* and *I. splendens* resulting from ITS1/ITS4 amplification showed length of 690 pb and 710 pb respectively. No differences were found between sequences obtained from mycorrhizas and from the respective fruiting body of each *Inocybe* species. The sequences resulting from ectomycorrhizae and fruiting body of *I. rufuloides* showed 93% level of similarity (510/544 nt) with a nearly complete ITS1-5.8S-ITS2 sequence of an uncultured ectomycorrhiza of *Inocybe* (accession number AY825514) described by Richard et al. (2005). Instead, the sequences resulting from ectomycorrhizae and fruiting body of *I. splendens* showed 90% level of similarity (580/640 nt) with a nearly complete ITS1-5.8S-ITS2 sequence of an uncultured fungus from ectomycorrhizal root isolate (accession number AY702725) described by Izzo et al. (2005). Lower similarities were obtained with the *Inocybe* sequences in the UNITE database.

## Discussion

The molecular and morphological characterization of *I. rufuloides* and *I. splendens* mycorrhizas provide reliable instruments for the identification of these two fungi that are potential contaminants in natural and cultivated truffières.

Many species of ectomycorrhizal fungi are able to contaminate cultivated truffières such as less valuable *Tuber* species, *Cenococcum* spp., *Hymenogaster* spp., *Tomentella* spp., *Scleroderma* spp., *Hebeloma* spp., and several, as yet, unidentified fungi known only as morphotypes (Donnini & Bencivenga 1995, De Miguel & Sáez 2005). This replacement of inoculant fungi in truffières by more aggressive competing fungi is one of the most important problems during the cultivation of truffles (Sourzat 2001). These ectomycorrhizal species usually either have a broad range of adaptability to different ecological conditions or ecological requirements similar to cultivated truffles (Dalberg 2001, Giovannetti 1983). *I. rufuloides* is reported to grow in association with *Pinus*

*maritima* on sandy soil along the Mediterranean coasts while *I. splendens* is associated with broad-leaf trees on calcareous sand and clay soils in Europe (Kuyper 1986). These are the same habitats where *T. borchii* and *T. aestivum* grow respectively. The discovery of *Inocybe* spp. in cultivated truffières and natural *Tuber magnatum* (Giovannetti 1983, Pirazzi 2001), *T. aestivum* (Chevalier & Frochot 1997) and *T. borchii* (Zambonelli et al. 2002) truffières, and their ecological requirements seems to confirm that members of this genus, might be important competitors.

The morphological characterization of *I. rufuloides* and *I. splendens* mycorrhizas confirms that some characters such as the plectenchymatus mantle structure, the form of the fusion point of clamp connections can be useful to distinguish the mycorrhizas formed by the species belonging to the genus *Inocybe* (Agerer 1987-2002). However, the only morphological characters don't allow identification at species level. Moreover, colour and form of the mycorrhizas can vary with their age; the form of the mantle cells, which is a quite important taxonomic character, can show some differences depending on the fungal strain and the host plant (Giomaro et al. 2000, 2002; Sisti et al. 2003).

The early identification of the presence of contaminant fungi in cultivated truffières offers growers the possibility to promptly introduce cultivation procedures that favour the establishment and development of *Tuber* ectomycorrhizas such as correct irrigation rates and mulching (Zambonelli et al. 2005).

Molecular analyses have allowed us to unequivocally identify *I. rufuloides* and *I. splendens* mycorrhizas by comparing their ITS sequences in the symbiotic and reproductive phases. The ITS sequence of *I. rufuloides* and *I. splendens* deposited in genbank will be a useful tool for other researchers who need to confirm the morphological identification of these fungi by the comparison of their sequences and, for example, will permit them to quantify the extent of problems caused by these fungi and develop specific strategies for their control.

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# *Russula* in Himalaya 1: A new species of subgenus *Amoenula*

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**Abstract**—*Russula mukteshwarica*, a species closely related to *R. violeipes*, is proposed here as new to science. Macro- and micromorphological characters of this species are described and illustrated in detail.

**Key words**—macrofungi, *Russulaceae*, taxonomy, phylogeny, India

## Introduction

Extensive investigation on Himalayan *Russulaceae* has been carried out by the authors (Das & Sharma 2003, Das et al. 2003, Das & Sharma 2004, Das et al. 2004, Das & Sharma 2005, Das et al. 2005) for the last eight years. During macrofungal surveys of Kumaon and Garhwal Himalaya, the authors came across a species of *Russula* which after thorough macro- and microscopic studies followed by molecular analysis appeared to be an undescribed taxon and is proposed here as *Russula mukteshwarica*.

## Materials and Methods

Macroscopic characters were noted from fresh material. Microscopic characterization was done from dried material by mounting free hand basidiome sections in 5% KOH, Melzer's reagent, Congo red, Lactophenol-cotton blue and Carbol Fuchsin. Colour terms follow Kelly & Judd (1955). Microscopic line drawings were made with the aid of a camera lucida at original magnification of 1500x for basidiospores, 1000x for other microstructures. Density of lamellae (L/cm) was measured at the margin of the pileus excluding lamellulae. Spore print colour follows Romagnesi (1967). Basidiospores length excludes the length of ornamentation. Basidium length excludes the length of sterigmata.

Quotient (Q = L/W) was calculated considering the mean value of length and width of 25 basidiospores. Herbarium names used follow Holmgren et al. (1990). Materials and methods for rDNA sequencing followed those of Miller & Henkel (2004).

## Description of the species

***Russula mukteshwarica*** K. Das, S.L. Mill., J.R. Sharma & R.P. Bhatt **sp. nov.**

**Figure 1**

*Etymology*: From Mukteshwar, referring to the type locality.

*Pileus 65–130 mm diam., planoconvexus, leviter depressus in centro, purpureus ad violaceus. Lamellae adnatae, confertae, luteae. Stipes 45–87 x 14–27 mm, cylindricus, purpureus. Sporae in cumulo albae, 7.6–9.3 x 7.3–8.2 µm, subglobosae vel globosae, amyloideae, verrucosae et incomplete reticulatae. Pleurocystidia 80–110 x 11–17 µm, fusiformia. Cheilocystidia 70–100 x 11–16 µm. Pileipellis bistrata. Typus: INDIA, Uttaranchal, Nainital, Mukteshwar, August 2003, leg. K. Das, KD2120 (HOLOTYPUS, BSD; ISOTYPUS, TUR-A)*

**Pileus** 65–130 mm diam., planoconvex, becoming umbilicate with depressed center at maturity; pileipellis dry, viscid when moist, pruinose to subvelvety, dark purple, deep to very deep purple or deep to dark violet, light to brilliant or very greenish yellow at the center; margin slightly decurved, almost plain at maturity, irregularly lobed, splitted, peeling up to 1/4$^{th}$ of the radius. **Lamellae** broadly adnate to adnexed, close (ca 7–9 per cm), forked from the base, brittle, cream to pale yellow or light greenish yellow; lamellulae present; edges even. **Stipe** 45–87 x 14–27 mm, cylindric to subclavate, dry, pruinose, light reddish purple at the top, gradually with white areas downwards but always with a white rim between the juncture of lamellae and stipe, pale greenish yellow (lemon yellow) at the base. **Context** stuffed, cream, changing light brown with phenol. **Taste** mild. **Spore print** white (Ia).

**Basidiospores** 7.6–9.3 x 7.3–8.2 µm, subglobose to globose (Q = 1–1.15, av. 1.05–1.10); ornamentation amyloid, composed of warts (=0.75 µm long) and ridges forming incomplete reticulum. **Basidia** 30–45 x 7–9 µm, subclavate to clavate, 4-spored; sterigmata =6.5–7 µm. **Pleurocystidia** 80–110 x 11–17 µm, abundant, emergent up to 40 µm, ventricose, subfusiform to fusiform, thick walled, content dense; wall up to 1.3 µm thick. Lamellae edge fertile, composed of basidia and cystidia. **Cheilocystidia** 70–100 x 11–16 µm, thick walled, same as pleurocystidia. **Subhymenium** layer narrow, up to 12 µm thick, cellular. **Pileipellis** two layered; upper layer composed of suberect to erect subulate, septate hyphae, 5–11 µm broad, pileocystidia absent; subpellis cellular.

**Ecology** – *Russula mukteshwarica* grows in close association with species of *Quercus*, *Rhododendron* and *Myrica* in moist deciduous to mixed subtropical to temperate (1800–2200 m) forests.

**OTHER SPECIMENS EXAMINED:** INDIA, Uttaranchal: NAINITAL, Mukteshwar, August 2003, leg. K. Das, KD2127; PITHORAGARH, Dafia Dhura, September 2001, leg. K. Das & J.R. Sharma, KD4082; PAURI, Nagdev forest on path to Snake Temple, June 2003, leg. P. Sharma & S.L. Miller, PR310; *ibid.*, June 2003, leg. S.L. Miller & P. Sharma, SLM 03-04, SLM 03-07; *ibid.*, Khirsu, June 2003, leg. S.L. Miller & P. Sharma, SLM 03-22.

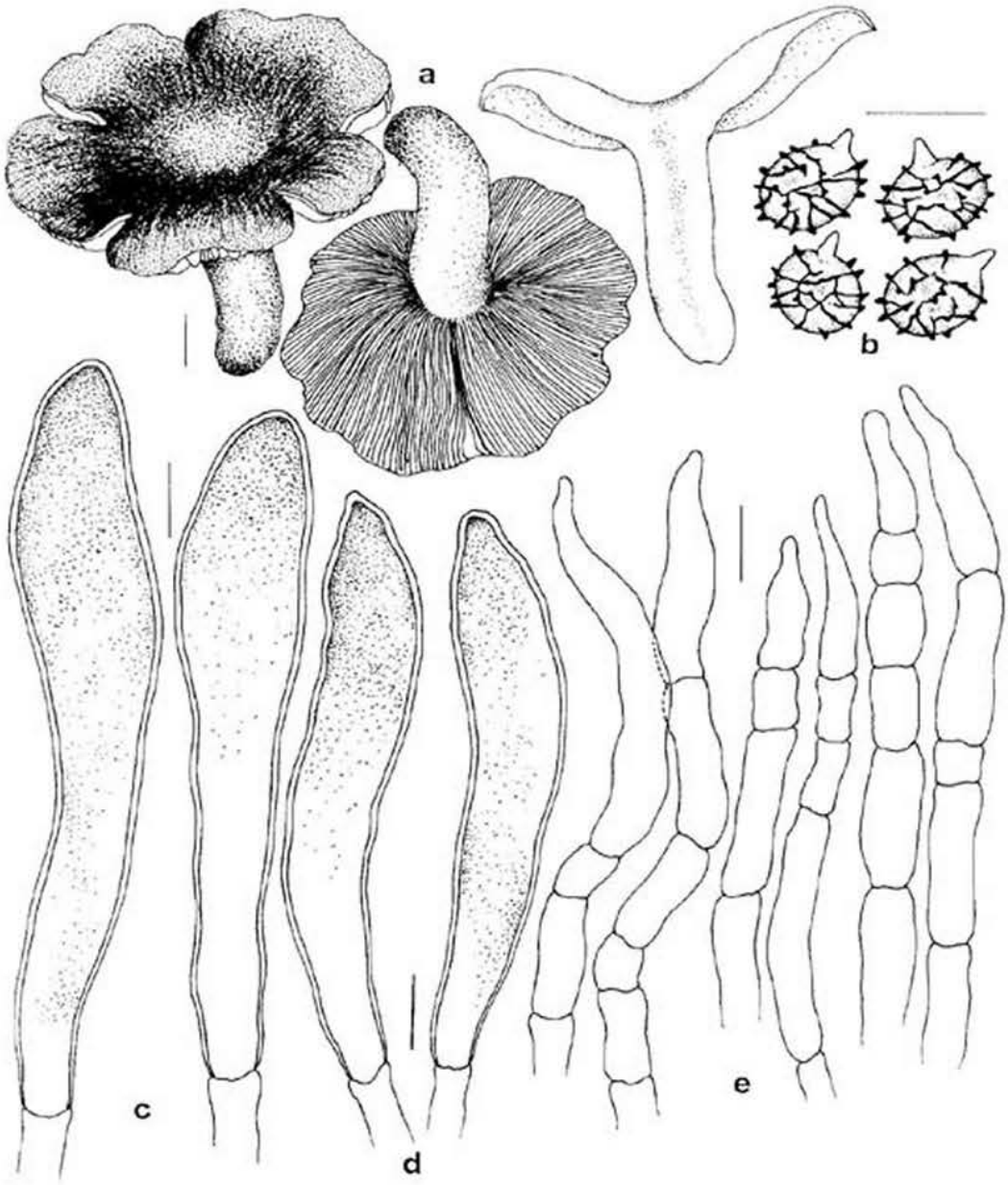


Fig. 1. *Russula mukteshwarica* (from holotype). a. Basidiomes. b. Basidiospores. c. Pleurocystidia. d. Cheilocystidia. e. Cross-section of pileipellis. Bars: a = 10 mm; b-e = 10 µm.

**Comments** – Typically subulate, up to 70 µm long hyphal ends of pileipellis and absence of dermatocystidia undoubtedly place the taxon in the subgenus *Amoenula* Sarnari. This species resembles *Russula amoenicolor* Romagn., *R. violeipes* Quél. and *R. amoena* Quél. from Europe. However, all these three species have a different spore print colour which varies from IIa–IIc (Romagnesi 1996), or from IIa–IIIa (Sarnari 1998). Moreover, the red to purple colour of stipe base in *R. amoenicolor*; presence of distinctly globose lower cells of the pilear hyphae in *R. violeipes* and the comparatively narrower pleurocystidia and reddish stipe base in *R. amoena* further separate these three species from *R. mukteshwarica*. Molecular analysis gathered from rDNA sequencing of the ITS gene

region (not shown) has also confirmed that the new species belongs to the subgenus *Amoenula* and is closely related to *R. violeipes*.

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# *Streptopodium passiflorae* comb. nov. on *Passiflora rubra*

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**Abstract** — Reexamination of *Ovulariopsis passiflorae*, the causal agent of a powdery mildew on *Passiflora rubra*, revealed that the fungus has dimorphic conidia and conidiophores formed from the external mycelium, a combination of features typical of *Streptopodium*. An emended description of this species is provided and the new combination *Streptopodium passiflorae* is proposed.

**Key words** — *Erysiphaceae, Phyllactinieae*

## Introduction

*Ovulariopsis passiflorae* Syd. (*Erysiphaceae*) was described on *Passiflora rubra* L. based on a specimen collected in Venezuela by Sydow (1930). Its published description suggested that its maintenance in *Ovulariopsis* Pat. & Har. under the present concept for the genera in the *Phyllactinieae* (Braun et al. 2002) might be inadequate. A re-examination of the type specimen was made, confirming this suspicion. This paper reports the results of this new study of the type material and the nomenclatural change that resulted from it.

## Material and Methods

In order to elucidate whether the conidiophores were produced from external or internal mycelium, a critical feature for separating genera in the *Phyllactinieae*, a whole leaf clearing and staining technique was used. Leaf pieces were immersed in solution of 50 g chloral hydrate in 20 mL of distilled water and left in stoppered glass vials at room temperature, for 24 h. The leaf pieces were then mounted on microscope slides in 85% lactic acid with aniline blue 1 g/L (Liberato et al. 2005). Only turgid and mature conidia (those unattached to conidiophores) were measured.

## Results

Dimorphic conidia were found, feature that excludes the possibility of this fungus belonging to *Ovulariopsis*. The leaf clearing and staining technique enabled the visualization of superficial hyphae entering the leaves through stomata, an indication

that the fungus has hemiendophytic mycelium (partly external and partly internal). With a single exception [species belonging to Cystotheca (Braun 1987)], this feature is exclusive to the tribe *Phyllactinieae* (Braun et al 2002). This technique also enabled the visualization of conidiophores arising from external mycelium. This combination of characters clearly indicates that the fungus belongs in the genus *Streptopodium* R.Y. Zheng & G.Q. Chen emend. (Liberato et al. 2004).

## Taxonomic Description

***Streptopodium passiflorae*** (Syd.) Liberato & R.W. Barreto **comb. nov. (emended)**

**Figs. 1-8**

= *Ovulariopsis passiflorae* Syd. Ann. mycol. 28(1-2): 199, 1930.

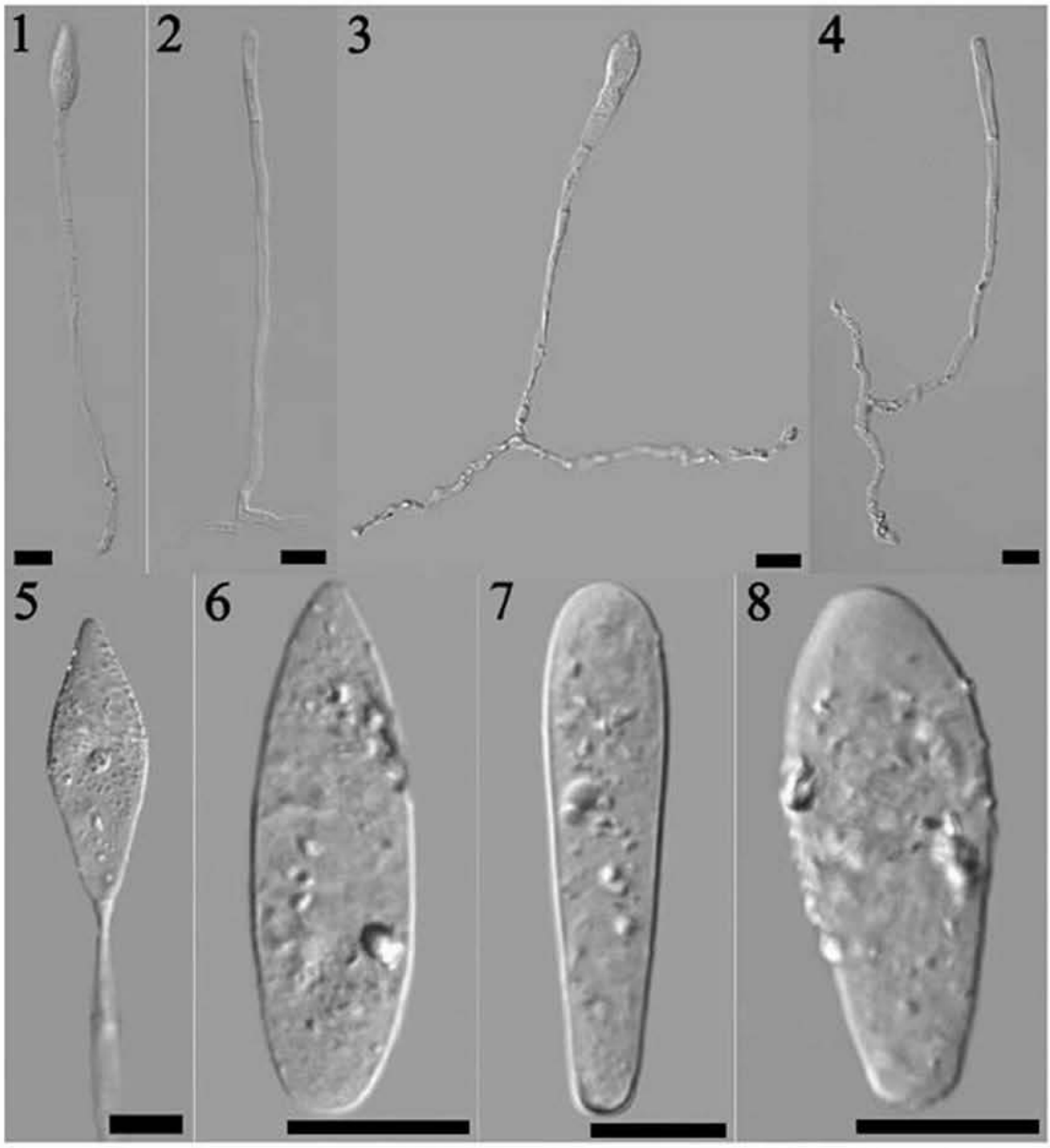
On living leaves. *Mycelium* hypophyllous. Superficial *hyphae* branched, septate, hyaline, flexuous, 4–6 µm wide, mycelial appressoria indistinct. *Conidiophores* produced from the external mycelium, cylindrical, hyaline, smooth, unbranched, septate, up to 264 x 5-7 µm, foot-cells mostly straight or somewhat slightly sinuous, very long, followed by 1-2 shorter cells. Conidia single, dimorphic: primary conidia lanceolate, apically pointed, 54–84 x 14–28 µm, l/w ratio 1.9–5.1; secondary conidia cylindrical to clavate with apically rounded, basally subtruncate ends, 50–76 x 16–26 µm, l/w ratio 1.9–4.8, aseptate, hyaline, smooth. One germ tube per conidium, up to 2 × the length of the conidium, with indistinct appressoria. Primary conidium with germ tube at base or at apex, secondary conidium with germ tube arising from the conidial shoulder. Teleomorph: not found.

SPECIMENS EXAMINED – VENEZUELA, La Victoria, Aragua, on *Passiflora rubra* L., 31 Jan 1928, H. Sydow (**HOLOTYPE**: BPI 414143; **ISOTYPE**: K(m) 131785).

## Discussion

There are few reports of powdery mildew on *Passiflora*. *Oidium passiflorae* Politis was described from Greece (Politis 1938) and also reported in Germany (Braun 1998) and Australia (Liberato 2006). Anamorphic *Leveillula taurica* (Lév.) G. Arnaud was reported in Australia (Liberato 2006). Amano (1986) listed *Passiflora* spp. as hosts of these three species in some countries, although this author did not provide the original references from such records. The status of the two South African specimens, listed as *Ovulariopsis passiflorae* (Doidge 1950), and of *Ovulariopsis* sp. reported in Colombia (Tamayo & Pardo Cardona 2000) remain to be clarified. Misplacement of members of *Streptopodium* in *Ovulariopsis* appears to have occurred with some frequency in the past. For instance the powdery mildew of *Tabebuia serratifolia* (Vahl) G. Nicholson, originally identified by Ferreira (1989) as *Ovulariopsis* sp. was recently recollected and described as the new species *Streptopodium tabebuiae* Liberato & R.W. Barreto (Liberato & Barreto, 2005). This work represents part of an ongoing study aimed at elucidating the status of some dubious *Ovulariopsis*.

Now, there are five species included in *Streptopodium*: *S. bonariense* (Speg.) R.Y. Zheng & G.Q. Chen (teleom.: *Pleochaeta polychaeta* (Berk. & M.A. Curtis) Kimbr. & Korf) (Zheng & Chen 1978), *S. caricae* Liberato & R.W. Barreto (Liberato et al. 2004), *S. diospyri* G.J.M. Gorter (Gorter 1988), *S. passiflorae* and *S. tabebuiae* (Liberato &




**Figs 1-8.** *Streptopodium passiflorae* (from isotype) (bar = 20 µm). **Figs 1-4.** Conidiophores. **Figs 5-6.** Primary conidia. **Figs 7-8.** Secondary conidia.

Barreto 2005). Moreover, three other species of *Pleochaeta* Sacc. & Speg. emend. have unnamed anamorphs belonging in *Streptopodium*: *P. indica* N. Ahmad, A.K. Sarbhoy & Kamal (Ahmad et al. 1995), *P. prosopidis* (Speg.) U. Braun (Braun 1987) and *P. shiraiana* (Henn.) Kimbr. & Korf (Zheng & Chen 1978, Gorter & Eicker 1983).

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# Type revision of three *Termitomyces* species from India

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**Abstract**—Taxonomic status of three *Termitomyces* species described from India, viz. *T. longiradicatus, T. quilonensis* and *T. poonensis,* is discussed in this paper based on examination of the type material. *Termitomyces longiradicatus* is proved to be a synonym of *T. heimii*, and *T. quilonensis* and *T. poonensis* are conspecific with *T. eurhizus*.

**Key words**—*Tricholomataceae*, termite symbionts, nomenclature, taxonomy

## Introduction

*Termitomyces* R. Heim is a paleotropical genus of agarics obligately symbiotic with termites belonging to the subfamily *Macrotermitinae* (*Isoptera*). The *Termitomyces* species are usually characterised by the termite association, pinkish spore print, prominent perforatorium on the pileus and the subterranean pseudorhiza connected to the comb in the termite nest. *Termitomyces* has been proved to be a monophyletic clade in *Agaricales* based on molecular phylogenetic analyses (Moncalvo et al. 2000, Aanen et al. 2002, Rouland-Lefevre et al. 2002, Frøslev et al. 2003).

*Termitomyces* was established with ten species and six forms in 1942 (Heim 1942a), and more species and combinations were subsequently proposed by Heim (1942b, 1948, 1951, 1952, 1958) and by Otieno (1964, 1968). The genus was monographed by Heim (1977) with 28 taxa, including 20 species and eight forms from Africa and Asia. In the following decades, many new taxa were introduced, e.g. four from China (He 1985, Zhang and Ruan 1986, Wei et al. 2004), seven from India (Natarajan 1975, 1977, 1979, Sathe & Daniel 1980, Sathe & Deshpande 1980, Dhancholia et al. 1991), three from South Africa (Reid 1975, Van der Westhuizen & Eicker 1990), one from Zambia (Pegler & Piearce, 1980) and another from Tanzania (Saarimäki et al. 1994), eight from Cameroon (Mossebo et al. 2002) and five from South America (Gómez 1995; this report requires confirmation because South America is beyond the distribution

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range of *Macrotermitinae*). In total, 68 taxa have been published in *Termitomyces*, with 81 names including combinations and autonames. The genus is currently placed in *Tricholomataceae* R. Heim ex Pouzar (Kirk et al. 2001).

The eight new taxa, including seven species and one form described from India are *T. albidolaevis* Dhanch. et al., *T. heimii*, *T. indicus* Natarajan, *T. longiradicatus*, *T. quilonensis*, *T. poonensis*, *T. radicatus* Natarajan and *T. microcarpus* f. *santalensis* R. Heim. Among these names, *T. albidolaevis* was invalidly published according to Art. 37.5 of International Code of Botanical Nomenclature (Greuter et al. 2000) and *T. indicus* was considered to represent *T. microcarpus* f. *santalensis* (Pegler & Vanhaecke, 1994). Many species of *Termitomyces* reported in India, e.g. *T. clypeatus* R. Heim (Purkayastha 1985), *T. eurhizus* (Purkayastha 1985), *T. heimii* (Natarajan 1979), *T. microcarpus* (Berk. & Broome) R. Heim (Natarajan 1983), *T. radicatus* (Natarajan 1977) and *T. striatus* (Beeli) R. Heim (Purkayastha 1985), are also reported from China (Wei & Yao 2003).

In an ongoing project of *Termitomyces* species in China and worldwide, collections from India, including the type material of *T. longiradicatus*, *T. quilonensis* and *T. poonensis*, were kindly made available for study by the curator of the Herbarium of Mycology and Plant Pathology Department, Agharkar Research Institute, Pune, India (AMH). Examination of those specimens, compared with collections preserved in Mycological Herbarium, Academia Sinica (HMAS), Herbarium of Cryptogams, Kunming Institute of Botany, Academia Sinica (HKAS) and Herbarium, Royal Botanic Gardens, Kew (K), reveals that *T. longiradicatus*, *T. quilonensis* and *T. poonensis* are later synonyms of other species of *Termitomyces*. The results of this study are presented in this paper.

## Materials and Methods

Dried specimens from the herbaria listed above were examined both macroscopically and microscopically. The dried specimens were photographed and dimensions measured. Most of the type specimens have been damaged, especially that of *T. longiradicatus*. The following description is based on the examination of the herbarium material. For microscopic studies, free-hand sections of dried basidiomata, including lamellae and cutis, were prepared using a razor-blade and mounted in a 5% KOH solution. Size ranges of basidiospores, basidia, lamella hyphae and cutis were measured using an ocular micrometer. At least 30 basidiospores and 20 basidia of each mature specimen were measured except otherwise specified. The microscopic characters were drawn with the aid of a camera lucida.

## Taxonomy

***Termitomyces longiradicatus*** Sathe & J. T. Daniel [as '*longiradicata*'] in MACS Monograph No. 1. *Agaricales* (Mushrooms) of South West India (Pune): 102 (1981) [as 1980].

**Figs 1 & 2**

*Pileus* 1.2–5.0 cm diam., campanulate or convex when young and then becoming plano-convex with an umbonate perforatorium; surface pale brown at the center, greyish white elsewhere. *Lamellae* cream, 3.0–4.0 mm broad, sinuate, crowded with lamellulae. *Stipe* 2.0 cm or longer, 0.5–0.8 cm diam., central, cylindric; surface pale brown. *Pseudorhiza*

0.5–0.7 cm diam., over 6.0 cm long; surface pale brown; smooth. *Annulus* broad and thick, greyish white, double-ringed, persistent on the upper part of the stipe; consisting of narrow, parallelly arranged hyphae of 2.0–5.0 μm diam. *Context* fleshy, consisting of thin-walled, inflated hyphae, 3.0–20 μm diam. *Basidiospores* 7.0–8.5 × 4.0–6.0 μm, ellipsoid, inamyloid. *Basidia* 18.5–24 × 8.5–9.5 μm, tetrasporic, clavate. *Lamella-edge* heterogenous. *Cheilocystidia* 21–56 × 10.5–27 μm, pyriform to inflated pyriform, hyaline. *Pleurocystidia* 28–67 × 14.5–28 μm, numerous, thin-walled, pyriform, hyaline. *Hymenophoral trama* regular, with parallel, thin-walled, hyaline hyphae, 3.0–20 μm diam. *Subhymenial layer* narrow. *Pileipellis* an epicutis of radially parellel, repent hyphae, up to 5.5 μm diam.

*Specimen examined*: **India:** Kerala State, Peechi, solitary, associated with termite nest, sine date, sine collector, AMH 4522 (holotype).

The color of pileus, stipe and pseudorhiza was observed from the dried material of the holotype, but it was described in the protologue as 'white-cream with pale grey tint' for the pileus, 'white becoming creamish white' for the stipe and 'white' for the pseudorhiza, presumably from the fresh material. Pileus context and lamellae in the holotype are seriously damaged by insects. There were only a few lamellae, basidiospores, basidia and cystidia available for examination (Fig. 2), but the lamellae and the range of the size of microscopic characters were described according to the original description. The tissue of stipe and pseudorhiza is also badly damaged in the holotype and the interior structure cannot be determined.

*Termitomyces longiradicatus* was described as close to *T. heimii* but different from the latter in having much longer radicating stipe (25–32 cm when fresh, with pseudorhiza up to 29 cm below the ground) and well-formed cheilocystidia (Sathe & Daniel 1981). From the original illustration and the present observation, the length of the stipe described for the holotype by Sathe & Daniel (1981) must include the pseudorhiza. However, the long stipe (5–9 cm) and pseudorhiza (7–36 cm), together with numerous cheilocystidia in the hymenium, have been reported in *T. heimii* (Pegler & Vanhaecke 1994). The length of the pseudorhiza is determined by the depth of the termite comb and the cystidia may be absent or present in species of *Termitomyces*. These are, therefore, not constant taxonomic characters. Specimens with long pseudorhiza and numerous cheilocystidia are found in many other collections from Asia, e.g.

**China:** Yunnan, Jinghong, bought on the local market, 3 Aug. 2003, T. -Z. Wei & Q. -B., Wang, W03-6, HMAS 77076; Mengla, Menglun, bought on the local market, 16 July 1990, J. -G. Shuai 1, HKAS 22668; the same locality, bought on the local market, 5 Aug. 2003, M. Li & T. -Z. Wei, W03-8, HMAS 77075. **India:** Andra Pradesh, Prakasam District, Kansukur, on termite hills, Nov. 1980, C. -P. Roo, K(M) 94757; Tamil Nadu, Madras, Chepauk, Madras University Campus, on termite nest, 8 Nov. 1977, K. Natarajan, KN129, K(M) 94755 (holotype of *T. heimii* Natarajan). **Malaysia:** Selangor, Serdang, University Patonian, symbiosis with Odontotermes sp., 9 July 1990, M. Vanhaecke T3, K(M) 16528. Pakistan: Daska, on termite nests, 6 Aug. 1980, Ahmad 27757, K(M) 94753; Islamabad, on termite nests, 6 Aug. 1977, Ahmad 27462, K(M) 94647.

All these collections share the same characters of *T. heimii*, as indicated by examination of the type of the species. The morphological characters described for *T. longiradicatus* by Sathe & Daniel (1981) and confirmed by the present study are within the range of variation of *T. heimii*.

*Termitomyces heimii* was originally described from Madras, Tamil Nadu, India (Natarajan 1979) and is widely reported from tropical Southeast Asia (Natarajan & Raman 1983, Purkayastha 1985, Pegler & Vanhaecke 1994, Turnbull & Watling 1999) and southern China (Wei & Yao 2003). It is recognized by its large, smooth, white (grey or brownish grey at the centre), subumbonate pileus and persistent annulus (Natarajan 1979, Pegler & Vanhaecke 1994). The large, white-cream (dark at the center), umbonate pileus and the thick, persistent, broad, double-ringed annulus (Sathe & Daniel 1981, and this study) make *T. longiradicatus* identical with *T. heimii*. Therefore, they are considered conspecific here and *T. longiradicatus* is a later synonym of *T. heimii* Natarajan in Mycologia 71: 853 (1979).

Most collections of *T. heimii* examined here were collected in July and August in China and Malaysia, while the collections from India, including the holotype, were made in November. There is another record of *T. heimii* from China, which was made in the late season of the year (Yunnan, Mengla, Menglun, bought on the local market, 12 Nov. 1989, Z. -L. Yang 984, HKAS 22118), but with the stipe enlarged at the position of annulus (Yang 1990). The late season in China and the enlarged stipe make the collection very interesting for further study to compare with other material of the species from India and other countries of Asia.

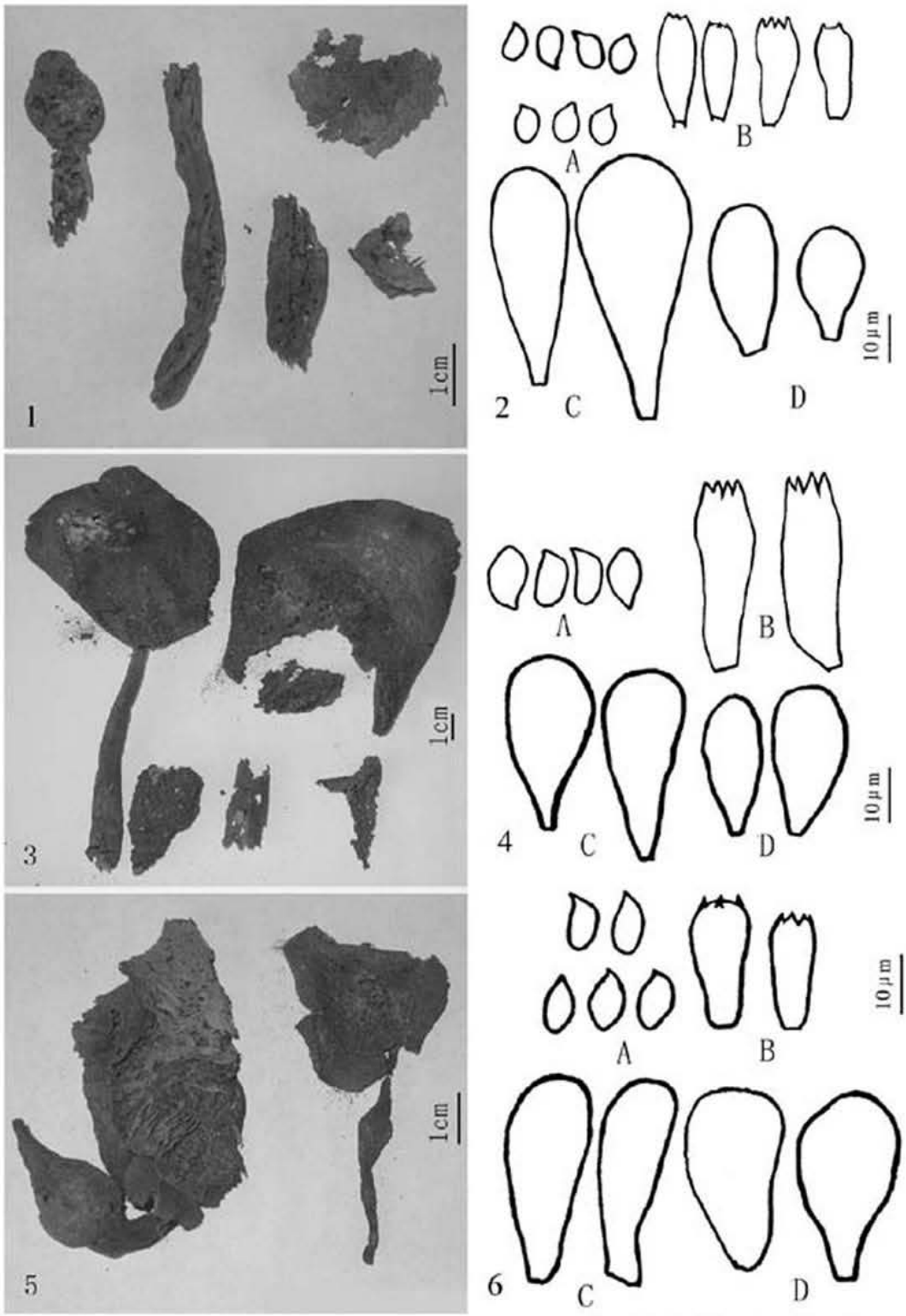
***Termitomyces poonensis*** Sathe & S. D. Deshp. in MACS Monograph No. 1. *Agaricales* (Mushrooms) of South West India (Pune): 36 (1981) [as 1980]. **Figs 3 & 4**

*Pileus* 6.0–7.8 cm diam., plano-convex with a prominently rounded perforatorium, margin incurved with radially-arranged fine grey striae and spots; surface dark brown at the centre, brown or orange brown elsewhere, smooth. *Lamellae* free, up to 4.0 mm wide. *Stipe* over 9.5 cm long, 0.4–1.0 cm diam., central, cylindric at upper part, thickening downward up to 1.8 cm diam. at ground level; surface grey, becoming orange brown downwards, smooth and glabrous. *Pseudorhiza* over 3.5 cm long, tapering downward; surface black. *Annulus* absent. *Context* fleshy, of inflated, hyaline, thin-walled hyphae, 3.0–25 µm diam. *Basidiospores* 7.0–9.0 × 4.0–6.0 µm, ellipsoid, subhyaline and thin-walled. *Basidia* 15.0–20 × 7.0–12.0 µm. *Lamella-edge* heterogenous. *Cheilocystidia* 21–54 × 17.0–25 µm., obovoid to pyriform, hyaline, thin-walled *Pleurocystidia* 25–46 × 13.0–28 µm, pyriform, hyaline, thin-walled. *Hymenophoral trama* regular, 40–60 µm wide, of hyaline, thin-walled hyphae, 2.5–15.0 µm diam. *Subhymenial layer* 10.0–15.0 µm wide, of branched hyphae, 2.0–5.0 µm diam. *Pileipellis* an epicutis of dense, radially parellel, repent hyphae, 2.0–5.5 µm diam.

*Specimen examined*—**India:** Poona, in groups, on termite nests, sine date, sine collector, AMH 4479 (holotype).

The color of basidiomata was observed from the herbarium specimen of the holotype, whilst the color of the pileus was described as 'smoke gray when young, becoming platinum blonde with age', the stipe as 'white' and the pseudorhiza as 'black' in the protologue, possibly based on fresh material.

Sathe & Deshpande (1981) proposed this new species on account of its 'perforatorium prominent and darker than pileus, monomorphic cheilocystidia, conidial elements on pileus'. However, the glabrous, exannulate stipe and the black pseudorrhiza are reminiscent of *T. eurhizus*. *Termitomyces eurhizus* is a species with a wide distribution




Figs 1–6. Photographs and line drawings of *Termitomyces* spp. from India. Figs 1–2. *Termitomyces longiradicatus* (AMH 4522, holotype). Fig. 1. Habit. Fig. 2. A. Basidiospores; B. Basidia; C. Pleurocystidia; D. Cheilocystidia. Figs 3–4. *Termitomyces poonensis* (AMH 4479, holotype). Fig. 3. Habit. Fig. 4. A. Basidiospores; B. Basidia; C. Pleurocystidia; D. Cheilocystidia. Figs 5–6. *Termitomyces quilonensis* (AMH 4546, holotype). Fig. 5. Habit. Fig. 6. A. Basidiospores; B. Basidia; C. Pleurocystidia; D. Cheilocystidia.

(Saccardo 1887, Pegler & Rayner 1969, Pegler 1977, Samajpati 1981, Pegler & Piearce 1980, Piearce 1987, Wei & Yao 2003), and its pileus color and form are variable. The color varies from grayish brown to dark brown and often darker in the center than elsewhere. The perforatorium is obtusely conical to mucronate (Pegler & Vanhaecke 1994) and often prominent (Singer 1949, Purkayastha & Chandra 1975, Samajpati 1981, Pegler 1986). The cheilocystidia are obovoid to pyriform (Pegler 1977, 1986, Pegler & Vanhaecke 1994) or usually pyriform (Purkayastha & Chandra 1975, Purkayastha 1985). There have been quite a few synonyms for this species, e.g. *T. cartilagineus* (Berk.) R. Heim (Pegler & Rayner 1969, Pegler 1977) and *Agaricus sparsibarbis* Berk. & Broome (Pegler & Vanhaecke 1994). *Termitomyces albiceps* S. C. He, based on some collections from Guizhou, China, was also suspected to be a synonym of *T. eurhizus* (Pegler & Vanhaecke 1994). The monomorphic cheilocystidia (Sathe & Deshpande 1981) of *T. poonensis* might be due to limited material examined, as the cheilocystidia of *T. albiceps* were also described as pyriform (He 1985). The 'transversely septate' cheilocystidia in the original description of *T. poonensis* were not found in the holotype by the present authors. The conidial elements on pileus, mentioned in the protologue, are possibly accidentally produced and have not been reported elsewhere for *Termitomyces*. Species of *Termitomyces* can produce sporodochia bearing conidiophores in the fungus garden within termite nest and the conidia produced by the conidiogenous hyphae were often similar in size and form (Botha & Eicker 1991b). The conidial elements mentioned in the protologue of *T. poonensis* are not a stable character and have never been used to distinguish species in the genus. Judged from the morphology of basidiomata observed from the holotype, *T. poonensis* is here considered a synonym of *T. eurhizus* (Berk.) R. Heim in Arch. Mus. Hist. Nat. Paris, Sér. 6, 18: 140 (1942).

To confirm the above determination, the following collections of *T. eurhizus* were also examined for comparison.

**India:** Bishnupur, Shillong, Meghalaya, 3 July 1984, R. -N. Verma, M107, K(M) 94642. **China:** Yunnan, Mengla, Menglun, Xishaungbanna Tropical Botanical Garden, 8 Aug. 2003, G. -R. Hu & T. -Z. Wei, W03-27, HMAS 88326; the same locality and date, G. -R. Hu & T. -Z. Wei, W03-21, HMAS 84723; the same locality, 4 Aug. 1988, Z. -L. Yang 262, HKAS 21785; Yunnan, Simao, bought on the local market, 10 Aug. 2003, T. -Z. Wei & Q. -B. Wang, W03-30, HMAS 85229; Sichuan, Chengdu, bought on the local market, 30 Aug. 2002, B. Wang 200252, HMAS 84529. **Malaysia:** Selangor, Serdang, Malesysen, Universitat Pertanian, 7 July 1990, M. Vanhaecke, T9, K(M) 16525. **Sri Lanka:** Kandy District, Peradeniya Botanic Garden, alt. 1600ft, 23 Oct. 1974, D. N. Pegler 2062, K(M) 94646.

***Termitomyces quilonensis*** Sathe & J. T. Daniel [as '*quilonesis*'] in MACS Monograph No. 1 *Agaricales* (Mushrooms) of South West India (Pune): 103 (1981) [as 1980].

Figs 5 & 6

*Pileus* 3.3–6.5 cm diam, convex or up-turned, with an obtusely conical perforatorium, slightly inflexed at the margin; surface dark brown at the centre and becoming paler toward the margin. *Lamellae* free, up to 3.0 mm wide. *Stipe* 5.0 × 0.2–1.0 cm, cylindrical, enlarged at ground level and forming a globose bulb up to 1.6 cm diam. below ground; surface dark brown to almost black, smooth; solid. *Pseudorrhiza* over 3.0 cm long, tapering abruptly downward below the bulb, solid; surface black. *Annulus* absent. *Context*

fleshy, of inflated, hyaline, thin-walled hyphae, 3.0–8.0 µm diam. *Basidiospores* 8.0–9.0 × 4.5–5.5 µm, ellipsoid, subhyaline and thin-walled. *Basidia* 17.5–30 × 6.0–8.5 µm, subhyaline, thin-walled, clavate, tetrasporic. *Lamella-edge* heterogenous. *Cheilocystidia* 25–41 × 12.0–27 µm, pyriform to inflated pyriform, hyaline, thin-walled. *Pleurocystidia* 28–52 × 14.5–28 µm, rare, broadly clavate or pyriform to inflated pyriform, hyaline. *Hymenophoral trama* regular, 50–60 µm wide, of hyaline, thin-walled hyphae, 2.5–15.0 µm diam. *Subhymenial layer* 10.0–15.0 µm wide, of branched hyphae, 2.0–5.0 µm diam. *Pileipellis* an epicutis of dense, radially parellel, repent hyphae, 2.0–5.5 µm diam.

***Specimens examined*:** India: Kerala State, Quilin, solitary, on termite nest, sine date, sine collector, AMH 4546 (holotype).

The color of pileus, stipe and pseudorhiza in the above description was taken from the dried material of the holotype. In the protologue, the pileus was described as 'gray towards margin, brownish gray towards center', the stipe as 'white' and the pseudorhiza as 'brownish black' possibly from fresh material.

According to Sathe & Daniel (1981), *T. quilonensis* differs from *T. poonensis* in the absence of conidia and in having bulbous base in the stipe. However, the stipe base of *T. poonensis* is also enlarged slightly in the holotype as observed by the present authors. As conidia are not a useful character to distinguish species in the genus (see above in the remark for *T. poonensis*), absence of conidia cannot make *T. quilonensis* a distinct species from *T. poonensis*. Further, as discussed above, *Termitomyces poonensis* is a synonym of *T. eurhizus*. In fact, *T. eurhizus* is a species variable in morphology, but can be easily recognized by its black-brown encrusted and cartilaginous pseudorhiza (Heim 1942, Pegler 1977, 1986, Pegler & Vanhaecke 1994). The stipe of *T. eurhizus* often expands at the base before attenuating into an elongate pseudorhiza (Pegler & Rayner 1969, Pegler 1977), often but not always with a bulbous base (Pegler 1986). The brownish gray pileus and the black pseudorhiza of *T. quilonensis* closely resemble *T. eurhizus*, and the microscopic characters are all within the range of those of *T. eurhizus*. Thus, *T. quilonensis* is considered as another synonym of *T. eurhizus*.

## Discussion

The above three species of *Termitomyces* were proposed based on minor morphological characters, e.g. cystidia, conidial elements, and the length or form of stipe or pseudorhiza (Sathe & Deshpande 1981). Cystidia may be absent or present in *Termitomyces* species (Van Der Westhuizen & Eicker 1990), and they could be found as either monomorphic or polymorphic based on different materials examined (Pegler 1977, Pegler & Vanhaecke 1994). Conidia are asexual spores produced in termite combs or in cultures. The size and form of conidia are similar in cultures, except for *T. microcarpus* which lacks conidiophores and conidia (Botha & Eicker 1991a) and the presence of conidia on the pileus surface might be accidental (Botha & Eicker 1991a). As the form of basidiomata in *Termitomyces* depends very much on the symbiosis, especially the identity and behaviour of the host termites (Piearce 1987), the length of pseudorhiza can vary with the depth of the termitaria. The swollen stipe base is present in several species of the genus, such as *T. umkowaani* (Cooke & Massee) D. A. Reid, *T. bulborhizus* T. Z. Wei et al. and *T. sagittiformis* (Kalchbr. & Cooke) D. A. Reid. However, the bulbous base is not stable in some species as discussed above in *T. eurhizus*.

Among the eight new taxa of *Termitomyces* described from India, two are well recognized, i.e. *T. heimii* with a wide distribution in Asia (Pegler & Vanhaecke 1994, Wei & Yao 2003) and *T. microcarpus* f. *santalensis* in India (Pegler & Vanhaecke 1994). However, in addition to *T. indicus* (=*T. microcarpus* f. *santalensis*, see Pegler & Vanhaecke, 1994), three more have been proved to be synonyms of other species in the genus by the present study, i.e. *T. longiradicatus*, *T. quilonensis* and *T. poonensis*. For the remaining two, *T. albidolaevis* and *T. radicatus*, they also need further examination. *Termitomyces albidolaevis* was proposed based on silvery white color of the pileus, considered close to *T. mammiformis* f. *albus* R. Heim but different in the absence of conical perforatorium and in having larger basidiospores (7.5–10.0 × 5.0–7.0 μm, Dhancholia et al., 1991), although the pileus was contradictorily described as 'umbonate' in the same publication. In fact, *T. albidolaevis* can be easily distinguished from *T. mammiformis* f. *albus* by the absence of the annulus and the size of basidiospores (6–8 × 3.5–4.5 μm in *T. mammiformis* f. *albus*, see Pegler 1977) according to the original description and illustration. *Termitomyces albidolaevis* is somewhat unique for its relatively large, white pileus (11–16 cm), the whitish pseudorhiza and large basidiospores. *Termitomyces citriophyllus* R. Heim is the most close species to *T. albidolaevis* in terms of the size of pileus (9–10 cm) and of basidiospores (9–11× 6.7–7.7 μm), but differs in the dark colored (ochreous gray) pileus, and much larger basidia (30–49 × 12–15 μm, compared with 17.0–27.5 × 5.0–7.5 μm in *T. albidolaevis*), although the surface color of pseudorhiza was never mentioned (Heim 1942a, 1977). Re-examination of the type material of *T. albidolaevis* may confirm its status. *Termitomyces radicatus* is distinguished from other species with small basidiomata, such as *T. microcarpus*, *T. medius* R. Heim & Grassé and *T. tylerianus* Otieno, by the dark colored spiniform perforatorium, a short pseudorhiza and the absence of pleurocystidia and cheilocystidia (Natarajan 1977). However, the pseudorhiza were found terminating at a sclerotized disk and scattered hymenial cystidia were detected in some material of *T. radicatus* from India and Pakistan (Pegler & Vanhaecke 1994). *Termitomyces radicatus* was also considered by Pegler & Vanhaecke (1994) to resemble *T. clypeatus* but much smaller and to differ from *T. microcarpus* mainly in the presence of a short pseudorhiza. The differences among *T. radicatus*, *T. medius* and *T. tylerianus* are also not very significant. *Termitomyces radicatus* was distinguished from the other two by the pseudorhiza termitnating at a sclerotized disc (Pegler & Vanhaecke 1994), but a disc-like structure in the base of the pseudorhiza was also observed in other species, such as *T. reticulatus* Van der Westh. & Eicker (Van der Westhuizen & Eicker 1990), although the latter is much larger in size. Observation of such a structure depends much upon the intactness of the collection. The terminal structure in the pseudorhiza of *Termitomyces* species may have been destroyed in most collections because the pseudorhiza is connected with the nest of termite. Detailed study of *Termitomyces* species with small basidiomata is required to reveal the relationships of those species. A molecular systematic study of these species is undergoing in this laboratory.

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# A review of the genus *Gyromitra* (Ascomycota, *Pezizales, Discinaceae*) in Mexico

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**Abstract**—*Gyromitra* has three species in Mexico: *G. ambigua, G. esculenta* and *G. infula*, of which *G. ambigua* is recorded for the first time. The three species are widely distributed primarily in forest of *Abies* and *Pinus-Quercus* and rarely in cloud forests. Some considerations of the *G. esculenta* complex are presented and a key to the recognized species is provided.

**Key words**—taxonomy, species complex

## Introduction

According to Kirk et al. (2001), the genus *Gyromitra* Fr. is composed of nearly fifteen species. Usually, it has been considered to be closely related to three genera: *Discina* (Fr.) Fr., *Neogyromitra* S. Imai and *Pseudorhizina* Jacz. These genera have a common excipular structure, four nuclei per spores and paraphyses that are very similar; differences in spore shape and ornamentation, ascoma shape and presence of a stipe are considered quantitative (Harmaja 1969a, 1973). The relationships among these and other genera are not clear (Harmaja 1969a). *Gyromitra* was placed first in the family Helvellaceae Dumort. (Saccardo1889; Seaver 1928; Raitviir 1965; Dennis 1978, Korf 1972, Kimbrough et al., 1990, Kimbrough 1991). However, O'Donnell et al. (1997) moved *Gyromitra* to the family Discinaceae Benedix emend. N.S. Weber, Trappe & O´Donnell, and they pointed out the necessity of doing phylogenetic studies within the genera of this family, including *Gyromitra* and argued, in agreement with Harmaja (1969a), that *Gyromitra* and *Discina* are congeneric (Eckblad 1968; Abbott and Currah 1997). Information on the position of the genus *Neogyromitra* (Harmaja 1969a) is still wanting. *Gyromitra* also needs a detailed survey to establish relationships among species. It may be composed of two (Harmaja, 1973) or more subgenera (Mcknight and Batra 1974; Abbott and Currah 1997).

*Gyromitra* sensu lato, that is including *Discina*, comprises species with inamyloid asci, elliptical, smooth ascospores, apothecia that are stipitate and bilobate or brain-like, as well as those species with subfusiform to fusiform ornamented ascospores, apothecia that are discoid, sessil or subsessil and medullary excipulum of textura intrincata. This is the generic concept presented by Harmaja (1969a, 1973) and Pfister (1980). In Mexico

only the stipitate, bilobate or brain-like species have been found to date. *G. esculenta* is variable in spore form and I follow Harmaja's (1969a) spore types in the discussion below to distinguish within this complex.

*Gyromitra* was recorded in Mexico for the first time by Herrera and Guzmán (1961), based on *G. infula* (as "*Helvella infula* Fr."), growing in coniferous forests in the State of Mexico and Federal District. There it has been known as an edible fungus, after being boiled and the water being discarded. Later *G. esculenta* was recorded from Nuevo León by Castillo et al. (1979). A third species *Gyromitra melaleuca* (Bres.) Donadini (as *Discina melaleuca* Bres.) was reported from Jalisco State by Guzmán-Dávalos et al. (2001).

## Materials and methods

Eighty five specimens from the following herbaria were considered in this study: ENCB, FCF, FCME, FH, H, IBUG, ITCV, K and XAL. Acronyms follow Holmgren et al. (http://sciweb.nybg.org/science2/ih/searchih.html) with the addition of ITCV (Instituto Tecnológico de Ciudad Victoria) and FCF (Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León). Microscopic observations were made on sections mounted in 10% KOH, for measurements of hymenial elements, and Melzer reagent and cotton blue in lactophenol for observation of spore ornamentation and apiculi.

## Results

*Gyromitra* in Mexico comprises three species: *G. ambigua*, *G. esculenta* and *G. infula*. *G. ambigua* has not previously been reported in Mexico. The report of *Gyromitra melaleuca* (as *Discina melaleuca*, Guzmán-Dávalos et al., 2001) is based on a misidentification. Study of reference material (*Tamayo & González* s.n., from Nevado de Colima mountain, in Jalisco, IBUG), showed a superficial similarity to *G. melaleuca*, i.e. apothecium discoid, with margin unevenly revoluted, and a short, nut-brown stipe; and spores 20-22 x 10-12 µm, with short spines and paraphyses 5-8 µm wide and clavate. Nevertheless, when mounted in Melzer reagent a positive reaction of the asci is observed indicating it is a member of the family *Pezizaceae*. It is worth note that the record of *G. melaleuca* from North America (Seaver 1928) is doubtful. Abbott and Currah (1997) suggest the collection from the United States represents a different species since they consider *G. melaleuca* to be restricted to Europe from where it was described.

## Key to the recognized species of *Gyromitra* in Mexico

1a. Apothecium gyrose or brain-like or rarely bilobate, thick, vinaceous brown, reddish or violaceous, spores elliptical to fusoid 18-25 (-27) x (8-) 10-12.5 µm . . . . . ***G. esculenta***

1b. Apothecium saddle shape or rarely gyrose and narrow, brown orange or reddish-brown. . . . . 2

2a. Spores elliptical, walls without thickened poles, 17.6-25 x 7.2-10 µm . . . . ***G. infula***

2b. Spores elliptical-fusoid, (23-) 25-33 x (8-)10-12 µm walls thickened at the poles forming apiculi up to 2.5 µm thick . . . . . ***G. ambigua***

*Gyromitra ambigua* (P. Karst.) Harmaja, Karstenia 9: 17. 1969. **Fig. 1-2**

= *Helvella ambigua* P. Karst., Meddelanden af Societas pro Fauna et Flora Fennica 5: 53. 1879.

= *Gyromitra infula* var. *apiculatispora* Raitv., Eesti NSV Teaduste Akadeemia Toimetised (Bioloogiline Seeria) 14 (3): 322. 1965 (fide Abbott and Currah, 1997)

The lobed or rarely brain-like, dark red-orange apothecia and fusoid spores, (23-) 25-33 x (8-) 10-12 µm, with thicker poles, as apiculi 2.5 µm high, are the diagnostic features. The ascoma of this species is very similar to *G. infula*, although the shape of some material studied resembles *G. esculenta*. We studied the holotype material and found the spores to measure 30-33 x 10-12 µm, with apiculi of 2 µm. Notes with the type material described spores up to 40 µm, but I have not seen spores longer than 33 µm. Mexican material has spores of (23-) 25-33 x (8-)10-12 µm, with longer apiculi (up to 2.5 µm) than as noted in the type specimen. The species was described in detail by Abbott and Currah (1997) and Harmaja (1969b) from the United States and Europe. These collections represent the first record of *G. ambigua* from Mexico. This species grows in soil or wood in *Abies religiosa* and *Pinus hartwegii* forests, at altitudes of around 2900 m.

**Studied material:** MEXICO. Michoacan. Villa Madero Municipality, Cerro Cruz Gorda, highway Pátzcuaro-Tacámbaro, 23/X/1979, *Sánchez 8* (XAL). MORELOS. Lagunas de Zempoala, road to Chalma, 12/X/1965, *Díaz y Carmona* s.n., (XAL, ENCB). CANADA. Prince Edward Island, Herb Ellis (FH). FINLAND. Tammela, 30/VII/1866, *Karsten 3289* (holotype of *H. infula* var. *similis*, H). UNITED STATES. Michigan, /IX/1979, *Beardslee* s.n. (FH).

*Gyromitra esculenta* (Pers.: Fr.) Fr., Summa VegetabiliumScandinaviae 2: 346. 1849. **Fig. 3-4**

= *Helvella esculenta* Pers.:Fr., Commentarius Schaefferi icones pictas p. 64. 1800.

= *Gyromitra bubakii* Velen., Česke houby p. 893. 1922 (fide Abbott and Currah, 1997).

The brain-like apothecia with vinaceous brown, reddish or violaceous tones are typical of this species. There is a great deal of variability, as reported by Harmaja (1979a), in *G. esculenta*, including hymenium folding, apothecial margins, color of the hymenium and stipe. Harmaja attributes this variation primarily to environmental factors and maturity of the material. He considered there to be three spores types within this group: type I (*G. esculenta* s. str.), spores 18-23 (-25) x 10-12.5 µm, elliptical to fusiform with apiculi up to 0.5 µm; type II (without any taxon assigned), spores 20-25 x 10-12.5 µm, subfusiform, apiculi up to 1 µm, and type III (*G. splendida* and *G. bubakii*?), spores 22-30 x 10-12.5 µm, fusiform, apiculi 0.6-1.2 µm.

I regard *G. esculenta* to be a species complex that includes *G. bubacii, G. esculenta* s. str., *G. longipes* Harmaja and *G. splendida* Raitv. All of these have very similar morphology and spore size. Raitviir (1974) described *G. splendida* with deep violaceous tones in the hymeniun and stipe, with a stipe longer than in *G. esculenta*, and subfusiform spores 23-28 x 11.5-13.5 µm, with a perisporium thickened up to 2 µm. This author pointed out that the long stipe and not so broad cap were different than *G. esculenta*, but in the field these species were indistinguishable. Harmaja (1979b) considered *G. longipes* to differ from *G. esculenta* s. str., in its darker pileus and long stipe with violaceous tones and in its fusiform spores 20-25 x 9-10 µm, with apiculi up to 2 µm. Huhtinen and Ruotsalainen (2004), studying specimens from Finland, suggested the conspecificity of *G. splendida*

Table 1. Characters of the species *G. esculenta* complex

| | Apothecium | Spore shape | Size (µm) | Apiculum |
|---|---|---|---|---|
| *G. esculenta* (I) | gyrose, vinaceous brown, reddish to violaceous tones stipe more or less long* | ellipsoid- | 18-23 (-25) x 10-12.5 | 0.5µm |
| *G. esculenta* (II) | | subfusiform | 20-25 x 10-12.5 | 0.5-1µm |
| *G. esculenta* (III) = *G. splendida*, *G. bubakii*? | | fusiform | 22-30 x 10-12.5 | 0.5-1.5µm |
| *G. longipes* | lightly gyrose, dark color with violet tones, long stipe | subfusiform | 20-25 x 9-10 | 1-2µm |
| *G. splendida* | gyrose, dark- brown to almost black with violet tones, long stipe | subfusiform | 23-28 x 11.5-13.5 | 1-2 µm |

*"long' = 2/3 of the total length of the ascocarp

and *G. longipes*. In fact, regarding morphological characters and measurements of spores (type III of Harmaja), *G. longipes* and *G. splendida* seem to be the same (see Table 1). *G. bubakii* (Velenovsky, 1922) has smaller fruitbodies, longer stipes (2/3 of the total length) and bigger spores 30-34 µm than *G. esculenta*. The type of *G. bubakii* was study by Moravec (1986), Abbott and Currah (1997) and Huhtinen and Routsalainen (2004). For the former *G. bubakii* is a variety of *G. esculenta*, while for Abbott and Currah it is conspecific with *G. esculenta*, and Huhtinen and Routsalainen considered it different from *G. esculenta*. Moravec (1986) showed a continuous wall on the spore, but not in the apiculi. The loss of apiculi is attributed to preservation in formaldehyde (Huhtinen and Rouatsalainen 2004), but Kempton and Wells (1973) have suggested that spores of *G. esculenta* four weeks old, lose their apiculi. Spores of *G. esculenta* seems to vary widely in size. Most of the material studied from Mexico has elliptical to subfusiform spores 18-20 x (8-) 10-12.5 µm, which correspond to type I. Nine of the studied specimens presented subfusoid spores of 20-25 (-27) x 10-12.5 µm with apiculi up to 1 µm (type II) and only one specimen (*Medel 11*, XAL) had very large fusoid spores, 27-33 x 11.8-13.5 µm, with apiculi of more than 1 µm. Mexican material shows great variability in ascoma shape, length and width of the stipe, but not in ascoma color. This variability was similar to that found in the materials from Canada, England, Finland and the United States. To understand this variability it will be necessary to study more material of *G. esculenta* from the whole geographical range, particularly in order to judge the importance of spore size in the taxonomy of the group. Regarding Mexican materials *G. esculenta* s. str. is considered to have the following characters: spores of 18-20 (-27) x (8-)10-12.5 µm, folded brain-like hymenia that are vinaceous brown, reddish or violaceous tones,

a stipe, elliptical spores, and apiculi up to 1 µm. This measurement falls in both spore type I and II of Harmaja and coincides with the measurements cited by diverse authors (Boudier 1905, Dennis 1978, Kempton and Wells 1973, De la Torre 1977, Weber 1988, Abbott and Currah 1997). Moravec (1986) found that the spores in *G. esculenta* have a wide continuous range of variation up to 29.5 µm. I have not seen Mexican material with spores longer than 27 µm. Among the studied specimens, one of the collections (*Medel 11*, XAL) could represent another taxon, because of the subfusiform spores 27-33 x 11.8-13.5 µm with apiculi of 1-1.5µm.

At present there is no consensus about *G. esculenta* spore size. Moravec (1986) suggested that the original description of *G. esculenta* by Persoon was based on immature specimens. Harmaja (1979a) noted that the original concept of *Helvella esculenta* needed clarification and he mentioned several names that he thought were synonyms of *G. esculenta*. Abboth and Currah (1997) mentioned the type specimen is unknown, and so a neotype should be selected. Full descriptions of *G. esculenta* can be found in De la Torre (1977), Abbott and Currah (1997), Kempton and Wells (1973) and Weber (1988). The species is humicolous and lignicolous, and grows in *Abies religiosa*, *Pinus*, *Pinus-Quercus* and rarely in cloud forest at 2500-3600 m.

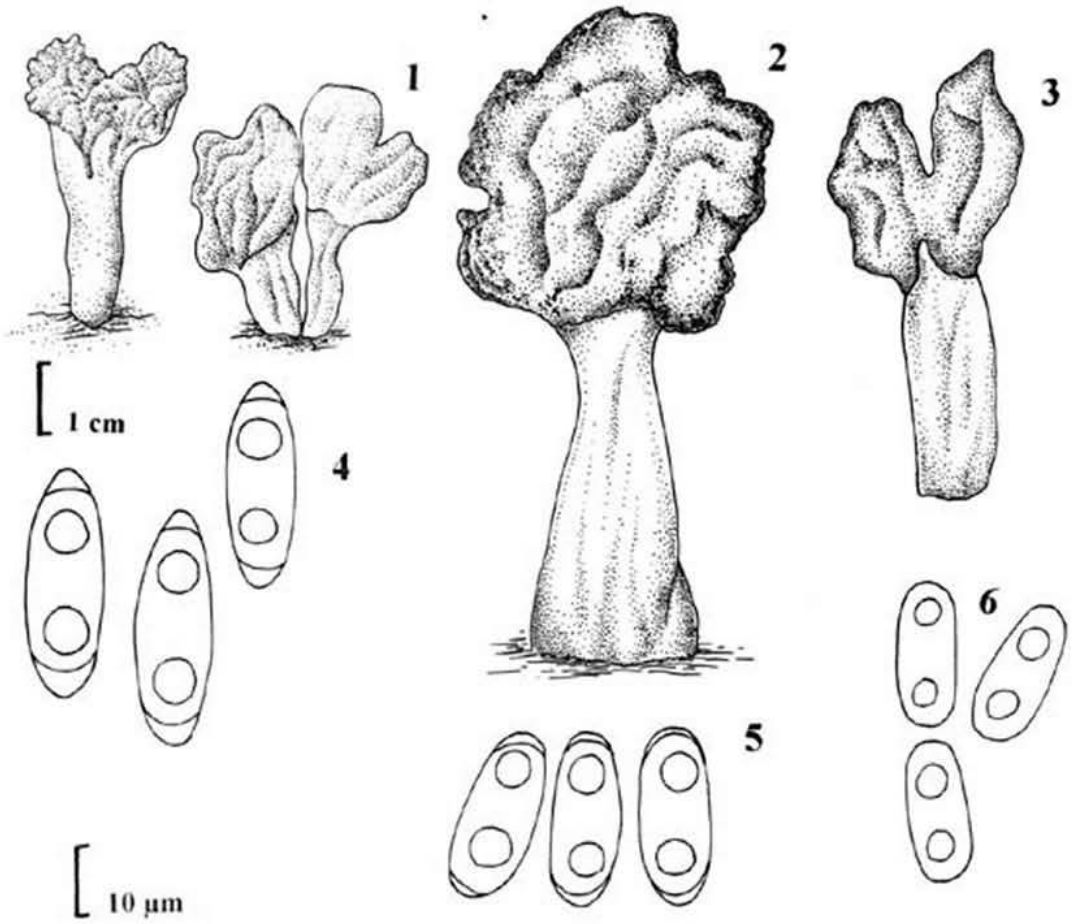
**Studied material:** MEXICO. COAHUILA. Arteaga Municipality, Las Carolinas, 27/VII/1986, *García* s.n. (ITCV); Arteaga Municipality, La Siberia, 19/X/2001, *Medel* s.n. (FCF). DURANGO. Highway Durango-Mazatlán, 28/VII/1984, *Garcia 434* (ITCV). ESTADO DE MÉXICO. Los Saucos, deviation to Valle de Bravo, highway Toluca-Temascaltepec, 15/VIII/1982, *Chacón 345* (ENCB); National Park Lagunas de Zempoala, highway to Chalma, 1/VIII/1982, *Chacón 287* (ENCB); Villa de Allende Municipality, San Cayetano N of Agua Escondida, 5/IX/1982, *González 42* (ENCB); West side Paso de Cortés, Popocatepetl Volcano, 26/IX/1986, *Chacón 570* (ENCB); Escualango, East side of San Rafael Atlixco, 7/X/1983, *Hernández 136* (ENCB). GUERRERO. Chilpancingo de los Bravo Municipality, Cerro Palo Hueco, Omiltelmi, *14/8/1984, Gutierrez* s.n., (FCME); Km 2.5 desviation to Puerto del Gallo, 27/VIII/1983, *Tapia* s.n., (FCME). HIDALGO. National Park El Chico Sierra de Pachuca, 31/10/1981, *Ramos* s.n. (ENCB); El Chico 13/8/1965, *Salas* s.n., (ENCB). MICHOACÁN. Zinapécuaro Municipality desviation to Eréndira, San Pedro Jacuaro, *Hernández y Villegas 924* (FCME).TLAXCALA. National Park La Malinche, 15/IX/1970, *Rodríguez-Martínez 43* (ENCB). VERACRUZ. Xico Municipality, Los Gallos, East side Cofre de Perote, *Rico 951* (XAL); Xico Municipality, near El Llanillo road Las Vigas-Tembladeras North side Cofre de Perote, *Bandala 2654* (XAL); Calcahualco Municipality, near Tlacoteopa, road La Jicara *Medel 891, 892, 893, 894, 895* (XAL); Cofre de Perote region, road Valle Alegre Park to Tembladeras, *Ramírez-Guillén 222* (XAL); IV Exposición de Hongos del INIREB, 31/VIII/1986, *Medel 11* (XAL); Cofre de Perote region, Valle Alegre Park, 13/VIII/2003, *Guzmán 35515* (XAL).

***Gyromitra infula*** (Schaeff.: Fr.) Quél, Enchiridion Fungorum 272, 1886. **Fig. 5-6**

≡ *Helvella infula* Schaeff.: Fr., Fungorum qui in Bavaria et Palatinatu nascuntur icones 4:105. 1774.

= *Helvella friesiana* Cooke, Mycographia seu icones Fungorum 1: 195, 1878.

The bilobate or saddle-shape, brown-orange apothecia and elliptic non-apiculate spores, 17.6-25 x 7.2-10 µm, are characteristic of *G. infula*. This species has the widest distribution of all the species reported in Mexico and is morphologically close to *G. ambigua*. Spore size and the lack of apiculi are diagnostic features. Our material has spores of 17.6-20




**Figs. 1-6.** Figs. 1-2. *Gyromitra ambigua*. 1: ascoma; 2: spores (showing apiculum). Figs. 3-4. *G. esculenta*. 3: ascoma; 4: spores (with a short apiculum). Figs. 5-6. *G. infula* 5: ascoma; 6: spores

(-25) x 7.2-10 µm. This is smaller than reported in the neotype specimen of *G. infula* (designated by Harmaja, 1969b) that is also the lectotype of *H. friesiana* at K, in which the spores reach a size of 20-25 x 8-10 µm, according to my observations. Additional descriptions of this species can be found in Abbott and Currah (1997), Kempton and Wells (1973), De la Torre (1979) and Weber (1988). The species grows on wood, humus or on burned soil, in forests of *Abies religiosa*, *Pinus*, and *Pinus-Quercus* and rarely in cloud forests, at 2700-3500 m.

**Studied material.** MEXICO. COAHUILA. Arteaga Municipality, La Siberia, 15/8/1973, *Guzmán 11248, 11251* (ENCB), 21/IX/1980, *Chacón 98* (ENCB). DISTRITO FEDERAL. El Ajusco, near Pico del Aguila, 25/IX/1966, Fagoaga 15c (ENCB). DURANGO. Pueblo Nuevo munipality, El Mil Diez, *García 3470* (ITCV). ESTADO DE MEXICO, highway Toluca-Temascaltepec, deviation to Valle de Bravo, 23/X/1986, *Santillán 540* (ENCB); Texcoco Market, 8/IX/1973, *Velásquez 832* (ENCB); El Capulín, highway to Sultepec, Nevado de Toluca, Nacional Park, 25/9/1983, *González 458* (ENCB); Nevado de Toluca, highway to Sultepec, 2/IX/1983, *Colón 358* (ENCB); Nevado de Toluca, cerro El Calvario, 24/IX/1983, *Colón 378-A*, 382 (ENCB); Villa Nicolás Romero Market, 17/10/1976, *Baca s.n.* (ENCB); Laguna de Ojotongo, highway Zempoala-

Chalma, 15/VII/1963, *Guzmán 5086*, (ENCB); Río Frío near Llano Grande, highway México-Puebla, 13/VII/1968, Díaz 9, Guzmán 1538 (ENCB); Temoaya Municipality, Las Navajas, Kong-Luz 54 (ENCB) GUANAJUATO. El Zamorano, near Tierra Blanca, 27/VIII/2000, *Landeros 7-10B* (XAL). MORELOS. Lagunas de Zempoala, *Rocha 10* (ENCB); Huitzilac Municipality, Lagunas de Zempoala National Park, 26/7/1982, *Aguilar s.n.* (FCME). GUERRERO. Chichihualco Municipality, between Carrizal and Puerto del Gallo, 12/7/1980, *Lugo s.n.*, 12/VII/1980, Villarías 52, 20/VIII/1983, *Perez-Ramírez 467, Herrera s.n.* (FCME); Chilchihualco Municipality, between El Carrizal and Atoyac, *Lugo 18* (FCME), Chilpancingo de los Bravo Municipality, Palo Hueco, Omiltelmi, 14/8/1984, *Gutierrez s.n.* (FCME). MICHOACÁN. Ciudad Hidalgo Municipality, National Park Cerro Garnica, 18/8/1983, *Arrieta s.n.* (FCME). MORELOS. Cuernavaca, region of the Lagunas of Zempoala, 19/VIII/1982, *Mora 429, Chacón 3613* (XAL). NUEVO LEON. Zaragoza Municipality, La Encantada, *García 2414, 8195* (ITCV); Zaragoza Municipality, El Viejo Hill, García 2583 (ITCV): OAXACA. Highway Tuxtepec-Oaxaca, between La Esperanza and Oaxaca, 25/VII/1977, *Pérez-Ortíz 695*, (ENCB). PUEBLA. Chiautzingo Municipality, San Juan Tetla, Cañada Chamier, 27/10/1982, *Gauzín s.n.* (ENCB); South of Hidalgo, Medel 680 (XAL). VERACRUZ. Municipality de Xico, West side Cofre de Perote, Los Gallos near El Rosario, *Rico 934, 949, Montoya 240, Bandala 465, Pérez-Moreno 397, 477, Villarreal 1334, 2191, 2618, 2580, 2562, 2618* (XAL); Xico Municipality, El Rosario near El Revolcadero, *Montoya 330-A, 381, Bandala 357, 406,464, Villarreal 631-A, 646,664,1465, 1546, 2251* (XAL), Calcahualco Municipality near Tlacoteopa, La Jicara road, 10/X/1998, *Medel 890* (XAL). SWEDEN. Uppsala (ex Herb. Berkeley lectotype of H. friesiana, K).

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# A new species and a new record of *Lepiota* occurring in the Gulf of Mexico area

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**Abstract** — Two species of *Lepiota* are described from material collected in the central area of Veracruz State, viz. *L. trichroma*, a new species proposed here, and *L. hemisclera*, an unreported species from Mexico. Samples of both taxa were found in the cloud forest (mesophytic or subtropical forest) surrounding Xalapa. Descriptions and discussions, including illustrations of macro- and microscopic morphology of both taxa based on the Mexican collections are provided. Microscopical observations after studying the lectotype of *Agaricus hemisclerus* from Cuba, as well as material of *Lepiota subcitrophylla* from Japan are included.

**Key words** — *Agaricaceae*, *Stenosporae*

## Introduction

During recent fieldwork to monitor the macromycetes in the central region of Veracruz state, several collections of two species of *Lepiota*, previously unrecorded in the region, were found. One of these species, *L. trichroma*, presents a combination of macro- and micromorphological characters that places it in an undescribed taxon belonging to the group of spurred-spored species close to *L. bichroma* E. Horak, *L. subcitrophylla* Hongo, and *L. citrophylloides* Sathe & S. M. Kulk. The other species corresponds to *L. hemisclera* of which the present collections document the information of its morphological variation and distribution range. The two *Lepiota* species studied were found at Santuario del Bosque de Niebla (cf. Parque Ecológico Fco. J. Clavijero in previous publications) and the native wooded area of the Botanical Garden Francisco Javier Clavijero, both corresponding to a cloud forest (subtropical or mesophytic forest according to Rzedowsky 1978) protected by the Instituto de Ecología (Xalapa), consisting of a mixed association dominated by *Quercus* spp., *Carpinus caroliniana* Walter, *Clethra mexicana* DC., *Ostrya virginiana* (Mill.) K. Koch and *Liquidambar styraciflua* L. var. *mexicana* Oersted, which has been visited weekly throughout the year since 2002.

## Materials and Methods

Macroscopic descriptions are based on the study of fresh material. Alphanumeric color codes indicated in the descriptions refer to Kornerup and Wanscher (1967) and Munsell

(1994) (bold codes). Microscopic structures are described from hand sections of revived tissues mounted in 3% KOH and Congo Red 1% aqueous solution. Measurements and colors of the structures were observed in 3% KOH. The basidiospores were measured in side view, 30 spores randomly selected from hymenophoral tissues were evaluated in a single element per collection. When estimating spore dimensions part of the recommendations by Heinemann and Rameloo (1985) were used. The first range reported in the descriptions describes the variation within the collections and extreme values are indicated in brackets (highest values of mean ±2 SD in each sample). The means of spore length, width and quotient Q (ratio of basidiospore length/basidiospore width) were calculated in each collection, then $\bar{x}$ corresponds to the range of means of length and width, and **Q** to the range of the mean values of Q of **n** collections. Line drawings were made with the aid of a drawing tube. Herbarium acronyms are according to Holmgren et al. (1990).

## Taxonomy

### *Lepiota trichroma* Montoya & Bandala sp. nov.

FIGURES 1-3

***Pileus*** *8–35 mm, convexus vel plano-convexus, interdum tenui umbonatus, pallide flavovirens, caerulescens, saepe rubigineomaculatus, subglaber vel minute subsquamulosus (sub lente), squamulis obscurioribus.* ***Lamellae*** *liberae, confertae vel subdistantes, flavidae, saepe rufomaculatae, caerulescens.* ***Stipite*** *22–70 × 1–4 mm, cylindricus, pileo concolor, caerulescens et rufomaculatus, fibrillosus vel minute subsquamulosus, squamulis obscurioribus, ad basim interdum rhizomorphis rubescens observatis.* ***Cortina*** *fibrillosa, mox destituta, interdum fibrillis apud annulus zonis instructo, raris ad pileus margin.* ***Caro*** *flavidae, caerulescens, rufo maculatae (stipite).* ***Basidiosporae*** *6–8.5 (–9) × 3–4 (–4.3) µm, elongatae, leves, calcaratae.* ***Pleurocystidia*** *nulla.* ***Cheilocystidia*** *13.5–30 × 6–12 µm, clavata, ventricosa, vel utriforme.* ***Pileipellis*** *ex hyphis cilindraceis cutem formantibus, elementa terminalis clavata, decumbentes vel ascendentibus, dispersus, in squamules saepe inflata pileocystidia similis, 7–40 × 8–25 µm, cumulus formantibus.* ***Fibulae*** *presentes. MEXICO, Veracruz, Xalapa Co., km 2.5 via Xalapa-Coatepec, Instituto de Ecología, Santuario del Bosque de Niebla, 3 July 2003, Bandala 3776 (XAL Holotypus).*

*Etymology:* Because of the yellow basidiomes which stain reddish and blue.

***Pileus*** 8–35 mm diam., convex to plane-convex, sometimes weakly umbonate, occasionally depressed at center, at first faintly tomentose, becoming variably minutely squamulose (under lens) especially in the center, smooth or slightly rugulose, dry, pale yellow (2A4, 4B5), lemon-yellow (1-2A4, 30A4-5), or sulphur-yellow (2B5, 30A3-4) (5Y 8/6-8), caerulescent (especially on handling), with pale pinkish tinges, finally with red or orange-oxide scattered stains, squamules brownish on yellow ground, these in groups giving a dark (brown) coloration to the disc center; margin straight, entire or somewhat eroded in age, obscurely striate, occasionally in parts bearing minute, fine fibrillose veil remnants. ***Lamellae*** close to subdistant, rounded-free or faintly adnate, at times rather seceding, subventricose, 3–4 (–5) mm broad, yellow (2A2, 3A4-B7) to sulphur-yellow (30A3-A4), caerulescent, frequently with red or orange-oxide stains, finally stained dark brown, edge entire, somewhat paler than the surfaces; lamellulae up to 4 different lengths.

Fig. 1. *Lepiota trichroma.* Basidiomes (*Bandala 3776*). Bar = 17 mm.

***Stipe*** 22–70 × 1–4 mm, cylindric, straight, often slightly curved downwards, yellow (2A2-2B5, 3A4, 4B4), concolorous with pileus, often with a brighter sulphur-yellow color near lamellae attachment, commonly stained blue (or when handling), whilst other areas irregularly stained red or reddish-brown to orange-oxide, finally becoming brownish or dark brownish spotted; apex pruinose, fibrillose to minute squamulose downwards (under lens), squamules scattered, darker; partially fistulose, filled with whitish fibers; base concolorous, at times stained orange-oxide, more or less strigose by the presence of white to pale yellowish mycelium, this often forms moderately coarse rhizomorphs. ***Veil*** fibrillose, soon disappearing with age, occasionally as scant fibrils remaining at annular zone or rarely at pileus margin. ***Context*** yellow, hygrophanous, caerulescent, stained reddish-brown or reddish mainly in the inferior two thirds of stipe including the rhizomorphs. ***Odor*** to rotten potatoes or herbs. ***Taste*** mild.

***Basidiospores*** 6–8.5 (–9) × 3–4 (–4.3) µm; $\bar{x}$ = 7.1–7.6 × 3.3–3.5 µm, $Q$ = 2.1–2.2, elongate, spurred on lateral view and with a dorsal depression, pale yellowish-green to hyaline, wall slightly thick, dextrinoid. ***Basidia*** 15–25 × 5–6.5 µm, clavate, bi- or tetrasporic, thin walled, hyaline. ***Pleurocystidia*** absent. ***Cheilocystidia*** 13.5–30 × 6–12 µm, clavate, broadly clavate or more or less narrowly utriform, often somewhat ampulliform, hyaline, thin walled, numerous. ***Pileipellis*** a cutis composed of interwoven, cylindric hyphae 4–7 µm broad, often ramified, clamped, at times ending in a projected or periclinally oriented claviform element, often the scales with mounds of compactly arranged clavate,

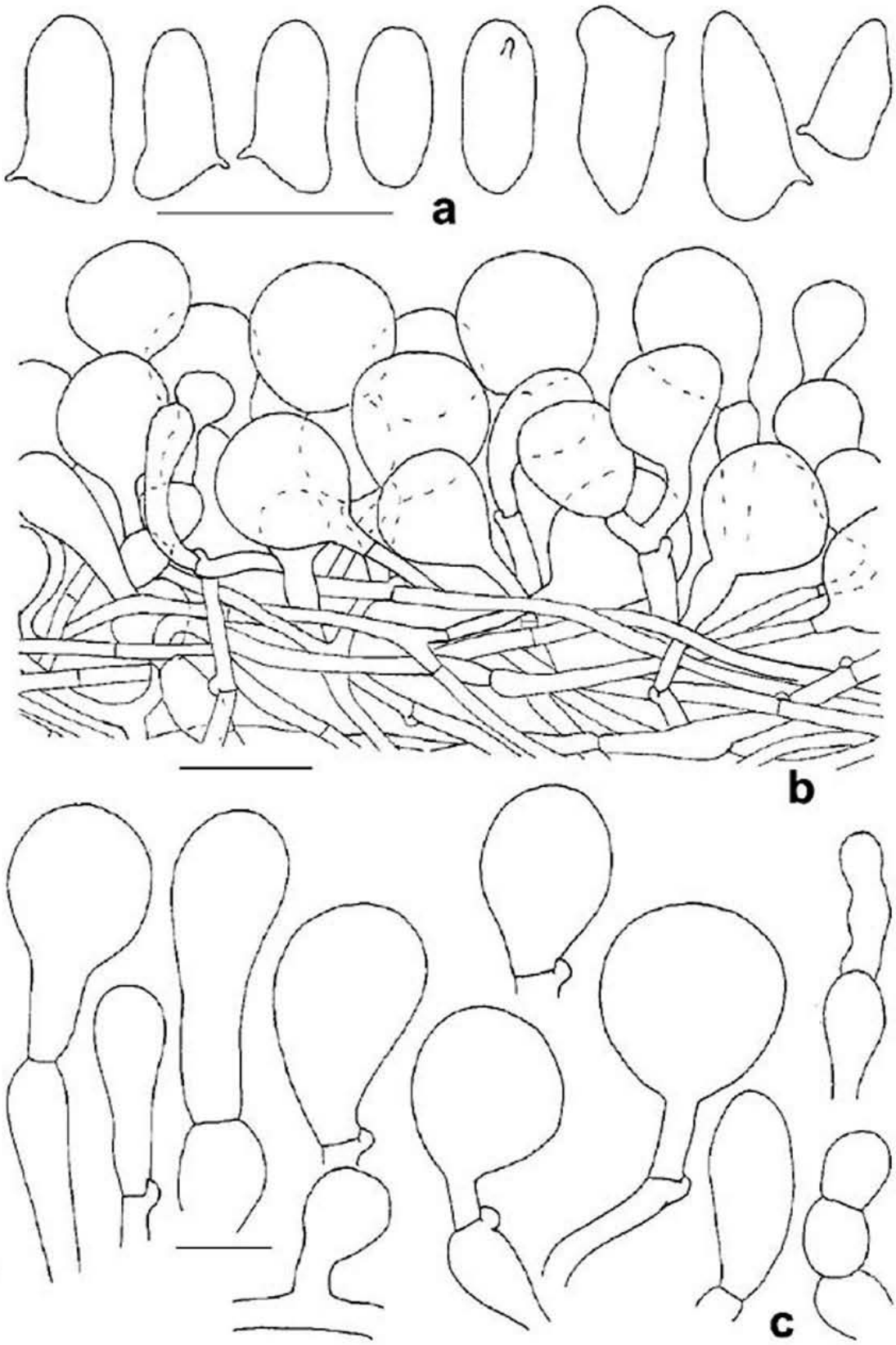


Fig. 2. ***Lepiota trichroma***. a: basidiospores; b: elements of the pileipellis and scales; c: scale elements (*Bandala 3776*). Bar a, c = 10 µm, b = 20 µm.

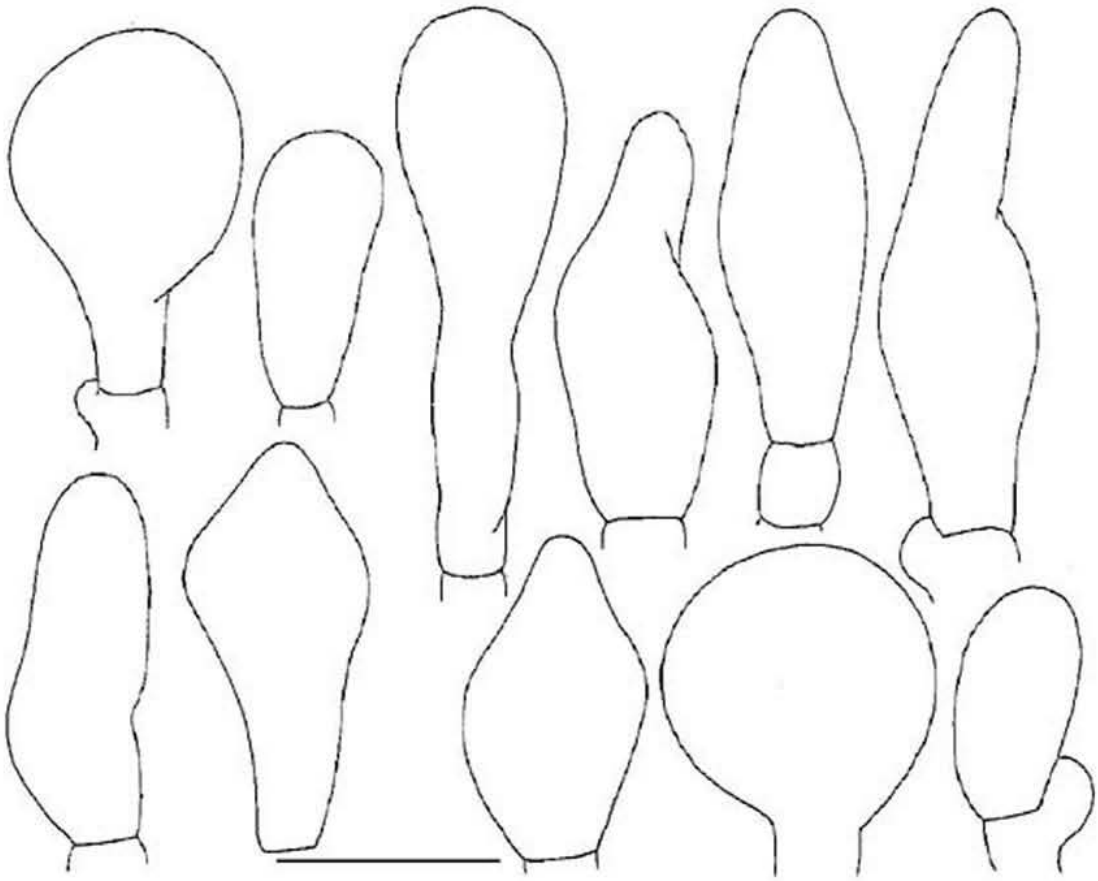

Fig. 3. ***Lepiota trichroma***. Cheilocystidia (*Bandala 3776*). Bar = 10 µm.

broadly clavate or sphaeropedunculate pileocystidia-like elements, 7–40 × 8–25 µm, 4–7 µm diam. at base, some more or less disposed in chains of 2–3 subisodiametric cells, all terminal elements with yellowish wall and with yellowish-brown contents, slightly thick walled (up to 0.5 µm thick), at times with incrustations. ***Context*** hyphae 4–13 µm diam., yellowish to yellowish-brown, thin walled, clamped. ***Hymenophoral trama*** subregular, hyphae 2.5–18 µm diam., cylindric to inflate, broad or sausage shape. ***Stipe trama*** with somewhat parallel hyphae 5–8 µm diam., thin or somewhat thick walled, yellowish in KOH, clamped. ***Stipitipellis*** a cutis of hyphae 4–8 µm diam., at times ending in a sphaeropedunculate element 19–40 × 8–12 µm or a subisodiametric cell up to 30 µm diam., other hyphae with short lateral outgrowths, clamped. ***Clamp connections*** present. A yellow sap is dissolved in KOH slides.

**Habitat.** Solitary to gregarious, at times caespitose, in small troops, on soil or among fallen leaves or grass, near *Carpinus caroliniana*, at 1300 m.

**SPECIMENS EXAMINED** — MEXICO. VERACRUZ: **Xalapa** Co., km 2.5 old road Xalapa-Coatepec, Instituto de Ecología, Santuario del Bosque de Niebla, 26.VI.2002, *Corona 68*; 21.X.2002, *Bandala 3714*; 25.X.2002, *Montoya 3958*; 14.XI.2002, *Corona 125*; 26.VI.2003, *Corona 263, 264*; 3.VII.2003, *Bandala 3776* (*Holotype*, XAL). Instituto de Ecología, Botanical Garden Francisco J. Clavijero, native wooded area, 4.VII.2002 *Montoya 3837, 3838*; 27.IX.2002, *Montoya 3922*; 13.VII.2005 *Bandala 3981* (all at XAL).

**Other material studied** — *Lepiota subcitrophylla*. JAPAN. Ooshimizu, Ibuki-sho, Shiga Pref., on soil in *Pinus densiflora-Quercus serrata* forest, 1.IX.1973, leg. *Z. Sugiyama* (*TMI 1458*); Kokoge, Tottori City, Tottori Pref., on soil in mixed Wood of *Camellia, Cinnamomum, Aphananthe, Phyllostachys*, etc., 12.XI.1974, leg. *E. Nagasawa* (*TMI 1847*).

**Remarks.** *Lepiota trichroma* is readily recognized by its yellow, caerulescent basidiomes developing pale pinkish, red or orange-oxide stains, the spurred spores, broadly clavate or ventricose cheilocystidia and pileipellis irregularly covered with subisodiametric or sphaeropedunculate terminal elements forming the scales of the pileus. The microscopic characteristics (pileipellis structure, basidiospores, cheilocystidia) in combination with color variation of the basidiomes, taxonomically separate *L. trichroma* from phenotypically similar taxa in Section *Stenosporae* (J. Lange) Kühner (Bon 1981; Candusso & Lanzoni 1990; Vellinga & Huijser 1993).

The bluing of the basidiomes is a characteristic rarely found among the species of *Lepiota* with spurred spores (Akers et al. 2000; Horak 1980). *Lepiota subcitrophylla* described from Japan (Hongo 1956) and depicted in Imazeki and Hongo (1983) iconography, superficially recalls *L. trichroma*. The distinctive reddish colors of the basidiomes as observed in the Mexican taxon, however were not described for *L. subcitrophylla* (Hongo 1956; Imazeki and Hongo 1983) and there are also taxonomically important morphomicroscopical differences. A re-examination of two Japanese collections of this latter species (one of them, *TMI 1458*, determined by Hongo), revealed a pileipellis of a trichodermis kind, composed of a disrupted layer of elongated (narrowly claviform, subcylindric or narrowly lageniform) terminal elements 35–145 (–160) × 6–14 (–16) µm, slightly bigger basidiospores [7–10 (–11) × 3–4 (–4.5) µm, $\bar{x}$ = 8.7 × 3.3 µm, $Q$ = 2.67] and lamellae edges lacking cheilocystidia (fig. 4). Hongo (1956) reported, however, cheilocystidia crowded, clavate to subcylindric or somewhat fusoid, 19–31 × 6–14 µm and dermatocystidia cylindric to clavate, 35–70 × 9.5–16 µm.

When describing the Australian *L. bichroma*, Horak (1980) observed a resemblance between it and *L. subcitrophylla*. The basidiomes of *L. bichroma*, as emphasized by Horak, are not caerulescent, instead they are distinguishable by the deep lilac colors produced by minute squamules or wart-like squamules of the pileus and stipe, respectively, on a pale yellow-orange background, therefore differing macroscopically from the Japanese taxon and the Mexican *L. trichroma*. Furthermore, microscopically *L. bichroma* differs from *L. trichroma* in its longer basidiospores (9–12 × 3–4 µm) and the pileipellis characteristics (a palisade of clavate to subfusoid elements 30–80 × 5–16 µm, having a pale brown encrusting and plasmatic pigment) (Horak 1980). Another species close to *L. trichroma* is *L. citrophylloides* described from India (Sathe and Kulkarni 1980). Regrettably, it has not been possible to study the holotype housed at AMH. Based upon descriptive data provided by Sathe and Kulkarni (1980) *L. citrophylloides* can be separated from the Mexican taxon by its apparently not caerulescent basidiomes lacking reddish tinges and the umbonate pileus with olivaceous brown scales, white veil, crowded lamellae and cylindric to narrowly clavate cheilocystidia 21–27 × 6–7.5 µm.

The monitoring developed in a square plot of 640 m$^2$ in the cloud forest of Santuario de Niebla showed that during 2002 to 2003 the basidiomes of this taxon occurred in 6

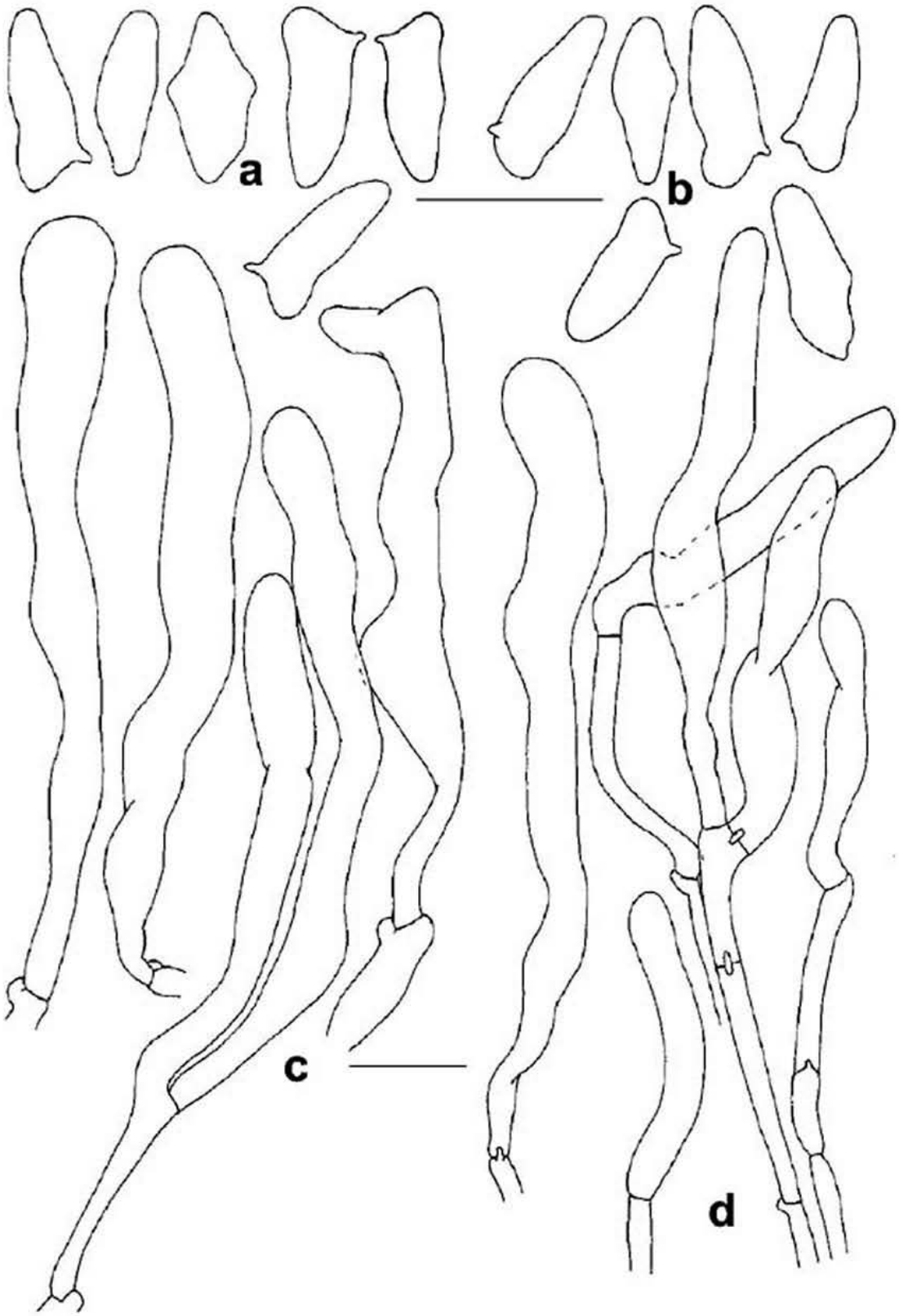


Fig. 4. ***Lepiota subcytrophylla***. a-b: basidiospores; c-d: pileipellis elements (a, c: *TMI 1847*; b, d: *TMI 1458*). Bar a-b = 10 µm; c-d = 20 µm.

subsquares (each of 1 $m^2$), growing from solitary to moderately gregarious. It presented a summer fruiting pattern, at times reaching the fall. Although the monitoring continued throughout 2004-2005, it was recorded again until September 2005, only in the Botanical Garden area.

***Lepiota hemisclera*** (Berk. & M.A. Curtis) Sacc., Syll. Fung. 5: 66, 1887.
= *Agaricus hemisclerus* Berk. & M.A. Curtis, Jour. Linn. Soc. Bot. 10: 283, 1868

**FIGURES 5-8**

***Pileus*** 40–75 mm diam., subglobose to subhemispheric when young, gradually convex, becoming plane-convex or plane, moderately umbonate, at times finally almost plane-concave, dry, somewhat hygrophanous, surface weakly wavy, with darker lines arranged in a striking reticulate pattern on all the surface, the paler ground areas yellow-brown to straw or wood yellow color (10YR8/6; 7/6), with the reticulum brownish, brownish-orange or grayish-orange (5C5-6, 5B5); surface covered with abundant pyramidal to echinate, blackish-brown, detersile scales, located often inside the mesh and distributed in a more or less concentric pattern throughout the pileus, with the pileus expansion they are more persistent towards the disc center where the mesh is denser, then this area appears more uniformly brownish-orange or at least darker; the darker lines glabrous in appearance but under lens faintly minutely fibrillose; margin at first more or less inflexed, becoming straight, often undulate (especially in very expanded pilei), not striate, at times minutely appendiculate in parts by veil remnants. ***Lamellae*** free, crowded, initially arcuate in unexpanded pileus to subventricose, at times rather linear, moderately narrow, 4–5 mm broad, some forked, with lamellulae of different size, whitish or yellowish-white, edge concolorous, somewhat irregular. ***Stipe*** 45–80 × 7–12 mm, cylindric, at times slightly widened towards the base, this latter often subbulbous, surface faintly striate in the area below point of lamellae attachment, appressed squamulose at half or two thirds of its inferior length, glabrescent upwards, whitish or yellowish-white, the area above and below the ring pinkish-brown to brownish-orange (5AB4); squamules brownish (5AB5); initially solid, with age becoming stuffed or fistulose; base with white, somewhat coarse rhizomorphs. ***Veil*** pendant, superior, membranous, double, whitish, more or less cottony-arachnoid below, persistent or partially persistent. ***Annulus*** attached or semi-moveable, persistent, thick, with coarse, flattened, brown or brownish, cremallation-like protuberances. ***Context*** whitish, unchanging, with yellow-brown stains at stipe base, hygrophanous, fleshy-fibrous. ***Odor*** fruity. ***Smell*** mild.

***Basidiospores*** 5–8.5 × 2.5–3 µm; $\bar{x}$ = 7.1–7.7 × 2.7–2.8 µm; $Q$ = 2.7–2.8 [in the *lectotype* 6–8 (–8.5) × (2–) 2.5–3 (–3.5) µm, $\bar{x}$ = 7.3 × 2.8 µm, $Q$ = 2.7], cylindric, somewhat basally truncate, with a faint suprahilar depression, attenuated and slightly curved towards the apex, hyaline to pale hyaline-greenish in KOH, yellowish in mass, at times with dense and weakly refractive contents, smooth, thin to slightly thick walled (up to 0.5 µm thick), dextrinoid or not dextrinoid. ***Basidia*** 13–16 × 5–6 µm, bi- or tetrasporic, clavate, thin walled, hyaline. ***Pleurocystidia*** absent. ***Cheilocystidia*** 14– 48 × 9–26 µm (28-63 × 7-17.5 µm in the *lectotype*), clavate to broadly clavate or broadly utriform, at times pedicellate, apically rounded and some appearing capitate, other at times nodulose, occasionally mucronate, projecting beyond the hymenial layer, hyaline to yellowish, frequently with dense, at times granulose, yellowish-brown contents (often appearing

Fig. 5. *Lepiota hemisclera*. Basidiomes and close up (below) of the annullus (*Bandala 3626*). Bar = 20 mm.

as a circular or irregular apically located vacuole), with some floating, refringent, crystal-like elements, wall up to 1 µm thick, numerous, clamped; apex at times with mucilaginous remnants as a hyaline incrustation. ***Pileipellis*** a loose trichoderm of approx. 450 µm thick, consisting of interwoven hyaline, hyphae 3–5 µm broad, thick-walled (0.5–1 µm thick), yellow-brown to yellow-green, clamped, at times there are some intercalar rows of subisodiametric, clavate or subcylindric clamped elements, which are similar to those forming the scales; pyramidal scales built of chains of versiform elements (subisodiametric, ventricose, pyriform, subellipsoid, mucronate), 10–63 × 7–13 µm, clamped, thick-walled (1–1.5 µm), yellow or yellowish-brown, the wall clearly

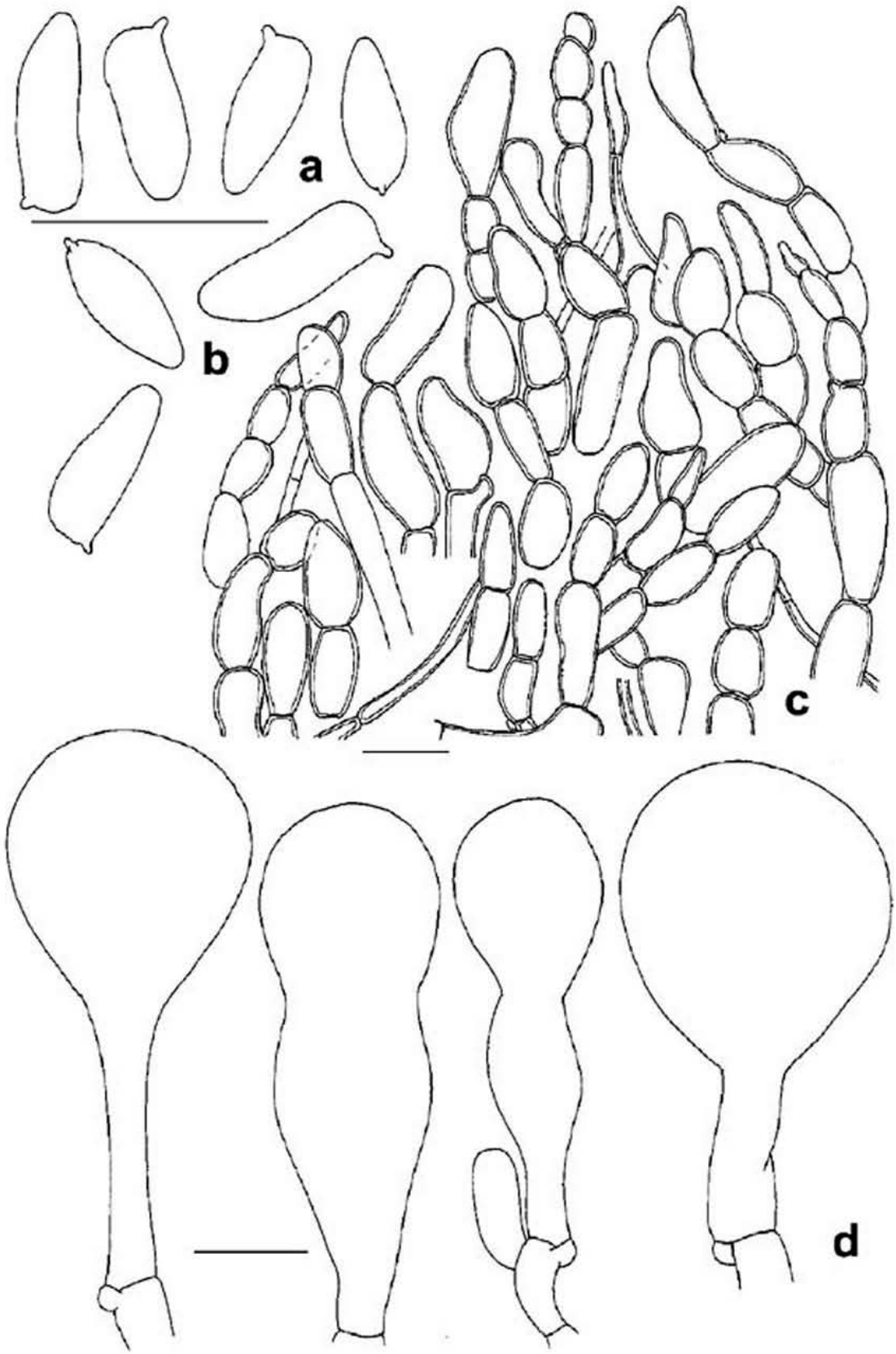


Fig. 6. ***Lepiota hemisclera***. a-b: basidiospores; c: elements of the scales on pileus; d: cheilocystidia (a, c: *Bandala 3626*; b, d: *Bandala 3769*). Bar a-b, d = 10 µm, c = 25 µm.

appears more yellow than the contents or almost yellowish-green. ***Context*** hyphae 2–10 µm diam., thin walled; with abundant sphaerocyte-like elements towards the hymenial region, these of 15–40 µm diam. ***Hymenophoral trama*** more or less regular, hyphae 2–16 µm diam., with sphaerocyte-like elements towards the connection with the context. ***Veil*** built by cylindric hyphae 2–12 µm diam., thin or thick-walled (0.5–1 µm thick), more or less hyaline, with a slight greenish tint, similar to that of the pileipellis elements. ***Stipe context*** with yellowish, somewhat parallel hyphae 4–16 µm diam., thin or somewhat thick-walled (< 1 µm thick), clamped. ***Stipitipellis*** a loosely arranged tissue, composed of hyphae 3–4 µm thick, septate, curled, branched, clamped, at times ending in a more or less clavate element of 6–7 µm diam. at apex. ***Clamp connections*** present.

**Habitat.** Gregarious, on soil, among leaf debris, at times among dead leaves and *Selaginella*, near *Platanus*, at forest edge, at 1300 m.

**SPECIMENS EXAMINED** — MEXICO. VERACRUZ: Xalapa Co., km 2.5 old road Xalapa-Coatepec, Instituto de Ecología, Botanical Garden Francisco J. Clavijero, native wooded area, 3.VII. 2002, *Montoya 4008*, *Bandala 3626*; 30.VI. 2003, *Bandala 3768*, *3769* (all at XAL).

**Other material studied** — CUBA. On logs, July 27 (year not mentioned), *J. Wright 120* (*Lectotype* of *Agaricus hemisclerus*, K).

**Remarks.** This *Lepiota* species macroscopically can be recognized by the entirely reticulate aspect of the pileus surface bearing pyramidal detersile scales, the persistent thick, superior, cog-wheel-like annulus, pendant veil, and the appressed squamulose inferior half of the stipe surface. These characteristics in combination with the cylindric basidiospores and both swollen cheilocystidia and elements of pileipellis, taxonomically distinguish *L. hemisclera*.

The diagnosis of this taxon is complemented with the above macro- and microscopic data recorded on fresh collections gathered in Xalapa, as well as those observed after re-examination of the lectotype of *Agaricus hemisclerus*. Two specimens collected in Cuba by Ch. Wright (no's *57* and *120*) were cited in the original description (Berkeley and Curtis 1868), one of them (*120*) selected later by Pegler (1987) as the lectotype. The Mexican specimens are indistinguishable from specimen *Wright 120*, this latter however, presents somewhat collapsed lamellae edges making difficult the study of cheilocystidia, which more or less recover when mounted in KOH (cf. figs. 6-8). It is also remarkable that the dextrinoid reaction of basidiospores is not always consistent in both the lectotype and Mexican samples. In the brief original description, based on the data recorded in fresh collections by Ch. Wright, Berkeley and Curtis (1868) described the annulus as "...amplo reflexo..." and with regard to the pileus surface they recorded it as "... sometimes prettily clouded...". The pileus of the lectotype, like that of Mexican collections, is certainly reticulate, the dry sample still exhibits a faint, wide mesh produced by paler and darker areas (it also presents scattered, pyramidal to echinate, detersile dark scales). The stipe bears a well preserved thick annulus (this holding a membranous-cottony almost pendant veil) which on its outer side presents brown, coarse, teeth-like protuberances. It should be pointed out that these two striking macroscopic features unique for *L. hemisclera* were not mentioned in subsequent cites after diagnosis of the species (Morgan 1906; Murrill 1911, 1914) including a more

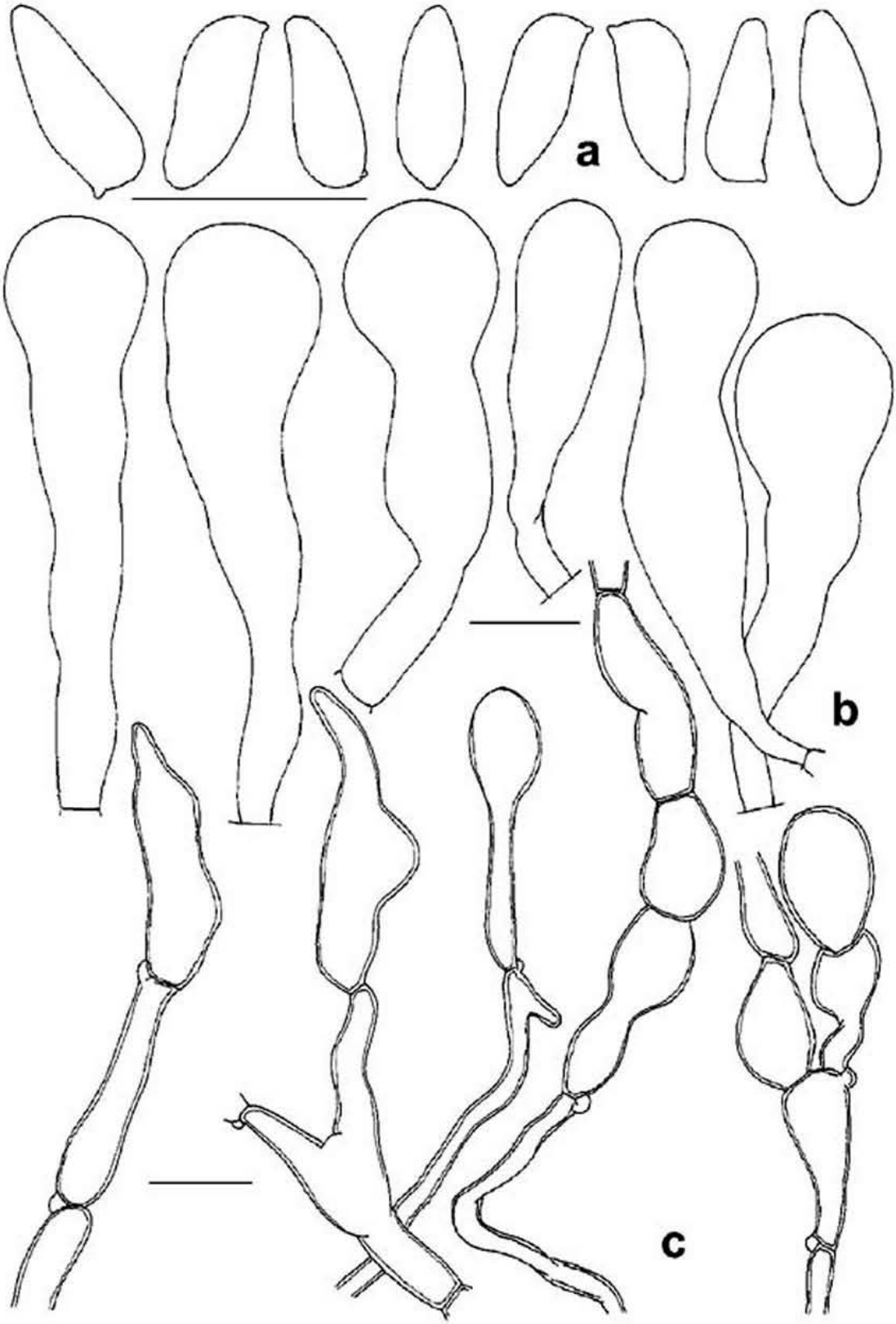


Fig. 8. ***Lepiota hemisclera***. a: basidiospores; b: cheilocystidia; c: elements of the scales on the pileus (*Wright 120*). Bar a-b = 10 µm, c = 20 µm.

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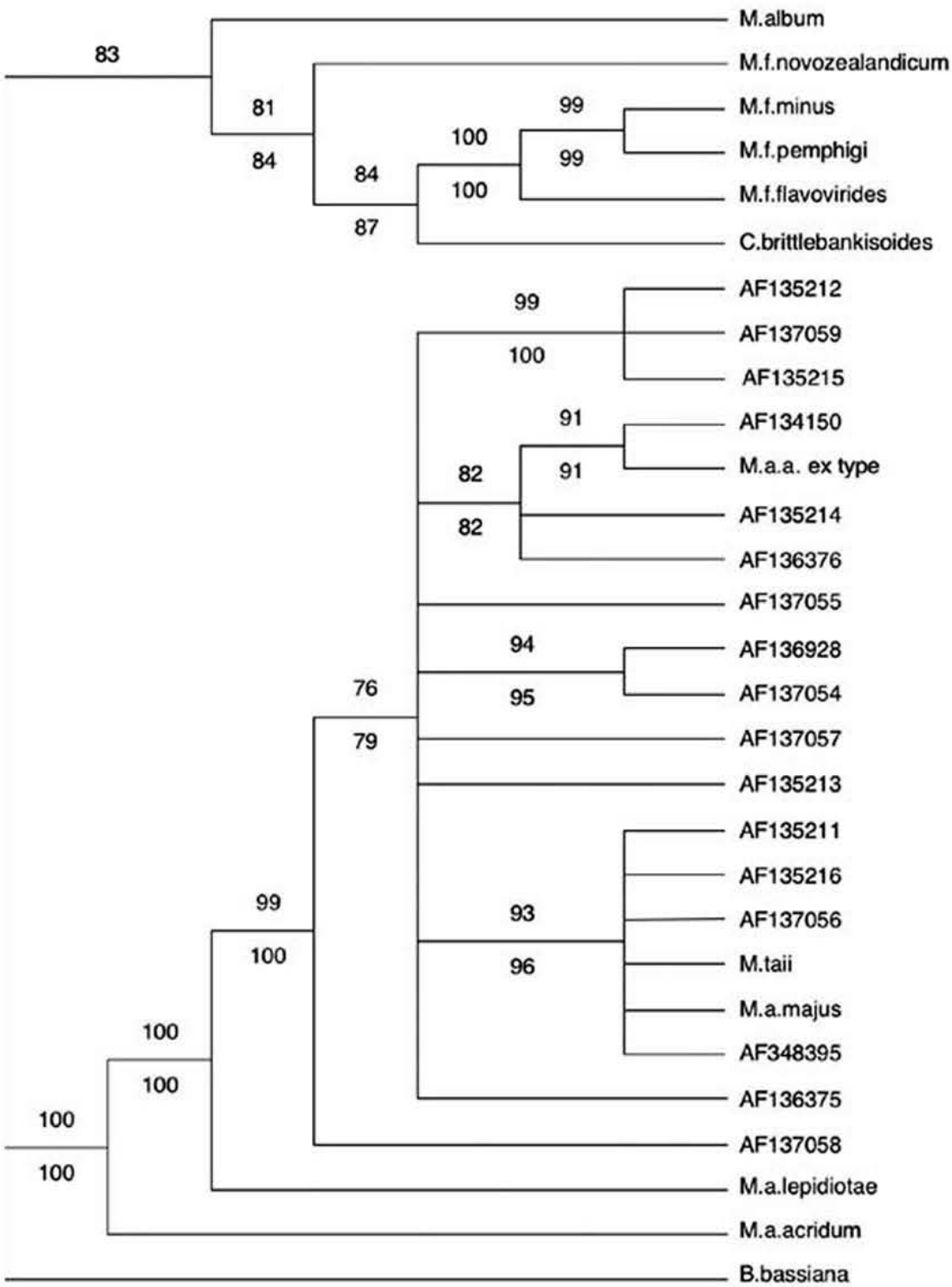


**Figure 3.** Strict consensus of 2864800 equally parsimonious trees based on ITS region sequence data from *Metarhizium* spp. Values above the branches indicate bootstrap support; posterior probabilities resulting from the Bayesian analysis are shown below the branches.

this connection was based on studies of secondary (microcyclic) conidiogenesis by discharged part spores. The data presented here show that *M. taii* cannot be maintained as an independent species and that the most reasonable treatment for this species is as a facultative (heterotypic) synonym of *M.a. anisopliae*:

*Metarhizium anisopliae* (Metschn.) Sorokīn var. *anisopliae*, [Plant Parasites of Man and Animals as Causes of Infectious Diseases] 2: 267 (1883) [in Russian]

**Synonym:** *Metarhizium taii* Z.Q. Liang & A.Y. Liu, Acta Mycol. Sinica 10: 260 (1991).

Because the present molecular and cultural evidence confirms unambiguously that the teleomorph of *Metarhizium taii* is *Cordyceps taii*, it must also now be recognized that the teleomorph of *M. anisopliae* var. *anisopliae* is *C. taii*.

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MYCOTAXON

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# *Vamsapriya indica* gen. et sp. nov., a bambusicolous, synnematous fungus from India

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**Abstract**—*Vamsapriya indica* gen. et sp. nov. is reported from decaying culms of bamboo, *Bambusa arundinacea* (*Gramineae* or *Poaceae*), collected at Yellapur, Uttara Kannada, Karnataka, India. The fungus is unique in producing catenate, phragmosporous conidia on synnematous conidiophores with non-cicatrized, monotretic conidiogenous cells. The novel genus is described and illustrated, and compared with two closely resembling genera, *Didymobotryum* and *Podosporium*.

**Key words**—conidial fungi, tropical biodiversity, hyphomycetes, taxonomy

## Introduction

Fungi occurring on monocot plants are fairly well documented (Ju & Rogers 1994; Fröhlich & Hyde 1999; Hyde & Alias 2000; Yanna et al. 2001). Among them, bambusicolous fungi have been well-studied (Petrini et al. 1989; Eriksson & Yue 1990; Hyde et al. 2002a) with a special interest on the pathogens (Samajpati 1984; Johnson 1985; Deka et al. 1990) and saprobes (Hyde et al. 2001; Zhou & Hyde 2002; Hyde et al. 2002b). Presently, more than 110 bambusicolous fungi have been recorded (Shenoy et al. 2005a).

During the course of studies on fungal diversity of Western Ghat forests (e.g. Pratibha et al. 2005; Shenoy et al. 2005b), we came across a unique, dematiaceous, synnematous hyphomycetous fungus on decaying culms of bamboo, *Bambusa arundinacea* (Retz.) Willd. (*Gramineae* or *Poaceae*). On careful examination, we observed a combination of morphological characters of the genera *Didymobotryum* Sacc. and *Podosporium* Schwein. Both *Didymobotryum*, lectotypified by *D. rigidum* (Berk. & Broome) Sacc. and *Podosporium* lectotypified by *P. rigidum* Schwein. are characterized by large synnemata with a stipe and apical head, branched conidiophores, monotretic conidiogenous cells and acrogenous conidia. However, the conidia of *Didymobotryum* are catenate and 1-septate whereas those of *Podosporium* are solitary and phragmosporous (Ellis 1971). The unique combination of catenate, phragmoporous conidia in a single fungus, with the other features of both *Didymobotryum* and *Podosporium* warrants placement of the bambusicolous fungus in a new species and a new genus. *Vamsapriya indica* gen. et sp. nov is described and illustrated in this paper.

## Taxonomic Description

*Vamsapriya* Gawas & Bhat **anam. gen. nov.**

*Ad fungos conidiales, hyphomycetes. Coloniae effusae, atro brunneae vel nigra. Mycelium substrato immersum, ex hyphis subhyalinis, septatis, ramosus, laevis. Conidiophora macronematica, synnematica, atrobrunnea, laevia, septata, ramosa, laxus apicem. Synnematica erecta, atro brunnea. Cellulae conidiogenae monotreticae, nunquan cicatricem, integratae vel discretae, terminalae, clavatae. Conidia sicca, catenulata, acrogenosa, brunnea, cylindrica, vermiformata, rotundata ad duo extremitas, phragmoseptata, angustus ad septa, acropetalibus.*

*Etymology*: In Sanskrit *Vamsa* – bamboo; *priya* – loving.

Conidial fungi, hyphomycetes. *Colonies* effuse, dark brown to black. *Mycelium* immersed, composed of subhyaline, septate, branched, smooth hyphae. *Conidiophores* distinct, macronematous, synnematous, dark brown, smooth, septate, branched, wider at the apex. *Synnemata* erect, dark brown, composed of compact parallel conidiophores, fertile in the upper half. *Conidiogenous cells* monotretic, non-cicatrized, integrated or discrete, terminal, clavate. *Conidia* dry, catenate, acrogenous, brown, cylindrical, vermiform, phragmoseptate, constricted at the septa, developing in acropetal chains.

Type species: *V. indica*.

*Vamsapriya indica* **Gawas & Bhat sp. nov.** **Fig. 1–12**

*Ad fungos conidiales, hyphomycetes. Coloniae effusae, atrobrunneae vel nigra. Mycelium substrato immersum, ex hyphis subhyalinis, septatis, tremes, laevis, 2.5–3.5 µm latis. Conidiophora macronematica, synnematica, atrobrunnea, laevia, septata, tremes, laxus, apicem, 3–4.5 µm lata. Synnematica erecta, rigidis, atro brunnea, 700– 870 µm longa, 80–90 µm lat. ad pessum, 28–42 µm lat. ad medius, 110–150 µm lat. ad apicem fertilis ora. Cellulae conidiogenae monotreticae, nunquam cicatricem, integratae vel discretae, terminaliae, clavatae, leviter curvatae extrinsecus, 4–12 x 2–4.5 µm. Conidia sicca, catenulata, acrogenosa, brunnea, laevia, simplicia, cylindrica, vermiformata, 2–12-septata, 10–80 x 4–6 µm, angustus ad septa, acropetalibus; conidia terminalia teres ad apicem, leviter truncata ad pessum; conidia alia leviter truncata ad duo extremitas.*

Holotype: On dead and decaying bamboo twigs, Yellapur, Uttara Kannada, Karnataka, India, coll. Puja Gawas, 27/9/2005, Herb. No. IMI 393674.

Fungus hyphomycete. *Colonies* effuse, dark brown to black. Mycelium immersed, composed of subhyaline, septate, branched, smooth hyphae, 2.5–3.5 µm wide. Conidiophores macronematous, synnematous, dark brown, smooth, septate, branched, wider at the apex, 3–4.5 µm wide. Synnemata erect, rigid, dark brown, composed of compact parallel conidiophores, 700–870 µm long, up to 80–90 µm wide at the base, 28–42 µm wide in the middle, up to 110–150 µm wide at the apical fertile region. Conidiogenous cells monotretic, non-cicatrized, integrated or discrete, terminal, clavate, slightly curved towards the exterior, 4–12 × 2–4.5 µm. Conidia dry, catenate, acrogenous, brown, smooth, simple, cylindrical, vermiform, 2–12-septate, constricted at the septa, 10–80 × 4–6 µm, developing in acropetal chains; terminal conidia rounded at the apex, slightly truncate at the base; other conidia slightly truncate at both ends.

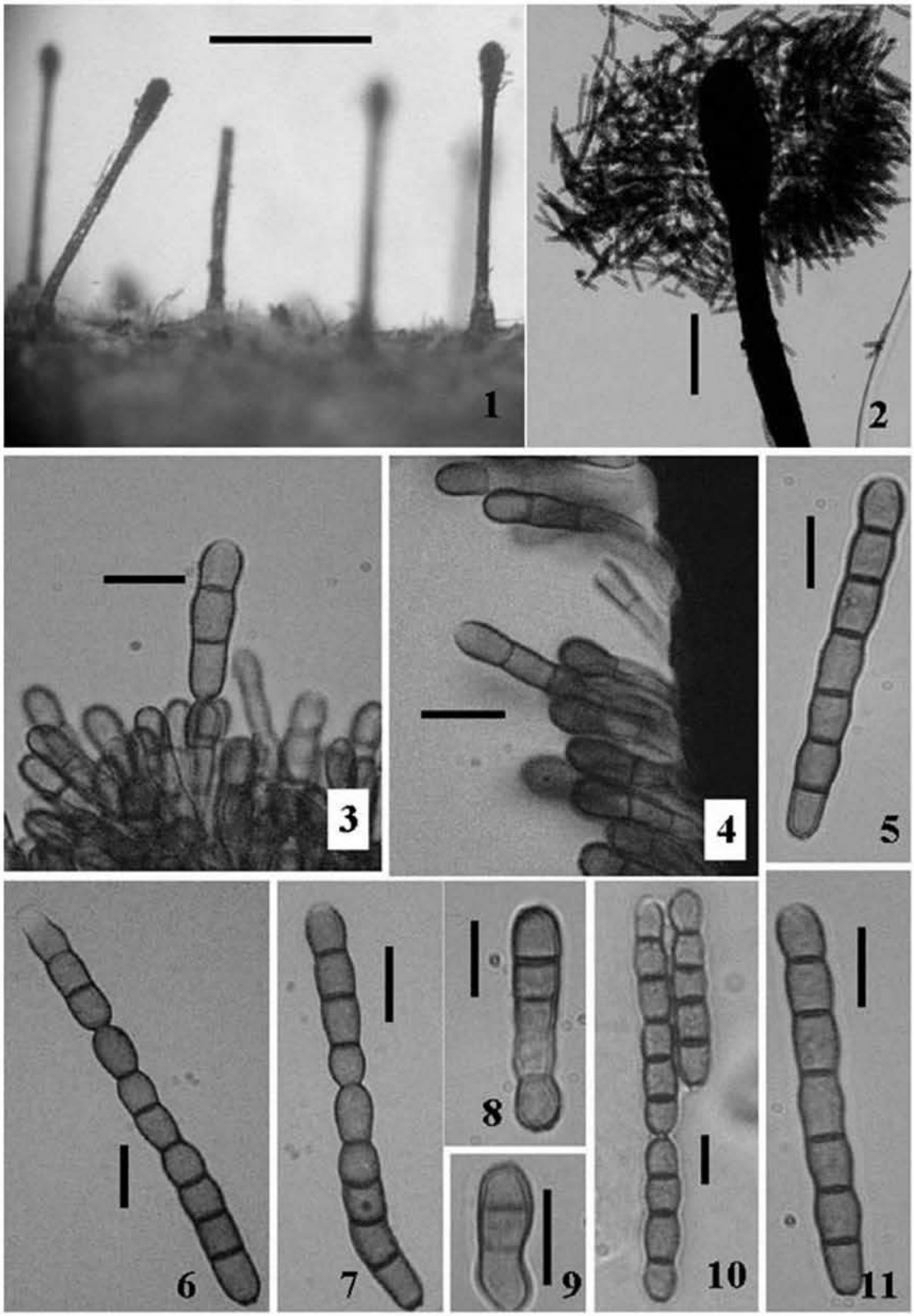


Fig. 1-11. ***Vamsapriya indica.*** 1. Stereomicroscopic image of the synnemata (bar = 0.5 cm), 2. Fertile apical zone (bar = 200 µm), 3,4. Conidiogenous cells showing monotretic conidiogenesis (bars = 10 µm), 5-11. Morphological variation in conidial size and display of catenate nature of the conidia (bars = 10 µm)

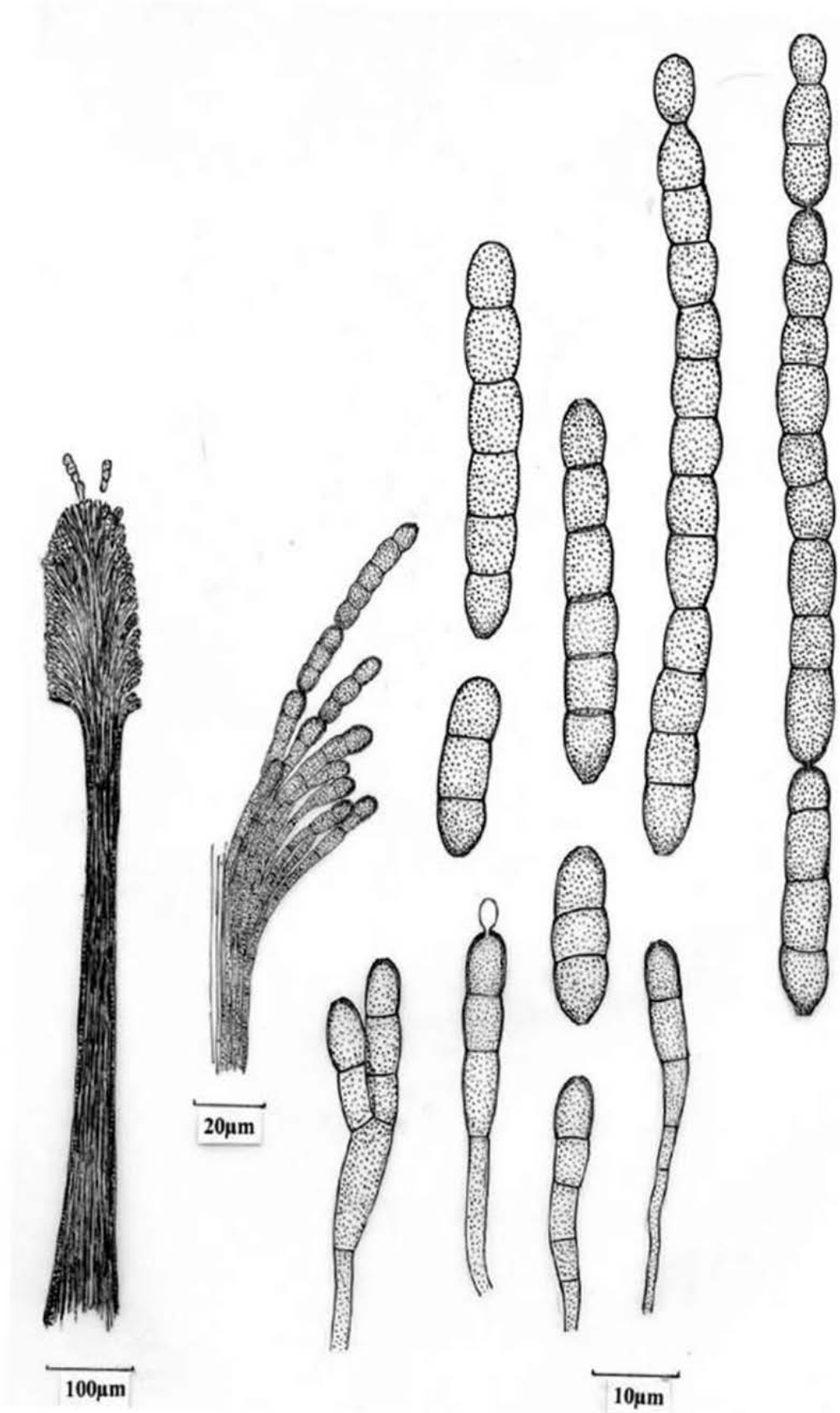


Fig. 12. *Vamsapriya indica*. Synnemata, conidiogenous cells and conidia

## Discussion

*Vamsapriya* is morphologically similar to *Didymobotryum* and *Podosporium*. All three genera have brown to black rigid synnemata with compactly arranged brown conidiophores, monotretic, integrated, clavate to cylindrical conidiogenous cells bearing dry, acrogenous, simple conidia. *Didymobotryum* differs from *Podosporium* in having catenate, ellipsoidal-cylindrical, 1-septate conidia. *Podosporium*, in contrast, has solitary, obclavate, multiseptate conidia (Ellis, 1971). *Vamsapriya* exhibits a combination of morphological characters of both these genera, bearing catenate, cylindrical to vermiform, multiseptate (phragmosporous) conidia. The catenate conidia do not support disposition of *Vamsapriya* in *Podosporium* and their phragmosporous character makes it difficult to accommodate in *Didymobotryum*.

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# Two new species of *Hypogymnia* (*Lecanorales*, Ascomycota) with pruinose lobe tips from China*

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**Abstract**—Two new species of lichens are described from China, viz. *Hypogymnia pseudopruinosa* (in Yunnan) and *H. subfarinacea* (in Sichuan and Yunnan). They are characterized by means of morphology and secondary chemistry. Latin diagnoses, English descriptions, and habitus photographs are provided.

**Keywords**—*H. macrospora*, *H. farinacea*, lichen substances

Until recently, forty species of *Hypogymnia* (Nyl.) Nyl. have been reported from China (Wei 1991; Chen 1994; McCune & Obermayer 2001; McCune & Tchabanenko 2001; McCune et al. 2003). During our studies on the lichen flora of China, two new species of the genus were collected from Yunnan and Sichuan Provinces, which are described in this paper. The gross morphology and anatomy were examined using the dissecting microscope (ZEISS Stemi SV 11) and compound microscope (OPTON ⊠). The lichen substances were detected by colour reagents and thin-layer chromatography (Culberson & Kristinsson 1970; Culberson 1972; White & James 1985).

***Hypogymnia pseudopruinosa*** X.L. Wei & J.C. Wei, sp. nov. **Plate I: A-B**

*Hypogymniae macrosporae similis, sed sporis minoribus, lobi pruina obductis et atranorina deest.*

Type: China. Yunnan, Dêqên county, alt. 4100 m, on dead branches of *Sabina* sp., X. Y. Wang, X. Xiao & J. J. Su 7606, 29 August 1981 (holotype, HMAS-L).

Thallus foliose, tightly appressed; with subdichotomously branched lobes of 1-2 mm wide and 5 mm long; upper surface dark brownish-yellow, partly black, with black margin, rugose, glossy, lacking isidia, soredia and lobules but with dense layer of pruina limited to the lobe tips; lower surface black, brown near the apices, rugose, glossy, and with round perforations at the lobe tips and on the lower surface.

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Upper cortex prosoplectenchymatous, pale yellow, 12-14.5 μm thick; algal layer 10-14.5 μm thick, consisting of green, subspherical cells of 7.5-9.5 μm in diameter; hyphae in medulla colorless, about 2 μm in diameter; lower cortex prosoplectenchymatous, pale yellow, 10 μm thick.

Apothecia rare, 1-3 mm in diameter, stipitate; disc yellow brown or red brown, glossy, concave at first and then slightly plane with entire and thin margin; epithecium brown, 7-9 μm thick; hymenium colourless, 32-36 μm thick; asci clavate, 8-14 × 23.5-25 μm, 8-spored; spores simple, colourless, ellipsoid to nearly spherical, 3.5-4 × 5.5-7 μm; paraphyses linear, septate, 2 μm wide, slightly swollen at the tips; hypothecium colourless, 27-36 μm thick. Pycnidia not seen.

*Chemistry.* Cortex K-, C-, P-; medulla K+ yellow, C-, P+ orange yellow → orange red; containing physodalic and physodic acids, and a pale spot in $R_f$ class 6 (solvent system C).

**Comments:** The new species resembles *H. macrospora* (J.D. Zhao) J.C. Wei (Zhao 1964; Wei 1991) at first sight, but differs by having a dense layer of pruina limited to the tips of the lobes and smaller ascospores, and by lacking atranorin.

***Hypogymnia subfarinacea*** X.L. Wei & J.C. Wei, **sp. nov.** **Plate I: C-E**

*Habitu cum Hypogymnia farinacea optime congruens, sed differt lobis pruinosis et acidum physodalicum continens.*

Type: China. Sichuan Province, Nanping County, Jiuzhai Gou, alt. 2151 m, on trunk of *Tsuga* sp., 10 June 1983, X. Y. Wang & X. Xiao 10582 (holotype, HMAS-L).

Thallus foliose, loosely appressed, with subdichotomously branched and separated lobes of 2 mm wide; upper surface gray, dull, slightly rugose to smooth with some pieces of upper cortex in the old lobes disintegrating, lacking isidia and lobules but bearing granular soredia coalescent in more or less sacciform structures, with thin layer of pruina limited to the lobe tips; lower surface black, pale brown near the apices, wrinkled, glossy, with large, round perforations of 2 mm in diameter.

Upper cortex prosoplectenchymatous, pale yellow, 14.5 μm thick; algal layer 20.5-22.5 μm thick, consisting of green and subspherical cells of 3-4 μm in diameter; hyphae in medulla colorless, septate, 1-2 μm in diameter; lower cortex prosoplectenchymatous, pale yellow, 12-14.5 μm thick. Apothecia and pycnidia unknown.

*Chemistry.* Cortex K-, C-, P-; medulla K+ yellow, C-, P+ orange yellow → orange red, containing physodalic, physodic, 3-hydroxyphysodic (conphysodic), protocetraric acids, and atranorin.

Other Material Examined: **China, Yunnan:** Lijiang County, Mt. Yulong shan, alt. 2900 m, on the bark of *Quercus* sp., 8 August 1981, X. Y. Wang, X. Xiao & J. J. Su 4892 (HMAS-L); alt. 3100 m, on the ground, 8 August 1981, X. Y. Wang, X. Xiao & J. J. Su 6591 (HMAS-L).

**Comments:** The new species resembles *H. farinacea* Zopf at the first sight, but differs by more separated lobes, presence of pruina limited to the lobe tips, and in containing physodalic acid.

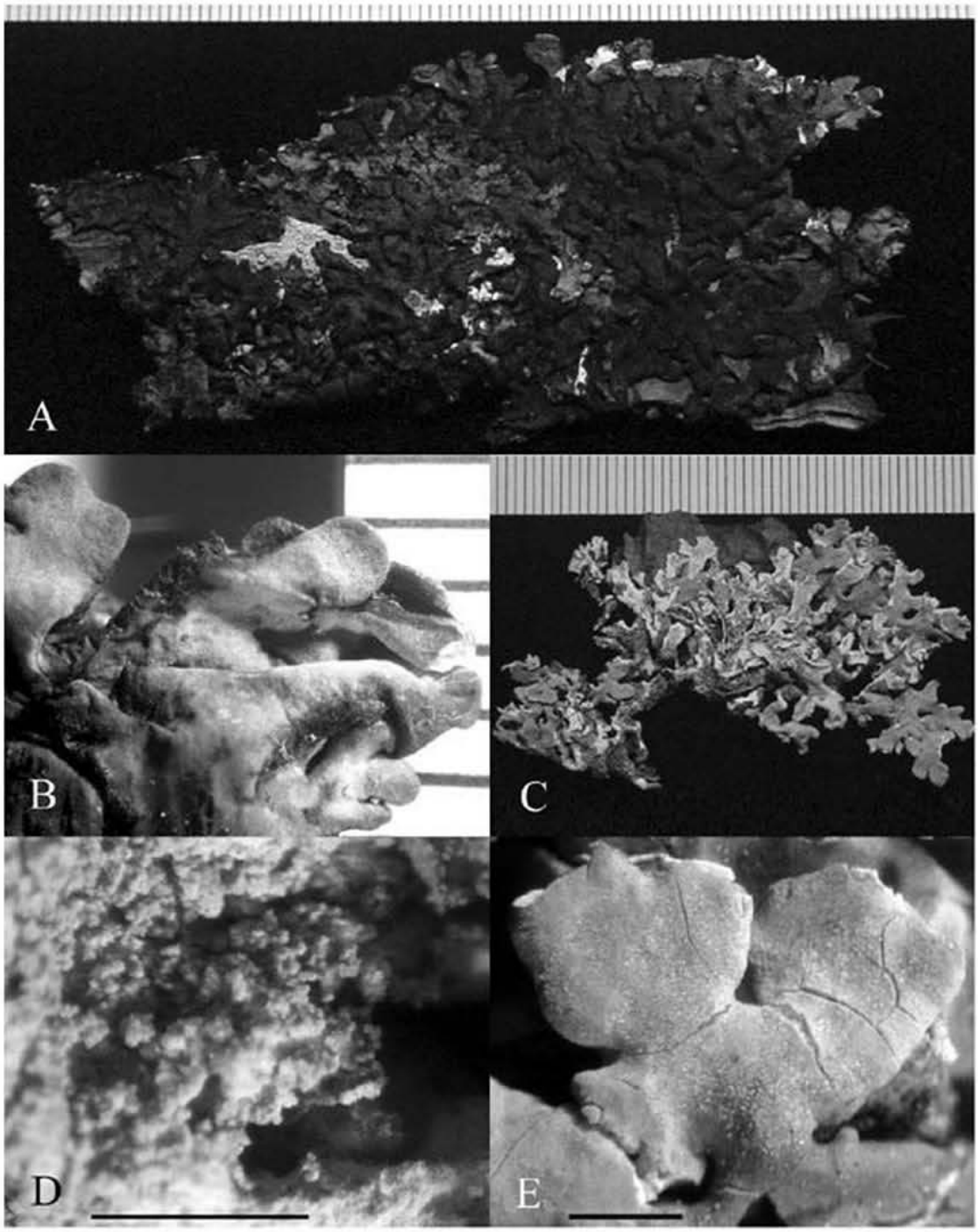


**Plate I**. A. *Hypogymnia pseudopruinosa*, Wang et al. *7606* (holotype in HMAS-L), showing general appearance of thallus. B. *H. pseudopruinosa*, Wang et al. *7606*, showing the pruina limited to the margin of lobes. C. *H. farinacea*, Wang et al. 10582 (holotype in HMAS-L), showing general appearance of thallus. D. *H. farinacea*, Wang et al. 10582, showing the pruina limited to the margin of lobes. E. *H. farinacea*, Wang et al. 10582, showing the soredia on the upper surface.

A-C: Scale in mm; D-E: Scale bar = 1 mm.

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# Discovery and description of a teleomorph for *Leptographium koreanum*

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**Abstract**—A *Leptographium* sp. unknown in Japan was isolated from Japanese red pine (*Pinus densiflora*) in this study. Pairing of different strains of the fungus gave rise to mature perithecia of an *Ophiostoma* sp. Characteristics of this teleomorph were similar to those of *Ophiostoma piceaperdum*, but the fungus had larger ascospores. The anamorph state had 2 to 3 primary branches, and was similar to *Leptographium truncatum*, *L. yunnanense*, *L. pini-densiflorae*, and particularly to *L. koreanum*. Comparisons of partial actin, β-tubulin, and rDNA sequences data showed that the Japanese fungus is *L. koreanum*. Pairing of strains from Japan with the ex-type culture of *L. koreanum* gave rise to mature perithecia confirming this identification. The teleomorph of *L. koreanum* is thus described here as *Ophiostoma koreanum*.

**Key words**—biological species, phylogenetic species, ophiostomatoid fungi, blue-stain fungi, DNA sequences

## Introduction

Species of *Leptographium* Lagerb. et Melin are best recognized as anamorphs of the ascomycete genus *Ophiostoma* Syd. et P. Syd. They include economically important agents of sap-stain as well as tree pathogens (Gibbs 1993, Seifert 1993). *Leptographium wingfieldii* M. Morelet and *L. wageneri* (W. B. Kendr.) M. J. Wingf. have relatively high levels of virulence, and the three varieties of the latter species are well-recognized root pathogens in the western United States (Cobb 1998, Solheim et al. 1993, Harrington 1988). These and other *Leptographium* spp., produce mitospores in slimy masses at the

apices of erect conidiophores that are specifically adapted to be carried by arthropods, particularly bark beetles (Coleoptera, Scolytinae, Jacobs and Wingfield 2001).

A recent monograph on *Leptographium* included 46 species (Jacobs & Wingfield 2001). The majority of these species occur in Europe and North America and it is well-recognized that this group of fungi has been poorly sampled in other parts of the world. This is particularly true for Asia where a large number of bark beetles are known to infest native conifers. This study represents part of an ongoing effort to catalogue the *Leptographium* spp. in Asia, and particularly in Japan.

Recent isolations from pine bark beetles and bark beetle-infested Japanese red pine (*Pinus densiflora* Siebold et Zucc.) and other pine species have yielded a *Leptographium* spp. that has previously not been collected in Japan. This fungus has 2-3 primary branches, relatively poorly developed rhizoids, and is morphologically similar to *L. truncatum* (M. J. Wingf. et Marasas) M. J. Wingf., *L. yunnanense* X. D. Zhou et al., *L. koreanum*, and *L. pini-densiflorae* Masuya et M. J. Wingf. (Jacobs & Wingfield 2001, Kim et al. 2005, Masuya et al. 2000, Zhou et al. 2000, Jacobs et al. 2005). However, unlike these fungi, isolates of the Japanese *Leptographium* sp. often produce protoperithecia in culture (Masuya et al. 1998). Masuya et al.(1998, 1999) have previously noted the fungus and reported it as an undescribed *Ophiostoma* sp. This fungus was isolated from 9 out of 13 investigated bark beetle species and appears to be widely distributed in Japan (Masuya et al. 2001). It was also found to be relatively virulent in inoculations on Japanese red pine where it produced longer lesions than various other blue-stain fungi (Masuya et al. 2003).

The unknown *Ophiostoma* sp. with a *Leptographium* asexual state from Japan is considered to be economically important for Japan thus an appropriate name for the fungus is required especially since its teleomorph is recognized. The aim of this study is to establish its identity.

## Materials and methods

### Fungal isolates

The fungal isolates used for morphological comparisons and mating experiments in this study are listed in Table 1. Five strains of the *Leptographium* sp. (MCC206, 214, 217, 364, 365) were used in the DNA sequence analyses. Comparisons of sequence data also included sequence data of other *Ophiostoma* and *Leptographium* species obtained from Genbank (Table 2).

### Morphology

A 5 mm-diam. plug of each isolate used in the morphological comparisons was placed in Petri dishes containing 2% malt extract agar (MEA, 20 g Difco malt extract, 15 g agar and 1000mL distilled water) and incubated at 20C in dark. After one month, two autoclaved pine twigs were placed on the surface of the medium to stimulate the development of fruiting structures. After an additional month of incubation, plates were inspected for the presence of perithecia. Where these structures were found, they were mounted on glass slides in 1% lacto-fucsin for microscopic examination. In addition, perithecia were mounted after having been bleached with Sodium hypochlorite (1% available chlorine) for detailed observation of the cell arrangement of perithecial

**Table 1.** Isolates used for morphological comparisons and mating experiments in this study.

| Species | Isolate No.* | Other No. * | Source | Collector | Origin |
|---|---|---|---|---|---|
| *Leptographium* sp. | MCC206 | DAOM234395, JCM11853, MAFF410963 | *Tomicus piniperda* on *Pinus densiflora* | H. Masuya | Japan |
| | MCC211 | JCM11857, MAFF410964 | *T. piniperda* on *P. densiflora* | H. Masuya | Japan |
| | MCC213 | JCM11855, MAFF410965 | *T. piniperda* on *P. densiflora* | H. Masuya | Japan |
| | MCC214 | DAOM234396, JCM11854, MAFF410966 | *T. piniperda* on *P. densiflora* | H. Masuya | Japan |
| | MCC215 | JCM11859, MAFF410967 | *T. piniperda* on *P. densiflora* | H. Masuya | Japan |
| | MCC217 | JCM11860, MAFF410968 | *T. piniperda* on *P. densiflora* | H. Masuya | Japan |
| | MCC364 | JCM11858, MAFF410961 | *Hylurgops interstitialis* in *P. parviflora* var. *pentaphylla* | H. Masuya | Japan |
| | MCC365 | JCM11856, MAFF410962 | *H. interstitialis* in *P. parviflora* var. *pentaphylla* | H. Masuya | Japan |
| *L. lundbergii* | CBS352.29 | CMW217, PREM50548 | *P. sylvestris* | T. Lagerberg/E. Melin | Sweden |
| *L. truncatum* | CMW21 | PREM45896 | Trunk of *P. radiata* | M. J. Wingfiled | New Zealand |
| | CMW28 | ATCC58099 | Root of *P. taeda* | M. J. Wingfiled | South Africa |
| | CMW30 | PREM45699 | Trunk of *P. strobus* | M. J. Wingfiled | New Zealand |
| *L. koreanum* | KUC2072 | DAOM234393 | *P. densiflora* infested with *T. piniperda* | J. -J. Kim & G. -H. Kim | Korea |
| | KUC2102 | DAOM234392 | *P. koraiensis* infested with *T. piniperda* | J. -J. Kim & G. -H. Kim | Korea |
| *L. pini-densiflorae* | MCC071 | JCM10479, MAFF410861, CMW5157 | *P. densiflora* infested with *T. piniperda* | H. Masuya | Japan |
| | MCC194 | JCM10480, MAFF410865, CMW5158 | *P. densiflora* infested with *T. piniperda* | H. Masuya | Japan |
| *L. yunnanense* | CMW5304 | | *T. piniperda* on *P. yunnanensis* | XD Zhou | China |

* Culture collection source: ATCC, American Type Culture Collection, U.S.A; CBS, the culture collection of Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CMW, culture collection of the Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DAOM, National Mycological Herbarium, Canada; JCM, Japan Collection of Microorganisms, RIKEN BioResource Center, Japan; KUC, the Korea University Culture Collection, Korean University, Korea; MAFF, Genetic Resources Center, Culture Collection of National Institute of Agrobiological Resources, Japan; MCC, culture collection of the senior author.

Table 2. Isolates used for molecular comparison.

| Species | Isolate No.*1 | Origin | GenBank No. | | |
|---|---|---|---|---|---|
| | | | ITS2 & LSU | Actin | β-tubulin |
| *Leptographium* sp. | MCC206 | Japan | AB222065*2 | AB222061*2 | AB222063*2 |
| | MCC214 | Japan | AB222066*2 | AB222062*2 | AB222064*2 |
| | MCC217 | Japan | | | |
| | MCC364 | Japan | | | |
| | MCC365 | Japan | | | |
| *L. koreanum* | KUC2072 | Korea | AY707196 | AY707174 | AY707183 |
| | KUC2102 | Korea | AY707197 | AY707175 | AY707184 |
| *L. lundbergii* | CBS352.29 | Sweden | AY707198 | AY707176 | AY707185 |
| *L. pini-densiflorae* | MCC071 | Japan | AY707199 | | AY707186 |
| | MCC194 | Japan | AY707200 | | AY707187 |
| *L. pyrinum* | DLS879 | USA | AY544604 | AY544586 | AY263185 |
| *L. terebrantis* | C418 | USA | AY544607 | AY544589 | AY263191 |
| *L. truncatum* | CMW21 | New Zealand | DQ062056 | | DQ061990 |
| | CMW28 | South Africa | DQ062052 | | DQ061986 |
| | CMW30 | New Zealand | DQ062054 | | DQ061988 |
| *L. wingfieldii* | CMW2095 | France | AY707204 | AY707177 | AY707190 |
| *L. yunnanense* | CMW5304 | China | AY707206 | AY707179 | AY707192 |
| *Ophiostoma aenigmaticum* | CMW2199 | Japan | AY553389 | | |
| | CMW2310 | Japan | AY553390 | | |
| *O. aureum* | ATCC16936 | Canada | AY544610 | AY544592 | AY263187 |
| *O. clavigerum* | ATCC18086 | Canada | AY544613 | AY544595 | AY263194 |
| *O. huntii* | UAMH4997 | Canada | AY544617 | AY544599 | AY349023 |
| *O. laricis* | CMW1980 | Japan | AF343691 | | |
| *O. piceaperdum* | C274 | USA | AY707209 | AY707182 | AY707195 |
| *O. robustum* | CMW668 | USA | AY544619 | AY544601 | AY263185 |

*1 Culture collection source: ATCC, American Type Culture Collection, U.S.A; C, Collection of T. C. Harrington, Iowa State University; CBS, the culture collection of Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; CMW, culture collection of the Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; DLS, culture collection of D. L. Six; KUC, the Korea University Culture Collection, Korean University, Korea; MCC, culture collection of the senior author; UAMH, University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton.

*2 Accession numbers of the sequences obtained in this study.

necks and outer layers of the peridium. Morphologically characteristic structures were measured and averages and ranges computed. Fifty measurements for each structure were made.

## Mating experiments

Isolates of the unknown *Leptographium* sp. were paired in all possible combinations. In addition, four isolates originating from single ascospores derived from a single perithecium from a cross between isolates MCC206 and MCC214, were paired with each other in all possible combinations. We use MCC206 and MCC214 as tester isolates and try to pair each one with other species listed in Table 1. Negative control mating experiments also were done.

Plugs from 2-wk-old cultures on 2% MEA were excised with cork borer (5mm diam.) and placed on 2% MEA. Plates were incubated at 15 °C in the dark for 2 weeks. Two autoclaved pine twigs or sapwood blocks were then placed on the agar surface and the plates were incubated for an additional 2 weeks. Donor cultures were flooded with 600ml sterile and deionized water and conidial suspensions were prepared. This suspension was poured on the twigs in the each recipient culture. Plates were then incubated at 15 C and these were regularly inspected over a period of two months for the mature perithecia.

### DNA sequence comparisons

Cultures for DNA sequence comparisons were incubated on 2% MEA plates for four weeks. DNA was extracted using the methods described by Kim et al. (2005). Oligonucleotide primers used for both amplification and DNA sequencing of the internal transcribed spacer (ITS) 2 and partial large subunit (LSU) regions of the ribosomal DNA operon, portions of the actin and β-tubulin genes were the same as those used by Kim et al. (2005). Methods for amplification and sequencing of each gene or gene region were also as described by Kim et al. (2005). Both strands of fragments were sequenced and sequences have been deposited in GenBank (Table 2, Fig. 1).

Obtained sequences were analyzed together with previously published sequences to provide a sufficiently broad taxon sampling (Table. 2). Overall, the ITS2 and LSUrDNA—D1 sequence data set included 23 sequences including those derived in this study. The actin and β-tubulin gene sequence data set was comprised of 16 and 22 sequences including those obtained in this study.

Sequences were aligned using Clustal X version 1.81 (Thompson et al. 1997). Alignments were manually adjusted using the program BioEdit version 5.0.9 (Hall 1999). The aligned data set was analyzed using the program PAUP*4.0 beta10 (Swofford 2002). A parsimony analysis was carried out using the heuristic search with simple stepwise addition, MAXTREE option set to 1000, and tree-bisection reconnection (TBR) option of the program. Gaps were treated as missing data and all characters were equally weighted. Bootstrap and jackknife values (each 1000 replicates) were also calculated.

## Results

### Morphology

The unknown *Leptographium* sp. from Japan was characterized by having mainly two primary branches and conidia with truncated bases. These characteristics are commonly found in various species of *Leptographium*. In particular, *L. truncatum*, *L. pini-densiflorae*, *L. koreanum*, and the *Leptographium* anamorphs of *Ophiostoma* spp. including *L. laricis* Van der Westh. et al., *L. aenigmaticum* K. Jacobs et al., *L. huntii* M. J. Wingf. and *L. piceaperdum* K. Jacobs & M. J. Wingf. have similar characters to those of the unknown *Leptographium* sp. from Japan. However, the stipe lengths of the unknown *Leptographium* sp. were longer than those of *L. piceaperdum*, *L. laricis* and *L. aenigmaticum*. Hyphal characteristics of the Japanese fungus were also different from those of *L. huntii*, which are typically serpentine, but could not be distinguished from all other species considered in this study. Primary branches of the Japanese *Leptographium* sp. were smaller than those of *L. truncatum*. Conidia of the *Leptographium* sp. were the

Table 3. Teleomorphic characters of the *Leptographium* species and morphologically similar species.

| Character | *Leptographium* sp. | *O. piceaperdum*[a] | *O. laricis*[b] | *O. aenigmaticum*[c] | *O. huntii*[d] |
|---|---|---|---|---|---|
| Perithecial diam. | 240–310 | (170–) 199–312 (–370) | 210–310 | 142–254 | 280–448 |
| Perithecial neck length | 520–1000 | (280–) 503–603(–850) | 400–1320 | 115–310 | 140–720 |
| Perithecial neck width at the base | 52–75 | (30–) 32–60 | 50–70 | 35–100 | 40–70 |
| Perithecial neck width near the tip | 25–33 | 20–30 | 20–50 | 20–45 | 21–42 |
| Ascospore shape | Hat-shaped to cucullate | Hat-shaped | oblong to ellipsoid | Hat-shaped with elongated brims | Hat-shaped |
| Ascospore size | 5.5–10.5 × 4.5–7.5 | (3–) 4–5 × 2–3 | 6–11 × 2–4 | 4–5 × 1.8–3.5 | 3–4 × 1.5–2 |
| Shape of perithecial outer wall composed cell | polygonal to irregularly shaped | – | – | – | elongate to oval |
| Size of perithecial outer wall composed cell | 10–22 × 8–21 | – | – | – | ca. 19.6 × 9.7 |

[a] Jacobs & Wingfield (2001), [b] Van der Westhuizen et al. (1995), [c] Jacobs et al. (1998), [d] Robinson-Jeffrey & Grinchenko (1964)

**Table 4.** Result of mating experiment of the Leptographium sp.

| | | recipient | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | donor | MCC206 | MCC211 | MCC213 | MCC214 | MCC215 | MCC217 | MCC364 | MCC365 | KUC2102 | KUC2072 | CBS352.29 | CMW21 | CMW28 | CMW30 | MCC071 | MCC194 | CMW5304 |
| *Leptographium* sp. | MCC206 | - | - | - | ++ | - | ++ | - | - | + | + | - | - | - | - | - | - | - |
| | MCC211 | - | - | + | - | - | - | - | - | NT | NT | - | - | - | - | - | - | NT |
| | MCC213 | - | + | - | ++ | - | - | + | - | NT | NT | - | - | - | - | - | - | NT |
| | MCC214 | ++ | - | + | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - |
| | MCC215 | - | - | - | + | - | - | - | - | NT | NT | - | - | - | - | - | - | NT |
| | MCC217 | - | - | + | - | - | - | - | + | NT | NT | - | - | - | - | - | - | NT |
| | MCC364 | - | - | ++ | - | - | - | - | ++ | - | - | - | - | - | - | - | - | - |
| | MCC365 | - | - | - | ++ | - | + | - | - | - | - | - | - | - | - | - | - | - |
| | KUC2102 | + | NT | NT | - | NT | NT | - | - | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| | KUC2072 | + | NT | NT | - | NT | NT | - | - | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| *L. lundbergii* | CBS352.29 | - | - | - | - | - | - | - | - | NT | NT | - | - | - | - | - | - | NT |
| *L. truncatum* | CMW21 | - | - | - | - | - | - | - | - | NT | NT | - | - | - | - | - | - | NT |
| | CMW28 | - | - | - | - | - | - | - | - | NT | NT | - | - | - | - | - | - | NT |
| | CMW30 | - | - | - | - | - | - | - | - | NT | NT | - | - | - | - | - | - | NT |
| *L. pini-densiflorae* | MCC071 | - | - | - | - | - | - | - | - | NT | NT | - | - | - | - | - | - | NT |
| | MCC194 | - | - | - | - | - | - | - | - | NT | NT | - | - | - | - | - | - | NT |
| *L. yunnanense* | CMW5304 | - | NT | NT | - | NT | NT | - | - | NT | NT | NT | NT | NT | NT | NT | NT | NT |

* – no perithecia produced, +: perithecia (n <50) produced, ++: perithecia (n >50) produced, NT: not tested.

same as those of *L. koreanum*, which are oblong to ovoid and relatively straight and thus, different to those of other species.

The morphology of the teleomorph structures produced as a result of the pairing of various strains was similar to those of *Ophiostoma piceaperdum* (Rumbold) Arx, *O. aenigmaticum* K. Jacobs et al., and *O. huntii* (Rob.-Jeffr.) de Hoog & R. J. Scheff. where ascospores were hat-shaped and perithecial necks had no ostiolar hyphae. Perithecia sizes and neck lengths overlapped between species and could not be clearly separated. However, the ascospores of the unidentified *Ophiostoma* sp. were larger than those of the other species with which it might be confused (Table 3).

## Mating experiments

Two isolates (MCC206 and MCC214) of the unidentified *Leptographium* sp. from Japan produced protoperithecia in culture, but none of them produced mature perithecia unless they were paired with other isolates. Pairing of isolates with themselves did not result in perithecia. The results of the pairing of isolates in all possible combinations showed that some isolates were able to produce perithecia (Table 4). In addition, an isolate of the Japanese *Leptographium* sp. produced perithecia, when it was crossed with the ex-type strain of *L. koreanum* (KUC2102), and not when it was paired with other fungal species such as *L. truncatum* and *L. pini-densiflorae*. The result of crosses between single ascospore isolates showed clearly that the mating behavior of this fungus was heterothallic with two mating types.

## DNA sequence comparisons

Phylogenetic analyses of sequences for the ITS2 and partial LSU of rDNA gene regions showed that isolates of the unknown *Leptographium* sp. from Japan is clearly distinct from *O. piceaperdum, O. laricis, O. aenigmaticum* and *L. pini-densiflorae* but that it reside a monophyletic group with isolates of *L. koreanum, L. truncatum, L. yunnanense* and other *Ophiostoma* spp. and *Leptographium* spp. (Fig. 1a). This result was supported by strong bootstrap/Jackknife values (83/78). From a total of 610 characters, 572 characters were constant, 13 variable characters were parsimony uninformative and 25 were informative. The heuristic search gave rise to 390 most parsimonious trees, of which one was chosen for presentation (Fig. 1a). The tree had a length of 44 steps with a Consistency Index (CI) of 0.9091, a Homoplasy Index (HI) of 0.0909, and a Retention Index (RI) of 0.9565.

Phylogenetic analysis of the β-tubulin gene sequences resulted in the most parsimonious tree shown in Fig. 1b. This analysis also showed that the unknown *Leptographium* grouped with high bootstrap/Jackknife support (92/82) in the clade containing *L. koreanum*, and not with *L. truncatum, L. yunnanense, L. lundbergii, O. piceaperdum*, and *O. aenigmaticum*. From a total of 360 characters, 235 characters were constant, 19 variable characters were parsimony-uninformative and 106 were informative. The heuristic search found 87 most parsimonious trees of which one was chosen for presentation (Fig. 1b). The tree had a length of 221, a Consistency Index (CI) of 0.8190, a Homoplasy Index (HI) of 0.1810, and a Retention Index (RI) of 0.8987.

The phylogenetic analysis of the actin gene sequences showed that the unknown *Leptographium* sp. from Japan resided in a monophyletic group with *L. koreanum* (Fig. 1c) with high bootstrap/Jackknife support (99/98). *Leptographium yunnanense*

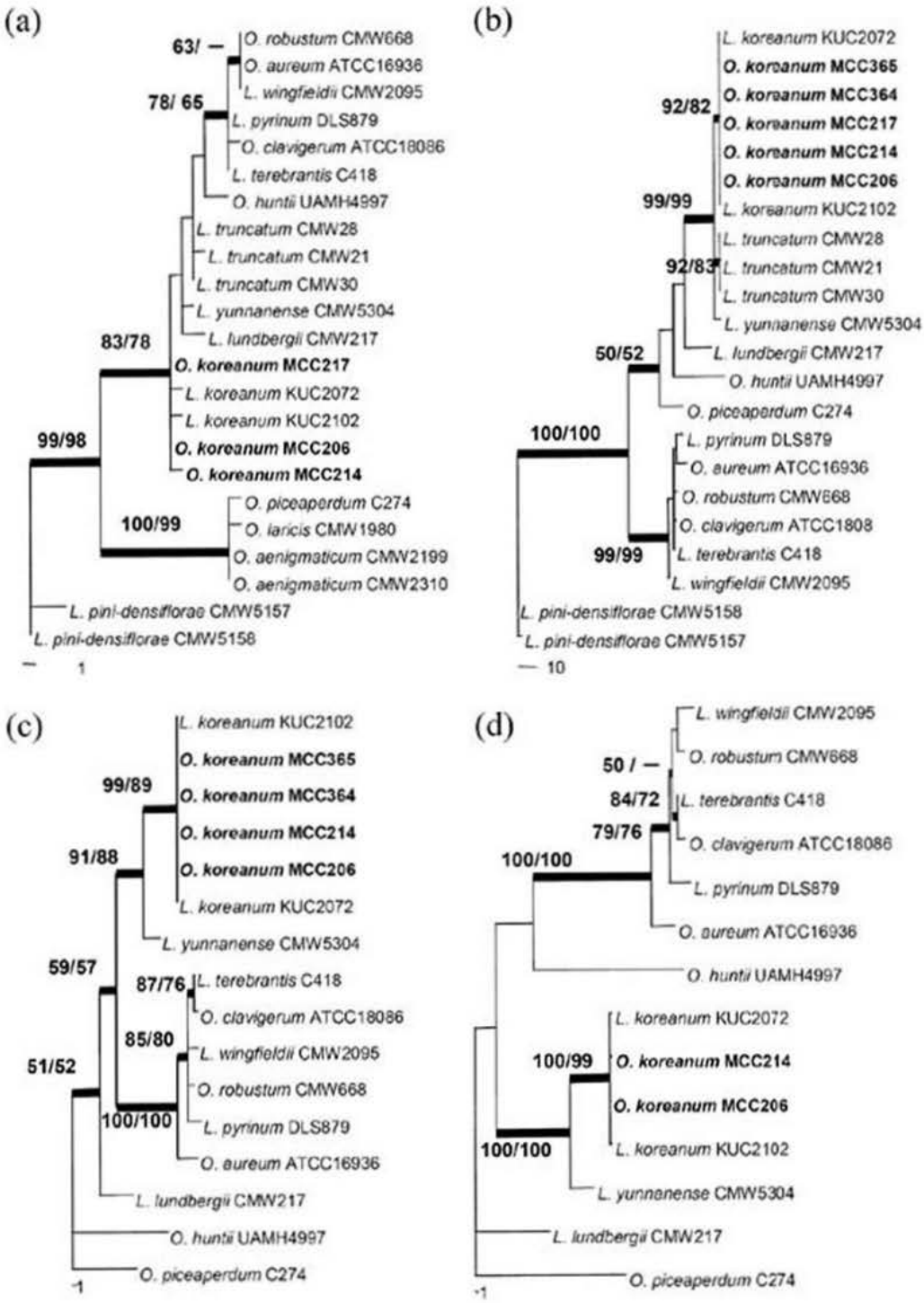


Fig. 1. The most parsimonious trees for each of the three nuclear gene datasets and the combined datasets: (a) rDNA, (b) β-tubulin, (c) actin, and (d) combined. The tree is unrooted. Bootstrap and Jackknife values > 50% with 1000 replications are indicated at the left of the branches (bootstrap value/Jackknife value).

appeared in a sister clade to *L. koreanum*. From a total of 763 characters, 668 characters were constant, 51 variable characters were parsimony-uninformative and 44 were informative. The heuristic search gave rise to two most parsimonious trees of which one (Fig. 1c) is presented. The tree length was 132 with a Consistency Index (CI) of 0.8788, a Homoplasy Index (HI) of 0.1212, and a Retention Index (RI) of 0.9171.

A combined sequence data set was also produced including 14 sequences representing the ITS2 and LSUrDNA—D1 domain, and parts of the actin, and β-tubulin genes. As was true for the analyses of the sequence data for the individual genes or gene regions, the phylogenetic analyses (Fig. 1d) showed that the unknown *Leptographium* sp. from Japan resided in a monophyletic group with *L. koreanum*, with a high bootstrap/Jackknife values (100/99). In this tree (Fig. 1d), *L. yunnanense* resided in a sister group to *L. koreanum*. From a total of 1728 characters, 1510 characters were constant, 110 variable characters were parsimony-uninformative and 108 were informative. The heuristic search found two most parsimonious trees of which one was chosen for presentation. The tree length was 305 with a Consistency Index (CI) of 0.8659, a Homoplasy Index (HI) of 0.1344, and a Retention Index (RI) of 0.9014.

## Taxonomy

Results of the DNA sequence and morphological comparisons show clearly that the *Leptographium* anamorph of unknown *Ophiostoma* species from Japan is conspecific with *L. koreanum*. Furthermore, crosses between isolates of this fungus and those of *L. koreanum* have given rise to its *Ophiostoma* teleomorph. Teleomorph characteristics are also distinct from other *Ophiostoma* spp. with *Leptographium* anamorphs. Thus, on the basis of the mating behavior, morphological characteristics and DNA sequence comparisons, we described the teleomorph of *L. koreanum* as follows:

***Ophiostoma koreanum*** Masuya, J.-J. Kim & M. J. Wingf. **sp. nov.** Figs. 2-7.

Anamorph: *Leptographium koreanum* J.-J. Kim & G.-H. Kim, Mycol. Res. 109(3): p. 275, 2005.

*Perithecia basi nigra, globosa vel subglobosa, 240—310 μm diam, Collum cylindraceum, curvatum vel rectum, 560—1000 μm longum, ad basim 52—75 μm latum, ad apicem 25—33 μm latum, apice obtusum vel truncatum, hyphis ostioli non praeditum. Asci evanescenti. Ascosporae hyalinae, aseptatae, aspectu laterali cuculatae, aspectu frontali triangulatae, vagina hyalina circumdantes, 5.5—10.5 × 4.5—7.5μm.*

***Etymology***: Derived from the name of anamorph, *Leptographium koreanum*.

**Perithecia** superficial or partly embedded in the substratum and medium. Basal part black, globose to subglobose, 240—310 (mean 290) μm diam. without hyphal ornamentation, outer layer of the peridium composed of thick-walled, more or less isodiametric, polygonal or irregularly shaped cells, 10—22 × 8—21 (mean 18 × 12) μm. Necks dark brown to black, broad at the base, becoming cylindrical or slightly tapered at the tip, straght or curved, 560—1000 (mean 680) μm long, 52—75 (mean 68) μm wide at base, 25—33 (mean 30) μm wide near the tip, composed of dark, thick-walled, squamous cells, 4—10 × 2—4 μm, terminating in an obtuse to truncate apex. Ostiolar hyphae absent. Asci evanescent, clavate when young, subglobose when mature, up to 25 × 20 μm. **Ascospores**, hyaline, one-celled, hat-shaped or cucullate in side view, triangular

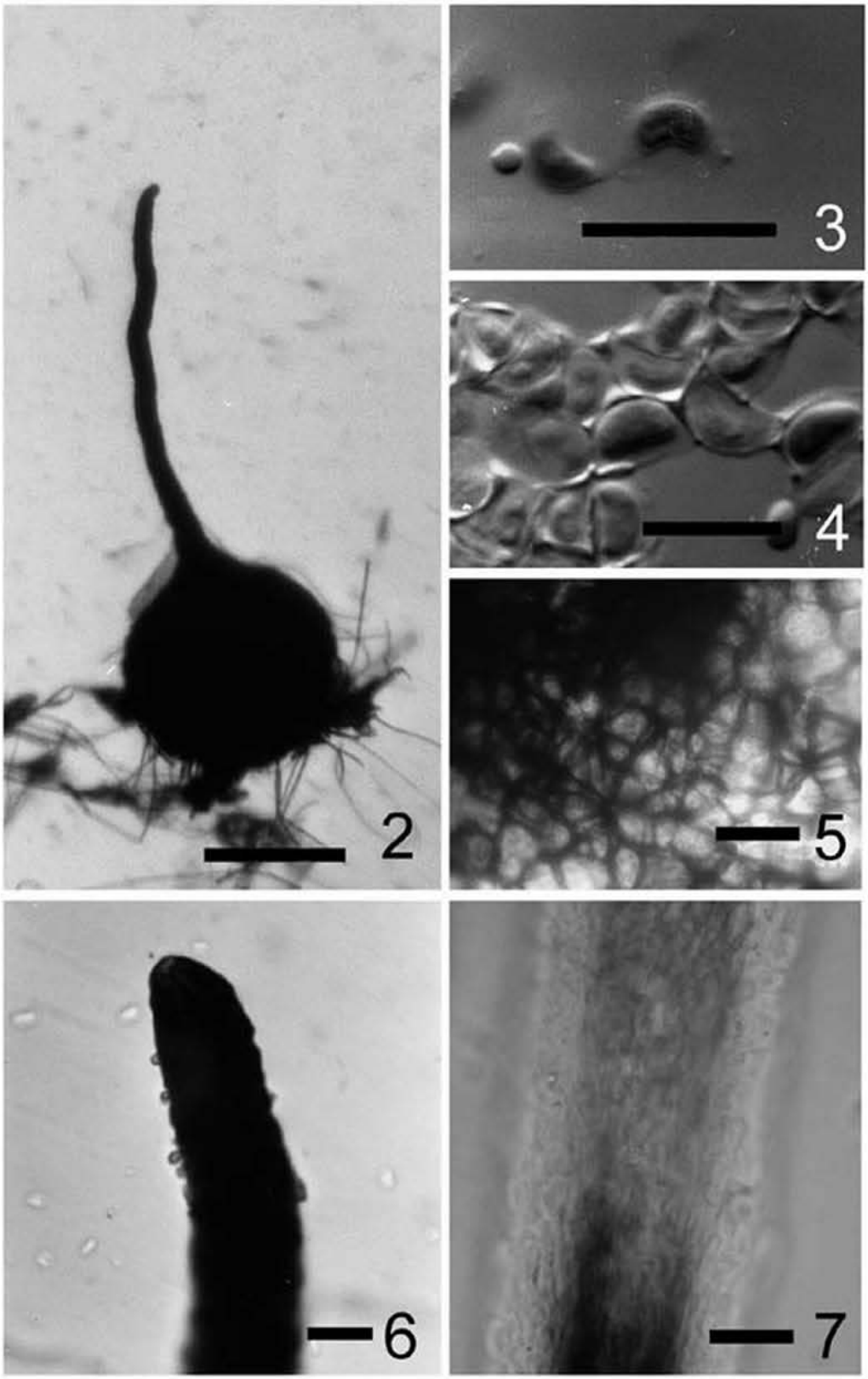


Fig. 2—7. *Ophiostoma koreanum*. Fig. 2. Perithecium. Fig. 3. Ascospore. Fig. 4. Ascospores. Fig. 5. Outer layer of the peridium composed of thick-walled, more or less isodiametric, polygonal or irregularly shaped cells. Fig. 6. Tip of perithecial neck. Fig. 7. Perithecial neck, composed of dark, thick-walled, squamous cells. Bars: Fig. 2 = 100μm, Figs. 3—5, 7 = 10μm, Fig. 6 = 5μm.

in front view, 5.5—10.5 × 4.5—7.5 (mean 7.5 × 5.5) µm, surrounded by a distinct hyaline wall, sometimes accumulating in a white mass at the tips of neck.

Morphology of anamorph is as described for *L. koreanum* (Kim et al. 2005).

**Hosts:** *Pinus densiflora*, *P. koraiensis* Siebold & Zucc. and other *Pinus* spp.

**Isolated from following insects:** *Tomicus piniperda* L., *Hylurgops interstitialis* (Chapuis), *Hylastes paralleus* Chapuis, *Hylastes plumbeus* Blandford and other bark beetle species.

**Known distribution:** Korea, Japan.

CULTURES AND SPECIMENS EXAMINED—**HOLOTYPE:** DAOM234414, dried culture derived from the pairing of the cultures KUC2102 (MAT-1) (DAOM234392) (KOREA. YEOJU: Sawmill, Central Forest Products Processing & Marketing Center, *P. koraiensis* log, 10 Aug 2000, *J. J. Kim & G. H. Kim*) and MCC206 (MAT-2) (DAOM234395, JCM11853, MAFF410963) ( JAPAN. IBARAKI: Tsukuba, *T. piniperda* adult, 4 Apr 1995, *H. Masuya*) on *P. contorta* Dougl. sapwood block. **PARATYPES:** FPH (= TFM) 7605, dried culture from pairing between isolates MCC206 (MAT-2) (DAOM234395, MAFF410963) and MCC214 (MAT-1) (DAOM 234396, JCM11854, MAFF410966) ( JAPAN. YAMANASHI: Masuho, *T. piniperda* adult, 15 May 1996, *H. Masuya*) on *P. densiflora* twigs, FPH (TFM) 7606, dried culture from pairing between isolates MCC213 (MAT-2) (JCM11855, MAFF410965) (JAPAN. FUKUSHIMA: Amasakae, *T. piniperda* adult, 24 Jun 1996, *H. Masuya*) and MCC214 (MAT-1) on *P. densiflora* twigs, FPH (TFM) 7607, dried culture from pairing between isolates MCC365 (MAT-2) (JCM11856, MAFF410962) (JAPAN. IWATE: Matsukawa, *H. interstitialis* adult, 1 Jun 2000, *H. Masuya*) and MCC214 (MAT-1) on *P. densiflora* twigs. **Additional cultures examined.** KOREA. YEOJU: Sawmill, Central Forest Products Processing & Marketing Center, *P. koraiensis* log, 10 Aug 2000, *J. J. Kim & G. H. Kim* (KUC2072, MAT-1, DAOM 234393, CMW14199). Bongwha: Sawmill, National Forestry Cooperatives Federation, *P. densiflora* log, 9 Aug 2000, *J. J. Kim & G. H. Kim* (KUC 2078, MAT-1, CMW 14201, PREM 58261). JAPAN. IWATE: Ichinoseki, *T. piniperda* adult, 19 Jun 1996, *H. Masuya* (MCC211, MAT-1, JCM11857, MAFF410964), HOKKAIDO: Yamabe, bark of *P. sylverstris* dead tree, 7 Nov 1996, *H. Masuya* (MCC215, MAT-2, JCM11859, MAFF410967), IBARAKI: Tsukuba, *T. piniperda* adult, 18 May 1995, *H. Masuya* (MCC217, MAT-1, JCM11860, MAFF410968), IWATE: Matsukawa, *H. interstitialis* adult, 1 Jun 2000, *H. Masuya* (MCC364, MAT-1, JCM11858, MAFF410961).

## Discussion

Results of this study have shown that the unidentified *Leptographium* sp. commonly isolated from pine bark beetles and bark beetle-infested *Pinus* spp. in Japan is morphologically and phylogenetically identical to *L. koreanum*. *Leptographium koreanum* was originally isolated from Korean (*P. koraiensis*) and Japanese red (*P. densiflora*) pine in Korea and was described as a new species by Kim et al. (2005). This fungus was frequently isolated from *T. piniperda* and it appears to be an important causal agent of blue-stain of conifer timber in Korea.

In this study, we have shown that *L. koreanum* in Japan is able to form a teleomorph when sexually compatible isolates are crossed. We have thus described the teleomorph of the fungus as *O. koreanum*. This fungus has been known in Japan for more than a decade where is was reported as an undescribed *Ophiostoma* sp. by Masuya et al (1998,

1999), frequently associated with the pine shoot beetle, *T. piniperda*. The Japanese *Leptographium* strains could be also paired with the ex-type strain of *L. koreanum* confirming that these two are biological heterothallic species. The distribution and ecology of *L. koreanum* and *O. koreanum* thus appear to be similar in Korea and Japan.

The teleomorph of *L. koreanum* described in this study is characterized by hat-shaped ascospores and long necks without ostiolar hyphae. In this regard it is similar to that of *O. piceaperdum* sensu Jacobs et al. (2000) and related species. Taxonomic status of *O. piceaperdum*, however, remains questionable. Morphological comparisons of the dried type specimens led Jacobs et al. (2000) to treat *O. europhioides* (E. F. Wright & Cain) H. Solheim and *O. pseudoeurophioides* (Olchow. & J. Reid) Georg Hausner et al. as synonyms of *O. piceaperdum*. However, recent DNA based comparisons by Hausner et al. (2000) showed that *O. europhioides*, *O. pseudoeurophioides* and *O. piceaperdum* reside in different clades. This suggests that the species concept of *O. piceaperdum* sensu Jacobs et al (2000) deserves reconsideration. Indeed, Olchowecki & Reid (1974) showed that the *Leptographium* anamorph of *O. pseudoeurophioides* has curved conidia, unlike *O. piceaperdum* illustrated by Jacobs et al. (2000). Because the ex-type cultures of *O. pseudoeurophioides* and *O. piceaperdum* are no longer available, it is not possible to consider their morphological characteristics or to make DNA based phylogenetic comparisons. These studies are required but must await further collections.

*Leptographium koreanum* does not have curved conidia and its LSU rDNA sequences do not correspond with those of *O. pseudoeurophioides* (GenBank accession No. AF155678). These species are, therefore, clearly different. In addition, our DNA sequence comparisons have shown that *O. koreanum* is not related to well-defined culture of *O. piceaperdum*. We are thus confident that the new species does not represent *O. piceaperdum*.

*Ophiostoma aenigmaticum* was described by Jacobs et al. (1998) from Japan. This species had previously been treated as *O. europhioides* by Yamaoka et al. (1997) but was differentiated from *O. europhioides* based on the characteristic of elongated brims of the ascospores (Jacobs et al. 1998). Because *O. koreanum* does not have ascospores with elongated brims and because *O. aenigmaticum* is homothallic, as opposed to the heterothallic *O. koreanum*, these species can easily be distinguished from each other. In addition, they also differ in their hosts and insect vectors. DNA based comparisons in this study have also shown that *O. aenigmaticum* is more closely related to *O. piceaperdum* than to *L. koreanum*.

The heterothallic mating behavior appears to be relatively uncommon in *Ophiostoma* with *Leptographium* anamorphs. While heterothallism has been noted for many ascomycetes including those from the genus *Ophiostoma* (Brasier 1993), *O. huntii* is the only *Ophiostoma* with a *Leptographium* anamorph that has previously been shown to display this mating behaviour (Jacobs et al. 1998). This is in contrast to species such as *O. piceaperdum* sensu Jacobs et al. (2000), *O. europhioides*, and *O. aenigmaticum* that have been reported to be homothallic. The fact that *O. koreanum* is heterothallic clearly distinguishes it from related *Ophiostoma* species with *Leptographium* anamorphs.

A relatively small number of *Leptographium* spp. have known teleomorphs and the majority of these appear to have hat-shaped ascospores (Jacobs & Wingfield 2001). In some cases such as that of *Leptographium wageneri* var. *ponderosae*, perithecia thought to represent an *Ophiostoma* state have been found in galleries of beetle vectors but these

have never been found in culture. This makes it difficult to confirm the anamorph/teleomorph connection. It is not uncommon to discover teleomorph states in fungi thought to exist only in the asexual form (Kuhls et al. 1996, Hodge et al. 1996, Chaverri et al. 2001). The discovery of the teleomorph of *L. koreanum* arising from crosses between different isolates suggests that some other *Leptographium* species might also be heterothallic and might have the ability to produce perithecia in crosses of sexually compatible isolates under suitable environmental conditions. This is clearly an area of research worth pursuing.

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# Two parasitic fungi on a new host, *Syringa* (*Oleaceae*)

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**Abstract**—*Thedgonia ligustrina*, the agent of *Ligustrum* leaf-spot, and *Gloeosporidiella turgida*, known as a parasite of *Fraxinus*, are reported for the first time on *Syringa* spp. Both fungi were found in Sweden, the first in a tree nursery in the south, and the second on plants cultivated in Uppsala. Brief descriptions and illustration are provided and the distribution of *T. ligustrina* is reviewed.

**Key words**—anamorph, Ascomycota, Lithuania, taxonomy

## Introduction

Several species of *Syringa* (lilac) are cultivated in Sweden for ornamental purposes (Nitzelius 1964). One parasitic fungus found on *S. vulgaris* L., presented for identification to the first author in 1985, and another one found on *S.* ×*chinensis* Willd. in 2003-2005 by V.M. and O.C., were identified as parasites of *Ligustrum* and *Fraxinus*, respectively, all members of the *Oleaceae*. These fungi were also isolated in culture, using the procedure described by Constantinescu (1988). Because these fungi have been described and illustrated in detail by previous authors, only short descriptions are provided and few, significant details are illustrated in this paper. The nomenclature of colony colour follows Kornerup & Wanscher (1983), the abbreviation of herbaria those from Holmgren et al. (1990), and UPSC is the acronym of Uppsala University Culture Collection of Fungi.

## *Thedgonia ligustrina* (Boerema) B. Sutton (Figs 1a & 2a)

A leaf-spot disease of *Syringa vulgaris* cultivated in a tree nursery in southern Sweden was observed in 1985. In late autumn the same year several leaves showing symptoms and the presence of a fungus were received for identification at UPSC.

On the upper leaf surface the fungus produces small, 0.5-1.5 mm round to irregular, silvery spots, surrounded by a narrow, reddish brown margin. When the spots coalesce, larger portions of the surrounding tissue become brown, necrotic. On the corresponding lower surface, an ochreous tuft develops composed of conidiophores emerging from colourless stromata and conidia. The conidiophores are agglomerated in fascicles,

colourless, 30-75 µm long, 4-6 µm wide, with 1-2 (-3) flat, not thickened scars located at the tip or slightly below. The conidia are colourless, more or less cylindrical, 35-75 × 4-6 µm, 0-4 septate.

Cultures initiated from conidia: on 2% malt-extract agar, the fungus grows very slowly, forming colonies of 3-4 mm diam in 10 days at ca. 20°C; the colonies appear umbonate, Brownish Orange to Light Brown (6CD 4-5), reverse Dark Brown (8F 4-5), margin narrow, deep; exudate and diffusible pigment absent; the surface is velvety and composed of erect hyphae Advancing hyphae are colourless, straight to slightly curved, 3-3.5 µm diam, septate every 15-25 µm, sparingly branched, branches at ca. 50-70°, usually arising just below the septum of the subtending hypha; older hyphae yellowish to brownish, 5-8 µm diam, constricted at septa, later becoming moniliform to chlamydospore-like and then up to 13 µm diam.

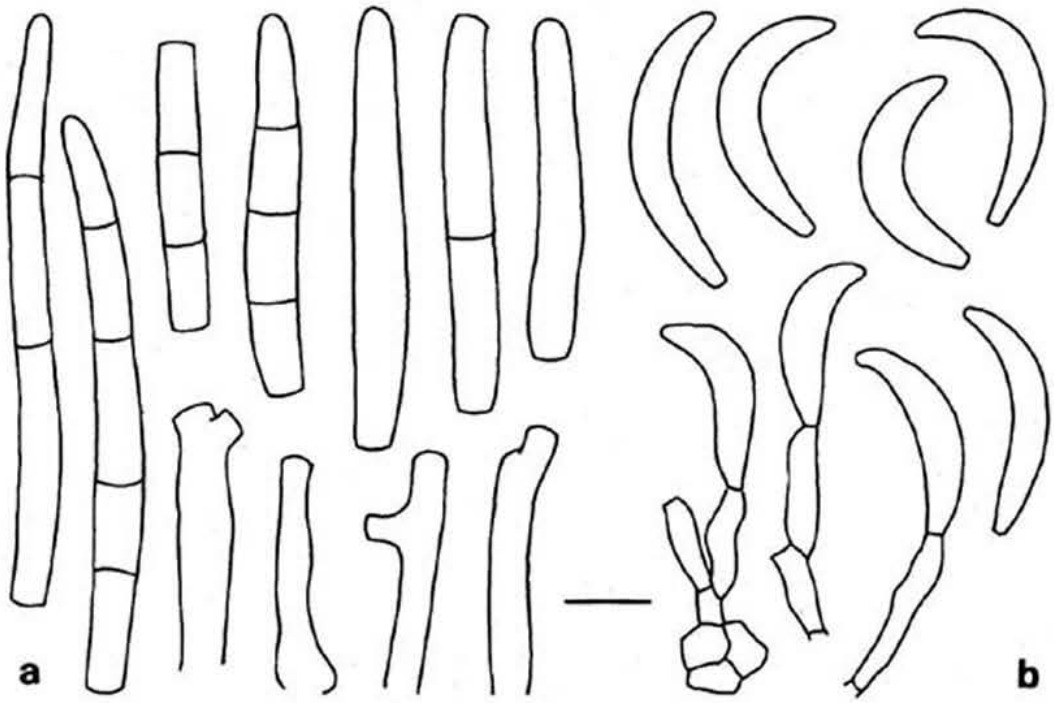


**Fig. 1.** Fungi on *Syringa*; conidiogenous cells and conidia of **a** - *Thedgonia ligustrina* (*UPS F-117834*) and **b** - *Gloeosporidiella turgida* (*UPS F-118065*). Bar = 10 µm).

SPECIMENS EXAMINED — On *Syringa vulgaris*: SWEDEN. **Skåne,** Veberöd, Björkhaga tree nursery (55°38'N 13°28'E), XI. 1985, coll. L. Nilsson, det. O. Constantinescu *UPS F-117834*; living culture *UPSC 1699* (now at *CBS 113536*); dried culture *UPS F-118064*. On *Ligustrum vulgare*: GERMANY. **Bayern, Oberbayern**, Bad Reichenhall, 'Thumsee' (47°43'N 12°50'E), 13 VIII 1996, U. Braun in Triebel, Microfungi exsiccati 348 *UPS F-11959*. **KOREA. Suwŏn**, Guundong street, near the entrance to NIAST (37°15'N 127°00'E), 25 X 2003, V. Mel'nik *LE 214697*. LITHUANIA. **Alytus**, Punia Forest (54°32'N 24°04'E), 7 IX 2000, A. Treigienė *BILAS 27697*; environs of Vilnius, Pašilaičiai (54°44'N 25°42'E), 15 VII 2002, A. Treigienė *BILAS 27698*. ROMANIA. **București**, Mogoşoaia Castle park (44°31'N 26°00'E), 26 X 1968, O. Constantinescu in Herb. Mycol. Romanicum 2010 *BUCM*; *UPS*; living culture *CBS 547.71*.

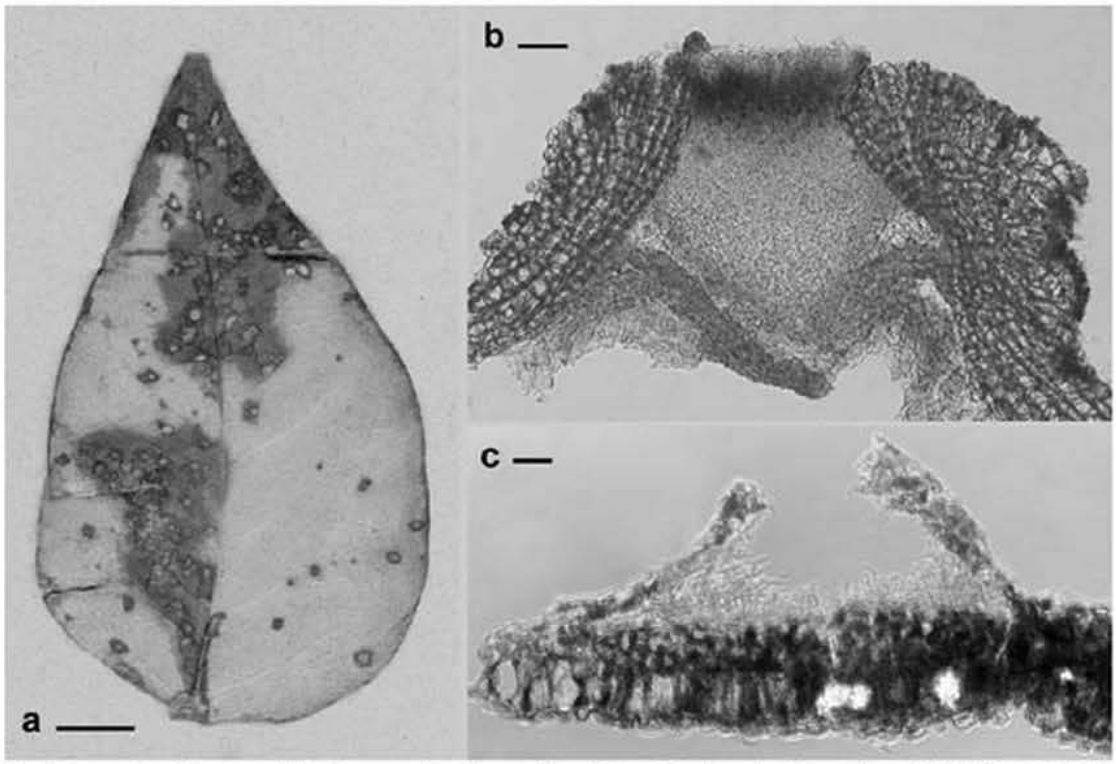


**Fig 2. a** - Leaf spot of *Syringa vulgaris* produced by *Thedgonia ligustrina* (*UPS F- 117834*). **b** - Stromatic conidioma of *Gloeosporidiella turgida* on twigs of *Syringa ×chinensis* (*UPS F-118065*). **c** - Acervular conidioma of *Gloeosporidiella ribis* on leaves of *Ribes rubrum* (*UPS F-120164*). Bars = 1 cm in **a**, 75 µm in **b**, and 25 µm in **c**.

**Discussion** — The characters of the fungus present on *Syringa* agree well with the detailed descriptions and illustrations of *Thedgonia ligustrina* from *Ligustrum* in Sutton (1973), Yoshikawa & Yokoyama (1992), and Braun (1995). Our specimen was also compared with several herbarium specimens.

*Thedgonia ligustrina* was recorded previously only on various species of *Ligustrum*, such as *L. japonicum* Thunb., *L. obtusifolium* Siebold & Zucc., *L. ovalifolium* Hassk., and *L. vulgare* L. *Syringa* is thus a new host record. Both *Ligustrum* and *Syringa* belong to the family *Oleaceae*. The presence of *T. ligustrina* on *Syringa*, particularly in a tree nursery, seems to be the result of a 'host jump' in the sense of Parlevliet (1979).

With the recent records by Stakvilevičienė (2004) and Mel'nik et al. (2005), the distribution of *T. ligustrina* includes Austria, Denmark, France, Holland, Germany, Japan, Lithuania, Korea, Romania, Sweden, United Kingdom and USA.

Attempts by Yoshikawa & Yokoyama (1992) to isolate this fungus failed. *Thedgonia ligustrina* has been tested as a possible agent of biological control of *Ligustrum* in La Réunion (http://www.cabi-bioscience.org/Html/activitiesDevelopinCountries.htm).

### *Gloeosporidiella turgida* (Berk. & Broome) B. Sutton (Figs 1b & 2b)

The fungus was found on dry, dead, ca. 2-3 mm thick twigs of *Syringa ×chinensis*. It appears as 0.2-0.5 mm pustules emerging through the bark, appearing ochre, later becoming brownish. Each conidioma is seated on a basal stroma, ca. 200 µm wide × ca. 150-200 µm high; conidiophores are colourless, simple or sparingly branched,

conidiogenous cells are 10-20 µm long, 3-4 µm broad; conidia are colourless, curved, falcate to sickle-shaped, 22-33 × 4-5 µm, narrowing to the rounded tip and the more or less truncate base.

Colonies initiated from conidia: on 2% malt-extract agar slow growing, attaining a diam of 5-7 mm in 10 days and 12-13 mm after 25 days at ca. 20°C, appearing flat, centrally umbonate, surface lanose, Brownish Orange (7C 3-4) to Reddish Grey, to Brownish Grey (8B, C 2); reverse vivid Brownish Orange (6C 8; 7C 8), margin narrow, entire in young colonies but fimbriate in older ones, exudate absent, diffusible pigment Brownish Orange. Advancing hyphae colourless, wavy to geniculate-like, ca. 2-3 µm diam, sparingly and irregularly branched at ca. 35-40° angle, septate every 18-22 µm, older hyphae yellowish and slightly wider. Spherical, 0.5-1 mm diam sclerotium-like bodies, composed of interwoven hyphae are formed in fresh cultures. These may represent young stages of conidiomata but they did not develop further after 6 months. These sclerotial bodies are not formed in later subcultures.

**SPECIMENS EXAMINATED**: On *Syringa ×chinensis*: SWEDEN, **Uppland**, Uppsala, Källparksgatan 12 (59°53'N 17°39'E), 22 VI 2003, V. Mel'nik *LE 214424; UPS F-118065*; dried culture *UPS F-118066*; living culture *CBS* 116473; ditto, 15 IX 2004, O. Constantinescu *UPS F*-118067; ditto, 15 VI 2005 *UPS F-120165*. On *Fraxinus excelsior*: **LITHUANIA, Lazdijai Distr.**, environs of Gerdašiai (53°57'N 23°54'E), 25 VII 1995, A. Treigienė *BILAS 27694*; **Kėdainiai Distr.**, Berunkiškiai Forest (55°13'N 24°08'E), 18 VI 1999, A. Treigienė *BILAS 27695*. ditto, Stebuliai Forest (55°19'N 24°06'E), 31 V 2000, A. Treigienė *BILAS 27696*.

**Discussion** — The characters of the fungus on *Syringa* agree well with the description and illustration of *Gloeosporidiella turgida* provided by Pirozynski & Morgan-Jones (1968) under *Cryptosporiopsis turgida* (Berk. & Broome) Piroz. & Morgan-Jones. *Gloeosporidiella turgida* is a known parasite of *Fraxinus* and was recorded from United Kingdom (Pirozynski & Morgan-Jones 1968). It was also found in Lithuania (A. Treigienė *in litt.*). This fungus was indicated as the agent responsible for canker of ash in the USA (Adams 2001) but, according to M. Putnam (*in litt.*), a more thorough investigation revealed that the fungus involved was *Phlyctema vagabunda* Desm. (Rossman et al. 2002). *Syringa* is a new host record for *G. turgida*. As in the case of *Thedgonia ligustrina*, the new host belongs to the same family.

*Gloeosporidiella turgida* is based on *Cryptosporium turgidum* Berk. & Broome. The placement in *Gloeosporidiella* was accepted by Verkley (1999) in his monograph of *Pezicula*. However, there are important morphological discrepancies between *C. scutellata* (G.H. Otth) Petr., the type species of *Cryptosporiopsis* and anamorph of *Pezicula ocellata* (Pers. : Fr.) Seaver (syn. *Ocellaria ocellata* (Pers. : Fr.) J. Schröt.), and *G. ribis* (Lib.) Petr., the type species of *Gloeosporidiella* and anamorph of *Drepanopeziza ribis* (Kleb.) Höhn. In the former the conidioma is stromatic whereas in the latter it is acervular, subcuticular and lacks stroma (Figs 2b,c). Consequently, *G. turgida* seems to be better accommodated in *Cryptosporiopsis*, but it should be noted that its teleomorph is unknown. Other *Cryptosporiopsis* anamorphs are associated with two phylogenetically distinct teleomorph genera, *Pezicula* and *Neofabraea* p.p., and also closely related to *Phlyctema* (teleomorph *Neofabraea alba* (E.J. Guthrie) Verkley), and *Foveostroma* anamorphs of *Dermea* (Abeln et al. 2000).

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Stakvilevičienė S. 2004 *Thedgonia ligustrina* (Boerema) B. Sutton - naujas paprastojo ligustro *(Ligustrum vulgare)* L. dėmėtligės sukėlėjas Lietuvoje [*Thedgonia ligustrina*. a new agent of brown leaf spot of *Ligustrum vulgare* in Lithuania]. Dendrologia Lithuaniae 7: 123-127. In Lithuanian.

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# Further notes on the molecular taxonomy of *Metarhizium*

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**Abstract**—The ITS1-5.8S-ITS2 rDNA regions from *Metarhizium guizhouense*, *M. pingshaense*, *M. iadini*, and four other *Metarhizium* isolates were amplified and sequenced. Bayesian analyses showed that *M. pingshaense* should be treated as synonym of *M. anisopliae* var. *anisopliae*; that *M. guizhouense* should be treated as a synonym of *M. anisopliae* var. *anisopliae*; and that *M. iadini* is a later synonym of *M. flavoviride* var. *pemphigi*. Several previously unidentified isolates, RCEF0898, ACCC30130 and RCEF1259, the taxonomic positions of which have been unclear or suspicious, were identified as *M. anisopliae* var. *anisopliae*, *M. anisopliae* var. *anisopliae* and *M. flavoviride* var. *pemphigi*, respectively.

**Key words**—entomopathogenic fungi, *Clavicipitaceae*, synonymy

## Introduction

Under natural conditions, fungi are frequent and often important natural mortality factors in insect populations (Milner, 2000). Entomopathogenic fungi may exhibit very narrow or very broad host ranges, depending on the species and isolate. *Metarhizium anisopliae* var. *anisopliae* (abbreviation: *M.a. anisopliae*) has one of the broadest host ranges, having been found to naturally infect over 200 insect species (Milner, 2000). Because of the absence of detrimental environmental effects, lack of residues in meat or crops, and ease of mass production, some species of *Metarhizium* can be used for the control of many insect pests as an environmentally acceptable alternative to chemical insecticides. Some *Metarhizium* mycoinsecticides are now registered in Australia, Europe and the USA as well as being used in countries such as Brazil and China, and have played important roles in controlling agricultural and forest insect pests.

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Two *Metarhizium* species and two varieties were accepted by Tulloch (1976) in her important review. Most recently, 3 species and 4 varieties have been accepted (Rombach et al. 1986) based on morphological data. Molecular phylogenetic studies of large and small subunit rDNA by Liu et al. (1994) addressed some Chinese *Metarhizium* strains. Later, Driver et al. (2000) proposed many new varieties based on their study of ITS sequences. Information from additional genetic loci and additional isolates is needed to fully resolve *Metarhizium* taxonomy.

The status of five *Metarhizium* species described from China has been unclear, largely because cultures were difficult to obtain. These are *Metarhizium cylindrosporae* Q.T. Chen & H.L. Guo, *M. guizhouense*, *M. pingshaense*, *M. taii*, and *M. iadini*. Recently Huang et al. (2004) showed that *M. cylindrosporae* is correctly placed in *Metarhizium*, rather than in *Nomurea* as proposed by Tzean et al. (1993), and that *Nomuraea viridula* Tzean et al. also belongs in *Metarhizium*. Huang et al. (2005) proposed that *M. taii* should be considered a synonym of *M.a. anisopliae* and revealed that *Cordyceps taii* Z.Q. Liang & A.Y. Liu is the teleomorph of *M.a. anisopliae*. This study deals with the taxonomy and identity of the remaining three species based on *ex type* cultures. The identity of several interesting *Metarhizium* isolates is also addressed. ITS sequences were used as molecular markers to address these problems.

## Materials and Methods

**Fungal isolates and culture**—Four isolates of *Metarhizium* deposited at the Institute of Soil and Fertilizer Sciences, Chinese Academy of Agricultural Science (ACCC) were provided by Li Sigui, the others were conserved in the Anhui Provincial Key Laboratory for Microbial Pest Control, Anhui Agricultural University (RCEF). Table 1 lists the hosts, identifications, and collecting locations of studied isolates. Those isolates that are included in this study only by means of sequence data obtained from GenBank are shown in Figure 1.

Table 1. Original identification, insect hosts, and geographic origins of six isolates of *Metarhizium* spp. studied here.

| GenBank Accession | Strain No. | Original Identification | Host | Location |
|---|---|---|---|---|
| DQ177434 | RCEF0898 | *M. anisopliae* | Soil | Anhui |
| DQ177428 | ACCC30105 (ex type) | *M. pingshaense* | *Alissonotum* sp. / Coleopt. | Guangdong |
| DQ177430 | ACCC30115 (ex type) | *M. guizhouense* | *Hepialus* sp. / Lepidopt. | Guizhou |
| DQ177429 | ACCC30124 (ex type) | *M. iadini* | *Rhynchites coreanus* / Coleopt. | Guizhou |
| DQ177432 | ACCC30130 | *M. flavoviride* var. *minus* | Uncertain | Uncertain |
| DQ177435 | RCEF1259 | *Metarhizium* sp. | Larva of Lepidoptera | Anhui |

For DNA extraction, spores or mycelium were transferred to a Petri dish containing potato dextrose agar medium overlaid with a disk of autoclaved cellophane. The Petri dish was sealed with Parafilm, and placed in an incubator at 25°C for about 1 week. Mycelium was then scraped from the cellophane, then stored at -20°C prior to DNA extraction.

**DNA extraction, amplification and sequencing**—Total genomic DNA was extracted from frozen mycelium by using a benzyl chloride method (Zhu et al. 1994). DNA pellets were air-dried and resuspended in 200 µl sterilized TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The quantity and quality of total genomic DNA was observed on a 0.8% TBE agarose gel stained with ethidium bromide. DNA for sequencing was amplified enzymatically using the polymerase chain reaction. The internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal DNA operon, including the 5.8S gene, were amplified using primers ITS4 and ITS5 (White et al. 1990). The PCR reaction was performed with 0.5 µl of the dissolved total DNA in a 50 µl reaction volume containing 200 µM of each dNTP, 2 mM $MgCl_2$, 0.1 mM of each primer, and 2 units *Taq* DNA polymerase with PCR buffer (Sangon, China). The PCR reactions were placed in a thermal cycler (Techine, UK) under the following temperature-cycling parameters: Step 1) 5 min at 95°C; Step 2) 35 cycles of 1 min at 94°C, followed by 1 min at 54°C, and 1 min at 72°C; Step 3) 10 min at 72°C. The resulting products were examined on a 1.2 % TBE agarose gel stained with ethidium bromide. PCR fragments were purified using the Wizard™ PCR Preps DNA Purification System Kit (Promega Co., France). PCR products were sequenced using the above-mentioned primers on an ABI 3700 automated sequencer at Shanghai Genecore Biotechnologies Company. Products were sequenced in both 5' to 3' and 3' to 5' directions.

**Phylogenetic analyses**—DNA sequences generated by us and downloaded from GenBank (see Table 1 in Huang et al. 2005) were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignment was refined by eye. *Beauveria bassiana* (Bals.-Criv.) Vuill., a related clavicipitaceous anamorph, was used as the outgroup. Parsimony analysis performed in PAUP* version b10 (Swofford 2002) using a heuristic search, but it failed to complete using available memory, apparently because of a high number of equally parsimonious trees arising from the similar sequences among isolates of *M.a. anisopliae*. Bayesian analysis was carried out using MrBayes 3.0 b4 (Huelsenbeck 2000, Huelsenbeck et al. 2001). We used a 6 parameter model to run four chains for 500,000 generations, sampling every 100 generations. The first 500 trees were discarded (burn in), and the remaining trees were saved to a file. A 50% majority rule consensus tree (Fig. 1) was then calculated using PAUP*.

## Results and Discussion

***Metarhizium pingshaense* and *Metarhizium guizhouense***—*M. pingshaense* is mainly distinguished from *M. anisopliae* var. *anisopliae* in the color of the colony and in that the spore chains are formed of individual spores connected eccentrically (Guo et al. 1986). Phylogenetic analysis reveals that the *ex type* isolate of *M. pingshaense* belongs to a clade that includes 17 isolates of *M.a. anisopliae*, including the *ex neotype* isolate FI1029. *M. pingshaense* differs from the *ex neotype* isolate by three base pairs in the ITS

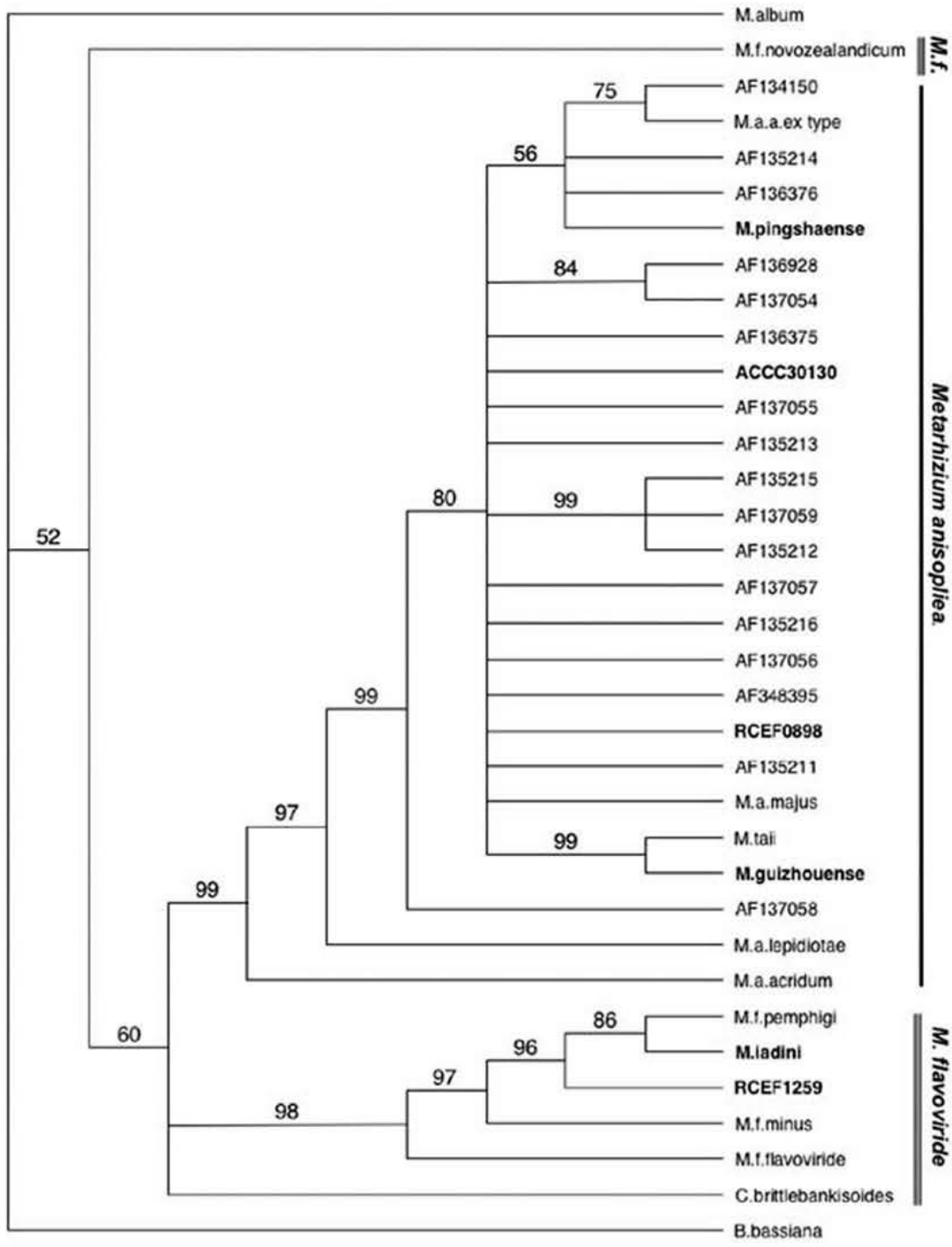


**Figure 1.** Bayesian phylogeny of *Metarhizium* spp. based on the analysis of ITS sequence data. Posterior probabilities (>50%) are given as percentages above the branches. (*M.f.* = *M. flavoviride*, and the six isolates in this study are indicated in bold face.)

sequence. We therefore propose that *M. pingshaense* should be regarded as a synonym of *M.a. anisopliae*. The conclusion is supported by the earlier conclusion of Liu et al. (1994) based on identical partial sequences from small (18S) and large (28S) subunit rRNAs. Negligible differences in serology among *M. pingshaense* and several strains of *M.a. anisopliae* (Hu et al. 1996) provide further support. There can be no question from

the integrative data here that *M. pingshaense* cannot be maintained as an independent species, and it should be treated as a synonym of *M.a. anisopliae*.

The *ex type* isolate of *M. guizhouense* also fell in the clade including all isolates of *M.a. anisopliae* except isolate FI379 (Fig. 1). Compared with the *ex neotype* isolate FI1029, *M. guizhouense* differed in 6 base positions in the ITS regions. Two per cent divergence in the ITS between *M. guizhouense* and isolate FI1029 falls within the scope of variation among *M.a. anisopliae* isolates (0-2.3%). According to Liu et al. (1986), conidial length in *M. guizhouense* measured 6.7-7.3 μm, while Driver et al. (2001) gave 5–8.0 μm as the range for conidial length in *M.a. anisopliae*.

Liu et al. (2001) suggested that *M. taii* and *M. guizhouense* were the same fungus based on their morphology and type of esterase isoenzyme. The ITS sequences of these two species are similar, differing in only two positions. Both species attack the larvae of Lepidoptera. Huang et al. (2005) have demonstrated that *M. taii* must be treated as a synonym of *M.a. anisopliae* based on its morphology and ITS sequence. We also consider that *Metarhizium guizhouense* is a synonym of *M.a. anisopliae*, based on morphological characters, ITS sequence and the conspecificity of *M. taii* and *M. guizhouense*.

*Metarhizium anisopliae* (Metsch.) Sorokīn var. *anisopliae*, [Plant Parasites of Man and Animals as Causes of Infectious Diseases] 2: 267 (1883) [in Russian].

**Synonym:** *Metarhizium pingshaense* Q.T. Chen & H.L. Guo, Acta Mycol. Sinica 5: 179 (1986) [in Chinese].

**Synonym:** *Metarhizium guizhouense* Q.T. Chen & H.L. Guo, Acta Mycol. Sinica 5: 181 (1986) [in Chinese].

**Synonym:** *Metarhizium taii* Z.Q. Liang & A.Y. Liu, Acta Mycol. Sinica 10: 260 (1991) [in Chinese].

***Metarhizium iadini* and identification of isolate RCEF1259**—According to Guo (1991), *M. iadini* is differentiated by its light green colonies in culture, its ovoid conidia with light plain base, (4-)5.7(-6)×(1.8-)2.2(-2.4)μm, and its coleopteran host, *Rhynchites coreanus*. *Metarhizium flavoviride* var. *pemphigi* (abbreviation: *M.f. pemphigi*) is similar, with ovoid to elongate conidia measuring 5.4×2.4 μm, a light green conidial mass, and a root aphid host. In our analysis (Fig. 1) the *ex type* isolate of *M. iadini*, *M.f. pemphigi* and strain RCEF1259 grouped together in a strongly supported clade. Among them there are 8 nucleotide differences in the ITS regions. *Metarhizium iadini* and *M.f. pemphigi* were distinguished by only small differences in host and growth rate at low temperature (the former reaches 15 mm, and the latter 40 mm at 10°C after 3 weeks). However, they share other characteristics such as conidial shape and size, and color of colony. Also, these two species display similar sequences in the ITS region. We conclude that *M. iadini* and *M.f. pemphigi* are conspecific. The former name is legitimate, but the lack of a Latin diagnosis makes it invalidly published (Art. 42). Accordingly, the isolate ACCC30124, originally identified as *M. iadini*, is properly identified as *M.f. pemphigi*.

Strain RCEF1259 was isolated from an entomogenous fungus specimen collected from Anhui Province in China. A synnemata formed on a Lepidoptera larva is 5 cm long, and green at the apex. Conidial size and shape are similar to those of *M.f. pemphigi*. This isolate formed a few light green conidia, and a floccular mycelium on PDA after 10 days. Because synnemata are unusual in *Metarhizium*, the isolate was included in this study to assess its relationships and identification. Compared to *M.f. pemphigi*, RCEF1259 differs

in 4 positions in ITS region, and also shows 3 deletions. We conclude that it should be recognized as *M.f. pemphigi* based on morphological and molecular data.

Before the current study, only two isolates of *M.f. pemphigi* had been recorded. Similarities among these newly recorded isolates provide evidence that it is reasonable for *M.f. pemphigi* to be recognized as a distinct variety. The light green color of the conidial masses is apparently an important identifying morphological character for this variety.

*Metarhizium flavoviride* var. *pemphigi* Driver & Milner, Mycol Res: 104: 144 (2000).
**Synonym:** *Metarhizium iadini* H.L. Guo, The Study and Application of *Metarhizium*: 27 (1991) [in Chinese].

**Identification of isolates ACCC30130 and RCEF0898**—Isolate ACCC30130 was identified as *M. flavoviride* var. *minus* in a catalogue of Chinese agricultural isolates (Guo & Ning, 1991). According to Liu et al.'s 1994 study, *M. pingshaense*, ACCC30130 and *M.a. anisopliae* shared similar 18S and 28S DNA sequences. However, the present study based on morphology and ITS sequences suggests that ACCC30130 should be attributed to *M.a. anisopliae*. The strain differs from the *M.a. anisopliae ex neotype* isolate by only 3 positions in the ITS regions.

The strain RCEF0898 differs by 6 nucleotides from the neotype strain of *M.a. anisopliae*; it falls into the same clade as most isolates of the latter (Fig. 1). The morphology of RCEF0898 is similar to *M.a. anisopliae* except that some large conidia measured up to 9×3.5 μm. Although *Metarhizium taii* has been reported to produce some large conidia, it was synonymized with *M.a. anisopliae* in a recent study (Huang et al. 2005). This small difference in conidial length is not considered taxonomically significant. Therefore, strain RCEF0898 is also identified as *M.a. anisopliae*.

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# The genus *Hymenochaete* (Basidiomycota, Hymenomycetes) in the Hawaiian Islands

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**Abstract** — Eighteen species of the genus *Hymenochaete* found in the Hawaiian Islands are described, including new species *H. flexosetosa, H. geniculata* and *H. legeri. H. attenuata* sensu G. Cunn., found in New Zealand, is different from *H. attenuata* (Lév.) Lév.; it is described as a new species *H. nothofagicola*. Main source of the origin of Hawaiian *Hymenochaete* biota seems to be Australasia.

**Key words** — New Zealand, distribution, spore variation

## Introduction

In his monograph of *Hymenochaete*, Burt (1918) did not mention any collections from the Hawaiian Islands nor did Cunningham (1957) in his paper on New Zealand and Australian "Thelephoraceae" or Léger (1998) in his world monograph of the genus *Hymenochaete*. Burt (1923) in a paper on higher fungi of the Hawaiian Islands mentioned three species: *H. tenuissima* Berk. (a synonym of *H. rheicolor)* was collected "on decaying wood, F.L. Stevens, 118, 967", but the specimens were not found by us in BPI or NY; *H. cinnamomea* (Pers.) Bres. and *H. spreta* Peck have been misidentified by Burt (see below under *H. semistupposa* and *H. tomentelloidea*). Gilbertson & Adaskaveg (1993) mentioned *H. anomala* and *H. corrugata* collected by them in 1990. Gilbertson & Hemmes (1997) described a new species, *H. tomentelloidea* from South Hilo District; Gilbertson et al. (2002) listed four species of *Hymenochaete (H. anomala, H. corrugata, H. mougeotii* and *H. tomentelloidea)* collected in Hawai'i, Kaua'i and Maui Islands in their check list of wood-rotting Basidiomycetes. Apparently no other data on Hawaiian *Hymenochaete* have been published.

## Materials and methods

In the National Fungus Collections (BPI), there are specimens of eight *Hymenochaete* species, mainly previously unidentified, collected by C.L. Shear and N.E. Stevens from Hawai'i. In the Herbarium of the New York Botanical Garden (NY), one specimen of *H. attenuata* collected by J. Kliejunas was found. Collections by R.L. Gilbertson, also by J.E. Adaskaveg and D.E. Hemmes from 1990 to 2002 contain 114 specimens of *Hymenochaete* reported in this paper. Collections are deposited in ARIZ and their duplicates in TAA. For comparison, some specimens collected by S.H. Wu in Taiwan and by the senior author in India (both in TAA) have also been examined.

Authors of fungal names follow Kirk & Ansell (1992). Herbarium acronyms are after Holmgren, Holmgren & Barnett (1990). Names of (phyto)geographical regions are given according to the Plant Taxonomic Database Standard No. 2, Edition 2 (Brummitt, 2001). Colour of the basidiomata of *Hymenochaete* species is very changeable during their development, and most descriptions published until now are saturated with different colour terms. To simplify the descriptions, colour names are given as used in "A mycological colour chart" by Rainer (1970). In parentheses, the additional and more precise colour notations are given according to Munsell, 1976 (M) and Kornerup & Wanscher, 1973 (K & W). In this genus, it is difficult to study spores due to their small measurements and Brownian movement when an ordinary microscope is used. That is why measurements and figures of spores were made with the aid of a Sony CCD Video Camera attached to a Nikon Labophot 2 microscope and analyzed by Global Lab Image (Data Translation Inc.) software. All descriptions given below are based on Hawaiian specimens; when these differ significantly from the descriptions published for other regions, the differences are indicated.

## Results and discussion

18 species of the genus *Hymenochaete* have been found in Hawaiian Islands. Only one of these *(H. tomentelloidea)* was definitely known until this study. The propagules of these fungi are small and easily transportable by winds (basidiospore length of most Hawaiian species is in the limits of 2.5–8 µm). Nevertheless, the number of species is rather low. For comparison, of the more than 120 species known, 32 have been found in New Zealand. The Hawaiian Islands have an isolated position far from mainlands. Their age – about 0.5–5 mln years (Carson & Clague, 1995) – is perhaps rather short time for invasion of *Hymenochaete* species from Americas, Asia or Australasia. Only two or three species are of pantropical distribution *(H. attenuata, H. unicolor* and possibly also *H. legeri).* Three species are common with South or Central America *(H. berteroi, H. cervina, H. minuscula),* but these have also been found in Australasia. Six Hawaiian species are common with New Zealand (*H. dissimilis, H. innexa, H. semistupposa, H. separata, H. unicolor, H. vaginata*). However, there are almost no data on distribution of *Hymenochaete* species in Papuasia and Southwestern Pacific, i.e., in the regions between Australasia and Hawaiian Islands. One species *(H. muroiana)* has been found in Eastern Asia (Japan, China) and Malesia, and one *(H. separabilis)* in Tropical Africa. According to the currently known distribution, the main source of the origin of Hawaiian *Hymenochaete* biota seems to be Australasia.

The interval of time up to five million years was long enough to cause intensive speciation of plants and insects; about 45% of mosses and 42% of angiosperms are endemic (Eldredge & Evenhuis, 2003). Speciation is possibly more slow in fungi than in other groups of living beings. In Hawai'i, the percentage of endemic fungi (lichenized species excluded) is about 13 (Eldredge & Evenhuis, 2003). Among *Hymenochaete*, four species are endemic: *H. flexosetosa* (a species possibly closely related to pantropical *H. attenuata* and *H. rheicolor* (Mont.) Lév.), *H. geniculata* (closely related to the New Zealand's *H. stratura* G. Cunn.), *H. subdissimilis* ad int. (if different from *H. dissimilis)* and *H. tomentelloidea* (closely related to *H. separabilis).* All these endemic taxa are closely related to their siblings.

Of the 18 species found, only one is common in the Hawaiian Islands *(H. legeri),* two occur occasionally *(H. attenuata* and *H. semistupposa),* all other species are very rare or found only once.

## Taxonomy

### Key to Hawaiian *Hymenochaete* species

(Some other species, common in Tropical Asia and Australasia are included)

1. Basidiome pileate or effused-reflexed ..... 2
1. Basidiome effused (resupinate) ..... 6
2. Setae 20–50 x 4–8 µm ..... 3
2. Setae 45–110 x 7–15 µm ..... 5
3. Cortex present; setae 30–50 x 5–8 µm; pileus flexible ..... 4
3. Cortex absent; setae 20–35 x 4–5 µm; pileus brittle .... ***H. adusta*** (Lév.) Har. & Pat.
(Found in Japan, Philippines, Vietnam, Australasia)
4. Numerous brown thick-walled hyphidia in hymenium, 2.5–3.5 µm in diam; basidiome up to 500 µm thick but tomentum thin (up to 150 µm)
***H. luteobadia*** (Fr.: Fr.) Höhn. & Litsch.
(Found in Philippines, Vietnam, Australasia)
4. Thick-walled hyphidia absent; tomentum up to 1000 µm thick, later disappearing and then pileal surface black..... ***H. villosa*** (Lév.) Bres.
(Pantropical. Figs. 1, *1;* 2, *3)*
5. Part of the setae L-shaped, arising from a short thin setal hypha, 40–70 x 7–12 µm; spores (4.8–)5–5.8(–6.2) x 3-3.5(–3.7) µm ..... **5. *H. flexosetosa***
5. All setae "normal", 45–70(–80) x 7–12 µm, spores 3.5–5(–5.5) x 1.7–2.2 µm; hyphae of the context above the hymenium densely compacted in parallel1. ***H. attenuata***
6. Setae in apical part with small thorns or teeth (visible at high magnification) .... 7
6. Setae without thorns ..... 8
7. Setae 30–45 x 5–7(–8) µm, with few (1–3) low broadly conical teeth or protuberances up to 0.5(–1) µm long..... **13. *H. separabilis***
7. Setae (35–)40–70 x 5–7 µm, with 5–10 broadly conical teeth 0.8-1.5(-2) µm long ..... **16. *H. tomentelloidea***
8. Brown or brownish hyphidia 2–4 µm in diam with thickened or thick walls present, but never monilioid ..... 9

8. Hyphidia absent, or monilioid brown hyphidia or subhymenial hyphae present 12
9. Hyphidia simple, not dendroid or arbusculoid ..... 10
9. Hyphidia dendroidly branched..... **6. *H. floridea***
10. Spores broad, (5.7–)6–7 x (4.0–)4.3–5(–5.2) µm; basidiomata thick (up to 500–1100 µm) ..... **3. *H. cervina***
10. Spores 1.8–3.2 µm broad; basidiomata very thin or up to 300 µm thick ..... 11
11. Spores cylindrical, 5.5–7(–7.5) x 1.8–2.5 µm; basidiomata 50–100 µm thick..... ..... **8. *H. innexa***
11. Spores ellipsoidal, 6.5–9.5 x 2.4–3.2 µm; basidiomata up to 300 µm thick ..... ..... **18. *H. vaginata***
12. Many hyphae or hyphidia monilioid, with thickenings on walls; context soft membranaceous, of loosely interwoven hyphae ..... 13
12. Hyphae and hyphidia (when present) not monilioid; context absent or its hyphae densely packed ..... 14
13. Setal hyphae present in context; spores 3.7–4.8 x 2.2–2.7 µm ..... **2. *H. berteroi***
13. Setal hyphae absent; spores 3.2–4.5 x 1.7–2.2 µm ..... **12. *H. semistupposa***
14. Setae large, (60–)75–100(–120) x 10–15 µm; spores 6–8 µm long .... **9. *H. legeri***
14. Setae smaller, 30–80 x 4–9 µm; spores 3.5–6.5 µm long ..... 15
15. Numerous conglomerates of crystals 8–50 µm in diam present in the context (setal or hyphal layer) ..... 16
15. Conglomerates of crystals absent, crystals in hymenium sometimes present ... 17
16. Setae 40–60(–65) x 7–10 µm; hyphal layer 30–150 µm thick,indistinctly different from the setal layer; spores 3.7–4.4(–5.0) x 1.8–2.5 µm ..... **15. *H. subdissimilis***
16. Setae smaller, (30–)35–45(–55) x 6–7(–8) µm; hyphal layer absent; spores ellipsoid, 5–6.5 x 3–3.5 µm ..... **14. *H. separata***
17. Setae partly angled (geniculate) near the base, narrowly fusoid, (35–)40–60 x 4–7 (–8) µm; setal hyphae (embedded horizontal setae) present .... **7. *H. geniculata***
17. Setae never geniculate; setal hyphae absent ..... 18
18. Setae partly bifurcate at base, with slightly curved tip, (30–)40–50(–60) x (5–)6–8 (–9) µm; spores 4–6 x (2.5–)3–3.5 µm; on bamboo ..... **11. *H. muroiana***
18. Setae never with bifurcate base; spores 2.2–3.2 µm broad; on other substrata .. 19
19. Setae straight, (35–)45–80 x 5–9 µm; spores ellipsoid, 4.2–5.4 x 2.5–3.2 µm ..... ..... **4. *H. dissimilis***
19. Setae smaller, straight, slightly curved or undulate, (30–)35–60(–65) x 4–7 µm; spores almost subcylindrical ..... 20
20. Spores 4.2–5.2 x 2.2–2.5 µm; hymenium ochraceous or yellowish brown ..... ..... **10. *H. minuscula***
20. Spores 3.4–4.8 x 2.2–2.8 µm; hymenium umber with fulvous, Sienna or grayish tint ..... **17. *H. unicolor***

## Annotated list of species

**1. HYMENOCHAETE ATTENUATA** (Lév.) Lév., Ann. Sci. Nat. Bot. III 5: 152. 1846 **not sensu** G. Cunn., 1957. Fig. 1, *6*; 2, *9*

**Basidiomata** annual (?), pileate or seemingly effused-reflexed (then umbonate), closely or loosely agnate, soft, papery-coriaceous and flexible when dry; pilei flabelliform to dimidiate, 0.5–2 cm long, 200–500 µm thick, usually imbricate or in rosettes, confluent (up to 5 cm wide); pileal surface velutinous or tomentose when young or near margin, then densely concentrically sulcate and zonate, coarsely radiately fibrillate (silky-fibrous), dull or shiny, Sienna to Umber or dark Cinnamon (M: 7.5 YR 5/8–7, when old 5 YR 4/6; K & W: 6 D 6–8, when old 6 E 6); margin thin, lobate, sometimes plicate, concolorous with the pileal surface or lighter coloured (when young); hymenium smooth, in very old specimens sometimes with low radial folds, not cracked, dark Sienna to light Umber or dark Cinnamon (M: 7.5 YR 6/8, when old 7.5 YR 5/6; K & W: 6 D 5–6 or 5 C 5).

Tomentum present as a layer of loosely interwoven hyphae or as hyphal tufts, (50)–100–450 µm thick; cortex absent; hyphal layer composed of loosely almost radiately interwoven hyphae in upper part and an indistinctly delimited layer 100–150(–250) µm thick of hyphae more densely arranged in parallel above hymenial layer; setal layer present in older specimens when setae are in two indistinct rows.

Hyphal system monomitic; hyphae with thickened walls (in tomentum thick-walled), brownish, sparsely branched, with septa, in hyphal layer 2.5–3.5 µm, in tomentum 4–5 µm in diam; setae sparsely situated or locally numerous, narrowly conical or slightly fusoid, straight or some with curved tip, bluntly acute, (35–)40–70(–80) x 7–12(–14) µm, emerging 20–45 µm above the hymenium, with a thin hyphal sheath, without incrustation; basidioles with thickened and brownish at base rough (finely encrusted) walls, 18–25 x 3–4.5 µm; basidia 20–25 x 3.5–4 µm, with 4 thin sterigmata; spores short cylindrical with one side slightly concave (suballantoid), 3.5–5(–5.5) x (1.5–) 1.6–2.2 µm.

HAWAI'I. J. Kliejunas 18a, det. G.A. Escobar, Kahalu'u Forest Preserve, Kona (NY); RLG 17007, 18112 on *Metrosideros polymorpha,* Mile 18, Saddle Rd. KAUA'I. RLG 21081, 21082, 21123 on *Acacia koa,* Nualolo Trail, Koke'e State Park; RLG 20558, 21063, 21067, 22745A on *Eucalyptus* sp., RLG 22735, 22743 on *Eucalyptus globulus,* Makaha Rd.; RLG 21182, on *Psidium guajava,* Ohelo Berry Flat Trail, Koke'e State Park. MOLOKA'I. RLG 19253 on *Acacia mearnsii* sp., Kamakou Rd.

**Comments.** This species belongs to the *H. rheicolor*-group of sibling species; their difference is not always clear, and most of the herbarium specimens including types of all species and their synonyms have very few or no spores.

Bresadola has synonymized *H. attenuata* with *H. rheicolor* in one of his later papers (1915: 301) but the two species are considered distinct by other authors. According to Léger (1998), who has studied the types of both species, the main difference is in size of setae and spores: 45–75 x 6–9(–11) µm and 3.5–4.5(–5.5) x 1.5–2(–2.2) µm in *H. attenuata,* 60–105(–120) x (8–)9–12(–13) µm and 5–6 x 1.8–2.5 µm in *H. rheicolor.* Tomentum is well developed in *H. attenuata,* thin and formed of less differentiated hyphal tufts in *H. rheicolor.* We have studied specimens of both species collected in Argentina, Brazil, China (Yunnan), Dominica, Guatemala, Guyana, India, Mexico,

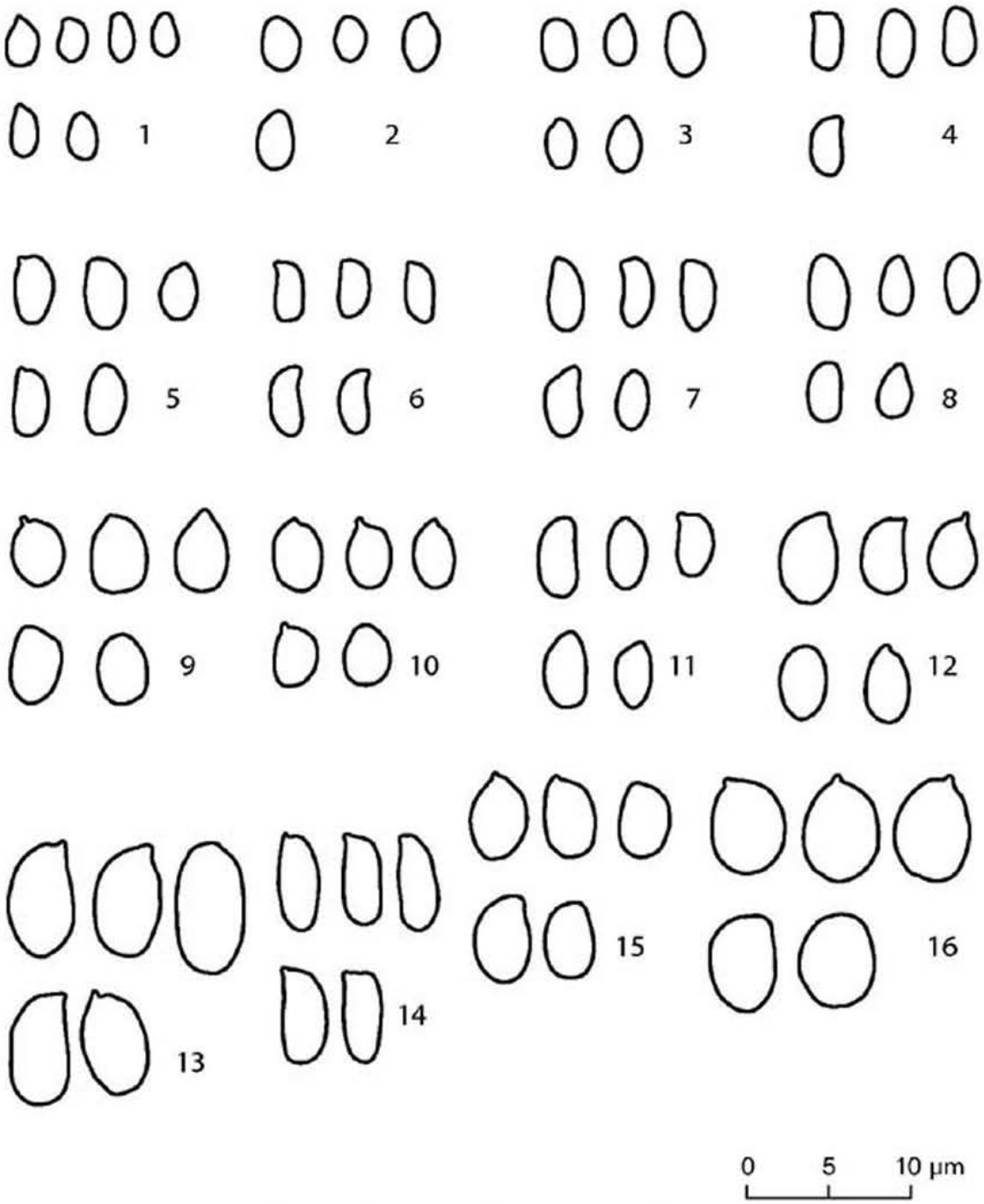


**Fig. 1.** Spores: *1 – Hymenochaete villosa, 2 – H. berteroi, 3 – H. subdissimilis, 4 – H. semistupposa, 5 – H. minuscula, 6 – H. attenuata, 7 – H. tomentelloidea, 8 – H. separabilis, 9 – H. geniculata, 10 – H. dissimilis, 11 – H. lignosa, 12 – H. flexosetosa, 13 – H. nothofagicola, 14 – H. legeri, 15 – H. muroiana, 16 – H. cervina*

Puerto Rico and Thailand, and found the variability of setal size to be much broader and sometimes overlapping in these species. Spore size is similar in the two species. We have seen only two specimens of "typical" *H. rheicolor* with spores bigger than in *H. attenuata* (see table below), and this difference is statistically insignificant when compared with spores of *H. attenuata*. However, most specimens of both species in various herbaria are sterile, and in the fertile specimens spores are usually partly damaged.

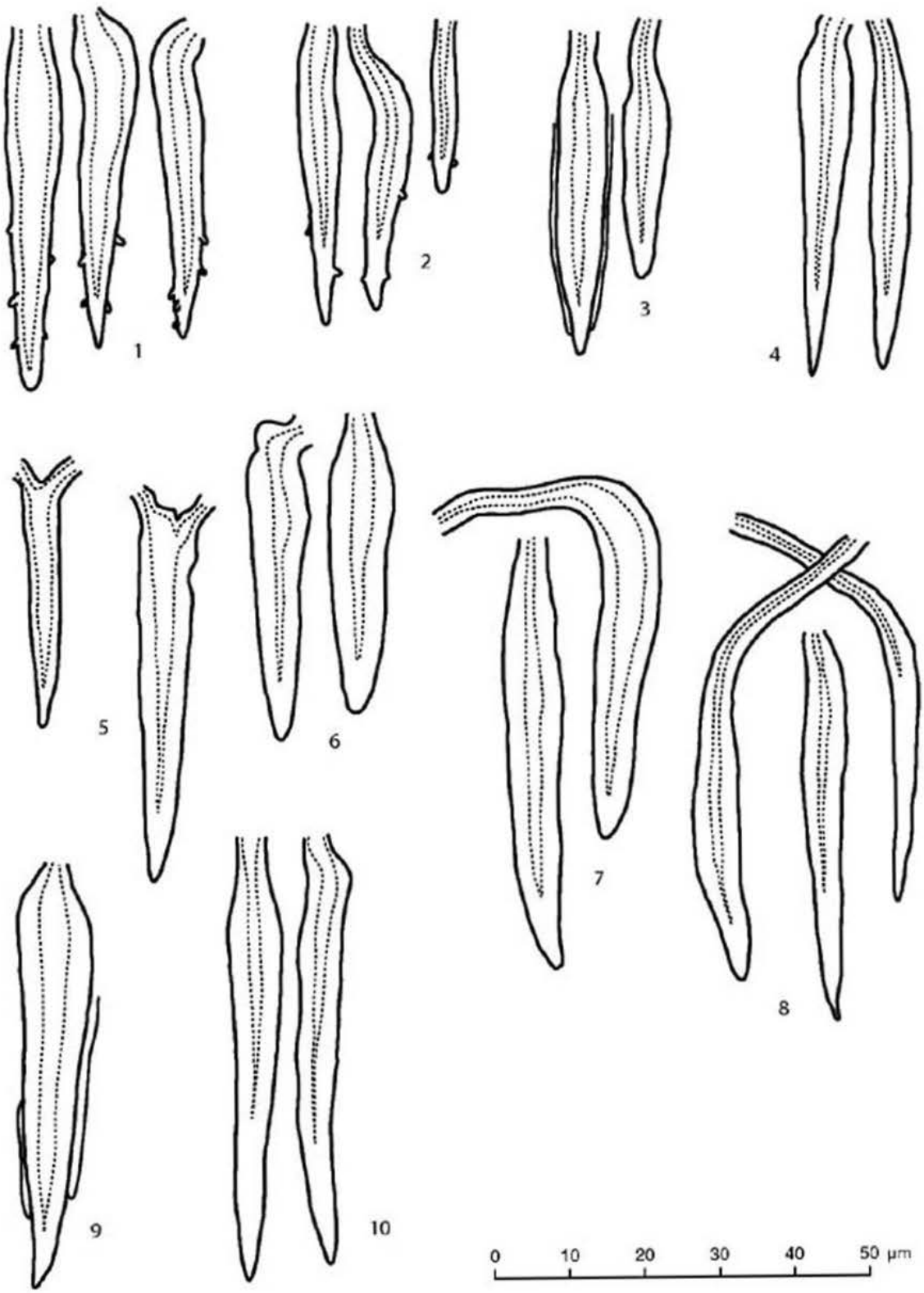


**Fig. 2.** Setae: *1 – Hymenochaete tomentelloidea, 2 – H. separabilis, 3 – H. villosa, 4 – H. lignosa, 5 – H. muroiana, 6 – H. subdissimilis, 7 – H. flexosetosa, 8 – H. geniculata, 9 – H. attenuata, 10 – H. minuscula*

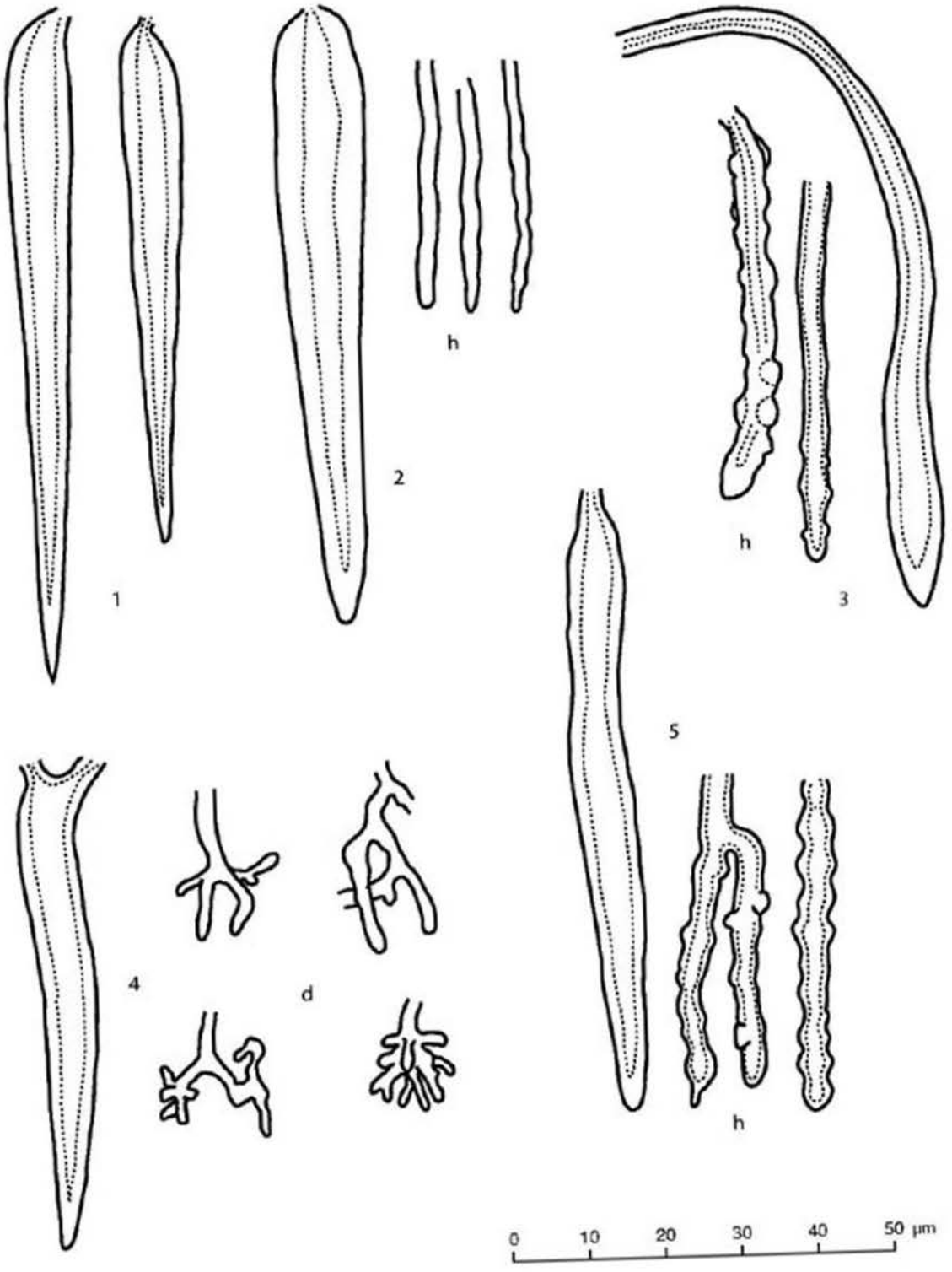


**Fig. 3.** Setae and hyphidia: *1 – Hymenochaete dissimilis, 2 – H. innexa* (*h* – hyphidia), *3 – H. berteroi* (*h* – monilioid hyphidia), *4 – H. floridea* (*d* – dendrohyphidia), *5 – H. semistupposa* (*h* – monilioid hyphidia)

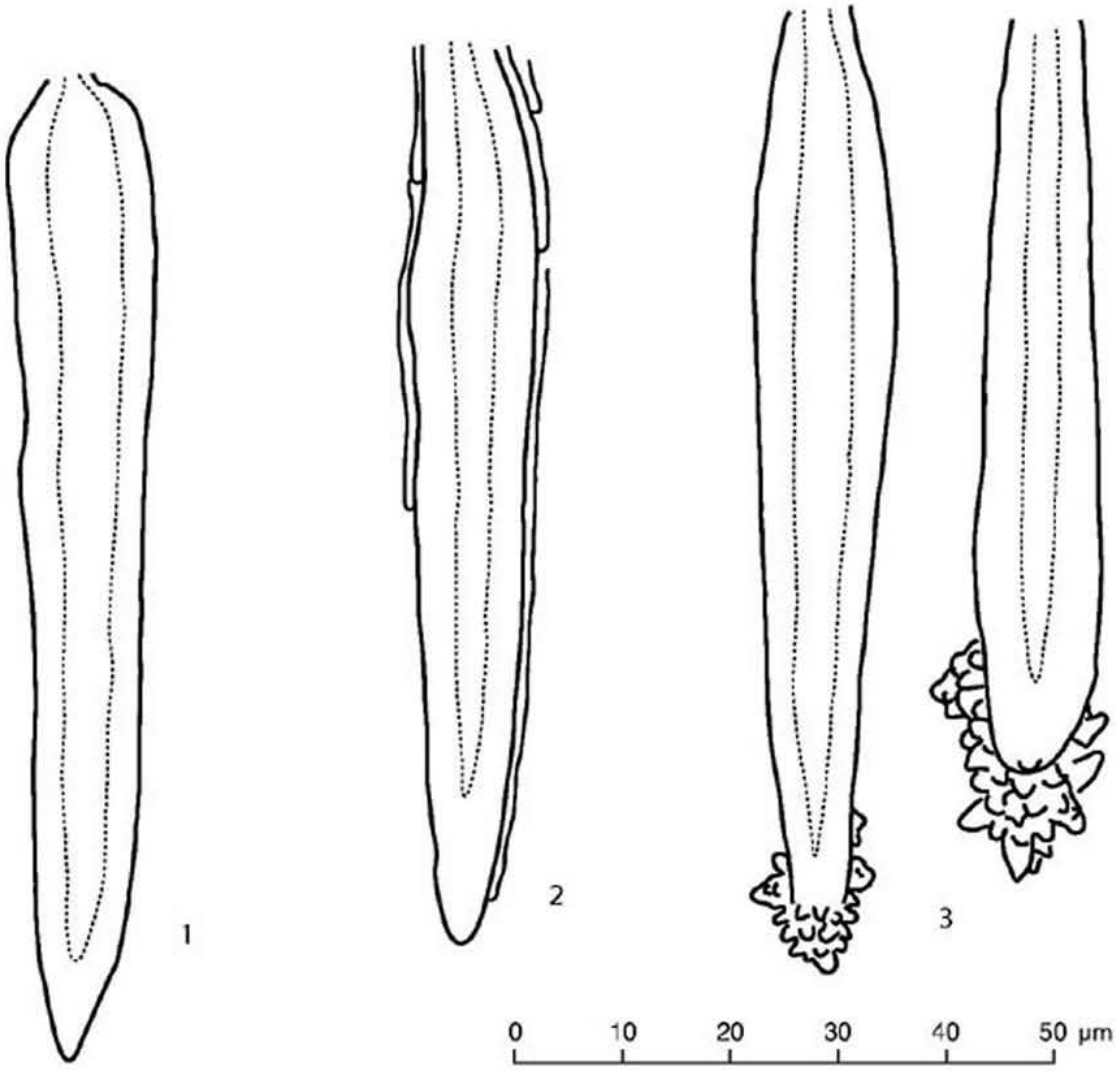


**Fig. 4.** Setae: *1 – Hymenochaete cervina, 2 – H. nothofagicola, 3 – H. legeri*

One of the additional characters of *H. attenuata* is differentiation of a hyphal layer near the hymenium. Hyphae of this layer are densely compacted in parallel, whereas in *H. rheicolor* they are interwoven.

It is possible to distinguish "typical" specimens of *H. attenuata* and *H. rheicolor*, but there are several "untypical" specimens with overlapping characters in herbaria, and the independence of these two species is doubtful.

Mean spore size and mean *Q* value in Hawaiian specimens of *H. attenuata* (*Q* value is the ratio of mean spore length to mean spore width):

| | | |
|---|---|---|
| 3.72 x 1.90 | 1.95 | RLG 21082 |
| 4.05 x 1.79 | 2.26 | RLG 22743 |
| 4.09 x 1.95 | 2.10 | RLG 21067 |
| 4.47 x 2.01 | 2.22 | RLG 19253 |
| 4.48 x 1.88 | 2.38 | RLG 17007 |
| 4.69 x 1.93 | 2.43 | RLG 21081 |
| 4.96 x 1.90 | 2.61 | RLG 18112 |

For comparison, the mean spore size and mean Q value in other specimens studied:

| | | | |
|---|---|---|---|
| *H. rheicolor* | 4.27 x 1.94 | 2.20 | Mexico, BPI 278538 |
| *H. attenuata* | 4.34 x 1.85 | 2.35 | Chile, BPI 330072 |
| *H. attenuata* | 4.34 x 1.86 | 2.35 | Chile, BPI 33758 |
| *H. attenuata* | 4.54 x 2.00 | 2.27 | India, TAA 103213 |
| *H. rheicolor* | 4.75 x 2.09 | 2.27 | Guyana, BPI Lloyd 6734 |
| *H. rheicolor* | 4.80 x 1.93 | 2.49 | Mexico, TN 18397 |
| *H. rheicolor* | 4.85 x 1.94 | 2.50 | Mexico, XAL 1588 |
| *H. rheicolor* | 4.87 x 2.13 | 2.29 | Mexico, TN 18369 |
| *H. attenuata* | 5.05 x 2.01 | 2.51 | Thailand, O 18025 |
| *H. rheicolor* | 5.17 x 2.10 | 2.46 | Dominica, BPI 297738 |
| *H. rheicolor* | 5.33 x 1.99 | 2.68 | Puerto Rico, BPI 278545 |

*H. attenuata* sensu G. Cunn. found in New Zealand on dead twigs of *Nothofagus* spp. is a different species characterized with setae (55–)65–100 x (6–) 7–11(–14) µm, broadly ellipsoid or subcylindric spores with one side flattened, (5.5–) 6.0–8.0(–8.5) x (3–)3.5–4.5(–5.0) µm. Sparse dark brown thick-walled hyphidia 2.5–3 µm in diam are present in the hymenium. The New Zealand species will be described as a new one *(H. nothofagicola)* in the Appendix of this paper. Description of *H. attenuata* by Job (1991: 7–8) combines the data on *H. attenuata, H. attenuata* sensu G. Cunn. and a species collected in Switzerland and described by Job and Keller (1988).

**2. HYMENOCHAETE BERTEROI** Pat., Bull. Soc. Mycol. France 10: 78. 1894.

**Description:** see Parmasto, 2005. **Figs. 1,** *2*; **3,** *3*

O'AHU. C.L. SHEAR 113 (BPI 1100595), on *Acacia koa*, Pupukea Forest Reserve, 31 Jan (1928?).

**Comments.** Closely related to *H. semistupposa*, which differs by absence of setal hyphae and having spores 3.2–4.5 x 1.7–2.2 µm.

**3. HYMENOCHAETE CERVINA** Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10: 334. 1868.

**Figs. 1,** *16*; **4,** *1*

**Basidiomata** perennial, effused, adnate, woody hard, up to 500–1100 µm thick, as rounded patches up to 5 cm or more in length. Hymenium even, umber (M: 5–7.5 YR 4/6 to 5/4; K & W: 7 E 4 – 7 F 5); margin abrupt.

Cortex present but not easily distinguishable, usually observable as a dark line near the substrate; hyphal layer absent; setal layer very thick; dark line above the hymenium absent.

Hyphal system subdimitic; (pseudo)skeletal hyphae ascending or vertically interwoven, densely agglutinated, with thick walls, brown, sparsely branched, 3–5 µm in diam; generative hyphae innumerous, thin-walled, branched, subhyaline, 3–5 µm in diam; setae numerous, narrowly conical or spine-like, at base arising from a dense node of thick-walled hyphae, straight, 70–100(–120) x 7.5–12(–14) µm, emerging up to 40 µm above the hymenium, with a hyphal sheath which is finely encrusted in old setae; cystidia absent; hyphidia scattered, cylindrical, with brown thickened walls, in upper part sometimes slightly sinuous, 2.5–3.5 µm in diam; basidioles present, thin-walled; basidia clavate or slightly subutriform, 20–25(–30) x 7–8.5 µm, with 4 thin sterigmata

about 4 µm long; spores broadly ellipsoid, with one side slightly flattened, mostly with a large guttule, (5.7–)6–7 x (4.0–)4.3–5(–5.2) µm.

Causes a fibrous white rot of wood.

KAUA'I. RLG 20644 on *Cryptomeria japonica*, Lehua puhi Trail, Koke'e State Park.

**Comments.** This Hawaiian specimen is typical for *H. cervina*. Presence of crystalline matter in the setal layer indicated for this species by Léger (1998: 86) is a variable character in *H. cervina*. Spores have rarely been observed in specimens kept in world herbaria but the Hawaiian collection has numerous spores. Their mean size is 6.39 x 4.60 µm and the length/width quotient is 1.39 (average of 22 spores measured). *H. cervina* has been found in Australasia (New Zealand), South America, Caribbean and North America. Data on occurrence in East Tropical Africa (Uganda) are doubtful.

**4. HYMENOCHAETE DISSIMILIS** G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 44. 1957. Figs. 1, *10*; 3, *1*

**Basidiomata** perennial, effused, closely adnate, hard when dry, up to 1500 µm thick; hymenium smooth or tuberculous, deeply cracked, when young grayish Cinnamon (M: 5.5 YR 5/6; K & W: 6 D 4), then dark Vinaceous Buff (M: 7.5 YR 5.5/4; K & W: dark 6 D 3); margin indistinct, then abrupt.

Tomentum and cortex absent, hyphal layer thin and soon disappearing; context composed of thickening, indistinctly stratose setal layer.

Hyphal system monomitic; hyphae densely agglutinated with brown resinous matter, 2.5–4.5 µm in diam, brownish to brown, thick-walled, septate, branched; in context and hymenium crystalline matter usually present; setae numerous, of variable size, (35–)45–80 x 5–9 µm, projecting to 50 µm above hymenium, subulate, with acute tip, straight, usually with a thin hyphal sheath, without incrustation; cystidia and hyphidia absent; basidioles with thin walls at base slightly thickened and yellowish, 18–25 x 3.5–4.5 µm; basidia subutriform, (15–)18–22(–25) x 4–5 µm, with 4 sterigmata about 4 µm long; spores ellipsoid, with one side flattened, 4.2–5.4 x 2.5–3.2 µm.

HAWAI'I. RLG 17624, 17741 on *Acacia koa*, Keanakolu Rd.

**Comments.** This species has been described from New Zealand and found later only in Réunion. All three specimens collected on Hawai'i differ from the previously published descriptions by lack of crystalline masses in the setal layer. We do not consider this character to be a taxonomically important one.

*H. dissimilis* is externally very similar to *H. unicolor*. Both may have very thick cracked basidiomata. The last named species differs in having smaller setae (30–)35–50 x 5–7 µm and spores 3.4–4.8 x 2.2–2.8 µm.

Mean spore size and mean *Q* value of *H. dissimilis*:

| | | |
|---|---|---|
| 4.66 x 2.81 | 1.66 | RLG 17624 |
| 5.03 x 2.90 | 1.74 | RLG 17741 |

**5. HYMENOCHAETE FLEXOSETOSA** Parmasto, sp. nova Figs. 1, *12*; 2, *7*

*Basidiomata annua, pileata, flabelliformes, imbricata, ad 1 cm longa et 450 µm crassa. Tomentum et stratum hypharum adsunt, cortex et textus setarum desunt. Systema hypharum monomiticum; hyphae tunicis incrassatis, ramosae; setae subulatae, 40–70*

*x 7–12 µm, subacutae, sine incrustatione, setae nonnullae in parte basale ex hyphis setoideis breves 20–40(–60) x 3–5 µm adscedentia; cystidia et hyphidia desunt; basidioli tunicis incrassatis basim tenuiter granulosis; basidia 20–25 x 4–5 µm; basidiosporae late ellipsoideae, (4.8–)5–5.8(–6.2) x 3–3.5(–3.7) µm.* A H. attenuata *et* H. rheicolore *hyphis setoideis praesentis atque sporis latis differt.*

**Holotypus:** Hawai'i, on *Psidium guajava,* R.L. Gilbertson 18932, Honokaia Boy Scout Camp (ARIZ; isotypus: TAA).

**Etymology:** *flexuosus,* flexuous; *seta,* seta.

**Basidiomata** annual, pileate, soft, papery-coriaceous and flexible when dry; pilei flabelliform, 0.3–1 cm long, 200–450 µm thick, imbricate, sometimes seemingly effused-reflexed but then umbonate (attached in one point); pileal surface velutinous or tomentose, concentrically sulcate and zonate, coarsely radiately fibrillate (silky-fibrous), dull Fulvous Umber (M: 7.5 YR 4/6; K & W: 6 D 5); margin thin, lobate, concolorous with the pileal surface or lighter coloured; hymenium smooth, not cracked, grayish Fulvous (M: 7.5 YR 5/6; K & W: 6 D 4).

Tomentum present as loosely interwoven hyphae, 80–200 µm thick; cortex absent; hyphal layer composed of radiately interwoven generative hyphae and few short setal hyphae bending to setae; setal layer absent.

Hyphal system monomitic; hyphae with thickened walls, brownish (in tomentum thick-walled and brown), sparsely branched, with septa, in hyphal layer 2.5–3.5 µm, in tomentum 3.5–5 µm in diam; setae innumerous or numerous, subulate, straight, bluntly acute, 40–70 x 7–12 µm, emerging 10–25 µm above the hymenium, with a thin hyphal sheath, without incrustation; some setae are L-shaped, originating in the context from a short setal hypha like horizontal part 20–40(–60) µm long and 3–5 µm in diam; this continues as a usual thick-walled generative hypha; basidioles with thickened and brownish base with rough (finely encrusted) walls, 25–30 x 3.5–4 µm; basidia 20–25 x 4–5 µm, with 4 thin sterigmata; spores broadly ellipsoid, with one side flattened, (4.8–)5–5.8(–6.2) x 3–3.5(–3.7) µm.

Causes white fibrous rot of wood.

HAWAI'I. RLG 18932 on *Psidium guajava,* Honokaia Boy Scout Camp.

**Comments.** This new species is externally very similar to *H. attenuata* and *H. rheicolor* which differ in spore form and width (1.7–2.3 µm) and absence of setal hypha-like base of L-shaped setae characteristic for *H. flexosetosa.* Mean size of spores of the type of *H. flexosetosa* is 5.40 x 3.21 µm; $Q = 1.68$.

An almost identical specimen has been collected 18 Sep 1956 by S. Ahmad from Pakistan on branches of *Viburnum* sp. and identified by him as *H. rheicolor* (LY 2284). It is sterile, with no spores.

**6. HYMENOCHAETE FLORIDEA** Berk. & Broome, J. Linn. Soc., Bot. 14: 68. 1873.

Fig. 3, *4*

**Basidiomata** effused, closely adnate, 100–200 µm thick (up to 500 µm according to Léger, 1998: 140); hymenium smooth, usually not cracked, bright Sienna (or reddish brown) (M: 5 YR 5-6/8; K & W: 7 (C–D) 7; specimens described by Léger M: 5 YR 4/3–4); margin very narrow.

Tomentum and cortex absent; hyphal layer present, of densely interwoven hyphae.

Hyphal system monomitic; hyphae interwoven, distinct, 2.5–4.5 µm in diam, brown, with thick walls, septate, branched; setae numerous, (55–)60–85(–100) x 6–9(–10) µm, broadly subulate, with bluntly acute tip, straight, usually with a thin hyphal sheath, some encrusted with granules, projecting to 40 µm above the hymenium, at base usually surrounded with arbuscular hyphidia; cystidia absent; hyphidia very numerous, with brown thick-walled stem 3–4 µm in diam, in subhymenium and hymenium dendroid, repeatedly branched, in setal layer very numerous head-like brown arbusculi 10–25 µm in diam with very numerous short side branches about 1 µm in diam; basidia not seen but according to Léger (1998: 140) 15 x 4 µ; spores cylindrical or ellipsoid, 6–7 x 2.5–2.8 µm.

HAWAI'I. RLG 19000: on *Metrosideros polymorpha*, Thurston Lava Tube, Hawai'i Volcanoes Nat. Park (HVNP).

**Comments.** This is the only *Hymenochaete* species with heavily branched dendrohyphidia in Hawai'i. Somewhat similar is *H. sphaericola* Lloyd (*H. cruenta* (Pers.: Fr.) Donk *p.p.*), a species widely distributed in East and South-East Asia and Australasia that has dendrohyphidia with innumerous long (5–25 µm) branches never forming arbusculoid heads. *H. floridea* has Asia Tropical–North-Central Pacific distribution.

### 7. HYMENOCHAETE GENICULATA Parmasto, sp. nova Figs. 1, 9; 2, 8

*Basidiomata perennia, effusa, coriacea, 100–250, deinde ad 500 µm crassa. Hymenium laeve vel leviter colliculosum, atro-ochraceum, vetustus crevisum. Tomentum et cortex adsunt; stratum hypharum crassum, ad stratum setarum indistinctum transiens, parte basale cum setis adscedentis atque hyphae setales vel setae hyphales nonnullis ad 80 x 6 µm. Systema hypharum subdimiticum; hyphae tunicis incrassatis brunneis, leviter ramosae non densiter intermixtae; setae numerosae, anguste fusoideae, 40–70 x 7–12 µm, partim breve stipitatae, aliae basim geniculate arcuatae, acutae, sine incrustatione; basidioli numerosi, subhyalini; basidia cylindraceo-clavati, 20–30 x 4–5 µm; basidiosporae breve cylindraceae vel subellipsoideae, 4.5–6 x 2.3–3(–3.3) µm. Species similis H. stratura stratis hypharum hyphis paralleliter densiter contextis atque sporis minutis 3.5–4 x 2–2.5 µm differt.*

**Holotypus**: Kaua'i, Koke'e St. Park, on *Metrosideros polymorpha*, 5 Jan 2000, R.L. Gilbertson 22797 (ARIZ; isotypus: TAA).

**Etymology**: *geniculatus*, (setae) bent like a bent knee.

**Basidiomata** perennial, effused, adnate, when old detachable as small pieces, coriaceous, 100–250, later up to 500 µm thick, as rounded patches, then confluent and up to 10 cm long. Hymenium even or slightly tuberculose, yellowish brown, bronze brown or dark ochraceous with an isabelline tint (M: 10 YR 5/8, 10 YR 5/6, when old umber – 7.5 YR 4/4); K & W: 5 (D–E) 5, when old 6 F 6), later with a few deep crevices; margin indistinct and more lightly coloured, then abrupt.

Tomentum and cortex absent; hyphal layer thick, in lower part with scattered setae (with gradual transition to the indistinct setal layer), with a few horizontal setae or short setal hyphae, in old specimens more dense and may be called setal layer.

Hyphal system subdimitic; hyphae in the hyphal layer almost loosely interwoven, with thickened brownish walls (some hyphae thin-walled and subhyaline), sparsely

branched, with septa, 2.5–3.5(–4) µm in diam; setae numerous, narrowly fusoid, (35–)40–60 x 4–7(–8) µm, partly with a slightly sinuous thick-walled stipe, others angled (geniculate) near the base, with an acute tip, naked or covered with a thin hyphal sheath, projecting up to 20 µm above the hymenium, setae embedded near to the substrate ascending and up to 75 µm long; setal hyphae (embedded horizontal setae) 40–80 µm long, 3.5–6 µm in diam; basidioles numerous, subhyaline, 20–30 x 3.5–4.5 µm; basidia cylindrical-clavate, 20–30 x 4–5 µm, with 4 sterigmata 2.5–3.5 µm long; spores short-cylindrical or almost ellipsoid, some with one side concave, 4.5–6 x 2.3–3(–3.3) µm.

Causes fibrous white rot of wood.

HAWAI'I. RLG 20478, on *Coprosma montana*, Kipuka Puaulu, HVNP; RLG 17378, 22365 on *Sapindus saponaria*, Kipuka Ki, HVNP. KAUA'I. RLG. 22797 (holotype), on *Metrosideros polymorpha*, Lehua Puhi Trail, Koke'e State Park.

**Comments.** The species is possibly closely related to the *H. stratura* G. Cunn., found twice in New Zealand on wood of *Podocarpus* species; *H. stratura* differs in having a hyphal layer composed of densely parallel hyphae and small spores 3.5–4 x 2–2.5 µm.

Mean spore size and mean *Q* value of *H. geniculata:*

| | | |
|---|---|---|
| 4.98 x 2.81 | 1.77 | RLG 22797 |
| 5.30 x 2.75 | 1.93 | RLG 17378 |
| 5.55 x 2.55 | 2.17 | RLG 22365 |

**8. HYMENOCHAETE INNEXA** G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 47. 1957.

Fig. 3, *2*

**Basidiomata** perennial, effused, closely adnate, hard when dry, 50–100 µm thick; hymenium smooth, not cracked, light Umber (M: 5 YR 5/4; K & W: 6 D 4; New Zealand specimens described by Léger (1998: 165) as reddish brown, M: 5 YR 4/4); margin very narrow, thin, arachnoid-fibrillose, Sienna (M: 7.5 YR 6/6), then indistinct.

Tomentum, cortex and hyphal layer absent.

Hyphal system monomitic; hyphae interwoven, distinct but partly agglutinated with brown resinous matter, 2–3 µm in diam, brownish, with thickened walls, septate, branched; setae numerous, 50–85 x (7–)8–13(–14) µm, broadly subulate, with bluntly acute tip, straight, usually with a thin hyphal sheath, some encrusted with granules, projecting to 40 µm above the hymenium; cystidia absent; hyphidia with brown thickened walls (but when new basidia are developing, then hyaline or absent), some slightly sinuous, 2–3 µm in diam; basidia subutriform, with slightly thickened walls at base, 15–20 x 4–5 µm, with 4 thin sterigmata; spores cylindrical, straight or slightly curved, 5.5–7(–7.5) x 1.8–2.5 µm.

Causes white pocket rot of wood.

O'AHU. RLG 18411 on *Eucalyptus* sp., Manoa Falls Trail.

**Comments.** There is only one other *Hymenochaete* species with non-monilioid brown hyphidia in Hawai'i, *H. cervina,* but it has broadly ellipsoid spores (5.7–)6–7 x (4.0–)4.3–5(–5.2) µm. *H. innexa* has been previously found in China, Japan, India, Australia and New Zealand; isotypes in BPI studied by E. P. The Hawaiian specimen has only few spores; mean size of 11 spores measured is 6.67 x 2.16 µm; *Q* = 3.1.

**9. HYMENOCHAETE LEGERI** Parmasto, **sp. nova** Figs. 1, *14*; 4, *3*

*A* H. corrugata *(Fr.: Fr.) Lév. setis longis robustis (60–)75–100(–110) x 10–15 µm apicis crystallis multis tectis, sporis grandis cylindricaceis vel suballantoideis 5.5–8.2 x 2.2–3.2 µm nec non hymenio pallide griseo vel albido differt.*

Holotypus: Hawaiian Islands, Moloka'i Is., Kamakou Rd., on *Eucalyptus* sp., 9 Jan 2000 R.L. Gilbertson 23032 (ARIZ; isotype in TAA).

Etymology: Jean-Claude Léger (2 Oct 1938 – 16 July 1999), distinguished French mycologist, author of a fundamental monograph of world *Hymenochaete*.

**Basidiomata** annual, effused, closely adnate, crustose, woody hard, 100–500 µm thick (when old up to 1 mm and sometimes indistinctly stratified), as rounded patches 0.5–2 cm long, then confluent and up to 10 cm long; hymenium even or somewhat tuberculose when old, later with some irregular deep fissures, pale Mouse Gray, Vinaceous Buff, pale Vinaceous Buff or whitish (M: 5–10 YR 7–8/2 to 8/1–3; K & W 5 B 2 or (5–6) (B–C) 2), when old and sterile dark Fulvous to Umber or Sepia (M: 5 YR 4–5/4–6; K & W 6 D 6); margin clearly delimited, when young narrowly white and fimbriate, soon abrupt, usually with a narrow brown(ish) strip (M: 5–7.5 YR 4/6; K & W: 6 D 6 – 6 E 7).

Tomentum absent; cortex absent or present and then 10–20 µm thick; hyphal layer 30–80 µm thick, sometimes almost absent; setal layer thickening; dark line above the hymenium absent.

Hyphal system subdimitic; hyphae in hyphal layer at first not densely, then densely interwoven; generative hyphae with thin or slightly thickened walls, subhyaline, sparsely branched and septate, 2.5–4 µm in diam; skeletoids sparsely branched, with thickened walls, brownish, 2.5–4 µm in diam; setae numerous or crowded, hymenium composed of basidioles, basidia and innumerous thin- or thick-walled hyphidia; setae broadly conical, at base arising from a dense coralloid node of brown thick-walled hyphae, straight, (60–)75–100(–120) x 10–15 µm, projecting 30–60 µm above the hymenium, covered with a hyphal sheath, acute or almost blunt tip usually covered with a conical conglomerate of crystals; hyphidia rare, cylindrical, brownish, with thickened walls, 2–3 µm in diam; basidioles thin-walled; basidia subutriform, 20–25 x 4.5–6 µm, with thin hyaline walls, with (2) 4 sterigmata 3–4 µm long; spores cylindrical or suballantoid, 6–8 x 2.2–3.2 µm.

Causes white pocket rot of wood.

HAWAI'I. RLG 17184 on hardwood, RLG 17193 on *Grevillea robusta*, RLG 17194 on *Psychotria hawaiiensis*, RLG 18651 on *Psidium guajava*, Kalopa State Park; RLG 17553 on *Casuarina* sp., RLG 17806, 17858, 18202 on *Psidium guajava*, RLG 17859, 18242, 22239 on *Eucalyptus* sp., RLG 18924 on *Toona ciliata*; Honokaia Boy Scout Camp; RLG 20977, 20989 on *Toona ciliata*, Stainback Highway; RLG 17246 on *Mangifera indica*, Rainbow Falls; RLG 19428 on *Mangifera indica*, Kapoho Rd.; RLG 20727 on *Acacia koa*, Keanakolu Rd.; RLG 23308 on *Coprosma montana*, Kipuka Puaulu, HVNP; RLG 16826 on *Sapindus saponaria*, Kipuka Puaulu, HVNP; RLG 22250 on *Schinus terebinthifolia*, RLG 22254 on *Cerecropia obtusifolia*, Manuka State Park. KAUA'I. RLG 21072 on *Acacia koa*, Makaha Rd.; RLG 21198 on *Metrosideros polymorpha*, Lehua Puhi Trail, Koke'e State Park; RLG 17945, 21260, 22726 on *Pinus elliottii*, Makaha Rd.; RLG 20617, 21055 on *Sequoia sempervirens*; Koke'e State Park; RLG 21044 on *Eucalyptus* sp., Kukiolona Park, Kalaheo; RLG 21168: on *Metrosideros polymorpha*, Ohelo Berry Flat Trail, Koke'e State Park; RLG 21226 on *Acacia koa*, Makaha Rd.; RLG 22727 on hardwood, Makaha Rd.;

RLG 22787 on *Nestegis sandwicensis*, RLG 22803 on *Metrosideros polymorpha*, Lehua Puhi Trail, Koke'e State Park; RLG 22845 on *Acacia melanoxylon*, Ohelo Berry Flat Trail, Koke'e State Park; RLG 21251 on *Pinus elliottii*, Miloli'i Rd. MAUI. J.E. Adaskaveg 1469 on *Spathodea campanulata*, Mile 4, Hana Highway; RLG 19172 on *Psidium guajava*, Maluhia Boy Scout Camp; RLG 21475, on *Pinus pinaster*, Waihou Spring Trail, Olinda; RLG 21524 on *Aleurites molluccana*, Makemakeole Trail, West Maui; RLG 23150, 23158 on *Araucaria heterophylla*, Maluhia Boy Scout Camp. MOLOKA'I. RLG 21449 on *Casuarina* sp., Pala'au State Park; RLG 21337, 23032, 22965, 22978, 23044, 23048, 23053 on *Eucalyptus* sp., Kamakou Rd.; RLG 19204 on hardwood, Kamakou Rd.; RLG 19353 on *Acacia mearnsii*, Kamakou Rd.; RLG 23002 on *Araucaria heterophylla*, Kamakou Rd.; RLG 21411 on *Pinus pinaster*, Kamakou Rd.; RLG 22947 on *Casuarina* sp., Kalaupapa Overlook. O'AHU. C.L Shear, BPI 11006101, on *Hibiscus tiliaceus*, Tantalus Rd.; Don Hemmes 65 on *Syzygium cumini*, Pali; RLG 16931 on hardwood, Manoa Falls Trail; RLG 18331, on *Schinus terebinthifolia*, Pu'u Ohia Trail; RLG 18346: on *Psidium guajava*, Manoa Cliffs Trail.

Found also in INDIA (Tamil Nadu St., Kalakad Sanctuary, 13 Feb 1979 E. Parmasto, TAA 103250), TAIWAN (Wu 880501/26, TAA) and AZORES (on *Cryptomeria*, B. Spooner, K).

**Comments.** The new species is well characterized by broad setae with a tip usually heavily covered with crystals, relatively big suballantoid spores and (when not very old or dead) alabaster or marble white hymenium. It seems to be closely related to the widespread (but not reported in Hawai'i) species *H. corrugata* that also has relatively big setae with nodose hyphal base and granulose tip, but has a much darker pinkish gray hymenium. That species has setae 35–80(–100) µm long, spores of similar form but smaller (4.5–6.5 x 1.5–2.3 µm). Several species of *Hymenochaete* have whitish gray hymenium covered with numerous crystals when basidiomata are old, sterile or half-dead. In *H. legeri*, pale colour of hymenium caused by abundant crystals is a sign of intensive growth and in many cases also of presence of ripe basidia and spores.

In old specimens of *H. legeri* the hyphal layer is almost indistinguishable from the setal layer, and cortex is hardly observable. In sterile and no longer growing specimens the hymenium turns brown and the characteristic pale (whitish) colour fades off.

*H. legeri* has Macaronesia–Asia Tropical–North-Central Pacific distribution.

Mean spore size and mean *Q* value of *H. legeri:*

| | | |
|---|---|---|
| 6.30 x 2.71 | 2.32 | RLG 18346 |
| 6.31 x 2.64 | 2.39 | RLG 18331 |
| 6.31 x 2.48 | 2.54 | RLG 19172 |
| 6.34 x 2.41 | 2.63 | RLG 23032 |
| 6.48 x 2.56 | 2.53 | RLG 17194 |
| 6.56 x 2.36 | 2.78 | RLG 18924 |
| 6.68 x 2.58 | 2.60 | RLG 22978 |
| 6.77 x 2.68 | 2.53 | Azores (K) |
| 6.92 x 2.57 | 2.69 | RLG 21475 |
| 6.94 x 2.49 | 2.78 | RLG 18924 |
| 7.03 x 2.64 | 2.67 | BPI, Shear 1100610 |
| 7.05 x 2.61 | 2.70 | RLG 23048 |
| 7.17 x 2.63 | 2.73 | RLG 23044 |
| 7.18 x 2.57 | 2.79 | RLG 22965 |
| 7.18 x 2.63 | 2.73 | RLG 21055 |

| | | |
|---|---|---|
| 7.22 x 2.63 | 2.75 | RLG 22947 |
| 7.25 x 2.53 | 2.87 | RLG 21449 |
| 7.37 x 2.68 | 2.75 | RLG 21044 |
| 7.37 x 2.87 | 2.56 | Adaskaveg 1469 |
| 7.92 x 2.67 | 2.97 | RLG 17184 |

**10. HYMENOCHAETE MINUSCULA** G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 48. 1957. **Figs. 1, *5*; 2, *10***

**Basidiomata** perennial, effused, adnate, woody hard, 150–450 µm thick (50–150 µm in specimens found in New Zealand, Africa and America), when old with innumerous deep cracks. Hymenium smooth, dark grayish Ochreous (M: 10 YR 5/6; K & W: 6 D 4); margin abrupt.

Cortex, tomentum and hyphal layer absent; setal layer indistinctly stratose, with scattered crystals; dark line above the hymenium absent.

Hyphal system monomitic; hyphae distinct, densely interwoven, with thin or thickened brownish walls, sparsely branched, septate, 2.5–4 µm in diam; setae numerous, subulate, acute, straight or some slightly curved and undulate, 35–60(–65) x 4–7 µm, usually with a thin hyphal sheath, emerging up to 40 µm above the hymenium; cystidia and hyphidia absent; basidioles 18–25 x 3–3.5 µm, with thickened base walls encrusted with granules; basidia subutriform, 15–22 x 3.5–4 µm, with 4 thin sterigmata; spores ellipsoid or short-cylindrical, with one side flattened or slightly concave, 4.2–5.2 x 2.2–2.5 µm.

Causes a fibrous white rot of wood.

HAWAI'I. RLG 17722: on *Acacia koa*, Keanakolu Rd..

**Comments.** Ochraceous or yellowish brown colour characterizes this species. It has been found also in New Zealand, Western Indian Ocean region of Africa, and in South America, i.e, has a Gondwanan distribution type.

Mean spore size and mean *Q* value of *H. minuscula:*

| | | |
|---|---|---|
| 4.67 x 2.37 | 1.98 | RLG 17722 |

**11. HYMENOCHAETE MUROIANA** I. Hino & Katum. in Hino, Icones Fung. Bambus. Japan 237. 1961. **Figs. 1, *15*; 2, *5***

**Basidiomata** annual, effused, adnate, when older detachable as small fragile pieces, coriaceous, hard when dry, 30–50(–100 ?) µm thick, as rounded patches 2–5 cm long, later sometimes confluent and up to 15 cm long. Hymenium even, vinaceous buff, fawn or dark fawn, when old dark fawn or grayish umber (M: 5 YR 5/4 or 6/1 – 7/2, when old and sterile 5 YR 4.5/2; K & W: 7 C 2 – D 3, when old 7 E 4–5); margin thin, indistinct, then distinctly delimited, concolorous with the hymenium.

Tomentum, cortex and hyphal layer absent; setal layer very thin, 10–20 µm thick; dark line above the hymenium absent.

Hyphal system monomitic; hyphae in setal layer densely interwoven, agglutinated, with thickened or thick walls, brownish, with rare septa and branches, (2–)2.5–4 µm in diam; hyphal layer composed of loosely interwoven, richly branched and septate brownish hyphae with thickened or thick walls, 2.5–4.5 µm in diam; setae numerous; hymenium composed of basidioles and basidia; cystidia and hyphidia absent; setae

narrowly (sometimes broadly) subulate, usually partly bifurcate at base, with slightly curved upper part and acute or almost blunt tip, (30–)40–50(–60) x (5–)6–8(–9) µm, naked (without a hyphal sheath), projecting up to 20(–30) µm above the hymenium; basidioles thin-walled; basidia subutriform, 15–20 x 4.5–6 µm, with thin hyaline walls, with 4 sterigmata 3–4 µm long; spores broadly ellipsoid, 4–6 x (2.5–)3–3.5 µm.

Causes fibrous white rot of wood.

HAWAI'I. RLG 21011, 21019, 21028 on *Bambusa vulgaris*, Waipio Ridge Trail; RLG 21013, 21017 on *Phyllostachys nigra*, Waipio Ridge Trail. MAUI. C.L. Shear and N.E. Stevens, BPI 1199597; West Maui, Pogues Ditch, 2 Jan 1928, no other data (BPI 1100597).

**Comments.** This species has been described from Japan, and found later only in Taiwan (NMNS, S.H. Wu 880501-26, unpublished data), Indo-China and Malesia (Parmasto, 2005). Most collections are from dead (fallen) stems of bamboo.

In the original description of *H. muroiana* and its synonym *H. iriomotensis* I. Hino & Katum. (Hino & Katumoto, 1964) as well as in the redescription of the types by Léger (1998: 199), the spores are described as (3–)3.5–4(–4.5) x (1.8–)2–2.6 µm and setae as very numerous and smaller than in most of our specimens (Léger: (16–)18–35 x (4.5–)5–6(–6.5) µm). However, some old sterile specimens collected from Taiwan and Hawai'i (BPI 1100597) studied by us have densely crowded, short conical setae 30–40 x 7–12 µm; setal variation seems to be related to the state of development of the basidiomata. The types of this species were described by Léger (1998: 198, 200) as soot brown *(brun suie,* 7.5 YR 5/2); as in most species of *Hymenochaete,* the colour depends on presence or absence of basidia and basidioles. In the Hawaiian collections, both sterile umber fawn and fertile vinaceous buff specimens are represented.

One of the Hawaiian specimens (RLG 21011) differs from the others by thickness (up to 250 µm), dark brown colour (7.5 YR 4/6), setae 35–50(–55) x 5–7(–8) µm, small spores 3–4 x 1.9–2.5 µm (mean size: 3.47 x 2.15 µm, $Q$ = 1.62) and substrate *(Coprosma montana* of the family *Rubiaceae).* According to the spore size, this collection is close to the Japanese and Sulawesi specimens of *H. muroiana.* It may be possible that there is more than one species in this group.

Mean spore size and mean $Q$ value of *H. muroiana:*

| | | |
|---|---|---|
| 5.36 x 3.50 | 1.54 | RLG 21028 |
| 5.70 x 3.65 | 1.39 | RLG 21019 |

**12. HYMENOCHAETE SEMISTUPPOSA** Petch, Ann. Roy. Bot. Gard., Peradeniya 9: 278. 1925. **Figs. 1,** *4*; **3,** *5*

**Basidiomata** annual (but sometimes layered), effused, adnate but easily detachable, soft membraneous, 200–500 µm thick; first 3–10 mm in diam, with pennately fimbriate margin, then confluent. Hymenium even (under looking glass pubescent), in old specimens sometimes minutely cracked, sepia to dark brown (M: 2.5–5 YR 4/3, K & W: 6 E 6); margin when young pennate-fimbriate with fibrils 1–2 mm long, not attached to the substrate, margin and very young basidiomata sienna or fulvous to sienna (M: 5 YR 5–6/8; K & W: Cinnamon or Raw Sienna, 6 D (6–7)).

Tomentum absent or present as abhymenial hairs; cortex present, 40–150 µm thick; context composed of cinnamon or fulvous hyphal layer 100–200 µm and thin to thick setal layer up to 200 µm thick; dark line above the hymenium absent.

Hyphal system monomitic; hyphae of the fibrillose margin parallel, with thickened walls, yellow, rarely septate, some slightly flexuous; cortex composed of densely agglutinated parallel brown, thick-walled hyphae 3.5–5 µm in diam; hyphal layer composed of loosely interwoven, richly branched, septate brown hyphae with thickened or thick walls, 2.5–4.5 µm in diam, many hyphae slightly monilioid and with punctually thickened walls; setal layer made of thickening hymenium, between setae are vertically situated, densely parallel, frequently septate, yellow or brownish, slightly monilioid hyphae; hymenium composed of setae, numerous hyphidia and basidioles, and few basidia; setae numerous, conical, straight, with acute tip, (40)45–70(–80) x (6–)7–9.5(–12) µm, mostly with a hyphal sheath, projecting up to 40 µm above the hymenium; hyphidia, basidioles and basidia similar to each other, 18–25 µm long and 2.5–4 µm (basidia up to 6 µm) in diam, with walls thickened and brownish at base, developing from hyphae with numerous septa; many hyphidia monilioid and/or with thickenings on walls; basidia cylindrical, with 4 sterigmata 3–4 µm long; spores ellipsoid or short cylindrical, one side slightly concave or flattened, 3.2–4.5 x 1.7–2.2 µm.

HAWAIIAN Is. 1921 F.L. Stevens 871, 878 (BPI 277727, 277717, identified as *H. cinnamomea*). HAWAI'I. RLG 18934 on *Eucalyptus* sp., Honokaia Boy Scout Camp; RLG 17442 on *Metrosideros polymorpha*, Thurston Lava Tube, HVNP; RLG 16978, 16983 on *Metrosideros polymorpha*, Mile 21, Saddle Rd.; 18085, 20746, 20757, 20763 on *Metrosideros polymorpha*, Mile 18, Saddle Rd.; RLG 19047 on *Metrosideros polymorpha*, Manuka State Park. KAUA'I. RLG 20580 on *Acacia melanoxylon*, Ohelo Berry Flat Trail, Koke'e State Park; RLG 22828 on hardwood, Lehua Puhi Trail, Koke'e State Park. O'AHU. C.L. Shear (BPI 1100598) on *Metrosideros polymorpha*, Manoa Valley.

**Comments.** Cunningham (1957) and Job (1987) found that the basidiomata of this species may be layered. In several Hawaiian specimens new basidiomata are growing on old (dead?) ones, in the beginning as small isolated "islands". The "layers" (basewalls) of the basidiomata are not the result of uniform thickening of old ones.

*H. semistupposa* has been found in Southern Africa, East Tropical Africa (Kenya), Asia Tropical (India and Sri Lanka) and Australasia. Almost all known substrates belong to the tropical family *Myrtaceae*. These data suggest that this fungus belongs to the Gondwanan historical type of distribution.

Mean spore size and mean *Q* value of *H. semistupposa:*

| | | |
|---|---|---|
| 3.56 x 1.83 | 1.95 | RLG 16983 |
| 3.57 x 1.81 | 1.97 | RLG 18934 |
| 3.75 x 1.93 | 1.94 | RLG 20580 |
| 3.93 x 1.82 | 2.17 | RLG 18085 |
| 4.13 x 1.93 | 2.14 | RLG 22828 |

**13. HYMENOCHAETE SEPARABILIS** J.C. Léger, Bull. Soc. Mycol. France 97 (1): 7. 1981.
Figs. 1, *8*; 2, *2*

**Basidiomata** annual or perennial, effused, adnate but separable as pieces when dry, soft or coriaceous, when young about 60 µm thick, then 200–400 µm, as rounded patches 0.5–5 cm in diam, later confluent and up to 10 cm long; hymenium even, in older parts irregularly cracked; light Umber (M: 5 YR 4.5–5/3–4; African specimens 10 YR 6/8, later 7.5 YR 5/6; K & W: 7 E 6); margin thin, slightly fibrillose, then abrupt, distinct, lighter

coloured than hymenium; context lighter coloured than hymenium, dark Sienna (M: 5 YR 5/7; K & W: 7 D 6).

Tomentum and cortex absent; hyphal layer present but weakly differentiated from the thickening setal layer; dark line above the hymenium absent.

Hyphal system monomitic; hyphae brownish, with thickened walls, occasionally branching, septate, 2–4 µm in diam, in hyphal layer loosely, later densely interwoven; setae numerous, subulate, subfusoid, with acute tip, 25–45 x 5–7(–8) µm, straight or some slightly curved, not encrusted, usually with a thin hyphal sheath, projecting up to 30 µm above the hymenium, in upper quarter with few (1–4, rarely up to 8) low broadly conical teeth or protuberances up to 0.5(–1) µm long; cystidia and hyphidia absent; basidioles 12–15 x 3–4 µm; basidia subutriform, 15–35 x 4–5 µm, walls of basal part slightly thickened and encrusted with granules (as are the basidioles, too), with 4 thin sterigmata 3–4 µm long; spores broadly ellipsoid, with one side slightly flattened, 2.7–3.5(–4.0) x 1.8–2.5 µm (in African specimens 2.8–4.5 x 1.5–2.5 µm).

Causes white fibrous rot of wood.

HAWAI'I. F.L. Stevens 877 (BPI 278618) on frondose wood, identified by Burt as *H. spreta* (see Burt, 1923: 186); RLG 20978 on *Toona ciliata*, Stainback Hwy. KAUA'I. RLG 21190 on *Metrosideros polymorpha*, Lehua Puhi Trail, Koke'e St. Park. MOLOKA'I. RLG 23023 on *Araucaria heterophylla*, RLG 23026 on *Cupressus macrocarpa*, Kamakou Rd.

**Comments.** The very small protuberances (denticles) on upper part of setae are visible only when great magnification (x 1000 or more) is used. The similar species *H. tomentelloidea* has setae (35–)40–70 x 5–10 µm with 5–10 broadly conical teeth 0.8–1.5(–2) µm long and more elongated spores 3.1–4.5 µm long. *H. separabilis* is a species of Tropical African–Northwestern Pacific distribution and possibly of Gondwanan origin. The specimens found in Africa (Central African Republic, Gabon, Madagascar, Mauritius, Réunion) are all thin (about 60 µm), possibly young, and with better developed hyphal layer with loosely interwoven thin-walled hyphae (see Léger, 1998: 252–254).

Mean spore size and mean *Q* value of *H. separabilis:*

| | | |
|---|---|---|
| 2.99 x 2.23 | 1.34 | RLG 20978 |
| 3.17 x 2.24 | 1.41 | RLG 23026 |

**14. HYMENOCHAETE SEPARATA** G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 50. 1957.

**Basidiomata** perennial, effused, closely adnate, very hard when dry, as rounded patches 0.5–2 cm long, later confluent, 200–500, later up to 750 µm thick; hymenium coarsely low-tuberculate or smooth, with some crevices, dark Hazel to Fawn (M: 7.5 YR 5/4 or almost 7.5 YR 4/4; K & W: 7(–6) E , brownish terra cotta); margin abrupt, concolorous.

Tomentum, cortex and hyphal layer absent, setal layer sometimes indistinctly 2-layered.

Hyphal system subdimitic; skeletoids ascendant, with thickened brownish walls, 2.5–3.5 µm diam., densely agglutinated with brown resinous matter; generative hyphae few, subhyaline, with septa, 3–4 µm diam; setal layer and hymenium with numerous conglomerates of crystals 8–30 µm diam; setae numerous, broadly subulate, (30)35–45(–55) x 6–7(–8) µm, partly covered with a thin hyphal sheath, without incrustation or

with a few crystals; cystidia and hyphidia absent; basidioles numerous, brownish, 22–28 µm long; spores (according to Léger, 1998: 255) ellipsoid, 5–6.5 x 3–3.5 µm.

HAWAI'I. RLG 20489, 23311 on *Coprosma montana*, RLG 23297 on *Pisonia sandwicensis*, Kipuka puaulu, HVNP.

**Comments.** All Hawaiian specimens are sterile; they may belong to one of the two Australasian species, *H. separata* or *H. vallata*. The last named species has spores about 4 x 2 µm and setae (23–)25–45(–55) x (4.5–)5–7.5 µm, but these are all covered with crystals. Setae of *H. separata* are bigger, described as (37–)45–55(–57) x (5.5–) 6–8 µm by Léger (1998: 255). *H. separata* has been found previously in New Zealand and in Réunion.

### 15. HYMENOCHAETE SUBDISSIMILIS *ad int.* Figs. 1, *3*; 2, *6*

**Basidiomata** perennial, effused, closely adnate, hard when dry, detachable as small pieces, 100–400 µm thick; hymenium tuberculate, rather densely deeply cracked, Grayish Sepia (M: 5 YR 5/2; K & W: 7 (D–E) 3 – 8 E 3), margin abrupt (?).

Tomentum and cortex absent, hyphal layer 30–150 µm thick, pseudoparenchymatous, indistinctly different from the setal layer; setal layer thickening, not clearly stratose.

Hyphal system monomitic; hyphae very densely agglutinated with brown resinous matter, indistinct, 2–3.5 µm in diam, brown, thick-walled; crystalline matter present in context and hymenium, in context as conglomerates 15–50 µm in diam; setae numerous, broadly subulate, 40–60(–65) x 7–10 µm, projecting to 30 µm above the hymenium, with bluntly acute tip, straight, usually with a thin hyphal sheath, irregularly encrusted; cystidia and hyphidia absent; basidioles present; basidia subutriform, 12–16 x 4–5.5 µm, with 4 sterigmata 3–4 µm long; spores elongated-ellipsoid or short-cylindrical, slightly curved or with one side flattened, 3.7–4.4(–5) x 1.8–2.5 µm.

Causes white fibrous rot of wood.

HAWAI'I. RLG 19028 on *Psidium guajava*, Manuka State Park. MAUI. RLG 16777 on *Syzygium jambos*, Mile 6, Hana Highway (identified in Gilbertson et al., 2002: 227 as *H. anomala* Burt).

**Comments.** This possibly new species is externally very similar to *H. dissimilis* as described by Léger (1998: 126–127) but differs in having a tuberculate hymenium, an indistinct hyphal layer, shorter setae and distinctly smaller spores. However, *H. dissimilis* described by Job (1990: 19–20) has setae similar to those in *H. subdissimilis* (45–65 x 6–8 µm). Another possibly closely related species, *H. unicolor*, differs in having a smooth (even) brown hymenium, smaller nonencrusted setae (30–)35–50 x 5–7 µm, basidioles with thickened walls at the base, and broader spores 3.4–4.8 x 2.2–2.8 µm.

Among specimens collected in Hawai'i, one (BPI 1103516, on *Cyathodes* sp., Maunahui, Moloka'i; Degener Otto 2823, 15 Apr 1928) is very similar to *H. subdissimilis* but has longer spores 4.2–5.2 x 1.7–2.4 µm (mean size: 4.80 x 2.08 µm, $Q$ = 2.31). Its hymenium is Vinaceous Buff–Grayish Sepia (M: 2.5 YR 5.5/4). After more collections (including young and thin ones) are found, possibly all three species (*H. dissimilis*, *H. subdissimilis*, and *H. unicolor*) may be synonymous, and only one species with variable spore size exists.

Mean spore size and mean *Q* value of *H. subdissimilis*:

| | | |
|---|---|---|
| 4.05 x 2.05 | 1.97 | RLG 19028 |
| 4.19 x 2.16 | 1.94 | RLG 16777 |

**16. HYMENOCHAETE TOMENTELLOIDEA** Gilb. & Hemmes, Mycotaxon 62: 476. 1997.
Figs. 1, *7*; 2, *1*

**Basidiomata** annual, effused, adnate, soft or coriaceous, when young 60–70 µm thick, then 200–450 µm, as rounded patches 0.5–2 cm in diam, later confluent and up to 10 cm long; hymenium even, in older parts irregularly cracked; grayish dark Cinnamon or Sienna-Umber (M: 7.5 YR 5–6/8 or 6/6; K & W: 6 D (6–7), 6 E 5 or 6 D 4); margin thin, indeterminate, lighter coloured than hymenium (sometimes M: 7.5 YR 6/10), slightly fibrillose, then distinct and concolorous with the hymenium.

Tomentum absent, cortex sometimes present but then indistinct, 20–40 µm thick; hyphal layer up to 300 µm thick; setal layer (when present) later thickening and up to 100 µm thick; dark line above the hymenium absent.

Hyphal system monomitic; hyphae brown, with thickened or thick walls, occasionally branching mainly at less than right angles, septate, 2–3.5 µm in diam, in hyphal layer loosely, then densely interwoven; setae numerous, subulate, with acute tip, (35–)40–70 x 5–7 µm, straight or some slightly curved, not encrusted, usually with a thin hyphal sheath, in upper third with few (5–10) broadly conical teeth 0.8–1.5(–2) µm long; projecting up to 20 µm above the hymenium; cystidia and hyphidia absent; basidioles 15–20 x 3.5–5 µm; basidia subutriform, 15–20 x (3.5–)4–5 µm, with lower part slightly thickened and encrusted with brown granules, with 4 thin sterigmata 3–4 µm long; spores ellipsoid, with one side slightly flattened, 3.1–4.5 x 1.8–2.5 µm.

Causes white fibrous or pocket (?) rot of wood.

HAWAI'I. RLG 20807 (holotype) on *Cibotium chamissoi*, Stainback Highway; RLG 18075, 18123 on *Metrosideros polymorpha*, Mile 18, Saddle Rd. KAUA'I. RLG 21106 on *Acacia koa*, Nualolo Trail, Koke'e State Park. MOLOKA'I. RLG 22952, on *Casuarina* sp., Kalaupapa Overlook.

**Comments.** The innumerable small denticles on upper part of setae are clearly visible only when great magnification (x 1000 or more) is used. Differences between this species and the very similar *H. separabilis* are described in the comments under that species. *H. separabilis* and *H. tomentelloidea* are possibly two closely related sibling species of Gondwanan origin, *H. tomentelloidea* being endemic.

Mean spore size and mean *Q* value of *H. tomentelloidea*:

| | | |
|---|---|---|
| 3.40 x 2.14 | 1.59 | RLG 18123 |
| 3.74 x 2.35 | 1.58 | RLG 21106 |
| 4.11 x 2.43 | 1.69 | RLG 18075 |
| 4.14 x 2.01 | 2.06 | RLG 22952 |

17. HYMENOCHAETE UNICOLOR Berk. & M.A. Curtis, J. Linn. Soc., Bot. 10: 335. 1868.
Figs. 4, *11*; 2, *4*

**Basidiomata** perennial, effused, adnate, hard to woody when dry, when young 60–120 µm thick, when old up to 700 µm or more, as rounded patches 0.5–2 cm in diam,

later confluent and up to 5 or more cm long; hymenium even, soon cracked, when old with deep cracks (fissures), Fulvous Umber or Sienna-Umber (M: 5 YR 4.5/6 to 5/4; K & W: 6 E 6), when old light grayish Umber (M: 5–7.5 YR 6/4; K & W: 6 (E–D) 4); margin distinctly delimited, lighter when young, soon abrupt and concolorous with the hymenium.

Tomentum and cortex absent; hyphal layer 30–50 µm thick, with some included setae, hardly distinguishable from the setal layer (with some setae), later disappearing; setal layer thickening, with indistinct layers; dark line above the hymenium absent.

Hyphal system monomitic; hyphae brownish, with thickened walls, scarcely septate, 2–3 µm in diam, in hyphal layer interwoven, in setal layer tightly agglutinated and encrusted with brown resinous matter; setae numerous, subulate, some slightly curved, with acute tip, (30–)35–50 x 5–7 µm, not encrusted, usually with a thin hyphal sheath, projecting up to 20 µm above the hymenium; hymenium composed of basidioles and basidia, no cystidia or hyphidia; basidioles almost cylindrical, with basal part thickened and encrusted with resinous matter (uneven), 15–25 x 3.5–5 µm; basidia subutriform, 15–25 x 4–5 µm, with 4 thin sterigmata; spores ellipsoidal with one side flattened or almost short-cylindrical, 3.4–4.8 x 2.2–2.8 µm.

Causes white fibrous rot of wood.

HAWAI'I. RLG 17718 on *Acacia koa*, Keanakolu Rd. KAUA'I. RLG 20601 on *Acacia koa*, Nualolo Trail, Koke'e State Park; RLG 21204 on *Myrica faya*, Lehua Puhi Trail, Koke'e State Park.

**Comments.** Identity of the specimens studied by us is not certain. Types of both *H. unicolor* and its synonym *H. lignosa* G. Cunn. are old thick specimens with only a few small spores, and most collections kept in herbaria under these names are sterile. Spores of the Hawaiian specimens are smaller than those described by Cunningham (1957), Reeves & Welden (1967), Job (1990) and Léger (1998), who all indicated the same (!) size, 4.5–5.5 x 3–3.5 µm. Asian and African specimens studied by Parmasto (2005) have spores 4.5–5.7 x 2.5–3.3 µm. The species complex including *H. unicolor*, *H. dissimilis*, *H. minuscula* and *H. subdissimilis* is in need of further study.

Mean spore size and mean *Q* value of *H. unicolor:*

| | | |
|---|---|---|
| 3.65 x 2.33 | 1.56 | RLG 20601 |
| 3.75 x 2.51 | 1.50 | RLG 21204 |
| 4.41 x 2.32 | 1.90 | RLG 17718 |

**18. HYMENOCHAETE VAGINATA** G. Cunn., Trans. Roy. Soc. New Zeal. 85 (1): 30. 1957.

**Basidiomata** effused, adnate but detachable as pieces, coriaceous, 120–300 µm thick, as rounded patches 0.5–1 cm long, then confluent; hymenium smooth (or tuberculate), Umber (M: 7.5 YR 4/6); margin Ochraceous (M: 7.5 YR 7/6–7).

Tomentum thin; cortex, hyphal and setal layers present but not easily distinguishable in young specimens; dark line above the hymenium absent.

Hyphal system monomitic; hyphae densely or almost loosely interwoven, with thickened walls, brownish, sparely branched, septate, 3–4 µm in diam; setae numerous, conical-subfusiform, 90–130 x 10–17(–20) µm, emerging up to 75 µm above the hymenium, with a hyphal sheath, not encrusted; hyphidia innumerous, brownish, with thickened

walls, 2–3 µm in diam, some slightly flexuous; basidia subcylindrical, 18–25 x 4.5–5.5 µm, with 4 sterigmata; spores cylindrical, about 6.5–9.5 x 2.4–3.2 µm.

O'AHU. C.L. Shear (BPI 1100612) on *Scaevola* sp., Pupukea Paumalu Forest Reserve, 15 Feb [1928 ?]. – **Isotype studied:** NEW ZEALAND, J.M. Dingley (K (M) 57642) on *Phyllocladus alpinus*, Wellington, Whakapapa, Mt. Ruapeku, 20 Oct 1949.

**Comments.** Until now the species was known only from a single New Zealand collection; its variation is unknown. The Hawaiian collection is a small young specimen but with big setae and spores characteristic for this species, and brownish hyphidia. According to Job (1991: 46) and Léger (1998: 288), the basidiomata of this species may be up to 800–1000 µm thick with thickening stratose setal layer. Isotype of this species is almost sterile with only few, partly collapsed spores (6–)6.8–8 x 2.4–3.2 µm (mean of 12 spores: 7.24 x 2.79 µm; $Q$ = 2.60). The Hawaiian specimen has few cylindrical curved spores 7–9.5(–10) x 2.5–2.9 µm; mean of 14 spores: 8.37 x 2.64 µm, $Q$ = 3.17.

## Appendix

**HYMENOCHAETE NOTHOFAGICOLA** Parmasto, **sp. nova.** Fig. 1, *13*; 4, *2*

– *H. attenuata* sensu G. Cunn., Trans. R. Soc. New Zeal. 85 (1): 33. 1957.

*A* H. attenuata *(Lév.) Lév. setis longis (55–)65–100 x (6–)7–11(–14) µm, sporis grandis (5.5–)6.0–8.0(–8.5) x (3–)3.5–4.5(–5.0) µm nec non pileis anguste reflexis ad 3 mm longis differt.*

**Holotypus:** NEW ZEALAND, North Canterbury Distr., Arthur's Pass, 2500 ft, on *Nothofagus solandri*, Jan 1956, J.M. Dingley (PDD 16989).

**Etymology:** *nothofagicola*, growing on *Nothofagus* spp.

**Basidiomata** effused-reflexed with narrow reflexed part (pileus) 1–3 mm broad, adnate but removable in pieces, soft coriaceous, 200–350 µm thick; pileal surface radially fibrillose or substrigose, then almost glabrous, with one or two concentric zones, brown (M: 5 YR 5/4–6; K & W: 6 (D–E) 5, Sahara); hymenium smooth but uneven, soon minutely radially or plumosely cracked, Fulvous or Sienna-Umber when young (M: 7.5 YR 5/8 to 5 YR 5/6; K & W: 6 D 7 to 6 D (4–5), raw Sienna, Sunburn or Camel), then Umber (M: 5 YR 4/5–6; K & W: 6 E (4–5), brown when old M: 5 YR 4/4); margin 0.5–1 mm broad, thin, sometimes torn, more lightly coloured (7.5 YR 7/10; K & W: 5 B 6, apricot yellow), soon concolorous with hymenium.

Tomentum present but in old specimens almost indistinct; cortex absent; context composed of hyphal layer gradually transiting to tomentum, and setal layer.

Tomentum 50–150 µm thick, hyphae loosely arranged; hyphal system subdimitic; setal hyphae absent; skeletoids 2–4 µm in diam, brownish, with thickened walls; generative hyphae yellowish, thin-walled, with rare septa; aggregates of crystals sometimes present in hyphal layer; setae numerous, (55–)65–100 x (6–)7–11(–14) µm, fusiform, with almost blunt or acute tip, straight or in upper part slightly curved, naked, in old specimens with hyphal sheaths, without incrustation; basidioles not numerous, similar to basidia; basidia broadly clavate, 18–25 x 6.5–8 µm, with 4 sterigmata; spores broadly ellipsoid, with one side flattened, or subcylindric, sometimes with a large guttule, (5.5–)6.0–8.0(–8.5) x (3–)3.5–4.5(–5.0) µm; in old specimens small crystals are present in hymenium.

Causes white fibrous rot of wood.

**Specimens studied.** Holotype (see above); **cotypes:** NEW ZEALAND: Taupo Distr., Kaimanawa Ranges, 2000 ft, on *Nothofagus solandri* var. *cliffortioides*, Dec 1946, G.H. Cunningham (PDD 4959); Kaimanawa Ranges, Upper Mohaka River, on *N. menziesii*, May 1953, J.M. Dingley (PDD 12542); same locality, on *N. fusca*, May 1953, J.M. Dingley (PDD 12593); Taupo Distr., Poronui, Upper Homestead, on *N. menziesii*, Jun 1953, J.M. Dingley (PDD 12596); Buller Distr., Totara Flats, Glandville Forest, on *N. fusca*, Apr 1955, J.M. Dingley (PDD 16595); all specimens determined by G.H. Cunningham as *H. attenuata* (Lév.) Lév.

**Comments.** This species was identified as *H. attenuata* by Cunningham. The true *H. attenuata*, which has not been found in Australasia, differs by densely parallelly compacted hyphae above the hymenium, shorter setae (35–)45–70(–80) µm long and small spores 3.5–5(–5.5) x (1.5–)1.7–2.2 µm. Description of *H. attenuata* by Job (1990: 7–8) combines the data on *H. attenuata*, *H. attenuata* sensu G. Cunn. (= *H. nothofagicola*) and a species collected in Switzerland and described by Job & Keller (1988).

Another species growing on *Nothofagus* spp. and closely related to *H. nothofagicola* is *H. australis* Parmasto & Gresl. which has been found in Southern South America where it is common in Tierra del Fuego. It differs in having a cortex and cylindrical spores 7–9 x 2.4–3.2 µm. The two sibling species possibly have common Gondwana origin. Both are superficially very similar to *H. tabacina*, which differs in presence of setal hyphae and smaller spores 4.2–6.8 x 1.3–2.2 µm.

Three of the specimens of *H. nothofagicola* studied by me are almost sterile, with a few damaged spores, the other three (including the holotype) have limited numbers of spores. In only one specimen (PDD 4959) was it possible to measure 25 spores. Their mean size is 7.23 x 4.28 µm; $Q = 1.69$.

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MYCOTAXON

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# A new predatory fungus from China

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**Abstract**—*Dactylellina illaqueata*, a new predacious fungus capturing nematode by stalked adhesive knob in combination with non-constricting ring, is reported from Yunnan Province, China. The fungus is characterized by its simple, unbranched conidiophores singly bearing elongate fusiform conidia with 3-8 septa (usually 5) on the tip.

**Key words**—orbiliaceous fungi, nematode-trapping fungi

While surveying predacious fungi in Yunnan Province, soil samples were collected and spread on plates containing 2% corn meal agar medium. Nematodes (*Panagrellus redivivus*) were added in the plates as bait for predacious fungi. After 20 d for incubation at room temperature (about 20-28°C), predacious fungi were isolated under a dissecting microscope and identified according to the taxonomic system of Scholler et al. (1999). A new taxon, named *Dactylellina illaqueata*, is described here.

***Dactylellina illaqueata*** D. S. Yang & M. H. Mo **sp. nov.** **(Figures 1-15)**

*Mycelium effusum, hyphis sterilibus hyalinis, septatis, plerumque1.8-2.5 µm crassis. Conidiophoris hyalinis, septatis, erectis, simplicis, plerumque 95-250 µm altis, basi 2.2-2.6 µm crassis, apice1.8-2.1 µm crassis. Conidiis hyalinsi, elongato fusiformibus, apice rotundatis, basi truncates, 3-8 septatis (plerumque 5-septatis), 25.5-117.5 µm (saepe circa 66.5 µm) longis, 5.5-15.2 µm (saepe circa 4.8 µm) crassis. Chlamydosporis in culturis vetustioribus, globusis ad ellipsoidis.*

**Etymology:** The species epithet refers to the species capturing nematodes by trapping devices.

**Holotype:** YMF1.01846D, Simao, Yunnan, China, Oct 2005, DongSheng Yang. The holotype and its living culture (YMF1.01846) were deposited in the Laboratory for Conservation and Utilization of Bio-resources, Yunnan, P. R. China.

Mycelium scanty, spreading, vegetative mycelium colorless, septate, mostly 1.8-2.5 µm wide. Conidiophores (Figs 1-3) colorless, erect, unbranched, often 95-250 µm high, 2.2-2.6 µm wide at base, and gradually tapering upward to a width of 1.8-2.1 µm

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at tip, bearing a single conidium on the tip, occasionally two conidia. Conidia (Figs 4-12) colorless, elongate fusiform, narrowly obtuse at the distal end, truncate at the base, the middle cell swelling obviously, 25.5-117.5(66.5)×5.5-15.2 (14.1) μm, 3-8 septa, mainly 5 septa. The proportion of conidia with 3,4, 5, 6, 7 and 8 septa accounts for 9.1%, 15.2%, 63.6%, 6.1%, 3.0% and 3.0%, respectively. When induced with nematodes, the fungus produced non-constricting ring (Fig 13) and stalked adhesive knob (Fig 14). Chlamydospores (Fig 15) spherical to ellipsoidal, intercalary.

Based on phylogenetic analysis of 18s rDNA, a new genus concept was proposed for predatory anamorphic *Orbiliaceae* by Scholler et al. (1999) in which the trapping device is the main morphological criterion for generic delimitation. In this taxonomic system, the genus *Dactylellina* M. Morelet emend. M. Scholler et al. includes three species capturing nematode by stalked adhesive knob in combination with non-constricting ring, *D. leptospora* (Drechsler) M. Morelet (Drechsler, 1937), *D. lysipaga* (Drechsler) M. Scholler et al. (Drechsler, 1937) and *D. yunnanensis* (K. Q. Zhang et al.) M. Scholler et al. (Zhang et al. 1996). *D. illaqueata* described here is mainly characterized by its 5-septate conidia singly bearing on the unbranched conidiophores and this species resembles *D. yunnanensis* and *D. lysipaga* in conidial shape. However, *D. yunnanensis* usually forms short denticles on tip of conidiophores and bears 2-5 conidia, and *D. lysipaga* produces the conidia mainly with 2-4 septa. In comparison with *D. leptospora*, the conidia of *D. illaqueata* usually have a wider middle cell (average 14.1 μm) than that of *D. leptospora* (4.0-5.8 μm). In addition, conidia of *D. leptospora* have more septa (5-15) than those of *D. illaqueata* (3-8, mainly 5 septa).

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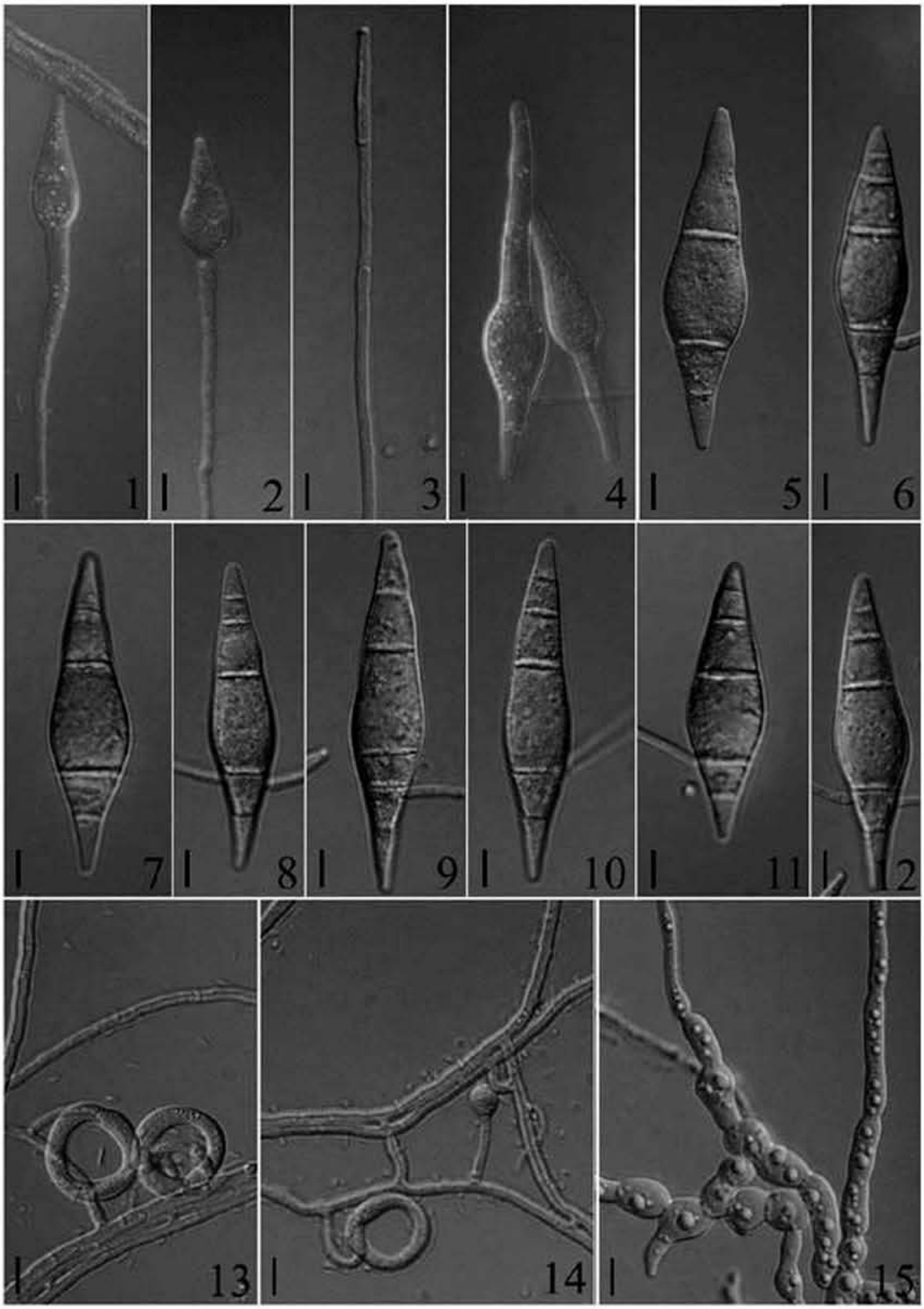


Figs 1-15. *Dactylellina illaqueata*. 1-3. Conidiophores and immature conidia. 4. Immature conidia. 5-12. Mature conidia. 13. Non-constricting rings. 14. Adhesive knobs and non-constricting rings. 15. Chlamydospores. Bar=10μm.

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# New species of sterile crustose lichens from Australasia

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**Abstract**—*Hypocenomyce isidiosa* and *Lepraria lobata* from Australia, *Leprocaulon australasicum* from Australia and Norfolk Island and *Pertusaria cyathicola* from Norfolk Island are described as new to science. The species are distinguished by their characteristic chemistries and vegetative propagules.

**Key words**—chromatography, morphology, taxonomy

## Introduction

Sterile crustose lichens are, in general, very poorly known although Tønsberg (1992), made a very significant contribution to the understanding of the Norwegian species. Like Tønsberg, I consider that the vegetative dispersal units (soredia, isidia, lobules, phyllidia and pseudopodetia) and the presence of diagnostic lichen substances to be important taxonomic characters. In this paper I describe four new species from Australia and Norfolk Island.

## Materials and Methods

The morphology of the lichen specimens was examined using a Zeiss Stemi 2000C stereomicroscope, and a Zeiss Axiolab compound microscope. Chemical constituents were identified by thin layer chromatography (Elix & Ernst-Russell 1993), high performance liquid chromatography (Elix et al. 2003) and comparison with authentic samples.

## Taxonomic Descriptions

### *Hypocenomyce isidiosa* Elix, sp. nov.

*Similis* Hypocenomyce friesii *(Ach.) P. James & Gotth. Schneid. sed sterilis et superficie dense isidiatis differt.*

Etymology: The specific epithet is derived from the dense isidia characteristic of this species.

Type: AUSTRALIA. Western Australia, Charles Gardner Flora Reserve, central track, 20 km SW of Tammin along old York Road, 31°47'24"S, 117°28'07"E, 305 m, on dead, charred wood in *Eucalyptus* woodland with *Casuarina* and *Acacia* in shallow gully, *J. A. Elix 31849*, 22 Apr. 2004; holo: PERTH, iso-CANB.

KEY CHARACTERS — **Thallus** lignicolous, crustose to subsquamulose, scattered granules or squamules 0.1–1.2 mm wide, adnate, separate, not proliferating, green-brown, margin somewhat crenulate and ±upturned, squamules soon developing dense granular isidia which dominate the thallus, soredia absent. **Granular isidia** globose to subglobose, 0.03–0.05 mm high, with conspicuously blackened apices, becoming coralloid and densely crowded, forming cerebriform, conglomerate clusters, 0.5–2.0 mm wide. Apothecia and pycnidia not seen. **Chemistry** — K-, C-, KC-, Pd-, UV+ faint white; containing confriesiic acid (major), friesiic acid (minor).

**Distribution** — At present this species is only known from the type locality in Western Australia.

**Ecology** — This species is lignicolous on dead, charred *Eucalyptus* logs. Common associated species include *Flavoparmelia rutidota* (Hook. f. & Taylor) Hale, *Hertelidea pseudobotryosa* R.C. Harris et al., *Hypocenomyce australis* Timdal, *H. foveata* Timdal, *H. scalaris* (Ach.) M. Choisy, *Punctelia subalbicans* (Stirt.) D.J. Galloway & Elix and *Thysanothecium scutellatum* (Fr.) D.J. Galloway.

COMMENTS — *Hypocenomyce isidiosa* is a rare species characterized by the dominant, black-tipped granular, globose to subglobose isidia which coalesce to form cerebriform clusters largely obscuring the primary squamules and the presence of confriesiic and friesiic acids as lichen substances. Friesiic and confriesiic acids are very rare depsido-depsones so far only known from the genus *Hypocenomyce* (Elix et al. 2004; Timdal 1984). Both *Hypocenomyce friesii* and *H. caradocensis* (Leight. ex Nyl.) P. James & Gotth. Schneid. contain friesiic acid (major) and confriesiic acid (minor or trace). The new metabolite confreisiic acid shows an identical ultraviolet spectrum to freisiic acid, but appears to be a higher homologue with standard TLC $R_F$ values: $R_F$ (A) 0.12; $R_F$ (B') 0.23; $R_F$ (C) 0.16; standard HPLC RT=24.5 min. The present species is considered to belong to *Hypocenomyce* because of the unique chemistry and substrate preference, both of which are typical of this genus. Although common at the type locality, no fertile material of *H. isidiosa* was found. The squamules of *H. isidiosa* resemble those of *H. friesii* but are scattered and separate rather than being dense and contiguous to subimbricate. Furthermore, *H. friesii* differs chemically and lacks isidia.

### *Lepraria lobata* Elix & Kalb, **sp. nov.**

*Similis* Lepraria jackii *Tønsberg sed thallo crassiore et lobato differt.*

Etymology: The specific epithet refers to the characteristic lobate margins of this species.

Type: **AUSTRALIA.** Western Australia, slopes of Angwin Peak, Porongurups Range, Porongurups National Park, 19 km ESE of Mt. Barker, 34°40'S, 117°51'E, 360 m, on granite rocks in low sclerophyll forest with heath and numerous granite outcrops, *J. A. Elix 41327, H. T. Lumbsch & H. Streimann*, 16 Sept. 1994; holo: PERTH.

KEY CHARACTERS —**Thallus** leprose-sorediate, granulose, whitish grey to greenish or bluish grey, usually delimited, forming extensive, irregularly spreading patches to 10 cm wide, or in small, irregularly roundish-colonies 0.5–1 cm wide that eventually coalesce; often with well-defined marginal lobes, 1–2 mm wide, ±raised at margins, covered

with granules, thick (up to 250 µm), medulla white, distinct; hyphae 1.6–3 µm thick; soredia farinose, dispersed or forming a thick, continuous layer, ±roundish, 20–75 µm wide, commonly aggregated in roundish clumps (consoredia) up to a 350 µm wide, usually with short projecting hyphae to 20(–100) µm long; photobiont chlorococcoid, with individual cells 7–12 µm wide. Hypothallus not apparent. **Chemistry** — K+ yellow, C-, Pd+ pale yellow; containing atranorin (major), zeorin (major or minor, very rarely absent), rangiformic/jackinic acid (major) or roccellic/angardianic acid (major), ±norrangiformic/norjackinic acid (minor or trace), ±pallidic acid (minor), ±conpallidic acid (minor), ±3'-demethylatranorin (trace), ±3,7-di-O-methylstrepilin (trace), ±unknown dibenzofurans (minor), ±fragilin (trace), ±7-chloroemodin (trace), ±ursolic acid (minor).

**Distribution** — At present this species is known from Western Australia and the Australian Capital Territory.

**Ecology** — This species is corticolous on *Leptospermum* or muscicolous or terricolous in sheltered rock ledges. Common associated species on terricolous substrata include various *Cladonia* and *Xanthoparmelia* species, *Buellia substellulans* Zahlbr., *Lecanora farinacea* Fée and *Parmelia signifera* Nyl., and on corticolous substrata, *Candelariella xanthostigmoides* (Müll. Arg.) R. W. Rogers, *Flavoparmelia rutidota*, *Hypogymnia mundata* (Nyl.) Rass., *Parmelia pseudotenuirima* Gyeln., *Parmelina endoleuca* (Taylor) Hale, *Parmotrema reticulatum* (Taylor) M. Choisy and *Usnea inermis* Motyka.

COMMENTS — *Lepraria lobata* is a scattered species characterized by a relatively thick, grey-white, leprose thallus which typically becomes lobate at the margins and by the presence of atranorin, ± zeorin and fatty acids as the major lichen substances. Chemically *L. lobata* closely resembles *L. jackii* (Tønsberg 1992) in containing atranorin and fatty acids (i.e. jackinic/rangiformic acid, norjackinic/norrangiformic acid and/or roccellic, angardianic and toensbergianic acids) but is distinguished by usually containing high concentrations of zeorin (*L. jackii* rarely contains trace amounts of zeorin). Morphologically *L. lobata* differs from *L. jackii* in having a much thicker and compact thallus, a medullary layer and distinct, lobate margins. *Lepraria jackii* is normally unstratified and lacks lobate margins. *Lepraria borealis* Loht. & Tønsberg is similar to *L. lobata* since it forms lobed, rosette-forming thalli with a more or less granular upper surface and contains atranorin, rangiformic acid and ±roccellic acid. However, *L. borealis* never contains zeorin or pigments and the lobed margins are obscure.

SPECIMENS EXAMINED — **AUSTRALIA.** Australian Capital Territory, S of Paddys River near Murrays Corner, 35°24'S, 148°57'E, 550 m, on bark of *Leptospermum* in thicket, *J. A. Elix 647*, 13 Mar. 1975 (CANB). Western Australia, Gorge Rock, c. 24 km SE of Corrigin, 32°25'S, 118°00'E, 300 m, over moss on sheltered granite ledge, *K. & A. Kalb 35287*, 18 Aug. 1994 (CANB; herb. Kalb).

### *Leprocaulon australasicum* Elix, sp. nov.

*Similis* Leprocaulon microscopicum *(Vill.) Gams ex D. Hawksw. sed superfice cinerascens et acidum protocetraricum, acidum norsticticum et acidum salazinicum continente differt.*

Etymology: The specific epithet is derived from the distribution of this species.

Type: NORFOLK ISLAND. Norfolk Island National Park, West Palm Glen Track, 29°01'06"S, 167°56'33"E, 140 m, on base of *Cyathea* in subtropical forest, *J. A. Elix 29042*, 16 June 1992; holo: CANB.

KEY CHARACTERS — **Primary thallus** crustose, arachnoid-tomentose, thin, persistent, white to off-white or pale cream, irregularly thickening, ±glabrous, becoming verruculose and somewhat leprose-granular, granules scattered, pale orange-brown, 20–40 µm wide. **Secondary thallus** of numerous, pale yellow-orange to orange-brown pseudopodetia, **pseudopodetia** globose at first but then elongate-cylindrical, delicate, fragile, simple or coralloid-branched and entangled, erect or ±decumbent, 0.1–1.0 mm high, 0.1–0.15 mm thick, bearing small, leprose-arachnoid granules, 20–70 µm wide, often with dense, projecting hyphae up to 20 µm long; photobiont chlorococcoid, with individual cells 7–10 µm wide. Hypothallus not apparent. Apothecia and pycnidia unknown. **Chemistry** — K+ yellow becoming dark red, C-, Pd+ orange-red; containing protocetraric acid (major), norstictic acid (minor), salazinic acid (minor), atranorin (minor).

**Distribution** — At present this species is known from three localities in subtropical rainforest in Queensland, New South Wales and Norfolk Island.

**Ecology** — This species is corticolous on the base of *Cyathea* or *Eucalyptus* or is lignicolous. Common associated species include various *Cladonia* species, *Cryptothecia bartlettii* G. Thor, *C. scripta* G. Thor, *Flavoparmelia euplecta* (Stirt.) Hale, *Lepraria atrotomentosa* Orange & Wolseley, *L. coriensis* (Hue) Sipman, *Parmotrema reticulatum*, *P. tinctorum* (Nyl.) Hale, and *Pseudocyphellaria poculifera* (Müll. Arg.) D.J. Galloway & P. James.

COMMENTS — *Leprocaulon australasicum* is a scattered species characterized by the conspicuous, pale yellow-orange to orange-brown pseudopodetia and the presence of protocetraric, salazinic and norstictic acids as the major lichen substances. Morphologically *L. australasicum* resembles *L. microscopicum* but the latter species differs in having a blue-green to yellow-grey upper surface with white pseudopodetia and in containing usnic acid, zeorin, ±atranorin, ±rangiformic acid (Lamb & Ward 1974). Furthermore, *L. microscopicum* grows on thin soil in rock crevices, peaty soils and only very rarely on the base of aged trees rather than predominantly on corticolous or lignicolous substrata.

SPECIMENS EXAMINED — **AUSTRALIA.** Queensland, Noosa Heads, Noosa National Park, along the Noosa Hill Track, 26°23'S, 153°06'E, 30 m, on base of *Eucalyptus* in coastal rainforest, *J. A. Elix 10369*, 31 July 1982 (CANB). New South Wales, Andersons Hill Road, Conglomerate State Forest, 21 km NW of Coffs Harbour, 30°04'S, 153°05'E, 240 m, on shaded rotting log in subtropical forest, *H. Streimann 60554*, 19 April 1998 (CANB). **NORFOLK ISLAND.** Norfolk Island National Park, track between Mt Pitt and Mt Bates, 29°00'50"S, 167°56'05"E, 270 m, on dead *Cyathea* in disturbed subtropical forest, *J. A. Elix 27367*, 15 June 1992 (B, CANB, NY); Norfolk Island National Park, West Palm Glen Track, 29°01'06"S, 167°56'33"E, 140 m, on base of *Cyathea* in subtropical forest, *J. A. Elix 29042*, 16 June 1992 (CANB); Pop Rock, near Mount Pitt Road, 29°01'23"S, 167°56'10"E, on dead *Cupressus* in disturbed subtropical forest, *J. A. Elix 29293*, 18 June 1992 (CANB); Norfolk Island National Park, Mount Pitt Road, 29°01'S, 167°56'E, 300 m, on treelet stem in disturbed subtropical forest, *H. Streimann 34843*, 10 Dec. 1984 (B, CANB).

***Pertusaria cyathicola*** Elix, **sp. nov.**

*Similis* Pertusaria erythrella *Müll. Arg. sed arthothelinum et thuringionum continente differt.*

Etymology: The specific epithet derives from *Cyathea* and the Latin *cola* (dweller) in reference to the substrate of the holotype.

Type: **NORFOLK ISLAND**. Norfolk Island National Park, West Palm Glen Track, 29°01'06"S, 167°56'33"E, 140 m, on base of *Cyathea* in subtropical forest, *J. A. Elix 29043*, 16 June 1992; holo: CANB.

KEY CHARACTERS — **Thallus** crustose, off-white to pale greyish white, slightly cracked and areolate, surface slightly wrinkled and dull, verruculose, soon developing soredia, lacking isidia. **Soralia** yellow to yellow-orange, numerous, conspicuous, disc-like or sub-hemispherical, often constricted at the base, 0.5–2 mm diam., soredia farinose. Apothecia and pycnidia unknown. **Chemistry** — stictic acid (major), constictic acid (minor), arthothelin (minor), thuringione (minor), 3-*O*-methylthiophanic acid (trace), peristictic acid (trace), substictic acid (trace), hypostictic acid (trace), novostictic acid (trace).

**Distribution** — At present this species is only known from the type locality on Norfolk Island.

**Ecology** — This species is corticolous on *Cyathea*. Common associated species include various *Cladonia* species, *Cryptothecia bartlettii*, *Flavoparmelia euplecta*, *Lepraria atrotomentosa*, *L. coriensis*, *Leprocaulon australasicum*, *Parmotrema reticulatum*, *P. tinctorum* and *Pseudocyphellaria poculifera*.

COMMENTS — *Pertusaria cyathicola* is a rare species characterized by the conspicuous yellow to yellow-orange soralia and the presence of arthothelin, thuringione, stictic acid and constictic acid as the major lichen substances. Although no fertile specimens have been seen, this species has typical pertusarioid chemistry comprising the stictic acid chemosyndrome and xanthones. Morphologically *P. cyathicola* closely resembles *Pertusaria erythrella* but the latter species has white soralia and contains lichexanthone and the norstictic acid chemosyndrome (Archer 1997).

## Acknowledgements

I thank Dr Alan W. Archer and Dr Patrick M. McCarthy for helpful discussions and amendments to the draft manuscript.

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MYCOTAXON

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# Some interesting pyrenomycetous fungi on bark of *Quercus* spp. from Spain

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**Abstract**—Four species of pyrenomycetous fungi (*Decaisnella mediterranea, Decaisnella mesascium, Ostreichnion nova-caesariense* and *Rosellinia tassiana*) growing on bark of *Quercus* spp were studied.

**Keywords**—*Pyrenulales*, Ascomycota

## Introduction

During the study of pyrenomycetous fungi from the National Park of Cabañeros (Ciudad Real, Spain), the bark of *Quercus* spp. has been demonstrated as a very good substrate for the development of this kind of fungus.

This National Park is located in the centre of Spain and has one of the best representations of Mediterranean flora on the Iberian Peninsula. Here, there are forests of *Quercus faginea* Lam., *Q. ilex* ssp. *ballota* (Desf.) Samp., *Q. suber* L. and *Q. pyrenaica* Willd., accompanied by *Arbutus unedo* L., heaths and others shrubs. We have compared this area with a holm oak wood situated in Retiendas (Guadalajara, Spain) and found that both places share some interesting species, which are new records for Spain or Europe.

This paper deals with four species [*Decaisnella mediterranea, Decaisnella mesascium, Ostreichnion nova-caesariense* and *Rosellinia tassiana*] and provides new data on their areas and habitats.

## Descriptions

***Decaisnella mediterranea*** Checa & M.N.Blanco, Mycotaxon 91:353 (2005)

**SPECIMENS EXAMINED— SPAIN:** Ciudad Real NATIONAL PARK OF CABAÑEROS: 'EL CARACOL', 30.V.2003, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 32203* (TYPUS). 30.IV.2004, *AH 33904* (PARATYPUS). 'EL LABRADILLO', 30.IV.2004, bark of *Quercus ilex* ssp. *ballota*, leg. J. Checa & M.N. Blanco, *AH 34188, 34186.*

**Comments-** The genus *Decaisnella* Fabre was reinstated by Barr (1979) who recognized two series of species on the basis of ascospore shape (Barr 1986).

*D. mediterranea* with fusiform ascospores, (12)-15-(17) transversal septa and 1-(2) longitunal septa, was described by us (Checa & Blanco 2005). It was found on *Quercus faginea* in the National Park of Cabañeros, and now we add other collections from *Quercus ilex* ssp. *ballota* in the same area.

***Decaisnella mesascium*** (De Not.) M.E. Barr, *N. Amer. Flora*, Ser. 2, 13: 80 (1990)

FIGURE 1, A-B

**Ascomata** scattered or clustered, erumpent to superficial, 0,5-1 mm diam., globose, papilla rounded, periphysate.

**Asci** clavate, with 8 biseriate ascospores, 185-215 x 35-40 µm; **pseudoparaphyses** trabeculate, 2 µm diam.; **ascospores** ellipsoid, 53-60-(64) x 17-22 µm, brown with pallid ends, verruculose, with 7-15 transverse septa and 2-3 longitudinal septa.

**SPECIMENS EXAMINED**— SPAIN: Ciudad Real NATIONAL PARK OF CABAÑEROS: 'ARROYO BREZOSO', 21.XI.2003, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 342224, 34225*. ibid. bark of *Quercus pyrenaica*, *AH* 34235. 'ARROYO MENGÜADOS', 30.IV.2004, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 34202*. 'EL CARACOL', 8.XI.03, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 34223*, 30.IV.2004, *AH 34210, 34226*. 'EL LABRADILLO', 30.IV.2004, bark of *Quercus ilex* ssp. *ballota*, leg. J. Checa & M.N. Blanco, *AH 34187*

**Comments**- Barr (1990) included *D. mesascium* in the group having oblong or ellipsoid ascospores with rounded or obtuse ends.

Although this species has been demonstrated to be very common on bark of *Quercus* spp., this is the first record in Mediterranean area; it seems to be chiefly European as Barr (1990) suggested.

***Ostreichnion nova-caesariense*** (Ellis) M.E.Barr, Mycotaxon 3:84 (1975).

FIGURE 1, C-E

**Ascomata** scattered, superficial, boat-like, 0,8-1,2 x 0,5-0,7 mm, with a longitudinal line of dehiscence.

**Asci** clavate, with 8 biseriate ascospores, 120-130 x 30-35 µm.; **pseudoparaphyses** trabeculate, in gel matrix; **ascospores** ellipsoid, 35-40 x 13-15 µm, sometimes constricted at the primary septum, 7-8-(13) transversal septa, Y-formed in the end cells and 1-3 longitudinal septa, brown, smooth or finely verruculose.

**SPECIMENS EXAMINED**— SPAIN: Ciudad Real NATIONAL PARK OF CABAÑEROS: 'ARROYO BREZOSO', 21.XI.2003, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 34236, 34237*. 'EL CARACOL', 30.V.2003, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 32201, 32202*. 8.XI.2003, leg. J. Checa & M.N. Blanco, *AH 34238*. 30.IV.2004, *AH 34203, 34205, 34244, 34248*. 5-XI-2004, *AH 34213*. 'LAGUNA DE LOS CUATRO MORROS', 5.XI.2004, bark of *Quercus faginea*, leg. J. Checa & M.N. Blanco, *AH 34212*.

**Comments**- The genus *Ostreichnion* Duby is characterized by the conchate ascomata and muriform ascospores. It was created for two species, *O. europaeum* Duby and *O. americanum* Duby, the first one is a synonym of *Hysterium pulicare* Pers. and the second

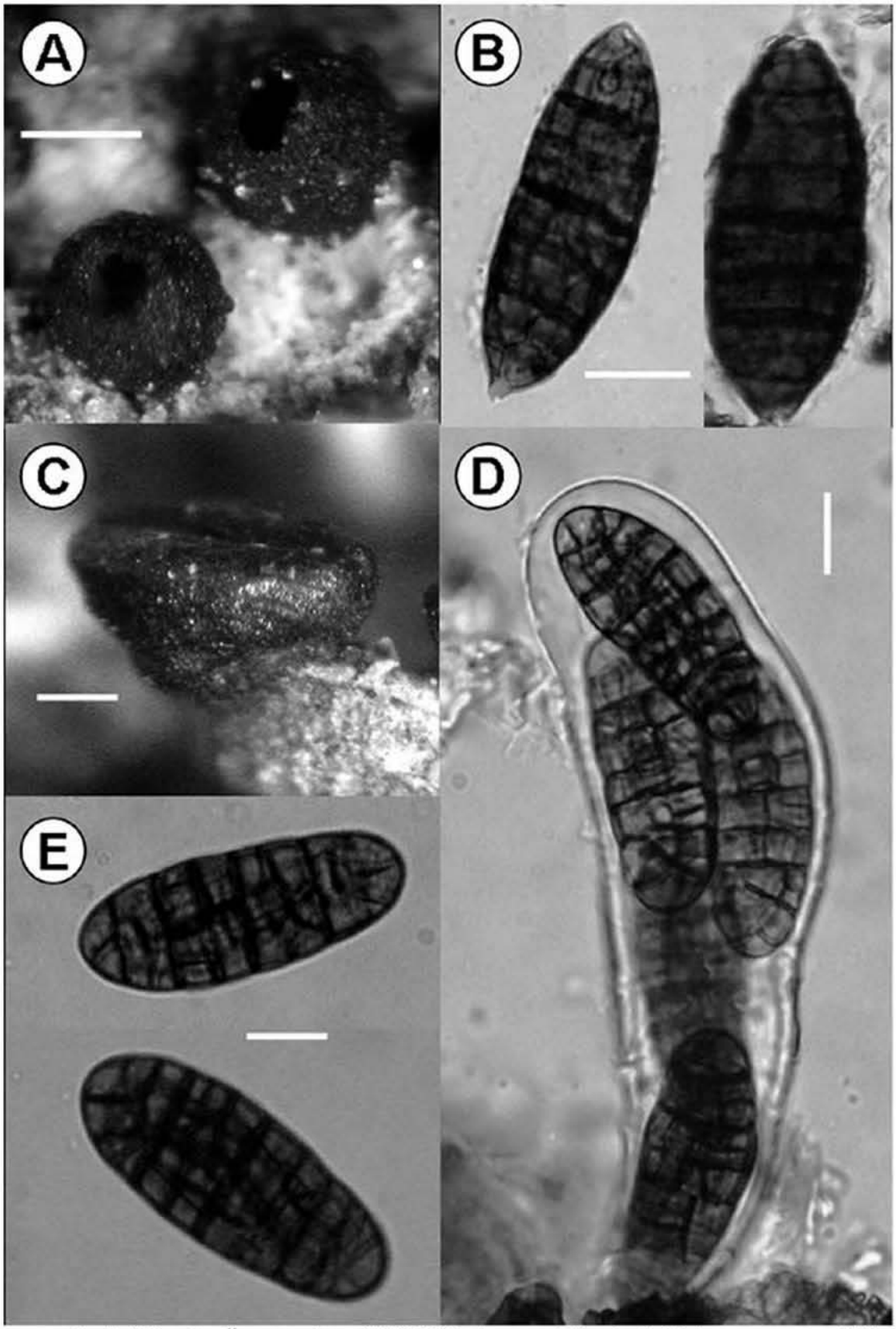


FIGURE 1.- A, B *Decaisnella mesascium*, (*AH 34223*). A.- pseudothecia. B.-Mature ascospores. C-E.- *Ostreichnion nova-caesariense* (*AH 34213*). C.- hysterothecia. D.- Ascus. E.- Ascospores.
Scale bars. A,C- = 0,5 mm. B, D, E = 10 µm.

considered to be the same as *O. sassafras* (Schwein.) M.E.Barr. At present it includes three species: *O. curtisii* (Duby) M.E.Barr, *O. nova-caesariense* and *O. sassafras* (Barr 1975, 1990). These species have been treated in different genera of the *Hysteriaceae*, but the morphology of ascomata and ascospores justifies their inclusion in the *Mytilinidiaceae*.

*O. nova-caesariense* was regarded as a synonym of *Hysterographium flexuosum* (Schwein.) Sacc. (Zogg 1962), however there are some differences between the two genera. The conchate ascocarps, prosenchymatous peridium and trabeculate pseudoparaphyses characterize *O. nova-caesariense* as a member of the *Mytilinidiaceae*.

Barr (1990) indicated the bark of *Pinus rigida* as the substrate for this species, known only from type locality. We add some collections from *Quercus faginea* that provide a wider habitat and distribution of it. Probably, the substrate is not too important to this saprotrophic fungus (Barr, com. pers.).

***Rosellinia tassiana*** Ces. & De Not., *Comm. Soc. crittog. Ital.* 1(4): 227 (1863)

FIGURE 2

**Stromata** scattered or clustered in groups of two or three from a common base, superficial, black, 1-1,5 mm diam., 2-2,5 mm long, subglobose, surface warty, with one papillate perithecium, 800-900 µm diam; ectostroma black, entostroma white.

Asci cylindric, 250-280 x 18-20 µm with 8 uniseriate ascospores; **paraphyses** 4-5 µm diam.; **ascospores** ellipsoid to ovoid, 30-37-(49) x 12-15 µm, dark brown, germ slit nearly spore length, straight, without appendages.

Cultures: Monosporic cultures were made in PDA, but they produced only sterile mycelium not the anamorph state.

**SPECIMENS EXAMINED— SPAIN:** Ciudad Real NATIONAL PARK OF CABAÑEROS: 'ARROYO BREZOSO', 6.XI.2004, bark of *Quercus ilex* ssp. *ballota*, leg. J. Checa & M.N. Blanco, *AH 34211*. **Guadalajara,** RETIENDAS: 30.I.2003, bark of *Quercus ilex* ssp. *ballota*, leg. J. Checa & M.N. Blanco, *AH 35129*. 12.II.2003, *AH 35125, 35126, 35127, 35128*.

**Comments**- This species commonly grows on *Quercus* spp., but until now it was only known from Italy (Petrini 1993). This author considered *R. tassiana* to be related to uniperitheciate *Xylaria* or *Kretzschmaria* species and excluded it from *Rosellinia*.

The anamorph state is not known and our cultures from ascospores in PDA and MEA only produced sterile mycelium.

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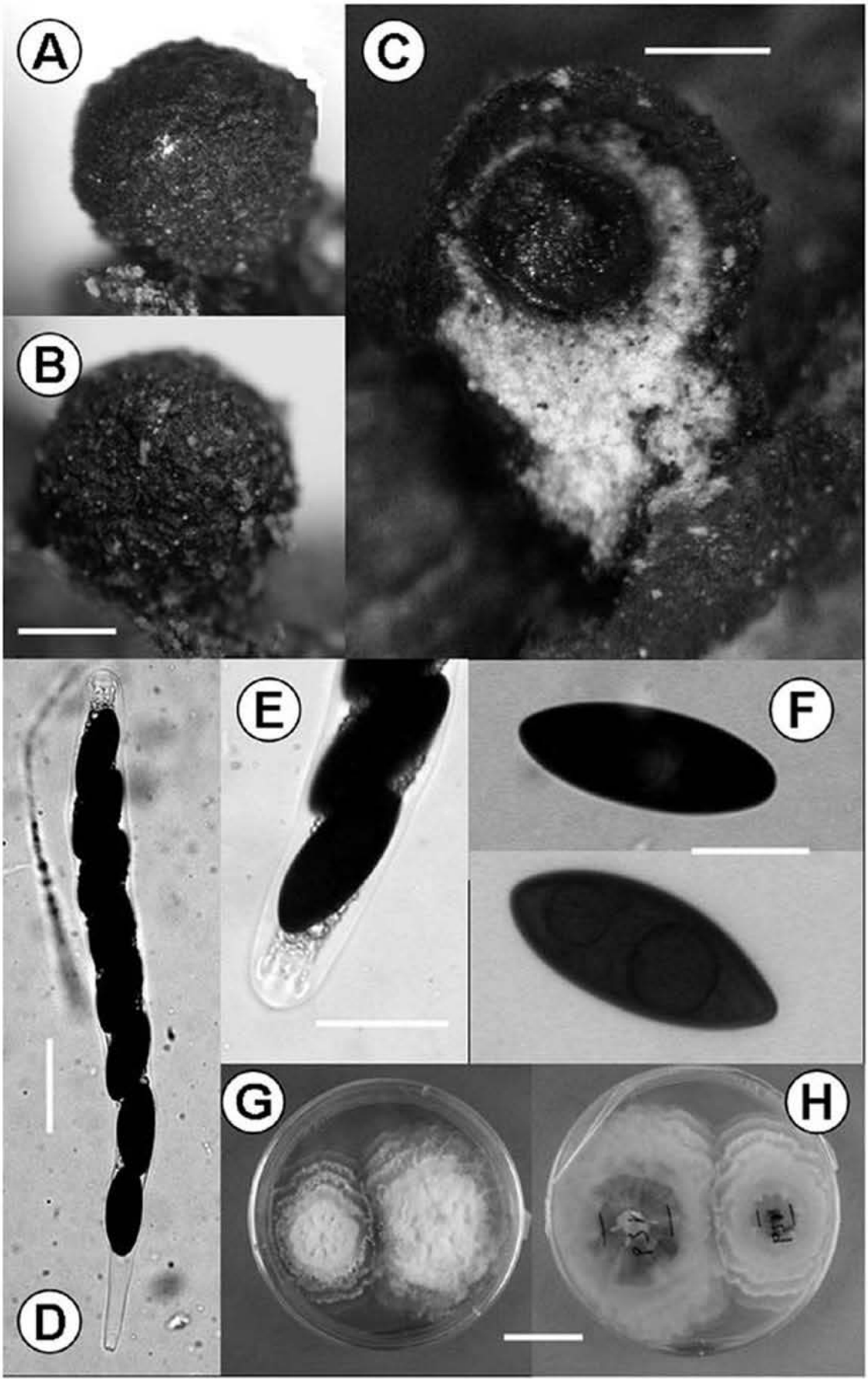
FIGURE 2.- *Rosellinia tassiana* (*AH 35127* ) A, B.- Stromata. C.- longitudinal section of stroma. D.- Ascus. E.- Ascus apex. F.- Ascospores. G, H.- cultures; G- obverse; H- reverse.
Scale bars. A-C = 0,5 mm. D-E 35 µm. F = 15 µm. G-H = 2 cm.


A
B
C
D
E
F
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MYCOTAXON

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# Biogeography and hosts of poroid wood decay fungi in North Carolina: species of *Fomes, Fomitopsis, Fomitella* and *Ganoderma*

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**Abstract**—Distribution and host species are given for two species of *Fomes*, one species of *Fomitella*, four species of *Fomitopsis* and five species of *Ganoderma*. A county distribution map is provided for eight species. Numerous new fungus-host plant associations are reported. Species checklist and figures can also be accessed at: http://www.cals.ncsu.edu/plantpath/people/faculty/grand/mycotaxon_4.pdf.

**Key words**—fungus distribution, polypores

## Introduction

The importance of biodiversity and biogeography of fungi, especially in unique ecosystems and specific regions, was previously addressed by Grand & Vernia (2004ab, 2005). Studies by Jung (1987), Vernia & Grand (2000) and Grand & Vernia (2002, 2003) reported on the occurrence of host plants of poroid wood decay fungi in North Carolina. The distribution and host plants in North Carolina of species of *Phellinus* and *Schizopora* (Grand & Vernia 2004a), *Ceriporia, Ceriporiopsis* and *Perenniporia* (Grand & Vernia 2004b) and *Coltricia, Coltriciella* and *Inonotus* (Grand & Vernia 2005) were previously addressed. This report is the fourth in a continuing study of poroid wood-decay fungi in North Carolina and deals with species of *Fomes, Fomitella, Fomitopsis* and *Ganoderma*.

## Materials and methods

Poroid wood-decay fungi were intensively collected in North Carolina over the past eight years (1997-2004). Collections, housed in the Mycological Herbarium, Department of Plant Pathology, North Carolina State University (NCSC), and records of the Plant Disease and Insect Clinic, Department of Plant Pathology, NCSU, were utilized in the results. Previous studies (Grand et al. 1975, Jung 1987) that contained data on county distributions were used in developing the distribution maps. Similarly, data from the BPI website (Farr et al. n.d.) provided some county data.

Collections were made of all species of *Fomes, Fomitella, Fomitopsis* and *Ganoderma* species on unusual hosts. Specimens were placed in paper bags in the field with a sample of decayed wood with most collections and field notes for all collections. Specimens

were examined in the laboratory and identified using existing taxonomic treatments (Gilbertson & Ryvarden 1986, Jung 1987, Overholts 1953).

Nomenclature and authorities are from Gilbertson & Ryvarden (1986) and Index Fungorum (CABI Biosciences et al.) for the fungi and Kartesz (1994) for the host plant species.

The majority of collection sites were in state parks, gamelands and natural areas, Nantahala, Pisgah, Croatan and Uwharrie National Forests, the Blue Ridge Parkway and the Great Smoky Mountains National Park. A county distribution map is provided for all species that were recorded in three or more counties (Figs. 1–8).

## Results and discussion

*Fomes fomentarius* (L. : Fr.) J. Kickx f. (Fig. 2) was found on six host species in 12 western counties, all in the Blue Ridge Mountains of the Southern Appalachian Mountain chain. *Fomes fasciatus* (Sw. : Fr.) Cooke (Fig. 1), a species with a distribution in the southern United States (Gilbertson & Ryvarden 1986), was collected for the first time in North Carolina, in three counties in the Coastal Plain and southern Piedmont regions. It appears that *F. fomentarius* reaches its southernmost distribution in the southern mountains of North Carolina and Tennessee and that *F. fasciatus* reaches its northernmost distribution in southern North Carolina.

*Fomitopsis cajanderi* (P. Karst.) Kotl. & Pouzar (Fig. 3) was found in 15 counties in the Blue Ridge Mountains and Piedmont regions and was recorded on five host species. *Fomitopsis pinicola* (Sw. : Fr.) P. Karst. (Fig. 4) was found in eight counties in the Blue Ridge Mountain region of western North Carolina.

*Fomitella supina* (Sw. : Fr.) Murrill, *Fomitopsis durescens* (Overh. ex J. Lowe) Gilb. & Ryvarden and *Fomitopsis spraguei* (Berk. & M.A. Curtis) Gilb. & Ryvarden were not collected frequently enough to determine any distributional patterns.

Five species of *Ganoderma* were recorded in this study. *Ganoderma applanatum* (Pers.) Pat. (Fig. 5) was found in 19 counties on 22 host species. *G. applanatum* is primarily distributed in the Blue Ridge Mountains of western North Carolina but collections were made in the eastern Piedmont and northern Coastal Plain regions as well. *Ganoderma tsugae* Murrill (Fig. 8), which is primarily found on *Tsuga canadensis* Carrière in North Carolina, was also found on *Abies fraseri* (Pursh) Poir. and *Pinus pungens* Lamb. *Tsuga caroliniana* Engelm. is most likely a host as well. Dead, needleless trees of *T. caroliniana* are difficult to distinguish from *T. canadensis*. *Ganoderma tsugae* is distributed in nine counties in the Blue Ridge Mountains in western North Carolina with a single report from a disjunct population of *T. canadensis* in the Piedmont.

*Ganoderma lucidum* (Curtis : Fr.) P. Karst. (*sensu lato*) is morphologically variable (Gilbertson & Ryvarden 1986) and considered by most taxonomists to be a species complex. With the exception of *Ganoderma curtisii* (Berk.) Murrill, *G. lucidum* was considered in the broad species concept in this study. *G. lucidum* (Fig. 7) is widely distributed in North Carolina and was found in 29 counties on 29 host species. The species concept of *G. curtisii* in this study was limited to those basidiocarps with a well-developed stipe, eccentric pileus and typically forming from underground roots near stumps. *G. curtisii* (Fig. 6) is widely distributed in North Carolina and was found in 11 counties on 14 host species.

Fig. 1. Distribution of *Fomes fasciatus* in North Carolina.

Fig. 2. Distribution of *F. fomentarius* in North Carolina.

Fig. 3. Distribution of *Fomitopsis cajanderi* in North Carolina.

Fig. 4. Distribution of *F. pinicola* in North Carolina.

Fig. 5. Distribution of *Ganoderma applanatum* in North Carolina.

Fig. 6. Distribution of *G. curtisii* in North Carolina.

Fig. 7. Distribution of *G. lucidum* in North Carolina.

Fig. 8. Distribution of *G. tsugae* in North Carolina.

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MYCOTAXON

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# Two new species of *Ramaria* from southwestern China

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**Abstract**—Two new species of the genus *Ramaria* collected from southwestern China are described and illustrated. They are *Ramaria luteoaeruginea* and *Ramaria pallidolilacina*. The former belongs to subgenus *Echinoramaria* while the latter to subgenus *Laeticolora*. Discussions on these two species and their affinities are furnished. The holotypes are deposited in the Cryptogamic Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (HKAS).

**Key words**—clavarioid fungi, taxonomy

## Introduction

Southwestern China, including Yunnan, Sichuan, Tibet and Guizhou, is rich in taxa of *Ramaria* Fr. ex Bonord. Several new species have been discovered and reported from this area previously (Petersen 1987a; Petersen & Zang 1986, 1989, 1990). While studying the *Ramaria* samples collected from the region, the authors have recently found two additional new species. They are reported herein.

Microscopic examinations were made with bright field and phase contrast optics. Measurements of spores were made in 5% KOH solution. The abbreviation [*n/m/p*] shall mean *n* basidiospores measured from *m* basidiocarps of *p* collections. Dimensions for basidiospores are given with notation of the form *(a)b–c(d)*. The range *b–c* contains a minimum of 90% of the measured values. Extreme values, *i.e.*, *a* or *d*, are given in parentheses. *Q* is used to mean 'length/width ratio' of a basidiospore in side view; *Q* means average *Q* of all basidiospores ± sample standard deviation. For demonstration

of spore ornamentation, aniline blue (cotton blue) staining was used following the method described by Kotlaba & Pouzar (1964). Color codes of the form "3B3" are from Kornerup & Wanscher (1981); Color names with first letter capitalized (e.g., Cream Color) are from Ridgway (1912).

## Taxonomy

**1. *Ramaria luteoaeruginea*** P. Zhang & Zhu L. Yang, **sp. nov.** **Figs. 1-4**

*Basidioma ramosum, ad 7 × 5 cm, obovatum ad obconicum;stipite ad 3 × 3 cm, crasso, singulari, albo, interdum aeruginescenti, cum ramulis abortivis, carne alba, non-gelatinosa; ramis crassis, luteis; apicibus acutis ad digitatis, aerugineis; basidiis 55–70 × 7–9 µm, clavatis, 4-sporigeris; sporis (9.5–) 10.5–12.5 (–13.0) × (4.5–) 5.0–6.0 (–6.5) µm, ellipsoideis vel elongato-lacrymiformibus, subechinatis. Fibulae praesentes.*

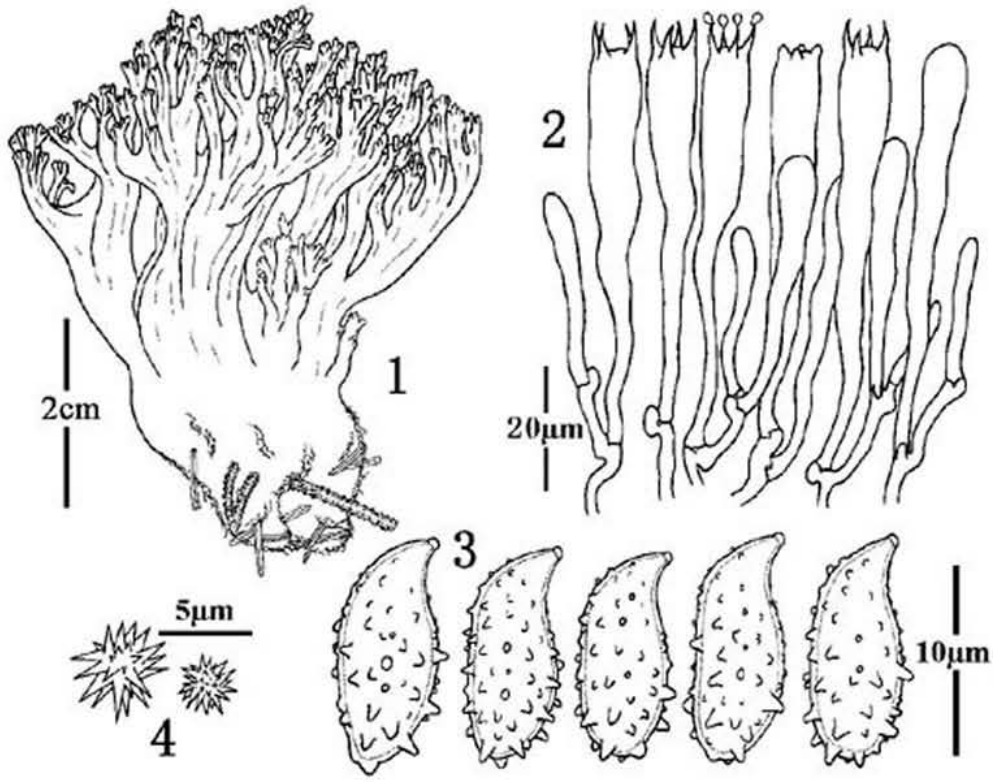
Holotype: Zhu L. Yang 4314 (HKAS 45693), 7. VIII. 2004, Changdu, Tibet, China.

Etymology: Referring to the colors of the yellow branches and more or less green apices.

*Basidioma* (Fig. 1) up to 7 cm high, up to 5 cm broad, repeatedly branched, usually obovate to obconic in outline. *Stipe* up to 3 × 3 cm, single or falsely fasciculate, smooth upward, tapering gradually downward into a tangle of white mycelia and slender rhizomorphs involving significant amounts of substrata, white [1A1], unchanging on drying, staining locally to intense verdigris [25C4-6, Montpellier Green, Light Porcelain Green] when fresh, remaining so after drying, with a few abortive stumps high on stipe; flesh white, solid, not gelatinous or slippery, dried flesh firm but easily penetrated. *Major branches* 4–6, stout, more or less terete, ascending, white below, upward concolorous with upper branches. *Branches* 4–5 ranks, ascending, polychotomous, longitudinally rugose; internodes diminishing gradually upward, buff-yellow [3A2-6, Straw Yellow, Warm Buff] below, gradually concolorous with apices; axils more or less rounded. *Apices* 1–2 mm long, acute to finger-like, dull blue-green [29D4-7, Dark Dull Yellow-Green]. *Taste and odor*, and *macrochemical reactions* not recorded.

*Stipe tramal hyphae* 2–8 µm wide, hyaline, thin-walled (wall up to 0.5 µm thick), parallel, clamped; ampulliform clamps occasional; gloeoplerous hyphae not observed. *Tramal hyphae of upper branches* 2–15 µm wide, hyaline, thin-walled, loosely parallel, clamped; ampulliform clamps occasional, up to 10 µm broad, thin-walled. Hyphae of basal mat 1.5–3.0 µm diam, thin-walled, clamped; stellate crystalline material (Fig. 4) often found between hyphae. *Subhymenium* rudimentary, hyphal. *Hymenium* thickening. *Basidia* (Fig. 2) 55–70 (–80) × 7–9 µm, clavate, clamped; contents homogeneous when young, becoming multiguttulate when mature, hyaline or slightly yellowish, slightly cyanophilous, 4-spored; sterigmata 4–6 µm long.

*Basidiospores* (Fig. 3) [40/2/1] (9.5–) 10.5–12.5 (–13.0) × (4.5–) 5.0–6.0 (–6.5) µm [Q = (1.90–) 2.00–2.36 (–2.40), Q = 2.19 ± 0.14], elongate pip-shaped, with apex mostly rounded, sometimes somewhat attenuate, with a distinct suprahilar depression, yellow-ocher under bright field; wall slightly thickened (up to 0.5 µm thick), moderately cyanophilous; apiculus not conspicuous, eccentric; ornamentation of numerous, scattered, strongly cyanophilius warts or obtuse spines up to 1 µm long.




Figs. 1–4: *Ramaria luteoaeruginea* (HKAS 45693, holotype).
1. Basidioma; 2. hymenium; 3. Basidiospores; 4. Crystal from basal mat.

**Habitat:** Gregarious on humus under *Picea*.

**Known distribution:** Only known from the type locality.

**Notes:** *Ramaria luteoaeruginea* is characterized by its stocky fruitbodies naturally staining blue-green both on stipes and apices in the field even without bruising, middle size spores [i.e., larger than those of *R. abietina* (Pers.: Fr.) Quél., but smaller than those of *R. grandis* (Peck) Corner], and subspinous spore ornamentation. It is a member of *Ramaria* subgenus *Echinoramaria* Corner (Corner 1950; Petersen 1981).

*Ramaria luteoaeruginea* is similar to *R. echinovirens* Corner et al., *R. glaucoaromatica* R.H. Petersen and *R. ochrochlora* Furrer-Ziogas & Schild. However, *R. echinovirens*, described from the Indian Himalayas, has orange yellow apices and was collected under *Quercus*. *Ramaria glaucoaromatica*, originally described from the Rockies, United States, can be separated from *R. luteoaeruginea* on its dull ocher apices and smaller spores (Petersen 1981). *Ramaria ochrochlora*, from the Alps in Switzerland, is distinguished from *R. luteoaeruginea* by its greenish yellow apices and smaller spores (Petersen 1981; Schild 1971).

These four species appear to be very closely related in having stocky fruitbodies, virescent color reactions and spinous or warty spores, and tending to occur in high elevation areas.

Singh (1977) reported a taxon from India, which is quite similar to *R. luteoaeruginea*. Branch tips of the specimens were noted as green. He identified the specimens as *R. ochrochlora*. The identification was dubious, because *R. ochrochlora* stained green only at the base, rather then on tips.

**2. *Ramaria pallidolilacina*** P. Zhang & Z. W. Ge, **sp. nov.** Figs. 5-7

*Basidioma ramosum, ad 13 × 10 cm, obovatum ad subglobosum; stipite ad 3 × 2 cm, attenuato, singulari, albo ad cremeo, carne alba, non-gelatinosa; ramis pallide lilacinis, subrugulosis vel rugulosis, apicibus scopiformibus vel brevi-digitatis, cum ramulis concoloris; basidiis 60–85 × 9–11 µm, clavatis, 4-sporigeris; sporis (10.0–) 10.5–13.0 (–14.0) × (4.5–) 5.0–6.0 (–7.0) µm, ellipsoideis, irregulariter verruculosis. Fibulae absentes.*

**Holotype:** Z. W. Ge 332 (HKAS 46112), 11. VIII. 2004, Leiwuqi, Tibet, China.

**Etymology:** Referring to the color of the branches.

*Basidioma* (Fig. 5) up to 13 cm high, up to 10 cm broad, repeatedly branched, usually obovate to subcircular in profile. Stipe up to 3 × 2 cm, single, tapering gradually downward, tomentose at base, smooth upward, without abortive branches, white to cream [1A1, 2A2], not changing color on bruising; flesh white, solid, not gelatinous or slippery. *Major branches* 4–6, stout, up to 1 cm thick, ascending, concolorous to branches above. *Branches* 4–5 ranks, ascending, more or less rugulose, polychotomous, pallid lilac [15A2-3; Light Vinaceous-Gray, Pale Light Vinaceous-Purple], slowly becoming purple-gray [14A2; Vinaceous-Gray] with spore deposit; internodes diminishing gradually upwards; axils more or less rounded. *Apices* obtuse, rather crowded, 0.5–1 mm long, molar-like when young, broom-form or short-digitate by maturity, concolorous with the branches. *Taste and odor*, and *macrochemical reactions* not recorded.

*Tramal hyphae of stipe* 3–15 µm wide, hyaline, thin-walled, clampless, interwoven; ampulliform septa and gloeoplerous hyphae not observed. *Tramal hyphae of upper branches* 3–10 µm wide, hyaline, thin-walled, loosely parallel, clampless; gloeoplerous hyphae not observed. *Subhymenium* rudimentary, hyphal. *Hymenium* unthickening. *Basidia* (Fig. 6) 60–85 × 9–11 µm, clavate, clampless, cyanophilous, 4-spored; sterigmata 6–8 µm long.

*Basidiospores* (Fig. 7) [60/2/2] (10.0–) 10.5–13.0 (–14.0) × (4.5–) 5.0–6.0 (–7.0) µm [Q = (1.62–) 1.85–2.40 (–2.60), **Q** = 2.14 ± 0.20], ellipsoid, more or less flattened adaxially, roughened in profile; wall up to 0.5 µm thick, cyanophilous; hilar appendix prominent; ornamentation of prominent, discrete, low warts and short ridges randomly placed.

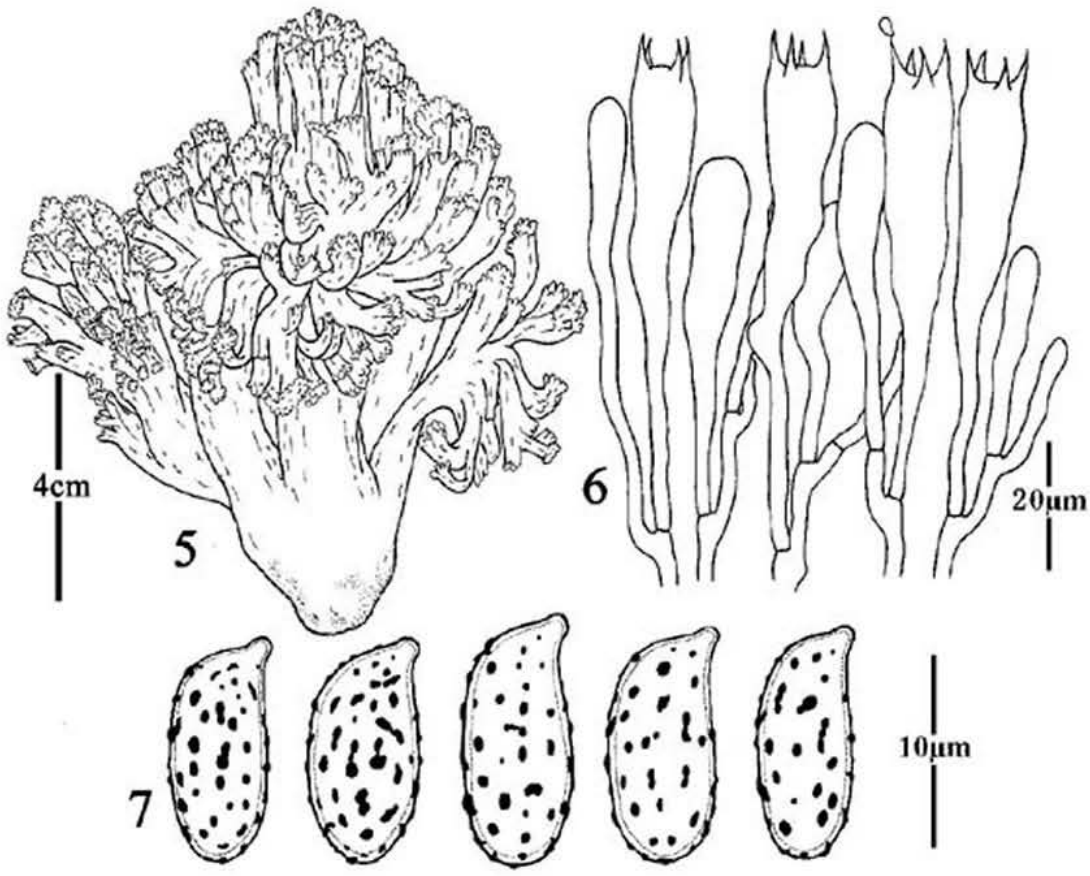
**Habitat:** Solitary on soil in *Picea* forest.

**Known distribution:** Only known from southwestern China.

**Additional material examined:** CHINA, Tibet, Jiangda County, near Tongpu village, alt. 3300m, 2-VIII-2004, Z. W. Ge 260 (HKAS 46040).

**Notes:** *Ramaria pallidolilacina* is characterized by its pallid lilac branches and apices, relatively small, white, tapering stipe, clampless basidia and coarsely ornamented spores. It belongs to *Ramaria* subgenus *Laeticolora* Marr & D. E. Stuntz (Marr & Stuntz 1973; Petersen 1987a, 1987b; Petersen & Zang 1989, 1990).

A group of taxa within *Ramaria* subgenus *Laeticolora* with lilac, violet or purple coloration has been described previously (Petersen 1987b). Several species included in this group such as *R. asiatica* (R.H. Petersen & M. Zang) R.H. Petersen, *R. cedretorum* (Maire) Malençon, *R. fennica* (P. Karst.) Ricken, *R. himalayensis* R.H. Petersen, *R. purpurissima* R.H. Petersen & Scates, *R. versatilis* Quél., can be easily separated from *R. pallidolilacina* by producing clamped basidia. Although *R. pallida* (Schaeff.) Ricken (=




**Figs. 5–7:** ***Ramaria pallidolilacina*** (HKAS 46112, holotype).
5. Basidioma; 6. hymenium; 7. Basidiospores.

*R. mairei* Donk) is a clampless species with purplish fruitbodies, its purple tints exhibit only at the apices. Furthermore, its stipe is more conspicuous than *R. pallidolilacina* (Petersen 1974). *Ramaria spinulosa* (Pers.: Fr.) Quél., reported from Europe and North America, with purple tinged fruitbody and clampless basidia, is also similar to *R. pallidolilacina*. But the former differs from the latter by its unique cinnamon tan stipe surface and pale beige stipe flesh (Petersen 1985; Schild 1990). *Ramaria subspinulosa* (Coker) Corner has smaller spores, and a tinge of lavender appearing only on the upper part of fruitbody when young (Coker 1923; Corner 1950; Petersen 1987b). *Ramaria fumosiavellanea* Marr & D. E. Stuntz, originally described from North America, with violet color component and clampless basidia, is distinguished from *R. pallidolilacina* by its darker (15-18D3) branches with grayish orange (5B3) apices and less ornamented spores (Marr & Stuntz 1973).

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# The world's second record of *Neoheteroceras flageoletii* reported from Turkey

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**Abstract**—*Neoheteroceras flageoletii*, a rare coelomycete with appendage-bearing conidia, is reported and illustrated from Turkey on *Tilia rubra* subsp. *caucasica*. It is a second locality of this fungus.

**Key words**— anamorphic fungi, morphology

## Introduction

Fungi of Turkey have not been extensively investigated. Most studies research macromycetes, generally agaricoid fungi. Reports on micromycetes, including anamorphic fungi, were made by Bremer et al. (1947, 1952) and Petrak (1953). Data concerning the anamorphic fungi were first published as information on diseases of plants (Karel 1958) or fungal diversity investigations (Göbelez 1967). Anamorphic fungi of Turkey have been studied extensively, however, during the last ten years (Altan & Tamer 1996, Hüseyinov & Selçuk 1999, Braun et al. 2000, Hüseyinov 2000, Hüseyin & Selçuk 2001, Hüseyinov et al. 2002, Hüseyin et al. 2003, Selçuk et al. 2003, Mel'nik et al. 2004, Kırbağ 2004).

## Material and Methods

The plant material for this paper was gathered from the forest of Rize province (Eastern Black Sea Region) in July 1998.

The host plant was identified according to Davis (1967). Specimens of the fungus were taken to the laboratory and microscopically examined under a Nikon compound microscope. Sections were hand cut using a razor blade. The fungus was identified following Nag Raj (1993). The specimen was deposited at GAZI.

## Results

In this study, we clearly demonstrated that the fungus is a species of *Neoheteroceras*, viz. *N. flageoletii*. The description and illustration below is based on the Turkish material.

*Neoheteroceras flageoletii* Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia, p. 539, 1993.

**Conidiomata** stromatic, subepidermal, innate-erumpent, broad conical in sectional view, 220–500 µm in diam in the base, uni- or bilocular, dark brown (Fig. 1A). **Conidiophores** cylindrical, simple, sparsely septate at the base, colourless. Paraphyses unbranched, filiform, septate, colourless, 50–65 x 2.5–3 µm. **Conidia** oblong to fusiform or stenoclavate, bearing appendages, 7– septate, faintly constricted at the septa, 45–58 x 7.5–10 µm including the apical and basal appendages; basal cell obconic with a truncate base, colourless, cellular appendage, 5–10 µm long; median cells dirty-brown to amber-brown 35–40 (–45) µm long, wall thick, smooth or slightly verruculose; apical cell conic, colourless, drawn out at the apex into an unbranched, attenuated, tubular appendage, 10–20 µm long; 1–2 sometimes 3 lateral appendages arising from the second, third and sixth median cells from the top, cellular, unbranched, colourless, sinuate, 7.5–22.5 x 2.5–3 µm (Fig. 1B).

Specimen examined—TURKEY, Rize Prov., Ardeshen district, near the Nursery of General Forest Manager, on dead branches of *Tilia rubra* DC. subsp. *caucasica* (Rupr.) V. Engler (*Tiliaceae*), 41°21'14"N, 41°13'50" E, 670 m, 30-VII-1998, Co. HUSEYIN E (EH 1281) and SELCUK F (FS 0206).

## Discussion

The morphological characters of *Neoheteroceras flageoletii* of Turkish sample show some distinctions from the original collection. According to Nag Raj (1993) the fungus has conidia 48–60 x 7–11 µm, basal cellular appendage 3–8 µm long, apical cellular appendage 14–22 µm long and conidia with 2–3 lateral appendages, 12–30 x 2–3 µm. The fungus is terrestrial saprotroph, which requires a high level humidity and inhabits the bark of dead branches 0.5-1 cm diam.

Saccardo (1915) first described this fungus on *Tilia europaea* from France, as *Heteroceras flageoletii*. However, the monotypic genus *Heteroceras* Sacc. is an illegitimate later homonym, and consequently the species name is invalid. When Nag Raj (1993) re-examined the holotype, he published *Neoheteroceras* as a nom. nov. for the genus and *N. flageoletii* as a nom. nov. for the species. Until now there has been no additional information about this fungus. We think that our report from Turkey is the second registration of this fungus in the world.

The teleomorph of this species is unknown.

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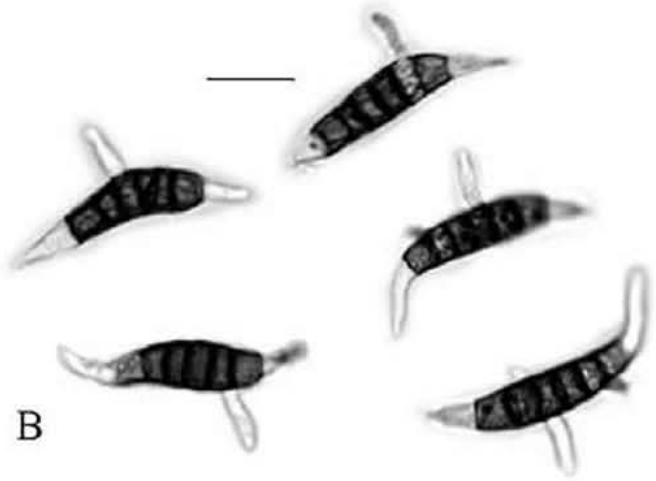


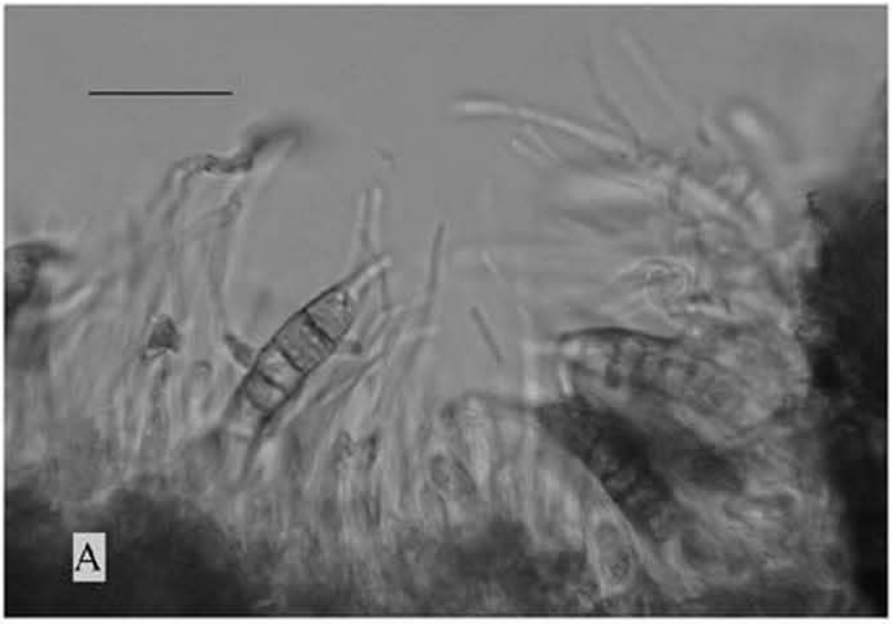


**Fig.1.** *Neoheteroceras flageoletii*: A.-vertical section of a conidioma. Scale bar=50 µm; B.-natural conidia. Scale bar=15 µm.

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# *Lewia chlamidosporiformans* sp. nov. from *Euphorbia heterophylla*

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**Abstract**—*Lewia chlamidosporiformans* found associated with the weed *Euphorbia heterophylla* (wild poinsettia) was collected in Brazil and is newly described and illustrated. It differs from the other species in the genus by having longer ascospores that are surrounded by a mucilaginous sheath and asci that have a different shape (obovoid to obpyriform). It produces abundant chlamydospores when grown in liquid media. Its pathogenicity to wild poinsettia was demonstrated.

**Key words**—*Pleosporaceae, Euphorbiaceae, Ascomycota*

## Introduction

During isolations of fungal pathogens of weeds occurring in Brazil, cultures of a fungus showing typical features of members of *Lewia* (Simmons 1986a) were obtained from leaf spots on wild poinsettia (*Euphorbia heterophylla* L). *Alternaria*-like conidia were also produced. Members of *Alternaria* are known to be anamorphs of *Lewia*, helping to confirm that the fungus newly collected belonged to *Lewia*. This paper describes the teleomorphic stage of this fungus.

Although there are 50 known species of *Alternaria*, according to Kirk et al. (2001), only 5 species of *Lewia* have been described. Nevertheless, the total number or species presently included in this genus is 7 namely: *L. intercepta*, *L. infectoria*, *L. photistica*, *L. avenicola*, *L. ethzedia*, *L. sauropi*, *L. scrophulariae* (Simmons 1986a,b; Zhang & David, 1996; Simmons 2002; Kwasna & Kosiak 2003). Barreto & Evans (1998) provided a complete list of the fungal pathogens known in association with wild poinsettia but the list does not include any species of *Lewia* or *Pleospora* (the genera from which *Lewia* was segregated). There are also no recent reports of species in these genera associated with this host. A comparison of the fungus on wild poinsettia with the other species in the genus led to the conclusion that this represents a new species, which is described below.

## Material and Methods

Cultures were obtained on VBA medium (vegetable broth-agar), a general-purpose medium (Pereira et al. 2003). Semi-permanent slides were prepared and mounted in lactophenol. Observations, measurements and photographs were made using an

Olympus BX 50 light microscope, fitted with a camera. Dry cultures of the fungus were prepared by following the methodology described by Rossman & Simmons (1999) and representative specimens of the fungus were deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC).

When grown in a liquid medium Jenkins-Prior (Fargues et al. 2001) under agitation at 180 rpm with a temperature of 25 ± 2 °C, the fungus formed abundant chlamydospores starting after 3 days of cultivation.

Confirmation of the pathogenicity of the isolate was made by utilizing a chlamydospore suspension, produced as described above, as inoculum. Inoculation of *E. heterophylla* plants was performed as follows: the concentration of the suspension was adjusted to 1 x $10^7$ chlamydospores/ml with the help of a hemocytometer. Tests plants were brush-inoculated with the suspension on both sides of all leaves of the plants. After the inoculation, the plants were maintained in a dew chamber at 25 °C for 48 hours and later transferred to a greenhouse where they were observed daily for the appearance of symptoms. Plants brushed with sterile water served as controls. The experiment was accomplished with three replicates, each replicate consisting of a pot containing a 20-day old plant.

## Taxonomic Description

***Lewia chlamidosporiformans*** B.S. Vieira & R.W. Barreto, **sp. nov.** FIGS 1-3

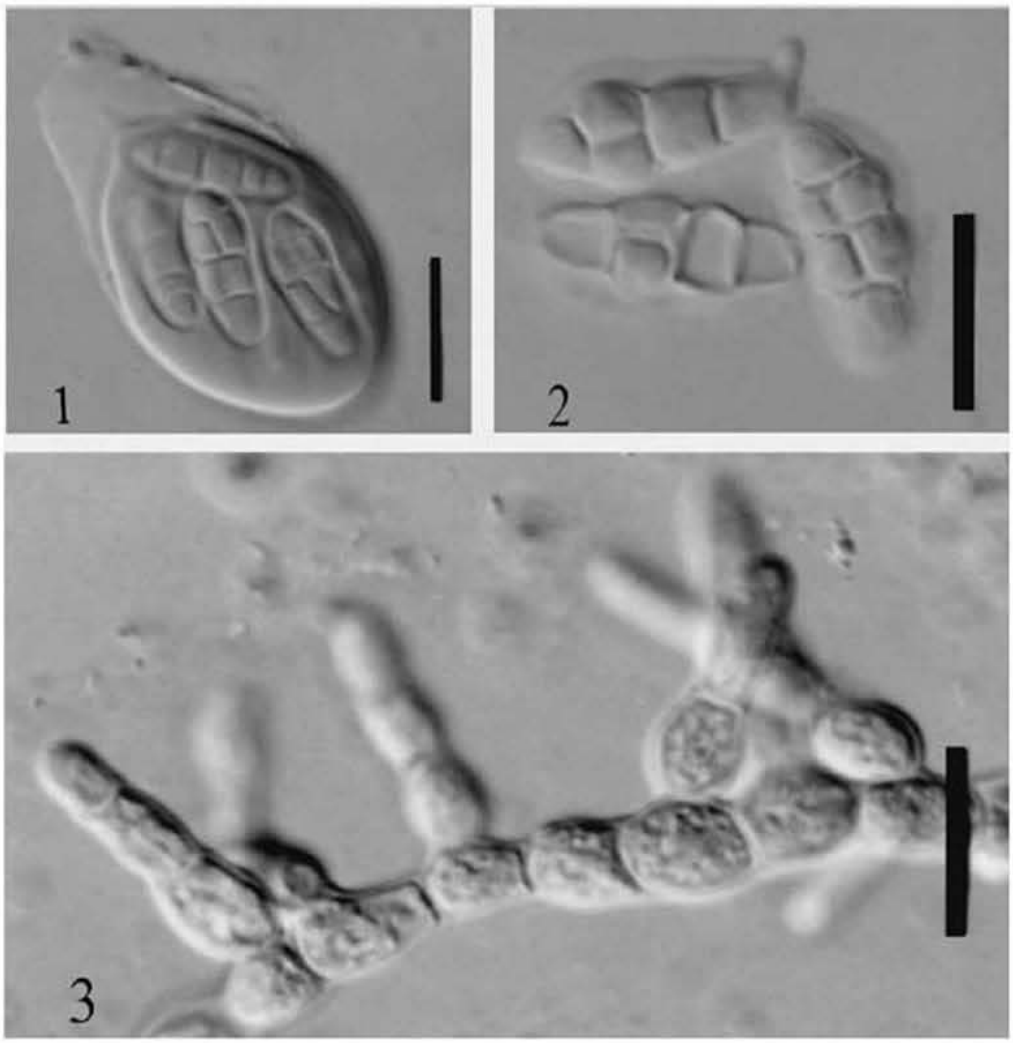
*Ab* Lewia photistica *Simmons; In VBA ascomata 145-190 x 115-160 µm, asci obovoidei vel obpiriformei, 80-120 x 37-44µm, ascosporae 25-37 x 10–12, cum tunica mucosa involutae, differens.*

Etymology: reference to the abundant formation of chlamydospores in liquid culture.

Holotype: on *Euphorbia heterophylla*, Brazil, Minas Gerais, Viçosa, January 2005, B.S. Vieira (VIC-28732).

*Colonies* on VBA (amended with 20 glucose g/L) 50 mm diam. after 7 days at 25 °C under a 12 hours photoperiod, cottony, grey, with some whitish areas, dark green reverse, with diurnal zonation with ascomata formation and sporulation. *Ascomata* pseudothecial, globose, dark brown, often immersed in the culture medium, 145-190 x 115-160 µm. *Asci* obovoid to obpyriform, thin-walled, bitunicate, 80-120 x 37-44 µm, 8 spored. *Ascospores* inordinate fusiform, often rounded at the apex and subacute at the base, usually constricted at the subapical transversal septum, 25-37 x 10-12 µm, subhyaline to very light brown, smooth, mature ascospores usually with 2 to 5 transverse septa and 1 or 2 longitudinal septa, surrounded by a distinct mucilaginous sheath. *Chlamydospores* formed abundantly in liquid culture media, in hyphae, intercalary, mostly in chains, 1-celled, subspherical, subcylindrical or irregular, 7 - 17 µm diam, thick walled, smooth, subhyaline to very light brown. *Anamorph*: a typical *Alternaria*.

Comments — Ascomata of *L. chlamidosporiformans* were produced *in vitro*, in synthetic media, namely: Kondryatiev medium and Goral medium (Fargues et al. 2001), submerged in those media, either singly or clustered. *L. chlamidosporiformans* resembles *L. avenicola* in this respect as described by Kwasna & Kosiak 2003 for ascomata produced on SNA slants. *L. sauropi* is also known to produce ascomata in culture (Zhang




**Figs. 1–3.** *Lewia chlamidosporiformans*. **Fig. 1.** Ascus with ascospores. **Fig. 2.** Mature ascospores (note the mucilaginous sheath surrounding the ascospores). **Fig.3.** Chain of chlamydospores with some undergoing germination. Bars = 24 µm (Fig. 1);32 µm (Fig. 2); 31 µm (Fig. 3)

& David, 1996). The other *Lewia* usually produce ascomata only on tissues of infected plants (Whitehead & Dickson 1952; Simmons 1986a,b). Sporadically, the formation of teleomorphs *in vitro* was observed by Bilgrami (1974) in *L. infectoria* (*Pleospora infectoria*) and Simmons (1986b) in *L. photistica*. Although *Alternaria* species and their teleomorphs may produce clumps of thick walled cells, they do not usually produce typical chlamydospores such as those of *L. chlamidosporiformans* (Ellis 1971; Ellis 1976). *L. avenicola* is known to produce chlamydospores arranged in chains or clusters on PDA and SNA (Kwasna & Kosiak 2003). *L. chlamidosporiformans* resembles *L. ethzedia* and *L scrophulariae* in ascomatal size (150-200 µm), but differs from the other species in this respect (Kwasna & Kosiak 2003). The sole species of *Lewia* known to attack a member of the *Euphorbiaceae* (namely *Sauropus androgynus* Merr.) is *L. sauropi*. This species differs from *L. chlamidosporiformans* in the following features: asci in *L. sauropi* have a different shape (obovoid, pyriform or ellipsoidal), asci are wider (47-57 µm) and no mention of chlamydospore formation was made in the original description (Zhang & David, 1996).

Three features are particularly useful for separating *L. chlamidosporiformans* from all other species in this genus, being unique for the new species: the ascus shape which is obovoidal to obpyriform (subcylindrical to subellipsoidal in all other species); the length of the ascospores is larger than described for other known species (25 -37 µm for *L. chlamidosporiformans* as compared to the the maximum length known for this genus until now *L. sauropi* - with a length of 29.5-35 µm); the presence of a mucilaginous sheath surrounding the ascospores (unknown for other members of *Lewia*).

Inoculation of *L. chlamidosporiformans* caused severe yellowing of leaves followed by a general necrosis and defoliation that finally led to plant death after 5 days of inoculation. Some diseased leaves were maintained in a dew chamber under a temperature of 25 °C for 24 hours and after this period abundant formation of conidia was observed.

## Acknowledgments

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# *Weddellomyces turcicus*, a new species on a grey *Acarospora* from Turkey

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**Abstract**—*Weddellomyces turcicus* is described as new from the thallus of an unidentified grey placodioid Acarospora in Turkey; it differs from other species of the genus in the asci being 2-spored when mature.

**Key words**—Ascomycetes, cephalothecoid exciple, lichenicolous fungi, *Dacampiaceae*

## Introduction

The genus *Weddellomyces* comprises 11 species occurring on saxicolous lichens. The species have been treated by Calatayud & Navarro-Rosinés (1998) and Navarro-Rosinés & Roux (1995). The perithecial wall in this genus is composed of cephalothecoid plates, but Alstrup & Hawksworth (1990) also included one species without such plates. A recent collection from Turkey differs significantly from previously described taxa, and is described here as a new species.

## Methods

Sections were prepared by hand, and examined in Congo Red (1% solution in water, mixed 1: 1 with 10% KOH), I (Lugol's Iodine: I 0.5 g, KI 1.5 g, water 100 ml), and Brilliant Cresyl Blue in water. The pigment of the ascomatal wall was investigated following the methods of Meyer & Printzen (2000).

## The species

***Weddellomyces turcicus*** Halici & Orange **sp. nov.** **Figs. 1–2**

*Fungus lichenicola. Ascomata globosa, 390–490 µm diam., nigra. Excipulum superum ex segmentis cephalothecoideis constructum. Hamathecium ex hyphis ramosis, anastomosantibus et septatis compositum. Asci maturi 2-spori. Ascosporae brunneae, 3-septatae, 50–61(–69) × (16.5–)18.5–21.5(–22) µm, verruculosae.*

Typus: Turkey, Kayseri, Aladaglar Milli Parki, Hacer Ormanlari, 37°47'N, 35°18'E, alt. 1648 m, on thallus of *Acarospora* sp. on exposed limestone rocks, 20 November 2004, M.G. Halici 0.2018 (NMW C.2005.013.1–holotypus).

Lichenicolous, on dead and decolourized areas of the host thallus. Ascomata perithecioid, immersed in host thallus, erumpent, the apex visible through an irregularly shaped split in the host cortex, later the upper half of the perithecium exposed by the break up of the dead host tissue; ascomata globose, 390–490 µm diam., black, matt, slightly roughened, without setae, apex opening by a minute irregularly shaped depression, often with a few irregular radiating cracks. Exciple in surface view of polygonal cephalothecoid plates 30–105 × 30–50 µm, comprising dark-pigmented areas separated by pale areas, plates becoming indistinct in the lower part of exciple; in vertical section exciple 24–40 µm wide at sides and base, 40–60 µm thick near apex; cells slightly tangentially compressed, mostly 5.5–16.5 × 4–15 µm, the walls 1–2.5 µm thick, outermost layers of cells dark-pigmented except at the junctions of the plates, pigment within cell wall, unevenly distributed in inner cells of the pigmented layer, obscuring cell outlines in outer cells; pigment dark (slightly reddish) brown in water, K + dark greyish brown, HCl + reddish brown; after treatment with N, the colour in K is similar to the colour in water (Atra-brown). Subhymenium c. 20 µm thick. Hamathecium of richly branched and anastomosing interascal filaments 2–3.5 µm wide, with frequent septa. Asci cylindrical, 140–160 × 23–27 µm, initially up to 8-spored, but 2-spored at maturity, wall gradually thickened towards apex, I –, ocular chamber present. Ascospores (2–)3-septate, 50–55.6–61(–69) × (16.5–)18.5–19.9–21.5(–22) µm, length/breadth ratio (2.3–)2.6–2.8–3.0(–3.2) [n = 33]; brown, terminal cells concolorous or very slightly paler than central cells; surface verruculose, perispore not detected in mature spores, torus inconspicuous at all stages; wall of semi-mature to mature spores I + dilute blue (partly masked by brown pigment).

## Discussion

The species of *Weddellomyces* are distinguished mainly by features of the ascospores, including size, shape, septation, and surface ornamentation; and by the identity of the host. *Weddellomyces turcicus* differs from all other members of the genus in the asci 2-spored at maturity, although the asci initially have 6-7 ascospores visible, and it is likely that 8 are present. It is also the first species in the genus to be recorded from *Acarospora*. It is known only from the type collection. *W. turcicus* is likely to be pathogenic, as it occurs on two small, bleached areas of host thallus, but further collections are necessary to confirm this. The currently unidentified host has a grey, placodioid thallus which is K + yellow, C –.

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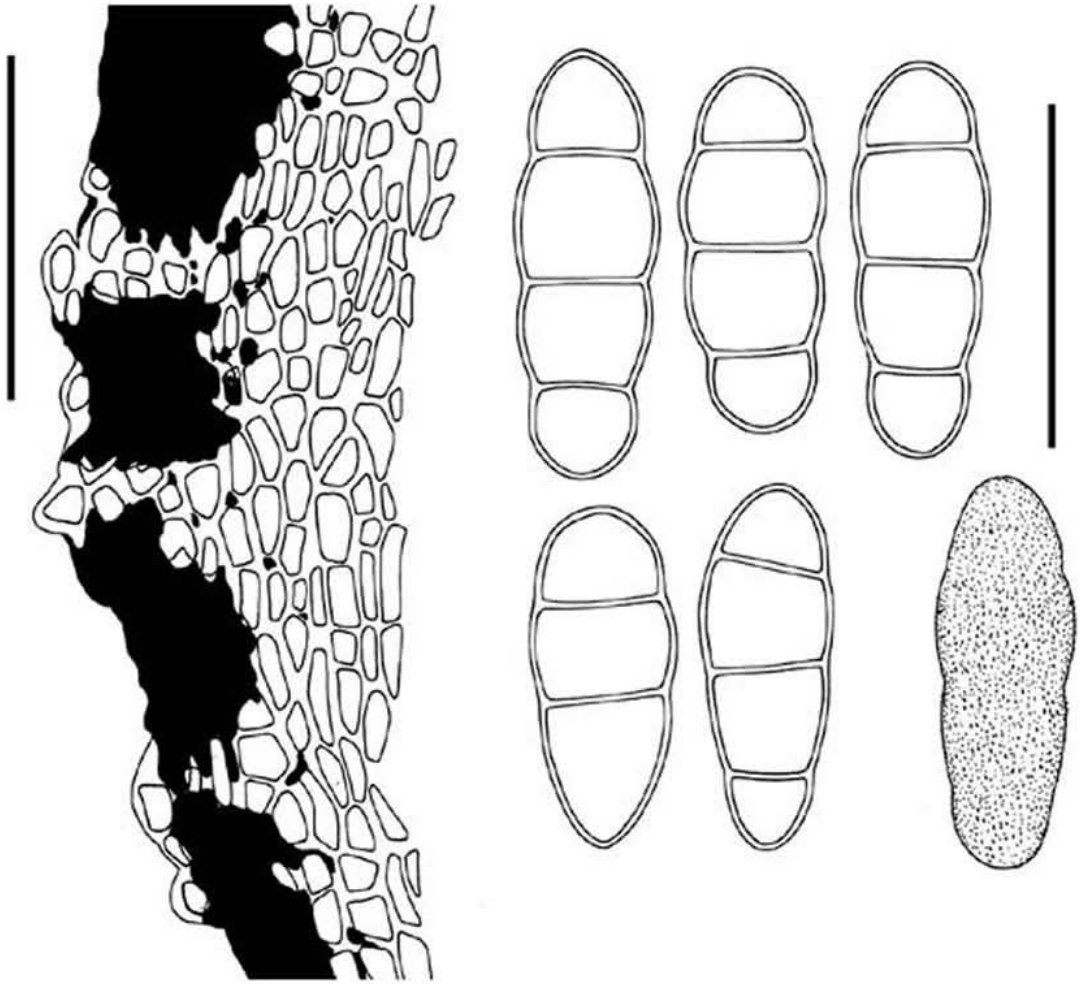

Fig. 1 – *Weddellomyces turcicus* (Holotype). Line drawings. a. section of part of upper exciple, showing parts of four cephalothecoid plates. b. ascospores (outlines). c. ascospore, showing surface ornament. Bar = 50 µm.

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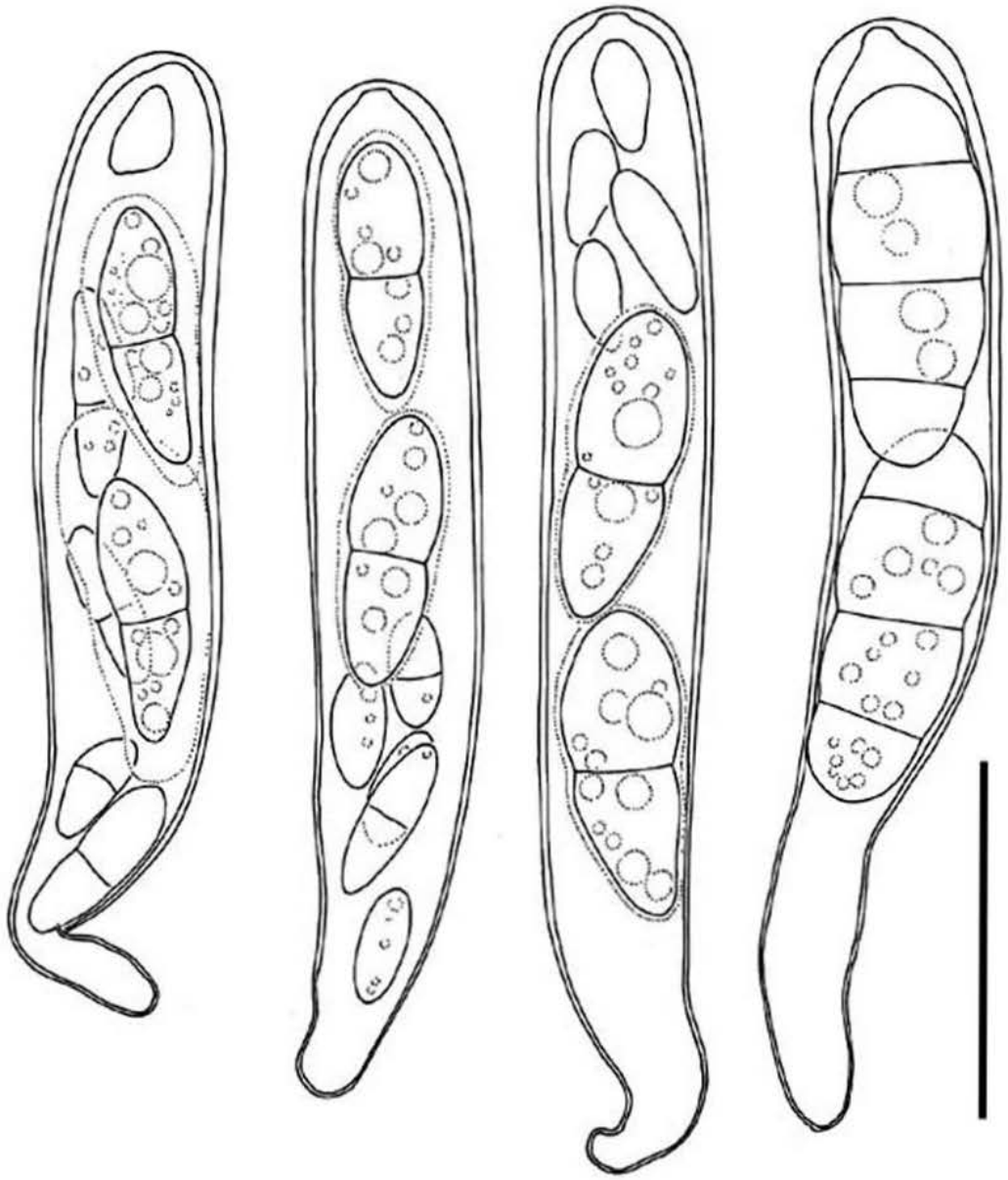

Fig. 2 – *Weddellomyces turcicus* (Holotype). Asci in water: three immature and one (far right) mature. Bar = 50 µm.

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# *Cordyceps spegazzinii* sp. nov., a new species of the *C. militaris* group

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**Abstract**—The proposed new species, *Cordyceps spegazzinii* sp nov., was collected on Barro Colorado Island, Panama. It is characterized by simple stromata with cylindrical to clavate heads, perithecia superficial to partially immersed, ascospores not fragmenting into part-spores and a *Evlachovaea* sp. anamorphic state. We compared *C. spegazzinii* to similar species in the *C. militaris* group. We determined that *C. spegazzinii* belongs to the *C. militaris* group, based on examination of morphological features and cultural characteristics, combined with phylogenetic analyses using LSU rDNA sequences.

**Key words**—*Clavicipitales*, taxonomy

## Introduction

Members of the family *Clavicipitaceae* (*Hypocreales*) display a wide range of life strategies ranging from insect endoparasites to pathogens of grasses and endophytes (Spatafora et al. 1993). The entomogeneous fungal genus *Cordyceps* (Fr.) includes approximately 450 described species (Sung 2004, Stensrud et al. 2005) that are known to be endoparasites of several orders of arthropods and parasitic on hart's truffles (*Elaphomyces* spp.) (Kobayasi 1982, Mains 1957) while other species live on the sclerotia of plant pathogenic *Claviceps* species. In addition, a few species are known to utilize seeds of higher plants (Fukatsu & Nikoh 2003).

The genus *Cordyceps* has also received significant attention due to the importance of some species in the production of bioactive compounds. The species *C. sinensis* (Berk.) Sacc. and *C. sobolifera* (Hill) Berk. & Broome have been used in traditional Chinese medicine (Liu et al. 2002) and *C. kyusyuensis* Kawam. has recently received attention due to production of bioactive compounds with anti-tumor activity (Sun et al. 2003). Other species, like *C. brongniartii* Shimazu and *C. bassiana* Z.Z. Li et al. have been connected with their anamorphic stages, *Beauveria brongniartii* (Sacc.) Petch and *Beauveria bassiana* (Bals.-Criv.) Vuill., respectively, both with potential for biocontrol of insects (Shimazu et al. 1988, Li et al. 2001, Rehner et al. 2005).

There have been several attempts to better characterize the genus (Kobayasi 1982). Four subgenera have been proposed based on morphological characters: subgenera *Ophiocordyceps*, *Eucordyceps*, *Neocordyceps* (Kobayasi 1982) and *Bolacordyceps* (Erikkson 1986). However, identification and classification of *Cordyceps* species based exclusively on morphological and cultural data is a difficult task due to the large number of species that compose the genus, possible synonymies, presence of several anamorphs and inability to locate type specimens (Sung 2004, Sung & Spatafora 2004, Stensrud et al. 2005). Increasingly, phylogenetic studies have employed sequence data to shed light on the phylogenetic relationships and species delimitation in the genus *Cordyceps* (Sung et al. 2001; Nikoh & Fukatsu 2000, Sung et al. 2004, Stensrud et al. 2005). Sung et al. (2001) employed nuclear rDNA sequences (SSU and LSU) in the revision of *Verticillium* sect *Prostrata*. They determined that *Cordyceps* was not monophyletic and recognized two distinct clades, *C. militaris* sensu stricto and *C. ophioglossoides* clade. Stensrud et al. (2005) using ITS rDNA sequences of 72 clavicipitalean taxa also found *Cordyceps* to not be monophyletic and recognized four separate *Cordyceps* evolutionary lineages.

In 2003 a specimen of *Cordyceps* was collected on Barro Colorado Island, Panama. Morphological examination and phylogenetic placement of our material revealed that its features are not consistent with previously described *Cordyceps* species, and a new species is proposed.

## Materials and Methods

**Field collection:** J.F. Bischoff and J.F. White in August 2003 made the collection on Barro Colorado Island, Panama. The material was associated with insect eggs on partially rolled leaves of an unknown dicotyledonous tree. The eggs were identified as belonging to Order *Diptera* by Karl Kjer (Department of Ecology and Evolution, Rutgers University). The material was brought to the field station and isolated on Potato Dextrose Agar (PDA; Difco, Inc.) with antibiotics (gentamicin 40 mg/L, streptomycin 40 mg/L; penicillin 20 mg/L). Stromata were kept in 90% alcohol. Specimens of the collections were submitted to the Rutgers University Plant Pathology Herbarium (RUTPP).

**Morphological observations:** Microscopic examinations of anamorphic characters were made from cultures maintained on PDA and potato carrot-agar (PCA), at room temperature (23°C) for 10 days. Slide preparations were mounted in lactic acid-cotton blue or water. Twenty measurements were made for each morphological feature.

To observe the structure of perithecia, specimens were fixed in 95% ethanol, dehydrated in 100% ethanol and infiltrated with LR White® acrylic embedding medium for 24 hours. Specimens were oriented in capsules containing embedding medium and cured in an oven (60°C) for 24 hours. Sections approximately 1 µm thick were made using glass knives and stained in aniline blue (0.1% aqueous) followed by toluidine blue (0.1% aqueous). Photographs were made using a Nikon Coolpix digital camera.

**Cultural studies:** Growth was measured on several media: PDA, PCA, malt-extract agar (MEA), Czapek cellulose agar (CCA) and corn meal dextrose peptone (CMDP). All media were inoculated using 7mm plugs cut from margins of colonies growing on PDA

plates. Plates were maintained at 23°C in dark and measured after 5 days. Representative cultures were sent to the ATCC in Manassas, Virginia (MYA-3684) and USDA-ARS Collection of Entompathogenic Fungal Cultures (ARSEF) (ARSEF 7850).

**Sequence data:** DNA extraction was carried on from fresh perithecia taken in the field and from fresh mycelium growing on PDA media overlaid with cellulose acetate sheets. Genomic DNA was extracted using the DNeasy® Plant Mini Kit (Qiagen). Internal transcribed spacer (ITS) regions 1 and 2, 5.8s, and the 5' end of the large subunit LSU rDNA were amplified from 4 µL of genomic DNA using primers ITS5 and ITS4 (Sullivan et al. 2000) in a 50 µL reaction. PCR reactions, sequencing reactions to amplify the ITS and LSU regions, and reaction analyses were performed as described by Sullivan et al. (2000). The rDNA LSU and ITS1-5.8s-ITS2 regions were submitted to GenBank (accession number DQ196435).

**Phylogenetic analysis:** Sequencher (Genecodes, Ann Arbor, MI) was used to analyze, edit and construct consensus sequence from sequence products. Forty LSU sequences to represent *Cordyceps* species and others representatives of Clavicipitaceae were selected. GenBank accession numbers are listed in Table 1. Members of the family *Hypocreaceae* were used as outgroup taxa based on the work of Spatafora et al. (1993) and Nikoh et al. (2000). Sequences were aligned by hand using the secondary structure of *Saccharomyces cerevisiae* (U53879) from the comparative RNA Web site (CRW) database (Cannone et al. 2002). The matrix was annotated as described by Kjer (1995). Sequences were analyzed using the program PAUP v4.0 (Swofford 2002) by maximum likelihood and Bayesian analysis. ModelTest v.3.06 (Posada & Crandall 1998) was used to select the best fitting model of sequence evolution determined by Akaike information criterion (Akaike 1974). The model selected was GTR with proportion of invariable sites (I) and gamma distribution (G). The parameters include base frequencies A=0.2804, C=0.2443, G=0.373, T=0.1680; rate matrix [A-C]=0.4817, [A-G]=1.4368, [A-T]=1.021, [C-G]=1.0609, [C-T]=7.4863; [G-T]=1; I=0.6130 and G=0.5932 and this model was incorporated into PAUPv.4.0. The most likely tree (-ln 3703.8447) is shown in Fig. 11. Bayesian inference was used to estimate branch support (posterior probability) under likelihood using Mr Bayes 3.0 (Huelsenbeck 2000) (Fig. 11). Bayesian analysis was run three times with four mcmc (Markov Chain Monte Carlo) chains for 1,000,000 generations, sampling every 100 generations. The 30,000 trees resulting from the three runs were pooled and 28,500 were imported into PAUP to construct a majority rule consensus tree after discarding the asymptotic trees (burn in).

Five complete ITS sequences were selected based on being included in *C. militaris* clade or morphological similarities. GenBank accession numbers are listed in Table 1. Sequences were aligned using ClustalX (Thompson et al. 1997) and their similarity compared (Table 2).

Table 1. Taxa used in LSU and ITS analyses

| Taxa | LSU | ITS |
|---|---|---|
| *Atkinsonella hypoxylon* | U57087 | |
| *Balansia henningsiana* | U57678 | |
| *Balansia strangulans* | U57679 | |
| *Beauveria brongniartii* | AB027381 | |
| *Claviceps fusiformis* | U17402 | |
| *Claviceps paspali* | U47826 | |
| *Claviceps purpurea* | U57085 | |
| *Cordyceps capitata* | U57086 | |
| *Cordyceps cardinalis* 1 | AY184965 | |
| *Cordyceps cardinalis* 2 | AY184964 | |
| *Cordyceps gunnii* | AF339522 | AJ536552 |
| *Cordyceps inegoensis* | AB027368 | |
| *Cordyceps japonica* | AB027367 | |
| *Cordyceps kanzashiana* | AB027371 | |
| *Cordyceps kyusyuensis* | AY465959 | AY781661 |
| *Cordyceps militaris* | AB027379 | AJ786573 |
| *Cordyceps ophioglossoides* | U47827 | |
| *Cordyceps prolifica* | AB027370 | |
| *Cordyceps pruinosa* | AB044635 | |
| *Cordyceps pseudomilitaris* | AF327376 | AJ786589 |
| *Cordyceps ramosopulvinata* | AB027372 | |
| *Cordyceps sinensis* | AB067737 | |
| *Cordyceps spegazzinii* | DQ196435 | DQ196435 |
| *Cordyceps subsessilis* | AF373285 | |
| *Cordyceps takaomontana* | AB044637 | AB044637 |
| *Cordyceps tuberculata* | AF327384 | |
| *Dussiella tuberiformis* | U57083 | |
| *Epichloe amarillans* | U57680 | |
| *Epichloe typhina* | U17396 | |
| *Hypocrea lutea* | AB027384 | |
| *Hypocrea schweinitzii* | U47833 | |
| *Hypomyces armeniacus* | AF160293 | |
| *Hypomyces orthosporus* | AF160241 | |
| *Lecanicillium lecanii* 1 | U17414 | |
| *Lecanicillium lecanii* 2 | U17421 | |
| *Neotyphodium coenophialum* | AF160241 | |
| *Paecilomyces farinosus* | DQ067297 | |
| *Paecilomyces tenuipes* | AB027380 | |
| *Tolypocladium cylindrosporum* | AF245301 | |
| *Tolypocladium inflatum* | AB103381 | |

## Taxonomic Description

***Cordyceps spegazzinii*** M.S. Torres, J.F. White & J.F. Bischoff **sp. nov.**

FIGURES 1-4, 5-10

*Stromata solitaria, simplicia in ovis insectorum (Diptera), 7-9 mm altus. Stipes tenuis, glabro. Zone fertilis terminalis, cylindricea vel clavata (1x2-3 mm). Perithecia agregata, laxe immersa, 400-460 x 200-260 µm ovata vel obclavata. Asci cylindracei, 200-250 x 2.5-3 µm, hyalini. Ascosporae hyalinie, filiformes, flexuosae, multiseptatae, 100-250 x 0.5-1 µm, in ascis numquam individuales in partes secedentes. Status anamorphicus in cultura PDA, celeriter crescens, mycelio aerio gossypino, hyphis vegetativis ramosis, septatis, albis, 1-2 µm latis, reverso cremeo. Cellulae conidiogenae monophialidicae, hyalinae, leves, 7-15 µm, ad septum 1-2. Conidia hyalina, aseptata, levia, ellipsoidea, 4-5 x 2 µm, catenulata Etymology: this species is named after the mycologist and naturalist Carlos L. Spegazzini.*

***Holotype***: Barro Colorado Island (BCI), Panama; insect eggs (Diptera) on leaves of unknown dicotyledonous plant; August 2003; J.F. Bischoff & J.F. White; Rutgers Mycological Herbarium (RUTPP).

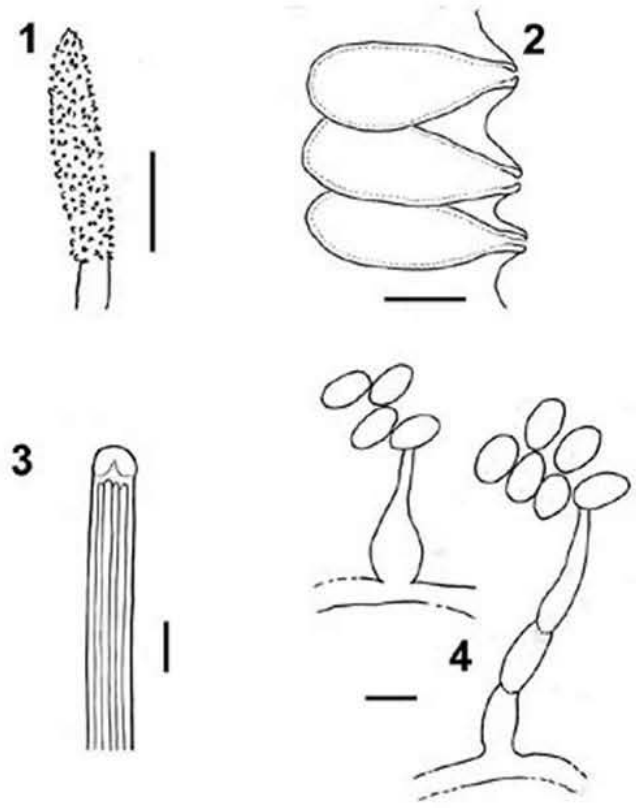
Stromata solitary, simple, associated to dipteran eggs, measuring 7-9 mm from base to tip, consisting of white, slender smooth stipe and yellowish, cylindrical to clavate head (measuring 1 x 3-4 mm) (Fig 1 and 5). Perithecia crowded, 400-460 x 200-240 µm, ovate to obclavate, superficial to partially immersed, with perithecial necks protruding; asci 200-250 x 2.5-3 µm (Fig. 6 and 7), cylindrical with a refractive apical cap (Fig. 3), containing 8 ascospores. Ascospores filiform, running in parallel, hyaline, irregularly multiseptate, 100-250 x 0.5-1 µm not fragmenting into partspores.

Colonies on PDA fast growing (20 mm in 5 days at 25°C; 14 mm in MEA, 15 mm in PCA, 18 mm in CMDP and 13 mm CCA), smooth surface, white, cottony, dense, with abundant sporulation; reverse cream to yellowish and smooth. *Evlachovaea* Borisov & Tarasov (Borisov & Tarasov 1999) anamorphic state developing in culture (Fig. 4, 8-10). Conidiogenous cells flask shaped (phialide), 7-15 µm long, swollen at the base, base 3 µm wide, and gradually narrowed to a distinct neck (Fig. 4). Phialides borne singly, grouped in pairs or in loose clusters (Fig. 8). Conidia hyaline, unicellular, smooth, oval to fusoid shaped, 4-5 x 2 µm, forming short dry chains with zipper-like morphology due to alternating oblique orientation of successive conidia when formed (Fig. 4 and 8).

## Discussion

The features of *C. spegazzinii* do not match any previously described species of *Cordyceps*. *Cordyceps spegazzinii* is comparable to *C. takaomontana* Yakush. & Kumaz, *C. memorabilis* (Ces.) Sacc., *C. pseudomilitaris* Hywel-Jones & Sivichai and *C. cardinalis* G.H. Sung & Spatafora, all morphologicaly similar and closely related species in the *Cordyceps* s.s clade (Sung & Spatafora 2004).

Nevertheless, *C. spegazzinii* is easily separated by morphological and microscopic characteristics from *C. takaomontana* and *C. memorabilis*, species that are characterized by capitate stromata and superficial perithecia while *C. spegazzinii* is non-capitate with a much less distinct fertile head. Further, the species *C. takaomontana*, and *C. memorabilis* are parasites of *Coleoptera* while *C. spegazzini* was found associated to *Diptera* eggs.




Figs. 1-4. Morphological features of *C. spegazzinii* and its *Evlachovaea* sp. anamorph. 1. Stroma (bar=1mm). 2. Arrangement of perithecia in the stroma (bar=160µm). 3. Tip of ascus showing the cap (bar= 3µm). 4. Typical phialides and conidia of the anamorph from culture on PDA (bar= 3µm).

*Cordyceps pseudomilitaris* shares many morphological features with *C. spegazzinii* such as small stromata, superficial to partially immersed perithecia, smooth filiform ascospores that do not break into partspores. Although Hywel-Jones (1994) stated in his description that there exists a wide variation in size and shape in the specimens collected, *C. pseudomilitaris* presents slightly longer and wider asci and elongated ellipsoid to elongated ovoid perithecia while *C. spegazzinii* is characterized by ovate to obclavate perithecia and slightly shorter and thinner asci. Also *C. pseudomilitaris* was associated with a *Hirsutella*-like anamorph (Hywel-Jones 1994), and leptidopteran host. *Cordyceps cardinalis* differs from *C. spegazzinii* in that the fertile area of the former is reddish orange to reddish, cylindrical, elliptical to fusiform and those of the latter are yellowish cylindrical to clavate. Conidial state of *C. cardinalis* and *C. spegazzinii* are closely similar. The anamorph of *C. cardinalis* is characterized by phialides with swollen basal portions and producing conidia in partially imbricate chains and it was best described as being *Clonostachys*-like or *Mariannaea*-like (Sung & Spatafora 2004). *Cordyceps spegazzinii* anamorph present flask shaped phialide, with conidia forming short dry chains with zipper-like morphology. Humber et al. (2002) proposed

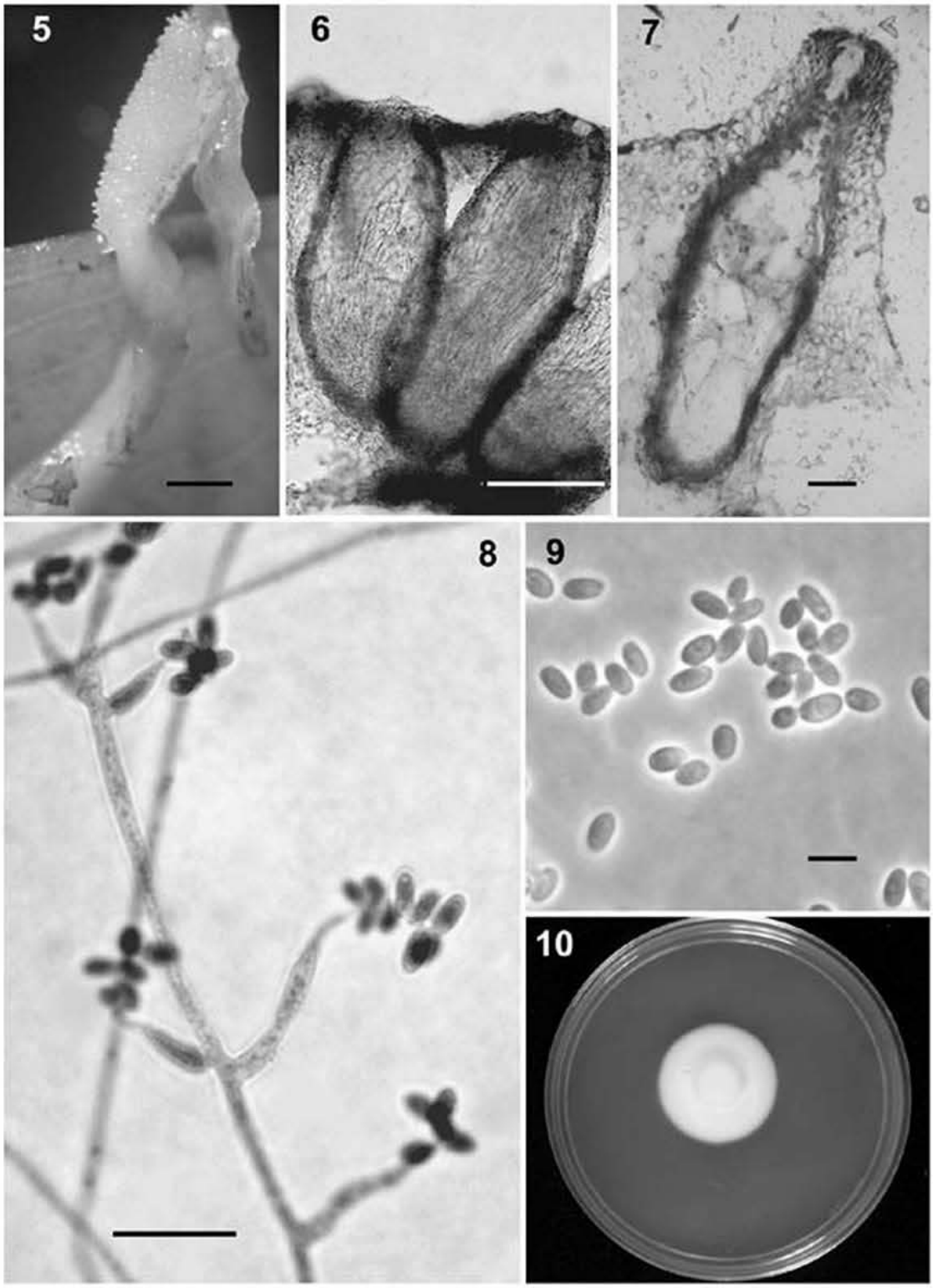



Fig. 5-10. *Cordyceps spegazzinii*. 5. Stroma (bar = 1 mm). 6. Cross section of stroma with perithecia. (bar = 150 µm). 7. Detail of perithecium (bar = 70µm). 8. Phialides (bar = 15µm). 9. Conidia (bar = 5 µm). 10. Seven-day-old colony on PDA.

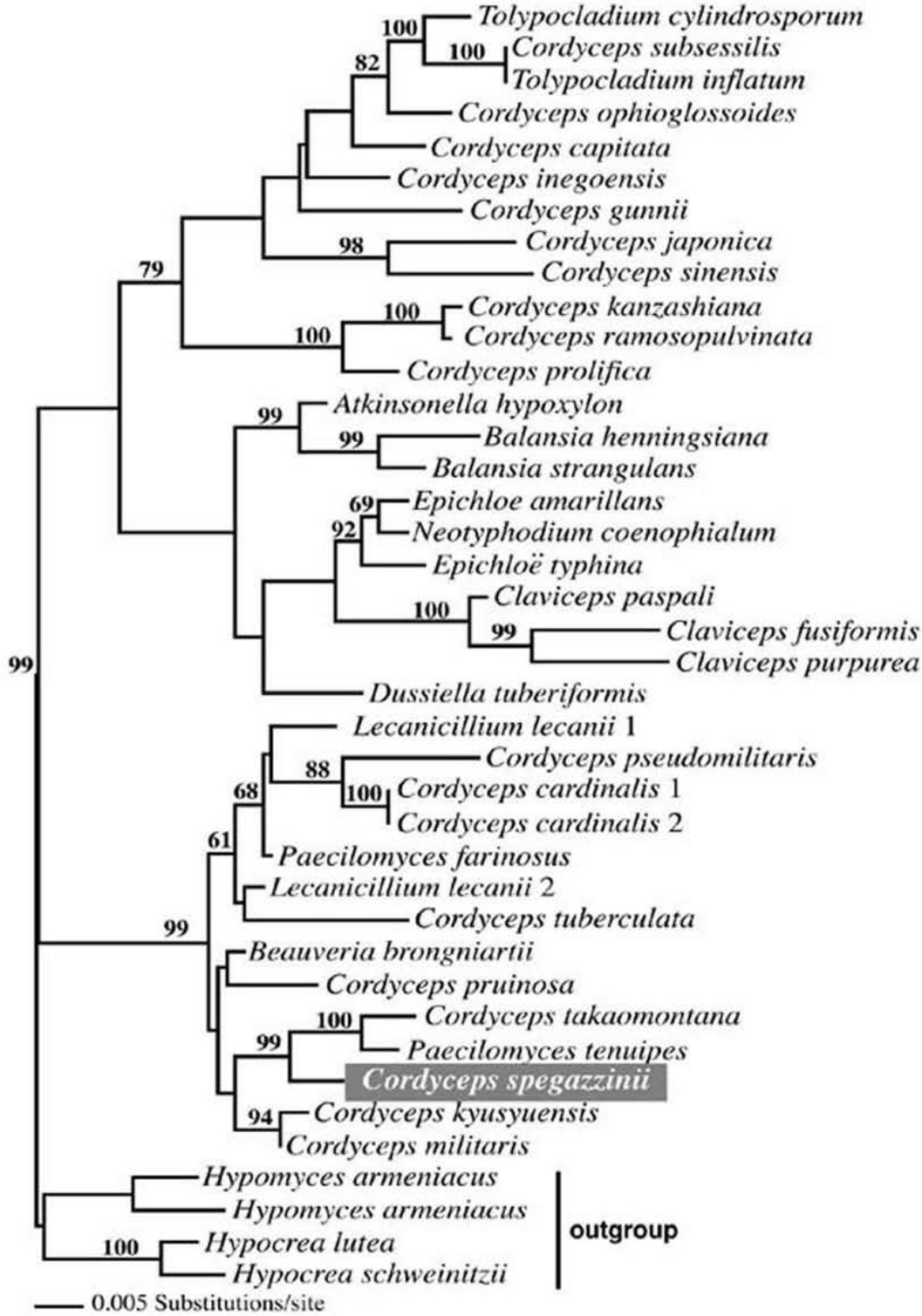



Fig 11. The most likely tree based on rDNA LSU sequence data analysis using GTR+I+G model of evolution. The numbers on the branches indicate posterior probabilities

that morphological characteristics and entomopathogenic habit of *Evlachovaea* could suggest a teleomorph connection with *Cordyceps* species and certainly this is the case for *C. spegazzinii*. *Cordyceps cardinals* anamorph belongs to *Evlachovaea* genus (R. Humber, personal communication).

Host range of *Cordyceps* species may be of low significance as a phylogenetic character (Stensrud et al. 2005). Several *Cordyceps* species have been previously reported on hosts from Order *Diptera*, including *C. dipterigena* Berk. & Broome, *C. sakishimensis* Kobayasi & Shimizu, *C. discoideocapitata* Kobayasi & Shimizu, *C. bicephala* Berk., *C. iriomoteana* Kobayasi & Shimizu and *C. novoguineensis* Kobayasi & Shimizu. However, none of these species are grouped in subsection *Pseudoimmersae* (Kobayasi 1982) where *C. spegazzinii* would be placed based on its stromatic features.

Although the interrelationship of groups changed to some extent our analyses recognized the same general clades reported by Sung et al. 2001 and Sung & Spatafora 2004 (Fig.11). The *Cordyceps* s.s. clade (99% posterior probabilities) was identical to that observed by Sung & Spatafora (2004) and Stensrud et al. (2005) except for the addition of *C. spegazzinii*. Within this clade, *C. spegazzinii* grouped closely (99% posterior probabilities) with *C. takaomontana* and its anamorph *Paecilomyces tenuipes* (Peck) Samson (Luangsa-ard et al. 2005) and it is closely related to *C. kyusyuensis* Kawam. and *C. militaris* (L.) Fr. *Cordyceps spegazzinii* was compared to *C. kyusyuensis* and *C. militaris*. Morphologically, *C. spegazzinii* is distinguished from *C. kyusyuensis* in having ovate to obclavate superficial perithecia, and *Diptera* host while the later present ovoid immersed perithecia, *Verticillium*-like anamorphic state (Sung, personal communication) and leptidopteran host. *Cordyceps militaris* differs form *C. spegazzinii* in that the ascospores of the former disarticulate and those of the latter do not disarticulate. Additionally, *C. militaris* is characterized by a *Lecanicillium* W. Gams & Zare anamorph and brightly colored stromata.

In our analysis of ITS sequences similarities (Table 2), *C. takaomontana* was 93.2% and *C. gunnii* was only 78.3% similar to *C. spegazzinii*.

**Table 2.** Similarity matrix derived from the sequence data of the ITS1 region in six *Cordyceps* species.

| Taxa | (1) | (2) | (3) | (4) | (5) | (6) |
|---|---|---|---|---|---|---|
| (1) *C. spegazziniii* | --- | | | | | |
| (2) *C. takaomontana* | 93.2 | --- | | | | |
| (3) *C. militaris* | 92.8 | 99.6 | --- | | | |
| (4) *C. kyusyuensis* | 92.7 | 99.4 | 99.7 | --- | | |
| (5) *C. pseudomilitaris* | 90.2 | 94 | 94.2 | 93.5 | --- | |
| (6) *C. gunnii* | 78.3 | 85 | 86.5 | 82.6 | 87.2 | --- |

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# A phylogeny of *Ramariopsis* and allied taxa

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**Abstract**—The phylogenetic relationships of *Ramariopsis* and related taxa were studied through a cladistic analysis of 36 morphological, cytological, and biochemical characters among 23 species in six genera. Two of these genera were directly studied as groups of interest, three as external taxonomic outgroups, and one as operative outgroup. Representatives of *Ramariopsis* sensu Corner formed a monophyletic group, supported by the cyanophilous nature of their basidiospores and derivation of their ornamentation from the tunica. The new combination, *Clavulinopsis antillarum*, is proposed.

**Key words**—*Clavariaceae*, Homobasidiomycetes, spore ultrastructure, outgroup sampling, taxonomy

## Introduction

*Ramariopsis* was described as a subgenus of *Clavaria* by Donk (1933), who selected *Clavaria kunzei* Fr. as the type species; the name refers to its macromorphological similarity to the genus *Ramaria* (Donk 1954). The taxa originally included the type species, *Clavaria angulispora* Pat. & Gaillard, *Clavaria pulchella* Boud., *Clavaria pyxidata* Pers. and *C. subtilis* Pers.

Corner (1950) elevated the taxon to genus level and included several fibulate species with branched, whitish basidiomes, monomitic hyphal systems, and echinulate spores. He retained *Clavaria kunzei* [= *Ramariopsis kunzei* (Fr.) Corner] as the type species, removing three species—*C. angulispora*, *C. pyxidata* and *C. subtilis*—and adding eight more for a total of ten species in the genus. Petersen (1964) added two more species after examining the type specimens of various species of the genus *Clavulinopsis*.

Petersen (1966) emended the original delimitation of the genus, to include taxa with smooth spores and hysterochroic basidiomes. Petersen considered the size of the basidia, the thickness of the spore wall, the composition of the ornamentation—when present—and the pattern of coloration of the basidiomes as the relevant characters for circumscribing the genus. He proposed dividing *Ramariopsis* into two subgenera: *Laevispora*, typified by *Ramariopsis minutula* (Bourdot & Galzin) R.H. Petersen, for species with smooth-spores, and *Ramariopsis*, consisting of species with echinulate spores.

Corner (1970) maintained the original circumscription of the genus, recognizing that it might be an artificial group, closely related to *Scytinopogon* and *Clavulinopsis*. Corner argued that his circumscription conformed to a homogeneous group that was of more utility for fieldwork.

Petersen (1978a) proposed a new delimitation for the genera *Ramariopsis*, *Clavulinopsis*, and *Clavaria* based on the size of the hilar appendix, the type of pigments present in the basidiome and the number of nuclei remaining in the basidium after the formation of spores. He transferred species with globose spores and a conspicuous hilar appendix from *Clavulinopsis* to *Ramariopsis*, and species with elongate spores and a small hilar appendix from *Clavulinopsis* to a new subgenus: *Clavaria* subg. *Clavulinopsis*. He also proposed designating *Clavaria corniculata* Schaeff. [= *Ramariopsis corniculata* (Schaeff.) R.H. Petersen] as the type species of *Ramariopsis*.

Based on Petersen's arguments, *Ramariopsis* should include hysterochroic species with branched or simples basidiomes, with whitish or bright coloration, globose or subglobose and smooth or ornamented spores, and a conspicuous hilar appendix (see Fig. 1). Petersen argued that this circumscription permits a continuum among related species, from smooth-spored species with a large basidiome, to species with a small basidiome and echinulate spores.

There are only a few additional contributions to this polemic. Jülich (1985) transferred all species of *Ramariopsis* to *Clavulinopsis* based on nomenclatural arguments, but this interpretation apparently has not been followed by the majority of taxonomists (Hawksworth et al. 1995, Kirk et al. 2001). Pegler & Young (1985), in an electron microscopy (EM) study of several species of *Ramariopsis*, *Clavulinopsis*, and *Scytinopogon*, described three ultrastructural patterns of spore ornamentation that corresponded with the three genera mentioned before. They also observed that several apparently smooth-spored species, such as *Ramariopsis californica* R.H. Petersen, actually possessed ornamentation. The observed ornamentation was very small and covered by a thin myxosporium, such that the spores appear smooth under a light microscope even at magnifications above 1000x. Pegler & Young (1985) recognized

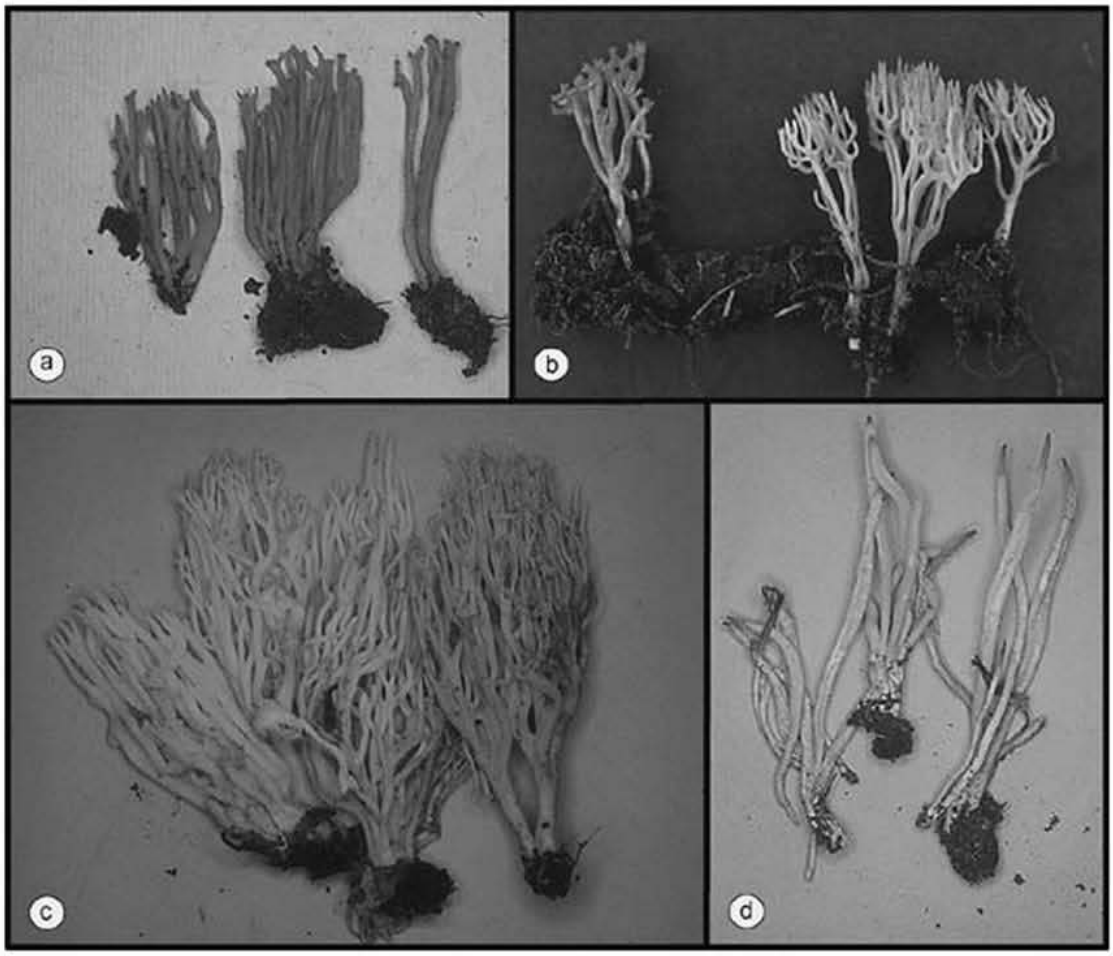


**Figure 1.** Four species representative of the variation observed in *Ramariopsis* and *Clavulinopsis*. a) *Clavulinopsis corniculata*, b) *Ramariopsis pulchella*, c) *Ramariopsis kunzei* and d) *Clavulinopsis fusiformis*. Photos a: J. Cifuentes, b: A. Estrada-Torres, c: J. Cifuentes, d: J. Cifuentes.

the delimitation proposed by Corner (1950) for *Ramariopsis*, but not the relationship between this genus and *Scytinopogon*.

The genus *Ramariopsis* has been included in the *Clavariaceae* in the majority of the treatments of this family (Donk 1964, Corner 1970, Jülich 1981, Hawksworth et al. 1995), with the exception of Petersen (1978a, 1988a) and Kirk et al. (2001), who placed *Ramariopsis* in *Gomphaceae*.

A phylogenetic study of *Gomphaceae* (Villegas et al. 1999), however, indicates that *Ramariopsis*—at least sensu Corner—should not be considered part of that family. Pine et al. (1999), on the other hand, studied the clavarioid and cantharelloid Homobasidiomycetes, and found that *Clavulinopsis fusiformis* (Sowerby) Corner [= *Ramariopsis fusiformis* (Sowerby) R.H. Petersen] nested within the euagaric clade, forming a monophyletic group with *Clavaria acuta* Sowerby, indicating that *Ramariopsis* subgenus *Laevispora* is related to *Clavaria*, or at least to representatives of *Clavaria* subgenus *Holocoryne*. Additionally results of Larsson et al. (2004) indicate close relationships among *Clavulinopsis helvola* (Pers.) Corner, *Clavaria argillacea* Pers.–*Clavaria* subgenus *Holocoryne*–and *Clavaria fumosa* Pers.–subgenus *Clavaria*–

on a monophyletic group nested in the euagaric clade. This results points to a close relationship between *Clavulinopsis* and at least some part of *Clavaria*.

There are currently only a few works, that have attempted to study the phylogeny of clavarioid and gomphoid macromycetes in general (Pine et al. 1999, Villegas et al. 1999, Humpert et al. 2001), and there is no consensus about the phylogenetic relationships of these taxa.

Given that there is no consensus delimitation for *Ramariopsis*, the number of species in this genus depends on the source consulted (i.e. Jülich 1981, 1985; Hawksworth et al. 1995; Kirk et al. 2001). This number varies from 24 to 45 species, with a distribution that stretches from sub-Arctic regions to the forests of New Zealand (Corner 1950, 1967a, 1970; Thin 1961; Petersen 1968, 1969, 1971a, 1978b, 1979, 1988a, 1989; Pilát 1971; Gómez 1972; García-Sandoval et al. 2002).

The principal objective of the present work is to suggest a more robust delimitation of *Ramariopsis*, based on a phylogenetic analysis of the available information and new morphological characters derived from direct observation of herbarium specimens. The use of morphological characters presents some advantages (see Jenner 2004, Wiens 2004, for an extensive up-to-date review) and for this particular case, these include the possibility of a wide sampling of species because of the availability of herbarium material and the opportunity to directly test the hypothesis of homology for diverse characters considered taxonomically relevant.

## Materials and Methods

*Selection of outgroups and taxonomic sampling.* The selection of outgroups was critical for the present study since a reference phylogenetic framework is lacking and an inadequate or insufficient selection of external groups could result in the artificial interpretation of monophyly of the group of interest (Nixon & Carpenter 1993, Hopple & Vilgalys 1999). Selection was based on three criteria: a) a phylogenetic survey of the family *Clavariaceae* sensu lato (results not shown), b) previous phylogenies of the clavarioid Homobasidiomycetes, and c) previous classification proposals that include the genus *Ramariopsis* in some specific family.

The phylogenetic survey was conducted based on diverse delimitations of the family *Clavariaceae* (Donk 1964, Corner 1970, Jülich 1981, Hawksworth et al. 1995). All of the genera included in these proposals were considered, and representatives of the observed variation were selected for study. A matrix of 26 taxa and 30 morphological characters was constructed, and an initial selection of taxonomic outgroups and an operative outgroup was performed based on the strict consensus of the trees obtained from the analysis. The selection of outgroups, especially the operative outgroup, was based partially on the phylogenetic analyses by Hibbett et al. (1997), Pine et al. (1999), Humpert et al. (2001) and Binder & Hibbett (2002). Based on Petersen's (1978a, 1988a) proposals of the phylogenetic affinities of *Ramariopsis*, one additional representative of *Gomphus* was selected to complete the taxonomic outgroups. Sampling of the ingroup was based on Petersen's (1978a) proposed delimitation of *Ramariopsis*, which includes the species considered by Corner (1950). Taxa representative of the observed variation, and with available herbarium specimens, were chosen for analysis.

For a few confusing species [*Clavaria sulcata* (Overeem) R.H. Petersen, *Clavaria vermicularis* Sw., *Clavaria amoena* Zoll. & Moritzi and *Clavaria aurantiocinnabarina* Schwein.], assignment of specific epithets and the concepts used to delimit species followed Petersen (1967, 1976, 1979, 1980a, 1988a).

*Analysis of characters and elaboration of the data matrix.* Morphological observations were analyzed and interpreted in the framework of cladistic ontology (Hennig 1966, Farris 1983, de Pinna 1991, De Luna & Mishler 1996). The selection and analysis of characters were based on the variation observed among sampled species, without excluding a priori any sources of information (Poe & Wiens 2000). Hypotheses of homology were elaborated based on the homology criteria proposed by de Pinna (1991), employing similarity, conjunction, independence, variability, and heritability as auxiliary criteria (Patterson 1988, Rieppel 1988, Brower & Schawaroch 1996, Hawkins et al. 1997, Rieppel & Kearney 2002).

Codification of characters followed the criteria proposed by de Pinna (1991) and later additions (Hawkins et al. 1997, Hawkins 2000, Kluge 2003, Grant & Kluge 2004). Characters were not ordered nor polarized a priori to avoid bias in the exploration of tree space (Hauser & Presch 1991). Similarly, no weighting scheme was applied a priori to avoid ad hoc hypotheses that would constrain the results (Farris 1983). Character states were analyzed by directly observing herbarium specimens from distinct collections (see Table 1); these data were complemented by previous descriptions (Coker 1923; Singer 1945, 1986; Corner 1950, 1957, 1966, 1967a, b, 1970; Thin 1961; Petersen 1964, 1965, 1966, 1967a, 1968, 1969, 1971a, b, 1978b, c, d, 1979, 1980b, 1984, 1985, 1988a, b, 1989; Petersen & Olexia 1967, 1969; Bataile 1969; Fiasson et al. 1970; Schild 1971; Kühner 1977; Hubbard & Petersen 1979; Claus 1983; Pegler & Young 1985; Gill & Steglich 1987; Hansen & Knudsen 1997; García-Sandoval et al. 2002; Gill 2003; Bertagnolli & Novello 2004). A matrix of 36 characters (see Appendices 1 and 2) was constructed, that included observations of macro- and micro-morphology, macro- and micro-chemical reactions, and biochemical, cytological, and ultrastructural characters. Information for the homology hypothesis came primarily from direct observation of herbarium specimens and only in few cases were based on previously reported data (see Appendices).

*Tree searches, robustness, and topology test.* A series of heuristic searches were performed with 1,000 replicates in PAUP* 4.0b10 (Swofford 2002), using TBR, random addition, and MAXTREE set to auto-increase. A branch and bound search was performed using as an upper limit the observed tree length from the heuristic searches, and characters were optimized with the ACCTRAN option.

Interpretation of the change of character states along phylogenies was made in WinClada (Nixon 2002), using one of the most parsimonious trees encountered in the branch and bound search.

Bremer's support (Bremer 1994) was calculated to evaluate the robustness of the observed clades. The analysis was conducted using AutoDecay 4.0 (Eriksson 1999) with 100 heuristic replicates per search, using random addition, MAXTREE set to auto-increase, the ACCTRAN option for optimization, and equally weighted characters. Bootstrap values (Felsenstein 1985) were also calculated using 10,000 replicates sampling all characters, with 10 heuristic searches for each bootstrap replicate, TBR branch rearrangement, and MAXTREE set to 100 trees.

Table 1. List of specimens examined.

| Species | | Specimens |
|---|---|---|
| *Clavaria amoena* | TO | Corner CLAVARIA–4 (E); Donk 13690 (L) |
| *Clavaria aurantiocinnabarina* | TO | Cifuentes 2004–94 (FCME); Corner RSNB–8376 (L); Corner RSNB–8378A (L); Corner ICTA–1501 (E) |
| *Clavaria gibbsiae* Ramsb. | TO | Corner 442 (L); Corner 24165 (L); Corner–Singer 24165 (E) |
| *Clavaria sulcata* | TO | Hongo 705 (L); Corner s.n. (E); Corner 1676 (E) |
| *Clavaria vermicularis* | TO | Brit. Mycol. Soc. 12099 (L); Kotlaba s.n. (L); Corner NG 192 (E); Corner RSS 1439 (E) |
| *Clavaria zollingeri* Lév. | TO | Corner s.n. (E); Corner s.n. (E) |
| *Clavariadelphus pistillaris* (L.) Donk | TO | Meyer 3700 (TENN); Petersen 4920 (TENN) |
| *Clavulinopsis corniculata* (Schaeff.) Corner | IG | Piepenbroek & Piepenbroek 876 (L); Mass Geesteranus 14580 (L); Villegas 1144 (FCME); López 782 (ENCB); Aranda-Breceda 4 (FCME); Corner & Thind 206 (E) |
| *Clavulinopsis fusiformis* | IG | Guzmán U-482 (XAL); Cooke & Cooke 45644 (XAL); Cooke & Cooke 39815 (XAL); Hongo 764 (L); Villegas 1313 (FCME); Villegas 1305 (FCME); Heredia 371 (XAL); Heredia 371 (XAL); Santillán s.n (XAL); Guzmán & Ventura 5835 (ENCB); Ventura 13281 (ENCB); Villegas 1438 (FCME) |
| *Clavulinopsis helvola* | IG | Bas 6730 (L); Maas Geesteranus 13887 (L) |
| *Clavulinopsis laeticolor* (Berk. & M.A. Curtis) R.H. Petersen | IG | Corner 452 (L); Donk 13896 (L); Villegas 1803 (FCME); Hernández 188 (IBUG); Altamirano 628 (TLXM); Villegas 1450 (FCME). |
| *Gomphus clavatus* (Pers.) Gray | OO | Petersen 1797 (TENN); Arias-Montes s.n (FCME) |
| *Gomphus floccosus* (Schwein.) Singer | TO | Cifuentes 111 (FCME); Moreno-Fuentes 418 (FCME); Villegas 1109 (FCME); Fajardo s.n (FCME). |
| *Lactarius indigo* (Schwein.) Fr. | TO | Mendoza 9–09–1983 (FCME) |
| *Ramariopsis californica* | IG | Petersen 3006 (TENN); Petersen 280109 (TENN) |
| *Ramariopsis crocea* (Pers.) Corner | IG | Locrakker s.n. (L); Jalink & Nauta 6384 (L); de Vries s.n. (L) |
| *Ramariopsis kunzei* | IG | Bas 5105 (L); Corner RSNB-8291; Petersen 3909 (TENN); Guzmán U-399 (ENCB); Petersen s.n. (TENN); Villegas 1804 (FCME); Pérez-Ramírez 280 (FCME); Rodríguez s.n. (ENCB); Guzmán-Dávalos 2848 (IBUG); Guzmán 22666 (ENCB); Guzmán 6969 (ENCB); Valenzuela 1197 (ENCB); Corner NG-237 (E); Corner NG-229 (E); Ruíz & Herrera 3494 (MEXU) |
| *Ramariopsis pulchella* (Boud.) Corner | IG | Corner NG-217 (E); Altamirano 148 (TLXM); Altamirano 157 (TLXM) |
| *Ramariopsis tenuiramosa* Corner | IG | Donk 11421 (L); Mass Geesteranus 9576 (L); Geesink 1504 (L); Corner NG-124 (E) |
| *Scytinopogon dealbatus* (Berk.) Corner | TO | Corner s.n. (E) |
| *Scytinopogon echinosporus* (Berk. & Broome) Corner | TO | Corner 1517 (E) |
| *Scytinopogon robustus* (Rick) Corner | TO | Cifuentes 676 (FCME); Cifuentes 2004–26 (FCME) |
| *Scytinopogon pallescens* (Bres.) Singer | TO | Martínez-C. s.n (ENCB) |

Herbaria: E = Royal Botanic Garden, Edinburgh, Scotland, United Kingdom; ENCB = Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México; FCME = Facultad de Ciencias, UNAM, México; IBUG = Instituto de Botánica, Universidad de Guadalajara, México; L = National Herbarium Nederland, Leiden University Branch, Nederlands; MEXU = Instituto de Biología, UNAM, México; TENN = University of Tennessee, Knoxville, EUA; TLXM = Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, México; XAL = Instituto de Ecología, A.C., Xalapa, México; IG = Ingroup (= group of interest); TO = Taxonomical outgroup; OO = Operative outroup.

These parameters were selected to allow for a large number of bootstrap replicates and a reasonably accurate search procedure for each replicate (as opposed to the "fast bootstrap" option), thus avoiding the underestimation of clade support (DeBry & Olmstead 2000; Mort et al. 2000).

Templeton's topology test (Templeton 1983) was used to evaluate differences between the observed phylogenetic hypothesis and that of Petersen (1978a), employing a two-tailed Wilcoxon's signed rank test following Templeton (1983). To conduct the test, a branch and bound search was performed constraining monophyly of the representatives of *Ramariopsis* sensu Petersen. To select a subgroup of equally parsimonious trees for topology testing, a second branch and bound search was conducted using ACCTRAN to optimize characters and successive weighting (Farris 1969) following Carpenter (1988, 1994), using the RI to calculate reweighting. Each of the most parsimonious trees thus encountered was compared with each of the most parsimonious trees from the branch and bound search with successive weights (see above) using the Templeton test implemented in PAUP*b10 (Swofford 2002), and the results were compared to tables of critical values of T for the Wilcoxon test.

Diverse methods exist to evaluate the stability of a phylogeny with respect to the inclusion/exclusion of taxa (see Grant & Kluge 2003 for an extensive review). The present study assessed the impact of taxonomic outgroups sampling with a selective inclusion/exclusion of those taxa, followed with branch and bound searches of all of the combinations of taxonomic outgroups: *Clavaria, Clavariadelphus, Gomphus, Lactarius,* and *Scytinopogon.*

## Results

Twenty-three species were chosen for analysis based on the criteria employed for outgroup selection, taxonomic sampling, and character analysis (Table 1). The branch and bound search resulted in 12 trees of 80 steps in length (CI = 0.4875, RI = 0.7153, RC = 0.3487). The strict consensus of these (see Fig. 2) shows *Ramariopsis* sensu Corner (1950, 1970) as a monophyletic group.

This group forms a monophyletic clade with the representatives of *Scytinopogon.* Bootstrap analysis indicated a generally low level of support across the observed clades (see Fig. 2); only the clades containing representatives of *Ramariopsis* Corner, and *Scytinopogon* showed bootstrap support above 50%. Interestingly, there was no significant support for the clade that includes most of the representatives of *Clavaria* sensu Petersen. Bremer support was also relatively low for most of the clades, and the highest values corresponded to the clade of *Scytinopogon.*

During the constrained analysis conducted for topology tests, 1540 equally parsimonious trees of length 85 (CI = 0.4535, RI = 0.6781, RC = 0.3075) were found, five steps longer than those in the unconstrained search.

The application of successive weightings allowed selection of a subset of 8 most parsimonious trees from those found in the original branch and bound search. Application of the topology test, however, did not indicate significant differences between the hypotheses. Comparison of all topologies resulted in no significant values of N = 16-14, T = 53-39.5, P = 0.4545-0.4220.

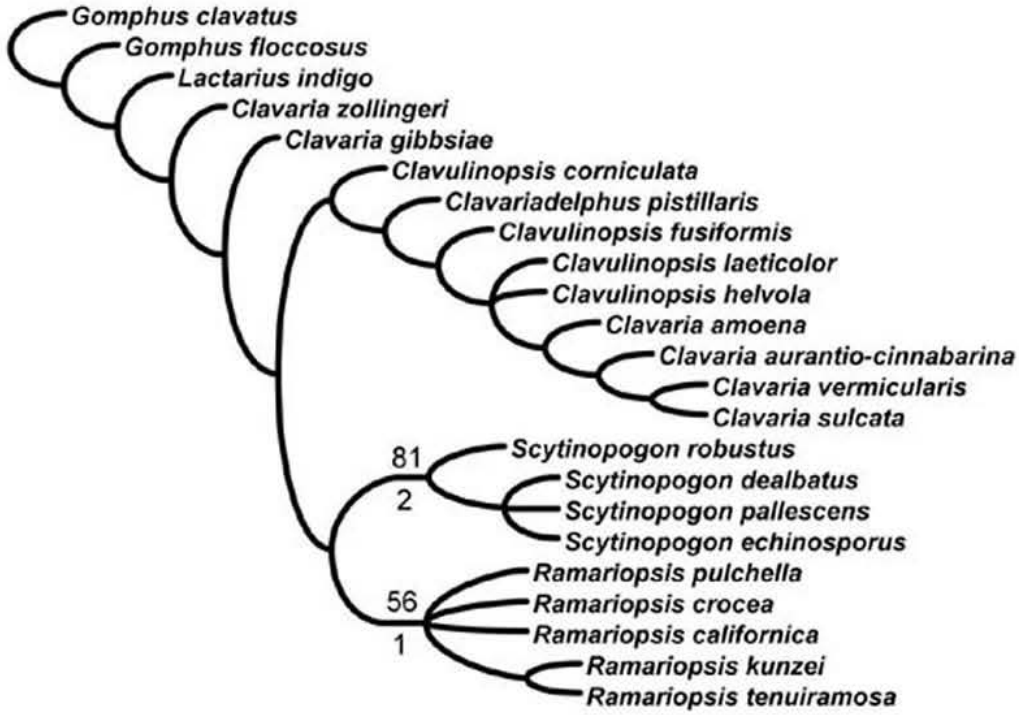


**Figure 2.** Strict consensus of the 12 trees of 80 steps (CI = 0.4875, RI = 0.7153, RC = 0.3487) found during the branch and bound search. Numbers below branches indicate Bremer support indices, and numbers above branches indicate bootstrap support values.

The combinations of selective inclusion/exclusion tested (not shown) did not modify the monophyly of *Ramariopsis* sensu Corner, but decreased the resolution of the topology.

## Discussion

The phylogenetic analyses performed support the monophyly of of *Ramariopsis* sensu Corner (1950), though with moderate bootstrap support (56%). Based on these results, *Ramariopsis* is limited to species with branched basidiomes, echinulate spores, and cyanophilous spore ornamentation derived from the tunica (Pegler & Young 1985).

The ultrastructural composition of the ornamentation is a synapomorphy for the group (see Fig. 3), but a large part of the cladogram was optimized as ambiguous for this character because of the lack of information for several species –e.g. information for any of the species of *Scytinopogon* included is not available. Although Corner did not include *Ramariopsis californica* in his most recent treatment of the genus (Corner 1970), this species exhibits all of the distinctive characters of the genus and the present results support its inclusion in this taxon. On the other hand, Petersen (1978a) included *Clavulinopsis helvola* in *Ramariopsis* [= *Ramariopsis helvola* (Pers.) R.H. Petersen], although this species has simple basidiomes and spores with thick tuberculous ornamentation. Pegler & Young (1985) established that this ornamentation is formed from growth of the corium, whereas ornamentation in *Ramariopsis* is formed via growth of the tunica. The results of the present study support the segregation of *C. helvola* from *Ramariopsis*.

The representatives of *Scytinopogon* form a well-supported group (81% bootstrap support), consistent with the original delimitation of Singer (1945) based on the presence of branched, thelephoroid basidiomes and verrucose spore ornamentation. In our results, *Ramariopsis* sensu Corner forms a monophyletic group with the representatives of *Scytinopogon*. These taxa all have cyanophilous spores with ornamentation partially derived from the tunica, though in *Scytinopogon* the ornamentation also seems to be composed of a thick core of corium (Pegler & Young 1985). It is worth mentioning that most of the species of *Scytinopogon* included in the present analysis have not been studied ultrastructurally—such data exist for only a single species of the genus, whose taxonomic status is in doubt.

The observed relationship between *Ramariopsis* and *Scytinopogon* was first suggested by Corner (1970), but this link should only be considered tentative as the taxonomic sampling of this analysis was designed to resolve a robust delimitation of the genus *Ramariopsis*, and not to identify its sister taxon. Furthermore, the clade *Ramariopsis+Scytinopogon* does not show bootstrap support. Thus, without a broader taxonomic sampling designed to establish the affinities of *Ramariopsis* with other taxa, it is preferable to consider these results as preliminary.

One of the principal consequences of this study is that *Ramariopsis* sensu Petersen is a paraphyletic group –i.e. a grade– because it was defined by simplesiomorphic characters. Petersen (1978a) used diverse sources of information for his delimitation, including the absence of carotenoid pigments. Pigment composition has been a frequently-used auxiliary character in fungal systematics (Arpin & Fiasson 1971, Tyler 1971, Gill & Steglich 1987, Frisvad et al. 1998, Gill 2003), but recent studies indicate that phylogenetic patterns inferred in the Homobasidiomycetes based on this type of character are often incongruent with the results obtained using other sources of information (Hibbett & Thorn 2001, Pine et al. 1999).

Nonetheless, the presence of certain types of pigments can be a very useful auxiliary character in studies aimed at generic delimitation (e.g. Feibelman et al. 1997, Weinstein et al. 2002), and the taxonomic relevance of this type of characters should not be completely discarded, but perhaps restricted to use at lower taxonomic levels. In the case of *Ramariopsis*, the delimitation proposed by Petersen (1978a) was based on the absence of carotenoid compounds, without specify the nature of the pigments present and without an explicit reference to a concrete character; the inferred pattern thus cannot be directly confirmed or refuted. In the present study this character was coded as the presence or absence of carotenoid pigments–character 11. (See Appendices for character argumentation.)

This character does not show evidence of homoplasy in the present study based on its observed distribution (CI = 1.0), although optimization of the character is not definitive due to the absence of information in several of the considered species (see Fig. 4). Additionally, the distribution of carotenoids in the present study supports the relationship among *Clavaria aurantiocinnabarina*, *C. amoena* and *C. sulcata*, and is congruent with a monophyletic group that includes representatives of *Clavaria* (see Fig. 2). This latter group is consistent with Petersen's (1978a, 1988a) delimitation of *Clavaria*. It is worth mentioning that the aforementioned results regarding *Clavaria* should be

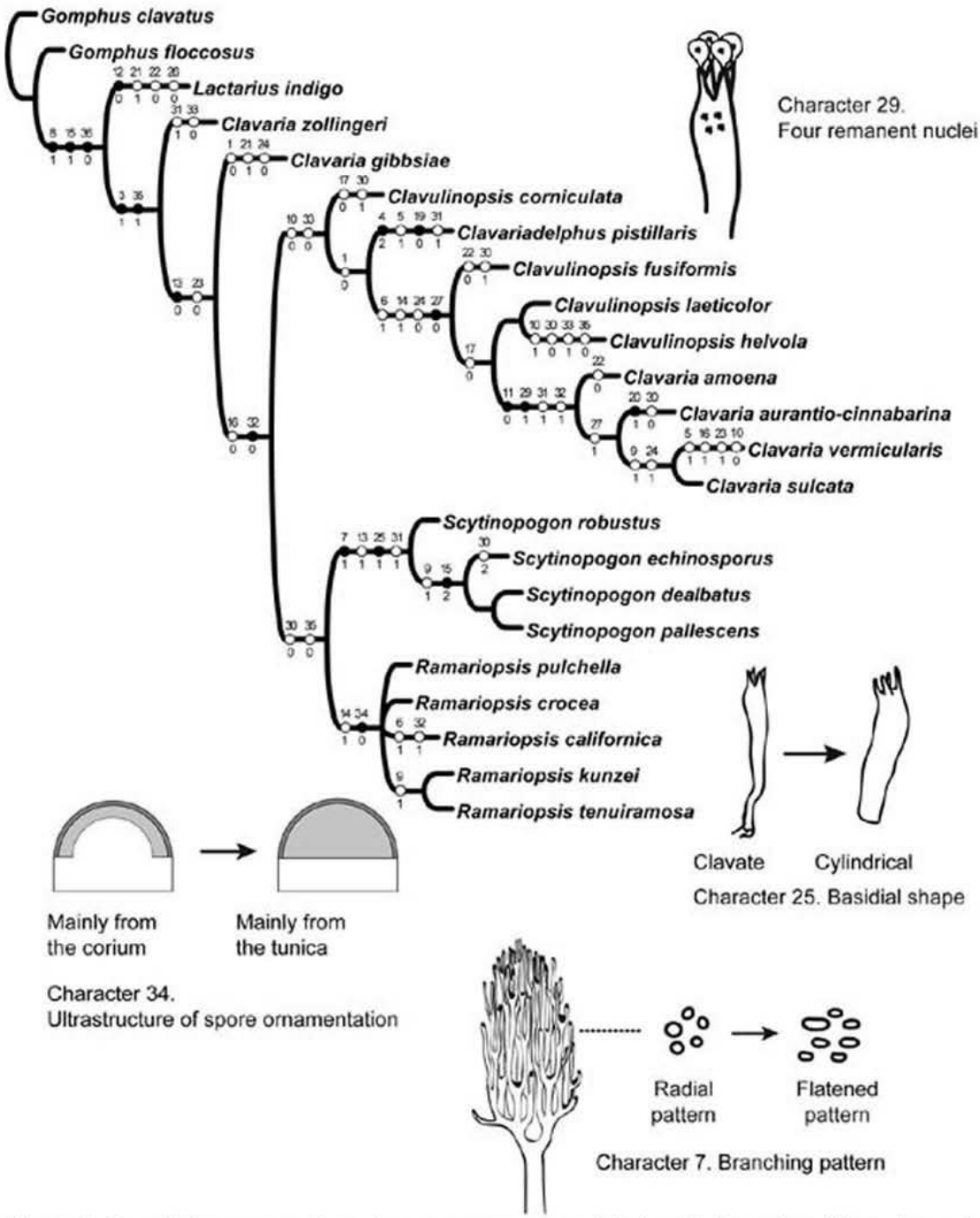


**Figure 3.** One of the most parsimonious trees encountered during the branch and bound search, showing the character states that can be unambiguously optimized. Numbers above dots indicate the character and numbers below dots the character state. Apomorphic states are shown in black dots and homoplastic states in white dots. Selected character transformations are illustrated close to the branch were change occur (see appendix 1 for character argumentation).

considered as preliminary due to the lack of bootstrap support and the taxonomic sampling of the present study.

Another relevant character used by Petersen (1978a) was the presence of spores with a conspicuous hilar appendix. Several species of *Clavulinopsis* subgenus *Cornicularia*

was transferred by Petersen to *Ramariopsis* based—in addition to the other mentioned character—on the presence of a conspicuous hilar appendix. In this study, this character was included (character 31) and their optimization is depicted in figure 4 (see also Appendices for character argumentation). The present results show it as a simplesiomorphy.

Farris (1991) provided an explicit criterion to recognize paraphyletic groups by tracing the status of the character used to define it; in the case of *Ramariopsis* prominent hilar appendix and absence of carotenoid pigments were used by Petersen in order to define the genus, both characters are plesiomorphic and shared –simplesiomorphic– (see Fig. 4). In the strict sense, the present results show *Ramariopsis* sensu Petersen as a paraphyletic group.

Petersen (1978a) also employed the presence of chiastic basidia with a post-meiotic mitotic division and four nuclei remaining in the basidia after spore formation as a cytological pattern that supported the delimitation of *Ramariopsis*. Recent phylogenetic studies (Hibbett et al. 1997, Pine et al. 1999) confirm the utility of cytological characters in the delimitation of taxonomic groups among the clavarioid and cantharelloid Homobasidiomycetes, but while the stictic pattern appears phylogenetically informative, the chiastic condition, which is widely distributed among the Homobasidiomycetes, does not seem to follow a clear phylogenetic pattern (Hibbett & Thorn 2001). As with the presence of carotenoid pigments, the utility of the chiastic condition in our analyses is noted, though more studies are necessary.

The pattern of four remaining nuclei reported for *Ramariopsis crocea* (Penancier 1961) results from a post-meiotic mitotic division. Post-meiotic mitotic divisions resulting in four nuclei remaining in basidia following spore formation are reported for a diversity of other taxa (Penancier 1961, Duncan & Galbraith 1972, Restivo & Petersen 1976, Kühner 1977, Mueller & Ammirati 1993). Both the meiotic pattern (chiastic/stictic) and the number of remaining nuclei (see Appendices for discussion and codification) were included as characters in the present study.

All of the species in our study for which data were available present a chiastic pattern, so it was not informative for addressing our questions. Very possibly this character could have relevance at other hierarchical levels when studying the taxonomic affinities of the genus *Ramariopsis*.

The number of remaining nuclei (character 29 CI = 1.0) did not present a homoplasious distribution, though, similar to the situation for carotenoid pigments, optimization of this character should be considered preliminary since data were not available for all species considered, and this lack of information results in a severely ambiguous optimization (results no shown).

Our finding of a monophyletic group that includes representatives of *Clavulinopsis* and representatives of *Clavaria* is congruent with the results of Pine et al. (1999). However, relationships among *Clavariadelphus pistillaris*, *Clavaria zollingeri* and *C. gibbsiae* are not consistent with previous classifications (see Fig. 2), and *Clavariadelphus* is found in a position incongruent with previous studies (Hibbett et al. 1997, Pine et al. 1999).

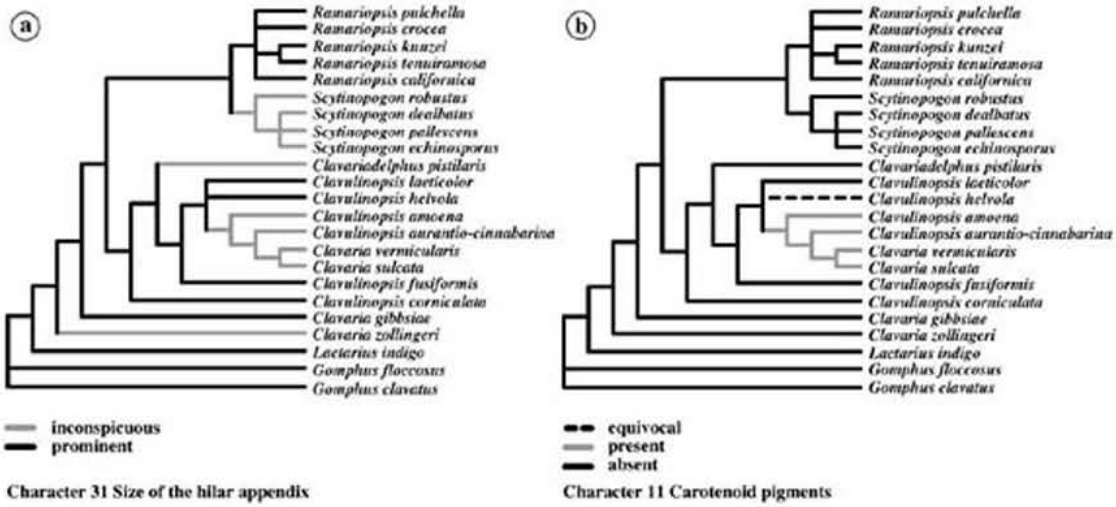


**Figure 4.** Optimization of two characters used by Petersen (1978a) to define *Ramariopsis*, onto one of the most parsimonious trees. a) Optimization of character 31 showing prominent hilar appendix as a simplesiomorphy; b) optimization of character 11 showing the absence of carotenoid pigments as a symplesiomorphy.

The position of *Clavariadelphus pistillaris* could be an artifact, due to the fact that the present sampling of taxonomic outgroups included distantly related groups –e.g. *Gomphus* in the clade Gomphoide-Phalloide sensu Hibbett & Thorn (2001).

To explore this possibility, a branch and bound search was performed excluding *Clavariadelphus*, and the monophyletic group of *Ramariopsis* sensu Corner+*Scytinopogon* obtained in the main analysis was recovered (results not shown).

It has not been possible to clearly elucidate the phylogenetic affinity of *Clavaria zollingeri*. This taxon was included in the analysis of clavarioid and cantharelloid Homobasidiomycetes by Pine et al. (1999), but their results were inconclusive and this species was located outside of any recognized clade in the strict consensus analysis of combined genes. In spite of this, indicated that the current delimitation of *Clavaria* (Corner 1970) –simple or branched basidiomes, monomitic hyphae in the context, clamp connections absent in the context, and present or absent of clamp connections at the base of the basidia– was not a monophyletic group. The results of our study are congruent with those of Pine et al. (1999).

*Clavaria gibbsiae* in the present results is located next to *C. zollingeri*, out of any large clade –e.g. *Clavaria* sensu Petersen or *Ramariopsis* sensu Corner. This species is traditionally included in *Clavaria* subgenus *Holocoryne* (Corner 1970, Petersen 1988a). We could expect a relationship between *Clavulinopsis* and representatives of *Clavaria* based on previous results (Pine et al. 1999, Larsson et al. 2004), but these studies also indicate that *Clavaria* is not a monophyletic group (Pine et al. 1999). Our results are congruent with these previous studies and show a core group that includes part of *Clavaria* and all the included representatives of *Clavulinopsis*. These results should be considered as preliminary due to the lack of bootstrap support for this group and the taxonomic sampling of the present study.

One important result from the present study is the proposal of nomenclatural changes in *Ramariopsis*. At the present time, phylogenies seem to be divorced from classifications since few phylogenetic hypotheses are used as the foundation for newer classifications. This may result in the undesirable situation in which robust phylogenetic papers have little impact on the daily practice of taxonomists (for a broader discussion on this issue see Wheeler 2004, Franz 2005). Phylogeneticists are frequently reluctant to introduce changes in the classification due to the nature of the phylogenetic research—e.g. occasionally the relative position of a clade undergoes modifications with the addition of new data. In the present case, we decided to make taxonomical decisions based on our phylogenetic results by introducing changes only when we felt confident to do it.

Our results are robust enough to restrict *Ramariopsis* to species with ornamented spores—which show the characteristic ultrastructural pattern. The only problem arises when we try to identify the species that meet those requirements since in some species spore ornamentation is difficult to see. There are cases in which some taxa originally described with smooth spores are demonstrated to have ornamented spores—one example of this is *Ramariopsis californica* R.H. Petersen (Pegler & Joung 1985). For this reason, and until we have more information—e.g. SEM and TEM studies of the spores—we avoid proposing new combinations for species with smooth spores originally described in *Ramariopsis* (see Appendix 3). We only recommend the use of combinations previously proposed, that are congruent with our results (see Appendix 3). The only exception is *Ramariopsis antillarum* (Pat.) R.H. Petersen. This taxon was originally described as *Clavaria fusiformis* var. *antillarum* Pat.; subsequently, Petersen (1988a) proposed to raise it to species rank based on the differences in the ontogenetic patterns of the basidiomes between yet the species and the variety. We concur with Petersen's proposal, yet we consider that the correct placement for this species is in *Clavulinopsis* based on the presence of simple club basidiomes and globose, smooth spores. Our current knowledge of this species leads us to propose the combination

***Clavulinopsis antillarum*** (Pat.) García-Sandoval & Cifuentes, **comb. nov.**

Basionym: *Clavulinopsis fusiformis* var. *antillarum* Pat., in Duss, Enum. Methodique des champignons recueilles a la Guadeloupe a la Martinique (Lons-le-Saunier): 14 (1903).

We have included a checklist of available species names for *Ramariopsis* and their correct combinations according to the present results (see appendix 3). The list is divided in three parts: a) species confidently placed in *Ramariopsis* sensu stricto; b) taxa once included in *Ramariopsis* that do not belong to *Ramariopsis* according to our results and available information; and c) species originally described in *Ramariopsis* that need further examination before a new combination be proposed. We think that this checklist provides practical applications, avoiding the proposal of unjustifiable new combinations that may result in unstable nomenclatural changes.

Two combinations are excluded from the list:

a) *Ramariopsis bizzozeriana* (Sacc.) Schild. (= *Clavaria bizzozeriana* Sacc.). *C. bizzozeriana* was recognized as a taxonomic synonym of *Ramariopsis pulchella* by Corner (1950); later the combination *Ramariopsis bizzozeriana* was incorrectly preferred over *Ramariopsis pulchella* by Schild (1972). This last combination should not be used

because *C. bizzozeriana* is currently considered a taxonomic synonym of *R. pulchella* (for details see Corner 1950, Petersen 1978b).

b) *Ramariopsis lentofragilis* (= *Clavaria lentofragilis* Atk.). Corner (1950 p. 640) considered *Clavaria lentofragilis* Atk. a taxonomic synonym of *Ramariopsis kunzei*, although he kept doubts. In his description of *Ramariopsis lentofragilis* f. *propera* (Bourdot) R.H. Petersen, Petersen (1969 p. 550) used the combination *Ramariopsis lentofragilis* without making any reference to the authority of the combination. In a subsequent article Petersen (1978a p.669) acknowledged Corner as the author of the combination. However, *Ramariopsis lentofragilis* was not considered by Corner (1950, 1970), who only referred to the species as a taxonomic synonym of *R. kunzei*. Whether *C. lentofragilis* is a synonym of *R. kunzei* or not is a matter that needs further investigation. For that reason we prefer to exclude that possible combination from the checklist.

In conclusion, the present study indicates that the delimitation of *Ramariopsis* proposed by Corner (1950, 1970) is robust, given currently available data. Although the topological comparisons did not find significant differences between this hypothesis and that proposed by Petersen (1978a), our analysis indicates that *Ramariopsis* sensu Corner represents a more parsimonious hypothesis (five steps shorter), in accordance with ultrastructural data on spore ornamentation and patterns of cyanophilous reaction in the spores. Additionally, the test of sensitivity of the taxonomic sampling indicated that the results obtained were not an artifact of taxon selection and are stable across various resamplings of the data. Relationships among taxa outside of the clade *Ramariopsis* sensu Corner should be taken as tentative, given that the sampling of the present study was designed for other objectives. Recently Dentinger & McLaughlin (2005) addressed the relationships of *Clavariaceae* and *Pterulaceae*; in their sampling they included representatives of *Ramariopsis* sensu Petersen and *Clavaria* sensu Petersen. Their results agree with our study and show *Ramariopsis* sensu Petersen as a paraphyletic group while also showing support for a clade congruent with *Clavaria* subgenus *Clavulinopsis*. In our results, we also find a clade congruent with the mentioned subgenus of *Clavaria*, but with non-bootstrap support. Future studies addressing *Clavariaceae* question are needed, but current findings (e.g. Dentinger & McLaughlin 2005) provide important insights about this questions.

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## Appendix 1. Characters and character states.

### Morphological characters of the basidiome

1.- *Simple clavate basidiome.* This type of basidiome corresponds to what Petersen (1988a) defined as clavarioid –holobasidiomycetes, in simple erect columns– excluding the branched forms which show a distinct ontogenetic pattern as described by Corner (1950). This includes intergradations from simple clavate forms to those with some amount of apical branching (bifurcated towards the apex). Within this general pattern are several ontogenetic variants (Corner 1950, Clémençon et al. 2004) that could be phylogenetically informative, but to date there are too few data to recognize discrete patterns; in the present study only the general pattern was considered. States: 0: present, 1: absent.

2.- *Profusely branched basidiomes.* This character corresponds to what Petersen (1988a) described as clavarioid (see character 1), but is confined to the branched forms, since this corresponds to an ontogenetic pattern distinct from the simple forms (Corner 1950). A basidiome was considered profusely branched when it exhibited three or more levels of branching coming from the middle or below the middle of the basidiome. States: 0: present, 1: absent.

3.- *Basidiome pileate-stipitate.* Corner (1966) defines the pileus as an apical expansion developed from a diageotropic growth that generates fan or umbrella shaped forms. This differs from the cantharelloids in the configuration of the hymenophore and the absence of a thickened hymenium. In the present study any basidiome exhibiting a pileus sensu Corner (1966) and a stipe sensu Kirk et al. (2001) was considered as pileate-stipitate, independent of the conformation of the hymenophore. States: 0: present, 1: absent.

4.- *Form of the hymenophore.* For the present study the hymenophore was defined following Clémençon et al. (2004) as the portion of the context that supports the hymenium –the layer of basidia, basidiospores, and sterile elements– in contrast to the proposal of Kirk et al. (2001), who considered the hymenophore the structure which supports spores –e.g. a basidiome. In the present study the hymenophore was considered to exhibit variation in form independent of that of the basidiome, and as such is an independent character (Mickevich 1982, Mickevich and Limpscomb 1991, Limpscomb 1992, Mabee 1993, O'Keefe & Wagner 2001) –against Clémençon et al. (2004) see character three– for example a smooth hymenophore can be present in a simple, clavate, or corticioid basidiome. The recognized states correspond in the case of gills to the description of Singer (1986), for a hymenophore in folds to Corner (1966) in cantharelloid fungi, for a smooth hymenophore to Clémençon et al. (2004), and for a wrinkled hymenophore to the description of some species of Clavariadelphus by Corner (1950) though with a lesser grade of organization. States 0: gills, 1: folds, 2: wrinkles, 3: smooth.

5.- *Longitudinally sulcate in simple clavate basidiomes.* This character corresponds to the description by Petersen (1988a) and represents those basidiomes that exhibit a furrow or longitudinal fold along the fertile part of the basidiome; it differs from a wrinkled hymenophore in that the furrow or fold is singular. States: 0: present, 1: absent.

6.- *Development of the context at the level of the hymenium.* This character corresponds to the presence or absence of the condition described as fistular or hollow by Kirk et al. (2001), but confined to the context at the level of the hymenium. The portion of the context below the hymenophore and subhymenium can exhibit distinct grades of development with two clearly recognizable states: when it is well developed, the basidiome exhibits a solid aspect in transverse section, but when poorly developed the basidiome appears hollow or fistulate in transverse section. States 0: fistulate, 1: solid.

7.- *Pattern of branching.* Profusely branched basidiomes exhibit different patterns of branching derived from differences in ontogenetic development (Corner 1950). The nomenclature and patterns described by Corner (1950) were followed for the present study. Only two states were observed among the included species in the present analysis. States 0: radial, 1: flattened.

8.- *Mycelial cords.* Aggregates of linear hyphae growing away from the basidiome and visible to the naked eye were considered mycelial cords. Clémençon et al. (2004) and Boddy (1999) distinguished between mycelial cords and rhizomorphs based on the level of organization and the type of growth of the structure. In the present study no distinctive apical growth was identified, and the observed structures were thus only characterized as mycelial cords in the general sense of Cairney et al. (1991). States: 0: present, 1: absent.

9.- *Mycelium at the base of the stipe.* Basidiomes growing from a patch or pillow of mycelium were considered as exhibiting mycelia at the base of the stipe. The mycelial growth was always conspicuous and found above the substrate; this mycelium covers the base of the stipe and exhibits different types of generative hyphae. Petersen (1988a) described subiculate as a patch of mycelia in the substrate from where the basidiome grows, but Clémençon et al. (2004) restricted the term subiculate to the thick layer of mycelia from which the corticioid basidiomes develop. We treat the character as equivalent to what Petersen (1988a) denominates subiculate, but since Clémençon et al. (2004) employed the term in a different manner, the descriptor subiculate is not used in the present study. States: 0: present, 1: absent.

10.- *Reaction of hymenium to iron salts.* The reaction to iron salts is a widely used character in the systematics of clavarioid fungi (Corner 1950; Donk 1964; Petersen 1978a, 1988a). The reagent contains ferric chloride in a 10% aqueous solution (Petersen 1988a), and is applied directly to the hymenium. A positive reaction is recognized by a color change to olive-green or gray-green. This reaction is considered indicative of the presence of the compound pistillarine (Steglich et al. 1984). A positive reaction to this reagent can exhibit other color changes due to the presence of distinct compounds (Gill & Steglich 1987, Singer 1986). In the present study only positive reactions that engendered olive-green or gray-green color changes were considered. Observations were made in dryed exemplars. In our experience species with positive reaction in fresh material also react when dry. States 0: positive, 1: negative.

11.- *Carotenoid pigments in the basidiome.* Along with sesquiterpinoids, carotenoids are the only pigments present in the macromycetes derived from the mevalonate pathway (Gill & Steglich 1987, Gill 2003). This character was coded as a nominal variable –sensu Hawkins (2000)– based on the aviable information for the species considered since other types of coding would require additional data about the specific metabolic pathways generating the compound (e.g. Barkman 2001). States 0: present, 1: absent.

## Micromorphological characters distinct from the hymenium and the spores

12.- *Lacticiferous hyphae.* This structure corresponds to what Singer (1986) described as lacticiferous in the strict sense –hyphae that produce latex. These hyphae can exhibit nuclei and septa and thus correspond to a specialized type of heteroplera sensu Clémençon et al. (2004). States 0: present, 1: absent.

13.- *Inflated hyphae.* Inflated hyphae are those generative hyphae that exhibit increased growth behind the point of lateral growth, widening and elongating significantly (Corner 1950, Kirk et al. 2001). They are recognizable by having a considerably greater diameter than the rest of the hyphae, and by having constrictions in the zone of the septa; they may or may not have clamp connections. Corner (1950) distinguished two types of monomitic contexts that present inflated hyphae based on the presence of secondary septa and clamps. In the present study this classification was not used, since it mixes two independently varying characters. States: 0: present, 1: absent.

14.- *Crystals in the hyphae of the basal mycelia.* The hyphae of the mycelia at the base of the stipe sometimes present amorphous crystals, similar to those reported in the context of the base of the stipe for *Ramariopsis pulchella* (Petersen 1988a) –5-20µm, hyaline or yellowish, and do not dissolve

in 5% KOH. The crystals are found covering the exterior surface of the hyphae and are not easily removed. States: 0: present, 1: absent.

15.- *Degree of thickening in the hyphal wall.* Thickening of the hyphal wall was considered only in the generative hyphae. The presence of generative hyphae with thickened walls has been a relevant systematic character in several genera (Corner 1966, Pegler 1996). In the present study three qualitative degrees of thickening were recognized due to the difficulty in making precise quantitative measurements. States: 0: none, 1: scarce, 2: conspicuous.

16.- *Simple fibulae.* Simple fibulae –clamp connections, clamp cells– are frequently observed in Basidiomycetes. Clémençon et al. (2004) recognized three types of simple fibulae, of which two were observed in the present study: closed and ring or medallion fibulae. Both types were considered equivalent since there were insufficient elements to determine discrete states, due the continuous variation observed. States: 0: present, 1: absent.

17.- *Geniculate fibulae.* Geniculate fibulae are those that exhibit a marked bend at the point of inflection, giving the appearance of a bent knee. The bend partially deforms the profile of the fibulae, allowing them to be easily differentiated from simple fibulae. These structures correspond to those described in various species of *Clavulinopsis* by Petersen (1968). Geniculate fibulae are not homologous to simple fibulae since both structures are found simultaneously in the same basidiome and are thus independent characters according to the conjunction test (Patterson 1988, Rieppel 1988, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). States: 0: present, 1: absent.

18.- *H connections.* These connections are structures formed by the union of two parallel hyphae through a third, transverse, hypha. They can be considered functional homologous –biologically homologous following Roth (1988)– to fibulae, but are not phylogenetically homologous sensu de Pinna (1991), since they are both present simultaneously with distinct types of fibulae. They should thus be considered independent characters following the conjunction test (Patterson 1988, Rieppel 1988, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). States: 0: present, 1: absent.

19.- *Ampulliform fibulae.* This type of fibula is characterized by the presence of a marked widening, giving the appearance of an inflated fibula similar to the inflated hyphae. This corresponds to the description by Petersen (1988a) as a characteristic of *Ramaria* subg. *Lentoramaria* Corner. This type of fibula is not phylogenetically homologous to the other types of fibulae described since it can be found present simultaneously with those other structures and should therefore be considered as an independent character by the conjunction test (Patterson 1988, Rieppel 1988, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). States: 0: present, 1: absent.

## Hymenial characters

20.- *Subhymenium clearly differentiated.* The subhymenium was considered to be the layer of generative hyphae growing below the hymenium (Kirk et al. 2001) and from which the hymenium forms (Petersen 1988a). Several distinct anatomical patterns of the subhymenium have been described (Clémençon et al. 2004), and in the case of the clavarioid fungi, Petersen (1988a) considered three types. The variation observed in the present study; did not permit differentiation of distinct types of subhymenium; only the conspicuous presence or absence of a subhymenium was considered. States: 0: present, 1: absent.

21.- *Thickening of the hymenium.* Corner (1950) described the thickening of the hymenium as a pattern resulting from the sympodial growth of the hyphae of the subhymenium, generating successive superimposed layers of hymenium, collapsing the preceding basidia. No additional patterns of variation of this type have been described to date, and in the present study no further variation was observed. States: 0: present, 1: absent.

22.- *Cystidia of the hymenium.* Cystidia are sterile hyphal apices, generally with a distinctive form and found in variable locations in the basidiome (Kirk et al. 2001, Clémençon et al. 2004). The form and anatomic disposition of the cystidia have been used as taxonomic characters, but only the cystidia in the hymenium were considered for the present study, as no other cystidia were observed in the species studied. States: 0: present, 1: absent.

23.- *Fibulate basidia.* Clamp connections on basidia are restricted to the base of the basidia. The presence on the basidia is independent of the presence of fibulae in the rest of the hyphae of the basidiome. As such, it was considered as an independent character. In the case of *Clavaria* subgenus *Holocoryne* the basidia exhibit a fibula described as broadly free (Corner 1950) or bifurcated (Petersen 1988a). States: 0: present, 1: absent.

24.- *Basidia with refringent contents.* This character refers to basidia with an oily, yellowish, appearance of its content, which is homogeneous and refringent in 10% KOH. It corresponds partially to what Petersen (1988a) described as gloeoplerotic, excluding the foamy appearance. Similarly, it corresponds partially to what Clémençon et al. (2004) described as oil-producing –resinous content– and to what Singer (1986) describes as oil-producing sensu Fayoid, but without the positive sulfovainillin reaction. States: 0: present, 1: absent.

25.- *Shape of the basidia.* The variation observed during the present study permitted recognition of two forms or general profiles of basidia: cylindrical and clavate. These terms correspond to the definitions of Kirk et al. (2001). States 0: clavate, 1: cylindrical.

26.- *Base of clavate basidia.* Clavate basidia exhibit variation in the size of the base. Variation of the size was coded as an independent character because it refers to a property or feature of an anatomical region particular to clavate basidia and is not homologous with terete basidia (de Pinna 1991, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). Additionally, this coding reflects the variation observed as it describes properties with independent variation. The size of the basidia exhibits variation logically independent of the form and thus can be coded as an independent character (Hawkins et al. 1997, Hawkins 2000, O'Keefe & Wagner 2001, Rieppel & Kearney 2002). States 0: short, 1: long.

27.- *Geniculate basidia.* These basidia exhibit a point of inflection in the middle part, and thus a marked bend giving the appearance of a flexed knee. This bend conspicuously deforms the profile of the basidia allowing them to be clearly differentiated. This pattern corresponds to that described in various species of *Clavulinopsis* by Petersen (1968). Geniculate basidia do not constitute a pattern or form homologous in the phylogenetic sense (de Pinna, 1991), since both structures are simultaneously present in the same basidiome and thus constitute independent characters by the conjunction test (Patterson 1988, Rieppel 1988, De Luna & Mishler 1996, Rieppel & Kearney 2002, Grant & Kluge 2004). States: 0: present 1: absent.

28.- *Orientation of the achromatic spindle.* Juel (1898) described two basic patterns of orientation of the meiotic spindle of basidia during meiosis: chiastic –transversal to the principal axis and situated in the apex– and stictic –parallel to the principal axis and situated in the middle. Boidin (1958) recognized an intermediate pattern that he called hemichiastic; Donk (1964) later qualified this as a homologous variant of the chiastic form. In the present study only the chiastic and stictic patterns were considered, given that those are the only patterns reported for the species studied. States: 0: chiastic, 1: stictic.

29.- *Four remaining nuclei.* After meiosis a third nuclear division sometimes occurs, producing a total of eight nuclei. In some species four of these nuclei disintegrate and are termed remaining nuclei (Penancier 1961). This pattern has been reported for several of the species considered in the present study and corresponds to that described as post-meiotic pattern "A" by Duncan & Galbraith (1972) and to that described by Kühner (1977). The data available for the species considered in

the present study only allowed recognition of the presence of a third division –exhibiting pattern "A"– and the absence of this division (only four nuclei form); this character was thus coded as a nominal variably (Hawkins 2000). States: 0: present, 1: absent.

## Characters of the basidiospores

30.- *Spore form.* The variation observed during the present study allowed the recognition of three spore forms: globose, subglobose, and elongate. States were assigned qualitatively, excluding the deformations produced by ornamentation when present, and correspond to the forms described by Kirk et al. (2001), except for the fusiform and ellipsoid forms, which are considered as elongated spores. States: 0: subglobose, 1: globose, 2: elongate.

31.- *Size of the hilar appendix.* The hilar appendix –also called the apicule, sterigmal appendix or apophysis (Kirk et al. 2001, Clémençon et al. 2004)– is the small conical or papilla-shaped projection, which is the point of connection between the spore and the sterigma. This structure is involved in the active liberation of the spores (Clémençon et al. 2004). Based on the observed variation in the species studied, two qualitative states were recognized to describe the size of the appendix. States: 0: prominent, 1: inconspicuous.

32.- *Thickness of the spore wall.* The species considered in the present study do not exhibit significantly thickened spore walls, but some species show a slight thickening. Based on the observed variation two qualitative states were recognized. States: 0: slightly thickened, 1: thin.

33.- *Smooth spores.* Spore ornamentation has been a relevant taxonomic character for *Ramariopsis* (Corner 1950), but several studies have shown that ornamentation can be derived from different layers of the spore wall. Treating the presence of ornamentation as homologous in different taxa could thus fail the test of similarity (Rieppel 1988, Nelson 1994, Rieppel & Kearny 2002). The presence of smooth spores –spores without modifications or deformations in the wall– was observed in preparations mounted in 5% KOH using a bright field light microscope at 1000x magnification. States: 0: present, 1: absent.

34.- *Ultrastructure of spore ornamentation.* The spore wall has been characterized in different studies using different sources of information –light and electron microscopy– that generated different terms to denominate the observed ultrastructural patterns (Clémençon et al. 2004). It is known that seemingly similar forms can exhibit different ultrastructural patterns (Clémençon et al., 2004), and as such regarding these forms as homologous sensu de Pinna (1991) would be incorrect by the test of similarity (Rieppel & Kearney 2002, Grant & Kluge 2004). Based on this knowledge, the ultrastructure of the ornamentation was coded instead by the morphological patterns observed. In the present study the nomenclature of Pegler & Young (1985) was used, which also corresponds to the descriptions of Hawksworth et al. (1995). States: 0: tunica, 1: corium.

35.- *Cyanophilous reaction of the spores.* For the present study a cyanophilous reaction was considered positive when the wall of the spore stains with cotton blue, following the nomenclature proposed by Kotlaba & Pouzar (in Donk 1964). During the present study the reagent was prepared dissolving 1.6 g of cotton blue in 10 ml of lactic acid. After adding the reagent, the preparation was heated until boiling and then left to cool for 10 minutes before observations were made. States: 0: positive, 1: negative.

36.- *Pattern of cyanophylly in the spores.* The positive reaction to cotton blue (cyanophylly) exhibits two patterns of coloring: homogeneous or more intense in the ornamentation. States: 0: homogeneous coloring, 1: ornamentation more cyanophilous.

## Appendix 2. Data matrix

**Table 2.** Data matrix for the 23 species and 36 characters.

? = missing data, – = inapplicable data. For description of the characters and character states see Appendix 1.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Clavaria amoena* | 0 | 1 | 1 | 3 | 0 | 1 | – | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| *Clavaria aurantiocinnabarina* | 0 | 1 | 1 | 3 | 0 | 1 | – | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Clavaria gibbsiae* | 0 | 1 | 1 | 3 | 0 | 0 | – | 1 | 0 | 1 | ? | 1 | 0 | ? | 1 | 1 | – | 0 |
| *Clavaria sulcata* | 0 | 1 | 1 | 3 | 0 | 1 | – | 1 | 1 | 1 | ? | 1 | 0 | – | 1 | 0 | 0 | 0 |
| *Clavaria vermicularis* | 0 | 1 | 1 | 3 | 1 | 1 | – | 1 | 1 | 1 | ? | 1 | 0 | – | 1 | 1 | – | 0 |
| *Clavaria zollingeri* | 1 | 0 | 1 | 3 | – | 0 | 0 | 1 | 0 | 1 | ? | 1 | 1 | 0 | 1 | 1 | – | 0 |
| *Clavariadelphus pistillaris* | 0 | 1 | 1 | 2 | 1 | 0 | – | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| *Clavulinopsis corniculata* | 1 | 0 | 1 | 3 | – | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| *Clavulinopsis fusiformis* | 0 | 1 | 1 | 3 | 0 | 1 | – | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| *Clavulinopsis helvola* | 0 | 1 | 1 | 3 | 0 | 1 | – | 1 | 0 | 1 | ? | 1 | 0 | 1 | 1 | 0 | 0 | ? |
| *Clavulinopsis laeticolor* | 0 | 1 | 1 | 3 | 0 | 1 | – | 1 | 0 | 0 | 1 | 1 | 0 | ? | 1 | 0 | 0 | 0 |
| *Gomphus clavatus* | 1 | 1 | 0 | 1 | – | 0 | – | 0 | 1 | 1 | 1 | 1 | 1 | – | 0 | 0 | 1 | ? |
| *Gomphus floccosus* | 1 | 1 | 0 | 1 | – | 0 | – | 0 | 0 | 1 | ? | 1 | 1 | 0 | 0 | 1 | – | ? |
| *Lactarius indigo* | 1 | 1 | 0 | 0 | – | 0 | – | 1 | 1 | 1 | ? | 0 | 1 | – | 1 | 1 | – | 1 |
| *Ramariopsis californica* | 1 | 0 | 1 | 3 | – | 1 | 0 | 1 | 0 | 1 | ? | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| *Ramariopsis crocea* | 1 | 0 | 1 | 3 | – | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| *Ramariopsis kunzei* | 1 | 0 | 1 | 3 | – | 0 | 0 | 1 | 1 | 1 | ? | 1 | 0 | – | 1 | 0 | 1 | 0 |
| *Ramariopsis pulchella* | 1 | 0 | 1 | 3 | – | 0 | 0 | 1 | 0 | 1 | ? | 1 | 0 | 1 | 1 | 0 | 1 | 0 |
| *Ramariopsis tenuiramosa* | 1 | 0 | 1 | 3 | – | 0 | 0 | 1 | 1 | 1 | ? | 1 | 0 | – | 1 | 0 | 1 | 0 |
| *Scytinopogon dealbatus* | 1 | 0 | 1 | 3 | – | 0 | 1 | 1 | 1 | 1 | ? | 1 | 1 | – | 2 | 0 | 1 | 0 |
| *Scytinopogon echinosporus* | 1 | 0 | 1 | 3 | – | 0 | 1 | 0 | 1 | 1 | ? | 1 | 1 | – | 2 | 0 | 1 | 0 |
| *Scytinopogon robustus* | 1 | 0 | 1 | 3 | – | 0 | 1 | 0 | 0 | 1 | ? | 1 | 1 | 0 | 1 | 0 | 1 | 0 |
| *Scytinopogon pallescens* | 1 | 0 | 1 | 3 | – | 0 | 1 | 1 | 1 | 1 | ? | 1 | 1 | – | 2 | 0 | 1 | 0 |

*Table 2, continued*

| | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Clavaria amoena* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 1 | 0 | ? | 1 | – |
| *Clavaria aurantiocinnabarina* | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | ? | 1 | – |
| *Clavaria gibbsiae* | – | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | ? | ? | 2 | 0 | 1 | 1 | ? | 1 | – |
| *Clavaria sulcata* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | ? | ? | 2 | 1 | 1 | 0 | ? | 1 | – |
| *Clavaria vermicularis* | – | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | ? | ? | 2 | 1 | 1 | 0 | ? | 1 | – |
| *Clavaria zollingeri* | – | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | ? | ? | 2 | 1 | 1 | 0 | ? | 1 | – |
| *Clavariadelphus pistillaris* | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | ? | 2 | 1 | 0 | 0 | ? | 1 | – |
| *Clavulinopsis corniculata* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | ? | 1 | – |
| *Clavulinopsis fusiformis* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | ? | 1 | – |
| *Clavulinopsis helvola* | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | ? | ? | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| *Clavulinopsis laeticolor* | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | ? | 1 | – |
| *Gomphus clavatus* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | ? | 2 | 0 | 1 | 1 | ? | 0 | 1 |
| *Gomphus floccosus* | – | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | ? | ? | 2 | 0 | 1 | 1 | ? | 0 | 1 |
| *Lactarius indigo* | – | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | ? | ? | 2 | 0 | 1 | 1 | ? | 0 | 0 |
| *Ramariopsis californica* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | ? | ? | 0 | 0 | 1 | 1 | 0 | 0 | 1 |
| *Ramariopsis crocea* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Ramariopsis kunzei* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | ? | ? | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Ramariopsis pulchella* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | ? | ? | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Ramariopsis tenuiramosa* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | ? | ? | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Scytinopogon dealbatus* | 1 | 0 | 0 | 1 | 0 | 1 | 1 | – | 1 | ? | ? | 0 | 1 | 0 | 1 | ? | 0 | 0 |
| *Scytinopogon echinosporus* | 1 | 0 | 0 | 1 | 0 | 1 | 1 | – | 1 | ? | ? | 2 | 1 | 0 | 1 | ? | 0 | 0 |
| *Scytinopogon robustus* | 1 | 0 | 0 | 1 | 0 | 1 | 1 | – | 1 | ? | ? | 0 | 1 | 0 | 1 | ? | 0 | 0 |
| *Scytinopogon pallescens* | 1 | 0 | 0 | 1 | 0 | 1 | 1 | – | 1 | ? | ? | 0 | 1 | 0 | 1 | ? | 0 | 0 |

## Appendix 3. Checklist of species names.

### Part 1. Species of *Ramariopsis* sensu stricto

*Ramariopsis asterella* (G.F. Atk) Corner
= *Clavaria asterella* G.F. Atk.
*Ramariopsis avellanea* R.H. Petersen
*Ramariopsis avellaneainversa* R.H. Petersen
*Ramariopsis biformis* (G.F. Atk.) R.H. Petersen
= *Clavaria biformis* G.F. Atk.
*Ramariopsis californica* R.H. Petersen
*Ramariopsis cinnamomea* R.H. Petersen
*Ramariopsis cinnamomipes* R.H. Petersen
*Ramariopsis citrina* Schild
*Ramariopsis clavuligera* (R. Heim) Corner
= *Clavaria clavuligera* R. Heim
*Ramariopsis costaricensis* L.D. Gómez
*Ramariopsis crocea* (Pers.) Corner
= *Clavaria crocea* Pers.
*Ramariopsis curta* (Fr.) Corner
= *Clavaria curta* Fr.
*Ramariopsis flavescens* R.H. Petersen
*Ramariopsis hibernica* Corner
*Ramariopsis kunzei* (Fr.) Corner
= *Clavaria kunzei* Fr.
*Ramariopsis longipes* R.H. Petersen
*Ramariopsis novahibernica* Corner
*Ramariopsis pulchella* (Boud.) Corner
= *Clavaria pulchella* Boud.
*Ramariopsis ramarioides* R.H. Petersen
*Ramariopsis rufipes* (G.F. Atk.) R.H. Petersen
= *Clavaria rufipes* G.F. Atk.
*Ramariopsis subarctica* Pilát
*Ramariopsis tenuicula* (Bourdot & Galzin) R.H. Petersen
= *Clavaria tenuicula* Bourdot & Galzin
*Ramariopsis tenuiramosa* Corner
*Ramariopsis tortuosa* R.H. Petersen
*Ramariopsis vestitipes* (Peck) Corner
= *Clavaria vestitipes* Peck

### Part 2. Species sometimes placed in *Ramariopsis* that belong in other genera

#### *Clavaria* L.

*Clavaria asperulospora* G.F. Atk.
= *Ramariopsis asperulospora* (G.F. Atk.) Corner

#### *Clavulinopsis* Overeem

*Clavulinopsis antillarum* (Pat.) Garcia-Sandoval & Cifuentes, comb. nov.
= *Clavaria fusiformis* var. *antillarum* Pat.
= *Ramariopsis antillarum* (Pat.) R.H. Petersen
*Clavulinopsis corniculata* (Schaeff.) Corner
= *Clavaria corniculata* Schaeff.

= *Ramariopsis corniculata* (Scaeff.) R.H. Petersen
*Clavulinopsis depokensis* (Overeem) Corner
= *Clavaria depokensis* Overeem
= *Ramariopsis depokensis* (Overeem) R.H. Petersen
*Clavulinopsis dichotoma* (Godey) Corner
= *Clavaria dichotoma* Godey
= *Ramariopsis dichotoma* (Godey) R.H. Petersen
*Clavulinopsis fusiformis* (Sowerby) Corner
= *Clavaria fusiformis* Sowerby
= *Ramariopsis fusiformis* (Sowerby) R.H. Petersen
*Clavulinopsis helvola* (Pers.) Corner
= *Clavaria helvola* Pers.
= *Ramariopsis helvola* (Pers.) R.H. Petersen
*Clavulinopsis holmskiodii* (Oudem.) Corner
= *Clavaria holmskiodii* Oudem.
= *Ramariopsis holmskiodii* (Oudem.) R.H. Petersen
*Clavulinopsis laeticolor* (Berk. & M.A. Curtis) R.H. Petersen
= *Clavaria laeticolor* Berk. & M.A. Curtis
= *Ramariopsis laeticolor* (Berk. & M.A. Curtis) R.H. Petersen
*Clavulinopsis luteo-ochracea* (Cavara) Corner
= *Clavaria luteo-ochracea* Cavara
= *Ramariopsis luteo-ochracea* (Cavara) R.H. Petersen
*Clavulinopsis luteotenerrima* (Overeem) Corner
= *Clavaria luteotenerrima* Overeem
= *Ramariopsis luteotenerrima* (Overeem) R.H. Petersen
*Clavulinopsis minutula* (Bourdot & Galzin) Corner
= *Clavaria minutula* Bourdot & Galzin
= *Ramariopsis minutula* (Bourdot & Galzin) R.H. Petersen
*Clavulinopsis subtilis* (Pers.) Corner
= *Clavaria subtilis* Pers.
= *Ramariopsis subtilis* (Pers.) R.H. Petersen
*Clavulinopsis umbrinella* (Sacc.) Corner
= *Clavaria umbrinella* Sacc.
= *Ramariopsis umbrinella* (Sacc.) R.H. Petersen

### *Ramaria* Fr.

*Ramaria lorithamnus* (Berk.) R.H. Petersen
= *Clavaria lorithamnus* Berk.
= *Ramariopsis lorithamnus* (Berk.) Corner

### *Scytinopogon* Singer

*Scytinopogon dealbatus* (Berk.) Corner
= *Clavaria dealbata* Berk.
= *Ramariopsis dealbata* (Berk.) R.H. Petersen

## Part 3. Species initially described in *Ramariopsis* that require further examination

*Ramariopsis agglutinata* R.H. Petersen
*Ramariopsis alutacea* R.H. Petersen
*Ramariopsis aurantio-olivacea* R.H. Petersen
*Ramariopsis bicolor* R.H. Petersen
*Ramariopsis cremicolor* R.H. Petersen

*Ramariopsis junquillea* R.H. Petersen
*Ramariopsis lignicola* R.H. Petersen
*Ramariopsis ovispora* R.H. Petersen
*Ramariopsis pseudosubtilis* R.H. Petersen
*Ramariopsis simplex* R.H. Petersen

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# A dichotomous key to *Scutellospora* species (*Gigasporaceae*, Glomeromycota) using morphological characters

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**Abstract**—A key to the genus *Scutellospora* (Order *Glomerales*, Phylum *Glomeromycota*) is compiled based on the protologues of species descriptions and information on type cultures of INVAM website.

**Key words**—arbuscular mycorrhizal fungi, *Glomerales*, identification, spore wall

## Introduction

The genus *Scutellospora* was separated from the genus *Gigaspora* by Walker & Sanders (1986) based on differences in germination characteristics, subcellular structure within spores, and morphology of extraradical auxiliary cells. Weight was assigned most heavily to germination properties, with germ tubes formed from the inner warty surface of the spore wall in *Gigaspora* and from a novel germination shield formed on flexible layers independent of the spore wall in *Scutellospora*. Etymology of *Scutellospora* affirms these criteria, as the name derives from *scutellum*, or small shield. The germination shield differentiates on a bi-layered flexible germinal wall unique to glomeromycotan fungi. When more than one of these germinal walls are present, the germination shield forms always on the innermost one. Cellular organization of spores consists of spore wall and one to three of these flexible germinal walls in *Scutellospora* while only the spore wall in *Gigaspora* species. Vegetative criteria for genus-level separation reside in auxiliary cells, which have a knobby surface in *Scutellospora* and distinctly echinulate surface in *Gigaspora*.

In their classification of arbuscular mycorrhizal fungi (AMF), Morton & Benny (1990) placed *Gigaspora* and *Scutellospora* within the family *Gigasporaceae*, suborder *Gigasporineae* and order *Glomerales*. Members of *Gigasporaceae* were grouped by densely staining arbuscules, knobby coiled hyphae, unique soil-borne auxiliary cells and formation of spores on unique sporogenous cells. *Gigasporaceae* currently is grouped in the order *Diversisporales* (Schußler et al. 2001) which consists of an eclectic

mix of families with no discernible morphological evolutionary pattern as classified. Therefore, at the order level, the morphological evolution conflicts with the 18S rRNA gene sequence phylogeny. Patterns and processes in spore ontogenesis (Franke & Morton, 1994; Morton, 1995) provided useful insights into the origins and boundaries of spore subcellular characters for the family *Gigasporaceae*. These works provided the foundation for reinterpretations and resolution of species-level characters. Stürmer & Morton (1999) subsequently described a new species, *Scutellospora rubra*, based on spore ontogenetic data.

Species of *Scutellospora* are geographically widespread with a pandemic distribution. Species of this genus have been reported from North America (Koske, 1987) South America (Siqueira et al. 1989; Stürmer & Bellei 1994; Yano-Melo et al. 2003; Silva et al. 2005), Europe (Blaszkowski 1989), Africa (Ba et al. 1996), Asia (Saito & Varga 1991) and Australia (Koske 1975). Although they are commonly associated with hosts in sand dunes, (Koske & Walker 1985, 1986; Koske & Gemma 1996; Blaszkowski 1991), they also are found in other natural ecosystems such as West Java tropical rain forests (Kramadibrata et al. 2000), Venezuela sclerophyllous shrublands (Herrera-Peraza et al. 2001), USA tall grassland (Hetrick & Bloom 1983) and Germany alpine ecosystem (Blaschke 1991), Brazil savanna-like "cerrado" (Walker & Diederichs 1989) and "caatinga" vegetation (Souza et al. 2003) as well as in brazilian degraded areas (Yano-Melo et al. 2003; Silva et al. 2005). Nevertheless, species of *Scutellospora* have also been registered from diverse agroecosystems (Hetrick & Bloom, 1983; Miller et al., 1985; Siqueira et al., 1989; An et al., 1993).

Early keys for identification of arbuscular mycorrhizal fungi were developed by Hall & Fish (1979) and Trappe (1982). Koske & Walker (1985) provided a key for a subset of *Scutellospora* species with ornamented spore walls. With a substantial increase in the number of new species described and the robust morphological delineation amongst species, we deemed it an appropriate time to erect a more comprehensive dichotomous key to *Scutellospora*.

## Material and Methods

Morphological characters of *Scutellospora* were obtained from species protologues and from descriptions of reference isolates in the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM, West Virginia University, WV) webpages (http://invam.caf.wvu.edu/speciesID.htm). Nomenclature in species descriptions was revised and standardized according to that proposed by Morton et al. (1995). Of 32 described *Scutellospora* species, 25 were incorporated in the key. The seven unincorporated species were *S. alborosea, S. gilmorei, S. minuta, S. nigra, S. reticulata, S. savannicola* and *S. tricalypta*, all of which were described before any standardized terminology for spore structures was developed (Walker 1983). For the 25 species considered in the key, characters of 14 species were obtained from INVAM web pages and 11 from published species descriptions. Wherever possible, color designations (*e.g.*, 0/20/40/0) were based on the CMYK model (Cyan, Magenta, Yellow and Black) used as the basis for the INVAM color chart (http://invam.caf.wvu.edu/otherinfo/articles/colorchart.htm). Spelling of names follows recommendations of Walker & Trappe (1993).

## Results

DICHOTOMOUS KEY TO SOME *SCUTELLOSPORA SPECIES*

1a. Spores with one germinal wall ..... 2

1b. Spores with more than one germinal wall ..... 8

2a. (1a) Surface of spore wall smooth ..... 3

2b. Surface of spore wall ornamented ..... 4

3a. (2a) Spores pale (0/0/5/0) to slightly darker cream (0/0/40/0) in color, with mean diameter of 180 µm (range 120-240 µm), second layer (L2) of the spore wall 4.5-6 µm thick (mean = 5.1 µm) ..... *S. fulgida*

3b. Spores chestnut (0/40/100/0) in color, with mean diameter of 300 µm, second layer (L2) of spore wall 10-25 µm thick ..... *S. castanea*

4a. (2b) Spore wall ornamented with warts ..... 5

4b. Spore wall ornamented with blunt tapering projections 1-3 µm wide and 2 µm high, L2 of the spore wall swelling in acidic mountants and turning dark red-brown in Melzer's reagent ..... *S. biornata*

5a. (4a) Color of mature spore ranging from pale-straw (0/10/10/0) to orange-brown (0/60/100/0) or pale to dark copper (0/60/100/0 to 20/80/60/0) ..... 6

5b. Color of mature spore darker than above, ranging from red-brown (20/80/100/10) to dark red -brown (40/80/80/0) ..... 7

6a. (5a) Spores pale to dark copper to darker cream color (20/60/70/10), 240-360 µm in diameter (mean = 313 µm) with surface covered by rounded warts 0.5 µm wide and 0.2-0.5 µm high ..... *S. persica*

6b. Spores pale straw to orange brown, 220-360 µm in diameter (mean = 308 µm), with surface covered with tightly packed warts < 1 µm apart, 0.5-1.0 µm wide and 0.5-1.2 µm high ..... *S. verrucosa*

7a. (5b) Spore size of 260-480 µm (mean = 362 µm), spore surface covered with flattened warts with angular margins (2-12 µm wide and 1-3 µm high) ..... *S. coralloidea*

7b. Spore size of 380-520 µm (mean = 473 µm), spore surface ornamented with rounded warts (11-26 µm wide and 3-7 µm high) ..... *S. gregaria*

8a. (1b) Spores with two germinal walls ..... 9

8b. Spores with three germinal walls ..... 24

9a. (8a) Surface of spore wall smooth ..... 10

9b. Surface of spore wall ornamented ..... 18

10a. (9a) Spores pale to dark orange-brown (0/60/100/0) to red-brown (20/80/100/0) ..... 11

10b. Spores lighter in color than above, color ranging from white/hyaline or pale yellow (5/0/20/0) to yellow (0/0/100/0) ..... 12

11a. (10a) Spore size of 140-220 µm (mean = 180 µm). Second layer (L2) of the spore wall 3.5-12 µm thick staining dark red black (20/80/70/10) in Melzer's reagent. Second layer of germinal wall 2 is 0.6-1.4 µm thick staining pale pinkish purple (0/20/20/0) to darker pinkish purple (20/40/20/0) in Melzer's reagent .. ***S. rubra***

11b. Spore size of (200-) 240 (-360) x (180-) 230 (-290) µm (mean = 235 µm). Second layer (L2) of the spore wall 0.8 -2.2 µm not reacting in Melzer's reagent. Second layer of germinal wall 2 is 3 - 4 up to 25 µm thick, with amorphous characteristics, staining reddish purple (20/80/20/0) in Melzer's reagent ..... ***S. hawaiiensis***

12a. (10b) Spore wall not reacting in Melzer's reagent .....13

12b. Spore wall reacting in Melzer's reagent .....15

13a. (12a) Spores pale yellow with a greenish tint (5/0/20/0). Spore size ranging from 120-240 µm. Second layer (L2) of spore wall finely laminated, ranging from 1.8-4.2 µm thick. Germinal wall 2 reacting in Melzer's reagent.....14

13b. Spores pale yellow (5/0/20/0) to yellow (0/0/100/0) without a greenish tint. Spore size ranging from 320-440 x 320-470 µm. Second layer (L2) of spore wall coarsely laminated, ranging from 6 -16 µm thick. Germinal wall 2 not reacting in Melzer's reagent ..... ***S. aurigloba***

14a. (13a) Spores with germinal wall 1 consisting of one hyaline layer 0.6-1.0 µm thick, staining light pink (0/20/20/0) in Melzer's reagent and often adherent to spore wall. Second layer (L2) of germinal wall 2 staining red-purple (20/80/20/0) to dark red-purple (40/80/60/0) ..... ***S. dipurpurescens***

14b. Spores with germinal wall 1 consisting of two hyaline adherent layers that together are 0.9-2.0 µm thick. Second layer (L2) of germinal wall 2 staining red-purple (20/80/20/0) to dark red-purple (40/80/60/0) ..... ***S. calospora***

15a. (12b) Spore color hyaline/white to pale pink (0/30/20/0) to deep pink (0/40/20/0) .....16

15b. Spore color apricot yellow and yellow brown (0/5/40/0) to orange brown (0/60/100/0).....17

16a. (15a) Spore color pale pink (0/30/20/0) to deep pink (0/40/20/0), turning hyaline when the pink color fades. Spore wall color turns yellowish with a greenish tint (0/0/80/). Second layer of germinal wall 2 turns pale pink (0/20/20/0) to light purple (20/40/20/0) in Melzer's reagent..... ***S. weresubiae***

16b. Spore color hyaline/white. Spore wall: first layer (L1), hyaline, 1.8-5.0 µm thick and second layer (L2) 3.0-8.8 µm thick. Spore wall turns dark red-purple (40/80/40/0) to reddish-black (60/80/50/10) in Melzer's reagent. Second layer (L2) of germinal wall 2 with characteristics "amorphous" staining red purple (20/80/20/0) to dark red purple (40/80/60/0) in Melzer's reagent ..... ***S. pellucida***

17a. (15b) Spore color apricot yellow to yellow brown (0/5/40/0). Spore wall turning garnet red in Melzer's reagent. Second layer (L2) of germinal wall 2 with characteristics "amorphous" staining red purple (20/80/20/0) in Melzer's reagent ..... ***S. armeniaca***

17b. Spore color pale yellow-brown (0/10/20/0 to 0/10/60/0) to orange brown (0/60/100/0). Spore wall turning dark red-brown in Melzer's reagent. Second layer (L2) of germinal wall 2 with characteristics "amorphous" staining dark reddish purple (40/80/40/0) in Melzer's reagent... ..... *S. arenicola*

18a. (9b) Spore color pale orange brown (0/60/80/0) and dark orange brown (0/60/100/10) to red brown (40/80/100/0) .....19

18b. Spore color lighter than above, ranging from hyaline/white, pale cream (0/10/40/0) to metallic gold (0/20/100/0) .....20

19a. (18a) Spore surface ornamented with short rounded warts 1.0-2.5 µm high. Second layer (L2) of spore wall 5-9 µm thick (mean = 7.4 µm), staining dark red brown (20/80/70/10) in Melzer's reagent..... *S. heterogama*

19b. Spore surface with two types of ornamentation: crowded small conical warts, 0.5-1 µm wide and 0.5-1.5 µm high and hyaline, blunt, bacilliform large projections 1.5-3 µm wide and 2-10 µm high. Second layer (L2) of spore wall 3-8 µm thick. Reaction of spore wall to Melzer's reagent unknown .....*S. dipapillosa*

20a. (18b) Spore wall reacting on Melzer's reagent turning dark red-purple (40/80/40/0) to reddish-black (60/80/50/10) .....21

20b. Not as above .....22

21a. (20a) Spore size of 220-380 µm (mean = 300 µm). Spore wall ornamented with papilla rarely > 1 µm high and 1-1.5 µm wide. Second layer (L2) of germinal wall 2 with characteristics of "amorphous" and stains red purple (20/80/20/0) to dark red purple (40/80/60/0) in Melzer's reagent .....*S. cerradensis*

21b. Spore size of 160-270 µm (mean = 240 µm). Spore wall ornamented with knobs 3.5 6.5µm high and 7-10.5 µm wide. Second layer (L2) of germinal wall 2 with characteristics of "amorphous" and stains dark red purple (40/80/60/0) in Melzer's reagent ..... *S. nodosa*

22a. (20b) Ornamentation of the spore wall 2-4 µm high .....23

22b. Ornamentation of the spore wall 3-6 µm heigh and 3.6-10 µm wide consisting of dome-like sub-polygonal papillae. Spore wall not swelling in acidic mountants. L2 of the germinal wall 2 turning red purple (20/80/20/0) in Melzer's reagent
..... *S. crenulata*

23a. (22a) Spore ornamented with columnar protuberances 2-4 µm long. Second layer (L2) of germinal wall 2 staining purple (40/60/20/0) in Melzer's reagent ..... *S. projecturata*

23b. Spore ornamented with dense blunt spines 2-4 µm long. Second layer (L2) of germinal wall 2 staining red purple (20/80/20/0) in Melzer's reagent..... *S. spinosissima*

24a. (8b) Spores hyaline/white to yellow brown (0/5/40/0) in older spores, 340-640 µm in diameter (mean = 495 µm), with dark yellow-brown germination shield highly contrasting with spore color. Spore wall not reacting in Melzer's reagent. Second

layer (L2) of germinal wall 3 staining red purple (20/80/20/0) to dark red-purple (40/80/60/0) in Melzer's reagent... ..... *S. scutata*

24b. Spores red-brown (20/80/60/0) to dark red-brown (20/80/100/10), subglobose to oblong, 160-320 µm diameter (mean = 240 µm). Spore wall not reacting in Melzer's reagent. Second layer (L2) of germinal wall 3 staining pinkish (0/40/20/0) to pinkish purple (0/60/30/10) ..... *S. erythropa*

## Discussion

This paper provides the first dichotomous key of *Scutellospora* since Koske & Walker's key of approximately two decades ago for species with roughened (ornamented) spore walls. The first character in the key is number of germinal walls present in the spore, which can be readily detected by both beginning and experienced researchers once they recognize the fundamental bi-layered organization of each wall. From there, properties of spore wall layers (color, presence and type of ornamentation, etc.) discriminate amongst species (Morton et al. 1995).

Spore wall structure was re-interpreted from published species descriptions of some species according to developmental patterns (Morton et al. 1995). For example, Koske & Walker (1985) described *S. coralloidea, S. gregaria, S. verrucosa* and *S. persica* as possessing a hyaline, membranous wall. Developmental patterns indicate this structure represents one germinal wall composed by two layers (Morton 1995). Based on this type of comparison, *S. hawaiiensis, S. aurigloba, S. weresubiae, S. dipapillosa, S. nodosa, S. projecturata, S. spinosissima, S. arenicola* and *S. crenulata* were considered to have two germinal walls. To support this interpretation, Melzer's reaction of germinal walls was also considered. *Scutellospora* species with only one germinal wall shows no reaction in Melzer's (Morton, 1995) while those with two or three germinal walls have the second layer of the innermost wall turning light to dark purple in Melzer's reagent. For instance, protologues of *S. arenicola* and *S. crenulata* are not straightforward about the number of germinal walls, however, both become red purple in Melzer's reagent; we therefore interpreted these species as having two germinal walls. Establishment of these organisms in single-species culture will help to clarify this interpretation.

The presence of one, two or three germinal walls defines groups of species within *Scutellospora*. Within each group, new subgroupings emerge when considering characteristics of spore wall and germinal wall. Therefore, within *Scutellospora* species with one germinal wall, two subgroups are formed relative to ornamentation of spore wall. One group is formed by *S. fulgida* and *S. castanea*, both of which have smooth spore walls, whereas another group is formed by *S. biornata, S. persica, S. verrucosa, S. coralloidea* and *S. gregaria* which produce a spore wall ornamented with blunt tapering projection and warts. We are not suggesting that they represent new taxa (*e.g.*, subgenus), but this grouping is very useful during species identification.

Within *Scutellospora* species with two germinal walls, subgroupings based on spore color and ornamentation also emerge. Therefore, there are species producing smooth spore walls with dark spore color (*e.g.*, *S. rubra* and *S. hawaiiensis*) and those with pale spore color (*e.g.*, *S. aurigloba, S. dipurpurescens, S. calospora, S. weresubiae, S. pellucida, S. armeniaca* and *S. arenicola*). The third subgroup includes species producing

ornamented spores (*S. heterogama*, *S. dipapillosa*, *S. cerradensis*, *S. nodosa*, *S. crenulata*, *S. projecturata* and *S. spinosissima*). The third large group within the genus includes species differentiating three germinal walls like *S. scutata* and *S. erythropa*, which can be readily separated from each other based on spore color and size (hyaline/white larger spores in the former and red-brown smaller spores in the latter).

Differences among some species now described in the genus *Scutellospora* can be small so that species boundaries are not clear. For example, the main difference between *S. dipurpurescens* and *S. calospora* is the presence of one or two layers in the first germinal wall, respectively. Spore size, color and Melzer's reaction of germinal wall 2 are identical between these two species. On the same token, differences between *S. heterogama* and *S. dipapillosa* reside mostly on the very small type of ornamentation occurring on the latter, a characteristic already noticed by Koske & Walker (1985). Some spores of *S. dipapillosa* are identical to *S. heterogama*, while others that show the variation described by Koske & Walker (1985) are present in parasitized spores – so the difference may be an artifact of parasitism rather than a heritable difference (J.B. Morton, personal communication). *Scutellospora pellucida* and *S. armeniaca* are also very similar and may represent the same species; both have the same range of spore size and Melzer's reaction of spore wall and germinal wall 2 are identical. Spore color can be used with caution to separate both species, as *S. armeniaca* produces spores apricot yellow and yellow brown while *S. pellucida* are hyaline. However, spores from the latter can be pale yellow brown when older or extracted from roots in pot cultures or from the field, the condition where spores from the former were described (J.B. Morton, personal communication).

We proposed this dichotomous key to help mycorrhizologists to identify *Scutellospora* species during field surveys or for establishing isolates in single-species cultures. We are aware that interpretation of spore wall structure for some species might not be adequate, especially those not established in monospecific culture. Therefore, we suggest that for further species description, spore wall characteristics should be described using nomenclature based on spore developmental model as proposed by Morton et al. (1995).

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# New species and phylogenetic relationships of *Hypoxylon* species found in Thailand inferred from the internal transcribed spacer regions of ribosomal DNA sequences

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**Abstract**—*Hypoxylon* specimens collected from different forest areas in Thailand were investigated and identified to species using both morphological and molecular characteristics. Fifteen species, including three new species, *H. kanchanapisekii*, *H. sublenormandii*, and *H. suranareei*, were recorded. The phylogenetic tree of the *Hypoxylon* examined was constructed using the neighbour-joining method based on the internal transcribed spacer (ITS) regions including 5.8S ribosomal DNA sequences. The molecular results revealed clear separation among *Hypoxylon* species including closely related species or on data based only on teleomorphic DNA. Three taxa in *Hypoxylon* sect. *Annulata*—*H. nitens*, *H. purpureonitens*, and *H. bovei* var. *microspora*—were grouped together, but the fourth species, *H.* cf. *archeri*, was separated. The phylogenetic tree does not support the monophyletic group of sect. *Annulata* in this study. In addition, these molecular data could be used to confirm the recognition of new species based on morphological features and they are valuable for the creation of the *Hypoxylon* DNA sequence database.

**Key words**—*Xylariaceae*, *Annulohypoxylon*, taxonomy

## Introduction

*Hypoxylon* Bull. is a large complex genus of the family *Xylariaceae* which is a well known family of the Ascomycota (Whalley 1996, Rogers 2000). They are distributed worldwide, being especially common in subtropical and tropical regions. Their habitat is on wood. Most *Hypoxylon* species are saprotrophs involved in the decay of wood or occasionally being weak parasites in stressed host trees (Whalley1996, Edwards et al. 2003). Moreover an increasing number of *Hypoxylon* species being reported as endophytes in a wide range of living plants (Rodrigues & Petrini 1997; Rogers 2000; Edwards et al. 2003).

Traditionally, *Hypoxylon* has been characterized by morphological characteristics based on stromatal structure, pigmentation, shape and features of the ascospore (Miller 1961; Martin 1968; Ju & Rogers 1996). Thus, the delimitation of the genus *Hypoxylon* has been the subject of several rearrangements since the monograph of Miller (Miller 1961). Miller's monograph divided the genus into four sections, *Hypoxylon*, *Annulata*, *Applanata* and *Papillata* strongly relying on stromatal form, texture, colour, and ostiole form (Miller 1961). Consequently, this monograph failed to recognize the relationships among species and groups of species. The section *Applanata* sensu Miller has since been redistributed between *Camillea* Fr. and *Biscogniauxia* Kuntze (Læssøe, Rogers & Whalley 1989; Whalley, Læssøe & Kile 1990; Ju et al. 1998) whereas members of the section *Papillata* subsection *Primocinerea* (Miller 1961) have been allocated to a range of genera including *Nemania* Gray emend. Pouzar (Pouzar 1985), *Rosellinia* De Not. (Petrini 1992), *Kretzschmaria* Fr. (Van der Gucht 1994), *Kretzschmariella* Viégas (Ju & Rogers 1994), *Entoleuca* Syd. (Rogers & Ju 1996), and *Euepixylon* Füisting (Læssøe & Spooner 1994). Recently, *Hypoxylon* was revised by Ju & Rogers (1996) using four major criteria to define the genus: *Nodulisporium*-like anamorphs, stromata unipartite, never erect, with a solid and homogenous basal tissue below the perithecial layer. They divided *Hypoxylon* into sections *Hypoxylon* and *Annulata* separating them on the basis of presence or absence of a layer of carbonaceous stromatal tissue enclosing perithecia. Additional major characters used to separate the two sections included the perispore, which if dehiscent, exhibits a visible thickened area positioned about one-third of the length of the ascospore and on the same side of the germination slit in members of the Annulata, and the presence or absence of an annular disc surrounding the ostiolum. Ju & Rogers (1996) accepted at least 130 species and varieties and have since recognized a further 16 species and one variety (Ju Rogers & Hsieh 2004). When revising the genus Ju & Rogers (1996) were able to utilize data absent from the monograph of Miller (1961), such as ascospore ornamentation using scanning electron microscopy, form of the apical apparatus of the ascus, germination slit morphology, and the colour of stromatal pigments extracted with 10% potassium hydroxide (KOH) solution. However, in spite of their revision identification of certain *Hypoxylon* species remains problematic with insufficient discriminatory morphological information to clearly delimit certain taxa and for the confident identification of individual species. This has proved to be most pronounced when considering tropical taxa of the *Annulata* especially *Hypoxylon nitens* (Ces.) Y.-M. Ju & J.D. Rogers, *H. moriforme* Henn., *H. bovei* Speg. var. *microspora* J.H. Miller and *H. purpureonitens* Y.-M. Ju & J.D. Rogers where, between them, there is considerable variation and overlap in their morphological features.

Molecular studies based on DNA sequences are recognized as more reliable methods to reveal genetic relationships, and can be used to evaluate the relationships of fungi at any taxonomic rank (Bruns et al. 1991). Fungal ribosomal DNA (rDNA) genes are frequently used because these genes contain highly conserved and variable regions, and they are found in multiple copies per genome (Gardes & Bruns 1993). In the rDNA genes, the regions of the internal transcribed spacers (ITS) 1 and 2 including 5.8S ribosomal DNA gene have been extensively studied to differentiate taxonomic entities at the species level in several fungal genera, such as *Fusarium* Link (O'Donnell 1992), *Penicillium* Link (Skouboe et al. 1999), *Xylaria* Hill *ex* Schrank (Lee et al. 2000), and *Hypoxylon* (Sánchez-Ballesteros et al. 2000, Mazzaglia et al. 2001). Sequence data of rDNA provides

a powerful tool for evaluating phylogenetic relationship among different taxa especially at higher taxonomic levels. Moreover DNA sequence data from ITS1-5.8S-ITS2 region has been used to develop specific oligonucleotide primers of pathogenic fungi such as *Penicillium marneffei* Segr., Capp. & Sur. (LoBuglio & Taylor 1995).

In this study we elected to investigate the genetic variation within *Hypoxylon* species in order to obtain correlations between their morphological and molecular characteristics using DNA sequences of the ITS1-5.8S-ITS2 region. It is our belief that the application of these data in conjunction with morphological features will provide a better definition of species delimitation of *Hypoxylon*. Since our study was completed Hsieh et al. (2005) have published on molecular phylogeny of *Hypoxylon* and closely related genera based on β-tubulin and α-actin gene sequences. They proposed a new genus *Annulohypoxylon* Y.-M. Ju, J.D. Rogers & H.-M. Hsieh to accommodate taxa originally assigned to *Hypoxylon* sect. *Annulata* (Ju & Rogers 1996).

## Materials and Methods

### Fungal materials

Eighty-nine collections of *Hypoxylon* specimens were collected from different forest areas in Thailand. They were classified and identified to species level using morphological characters (Ju & Rogers 1996; Ju Rogers & Hsieh 2004), and cultures were obtained whenever possible following ascospore discharge, germination and colony development. The stromatal pigments of *Hypoxylon* were extracted by using 10% potassium hydroxide (KOH) solution, left for one minute, and observed the colour compared to the colour chart of Rayner (1970). Nine *Hypoxylon* isolates were additionally obtained from the Royal Forest Department (Thailand). Two species (as teleomorphs) of *H. nitens* and *H. bovei* var. *microspora* were provided by Dr. Yu-Ming Ju (National University of Taiwan, Taiwan). Details of the representative cultures of the *Hypoxylon* species investigated together with the two Taiwanese collections are listed in Table 1. All isolates were routinely grown on potato dextrose agar at 25°C, and maintained in 15% glycerol at -20°C.

### DNA extraction, ITS1-5.8S-ITS2 region amplification and DNA sequencing

Genomic DNA was extracted from cultural mycelia and from stromatal herbarium materials by using the method of Lee & Taylor (1990) with some modifications. The internal transcribed spacer (ITS) regions 1 and 2 including 5.8S ribosomal nucleotide sequence were amplified by polymerase chain reaction (PCR) with the fungal specific primers ITS4 (5'-TCCTCCGCTTCTTGATAGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') (White et al. 1990). The PCR reaction was carried out in 50 µl mixtures containing 10 ng of DNA, 200 µM of each dNTP (dATP, dGTP, dCTP, and dTTP), 2.5 µM of each primer, 2 mM of $MgCl_2$, and 1 unit of *Taq* Polymerase (Sigma, U.S.A). The PCR cycle of ITS amplification consisted of 1 cycle of 95°C for 5 min; 35 cycles of 95°C for 30 sec, 53°C for 30 sec, 72°C for 1 min; and the final cycle of 72°C for 10 min. PCR reactions were carried out in an automated thermal cycler (BioRad, U.S.A).

Amplification products were purified through columns using the QIA quick PCR Purification kit (Qiagen, U.S.A) and sequenced using the PCR primers as sequencing

primers with the Big Dye DNA Sequencing kit (Applied Biosystems, U.S.A). All reactions were then run on a Perkin Elmer ABI PRISM™ 377 DNA Sequencer (Applied Biosystems, U.S.A).

### Phylogenetic analysis

All DNA sequences of rDNA were determined and trimmed to remove all 18S and 28S sequences according to sequence boundaries defined by Sánchez-Ballesteros et al. (2000). DNA sequences of ITS regions were analyzed and aligned manually using ClustalW (Thompson et al. 1994) and Bioedit program version 4.7.1 created by Tom Hall (Department of Microbiology, North Carolina State University, Raleigh, U.S.A). Genetic distances for the neighbour-joining and conditional clustering, Kimura 2 parameter distances (Kimura 1980), were computed with the Dnadist module of the PHYLIP software package (Felsenstein 1995). Selected ITS sequences of related species in the GenBank database (http://www.ncbi.nlm.nih.org) were included for comparison with sequences obtained in the current study. Strengths of internal branches of resulting trees were statistically tested by the bootstrap analysis of 1000 replications.

## Results

### Fungal collection and identification

Eighty nine *Hypoxylon* collections were classified and identified as belonging to 15 species including 3 unique new species, on the basis of their morphological characteristics (Table 1). Details of the new species are given below under taxonomic descriptions. The collections exhibited high variation in their morphological characteristics, e.g. stromatal surface colour, ascospore size and shape, and germination slit morphology. The morphological characteristics of representatives of each species recognized are presented in Table 2.

## Taxonomic Descriptions

Morphological features of the Thai collections identified as *H. anthochroum*, *H. bovei* var. *microspora*, *H. fendleri*, *H. haematostroma*, *H. lenormandii* (except for two collections on bamboo), *H. monticulosum*, *H. nitens*, *H. purpureonitens*, *H. stygium*, *H. atroroseum* and *H. rubiginosum* were all in very close agreement with the descriptions for those species (Ju & Rogers 1996) or with the characters of voucher specimens provided by Dr. Yu-Ming Ju. Four collections initially considered being close to *H. archeri* grouped together in clade III but were separated from the other annulate species suggesting that they represent the same taxon. The presence of a white fringe surrounding their ostioles and other minor deviations from typical *H. archeri* suggest that they all represent a new taxon. Three collections from Ratchaburi Province are described as *H. kanchanapisekii* named to honour the Royal Golden Jubilee Ph.D. Program, which provides the financial support for this study. Two collections similar to *H. lenormandii* but growing on bamboo are described as a new species named *H. sublenormandii*. Another new species, *H. suranareei*, is named following the location of specimen collections at Suranaree University of Technology, Thailand.

***Hypoxylon kanchanapisekii*** Suwannasai, Rodtong, Thienhirun & Whalley, **sp. nov.**

FIGURES 1A-F

*Stromata glomerata vel pulvinata, 0.5-2mm diam. X 0.1-0.2mm crassa; tumulis peritheciorum inconspicuis vel conspicuis, externe obscure rubro-brunnea vel umbrinis in KOH dissolutis; Perithecia globosa 0.1-0.2 mm diam. Ostiola plana vel parum elevata. Asci 105-120 µm longitudine tota x 3.8-5 µm crassa, partibus sporiferis 75-85 µm longitudine stipitibus 12.5-35 µm longitudine annulo apicali in liquore iodato Melzeri cyanescente, discoideo, 1.25 µm alto x 2.5 µm lato. Ascosporae brunneae, unicellulares, ellipsoideae cum apicibus angustatis, 10-11.5(-12.5) x (1-)3.5-5 µm, rima germinativa recta longa praeditae; perisporium in KOH indehiscens, leve; episporium leve.*

KEY CHARACTERS: **Stromata** glomerate to pulvinate, restricted and usually containing less than 20 perithecia, perithecia occasionally almost free, 0.5-2 mm x 0.1-0.2 mm thick, with perithecial mounds inconspicuous to 1/3 exposed, surface dull reddish brown with KOH extractable pigments brown vinaceous (84), or umber (9); **perithecia** spherical 0.1-0.2 mm diam.; **ostioles** slightly higher or the same as the stromatal surface; **asci** 105-120 µm total length x 3.8-5 µm broad, the spore bearing parts 75-85 µm long with stipes 12.5-35 µm; **ascospores** brown, unicellular, equilateral, with narrowly rounded ends, 10-11.5(-12.5) x (1-)3.5-5 µm, with straight 2/3 length germ-slit; **perispore** indehiscent in 10% KOH, smooth, **epispore** smooth; **colonies** on PDA covering 9-cm Petri dish in two weeks at room temperature, 23-28°C, at first cream then buff, velvety to felty, with concentric zones where aerial hyphal tufts develop; **anamorph** not formed.

**SPECIMENS EXAMINED:** THAILAND, Ratchaburi Province, the Royal Forest Department, on bamboo, 28 August 2003, Suwannasai, N. (Holotype *SUT069*); *SUT066*; *SUT068*.

**Comments:** *Hypoxylon kanchanapisekii* is close to *H. parksianum* Y.-M. Ju & J.D. Rogers (Ju & Rogers 1996) except that the stromatal form of *H. kanchanapisekii* is normally glomerate and contains less than 20 perithecia whereas *H. parksianum* is pulvinate to effused-pulvinate with inconspicuous to conspicuous perithecial mounds. The colour of granules, which beneath surface and between perithecia, of *H. kanchanapisekii* is dull reddish brown but it is blackish in *H. parksianum*. The perithecia of *H. kanchanapisekii* are spherical with 0.1-0.2 mm in diameter, whereas *H. parksianum* perithecia are spherical to tubular and larger size, 0.3-0.6 mm in diameter x 0.4-0.8 mm high. Asci of *H. parksianum* are longer, 147-174 µm total length x 6.5-7.5 µm broad, than *H. kanchanapisekii*, which are described above. Ascospores of *H. kanchanapisekii* are slightly smaller than *H. parksianum* (11-14.5 x (4.5-) 5-6 µm). Host of this new species is known only from bamboo clump while the substrate of *H. parksianum* is on corticated wood of *Hibiscus*.

***Hypoxylon sublenormandii*** Suwannasai, Rodtong, Thienhirun & Whalley, **sp. nov.**

FIGURES 2A-D

*A Hypoxylo lenormandii differt in ascosporis 8.9-11.3 x 3.4-4.7 µm et in rima germinativa recta longa praeditae.*

KEY CHARACTERS: **Stromata** glomerate to effused-pulvinate, often appearing almost rosellinioid but joined by thin stromal tissue, conspicuous perithecial mounds, surface

reddish brown, reddish brown granules immediately beneath surface and between perithecia, with KOH-extractable pigments brown vinaceous (84), or umber (9); **perithecia** spherical, 0.2-0.4 mm diam., ostioles slightly higher than the stromatal surface; **asci** 95-110 µm total length x 3.8-5 µm broad, the spore bearing parts 65-75 µm long with stipes 30-42.5 µm; **ascospores** brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, 8.9-11.3 x 3.4-4.7 µm, with straight germ slit spore-length; **perispore** dehiscent in 10% KOH, with inconspicuous coil-like ornamentation; **epispore** smooth; **colonies** on PDA covering 9-cm Petri dish in two weeks at room temperature, 25°C, at first cream-coloured white then brown, felty, azonate, with diffuse margins; **anamorph** not formed.

**SPECIMENS EXAMINED:** THAILAND, Kanchanaburi Province, Chong Kho Neab Forest, on bamboo, 14 December 2003, Suwannasai, N. (Holotype *SUT282*); Trad Province, Ta Gum Forest, on bamboo, 19 September 2003, Phosri, C. *SUT250*.

**Comments:** *Hypoxylon sublenormandii* is close to *H. lenormandii* (Ju & Rogers 1996) but the stromatal surface of this new taxon is strongly reddish brown while *H. lenormandii* is grayish sepia (106), fuscous (103), or brown vinaceous (84). Asci of *H. lenormandii* are longer, 123-170 µm total length x 6-9 µm broad, than *H. sublenormandii*, which are 95-110 µm total length x 3.8-5 µm broad. The germination slit form of *H. sublenormandii* is straight spore-length whereas in *H. lenormandii* it is slightly sigmoid spore-length. Ascospores of *H. lenormandii* are slightly larger, 9.5-15 (-16) x 4-6.5 (-7) µm, than those of *H. sublenormandii*. This new taxon is known only from bamboo clumps. Ju & Rogers (1996) discussed the occurrence of *H. lenormandii* on both monocot and dicot substrates suggesting that this wide host range and the broad range of ascospore size indicate that the taxon might be subdivided into subtaxa. *Hypoxylon suranareei* would therefore be one of these and according to Ju (pers. comm.) it might in the future prove to be conspecific with *H. disjunctum* Rehm. collected originally on bamboo in Philippines.

***Hypoxylon suranareei*** Suwannasai, Rodtong, Thienhirun & Whalley, **sp. nov.**

FIGURES 2E-H

*Stromata hemisphaerica vel effuso-pulvinata, tumulis peritheciorum conspicuis, externe ferruginea; sub superficie et inter perithecia granulis aurantiacis conspersa, granulis aurantiacis vel in KOH dissolutis; Perithecia globosa 0.2-0.4 mm diam. Ostiola plana vel umbilicata. Asci 90-120 µm longitudine tota x 3.8-5 µm crassa, partibus sporiferis 70-85 um, longitudine stipitibus 30-50 µm. longitudine annulo apicali in liquore iodato Melzeri cyanescente, discoideo. Ascosporae brunneolae vel brunneae, unicellulares, ellipsoideo-inequilaterales cum apicibus angustatis, (10-) 12.5-14 x 5-6.3 µm, rima germinativa recta longa praeditae; perisporium in KOH dehiscens; episprium leve.*

KEY CHARACTERS: **Stromata** hemispherical to effused-pulvinate, often appearing almost rosellinioid but joined by thin stromal tissue, conspicuous perithecial mounds, surface fulvous (43), or rust (39), orange granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange (7) or scarlet (5); **perithecia** obovoid, 0.2-0.4 mm diam; **ostioles** same or lower than the stromatal surface, with white substance; **asci** 90-120 µm total length x 3.8-5 µm broad, the spore bearing parts

70-85 µm long with stipes 30-50 µm; **ascospores** brown to dark brown, unicellular, ellipsoid-inequilateral, with narrowly rounded ends, (10-)12.5-14 x 5-6.3 µm, with straight germ slit spore-length; **perispore** dehiscent in 10% KOH, with inconspicuous coil-like ornamentation; **epispore** smooth; **colonies** on PDA covering 9-cm Petri dish in two weeks at room temperature, 25°C, at first cream-coloured white then pale grayish, alternate rings, velvety, with diffuse margins; **anamorph** not formed.

**SPECIMENS EXAMINED:** THAILAND, Nakhon Ratchasima Province, Suranaree University of Technology, on White Popinac (*Leucaena leucocephalade* Wit.), 17 November 2003, Suwannasai, N. (Holotype *SUT182*) *SUT183*, *SUT184*.

**Comments:** *Hypoxylon suranareei* is close to *H. anomalum* J.D. Rogers et al. (Ju et al. 2005) but their KOH-extractable pigments are different. *Hypoxylon suranareei* has orange (7) or scarlet (5) pigments but in *H. anomalum* they are luteous (12). The colour of granules beneath the surface and between the perithecia in *H. suranareei* is orange but in *H. anomalum* the colour is reddish brown. Asci of *H. anomalum* are considerably longer and broader, 140-160 µm total length x 10-11.5 µm broad, whereas in *H. suranareei* they are 90-120 µm total length x 3.8-5 µm broad. Notably, the perispore ornamentation of *H. suranareei* is inconspicuously coil-like whereas *H. anomalum* is smooth.

## DNA amplification and sequence analysis

Approximately 600 to 900 base pair (bp) of the amplified ITS1-5.8S-ITS2 fragments were achieved. Details of ITS sequences of each isolate are reported in Table 3. The greatest variation in length and sequence was observed in the ITS1 region which ranged from 156 bp (*H. fendleri* SUT280) to 506 bp (*H. atroroseum* SUT009). The ITS2 region ranged from 160 bp (*H. lendormandii*) to 169 bp (*H. purpureonitens*). The length of 5.8S region was constant at 155 bp in all *Hypoxylon* isolates and highly conserved. The extremely long ITS1 sequences (477 and 506 bp) were found in both isolates of *H. stygium* (SUT058 and SUT243) and in *H. atroroseum* (SUT009 and SUT010), and this is in agreement with previous findings for *H. stygium* (AJ390409) and *H. atroroseum* (AJ390397) respectively (Sánchez-Ballesteros et al. 2000). *Hypoxylon stygium* and *H. atroroseum* appeared to be closely related as shown by their 93% identity. The ITS1-5.8S-ITS2 sequence alignment of both species revealed the insertion and/or deletion sequences of 28 bp (5' ATCTGCTCGAATAAAATTGCTTCAATAT 3') within ITS1 region. This sequence fragment might be useful for the designer of a probes or markers for species specific detection.

## Phylogenetic relationships

Phylogenetic relationships among *Hypoxylon* specimens are presented from the neighbour-joining analysis of the aligned ITS1-5.8S-ITS2 sequences except for sequences of *H. stygium* (SUT058 and SUT243) and *H. atroroseum* (SUT009 and SUT010) because their extremely long ITS1 sequences could not be unambiguously aligned correctly. Support for specific branches of the trees was assessed by the percentage of the neighbour-joining trees of 1000 bootstrapped data sets forming the branch. In Fig. 3, the tree assigned the taxa to three main clades. Clade I consisted of two species,

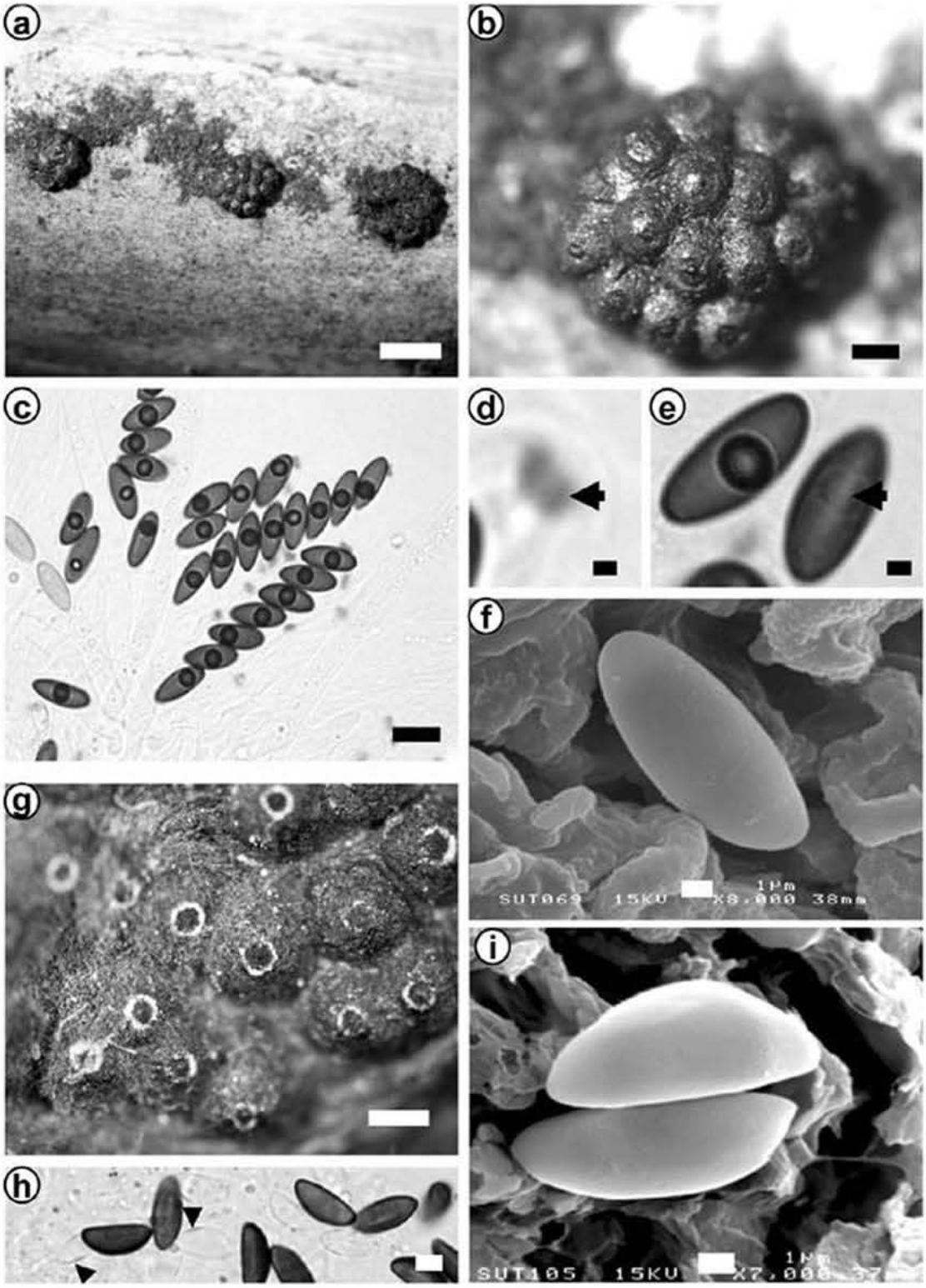


Fig. 1 *Hypoxylon kanchanapisekii* SUT069; a and b, stromatal form on bamboo (bars=1 mm and 0.2 mm respectively); c, ascospores (bar=10 μm); d, apical apparatus with discoid form (arrow) (bar=1 μm); e, straight 2/3 length of germ slit (arrow) (bar=1 μm); f, SEM micrograph of ascospore (bar=1 μm). *Hypoxylon* cf. *archeri* (SUT105); g, stromata with white fringe surrounding ostiolar disks (bar= 0.2 mm); h, ascospores with thickening of perispores (arrow) (bar=2 μm); and I, SEM micrograph of ascospores (bar=1 μm).

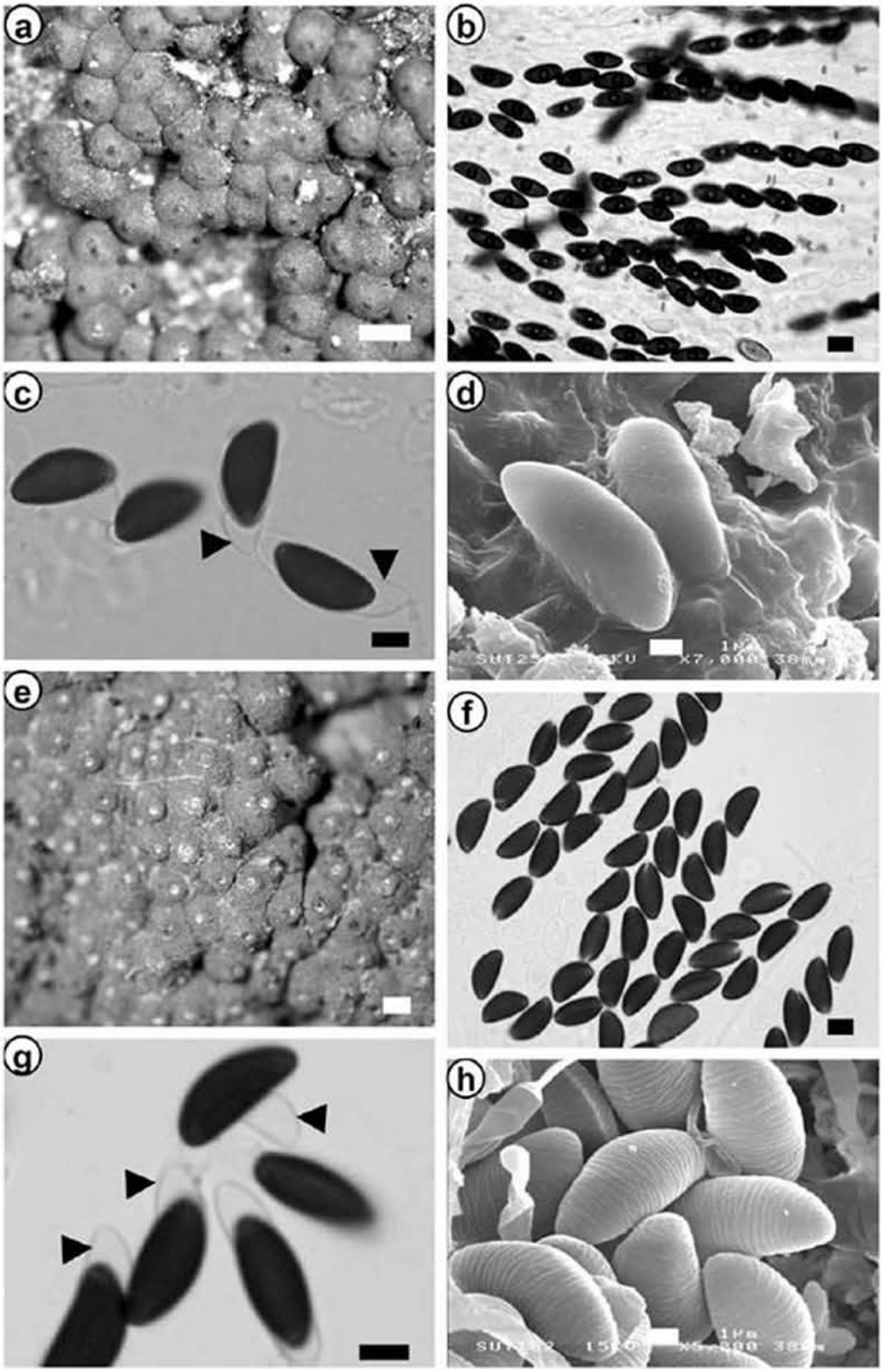


Fig. 2 *Hypoxylon sublenormandii* SUT250; a, stromatal form on bamboo (bar=0.2 mm); b, ascospores (bar=5 µm); c, ascospores with perispores dehiscing in 10% KOH (arrow) (bar=4 µm); d, SEM micrograph of ascospores (bar=1 µm). *H. suranareei* SUT182; e, stromatal form (bar=0.2 mm); f, ascospores (bar=4 µm); g, ascospores with perispores dehiscing in 10% KOH (arrow) (bar=5 µm); and h, SEM micrograph of ascospores (bar=1 µm).

Table 1. *Hypoxylon* species investigated in the present study.

| No | Species | Host | Location/Source |
|---|---|---|---|
| 1 | *Hypoxylon anthochroum* (SUT233) | Angiosperm wood | SUT's Herbarium (specimen collected from Trad Province, Thailand) |
| 2 | *H. anthochroum* (SUT240) | Angiosperm wood | SUT's Herbarium (specimen collected from Trad Province, Thailand) |
| 3 | *H.* cf. *archeri* (SUT103) | Angiosperm wood | SUT's Herbarium (specimen collected from Songkhla Province, Thailand) |
| 4 | *H.* cf. *archeri* (SUT105) | Angiosperm wood | SUT's Herbarium (specimen collected from Songkhla Province, Thailand) |
| 5 | *H.* cf. *archeri* (ST2333) | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 6 | *H.* cf. *archeri* (ST2527) | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 7 | *H. atroroseum* (SUT009) | Angiosperm wood | SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand) |
| 8 | *H. atroroseum* (SUT010) | Angiosperm wood | SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand) |
| 9 | *H. bovei* var. *microspora* (Taiwan) | No detail | Yu-Ming Ju's Herbarium (Taiwan) |
| 10 | *H. bovei* var. *microspora* (ST2406) | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 11 | *H. bovei* var. *microspora* (ST2575) | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 12 | *H. fendleri* (SUT061) | Angiosperm wood | SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand) |
| 13 | *H. fendleri* (SUT159) | Angiosperm wood | SUT's Herbarium (specimen collected from Yasothon Province, Thailand) |
| 14 | *H. fendleri* (SUT162) | Angiosperm wood | SUT's Herbarium (specimen collected from Yasothon Province, Thailand) |
| 15 | *H. fendleri* (SUT165) | Angiosperm wood | SUT's Herbarium (specimen collected from Yasothon Province, Thailand) |
| 16 | *H. fendleri* (SUT280) | Angiosperm wood | SUT's Herbarium (specimen collected from Kanchanaburi Province, Thailand) |
| 17 | *H. haematostroma* (SUT164) | Angiosperm wood | SUT's Herbarium (specimen collected from Yasothon Province, Thailand) |
| 18 | *H. haematostroma* (SUT292) | Angiosperm wood | SUT's Herbarium (specimen collected from Kanchanaburi Province, Thailand) |
| 19 | *H. haematostroma* (SUT293) | Angiosperm wood | SUT's Herbarium (specimen collected from Kanchanaburi Province, Thailand) |
| 20 | *H. kanchanapisekii* (SUT066) | Bamboo clump | SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand) |
| 21 | *H. kanchanapisekii* (SUT068) | Bamboo clump | SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand) |
| 22 | *H. kanchanapisekii* (SUT069) | Bamboo clump | SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand) |
| 23 | *H. lenormandii* (SUT016) | Angiosperm wood | SUT's Herbarium (specimen collected from Buriram Province, Thailand) |
| 24 | *H. lenormandii* (SUT180) | Angiosperm wood | SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand) |
| 25 | *H. lenormandii (ST2324)* | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 26 | *H. monticulosum (SUT042)* | Angiosperm wood | SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand) |

Note: SUT Suranaree = University of Technology.

*Table 1 concluded*

| No | Species | Host | Location/Source |
|---|---|---|---|
| 27 | *H. monticulosum* (SUT080) | Angiosperm wood | SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand) |
| 28 | *H. monticulosum* (SUT116) | Angiosperm wood | SUT's Herbarium (specimen collected from Songkhla Province, Thailand) |
| 29 | *H. nitens* (Taiwan) | No detail | Yu-Ming Ju's Herbarium (Taiwan) |
| 30 | *H. nitens* (ST2313) | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 31 | *H. nitens* (ST2473) | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 32 | *H. purpureonitens* (SUT004) | Angiosperm wood | SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand) |
| 33 | *H. purpureonitens* (SUT167) | Angiosperm wood | SUT's Herbarium (specimen collected from Yasothon Province, Thailand) |
| 34 | *H. purpureonitens* (SUT262) | Angiosperm wood | SUT's Herbarium (specimen collected from Trad Province, Thailand) |
| 35 | *H. purpureonitens* (ST2448) | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 36 | *H. purpureonitens* (ST2485) | Angiosperm wood | Royal Forest Department's Herbarium (Thailand) |
| 37 | *H. rubiginosum* (SUT148) | Angiosperm wood | SUT's Herbarium (specimen collected from Trad Province, Thailand) |
| 38 | *H. rubiginosum* (SUT187) | Angiosperm wood | SUT's Herbarium (specimen collected from Trad Province, Thailand) |
| 39 | *H. stygium* (SUT058) | Angiosperm wood | SUT's Herbarium (specimen collected from Ratchaburi Province, Thailand) |
| 40 | *H. stygium* (SUT243) | Angiosperm wood | SUT's Herbarium (specimen collected from Trad Province, Thailand) |
| 41 | *H. sublenormandii* (SUT250) | Bamboo clump | SUT's Herbarium (specimen collected from Trad Province, Thailand) |
| 42 | *H. sublenormandii* (SUT282) | Bamboo clump | SUT's Herbarium (specimen collected from Kanchanaburi Province, Thailand) |
| 43 | *H. suranareei* (SUT182) | White Popinac (*Leucaena leucocephalade* Wit.) | SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand) |
| 44 | *H. suranareei* (SUT183) | White Popinac (*Leucaena leucocephalade* Wit.) | SUT's Herbarium (specimen collected from Nakhon Ratchasima Province, Thailand) |

Note: SUT Suranaree = University of Technology.

Table 2. Morphological characteristics of the *Hypoxylon* species investigated.

| Species | Stromatal surface color | KOH-extractable pigment color | Ostiolar disc | Ascospore | Germ Slit | Perispore | Anamorph |
|---|---|---|---|---|---|---|---|
| *Hypoxylon* sect. *Annulata* | | | | | | | |
| *H.* cf. *archeri* Berk. | Blackish brown with white fringe | Hazel | *Truncatum*-type, 0.1-0.2 mm diameter | Ellipsoid-inequilateral, with narrowly rounded ends, 8.8-10(-11.5) x 3.8-5 µm | Straight full length | Smooth | *Nodulisporium*-like |
| *H. atroroseum* J.D. Rogers | Vinaceous gray | Greenish olivaceous | *Truncatum*-type, 0.1-0.2 mm diameter | Ellipsoid-equilateral, with narrowly rounded ends, 6.3-8.8 x 2.5-3.8 µm | Straight full length | Smooth | *Nodulisporium*-like |
| *H. bovei* var. *microspora* J.H. Miller | Black | Greenish olivaceous | *Bovei*-type, 0.3-0.7 mm diameter | Ellipsoid-inequilateral, with narrowly rounded ends, 7.5-10 x 3.8-5 µm | Straight full length | Smooth | *Nodulisporium*-like |
| *H. nitens* (Ces.) Y.-M. Ju & J.D. Rogers | Black | Greenish olivaceous | *Bovei*-type, 0.3-0.4 mm diameter | Ellipsoid-inequilateral, with narrowly rounded ends, 7.5-10 x 3.8-5 µm | Straight full length | Smooth | *Nodulisporium*-like |
| *H. purpureonitens* Y.-M. Ju & J.D. Rogers | Blackish with reddish brown tone, some shiny black | Vinaceous purple | *Bovei*-type, 0.3-0.4 mm diameter | Ellipsoid-inequilateral, with narrowly rounded ends, 7.5-10 x 3.8-5 µm | Straight full length | Smooth | *Nodulisporium*-like |
| *H. stygium* (Lév.) Sacc. | Blackish with reddish brown tone | Greenish olivaceous | *Truncatum*-type, 0.1-0.2 mm diameter | Ellipsoid-equilateral, with narrowly rounded ends, 3.8-6.3 x 2.5-3.8 µm | Straight full length | Smooth | *Periconiella*-like |

*Table 2, continued*

| Species | Stromatal surface color | KOH-extractable pigment color | Ostiolar disc | Ascospore | Germ Slit | Perispore | Anamorph |
|---|---|---|---|---|---|---|---|
| *Hypoxylon* sect. *Hypoxylon* | | | | | | | |
| *H. anthochroum* Berk. & Broome | Chestnut or brown vinaceous | Olivaceous | Lower than the stromatal surface | Ellipsoid-inequilateral, with narrowly rounded ends, 10.8-13(-14) x 4-6 µm | Straight full length | Dehiscent, with inconspicuous coil-like ornamentation | *Nodulisporium*-like |
| *H. fendleri* Berk. ex Cooke | Brown vinaceous or dark brick | Orange | Lower than the stromatal surface | Ellipsoid-inequilateral, with narrowly rounded ends, 8.5-11.5(-12.5) x 3.5-5 µm | Slightly sigmoid full length | Dehiscent, with inconspicuous coil-like ornamentation | *Nodulisporium*-like |
| *H. haematostroma* Mont. | Orange or rust | Orange | Lower than the stromatal surface | Ellipsoid-inequilateral, with narrowly rounded ends, 13-17.9 x 6.3-8.6 µm | Slightly sigmoid full length | Dehiscent, smooth | *Periconiella*-like |
| *H. kanchanapisekii* sp. nov. | Dull reddish brown | brown vinaceous , umber | slightly higher or the same as the stromatal surface | Ellipsoid-equilateral, with narrowly rounded ends, 10-11.5(-12.5) x (1-)3.5-5 µm | Straight full length | Indehiscent, smooth | Unknown |
| *H. lenormandii* Berk. & M.A. Curtis | Grayish sepia | Red | Slightly higher than the stromatal surface | Ellipsoid-inequilateral to equilateral, with narrowly rounded ends, 10-12.5 x 3.8-5 µm | Slightly sigmoid full length | Dehiscent, with inconspicuous coil-like ornamentation | *Nodulisporium*-like |

*Table 2, concluded*

| Species | Stromatal surface color | KOH-extractable pigment color | Ostiolar disc | Ascospore | Germ Slit | Perispore | Anamorph |
|---|---|---|---|---|---|---|---|
| *Hypoxylon* sect. *Hypoxylon* | | | | | | | |
| *H. monticulosum* Mont. | Rust, brown vinaceous then blackish when mature | Colorless or purple | Higher than the stromatal surface and minutely papillate | Ellipsoid-inequilateral, with narrowly rounded ends, (6.3-)7.5-8.8 (-11.3) x 3.8-5µm | Slightly sigmoid full length | Dehiscent, with smooth to inconspicuous coil-like ornamentation | Unknown |
| *H. rubiginosum* (Pers.) Fr. | Brown vinaceous | Rust | Lower than the stromatal surface | Ellipsoid-inequilateral, with narrowly rounded ends, (7.5-)8.8-10 x 3.8-5 µm | Straight full length | Dehiscent, with smooth to inconspicuous coil-like ornamentation | *Nodulisporium*-like |
| *H. sublenormandii* sp. nov. | Reddish brown | Brown vinaceous or umber | Slightly higher than the stromatal surface | Ellipsoid-inequilateral to equilateral, with narrowly rounded ends, 9-12 x 3.8-5 µm | Straight full length | Dehiscent, with inconspicuous coil-like ornamentation | Unknown |
| *H. suranareei* sp. nov. | Fulvous or rust | Orange or scarlet | Same or lower than the stromatal surface, with white substance | Ellipsoid-inequilateral, with narrowly rounded ends, (10-)12.5-14x 5-6.3 µm | Straight full length | Dehiscent, with inconspicuous coil-like ornamentation | Unknown |

*H. monticulosum* (SUT042, SUT080, and SUT116) and *H. suranareei* (SUT182 and SUT183). Two representatives of *H. monticulosum* isolates, SUT042 and SUT080, were without apparent KOH-extractable pigments as detailed by Ju & Rogers (1996) whereas *H. monticulosum* SUT116 had a purple coloured extract. This does however agree with Ju & Rogers (1996) who stated that "it is noteworthy that the purplish stromatal pigments dark livid to dark vinaceous of *H. monticulosum* and *H. submonticulosum* are easily detected in the young, rusty brown stromata but are hardly so in the mature, blackened stromata". The sequence alignment indicated 99% similarity and it is concluded that they represent the same taxon regardless of extractable pigment in KOH. *H. suranareei* (SUT182 and SUT183) isolates were separated from *H. monticulosum* with high bootstrap support, which confirmed this new taxon.

Clade II contained three *Annulata* species, *H. nitens* (Taiwan, ST2475 and ST2313), *H. bovei* var. *microspora* (Taiwan, ST2406 and ST2579), and *H. purpureonitens* (SUT004, SUT167, SUT262, ST2448, and ST2485). Additionally six species consisting of *H. lenormandii* (SUT016, SUT180, and ST2324), *H. sublenormandii* (SUT250 and SUT282), *H. haematostroma* (SUT164, SUT292, and SUT293), *H. anthochroum* (SUT233 and SUT240), *H. kanchanapisekii* (SUT066, SUT068, and SUT069), and *H. rubiginosum* (SUT215 and SUT221), were also grouped together. The three *Annulata* species are complex species and are very similar in morphological characteristics. The two isolates of *H. nitens* from Thailand exhibited a 95% similarity to *H. nitens* from Taiwan but they were closely linked, and they are therefore considered to be the same. *Hypoxylon purpureonitens* showed 28% divergence to *H. nitens*. This is in agreement with Ju and Rogers (1996) who reported that *H. purpureonitens* is very close to *H. nitens* in its teleomorphic morphology except KOH-extractable pigments. *Hypoxylon purpureonitens* is purple or livid whereas *H. nitens* is greenish olivaceous in 10% KOH.

Three isolates of *H. lenormandii* (ST2324, SUT016, and SUT180) closely matched the description by Ju & Rogers (1996) and all collections grew on dicotyledonous wood from different forest areas as detailed in Table 1. They were clearly separated from *H. sublenormandii* (SUT250 and SUT282), with both collections occurring on bamboo, with high bootstrap support (Fig. 3). They also differed in morphological characters such as spore size (9-12 x 3.8-5 µm), a more reddish brown stromatal surface color, and a straight germ slit and on the basis of this and the sequence data a new species is proposed. Three isolates of *H. haematostroma* (SUT164, SUT292, and SUT293) could also be separated from the other taxa in the same clade because of their distinctive teleomorphic characteristics having red or orange red stromatal granules. *Hypoxylon anthochroum* (SUT233 and SUT240), *H. kanchanapisekii* (SUT066, SUT068, and SUT069), and *H. rubiginosum* (SUT215 and SUT221) were separated from each other with high bootstrap support. Although *H. anthochroum* was considered to be a synonym of *H. rubiginosum* by Miller (1961), they differ in KOH-extractable pigment color. This phylogenetic tree also confirmed the separate identity of a further new species, *H. kanchanapisekii*, which was separated from other species examined.

Clade III consisted of three species, *H. fendleri* (AJ390400), *H.* cf. *fendleri* (SUT061, SUT159, SUT162, SUT165, and SUT280) and *H.* cf. *archeri* (SUT103, SUT105, ST2333, and ST2527). Initially, five *H.* cf. *fendleri* (SUT061, SUT159, SUT162, SUT165, and SUT280) collections had been identified as *H. fendleri* since their morphological

Table 3. Sizes of ITS1 and ITS2 regions and 5.8S ribosomal nucleotide sequences.

| No. | Species | ITS1 (bp) | 5.8S (bp) | ITS2 (bp) | Total (bp) | GenBank Accession number |
|---|---|---|---|---|---|---|
| 1 | *Hypoxylon anthochroum* (SUT233) | 180 | 155 | 162 | 497 | QD201125 |
| 2 | *H. anthochroum* (SUT240) | 180 | 155 | 162 | 497 | QD201126 |
| 3 | *H.* cf. *archeri* (SUT103) | 209 | 155 | 161 | 525 | QD201121 |
| 4 | *H.* cf. *archeri* (SUT105) | 209 | 155 | 161 | 525 | QD201122 |
| 5 | *H.* cf. *archeri* (ST2333) | 209 | 155 | 161 | 525 | QD201123 |
| 6 | *H.* cf. *archeri* (ST2527) | 224 | 155 | 160 | 539 | QD201124 |
| 7 | *H. atroroseum* (SUT009) | 506 | 155 | 164 | 825 | DQ223733 |
| 8 | *H. atroroseum* (SUT010) | 506 | 155 | 164 | 825 | DQ223734 |
| 9 | *H. bovei* var. *microspora* (Taiwan) | 226 | 155 | 167 | 548 | QD201127 |
| 10 | *H. bovei* var. *microspora* (ST2406) | 225 | 155 | 167 | 547 | QD201128 |
| 11 | *H. bovei* var. *microspora* (ST2579) | 226 | 155 | 167 | 548 | QD201129 |
| 12 | *H. fendleri* (SUT061) | 180 | 155 | 165 | 500 | QD201130 |
| 13 | *H. fendleri* (SUT159) | 183 | 155 | 164 | 502 | QD201132 |
| 14 | *H. fendleri* (SUT162) | 183 | 155 | 165 | 503 | DQ223735 |
| 15 | *H. fendleri* (SUT165) | 182 | 155 | 163 | 500 | DQ223736 |
| 16 | *H. fendleri* (SUT280) | 156 | 155 | 163 | 474 | DQ223737 |
| 17 | *H. haematostroma* (SUT164) | 176 | 155 | 161 | 492 | DQ223738 |
| 18 | *H. haematostroma* (SUT292) | 176 | 155 | 161 | 492 | DQ223739 |
| 19 | *H. haematostroma* (SUT293) | 176 | 155 | 161 | 492 | DQ223740 |
| 20 | *H. kanchanapisekii* (SUT066) | 209 | 155 | 162 | 526 | DQ223741 |
| 21 | *H. kanchanapisekii* (SUT068) | 209 | 155 | 162 | 526 | DQ223742 |
| 22 | *H. kanchanapisekii* (SUT069) | 209 | 155 | 162 | 526 | DQ223743 |
| 23 | *H. lenormandii* (SUT016) | 188 | 155 | 160 | 503 | DQ223744 |
| 24 | *H. lenormandii* (SUT180) | 188 | 155 | 160 | 503 | DQ223745 |
| 25 | *H. lenormandii* (ST2324) | 188 | 155 | 160 | 503 | DQ223746 |
| 26 | *H. monticulosum* (SUT042) | 171 | 155 | 165 | 491 | DQ223747 |
| 27 | *H. monticulosum* (SUT080) | 171 | 155 | 165 | 491 | DQ223748 |
| 28 | *H. monticulosum* (SUT116) | 171 | 155 | 165 | 491 | DQ223749 |
| 29 | *H. nitens* (Taiwan) | 158 | 155 | 166 | 479 | DQ223750 |
| 30 | *H. nitens* (ST2313) | 158 | 155 | 166 | 479 | DQ223751 |
| 31 | *H. nitens* (ST2473) | 158 | 155 | 166 | 479 | DQ223752 |
| 32 | *H. purpureonitens* (SUT004) | 225 | 155 | 169 | 549 | DQ223753 |
| 33 | *H. purpureonitens* (SUT167) | 225 | 155 | 169 | 549 | DQ223754 |
| 34 | *H. purpureonitens* (SUT262) | 225 | 155 | 169 | 549 | DQ223755 |
| 35 | *H. purpureonitens* (ST2448) | 225 | 155 | 169 | 549 | DQ223756 |
| 36 | *H. purpureonitens* (ST2485) | 225 | 155 | 169 | 549 | DQ223757 |
| 37 | *H. rubiginosum* (SUT215) | 178 | 155 | 164 | 497 | DQ223758 |
| 38 | *H. rubiginosum* (SUT221) | 178 | 155 | 164 | 497 | DQ223759 |
| 39 | *H. stygium* (SUT058) | 477 | 155 | 164 | 796 | DQ223760 |
| 40 | *H. stygium* (SUT243) | 477 | 155 | 164 | 796 | DQ223761 |
| 41 | *H. sublenormandii* (SUT250) | 198 | 155 | 161 | 514 | DQ223762 |
| 42 | *H. sublenormandii* (SUT282) | 198 | 155 | 161 | 514 | DQ223763 |
| 43 | *H. suranareei* (SUT182) | 199 | 155 | 162 | 516 | DQ223764 |
| 44 | *H. suranareei* (SUT183) | 199 | 155 | 162 | 516 | DQ223765 |

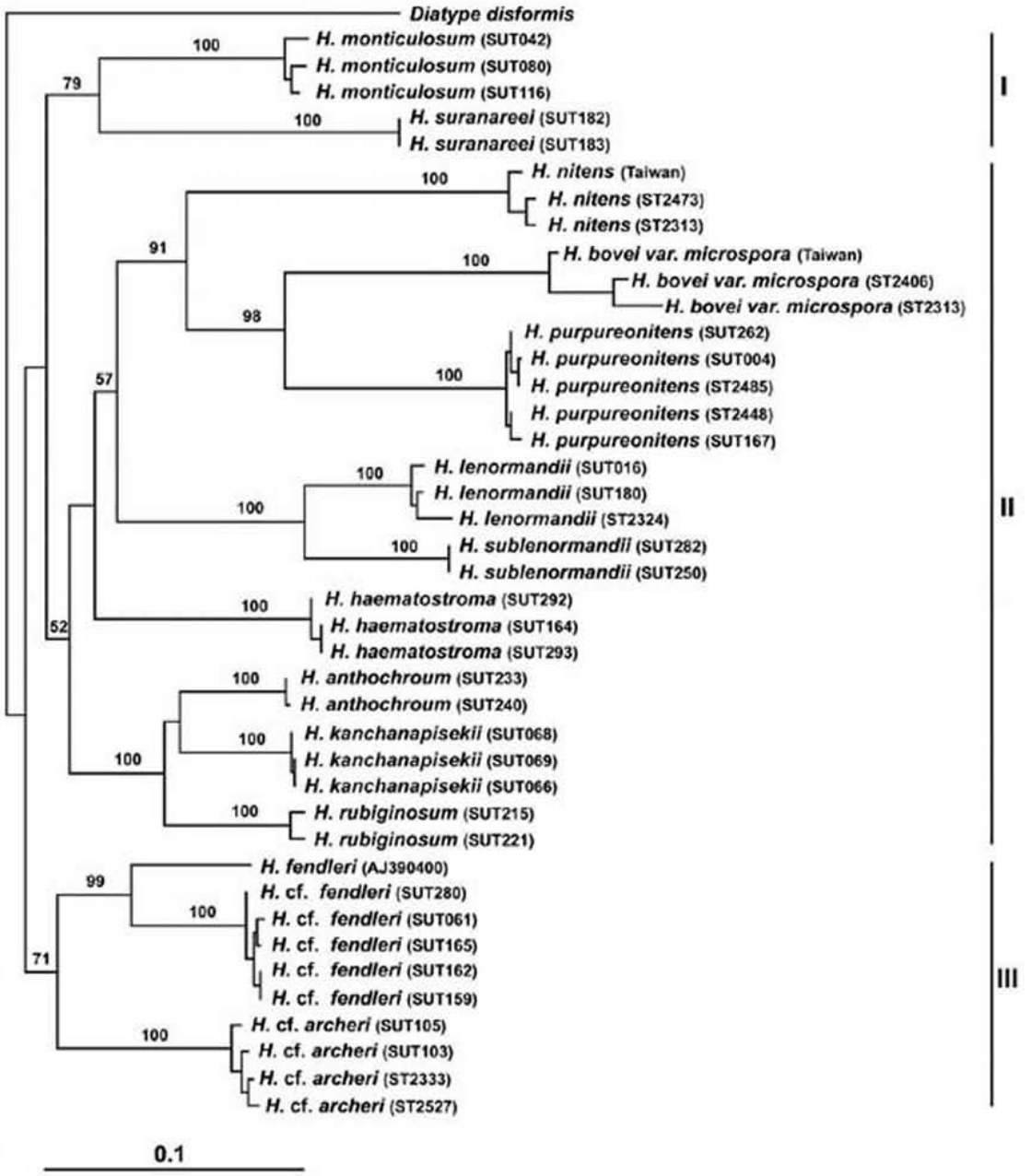


Fig. 3 Phylogenetic tree construction based on ITS1-5.8S-ITS2 sequences of *Hypoxylon* species. Branch lengths are scaled in terms of expected numbers of nucleotide substitution per site. Numbers on branches are bootstrap values from 1000 replications.

characteristics matched *H. fendleri* (Ju and Rogers 1996). However, Ju and Rogers (1996) pointed out that *H. fendleri* and *H. retpela* Van der Gucht & Van der Veken are very similar stating "These two fungi differ mainly in the conspicuousness of their perispore ornamentation". The ornamentation in *H. retpela* is described as very conspicuously coil-like. However all the Thai collections had similar coiling which was not noticeably conspicuous. Thus the description for *H. fendleri* (Ju & Rogers 1996) is the nearest match. The phylogenetic result showed that all the Thai isolates (SUT061, SUT159, SUT162,

SUT165, and SUT280) grouped together and were placed as a sister branch of *H. fendleri* (AJ390400) based on the GenBank database sequence with high bootstrap support. The percentage similarity of *H. fendleri* (AJ390400) to SUT061, SUT159, SUT162, SUT165, and SUT280 isolates was 85%, 85%, 85%, 85%, and 80% respectively. They are therefore quite different and as a result the Thai collections were recorded as *H.* cf. *fendleri*. This might be the result of a wide range of *H. fendleri* descriptions (morphological) or genetic variation within this taxon found in Thailand. More collections of specimens around the world are required for a better understanding of species delimitation and genetic variation within this taxon.

Four isolates of *H.* cf. *archeri* were grouped together. All of them had a small ostiolar disc around 0.1-0.2 mm in diameter, and belonged to the section *Annulata*. They appeared as a paraphyletic group with other isolates of the section *Annulata* also placed in Clade II. Its morphological features were similar to those of *H. archeri* Berk. and *H. michelianum* Ces. & De Not. Ascospore dimensions, stromatal form, and coloration are indicative of *H. archeri*, but the distinctive white fringe surrounding the ostioles is reminiscent of *H. michelianum*. However, *H.* cf. *archeri* examined, in the current study, grouped in Clade III and were thus separated from the other species of the *Annulata*, which grouped in Clade II. Unfortunately, no voucher material was available for DNA extraction or for morphological comparison, and no sequences were available in GenBank.

## Key to taxa of section *Hypoxylon* in this study

1a. Ascospores with perispore not dehiscent in 10% KOH ..... ***H. kanchanapisekii***

1b. Ascospores with perispore dehiscent in 10% KOH .....2

2a. (1b) Stromatal surface without vinaceous shades .....3

2b. Stromatal surface with vinaceous shades .....6

3a. (2a) Ascospores less than 12 µm long .....4

3b. Ascospores greater than 12 µm long .....5

4a. (3a) Stromatal glomerate to effused-pulvinate, rosellinioid; grayish sepia (106), or brown vinaceous (84), with KOH-extractable pigments sienna (8), cinnamon (62), fulvous (43); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with slightly sigmoid germ slit spore-length, 10-12.5 x 3.8-5 µm ..... ***H. lenormandii***

4b. Stromatal glomerate to effused-pulvinate, rosellinioid; reddish brown, with KOH-extractable pigments brown vinaceous (84), or umber (9); ascospore brown to dark brown, unicellular, ellipsoid-inequilateral, with straight germ slit spore-length, 9-12 x 3.8-5 µm ..... ***H. sublenormandii***

5a. (3b) Stromatal hemispherical to effused-pulvinate, plane or with inconspicuous to conspicuous perithecial mounds; surface orange (7), or rust (39); orange red granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange (7) or scaret (5); ascospores brown to dark brown,

unicellular, ellipsoid-inequilateral, with slightly sigmoid germ slit spore-length, 13-17.9 x 6.3-8.6 µm; perispore dehiscent in 10% KOH, smooth ..... ***H. haematostroma***

5b. Stromatal hemispherical to effused-pulvinate, with conspicuous perithecial mounds; surface fulvous (43), or rust (39); rust granules immediately beneath surface and between perithecia, with KOH-extractable pigments orange (7) or scaret (5); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with straight germ slit spore-length, (10-) 12.5-14 x 5-6.3 µm; perispore dehiscent in 10% KOH, inconspicuous coil-like ornamentation ..... ***H. suranareei***

6a. (2b) KOH-extractable pigments olivaceous (48), or gray olivaceous (107), or greenish olivaceous (90) ..... ***H. anthochroum***

6b. KOH-extractable pigments other than greenish or olivaceous, or without apparent pigments.....7

7a. (6b) KOH-extractable pigments purplish or without apparent pigments ..... ***H. monticulosum***

7b. KOH-extractable pigments orange (7), or rust (39) .....8

8a. (7b) KOH-extractable pigments orange (7); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with slightly sigmoid germ slit spore-length, 8.5-11.5 (-12.5) x 3.8-5 µm ..... ***H. fendleri***

8b. KOH-extractable pigments rust (39); ascospores brown to dark brown, unicellular, ellipsoid-inequilateral, with straight germ slit spore-length, (7.5-) 8.8-10 x 3.8-5 µm .....***H. rubiginosum***

## Key to taxa of section *Annulata* in this study

1a. Ostiolar disc not exceeding than 0.3 mm diameter.....2

1b. Ostiolar disc exceeding than 0.3 mm diameter .....4

2a. (1a) KOH-extractable pigments hazel (88) or honey (64) ..... ***H.* cf. *archeri***

2b. KOH-extractable pigments olivaceous (48) or greenish olivaceous (90) .....3

3a. (2b) Stromata blackish; conidiogenous structure *Periconiella*-like ..... ***H. stygium***

3b. Stromata rose-coloured with blackish; conidiogenous structure *Nodulisporium*-like ..... ***H. atroroseum***

4a. (1b) Ostiolar disc variable, 0.3-0.7 mm; ascospores 7.5-10 x 3.5-5 µm ..... ***H. bovei* var. *microspora***

4b. Ostiolar disc 0.2-0.5 mm; mature stromata shiny black .....5

5a. (4b) KOH-extractable pigments greenish olivaceous (90); ascospores 7.5-10 x 3.8-5 µm ..... ***H. nitens***

5b. KOH-extractable pigments vinaceous purple (101); ascospores 7.5-10 x 3.8-5 µm ..... ***H. purpureonitens***

## Conclusions

Fifteen *Hypoxylon* species identified from eighty nine collections from Thailand were recorded including three new species, *H. kanchanapisekii*, *H. sublenormandii*, and *H. suranareei*. The phylogenetic relationships between morphological and molecular data of the *Hypoxylon* species were achieved following sequence analysis of ITS1-5.8S-ITS2 rDNA regions. These molecular results showed clear separation within the *Hypoxylon* species studied and could be used to confirm the new species. In addition, molecular data could be used to resolve taxonomic problems based solely on morphology and has the potential to recognize the delimitation of other species in future study. Within the genus molecular data is valuable for resolving species complexes and for the recognition of relationships between species. The ITS sequences obtained in the current study do not support a clear separation of members of *Hypoxylon* and section *Annulata* and this is in agreement with the findings of Sánchez-Ballesteros et al. (2000). However, Hsieh et al. (2005) provided strong evidence for their separation based on different DNA sequences which is therefore in contrast with our findings and those of Sánchez-Ballesteros et al. (2000)

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# *Tricholoma equestre*, the correct name for *T. flavovirens* (*Agaricales*)

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**Abstract**—*Tricholoma equestre* is considered to be the correct name for the species often known as *T. flavovirens*. The history of the conservation of the generic name *Tricholoma* and its type, *T. flavovirens*, is reviewed and presented here. The author citation of *T. flavovirens* is also discussed and the upcoming correction in the ICBN is addressed.

**Key words**—nomenclature, synonym, *Tricholomataceae*

The type of *Tricholoma* (Fr.) Staude, *nom. cons.*, is *T. flavovirens* (for the author citation of this name, see the discussion below), as reported in successive editions of the International Code of Botanical Nomenclature (ICBN; see Voss et al. 1983; Greuter et al. 1988, 1994, 2000). However, much confusion has surrounded the correct name of the species to which this name has been applied because *T. equestre* and *T. flavovirens* are almost universally regarded as synonyms. Some researchers consider *T. equestre* as the preferred name with *T. flavovirens* its synonym (e.g. Bon 1987, Tkalčec & Mešič 2002, Massart 2003, Horak 2005), but many others still accept *T. flavovirens* as the correct name and reduce *T. equestre* to synonymy (e.g. Moser 1983, Singer 1986, Phillips 1988, Hansen & Knudsen 1992, Jordan 1995, Bruns et al. 1998, Noordeloos & Christensen 1999).

In their survey of *Tricholoma* species reported from China, Deng et al. (2004) noted that both *T. equestre* (e.g. Tai 1979, Tseng 1996, CAS 1996) and *T. flavovirens* (e.g. Ying & Zang 1994, Mao 2000, Wen et al. 2001, Wang et al. 2004) were cited in the Chinese literature, with the latter used more frequently, especially in recent years.

A second, more global, literature search was conducted to determine the correct name for this species. *Agaricus equestris* (=*T. equestre*) and *A. flavovirens* (=*T. flavovirens*) were consistently treated as synonyms by Fries (Fries 1821, 1828, 1874). The two names were also considered synonymous both by many of Fries' contemporaries (see Saccardo 1911) and more recent agaricologists (e.g. Singer 1962, 1975, 1986; Bon 1976, 1984; Hansen & Knudsen 1992, Noordeloos & Christensen 1999, Horak 2005).

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Although *A. equestris* was first treated as a synonym of *A. flavovirens* by Fries (1821), it was restored as an accepted name and the latter reduced to a synonym (Fries 1828, and 1832 in the index to *Systema Mycologicum*). According to the ICBN (Art. 15, including Note 1, in Greuter et al. 2000), both *A. equestris* and *A. flavovirens* were sanctioned by Fries (1821, 1828), but *A. equestris*, as the earlier name, has priority over *A. flavovirens*. This conclusion is also indicated in Ex. 4 of Art. 15, ICBN, although the date of Persoon's name is incorrect (see below). Therefore, if the two are regarded as synonymous, it is clear that *T. equestre* is the correct name for the species and *T. flavovirens* is a later synonym. The author citation and references of these two names are listed below:

***Tricholoma equestre*** (L. : Fr.) P. Kumm., Führ. Pilzk.: 130. 1871.

≡*Agaricus equestris* L., Sp. pl. Ed. 1, 1173. 1753. : Fr., Elen. fung. 1: 6. 1828.

=*Agaricus flavovirens* Pers. in Hoffmann, Abbild. Schwämme 3: t. 24. 1793. : Fr., Syst. mycol. 1: 41. 1821.

≡*Tricholoma flavovirens* (Pers. : Fr.) S. Lundell in Lundell & Nannfeldt, Fung. Exs. Suec., Fasc. 23–24: no. 1102. 1942.

Of the several proposals considered by the IAPT Special Committee for Fungi (Rogers 1953a), conservation of the generic name *Tricholoma* (Fr.) P. Kumm. 1871 against *Tricholoma* Benth. 1846 (the name of a plant genus in the *Scrophulariaceae*) was finally approved (Rogers 1953b) and formally included in the list of conserved generic names in the 1956 ICBN (Lanjouw et al. 1956) as:

'*Tricholoma* (Fries) Kummer, Führ. Pilzk. 25. 1871.
T.: *Agaricus flavovirens* A. & S. ex Fr. Syst. Mycol. 1: 41. 1821 (cf. *A. equestris* Fr. Elench. Fung. 1: 6. 1828).'

Donk (1962, 1968) subsequently determined that the author who first validly published the conserved *Tricholoma* was not Kummer, but Staude. Therefore, the authorship for the generic name in the 1972 ICBN (Stafleu et al. 1972) was changed from '(Fries) Kummer, Führ. Pilzk. 25, 129. 1871' to '(E. M. Fries) Staude, Pilze Mitt.-Deutschl. xviii, 25. 1857.' The type of the conserved generic name *Tricholoma*, namely '*Agaricus flavovirens* A. & S. ex Fr., Syst. Mycol. 1: 41. 1821 (cf. *A. equestris* Fr., Elench. Fung. 1: 6. 1828)', was also included in the proposal for conservation (Rogers 1953b) and accepted as thus in the 1956 edition of ICBN. Later editions of the ICBN, however, replaced the type synonymy—the part in brackets: '(cf. A. equestris Fr. Elench. Fung. 1: 6. 1828)'—with '[*A. equestris* Fries]'.

In the 1983 ICBN (Voss et al. 1983), the type was listed as '*T. flavovirens* (Albertini et Schweinitz : E. M. Fries) Lundell (*Agaricus flavovirens* Albertini et Schweinitz : E. M. Fries)', and in the most recent edition (Greuter et al. 2000) as '*Agaricus flavovirens* Alb. & Schwein. : Fr. (*T. flavovirens* (Alb. & Schwein. : Fr.) S. Lundel [sic!]). The synonym '*A. equestris*' no longer appeared in the list of conserved names. Singer, who cited *T. equestre* as the type of *Tricholoma* (Singer 1962, 1975), did list *T. flavovirens* as type in accordance with the 1983 ICBN in the 4th edition of *Agaricales* in Modern Taxonomy (Singer 1986). It is noteworthy that Bon, who first cited *T. flavovirens* with *T. equestre* as its synonym (Bon 1984), later reversed himself, citing *T. flavovirens* as synonymous to *T. equestre* (Bon 1987).

Although *T. flavovirens* has been designated as the type of *Tricholoma* in the ICBN list of conserved generic names, it is necessary to important to note that *T. equestre* is the correct name for the species to which the name *T. flavovirens* applies. In fact, it is not uncommon that the generic or specific name that indicates the type of the name of a family or genus is not the correct name for that genus or species. For example, the type of the family name *Caryophyllaceae* Juss. is *Caryophyllus* Mill. 1754 (non *Caryophyllus* L. 1753), but the accepted name for the genus to which *Caryophyllus* Mill. applies is *Dianthus* L. 1753 (Ex. 4 of Art. 18.3, ICBN).

Further, the authorship of *A. flavovirens*, 'A. & S. ex Fr.', was listed in the conservation of *Tricholoma* in the 1956 ICBN (Lanjouw et al. 1956). The authorship later changed to 'Alb. & Schwein. : Fr.' (Greuter et al. 1994, 2000), although 'Pers. (1801)' has been cited in Art. 15, Ex. 4 since 1994 (Greuter et al. 1994, 2000). However, in Fries (1821: 41), *A. flavovirens* was referred to '*Abb. d. Schw. – Pers. Syn. p.* 319.' The abbreviation *Abb. d. Schw.* stands for *Abbildungen der Schwämme*, now abbreviated as *Abbild. Schwämme* (Stafleu & Cowan 1979: 239), in which *A. flavovirens* was first published by Persoon (in Hoffmann 1793). It is unclear why this was mistaken as 'Alb. & Schw.' at least since Rogers (1953b), but the error may have arisen because the two were very well known authors who included a description of *A. flavovirens* in their major mycological publication (Albertini & Schweinitz 1805: 167). Although '*Pers. Syn. p. 319.*' (Persoon 1801) was also mentioned by Fries (1821), Persoon (1801: 319) explicitly mentioned the earlier publication of the taxon by Persoon (in Hoffmann 1793).

Accordingly, the author citation for *A. flavovirens* in both Ex. 4 of Art. 15 and the list of conserved names of the ICBN should be corrected to 'Pers. (1793)' in future editions. The authors have been informed that the authorship of the species name indicating the type of *Tricholoma* will be corrected in the upcoming ICBN (McNeill et al. 2006 in prep., Demoulin pers. comm. 2006) to: *T. flavovirens* (Pers. : Fr.) S. Lundell (*Agaricus flavovirens* Pers. : Fr.).

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# A new species of *Lecanicillium* isolated from the white pine weevil, *Pissodes strobi*

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**Abstract**—A species of *Lecanicillium* was isolated from the white pine weevil *Pissodes strobi* on Vancouver Island, British Columbia, Canada. Isolates were collected from adult weevil cadavers. The fungus shares some morphological traits with *L. attenuatum* although it differs in having a slower growth rate, larger conidial heads holding up to 50 conidia, a longer average phialide length and variable conidial size and shape. An analysis of the mitochondrial DNA and the β–tubulin gene showed the fungus to be distinct from other *Lecanicillium* species. The new species, *Lecanicillium pissodis*, is described and illustrated.

**Key words**—entomopathogen, Hyphomycete, *Picea*, Curculionidae, biological control, *Verticillium* section *Prostrata*

## Introduction

Numerous entomopathogenic Hyphomycetes have been isolated and described from various insect hosts worldwide (Butt et al. 2001). Entomogenous Hyphomycetes in the genera *Cephalosporium* Corda and *Verticillium* Nees were originally classified by isolates that formed either single conidiophores or verticillate conidiophores, respectively (Petch 1925, 1931, 1932, 1939; Bałazy 1973), and both authors had differentiated multiple taxa within the two genera based on morphological details. In 1971, Gams erected the new section *Prostrata* of the genus *Verticillium* and consolidated a range of *Cephalosporium* isolates that varied greatly in conidial shape and size under one name; *Verticillium lecanii* (Zimm.) Viégas. In 2001, Zare & Gams revisited *Verticillium* sect. *Prostrata* and, by using microscopic and molecular methods, they subsequently placed fifteen species into a new genus, *Lecanicillium* W. Gams & Zare. Their thorough work has resolved many of the difficulties of a broad array of variable isolates of this genus of entomogenous and fungicolous anamorphs of the *Clavicipitaceae*.

In 2003 live adult white pine weevils (*Pissodes strobi* Peck) were collected from Vancouver Island, British Columbia (BC) and while in rearing cages, dead weevils were noted and assayed for the presence of entomopathogenic fungi. The collected fungi were identified as *Lecanicillium muscarium* (Petch) Zare & W. Gams, with the exception of

three isolates. Microscopic examination and PCR-RFLP analysis determined that the three unidentified isolates did not fit the descriptions given for known *Lecanicillium* species.

In this paper we report on a new species of *Lecanicillium* that was isolated from cadavers of *P. strobi*. This fungus is represented by three collections. Both the morphological descriptions, restriction fragment length polymorphisms (RFLP) of mitochondrial DNA and the β-tubulin gene, as well as sequencing of the β-tubulin gene indicate they are sufficiently distinctive to warrant a description as a new species of the genus *Lecanicillium*.

## Materials and Methods

**Isolate collection:**
Live weevils were collected from the arboretum at the Pacific Forestry Centre (N48° 28' W124° 24') on Vancouver Island, BC. Weevils were reared on branches of Sitka spruce *Picea sitchensis* (Bong.) Carrière, in cages in a shade-house at the Pacific Forestry Centre. Cadavers were collected, incubated on moistened filter paper in a sealed Petri plate, and observed daily for the development of mycelium. Single spore isolates of *Lecanicillium* were produced by suspending a drop of sterile water from the end of a hypodermic needle, and upon touching a phialide the hydrophilic conidia migrated into the droplet. The droplet was spread over the surface of a *Lecanicillium*-selective medium (LSM) (Kope et al. 2006). Colonies from single germinated conidia were subcultured onto Sabouraud dextrose agar (SDA), grown for 14 days, and then stored at 5°C until needed.

**Microscopy:**
The identification of *Lecancillium* isolates from colony morphology was assessed on potato-dextrose agar (PDA, Oxoid) after 10 days incubation at 20°C in darkness (Zare and Gams 2001). Conidiophore branching and conidial arrangement was observed in open PDA Petri plates under a compound microscope at 50x magnification. Phialide length and conidial shape and size were observed in water mounts on glass slides under a compound microscope at 400-1000x magnification. Fifty conidia from four water mounts (200 conidia in total) for each of the three isolates were measured to determine conidial size. Photo-micrographs were taken with phase contrast optics. Colony growth of the three *Lecanicillium* isolates was measured on PDA after 10 days incubation at 24°C in darkness.

**DNA extraction:**
Mycelium for DNA extraction was harvested from potato-dextrose broth by filtration and then freeze-dried. The material was ground to a powder in liquid nitrogen, and the DNA was purified from 20-30 mg of the powdered mycelium using Qiagen DNeasy Plant Mini Kit following the supplied protocol. MtDNA was extracted from 2.0 grams of powdered mycelium using Epicentre's MasterPure™ Yeast DNA Purification Kit, following the supplied protocol to extract total DNA, followed by CsCl/EtBr density gradient equilibrium centrifugation (0.8 g $ml^{-1}$ CsCl and 0.4mg $ml^{-1}$ EtBr) in a Beckman Vti65 rotor at 250,000g for 16 hrs. The band was removed and extracted 3 times with isopropanol and dialyzed against 4L of TE (10mM Tris, 1mM EDTA, pH

8.0) twice for 4 hrs. The resulting DNA was separated into mtDNA and genomic DNA by CsCl/bisbenzimide density gradient centrifugation (1.0 g $ml^{-1}$ CsCl and 0.1 mg $ml^{-1}$ bisbenzimide) in a Beckman Vti65 rotor at 250,000g for 16 hrs. The mtDNA was removed and extracted 3 times with isopropanol and dialyzed against 4L of TE (10mM Tris, 1mM EDTA, PH 8.0) with 3 changes over 16 hrs.

**PCR and RLFP:**
PCR amplifications and RFLP analyses were carried out as described by Zare and Gams (2001), however, since we had difficulty discerning band patterns using total genomic DNA, we separated the mtDNA from the chromosomal DNA. The RFLP of the ITS region and the 3' partial β–tubulin gene were amplified using the primer sets, ITS–4/ITS–5 (White et al. 1990) and Bt1a/Bt1b (Glass and Donaldson 1995), respectively.

**Phylogenetic analysis and sequence alignment:**
PCR products of the ITS region and the 5' partial β–tubulin gene were sequenced after purification. To obtain additional β–tubulin sequence information, the 5' partial β–tubulin gene was amplified and sequenced using the primers Bt2E/Bt12 as described by Kim et al. (2003). Sequencing was performed on an ABI 3700 automated sequencer (Perkin-Elmer, Foster City, CA) at the DNA synthesis and sequencing facility, Macrogen (Seoul, Korea). A phylogenetic tree of the ITS region was built using sequences of other taxa obtained from NCBI. The ITS sequences of 16 taxa were aligned using ClustalX (Thompson et al. 1997) and manually adjusted in the PHYDIT program version 3.2 (*http://plaza.snu.ac.kr/~jchun/phydit/*). A phylogenetic tree of relatedness between the new species and related species was constructed by a neighbor-joining (NJ) method (Saitou and Nei 1987). The distances in the ITS region were determined by Kimura's two-parameter model. Branch stability was assessed by 1000 bootstrap replications implemented with PAUP*4.0b10 (Swofford 2001).

## Results

**Microscopy:**
On cadavers of adult *P. strobi*, mycelium can appear as soon as 4 days after infection and it is first seen along the length of the proboscis, around the eyes, at the joins between the thorax, abdomen and head, and at the intersegmented membranes of the legs (Figure 1). The first visible mycelium is white, long and silky, which over time, forms into filamentous mycelium ultimately enveloping the whole cadaver. Shiny globular droplets at the tips of phialides are visible to the unaided eye.

*Lecanicillium pissodis* can be distinguished from other described species in this genus by a number of morphological characters (Table 1). On agar, colony growth is radial, slightly raised, bright white in colour with a cream to pale yellow reverse and no pigment diffusion into the agar. Prostrate hyphae with phialides are visible at the leading edge of the colony, with phialide numbers increasing towards the center of the colony. Phialides are mostly single with some in a verticillate arrangement of 2-3 at intercalary nodes or at hyphal ends. Conidial heads at the tip of phialides are distinctly visible (Figure 2). When conidial heads are disturbed, conidia are dispersed (Figure 3) releasing up to and sometimes more than 50 conidia. The hydrophilic conidia are quickly spread via water. Conidia are hyaline, single celled, and variable in size, 4–9.2 x 1.6–2.4 μm, and shape, cylindrical to ovoid or oval (FIGURE 4).

TABLE 1. Morphological characteristics of some entomogenous *Lecanicillium* species*

| Species | Phialides (µm) | Conidial heads | Conidial size (µm) | Conidial shape |
|---|---|---|---|---|
| *L. aranearum* (Petch)Zare & W. Gams | 20-30 × 0.7-1.5 | — | 5.0-8.0 × 1.2-1.5 | slightly pointed at one or both ends, straight or curved |
| *L. attenuatum* Zare & W. Gams | 9-15.5 × 1-2 | 1-4 conidia in dry clusters | 4.5-6.5 × 1.5-2.0 | cylindrical, attenuate base uniform shape occasionally 2-celled |
| *L. lecanii* (Zimm.) Zare & W. Gams | 11-20(30) × 1.3-1.8 | globose heads | 2.5-3.5(-4.2) × 1.0-1.5 | short-ellipsoidal, uniform shape |
| *L. longisporum* (Petch)Zare & W. Gams | 20-40 × 1.2-2.7 | globose heads | 5.0-10.5 × 1.5-2.5 | ellipsoidal to oblong-oval |
| *L. muscarium* | (15-)20-35 × 1-1.7 | globose heads | (2-)2.5-5.5(-6) × 1.0-1.5(-1.8) | ellipsoidal or subcylindrical, irregular shape |
| *L. nodulosum* (Petch)Zare & W. Gams | 10-20 × 1.5 | globose heads | 2.5-4.5 × 1.2-1.5 | oval |
| *L. pissodis* sp. nov. | (16-)18-28(-38) × 1-2 | globose droplets with up to 50 conidia, or more | 4.0-9.2 × 1.6-2.4 | cylindrical to ovoid to oval, very variable shape |

*Characteristics for all species, except *L. pissodis*, taken from Zare and Gams (2001)

## Taxonomic Description

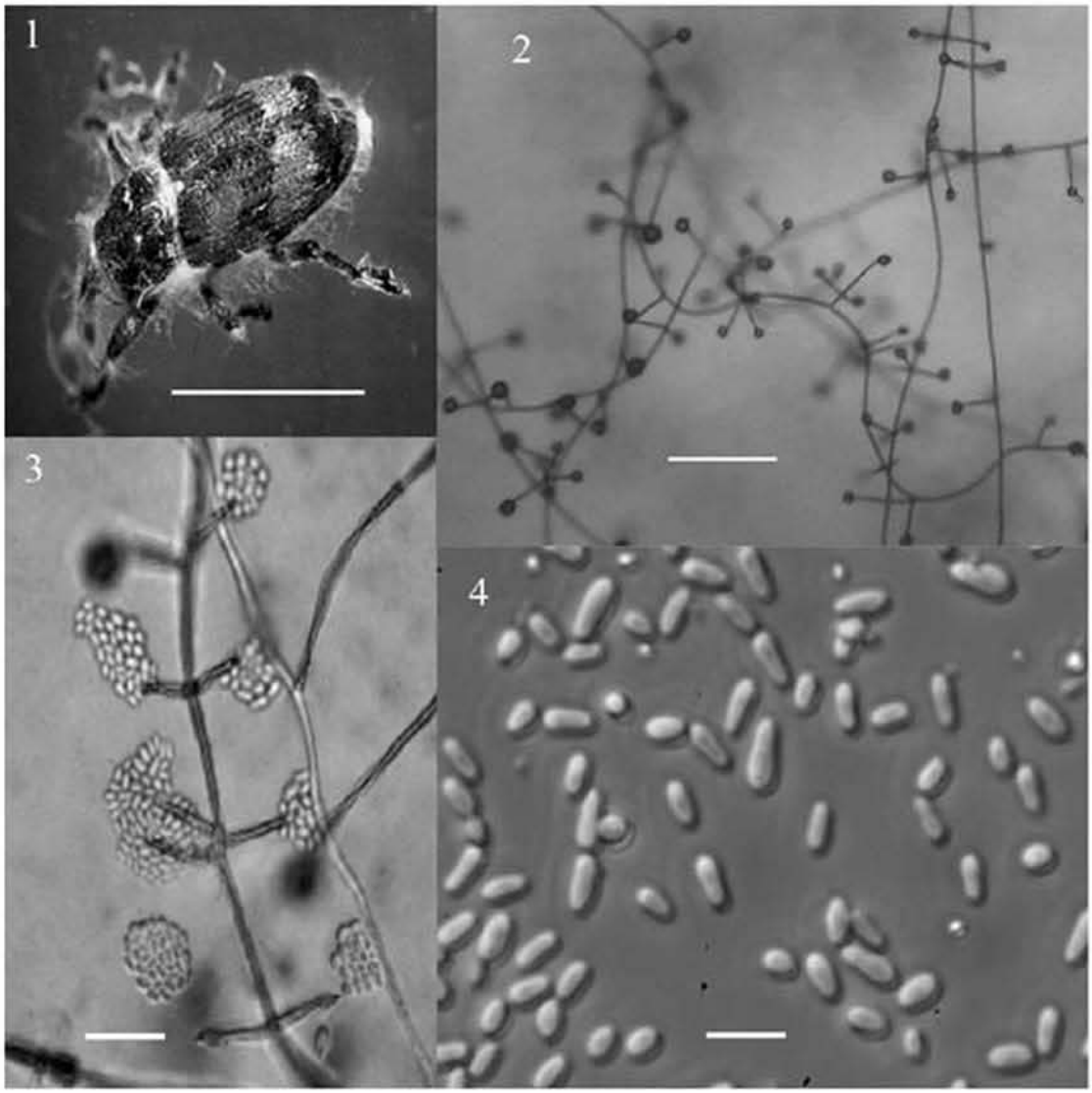
***Lecanicillium pissodis*** Kope & I. Leal, **sp. nov.** **FIGURES 1-4**

*Coloniae altae, albae, reverso cremeo vel brunnescente, ad 6–7 mm diam. post 10 dies in agaro PDA dicto. Phialides e hyphis prostratus oriundae, singulae vel 3 verticillatae, gradatim in apicem angustatae, 16–(18–28)–38µm x 1–2µm. Conidia in capitulis globosis cohaerentia, ovoidea vel cylindracea, 4–9.2µm x 1.6–2.4µm, magnitudine et forma inaequalia. Crystalla octaedrica copiosa.*

*Species ex Pissodes strobi ad Picea sitchensis in British Columbia Canada*

***Etymology:*** the specific epithet refers to the genus of the insect host.

Colony growth at 24°C on potato-dextrose agar (Oxoid, PDA) reaches 6–7mm in diameter after 10 days. Colony white, raised, abundant aerial mycelium, reverse cream to pale yellow. Phialides on prostrate hyphae, most as single, some double at right angles and in some instances a verticillate arrangement of up to 3 phialides per node or at the terminal end of hyphae, measuring 16–(18–28)–38µm x 1–2µm and tapered over their length. Conidia, up to and more than 50 formed in globose droplets at the tip of phialides, hyaline, single-celled, cylindrical to oval, to cylindrical with an attenuate end, very variable in size and shape, 4–9.2 x 1.6–2.4µm. Teleomorph unknown. Crystals present in agar medium, octahedral. Growth temperature optimum 20–25°C, with some growth occurring at 5°C and no growth at 30°C.




Figures 1-4. *Lecanicillium pissodis*. 1. Infected adult of *P. strobi*. 2. Prostrate hyphae, phialides with globose droplets holding conidia. 3. Dispersed droplets. 4. Varied shapes and sizes of conidia. Scale: 1 = 5 mm; 2 = 80 µm; 3 = 20 µm; 4 = 10 µm.

**Strains examined:**

DAVFP 29230, ex *Pissodes strobi* adult, Canada, 2003, H.H.Kope; DAVFP 29231, ex *Pissodes strobi* adult, Canada, 2003, H.H.Kope; DAVFP 29232, ex *Pissodes strobi* adult, Canada, 2003, H.H.Kope, **ex-type**.

**Holotype:**

DAVFP 29232, isolated from the cadaver of an adult *P. strobi* in BC, Canada, deposited in the Forest Pathology Herbarium, Pacific Forestry Centre, Victoria, BC, Canada. A living ex-type culture CBS 118231 (DAVFP 29232) is deposited in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. This isolate is also preserved at the Fungal Culture Collection of Plant Pests & Diseases Research Institute (Ministry of Agriculture), Tehran, Iran, under IRAN 945 C.

**PCR and RFLP:**

RFLP patterns of the ITS region, β-tubulin and mitochondrial DNA were compared to other *Lecanicillium* species. No differences in the ITS-RFLP pattern were seen between the three *L. pissodis* isolates and other *Lecanicillium* species. However, clear differences in the RFLP patterns of both the β-tubulin gene and mitochondrial DNA indicated that *L. pissodis* was distinct from other *Lecanicillium* species (Table 2). The β-tubulin gene RFLP-PCR patterns shown in Table 2 for *L. attenuatum* (CBS 170.76) and the three isolates of *L. pissodis* were based on β-tubulin sequencing analysis.

**Phylogenetic analysis and sequence alignment:**

The alignment of ITS sequences containing the ITS-1-5.8S-ITS region of 12 ITS sequences of other *Lecanicillium* species placed the three isolates of *L. pissodis* in a branch with *L. attenuatum* (Figure 5), which has a high bootstrap value in the NJ tree, and the BLAST search gave 100% identity. Accession numbers for the ITS region of *L. pissodis* 17, 18 and 19 are DQ364668, DQ364669, and DQ364670, respectively.

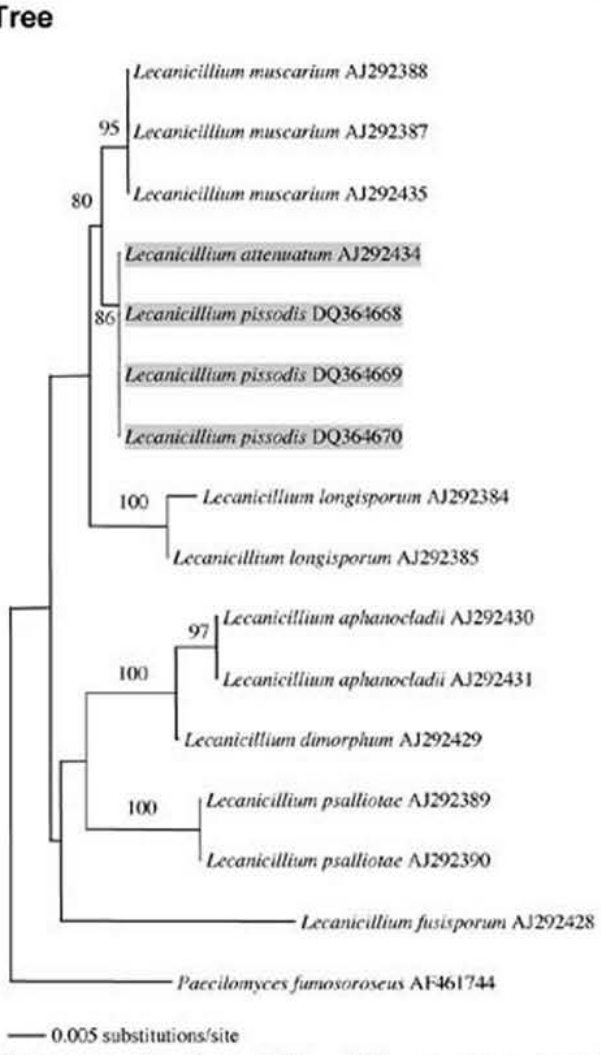



Figure 5. Neighbour joining tree for *Lecanicillium* ITS sequences rooted with *Paecilomyces fumosoroseus* showing the relationship between *L. attenuatum* and *L. pissodis*, and the position of this clade (shaded area) among other *Lecanicillium* species. Numbers on the branches are bootstrap values for major groupings in consensus trees.

Table 2. RFLP-PCR patterns of some taxa of *Lecanicillium* species*

| Taxa and accession code | RFLPs of ITS region | | | RFLPs of the ß–tubulin gene | | | | RFLPs of mt DNA |
|---|---|---|---|---|---|---|---|---|
| | *Hae* III | *Hinf* I | *Msp* I | *Alu* I | *Cfo* I | *Hae* III | *Hinf* I | *Hae* III |
| *L. attenuatum* (CBS 170.76) | 300, 160, 90, 70 | 300, 140, 100, 90 | 290, 140, 110, 60 | 444, 87 | 267, 194, 70 | 353, 178 | 360, 180 | 6.2, 5, 4.4, 3 |
| *L. muscarium** (IMI 068689) | 300, 160, 90, 70 | 300, 140, 100, 90 | 290, 140, 110, 60 | 450, 90 | 280, 200 | 360, 180 | 540 | 6.5, 4.4, 3 |
| *L. longisporum** (IMI 021167) | 300, 160, 90, 70 | 300, 140, 100, 90 | 290, 140, 110, 60 | 450, 90 | 280, 200 | 400, 140 | 540 | 6.8, 4, 3.6, 3.5, 3, 2.6 |
| *L. pissodis* 17 (DAVFP 29230) | 300, 160, 90, 70 | 300, 140, 100, 90 | 290, 140, 110, 60 | 444, 87 | 267, 194, 70 | 353, 178 | 225, 181, 119, 6 | 6.5, 4.2, 3.8, 2.8, 2.3 |
| *L. pissodis* 18 (DAVFP 29231) | 300, 160, 90, 70 | 300, 140, 100, 90 | 290, 140, 110, 60 | 444, 87 | 267, 194, 70 | 353, 178 | 225, 181, 119, 6 | 6.5, 4.2, 3.8, 2.8, 2.3 |
| *L. pissodis* 19 (DAVFP 29232) | 300, 160, 90, 70 | 300, 140, 100, 90 | 290, 140, 110, 60 | 444, 87 | 267, 194, 70 | 353, 178 | 225, 181, 119, 6 | 6.5, 4.2, 3.8, 2.8, 2.3 |

*The shaded area represents the RFLP-PCR fragments for *L. pissodis* in the ß-tubulin gene and mitochondrial DNA
RFLP patterns taken from Zare and Gams (2001)

Sequence alignment of the 3' end of the β-tubulin genes of *L. attenuatum* (CBS 170.76) and *L. pissodis* using primers Bt1a/Bt1b shows only a few sequence differences (Figure 6A). These sequences were deposited at EMBL as DQ364671 for *L. attenuatum* (CBS 170.76) and DQ364672, DQ364673 and DQ364674 for *L. pissodis* 17, 18, and 19, respectively. However, sequence alignment of the 5' end of the β-tubulin gene with primers Bt2E/Bt12 indicated major differences of the exon and intron regions between these two species (Figure 6B). The accession numbers for this part of the β-tubulin gene of *L. attenuatum* (CBS 170.76) is DQ364675, and for *L. pissodis* isolates 17, 18, and 19 are DQ364676, DQ364677, and DQ364678, respectively.

```
A                 1 2 2 2 2 3 3
                  8 0 1 5 8 0 4
                  7 1 6 2 8 9 2
L.attenuatum      T A A C T C T
L.pissodis -17    G G G T C T C
L.pissodis -18    G G G T C T C
L.pissodis -19    G G G T C T C
```

```
B                 0  0
                  1  2
                  8  5
L.attenuatum      T  GTAGCTTTTTAAGGCCCTACCCTGTACGAAATTAGATAGTGGTTCTGAGTATG
L.pissodis -17    C  GTGGTTTCCGACATTCGACTCCGAGACAAGAATTGGTGGATAGCGGTCCTGAC
L.pissodis -18    C  GTGGTTTCCGACATTCGACTCCGAGACAAGAATTGGTGGATAGCGGTCCTGAC
L.pissodis -19    C  GTGGTTTCCGACATTCGACTCCGAGACAAGAATTGGTGGATAGCGGTCCTGAC
```

```
                                  1111111111111222 2
                                  0011334455789001 2
                                  1806140967854568 2
L.attenuatum      TTTTGATAG-------- CGCCTTTTAGGATGTT GTTTGTTTTACGCACAAACAG
L.pissodis -17    TGTGCTTTTCTCGATAG TATTCCCCTCCGGACC GTTTGTTCTACCCGCGCAACG
L.pissodis -18    TGTGCTTTTCTCGATAG TATTCCCCTCCGGACC GTTTGTTCTACCCGCGCAACG
L.pissodis -19    TGTGCTTTTCTCGATAG TATTCCCCTCCGGACC GTTTGTTCTACCCGCGCAACG
```

```
L.attenuatum      CGCGCCTGTCGCGTCGCTAGCAATGTGGTGGATATTAACCATAATTCTCGTACAG
L.pissodis -17    CGTTGCTAGGCTTCGCTGTGGGTACTGACCGCGATTTTGCTCTAG----------
L.pissodis -18    CGTTGCTAGGCTTCGCTGTGGGTACTGACCGCGATTTTGCTCTAG----------
L.pissodis -19    CGTTGCTAGGCTTCGCTGTGGGTACTGACCGCGATTTTGCTCTAG----------
```

```
                  3333334444444444555555555555566666666666677777
                  4688990124555779011233567889904444567789002 2
                  8347065737039168134847517261492568465871280 6
L.attenuatum      TATAATTGCAATTACCCAAGTTCCCTTCAGCCATACCCGATCAT
L.pissodis -17    CTCGTCCTAGTCCGATTTGGCCCTTACCTATTGCCTTTCGCTGG
L.pissodis -18    CTCGTCCTAGTCCGTTTGGCCCTTACCGTATTGCCTTTCGCTGG
L.pissodis -19    CTCGTCCTAGTCCGTTTGGCCCTTACCGTATTGCCTTTCGCTGG
```

Figure 6. Distribution of β-tubulin sequence differences between *L. attenuatum* and *L. pissodis*. A. 3' end of β-tubulin gene amplified by primers Bt1a and Bt2a. B. 5' end of β-tubulin gene amplified by primers BT2E and BT12. The position of the polymorphism sites in the aligned sequence matrices is written vertically above columns. Bold-typed characters indicate the introns.

## Discussion

*Lecanicillium pissodis* is characteristic of the genus *Lecanicillium* with phialides borne on prostrate hyphae either singly or as multiples arranged in verticillate whorls at nodes, in pairs, or singly. The conidia formed in globose droplets at the end of phialides are single-celled and vary in shape from cylindrical to ovoid or oval.

Morphologically *L. pissodis* is distinct, but closest to *L. attenuatum*. It differs from *L. attenuatum* having a slower growth rate, longer phialides, a greater variability in conidial size and the production of droplets at the end of phialides containing up to 50 conidia. *L. pissodis* differs from *L. lecanii* having a slower growth rate, longer phialides, fewer phialides per whorl, and larger conidia. It also differs from *L. muscarium* and *L. longisporum* by size and conidial shape, respectively and having a slower growth rate than both species.

*Lecanicillium pissodis* differs in its RFLP banding patterns for both mitochondrial DNA and the β-tubulin gene when compared with other described *Lecanicillium* species. The isolates of *L. pissodis* had ITS sequences identical to that of *L. attenuatum*. The ITS region generally exhibits a high degree of variation between species, and it appears more highly conserved within species. Although, this ribosomal region can be very useful for determining relationships between fungal genera and species (Bruns et al. 1992), it might not allow for the differentiation of closely related taxa (Hermosa et al. 2000, Harrington et al. 2000, Jacobs et al. 2001). To address this, Kim et al. (2004) have suggested that sequences for the β–tubulin gene would be more useful. The sequence differences within the β–tubulin gene reported here separated *L. pissodis* from *L. attenuatum*. This result, coupled with the distinct morphological characteristics, provide strong support for describing *L. pissodis* as a new species.

The genus *Lecanicillium* contains entomogenous fungi with a cosmopolitan distribution occurring on a wide range of insect hosts. *L. muscarium* has been previously collected in British Columbia, Canada (CBS 113450 and 118576) and Kope et al (2000) demonstrated, after fulfilling Koch's postulates, that *L. muscarium* is an entomopathogen of *P. strobi*. The newly described isolate of *L. pissodis*, currently found only in British Columbia, is also pathogenic to *P. strobi* (H. Kope unpublished, fulfilling Koch's postulates). The occurrence and efficacy of *Lecanicillium* in the natural habitat of *P. strobi* would have an effect on weevil populations, making this entomopathogenic fungus a component to be considered in control strategies developed for *P. strobi*.

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# Two new species of *Hymenochaetaceae* from eastern China

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**Abstract**—*Coltricia tsugicola* and *Fomitiporia torreyae* (Basidiomycota, *Hymenochaetaceae*) are described as new from Fujian Province, eastern China. *Coltricia tsugicola* is characterized by small, stipitate and pendent basidiocarps, large and irregular pores and oblong ellipsoid basidiospores; its host tree is *Tsuga chinensis*. *Fomitiporia torreyae* has perennial, resupinate basidiocarps, small basidiospores, no hymenial setae, and grows on *Torreya grandis*.

**Key words**—polypore, wood-rotting fungi, taxonomy

## Introduction

During the study of poroid *Aphyllophorales* from Wuyi Mts. in eastern China, some novel wood-inhabiting fungi were found. Two such specimens were collected on *Tsuga chinensis*: fungi with small, stipitate and pendant basidiocarps, a monomitic hyphal structure without clamp connections, and oblong-ellipsoid, yellowish, smooth, and thick-walled basidiospores. These features fit well with *Coltricia* Gray, but with none of the existing taxa of the genus. Another specimen was collected on living *Torreya grandis*, a gymnosperm tree endemic to eastern China. This collection is a resupinate basidiocarp, a species with dimitic hyphal structure and simple septate generative hyphae. Its basidiospores are subglobose, hyaline, thick-walled, dextrinoid and cyanophilous. These characters indicate *Fomitiporia* Murrill (the *Phellinus robustus* complex), but no suitable species name is available for it. Here we describe these species as new in *Coltricia* and *Fomitiporia*.

## Materials and methods

The studied specimens are deposited at the Herbarium of the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Microscopic features, measurements and drawings were made from slide preparations stained with Cotton Blue and Melzer's reagent. The microscopic procedure fellows Dai (1999). Spores were measured from sections cut from the tubes. KOH = 5% potassium hydroxide, CB = Cotton Blue, CB+ = cyanophilous, CB– = acyanophilous, IKI– = both inamyloid and indextrinoid. In presenting the variation in the size of the spores, 5% of measurements were excluded from each end of the range, and are given in parentheses. In the text the following abbreviations are used: L = mean spore length (arithmetic mean of all spores), W = mean spore width (arithmetic mean of all spores), Q = variation in the L/W ratios between the specimens studied (quotient of the mean spore length and the mean spore width of each specimen), n = number of spores measured from given number of specimens.

## Descriptions

***Coltricia tsugicola*** Y.C. Dai & B.K. Cui, **sp. nov.** Fig.1

*Carpophorum annuum, stipitatum. Facies pororum auratum vel brunneum; pori rotundi vel sinuolati, 1–2 per mm. Systema hypharum monomiticum, hyphae generatoriae septatae, efibulatae, hyphae contexti 4–10 µm in diam. Sporae aureae, oblongo-ellipsoideae vel ellipsoideae, crassitunicatae, IKI–, CB(+), 8.5–11.9 × 5.6–6.9 µm.*

*Type.* — **China.** Fujian Prov., Wuyishan County, Wuyishan Nature Reserve, alt. 1700 m, on rotten wood of *Tsuga chinensis*, 21.X.2005 *Dai 7303* (holotype in IFP, isotype in H).

*Etymology.* — *Tsugicola* (Lat.): living on *Tsuga*.

*Fruitbody.* — Basidiocarps annual, centrally stipitate and pendent, solitary or gregarious, soft corky and without odour or taste when fresh, consistency soft corky to fragile and light in weight when dry. Pilei more or less circular or infundibuliform when fresh, becoming shrunken and irregular upon drying, up to 1 cm in diam., 5 mm thick at centre. Pileal surface yellowish to deep reddish brown when fresh, becoming cinnamon or yellowish brown to rust brown upon drying, azonate, velutinate or smooth; margin thin, obtuse, curving down when dry. Pore surface yellowish when fresh, becoming yellowish brown to rust brown upon drying; pores angular or sometimes sinuous to irregular, 1–2 per mm; dissepiments thin, entire. Context cinnamon to rust brown, coriaceous, up to 1 mm thick. Tubes yellowish brown, slightly paler than context, soft corky to slightly brittle when dry, up to 3 mm long. Stipe dark yellowish brown, corky, velutinate, up to 5 mm long, 1 mm in diam.

*Hyphal structure.* — Hyphal system monomitic; all septa without clamp connections; tissue darkening but otherwise unchanged in KOH.

*Context.* — Contextual hyphae pale yellowish to golden brown, thin- to fairly thick-walled with a wide lumen, occasionally branched, with frequent simple septa, fairly straight, loosely interwoven, IKI–, CB–, 4–10 µm in diam.; hyphae in the stipe golden yellowish to golden brownish, fairly thick-walled with a wide lumen, occasionally branched, with frequent simple septa, more or less straight, loosely interwoven, IKI–, CB–, 5–9 µm in diam.

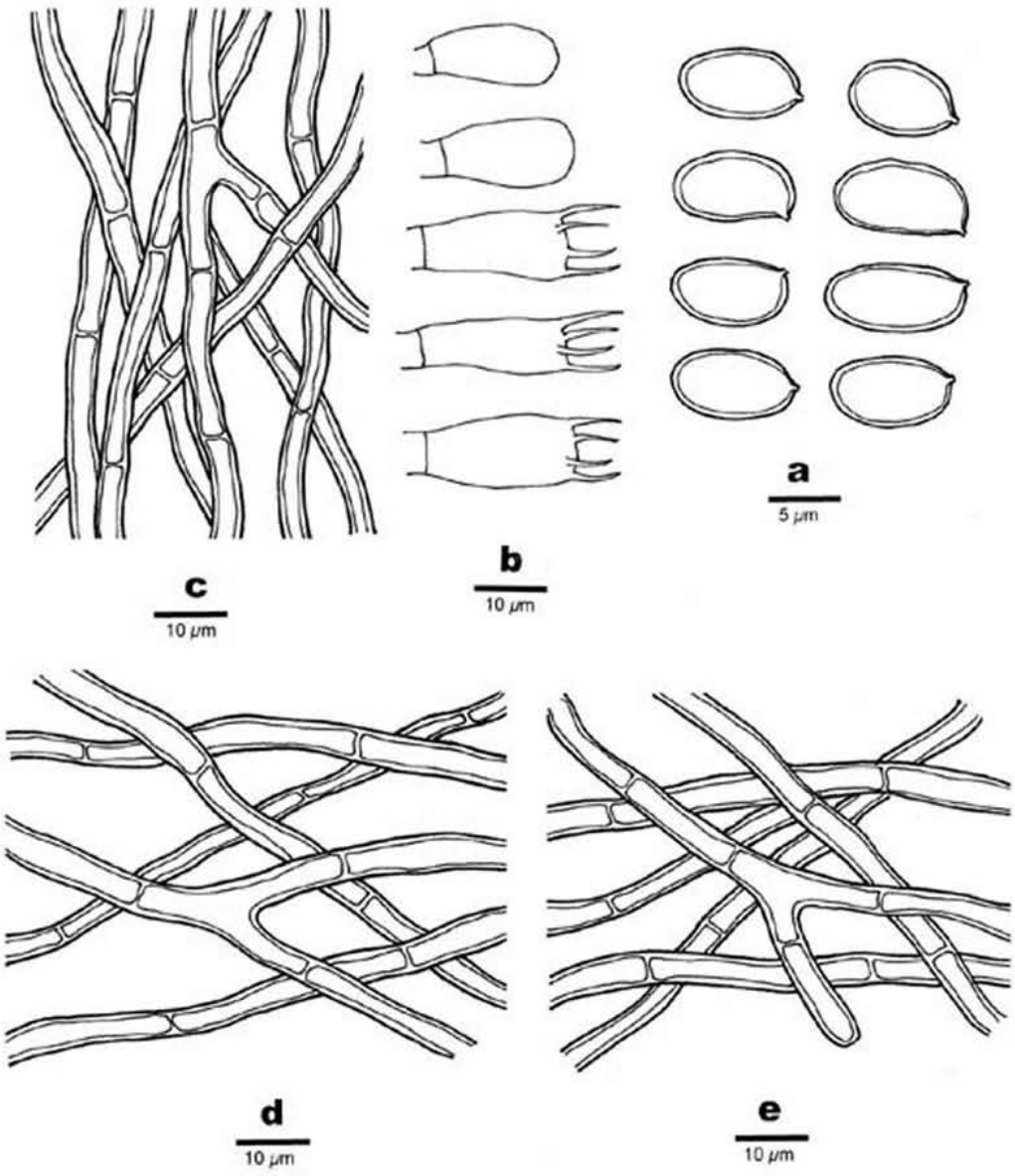


Fig. 1. Microscopic structures of *Coltricia tsugicola* (drawn from the holotype IFP *Dai 7303*). — a: Basidiospores. —b: Basidia and basidioles. —c: Hyphae from tube trama. —d: Hyphae from context. —e: Hyphae from stipe.

*Tubes.* — Tramal hyphae hyaline to pale yellowish or golden brownish, thin- to slightly thick-walled with a distinct lumen, frequently branched and simple septate, loosely interwoven, IKI–, CB– or very weakly CB+, 4–8 µm in diam. Basidia broadly clavate, with four sterigmata and a simple septum at the base, 14.6–23.8 × 5–8.4 µm; basidioles in shape similar to basidia, but smaller.

*Spores.* — Basidiospores ellipsoid or more often oblong-ellipsoid, yellowish, thick-walled, smooth, IKI–, CB– or weakly CB+ when juvenile, (8.2–)8.5–11.9(–13.2) × (5.2–)5.6–6.9(–7) µm, L = 9.62 µm, W = 6.31 µm, Q = 1.51–1.54 (*n*=60/2).

*Additional specimen (paratype) examined.* — China, Fujian Prov., Wuyishan County, Wuyishan Nature Reserve, alt. 1700 m, on base of living *Tsuga chinensis*, 21.X.2005 *Dai 7336*.

*Remarks.* — The species is characterized by small, stipitate and pendant basidiocarps, large and irregular pores, and oblong-ellipsoid basidiospores. *Coltricia tsugicola* was found in a *Tsuga chinensis* forest in the Wuyishan Nature Reserve. Forests of *Tsuga* are restricted to altitudes from 1100 to 1800 meters, close to the timberline which is at ca. 1900 m. Climatically the coniferous forests between these limits vary from warm temperate to almost boreal.

*Coltricia focicola* (Berk. & M.A. Curtis) Murrill resembles *C. tsugicola* by having oblong-ellipsoid and pale yellowish basidiospores, (7.5–)8–9.5(–10) × (3.8–)4–4.8(–5) μm, $L$ = 8.55 μm, W = 4.34 μm, Q = 1.97. However it has larger basidiocarps, lobed or incised at margin, lacerate to dentate dissepiments, and its spores are narrower than in *C. tsugicola*. In addition, *Coltricia focicola* grows on the ground, and it is evidently not a wood-rotting fungus (Ryvarden and Gilbertson 1993).

*Coltricia duportii* (Pat.) Ryvarden has laterally stipitate basidiocarps and ellipsoid basidiospores, and it may be related to *C. tsugicola*. Its growth habit is not pendent, and fruitbodies are larger, 2.5 cm in diam. and 1 cm thick; besides, its basidiospores are ellipsoid, rusty brown, 8–10 × 6–7 μm. It occurs on deciduous trees in tropical French Guyana and Brazil (Ryvarden 2004).

Basidiospores of the new species are similar to those of *Coltricia montagnei* (Fr.) Murrill which, however, has larger basidiocarps (up to 12 cm in diam. and 1–2 cm thick), poroid to concentrically lamellate hymenophore, and slightly dextrinoid basidiospores (Núñez and Ryvarden 2000, Ryvarden and Gilbertson 1993).

The minute, stipitate and pendant basidiocarps make *Coltricia tsugicola* confusingly similar to *Coltriciella dependens* (Berk. & M.A. Curtis) Murrill, but spores of *C. dependens* are finely verrucose, and it occurs in the tropics (Gilbertson and Ryvarden 1986, Ryvarden and Johansen 1980).

### *Fomitiporia torreyae* Y.C. Dai & B.K. Cui, sp. nov. Fig.2

*Carpophorum perenne, resupinatum, contextum atroumbrinum vel brunneum. Facies pororum ravide umbrina vel umbrina; pori rotundi vel sinuolati, 4–6 per mm. Systema hypharum dimiticum, hyphae generatoriae septatae, efibulatae. Sporae subglobosae vel globosae, crassitunicatae, dextrinoidae, CB+, 5–5.9 × 4.4–5.3 μm.*

*Type.* — China. Fujian Prov., Wuyishan County, Wuyishan Nature Reserve, alt. 800 m, on living *Torreya grandis*, 21.X.2005 *Dai 7320* (holotype in IFP, isotype in H).

*Etymology.* — *Torreyae* (Lat.): referring to the host tree genus.

*Fruitbody.* — Basidiocarps perennial, resupinate, inseparable, woody hard when fresh, without odour or taste, consistency woody hard and light in weight when dry, up to 20 cm or more in longest dimension, 10 cm wide, ca. 8 mm thick at centre; margin more or less receding, up to 2 mm wide, pale brown. Pore surface greyish brown when fresh, becoming pale brown to rust brown upon drying, shining, slightly cracked with age; pores circular to sinuous at oblique surface, 4–6 per mm; dissepiments thin, entire. Subiculum umber brown, woody hard, very thin, up to 0.5 mm thick. Tubes yellowish brown to rust brown, hard corky, up to 7 mm long, distinctly stratified.

*Hyphal structure.* — Hyphal system dimitic; all septa without clamp connections; skeletal hyphae IKI–, CB–; tissue darkening but otherwise unchanged in KOH.

*Subiculum.* — Tissue dominated by skeletal hyphae; generative hyphae hyaline to pale yellowish, thin- to fairly thick-walled, occasionally branched and frequently simple septate, 2–3.2 μm in diam.; skeletal hyphae golden brown to rust brown, thick-walled with a narrow to wide lumen, unbranched, interwoven, more or less agglutinated, 2.3–4.5 μm in diam.

*Tubes.* — Tramal hyphae dominated by skeletal hyphae; generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched and frequently simple septate, 1.8–3 μm in diam.; skeletal hyphae golden brown to rust brown, thick-walled with a narrow to wide lumen, unbranched, interwoven, slightly agglutinated, 2–4.2 μm in diam. Subhymenium indistinct. Hymenial setae absent, setae extremely rare in subiculum, subulate to ventricose, dark brown, thick-walled, 10–16 × 6–7 μm; cystidioles frequent, subulate, sharp-pointed or obtuse at apex, hyaline, thin-walled, 14–19.7 × 2.8–4 μm; basidia barrel-shaped to subglobose, with four sterigmata and a simple septum at the base, 8–13 × 6.8–9 μm; basidioles in shape similar to basidia, but slightly smaller; big rhomboid crystals abundant in hymenia and trama.

*Spores.* — Basidiospores subglobose to globose, hyaline, thick-walled, smooth, dextrinoid in Melzer's reagent, strongly CB+, (4.5–)5–5.9(–6) × (4–)4.4–5.3(–5.9) μm, L = 5.46 μm, W = 4.90 μm, Q = 1.11 (*n*=60/1).

*Remarks.* — The perennial growth habit, resupinate basidiocarp, lack of setae, presence of subulate cystidioles, small basidiospores, and growth on *Torreya grandis* characterize this species. We could not find hymenial setae in the type, and only a few setae were found in the subiculum and subhymenium. Even there they are extremely rare: 4 setae were observed from 5 microscope preparations. *Fomitiporia torreyae* is the third resupinate species in the genus in China, the other two being *F. punctata* (P. Karst.) Murrill and *F. bannaensis* Y.C. Dai (Dai 1999, Dai *et al.* 2001).

Four species in *Fomitiporia*—*F. aethiopica* Decock et al., *F. mediterranea* M. Fisch., *F. punctata* and *F. tabaquilio* (Urcelay et al.) Decock & Robledo—have resupinate basidiocarps, and lack hymenial setae (Decock *et al.* 2005). These species have distinctly larger basidiospores than *F. torreyae*, the length being more than 6 μm.

*Fomitiporia tenuis* Decock et al. was recently described from Africa (Decock *et al.* 2005). Its basidiospores are very similar to those of *Fomitiporia torreyae*. However, the former has a thinner fruitbody (up to 2 mm thick), smaller pores (10–11 per mm), and hymenial setae.

*Fomitiporia bannaensis* is found in China too but it is distinguished from *F. torreyae* by smaller pores (8–10 per mm) and abundant hymenial setae. Furthermore, *F. bannaensis* is a subtropical species, growing on angiosperm trees (Dai *et al.* 2001).

Besides the above-mentioned taxa, the remaining species of *Fomitiporia*, *F. sonorae* (Gilb.) Y.C. Dai, *F. pseudopunctata* (A. David et al.) Fiasson, *F. sublaevigata* (Cleland & Rodway) Y.C. Dai, have principally resupinate fruitbodies, but all have hymenial setae and grow on angiosperm trees. *Fomitiporia sonorae* has resupinate to reflexed basidiocarps, and its setae are long (35–55 × 5–8 μm, Gilbertson 1979, Valenzuela &

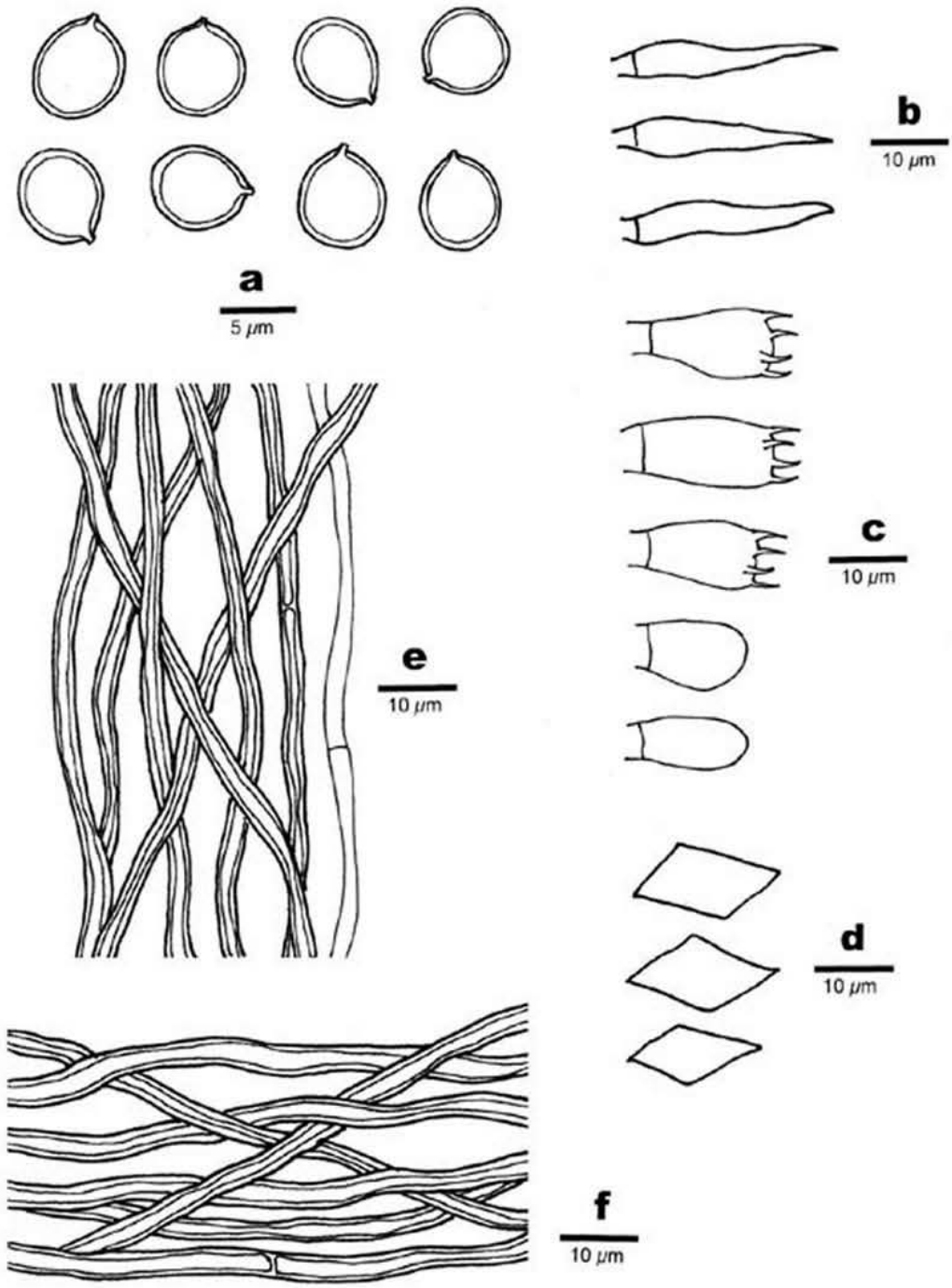


Fig. 2. Microscopic structures of *Fomitiporia torreyae* (drawn from the holotype IFP *Dai 7320*). —a: Basidiospores. —b: Cystidioles. —c: Basidia and basidioles. —d: Rhomboid crystals. —e: Hyphae from tube trama. —f: Hyphae from subiculum.

Chacón-Jiménez 1991). *Fomitiporia pseudopunctata* and *F. sublaevigata* have bigger spores (6.5–7.5 × 5.5–7 µm in *F. pseudopunctata*, 6.5–7 × 5–6 µm in *F. sublaevigata*, Decock et al. 2005).

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# Some entomogenous fungi from Wuyishan and Zhangjiajie Nature Reserves 2. Three new species of the genus *Hirsutella*[*]

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**Abstract**—Three new entomogenous fungi of the genus *Hirsutella*, *H. zhangjiajiensis*, *H. hunanensis* and *H. crinita*, collected from Wuyishan (Wuyi Mountains) and Zhangjiajie Nature Reserves, are described and illustrated. Some problems of the identification of these fungi are briefly discussed.

**Key Words**—hyphomycete, taxonomy, morphology

## Introduction

*Hirsutella* Pat. is one of the most abundant and important entomogenous fungi. In 1990, we reviewed the development, taxonomic characteristics of the genus, and proposed a key to 62 taxa. A few doubtful species were discussed briefly (Liang 1990a, b). Seifert & Boulay (2004) suggested that *Hirsutella* consists of 65 species. From our study, we refer 90 species around the world to the genus of *Hirsutella*.

The genus *Hirsutella* plays an important role in the natural control of pest insects (Evans 1974, 1982). *H. gigantea* Petch can infect many larvae and pupae of Lepidoptera in the Kuankuoshui Preserve in Guizhou, China (Liang 1991a). Besides this fungus, *H. rhossiliensis* Minter & B. L. Brady, discovered in the early 1980's, has a stronger lethiferous effect on many plant parasitic nematodes, such as *Ditylenchus dipsaci*, *Meloidogyne incognita*, *Aphelenchoides fragariae* and *Criconemella xenoplax* (Jaffee et al. 1982; Cayrol & Frankowski 1986; Cayrol et al. 1986).

It is known that many species in the genus *Hirsutella* are anamorphs of *Cordyceps* Fr. For example, an anamorph of the famous Chinese traditional medicine *C. sinensis* (Berk.) Sacc. is *H. sinensis* (Liu et al., 1989). Some valuable bioactive compounds have been recently discovered from members of *Hirsutella*. A protein toxic to insects, hirsutellin

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A (HtA), has been purified from the fungal mite pathogen, *H. thompsonii* F. E. Fisher (Mazet & Vey, 1995). HtA is considered to be one of many ribonuclease inactivator proteins (RIPs) (Maimala et al. 2002). Vongvanich et al.(2002) found hirsutellide A from a new species of *Hirsutella*, an interesting antimycobacterial cyclohexadepsipeptide. The synthesis of its key precursor has been studied (Xu et al. 2005).

From 1989 to 2001, Chinese mycologists reported seven new species of *Hirsutella* (Table 1). The present paper describes three additional new species of *Hirsutella* and their taxonomic position within the genus.

Table 1. Previously reported species of *Hirsutella* in China

| Fungal Name | References |
|---|---|
| *H. leizhouensis* H.M. Fang & S.M. Tan | Fang & Tan 1992 |
| *H. changbeishanensis* Z.Q. Liang | Liang 1991b |
| *H. polycolluta* Z.Q. Liang | Liang 1991b |
| *H. yunnanensis* Z.Q. Liang & A.Y. Liu | Liu et al., 1993 |
| *H. yunnanensis* var. *tenuisynnemata* Z.Q. Liang & A.Y. Liu | Liang et al.,1997 |
| *H. sinensis* X.J. Liu et al. | Liu et al., 1989 |
| *H. longissima* C.R. Li et al. | Li et al., 1999 |
| *H. huangshanensis* C.R. Li et al. | Li et al., 2005 |

## Materials and Methods

All collected specimens were routinely oven-dried at 40C to prevent growth of contaminant fungi. For examination by light microscopy, slide preparations were made of a snippet of outer layer tissue from the delaminated synnemata by mounting in lactophenol and cotton blue.

## Description of new species

***Hirsutella zhangjiajiensis*** Z.Q. Liang & A.Y. Liu **sp. nov.** **Figs. 1-1, 4**

*Stromatibus solitariis vel binariis, cylindraceis, simplicibus, 100 × 2 mm. Stipite et capitulo brunneo vel ochraceo. Phialides e ascosporis exorentibus, subulatiae graciles, 30-52 × 2-4.5 µm, vel basi inflata ellipsoida, 4.5 × 3µm. Conidia lanceolata or leviter curvata, 4.5-7.5 (-10) × 1.5-2.5 µm, mucigeri, lemoniformes, 10 × 4 µm. Holotypus: GZDXIFR98-7131.*

Stroma single or 2, cylindrical, 100 × 2 mm, not ramified, leathery. Stipe and fertile part brown to snuff-colored. Conidiogenous structure deriving from the microcycle conidiation of ascospores from *Cordyceps zhangjiajiensis*. Phialides slender awl-shaped, 30-52 × 2-4.5 µm or inflated ellipsoidal at basal portion, 4.5 × 3 µm. Conidia lanceolate or the shape of an orange segment, 4.5-7.5(10) × 1.5-2.5 µm, embedded in a mucous sheath, limoniform, 10 × 4 µm.

Specimen studied: GZDXIFR98-7131 was collected from Zhangjiajie Nature Reserve, Hunan Province by LIANG Zongqi, LIU Aiying et al. in VII 1998.

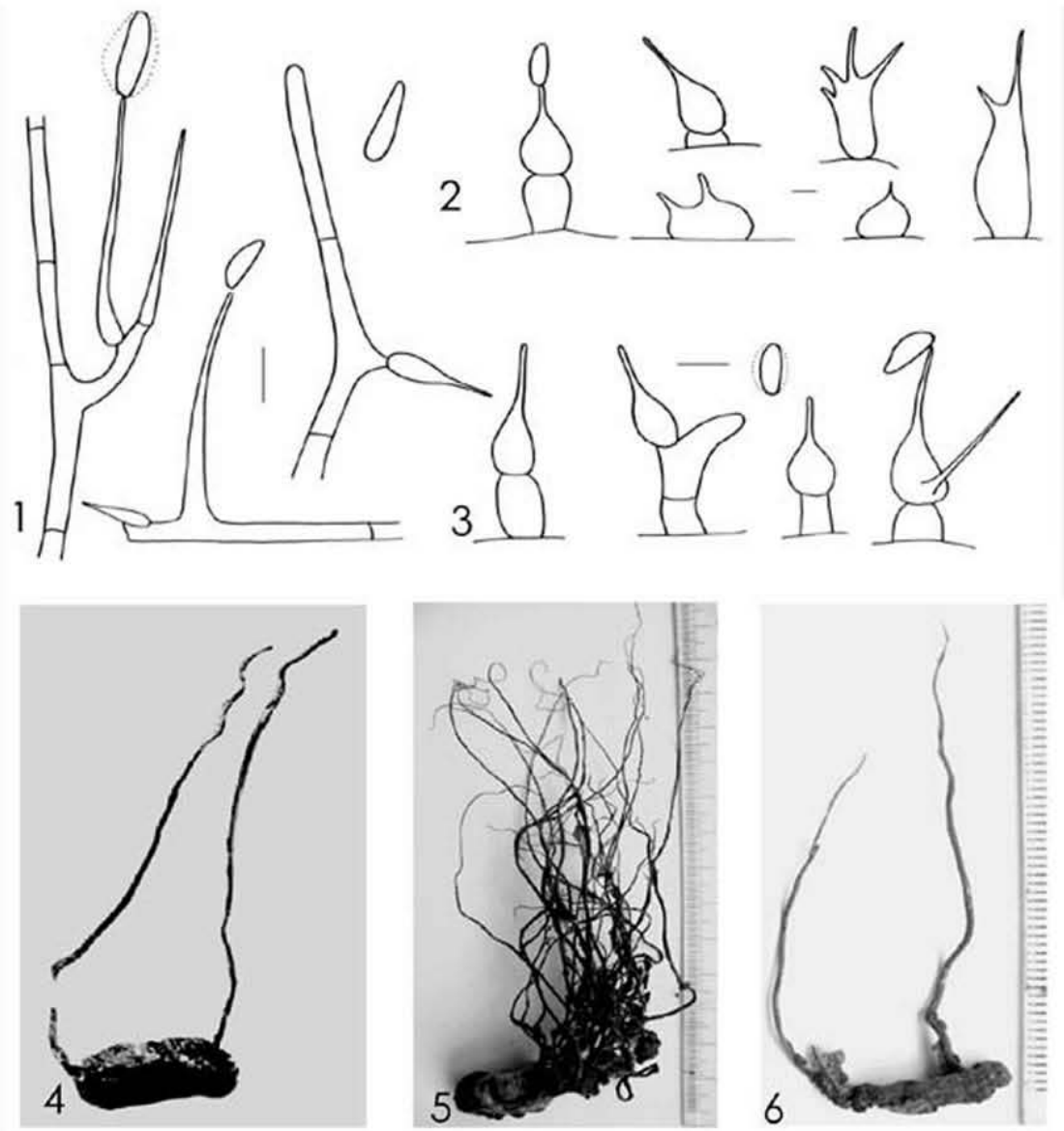


Fig. 1 Synnemata and conidiogenous structures of three species of *Hirsutella*. 1-1, 4: Conidiogenous structures and synnemata of *H. zhangjiajiensis*. 1-2, 5: Conidiogenous structures and synnemata of *H. crinta*. 1-3, 6: Conidiogenous structures and synnemata of *H. hunanensis*

Bars1, 2 =10μm, Bar3 =5μm

Habitat: Pupa of Lepidoptera.
Teleomorph: *Cordyceps zhangjiajiensis* Z.Q. Liang & A.Y. Liu (Liang et al. 2002).
Distribution—Zhangjiajie Nature Reserve: Hunan Province, China.

***Hirsutella hunanensis*** Z.Q. Liang **sp. nov.** **Figs. 1-3, 6**

*Synnemata erecta, filiformes, simplices, 5-55 mm longa, 1mm crassa, flexiles vel ligneae, nigro-brunneae. Phialides 7.5-9 × 4.5-5 μm, e basi inflate cylindrica vel pyriformibus in cillum 0.5-0.8 μm crassum angustatae, prolificis. Conidia ellipsoidea vel leviter curvata, 6 × 1.5-2 μm, mucigeri, ellipsoideis, 6 × 4.5 μm. Holotypus: GZDXIFR98-7132*

Synnemata filiform, erect, unbranched, 5-55 mm long, 1mm wide, flexible to ligneous, dark brown, arising from between head and thorax parts of host insect. Phialides solitary

or crowded along synnemata, mostly with cylindrical or pyriform inflated basal portion, 7.5-9 × 4.5-5 μm and with one or 2 slender, thin necks, 7.5-11 × 0.5-0.8 μm. Conidia long ellipsoidal to the shape of an orange segment, 6 × 1.5-2 μm; embedded in a mucous sheath, ellipsoidal, 6 × 4.5 μm. Teleomorph not observed.

Specimen studied: GZDXIFR98-7132. was collected from Zhangjiajie Nature Reserve, Hunan Province by LIANG Zongqi, LIU Aiying et al. in VII 1998.

Habitat: A larva of Lepidoptera.
Distribution: Zhangjiajie Nature Reserve—Hunan Province, China.

***Hirsutella crinita*** Z.Q. Liang **sp. nov.** Figs. 1-2, 5

*Synnemata erecta, filiformes vel cylindrica, simplices, 170 mm longa, 1-1.5 mm crassa, flexiles vel ligneae, nigro-brunneae, caespitosa. Phialides 4.5-6(-10) × 3.5-4.5 μm, e basi inflata globosa vel cylindrical in collum 0.3-0.5 μm crassum angustatae, prolificis. Conidia rara, clavata angusta, 6-9 × 1-1.5 μm, exmuca. Holotypus: GZDXIFR98-5231.*

Synnemata slender cylindrical or filiform, unbranched, flexible to ligneous, dark brown, caespitose, arising from the head and thorax parts of host insect, very long (up to 170 mm), slightly swollen in the basal region, 1.0-1.5mm wide, in middle part to upside, 0.5-1.0 mm wide. Phialides somewhat scattered, forming a loose hymenium, arising as lateral cells from the outer hyphae of the synnemata, hemispheric to globose or cylindrical, inflated at basal portion, 4.5-6(-10) × 3.5-4.5 μm, abruptly narrowing into a short thin neck, 1.5-4 μm long and 0.3-0.5 μm wide, always proliferating 2-4, sometimes forming inflated, sterile cylindrical hyphae, 15 × 4.5-5 μm. Conidia infrequent, narrowly anisomerous obclavate, 6-9 × 1-1.5 μm, absence of distinct mucus layer. Teleomorph not observed.

Specimen studied: GZDXIFR98-5231 was collected from Wuyishan, Hujian Province by LIANG Zongqi, LIU Aiying et al. in V 1998.

Habitat: A larva of Lepidoptera. 80-100 × 50-80 mm.
Distribution: Wuyishan, Fujian Province, China.

## Discussion

Evans & Samson (1982a,b, 1984) reported several species with phialides of two types (Table 2): A-phialides, which are lateral and compacted in a layer below the head, and B-phialides, which are terminal, compact, and awl-shaped. The new species, *H. zhangjiajiensis*, forms both A- and B-phialides during the ascosporic microcycle conidiation (Fig. 1-1) and produces B-phialides that are solitary and compacted. Species in the genus *Hirsutella* that have awl-shaped phialides more than 40 μm long include *H. aphidis*, *H. stilbelliformis* var. *stilbelliformis*, *H. stilbelliformis* var. *dolichoderi*, *H. sporodochialis*, *H. darwinii*, *H. guignardii*, *H. sinensis*, and *H. zhangjiajiensis*. Among them, species that have both A- and B-phialides and some phialides greater than 40μm long are *H. sporodochialis*, *H. stilbelliformis* var. *stilbelliformis*, *H. stilbelliformis* var. *dolichoderi* and *H. zhangjiajiensis* (Table 2). Possession of rough-walled hyphae and echinate phialides separates the three former species from *H. zhangjiajiensis*.

In the genus *Hirsutella*, some species that have proliferating phialides are *H. besseyi*, *H. guyana*, *H. versicola*, *H. verticillioides*, and *H. yunnanensis* var. *tenuisynnemata*. All

Table 2. A comparison of three new *Hirsutella* with related species

| Species | Phialides | | | Conidia (μm) | Reference |
|---|---|---|---|---|---|
| | Shape | Length in μm (max.) | Type | | |
| *H. aphidis* Petch | Awl | >40 | B | Cymbiform 9×1.5-2.5 | Petch 1942 |
| *H. besseyi* F.E. Fisher | Awl or cylindrical | >40 | B | Ellipsoid or limoniform 4.1-8.3×2.5-5.8 | Minter & Brady 1980 |
| **H. crinita* | Base inflated: subglobose | >10 | A | Narrowly clavate, 6-9 × 1-1.5 | This work |
| *H. darwinii* H.C. Evans & Samson | Awl | >40 | B | Fusiform 4.5-11.5×1.5-2 | Evans & Samson 1982a |
| *H. guignardii* (Maheu) Samson et al. | Awl | >40 | B | Ellipsoid or fusiform 7-13×4-6 | Samson et al. 1984 |
| *H. guyana* Minter & B.L. Brady | Base inflated: cylindric | >40 | A | Ellipsoid or an orange segment 8-12×3-7 | Minter & Brady 1980 |
| **H. hunanensis* | Base inflated: cylindric to pyriform | >10 | A | Ellipsoid or an orange segment 6 × 1.5-2 | This work |
| *H. minnesotensis* S.Y. Chen et al. | Base inflated: subglobose | >10 | A | Globose 4-6 (in length) | Chen et al. 2000 |
| *H. necatrix* Minter et al. | Base inflated: subglobose & awl | >5<br>>10 | A & B | Ovoid or ellipsoid 3-4×2.5-3<br>Globose, -3.5 | Minter et al. 1983 |
| *H. sinensis* Liu et al., | Awl | >40 | B | Reniform or ellipsoid 5.4.-14×3.2-5.4 | Liu et al., 1989 |
| *H. sporodochialis* H.C. Evans & Samson | Base inflated: flask-shaped or awl | >80 | A to B | Fusiform 10-27×3.5-4 | Evans & Samson 1984 |
| *H. stilbelliformis* H.C. Evans & Samson | Base inflated: ellipsoid echinate & awl | >10<br>>100 | A & B | Clavate 7-9×1.5-2.5<br>Ovoid 8-12×4-5 | Evans & Samson, 1982b |
| *H. stilbelliformis* var. *dolichoderi* H.C. Evans & Samson | Base inflated: ellipsoid echinate & awl | >40<br>>100 | A & B | Cylindrical to ovoid 6.5-9.5×3.5-4.5 | Evans & Samson, 1982b |
| *H. versicola* Petch | Base inflated: ellipsoid | >40 | A | Fusiform 4-7×1 | Minter & Brady 1980 |
| *H. verticillioides* Charles | Base inflated: cylindrical | >40 | A | Ellipsoid or an orange segment 6-8×3-5 | Minter & Brady 1980 |
| *H. yunnanensis* var. *tenuisynnemata* Z.Q. Liang & A.Y. Liu | Base inflated: ellipsoid or subglobose | >10 | A | Cylindrical or obclavate 4.5-7.2×1.5-1.8 | Liang et al.,1997 |
| **H. zhangjiajiensis* | Awl & base inflated: ellipsoid | >40 | A & B | Lanceolate or an orange segment, 4.5-10× 1.5-2.5 | This work |

of these species are similar to the new species *H. hunanensis* in fusiform, ellipsoidal and orange conidia. *H. hunanensis* can be distinguished from the above-mentioned species by phialides less than 10μm length and smaller conidia, around 6 × 1.5-2 μm.

Minter et al. (1983) described a new species, *H. necatrix*, that also has phialides with a subglobose or globose swollen base portion. Possession of ovate or ellipsoidal separates this fungus from *H. crinita*, which has narrowly clavate conidia (Table 2). The new species *H. crinita* is also closely related to *H. minnesotensis* and *H. yunnanensis* var. *tenuisynnemata* with similar phialides containing a subglobose basal portion. Globose conidia typically found in *H. minnesotensis* distinguish it from *H. crinita*. *H. yunnanensis* var. *tenuisynnemata* has fusiform or ellipsoidal conidia and can produce yellow caespitose synnemata.

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# *Paecilomyces parvosporus*, a new species with its relatives from Yunnan Province, China

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**Abstract**—Four *Paecilomyces* isolates were isolated from soils and insects infected in Yunnan Province, China. Amongst them, GZDX-IFR-A10.1 has been distinguished as a new species by its unique morphological characters including a light yellow colony on Czapek, small subglobose or globose conidia and long and thin phialidic necks as well as a phylogenetic analysis based on the nucleotide sequences of the ITS region. Meanwhile the other three isolates GZDX-IFR-A35.1, GZDX-IFR-466.6 and GZDX-IFR-468.2 were identified as *P. lilacinus*, *P. tenuipes* and *P. cateniannulatus*.

**Key Words**—taxonomy, morphology, fungi, rDNA sequence

## Introduction

China is rich in fungal species in *Paecilomyces*. Recently we have found several new species in *Paecilomyces* (Han et al. 2005a, b, Liang et al. 2005). In 2004, while carrying out a project of the National Natural Science Foundation of China (NSFC) to investigate the *Paecilomyces* species in Kunming, Nujiang, Lancangjiang Xishuangbanna (Tropical rain forest), Tengchong (Geothermal National Geopark) and Lijiang areas in Yunnan province, we collected many *Paecilomyces* samples from the soils and infected insects. Amongst them, quite a few species in *Paecilomyces* known in China, including *P. cateniannulatus*, *P. tenuipes*, *P. farinosus*, *P. cateniobliquus* (Liang 1981) and *P. amoeneroseus* (Liu & Liang 2003) were collected and isolated again. Moreover, a few *Paecilomyces* members with mesophilic and thermotolerant nature from soils have also been obtained (Liang et al. 2005).

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Table 1 DNA sequences used in the phylogenetic studies from Genbank

| Name | GenBank No. | Name | GenBank No. |
|---|---|---|---|
| *P. amoeneroseus* | AY624168 | *P. inflatus* | AB099943 |
| *P. carneus* | AY624170 | *P. javanicus* | AY624186 |
| *P. cateniannulatus* | AY624172 | *P. lilacinus* | AY624188 |
| *P. cateniobliquus* | AY624173 | *P. marquandii* | AY624193 |
| *P. cicadae* | AB085887 | *P. niphetodes* | AY624192 |
| *P. coleopterorum* | AY624177 | *P. penicillatus* | AY624194 |
| *P. farinosus* | AY624181 | *P. sinensis* | AJ243771 |
| *P. fumosoroseus* | AY624182 | *P. tenuipes* | AY491997 |
| *P. ghanensis* | AY624185 | *P. variotii* | AY247956 |
| *P. gunnii* | AY489650 | *P. viridis* | AY624197 |

The genus *Paecilomyces* Bainier shares many identical or similar morphological characters with its related genera including *Acremonium* Link, *Gabarnaudia* Samson & W. Gams; *Penicillium* Link, *Mariannaea* G. Arnaud, *Septofusidium* W. Gams and *Verticillium* Nees. It is difficult to distinguish them using only morphological characters, particularly species intermediate between *Penicillium* and *Paecilomyces*. Recently molecular biological techniques, including phylogenetic rDNA sequence analyses, have become efficient ways to identify the intermediate and doubtful species. In this paper, we used both morphology and phylogenetic analysis of rDNA sequences to identify a few *Paecilomyces* species isolated from soil samples and infected insects of Yunnan Province. The results showed that strain GZDX-IFR-A10.1 is a new species named here as *P. parvosporus*. The strain GZDX-IFR-A35.1 was identified as *P. lilacinus*. While the strains GZDX-IFR-466.6 and GZDX-IFR-468.10 were identified as *P. tenuipes* and *P. cateniannulatus* respectively.

## Materials and Methods

### Tested strains

Four tested strains, GZDX-IFR-A10.1, GZDX-IFR-A35.1, GZDX-IFR-466.1 and GZDX-IFR-468.10 were used in this paper.

### Collection and strain isolation

GZDX-IFR-A10.1 and GZDX-IFR-A35.1 were isolated from soil samples of Dali City, Yunnan Province. Two grams of soil were added to a flask containing 20ml sterilized water and glass beads. The soil suspension was shaken for about 10 min and then diluted to concentrations of $10^{-3}$-$10^{-1}$. 1 ml of soil suspension ($10^{-3}$) was mixed with Martin's medium in a sterilized 9cm diameter Petri dish and incubated at 25C for 5 days. Colonies with *Paecilomyces* conidiogenous structures were transplanted to Martin's slant for purification. GZDX-IFR-466.1 and GZDX-IFR-468.10 were isolated from infected insects from Xishan Park, Yunnan Province. A small amount of spore powder from insect surface was transplanted to Martin's medium by sterilized inoculation needle, and then incubated at about 25C for 3-5 days. The isolates were purified when their conidiogenous structures became the same as those on insects.

Fig. 1 Conidiogenous structures of *Paecilomyces parvosporus*. Bar = 10 μm.

**Strain identification**

The strains under study were transplanted on Czapek agar, potato dextrose agar (PDA), and Sabouraud agar according to Brown and Smith (1957) and Samson (1974). After incubation at 25°C for 7 days, the strains were identified based on colony features, conidiogenous structure and biological property. Type strains of GZDX-IFR-A10.1 and its holotypes GZDX-A10.1, the dried plate cultures on Czapek agar, were deposited in the Institute of Fungus Resources, Guizhou University, Guizhou province, China.

**Reagent**

Taq enzyme and dNTP were bought from Shanghai Sangon, Agarose Gel DNA Purification kit ver 2.0 from TAKARA Company®

**DNA extraction**

The four strains from Yunnan Province used for the molecular identification were incubated on Czapek agar and potato dextrose agar. Subsequently, the fresh sporulating cultures were used for DNA extraction according to Tigano-Milani et al. (1995), and then DNA were kept at -20°C.

**PCR amplification and determination of ITS rDNA sequences**

Polymerase chain reaction (PCR) amplification was performed according to the manufacturer's instructions, 50μL reaction system: 10× reaction buffer 5 μL, dNTP 1μL,

primer ITS4 1μL, ITS5 1μL, Pfu buffer 0.5 μl, 2μL of template DNA and $ddH_2O$ 39.5 μL. The amplification program: a first step of 94°C for 5 min; then 35 cycles consisted of 94°C for 40 s, 49°C for 40 s, and 72°C for 1 min; and a final step of 72°C for 10 min. To amplify ITS1-5.8S- ITS2 rDNA, the following primers were used: ITS4 (5'-TCCTCCGCTTATTGATATGC- 3') and ITS5 (5'-GGTGAGAG ATT TCTGTGC -3'). PCR products were purified using Agarose Gel DNA Purification kit ver 2.0 according to its procedure (TAKARA Company), and purified DNA samples were sent to Beijing Sunbiotech Co. Ltd. for sequencing. The rDNA sequences of ITS1-5.8s- ITS2 regions of the four strains from Yunnan Province were submitted to GenBank (DQ187951-DQ187954).

**Sequence alignment and phylogenetic analysis**

Sequences were aligned by Clustal X, and adjusted to maximize homology. Then the phylogenetic tree was constructed using Neighbour-Joining (NJ) and Maximum Parsimony (MP) methods in PAUP 4.0b10. Confidence values for individual branches were determined by bootstrap analysis (1000 replications).

## Results

### Taxonomy

***Paecilomyces parvosporus*** Y.F. Han & Z.Q. Liang **sp. nov.** **Fig.1**

*Coloniae in agaro Czapekii ad 20 mm diam, 14 diebus 25°C, primitus albae, tum leteus, olivaceus in margo; reversio citrinae. Hyphae hyalina, levia, 0.6-3.6 μm crassa. Conidiophora ramosa, 11.2-38.1 μm crassa, phialidibus 2 vel 5 terminatis. Phialides 4.8-9.6 × 1.2-1.8 μm, e basi inflata, cylindrical vel clavata in collum tenerum; Conidia continua, hyalina, levia, subglobulosa, vel globulosa, 1.2-2.4 μm. Chlamydospora ellipsoidea, 4.0-10.1 × 2.7-6.7 μm.*

*Typus: GZDX-IFR-A10.1 et cultara GZDX A10.1, isolatus e soli, Dali city, provincia Yunnan, China, VI, 2004, Y.F. Han & Z.Q. Liang. In Guizhou Univ, conservatur.*

Colony on Czapek agar, attaining a diameter of 20mm within 14 days at 25°C, floccose, at first white, then light yellow, green in margin, appearing evident radial furrow, round; reverse yellow. Vegetative hyphae hyaline, smooth-walled, septate, 0.6-3.6 μm in diameter. Conidiophores hyaline, smooth-walled, septate, branched, about 11.2-38.1 μm, bearing whorls containing 2-5 phialides. Phialides 4.8-9.6 × 1.2-1.8 μm, consisting of a cylindrical or claval basal portion, tapering into a distinct neck. Conidia in dry divergent chains, one-celled, smooth, subglobose to globose, 1.2-2.4 μm in diameter; Chlamydospores ellipsoidal, 4.0-10.1 × 2.7-6.7 μm.

Material examined—GZDX-IFR-A10.1 was isolated by Y.F. Han & Z.Q. Liang from vegetable soils collected in Dali, Yunnan Province, China, June, 2004.

Distribution—Yunnan Province, China.

### DNA sequencing

To analyze the ITS rDNA sequence of the tested strains and *Paecilomyces* species from NCBI (Table 1), PAUP was applied to construct the phylogenetic tree by the methods of

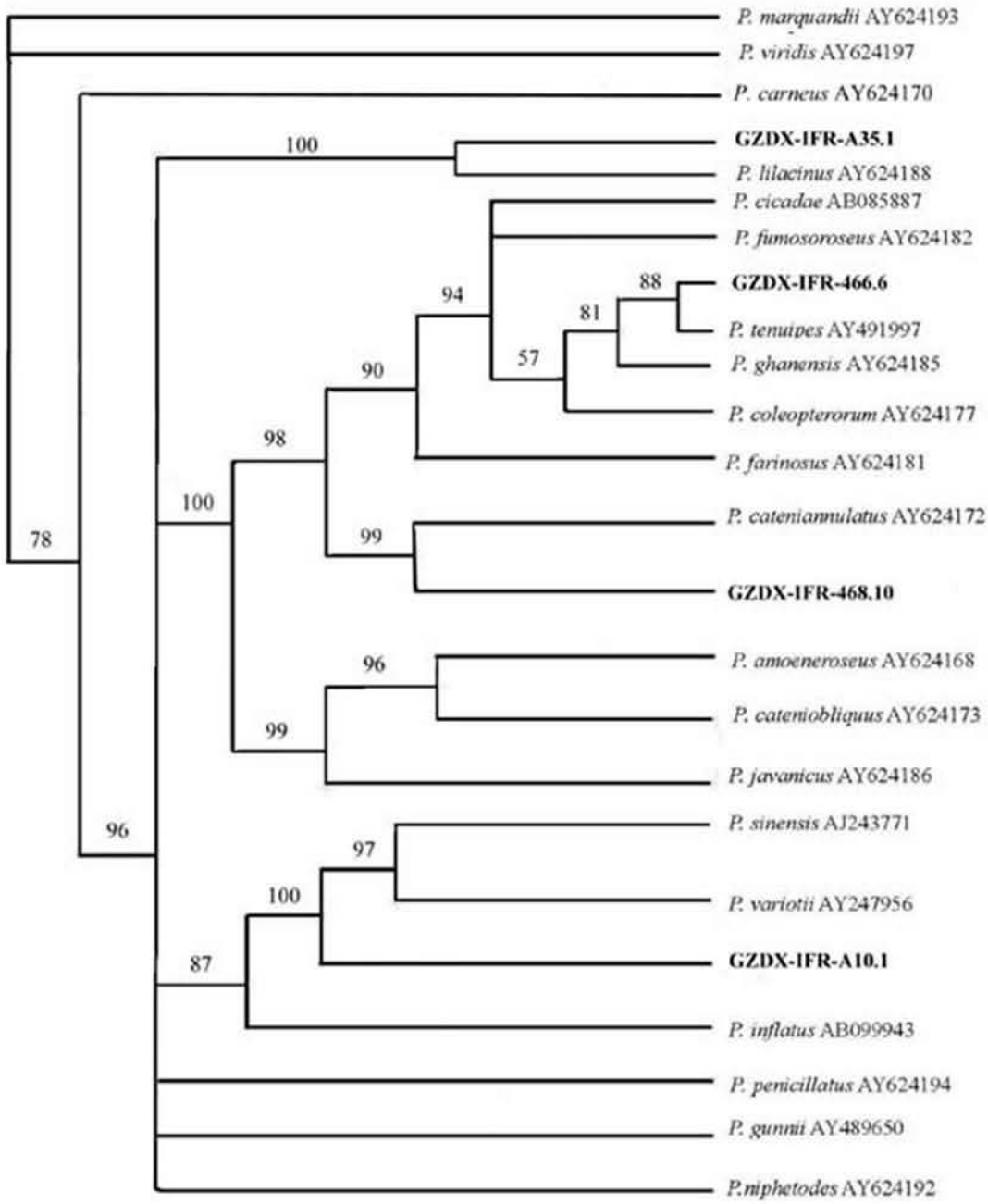


Fig.2 Phylogenetic tree based on aligned internal transcribed spacer sequences (ITS1-5.8S-ITS2 rDNA) of selected *Paecilomyces* species, showing the sister group relationship between a few species and the new species. (CI = 0.7284, HI = 0.2716, RI = 0.7577, RC = 0.5520)

NJ and MP. The results from NJ and MP methods are almost identical, so only the tree constructed by MP was shown in the paper. The phylogenetic tree inferred from the ITS sequence data (Fig.2) shows that the two strains GZDX-IFR-466.6 and GZDX-IFR-468.10 are clustered well with *P. tenuipes* AY491997 and *P. cateniannulatus* AY624172 from NCBI in two subclades (88% and 99% bootstrap supports), respectively. GZDX-IFR- A35.1 strain is very similar to *P. lilacinus* in the morphological characters, its ITS sequence is clustered very well with *P. lilacinus* AY624188 in the same subclade (100% bootstrap support), so GZDX-IFR- A35.1 strain is identified as *P. lilacinus*. The strain GZDX-IFR-A10.1 forms a separate branch close to *P. sinensis* AJ243771, *P. variotii* AY247956 and *P. inflatus* AB099943 is therefore identified as a new species.

## Discussion

In the genus *Paecilomyces*, based on the morphological characters, the accepted species with cylindrical phialides base and globose conidia are as follows: *P. carneus*. *P. marquandi*, *P. stipitatus* and *P. vinaceus*. The conidia of *P. carneus* are echinate. The colony of *P. marquandii* is red, but the colony of the new species is yellow to green. The phialides of *P. stipitatus* have septate and thin long stalks, and its colony is white. *P. vinaceus* is characterized by its vinaceous colony reverse. The new species can be distinguished from the above-mentioned species by its greenish colony, much smaller conidia and its very thin, long phialide necks.

The phylogenetic tree showed that the newly named *P. parvosporus* (GZDX- IFR-A10.1), *P. inflatus*, *P. variotii*, and *P. sinensis* were grouped in the same subclade. But *P. inflatus* has monophialides and fusiform conidia (Samson 1974). *P. sinensis* can produce obvious synnemata on the media and its conidia are fusiform to ellipsoidal, 4.5-7.5 × 1.2-3.8 μm (Q.T. Chen 1984). The 3.2-5 × 2-4 μm conidia and fulvous colony of *P. variotii* (Brown & Smith 1957, Samson 1974) distinguish it from the new species.

In addition, *P. parvosporus* was highly homologous with *Penicillium daleae* when blasted in NCBI Genbank. According to Raper & Thom (1949), *Penicillium daleae* is mainly characterized by coarsely roughened conidia that are ellipsoidal to subglobose, 3.0-3.5 × 2.5-3.0 μm. *P. parvosporus*, however, has smooth conidia that are globlose to subglobose, 1.2-2.4 μm. It can therefore be obviously distinguished from *Penicillium daleae*.

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# Notes on *Otidea* from Xinjiang, China

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**Abstract** — Newly collected specimens of *Otidea* from Xinjiang, China were examined. Five taxa of the genus are identified. Among them, *Otidea crassa* is described as a new species and *O. onotica* var. *brevispora* is treated a new variety.

**Key words** — taxonomy, new taxa

## Introduction

Species of the genus *Otidea* (Pers.) Bonord. is widely distributed in north temperate areas. They are characterized by apothecia of medium to large size, mostly terrestrial, ear-shaped, split down to base or discoid, with some shade of whitish, yellow, brown or ochre; asci operculate, not blued by iodine; ascospores elliptical to elliptical-fusiform, biguttulate; paraphyses slender, strongly curved at apex, capitate or with notches at apical portion. According to the CABI database,[2] 71 published species names are listed under *Otidea* and apparently 17 of them should be excluded from the genus. But many of the rest are either synonyms or doubtful members of the genus. About 15 species are commonly accepted in the world (Kirk et al. 2001). Regional studies have been carried out in China, India, Japan, Lithuania, United Kingdom, the Nordic countries, North America, etc. (Kanouse 1949, Nannfeldt 1966, Otani 1969, Thind & Waraitch 1974, Harmaja 1976, Dennis 1978, Breitenbach & Kränzlin 1984, Cao et al. 1990, Korf & Zhuang 1991, Dissing 2000, Kutorga 2000). Species diversity of the genus in each region seems not very high. In China, fifteen taxa of *Otidea* were previously reported by Cao et al. (1990) from Gansu, Heilongjiang, Jilin, Shaanxi, Shanxi, Sichuan, Yunnan, Xinjiang, and Xizang. *Otidea alutacea* var. *alutacea* [2 collections] and *O. propinquata* (P. Karst.) Harmaja [a single collection] in *Picea* forests were the only records known from Xinjiang, an area in the northwest occupying more than 1/7 of the China mainland territory. In our recent field trip to Xinjiang, 20 specimens of the genus were collected from Burqin, Jimsar, and Yining. Five taxa are recognized among them. *Otidea leporina* is the most common. *Otidea crassa* is described as a new species and *Otidea onotica* var. *brevispora* is proposed as a new variety.

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[2]http://www.indexfungorum.org/Names/Names.asp

## Taxonomy

***Otidea alutacea*** (Pers.) Massee, British Fungus Flora 4: 446, 1895.

Specimens examined: CHINA. Xinjiang, Urmuqi, on the ground, 12 VIII 1985, L. Fan 10, HMAS 88262; Xinjiang, Burqin, Hemuxiang, alt. 1100 m, 5 VIII 2003, on the ground, W. Y. Zhuang & Y. Nong 4719, 4722, 4685, HMAS 83559, 83560, 83562; *ibid.*, 6 VIII 2003, on the ground, W. Y. Zhuang & Y. Nong 4744, 4739, HMAS 83561, 83563.

Notes: The fungus is characterized by the truncate to cupulate apothecia with one side split to the base and up to 5–7.5 cm wide; hymenium surface beige, light cinnamon, cinnamon brown to brown when fresh; receptacle surface usually lighter than hymenium surface, beige, light cinnamon brown, brownish yellow to brown; ascospores ellipsoid with blunt ends, 13.5–15.8 × 6–8.5μm; paraphysis apices curved to hooked. The five known collections were all found in Burqin in northeastern Xinjiang. It was reported previously from Heilongjiang, Jilin, Xinjiang (Urmuqi), Shanxi, and Xizang of the country (Cao et al. 1990).

***Otidea cochleata*** (L.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23-24: 329, 1870.

Specimens examined: CHINA. Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on duff under *Picea* sp., W. Y. Zhuang & Y. Nong 4622, 4645, 4651, HMAS 83564, 83575, 83576.

Notes: This species is characterized by the medium-sized, cupulate to truncate apothecia with light brown, brown to dark brown hymenium surface and light brown, yellowish brown to brown receptacle surface when fresh; hymenium 240–270 μm thick, asci 12.5–15 μm wide; and ellipsoid to broad-ellipsoid ascospores 15–18 × 8.6–11.7 μm. The club-shaped cells of the outer excipulum arise from small angular cells which arise by a gradual transition from the textura intricata of the medullary excipulum.

*Otidea cochleata* was first reported from Milin, Xizang in southwestern China based on one collection (Cao et al. 1990). *Otidea umbrina* (Pers.) Bres. is a later synonym according to the CABI database.

As noted by Breitenbach & Kränzlin (1984), *Otidea cochleata* is similar to *O. alutacea* in apothecial shape and is distinguished from the latter in somewhat smaller and darker fruitbodies, as well as the larger ascospores.

*O. cochleata* might also be confused with *O. propinquata* (P. Karst.) Harmaja occurring in a similar niche. The latter possesses larger ascospores usually over 18 μm in length (Velenovský 1934, Kanouse 1949, Harmaja 1976). The single specimen from Xinjiang previously recorded as *O. propinquata* (Cao et al. 1990) has not been located. Another collection identified at the same time as *O. propinquata* from Shanxi turns out to be *O. cochleata*.

***Otidea crassa*** W.Y. Zhuang, sp. nov. Figs. 1–3

*Ab* Otidea leporina *surperficie hymenii flavo-brunneis vel brunneis, surperficie receptaculi dilute flavo-brunneis, ascis 178–192 × 9.5–12.5 μm, ascosporis late ellipsoideus, brevibus, 10.5–12.5 × 7.4–9 μm differt.*

Etymology: The specific epithet refers to the broadly ellipsoid ascospores produced by the fungus.

Apothecia ear-shaped, short-stipitate, 1.2–2 cm wide and up to ca 3 cm high, hymenium surface yellowish brown to brown when fresh and light brown to warm brown when dry, receptacle surface paler than hymenium when fresh, pale yellowish brown to pale brown when fresh and becoming orange-brown to warm brown when dry, stipe base whitish; ectal excipulum of textura angularis, 40–90 μm thick, cells more or less isodiametric, 8–30 μm diam.; medullary excipulum of textura intricata, 240–330 μm thick, hyphae hyaline, 3–12.5 μm wide; subhymenium 30–50 μm thick; hymenium 165–215 μm thick; asci 8-spored, subcylindrical, J– in Melzer's reagent, ca 178–192 × 9.5–12.5 μm wide; ascospores broadly ellipsoid with rounded or blunt ends, biguttulate, smooth, uniseriate, 10.5–12.5 × 7.4–9 μm; paraphyses filiform and curved at apex, 2–2.5 μm wide at apex and ca 1.5–2 μm below.

Holotype: CHINA. Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on mossy rotten wood, W. Y. Zhuang & Y. Nong 4647, HMAS 83571. Paratype: CHINA. Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on mossy rotten wood, W. Y. Zhuang & Y. Nong 4631, HMAS 83570.

Notes: The new species is similar to *Otidea leporina* (Kanouse 1949) except for the thinner flank, yellowish brown hymenium surface, and broadly ellipsoid ascospores. When the two collections of *O. crassa* were compared with eight of *O. leporina* from Xinjiang, distinctions have been found in spore size and shape [10.5–12.5 × 7.4–9 μm vs. 11.5–13.5 × 6.4–8 μm in the Chinese collections], width of medullary excipular hyphae (3–12.5 μm vs. 2.5–5 μm wide), size of ectal excipular cells (8–30 μm in diameter vs. 6–15 μm wide), and its occurrence on rotten wood instead of soil and duff.

In addition to the morphological characteristics, our 28S nrDNA partial sequence data (unpublished) support also its separation from *O. leporina* and show close relationship to *Otidea cantharella* (Fr.) Quél., a species with bright yellow pigment in fruitbodies and composed of only one kind of tissue in excipulum, viz. outer layer of *textura prismatica* changing gradually to inner layer of *textura intricata*, which was treated in a separate genus as *Flavoscypha cantharella* (Fr.) Harmaja (Harmaja 1974).

The apothecial shape and spore size of the new species are also similar to those of *O. felina* (Pers.) Bres. but the fruitbodies are smaller (1.2–2 cm wide and up to ca 3 cm high vs. 2–3 cm wide and 3–4.5 cm high), apothecial color is yellowish brown to brown when fresh instead of light grayish yellow to light flesh colored, ascospores are wider, shorter [10.5–12.5 × 7.4–9 μm vs. 11–13 × 6–8 μm according to Saccardo 1913)] and broadly ellipsoid with blunt ends instead of ellipsoid with slightly pointed ends (Boudier 1905-1910).

***Otidea leporina*** (Batsch) Fuckel, Jahrb. Nassauischen Vereins Naturk. 23–24: 329, 1870 var. ***leporina***

Specimens examined: CHINA. Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on the ground, W. Y. Zhuang & Y. Nong 4632, 4633, 4634, 4657, 4635, 4636, 4637, HMAS 83565, 83566, 83567, 83568, 83577, 83578, 83579.

Notes: The species seems very common in Xinjiang, as well as in other areas of China, such as Heilongjiang, Hunan, Jilin, Shanxi, Sichuan, Xizang, and Yunnan as reported by Cao et al. (1990) and Zang (1996). It is characterized by the ear-shaped apothecia 2–4 cm wide, with hymenium surface cinnamon, cinnamon brown, warm brown,

brown to grayish brown when fresh and receptacle surface concolorous, dark brown or grayish yellow; ectal excipulum of textura angularis, cells 6–15 μm wide, outermost cells sometimes short club-shaped; medullary excipulum of textura intricata, hyphae stable in width, ca 2.5–5 μm wide; hymenium 165–178 μm thick; asci 8–10 μm wide; and ascospores ellipsoid, 11.5–13.5 × 6.4–8 μm; paraphyses curved, 1.5–2.5(–3) μm wide at apical portion.

HMAS 83569 (Xinjiang, Yining, Guozigou, alt. 1800 m, 11 VIII 2003, W. Y. Zhuang, Y. Nong & S. Y. Guo 4845) has smaller, ear-shaped, brown to grayish brown fruitbodies up to 1.5 × 0.8 cm; elongate-ellipsoid, longer ascospores 12.5–16 × 6–8 μm; and paraphyses straight, bent or curved at apex and 2.5–3.2 μm at the widest, and is here tentatively treated as *Otidea* cf. *leporina*.

Kanouse (1949) accepted Saccardo's (1889) separation of *Otidea leporina* var. *minor* (Rehm) Sacc. from the original variety. The Chinese collections appear to have identical morphology to *O. leporina* var. *leporina*.

***Otidea onotica*** var. ***brevispora*** W.Y. Zhuang, **var. nov.** Figs. 4–6

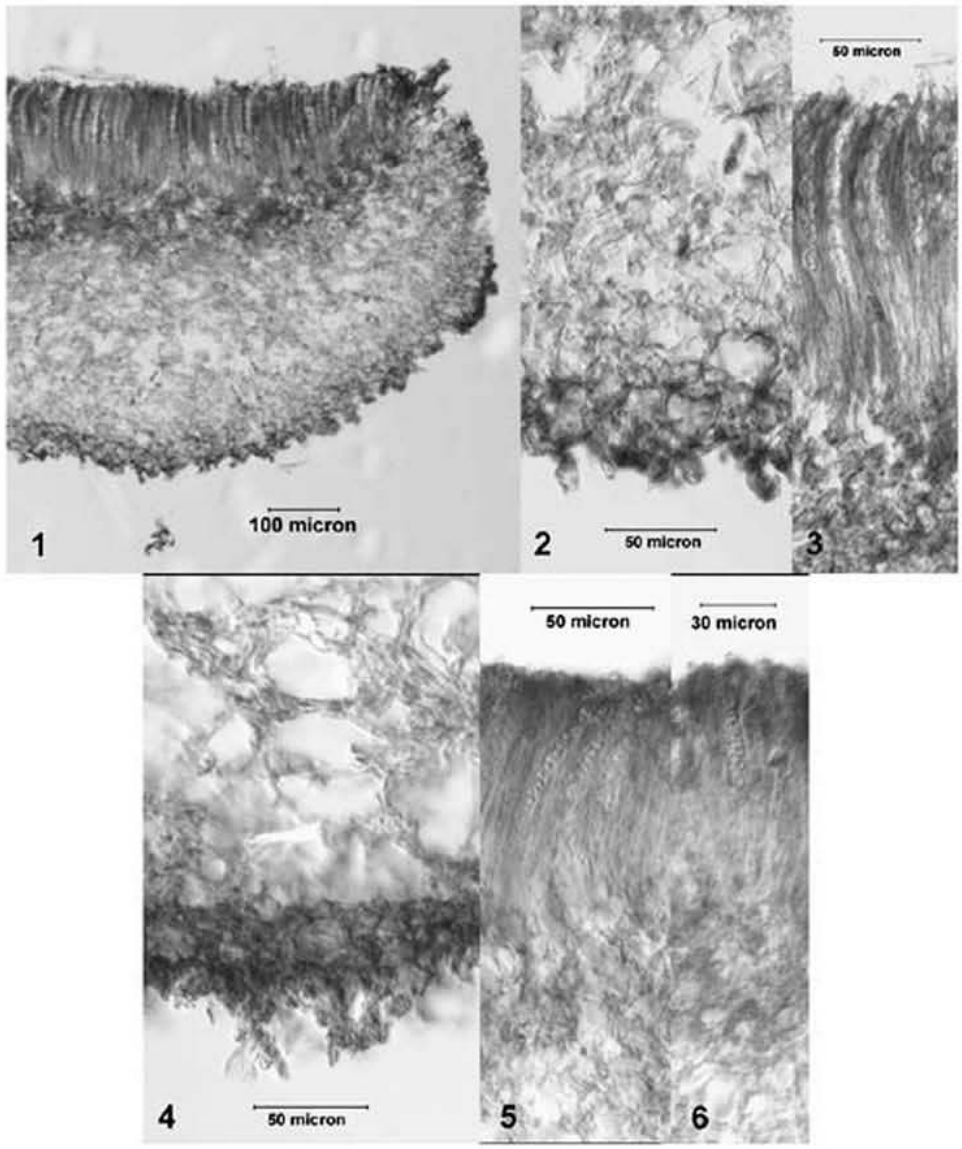
*Ab* Otidea onotica *var. onotica ascosporis brevibus, ellipsoideus vel late ellipsoideus, 8.5–10.5(–11) × 5.2–7 μm differt.*

Etymology: The specific epithet refers to the short ascospores produced by the fungus in comparison with those in *Otidea onotica* var. *onotica*.

Apothecia spoon- to ear-shaped, short-stipitate, 1–5 cm wide and 2.5–8 cm high, hymenium surface yellow to dull yellow when fresh, receptacle surface yellow or paler than hymenium when fresh, stipe base whitish; ectal excipulum of textura angularis, 50–80 μm thick, cells 13–20 × 5–12 μm and 9–26 μm in diameter if isodiametric, with the outer most cells somewhat club-shaped; medullary excipulum of textura intricata, 100–660 μm thick, hyphae hyaline, 2–8 μm wide; subhymenium 40–50(–70) μm thick; hymenium 140–180 μm thick; asci 8-spored, subcylindrical, J– in Melzer's reagent, (7–)8–9.5(–10.5) μm wide; ascospores ellipsoid to broadly ellipsoid with rounded or blunt ends, biguttulate, smooth, uniseriate, 8.5–10.5(–11) × 5.2–7 μm; paraphyses filiform and curved at apex, 2–2.8 μm wide at apex and ca 1.5 μm below.

Holotype: CHINA. Yunnan. Baoshan, alt. 1900 m, 24 VII 2003, Z. L. Yang 3854, HKAS 43003, isotype HMAS 83551. Paratypes: CHINA. Heilongjiang, Yichun, 6 IX 1989, on the ground under mixed woods, J. Z. Cao 821, HMAS 83572; Xinjiang, VIII 1994, on duff, J. Y. Wang 132, HMAS 69951 (filed as *Otidea cantharella*); Xinjiang, Jimsar, alt. 1700 m, 1 VIII 2003, on the ground, W. Y. Zhuang & Y. Nong 4655, HMAS 83573; *ibid.*, 2VIII 2003, on cone of *Picea* sp., W. Y. Zhuang & Y. Nong 4670, HMAS 83574.

Notes: The fungus is characterized by its ear-shaped, yellow apothecia and shorter ascospores 8.5–10.5(–11) × 5.2–7 μm than those of *Otidea onotica* var. *onotica* [(10–)12–13 × 5–6 (Dennis 1978), 12–14 × 6–7(–8) μm (Kanouse 1949)]. Our 28S rDNA partial sequence data (unpublished) also support its separation from *O. onotica* var. *onotica*.




Figs. 1-6. *Otidea* spp. 1-3. *Otidea crassa* (HMAS 83571): 1. Longitudinal section of apothecium. 2. Excipular structure. 3. Portion of hymenium showing asci and ascospores. 4-6. *Otidea onotica* var. *brevispora* (HMAS 83551): 4. Excipular structure. 5, 6. Portion of hymenium showing asci and ascospores.

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The author would like to thank Prof. R. P. Korf and Prof. D. H. Pfister for serving as pre-submission reviewers, Dr. Z. L. Yang for providing his own collection from Yunnan, Mr. Y. Nong for collecting jointly the specimens used in this work, Mr. C. Y. Liu for providing information about *Otidea* DNA sequences, and Ms. X. Song for making the sections.

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# Book reviews and notices

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## General

**Mycelium Running: How Mushrooms can help Save the World.** By Paul Stamets. 2005. Ten Speed Press, Box 7123, Berkeley, CA 94707, USA. Pp. x + 339, figs 360. ISBN 1 58008 579 2. Price: US $ 35.

This book by Paul Stamets, an entrepreneur and fellow fungus chauvinist, is not a systematic text, but is mentioned here as it is something that helps promote the importance of mycology in ecological processes and human settings. He draws parallels of mycelial networks with the internet, and stresses the potential of fungi in mycofiltration, mycoforestry, mycoremediation, and as mycopesticides. All this is written with a racy style and well-illustrated by colour photographs and drawings. The next two thirds of the book is devoted to the cultivation of mushrooms, and their nutritional and medicinal properties -- including many references to original sources. In summary, a great book to direct someone to who is unsure why on earth anyone should take an interest in mushrooms. A limited edition of 200 individually numbered, signed, boxed and hardbound versions is also available at US $ 195.

**Biodiversity of Fungi: Their Role in Human Life.** Edited by Sunil K. Deshmukh & Mahendra K. Rai. 2005. Science Publishers, Enfield, P. O. Box 699, NH 03784, USA. Pp. 461. ISBN 1 57808 368 0. Price: £ 48.40.

This volume comprises a hotchpotch of 18 chapters, some of which do emphasise biodiversity, others do concern their impact on the human environment, and some manage both at the same time. How some chapters, e.g. 'Fungal protoplast technology', fall under the umbrella title is not immediately obvious. The book is certainly a 'curate's egg', good in parts. Elizabeth Arnold's 'Diversity and ecology of fungal endophytes in tropical forests' was a highlight.

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[1]Books for consideration for coverage in this column should be mailed to the Book Review Editor (address above) in the first instance. Fax (+34) 91 857 3640; e-mail: myconova@terra.es.

Gunde-Cimarmans *et al.* 'Halotolerant and halophilic fungi', Deshmukh & Kushwaha 'Keratinophilic fungi on birds and their ability to decompose keratin', Wasser & Didukh's 'Mushroom polysaccharides in human health care', and Maldonades & Ibarra's 'Organic dyes from fungi and lichens' truly embraced the diversity theme. There are also useful chapters on the potential of ligninolytic basidiomycetes to degrade organopollutants, and on metal bioremediation (but why the latter needed three chapters I do not know).

There seems to be an increasing number of books that lump a set of loosely related chapters together under a catchall title, with the assumption that this provides a valuable contribution to the literature. Sadly, more often than not it does not work. Multi-author books need to provide more than the sum of the individual parts. This book is reasonably priced, but I will not be recommending it for purchase by the library.

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**Fungi in Forest Ecosystems: Systematics, Diversity, and Ecology.** Edited by Cathy L. Cripps. 2004. The New York Botanical Garden Press, 200th Street and Kazimiroff Boulevard, Bronx, New York, NY 10458-5126, USA (e-mail: nybgpress@nybg.org). Pp. xvi + 363. [Memoirs of the New York Botanical Garden Vol. 89.] ISBN 0 89327 459 3. Price: US $ 68.

This volume has been prepared as a tribute to Orson K. Miller Jr, 75 years young in 2005. Following a detailed, well-illustrated[2], and at times somewhat light-hearted life history, there are lists of theses prepared under his direction, new scientific names he has introduced, and a bibliography of his publications. The original papers contributed mainly by close friends and colleagues are placed in three groups: Systematics, Diversity, and Ecology. Those on systematics include ones on *Amanita* subgen. *Lepidella*, *Crepidotus*, *Entoloma*, and *Mycena*, between them with descriptions of 17 new species, mostly macromycetes. The diversity chapters cover different groups of fungi in areas from Papua New Guinea, through the European alps and Israel, to Oregon and the southern Appalachians. The contributions in the ecology group mainly concern ectomycorrhizas or wood-decay fungi. There are some fascinating items amongst the papers, from the relation of *Phellinus pini* to red-cockaded woodpecker cavity trees to macrolichen distribution patterns in the Montana and Yellowstone and Glacier National Parks, and an intriguing winter-fruiting helotialean anamorph (*Cadophora hiberna* sp. nov.). This is a work that all mycological libraries with a macromycete focus should endeavour to acquire, not least for its compilations of information on Orson's outstanding contributions.

**Fungi: Experimental Methods in Biology.** By Ramesh Maheshwari. 23 June 2005. CRC Press, Taylor & Francis Group, 6000 Broken Sound Parkway NW, Suite 300, Boca Raton, FL 33487-2742, USA. Pp. xii + 240. [Mycology Series Vol. 24.] ISBN 1 57444 468 9. Price: US $ 149.95, £ 85.

[2]I especially liked the photograph of Orson at the microscope under the caption "Girl Scout Leader".

While the subtitle might lead to the expectation that this was a hands-on book on methodologies, it is actually a textbook that arose from the teaching of graduate students in biochemistry in Bangalore. Those students would have clearly been especially favoured to have had such an exposure to mycology, but sadly much seems to be tailored to such students' perceived needs rather than providing a balanced introduction to the subject. The book is divided into six parts and an appendix. These address: The unique features of fungi (2 chapters), Integration of fungi with other organisms (2), Model fungi in research (4), Gene manipulation in fungi (1), Adaptations (3), and Populations (2). It would not be pertinent to consider these sections in depth in *Mycotaxon*, but I have to say that I was frankly aghast at the appendix on Naming, defining and broadly classifying fungi. Most of the appendix consists of crude illustrations evidently scanned from poor quality Xerox copies of earlier works, incorrectly uses subphylum terminations (i.e. '-*mycotina*) for the main groups labelled as at the rank of phylum (names in which have the termination '-*mycota*). Then, to compound matters, the author recognizes 'phylum *Deuteromycotina* (*Fungi Anamorphici*)! Just how out of touch can an author be with current thinking? The 2001 edition of *Ainsworth & Bisby's Dictionary of the Fungi* is cited at the end of the appendix, but clearly has not been taken note of.

Further, in the main body of the work, to find no detailed consideration of molecular phylogenetic approaches is almost unbelievable for a work with a 2005 date – there is only one tree from a paper of the author's from 2002 on Indian *Neurospora* strains accompanied by a mere 15 lines of discussion. Even with all the exciting current experimental work, mycorrhizas get a mere seven pages, and the part on interactions with other organisms does not have anything on insect-fungus relationships or medical aspects. There is even no treatment of endophytic fungi which I would have expected biochemistry students to want. In addition, there are numerous mistakes and out of date information in other sections, and I fail to see why the same photographs have been included twice in some cases, once in black and white and once in colour (e.g. Fig. 3.5, also not named in the legend, but probably *Caloplaca thallincola* growing on *Verrucaria maura*).

This is a stain in an otherwise really splendid series, and neither the text nor the figure quality can have been critically reviewed prior to publication. This is sad as the series would have benefited from counting a rounded text amongst its volumes, especially as there is still a shortage of authoritative introductions to the subject. Fortunately, the price of the book should prevent most students' having access to a copy.

**Revue du Cercle de Mycologie de Bruxelles.** Numéro 1. September 2001. Cercle de Mycologie de Bruxelles, c/o Yolande Mertens, Tomberg 116 bte 12, B-1200 Bruxelles, Belgium. Pp. 52. ISSN not indicated. Annual membership: 12.50 €.

This new journal is mentioned here as it is full of systematic papers, rather than just excursion reports and notes on interesting finds. And it deals no just with macromycetes. The first number has five original papers which concern, *Amanita vittadinii, Caloscypha fulgens, Podospora conica, Puccinia albescens,* and *Sarcoscypha jurana.* The second, issued in 2002, includes systematic contributions on *Ascobolus degluptus, Cordyceps* (with a key to 14 species and distribution maps), *Discinella lividopurpurea, Gymnosporangium sabinae,* and a key to long-stalked *Inocybe* species based on spore characters. Where

there are field meeting reports, these include notes on the features of interesting species. Colour photographs illustrate most articles. The journal is edited to a high professional standard, should be in all major mycological libraries, and merits coverage in abstracts databases.

**Encyclopedia of Fungi of Britain and Europe.** By Michael Jordan. 2004. Revised edn. Frances Lincoln, 4 Toriano Avenue, London NW5 2RZ, UK. Pp. 384, numerous col. photographs. ISBN 0 7112 2378 5 (hardback), 0 7112 2379 3 (paperback). Price: £ 27.50 (hardback), £ 15 (paperback).

When this work first appeared in 1995 it was warmly welcomed by the amateur mycological community because of its excellent colour photographs and concise descriptions. As in all identification guides covering a wide range or fungi, there were invariably some errors in the applied names, and this led to some most unfortunate and disproportionate published criticisms and dissociations (e.g. Emmett 1996; Henrici 1996). It is always hard for someone without the resources of a major institution behind them to produce such a major work, and such texts have to be seen in that context. For this revised edition, the author has endeavoured to address the criticisms and updated about 60 photographs and replaced or relocated some eight that were wrongly named.

The fungi are arranged by major group, and while "*Aphyllophorales*" and "*Gasteromycetes*" appear as categories for practical reasons, I was very pleased to see these names in inverted commas and the correct disposition of genera provided (e.g. *Lycoperdon* in *Agaricales*).

While the focus of the book is the photographs and descriptions, there are also helpful introductory chapters with notes on nomenclature, collecting, photography, chemical tests and conservation, as well as a colour chart and glossary. There is also a key and bibliography, although the latter sadly has not been updated and no post-1993 works included: further, *Ainsworth & Bisby's Dictionary of the Fungi* is given as "1986", ignoring the 1995 and 2001 editions.

Interestingly for a book aimed at amateur mycologists and mycophagists, English common names are generally omitted, as "for the vast bulk of fungi the Latin name is the only acceptable option" – I concur.

With so many fine photographs and at such a reasonable price in paperback, this revised edition will do much to promote serious mycological studies amongst the amateur community, those who are the key to the much deeper understanding of macromycete distribution and ecology that is so badly needed.

This is a fine achievement on which the author and publisher are to be congratulated, and those that might wish to pinprick from advantageous standpoints need to stand back and see the work in context. I did not consider it appropriate so to do, and look forward to a succession of future editions.

Emmett, E. E. (1996) A disclaimer. *Mycologist* **10**: 95.

Henrici, A. (1996) The Encyclopedia of Fungi. *Mycologist* **10**: 183.

**Die Pilzflora des Ulmer Raumes.** By Manfred Enderle. 2004. Manfred Enderle, Am Wasser 22, D-89340 Leipheim-Riedheim, Germany (e-mail: manfred.ender. Pp. 521, illustrated. ISBN 3 88294 336 X. Price: 24.50 €.

This beautifully presented and personal work aims to document the range of fungi so far found in the Ulm area of Baden-Württemberg in Bavaria, Germany. After a short but clear and well-illustrated introduction to what fungi are and characters used in macromycete identification, a detailed history of fungus exploration in the area follows. The first records go back to 1728, but the account goes through to document the activities of the local mycological society, the Arbeitsgemeinschaft Mykologie Ulm (AMU) founded in 1976, including data in publications and the personalities involved. There are also lists of localities with MTB grid square references, and sections on geology, soils, climate, and vegetation types – the latter with lists of species frequent in or characteristic of them within.

The core of the book is the species lists, which cover slime moulds, and straminipilous fungi as well as asco- and basidiomycetes. Sadly, a category *Fungi imperfecti* is retained, which for some inexplicable reason includes zygomycetes, and lichen-forming fungi are not covered at all. The total number of species treated is given as 2681 species, plus 72 that are unclear or partly described taxa. Most have been collected over the last 40 years, and detailed information is provided on localities, hosts, and for rarer species specific collections with date, collector, place where voucher material is held, or literature reference. Author citations are provided, but unfortunately do not follow those recommended by and used in the *Index fungorum* database – and there are inconsistencies in how the same author is abbreviated.

Fine colour photographs and line drawings are scattered throughout the body of the text, and in some cases full morphological and microscopical descriptions are provided, especially for species that either are in the Red List or described from the region. Critical collecting over an extended period has led to the discovery of a considerable number of novel taxa in the area, and a list of the 43 new scientific names introduced by the author over the years is provided (pp. 64-65). Further, several new taxa are introduced in the book: two new species of *Coprinus s. lat.*, a new variety of *Hebeloma vaccinium*, and a new combination for a form of *Tricholoma vaccinum*.

There are two separate sections of colour plates with integral descriptions at the end of the systematic treatment: one for the 50 most edible species with the outside margins of the pages with a green strip, and one for harmful ones with a red strip. The reference list and full index is preceded by a most delightful 20-pages of half-tone photographs of mycologists on excursions and in meeting rooms or laboratories. Not all are dated, but those that are cover the period 1980-2003 and include many well-known mycologists, several of whom are now dead. Additional snippets are ten rules for collectors, frequently consulted works, and a glossary.

The acknowledgements list is extremely extensive and includes not only different mycologists who have helped, but further several major companies in the region who made financial contributions to the cost of publishing this book – resulting in the most reasonable price for such a well-produced and lavishly illustrated local treatment.

The author should feel well-satisfied with what is very much a testimony and personal record of his four decades of collecting fungi in the Ulm region. It is a "must have" for those working in the region!

**Fungi of Northwestern China.** Edited by Wen-Ying Zhuang. 2005. Mycotaxon, P.O. Box 264, Ithaca, NY 1485-0264, USA. [Order from: Professor Wen-Ying Zhuang, P. O. Box 2714, Beijing 100080, Peoples' Republic of China (e-mail: zhuangwy@sun.im.as.cn).] Pp. vi + 430, figs 5. ISBN 0 930845 14 5. Price: US $ 40.

The region covered in this compilation embraces Gansu, Ningxia, Qinghair, Shaanxi, and Xinjiang provinces, more than a quarter of the People's Republic of China and including desert areas such as the Gobi desert, grasslands, mountains, and plateaux. In all, 3887 species distributed through 759 genera are treated, including straminipiles, lichen-fungi, and slime moulds. The data are presented as a series of 14 complementary chapters prepared by different specialists, but the editor clearly has insisted on a standard style, so that each entry includes a note on the host or substrate, provinces where the species has been found, and information as to the source (i.e. to a reference collection or publication). Especially helpful are the maps showing collecting sites in each province.

All chapters start with an overview of the study of a particular group in China, and there are comprehensive indices at the end by both host and fungal taxon. This is clearly an important regional synthesis, and will also be an aid to identification through the host index, but very many more species are to be expected. That the text is in English and with extensive references to the literature in Chinese is a real bonus for non-Chinese speakers, making otherwise inaccessible data available. My one frustration is the continual citing of authors of hosts names in the text that have surely never been verified by the authors. Further, abbreviations used for the authors of fungal names are inconsistent, sometimes even within the same chapter.

The work is a complement to *Higher Fungi of Tropical China* (Zhuang 2001; reviewed in *Mycotaxon* 87: 493-494, 2003), and it is to be hoped that in due course it will be followed by parallel treatments of the remaining regions of China.

Zhuang, W.-Y. (2001) *Higher Fungi of Tropical China*. New York: Mycotaxon.

**Fungi of the Antarctic: Evolution under Extreme Conditions.** Edited by G. Sybren de Hoog. 2005. Centraalbureau voor Scvhimmelcultures, P. O. Box 85167, 3508 AD Utrecht, The Netherlands (e-mail: info@cbs.knaw.nl). [Studies in Mycology No. 51.] Pp. vii + 79. ISBN 90 70351 55 2. Price: 40€.

The two systematic monographs that comprise volume 51 of *Studies in Mycology* provide an outstanding introduction to the world of non-lichenized fungal life under extreme Antarctic conditions. Both groups of fungi are poorly understood, but the results reported in these articles indicate they constitute a substantial part of the microbial diversity of these habitats. I much appreciate the inclusion of both reviews in the same volume since despite the very different strategies of the fungi involved, they share the ability to withstand the harsh conditions of the Antarctic desert, providing new insight into the limits of fungal life. At the end of each article, the authors provide hypotheses for the evolution of the fungal groups treated.

*Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert* (L. Selbmann, G. S. de Hoog, A. Mazzaglia, E. I. Friedmann and S. Onofri): The authors present an extensive treatment of Antarctic cryptoendolithic communities. These fungi of diverse phylogeny colonize rock cavities and thus avoid the inhospitable conditions of the rock surface. The article starts with an introduction in which the ecological niche of these fungi is described, along with some of their morphological and physiological characteristics. This is followed by a detailed description of the sampling sites and the methods employed in the study. The results include light microscopy and scanning electron microscopy descriptions of twenty-six strains of black, mostly meristematic, fungi from cryptoendolithic lichen-dominated communities of Antarctica, and the sequencing of their ITS rDNA region. The phylogenetic positions of these different strains and their survival strategies are discussed, and the new genus *Cryomyces* is introduced for two newly described species. A further new species of *Friedmanniomyces* is also named here.

*Evolution, taxonomy and ecology of the genus Thelebolus in Antarctica* (G. S. de Hoog, E. Göttlich, G. Platas, O. Genilloud, G. Leotta and J. van Brummelen): These authors report a high frequency of *Thelebolus* species in Antarctic "biomats" from several types of lake, and offer an explanation for this based on bird vectors. A short introduction to the genus is followed by a description of the sampling sites and methods used to sample, isolate, and analyze strains, and herbarium collections. The results include an extensive molecular analysis aimed at resolving the phylogeny of this adapted group of cup fungi using different methods, including microsatellite fingerprinting and the sequencing of several genes. Phylogenetic relationships, taxonomic positions and the evolution of this genus are discussed in the light of SSU and ITS rDNA and ß-tubulin sequence data. The article ends with a taxonomic section, which includes a key for the morphological identification of the four species described, two of which are described as new to science and appear to be endemic to Antarctica.

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## Basidiomycetes

**Les Réactifs Mycologiques.** Vol. 2. **Les Réactifs Microchimiques.** By Jacques Charbonnel. 2004. Edition Jean-Claude David-Rogeat, Rue Général Dufour 131, CH-2502 Bienne, Switzerland (e-mail: info@mycologie.ch). Pp. 289. ISBN 2 9700411 0 2. Price: 50 €.

This is the second part of *Aide pratiques à l'étude microscopique des Champignons* and follows a long French tradition for the use of chemicals in macromycetology, from Bataille to Josserand and Henry *et al.* Indeed 36 mycologists experienced in this field are thanked in the opening pages.

There are seven chapters, the first setting out the aims of the compilation, an introduction, abbreviations used therein, etc., and incorporating a preface by Francis

Quirin, President of the French Mycological Society. The next chapter tabulates the reagents, formulae, etc., which will be found in the pages which follow.

The largest part of the publication, 214 pages in all, brings together the reactions resulting from a specified, described and documented reagent when applied to a particular tissue or structure. For each case, where appropriate and possible, the same arrangement is used in the presentation so that direct comparisons can be made for the procedure and the reaction found in the same tissue in different reagents. Chapter 4 is particularly helpful because it deals with the application of selected chemicals to dried material, and in the following chapter discussion is focused on *Russula*, a genus in which chemicals have been used extensively in its systematics. The methods by which the reagents are made-up and applied are clearly given. Chapter 6 deals with naturally occurring pigments and their distribution within the sporomes. It is admirable that the author covers all the main groups of fungi when considering how to deal with the specimens in hand and their chemical characteristics.

A cross-referenced index follows, which is extremely helpful, especially as it cites the various species of fungi, or the genera which are mentioned under each reaction. The format for the whole publication parallels papers in *Documentes Mycologiques*, a journal in which Charbonnel regularly publishes. This is an excellent publication which will be of very great help to those undertaking this kind of work, and students should be encouraged to utilise the contents as the tests have a significant role to play in areas not attainable by so-called cutting edge science!

But for would-be readers, I suggest that on purchasing a copy it should be immediately rebound with a firmer spine, otherwise the pages will soon dismember.

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**Frontiers in Basidiomycote Mycology.** Edited by Reinhard Agerer, Meike Piepenbring & Paul Blanz. 2004. IHW Verlag, P.O. Box 119, D-85378 Eching, Germany (e-mail: dr.schmid@ihw-verlag.de). Pp. 430, figs 124, col. pl. 80. ISBN 3 930167 57 3. Price: 119 €.

Despite several requests to the publishers, no copy has been received. This implies that the publisher does not consider that the work merits coverage in *Mycotaxon*.

***Cortinariales.*** By Vincentas Urbonas. 2005. UAB 'Valstiečių Laikraštis', Vilnius, Lithuania. [Available from Institute of Botany, Žaliųjų ežerų 49, LT-08406 Vilnius 21, Lithuania. Pp. 288, figs 118, col. plates 32. [Mycota Lithuaniae Vol. 8 (5).] ISBN 9986 847 85 0. Price: 28 €.

This work is part of a series of books dealing with the mycobiota of Lithuania. It is written in Lithuanian, but contains a seven page long English summary with keys to the included genera.

In this book, the *Cortinariales* are subdivided into two families, *Cortinariaceae* and *Crepidotaceae*, and in total 291 species belonging to 13 genera assigned to these families are described. Keys are provided for all included species, but they are difficult to use

for people who do not know Lithuanian. The genera and numbers of species covered are: *Cortinarius* (124 spp.), *Crepidotus* (14), *Galerina* (27), *Gymnopilus* (12 ), *Hebeloma* (30), *Hebelomina* (1), *Inocybe* (65), *Leucocortinarius* (1), *Naucoria* (X), *Pellidiscus* (1), *Phaeocollybia* (3), *Rozites* (1), and *Simocybe* (4). *Cortinarius* is subdivided into five subgenera: *Cortinarius* (12), *Dermocybe* (12), *Myxacium* (10), *Phlegmacium* (37), and *Telamonia* (53). *Hebeloma* is divided into subgenera *Denudata* (18) and *Hebeloma* (12). *Galerina* is subdivided into subgenera *Phaeogalera* (24) and *Galerina* (3). And subgenera *Inocybe* (19) and *Inocybium* (46) are used for *Inocybe* species.

Basionyms, important synonyms, and iconography are provided for all included taxa. The nomenclature follows *Index Fungorum*, but is not always up to date. For example, *Rozites* is treated as a distinct genus, and the species epithet of *Cortinarius harcynicus* is given as '*hercynicus*'. Drawings of spores or other important microscopical structures are provided for selected taxa only, but they are more raw outlines than informative line drawings. An index of scientific names enables the reader to easily find all included taxa. The reference list includes a few interesting recent Lithuanian publications, but otherwise mainly includes publications up to the nineties; and more recent treatments (or iconography) of the included genera have not been considered in this work. The appendix of 32 colour plates provides either drawings or photographs of 163 of the species treated in the book. The colour drawings of the basidiomes are usually quite well done; but the quality of the photographs varies from quite good, to others which are out of focus or do not show the important features of the fungus. However, most pictures are supplemented by a line drawing.

In summary, this book is the first, well-investigated inventory of the cortinarioid fungi occurring in Lithuania. Those who understand the language will also find it a useful aid to the identification of fungi in Lithuania and surrounding countries, especially because pictures and line drawings are given in the same volume. Thus, it can be expected that with increasing sampling, the number of fungal species in this order known from Lithuania will rise. Consequently, in spite of a few shortcomings, this book will certainly help Lithuanian mycologists to study the brown-spored fungi of their beautiful country much more easily.

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**Checklist of Polish Larger Basidiomycetes.** By Władysław Wojewoda. 2005 ["2003"]. [Biodiversity of Poland Vol. 7.] W. Szafer Institute of Botany, Lubicz 46, PL–31–512 Kraków, Poland (e-mail: ed-office@ib-pan.krakow.pl). Pp. 812, fig. 1. ISBN 83 89648 09 1. Price: 58 €.

This is a welcome addition the literature of macromycetes of Europe. In the ninth edition of *Ainsworth & Bisby's Dictionary of the Fungi* (Kirk *et al.* 2001) works on the macromycetes of this continent were mentioned for Bulgaria, former Czechoslovakia, Denmark, France, Great Britain, Greece, Italy, Scandinavia, Spain, Sweden and Switzerland. However, Poland, the eighth largest country of Europe in area, was omitted.

The checklist of Polish larger basidiomycetes includes about 400 genera and 2650 species, although some of the listed names are invalid or otherwise contrary to the *Code*. The true number of nomenclaturally correct larger basidiomycete taxa known from Poland is actually about 2550.

Over 2100 species of agaricoid and boletoid fungi (Moser 1983) and about 2000 species of aphyllophoroid, gasteromycetoid, tremelloid, auricularoid, dacryomyceteoid and tulasnelloid fungi (Jülich 1984) are known in Europe. According to Wojewoda, these numbers are certainly too low and many more fungi occur in Europe. In the former German Federal Republic, 3150 species of basidiomycetes (Krieglsteiner 1991) were known through 1991, about a thousand more than the number known in Poland in 2003. Because Poland and the western part of Germany are comparable in terms of area, climate and nature preservation, a similar number of species might be expected to grow in Poland. In the years to come, Polish mycologists face the important task of finding these fungi.

Wojewoda includes data on threatened species compiled from the Red List of Threatened Macrofungi in Poland. To enable the data to be compared with information on threatened fungi in other European countries, categories of threat from the following countries are cited: Austria, Belgium, Bulgaria, Czech Republic, Estonia, Finland, Germany, Great Britain, The Netherlands, Lithuania, Latvia, Norway, Switzerland and Sweden.

The Polish names of the fungi given are derived from many authors of papers published between 1830 and 2003. The habitats in which the fungi grow, the plant community, substratum, mode of nutrition and fruiting time are detailed. Examples of species distribution in Poland, including the regions, form the most extensive part of the account of each species. For some very rare species, all or nearly all sites are given, while for fairly common species only selected sites are cited. For very common and common one (except a few like *Phallus impudicus* and *Langermannia gigantea*) no sites are given. Readers and all users of this new checklist can only congratulate the author on seeing the book published after 14 years of work.

Kirk, P.M., Connon, P.F., David, J.C., & Stalpers, J. (2001) *Ainsworth & Bisby's Dictionary of the Fungi*. 9th edn. Wallingford: CABI Publishing.

Moser, M. (1983) Die Röhrlinge und Blätterpilze (*Polyporales, Boletales, Agaricales, Russulales*). In: H. Gams (ed.) *Kleine Kryptogamenflora* II b/2: *Basidiomycetes*. 2. Teil 5., bearbeitete Aufl. VEB. Jena: G. Fischer.

Jülich, W. (1984) Die Nichtblätterpilze, Gallerpilze und Bauchpilze. *Aphyllophorales, Heterobasidiomycetes, Gasteromycetes*. In: H. Gams (ed.) Kleine Kryptogamenflora II b/1 *Basidiomyceten* I. Stuttgart: G. Fischer.

Krieglsteiner, G. J. (1991) Verbreitungsatlas der Grosspilze *Deutschland* (*West.*). Vol. 1. *Ständerpilze*. Teil A. *Nichtblätterpilze*. Teil B. *Blätterpilze*. Stuttgart: Verlag E. Eugen, Stuttgart, pp. 421–1016.

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**Guía de los hongos de Alcalá de Henares (Macromicetes).** By Michel Heykoop Fung-a-You & Rosa Antolín Bellver. 2005. Servicio de Publicaciones, Ayuntamiento de Alcalá de Henares, Concejalía de Medio Ambiente, Instituto de Planificación y Gestión Ambiental, Quinta de Cervantes, Calle Navarro y Ledesma 1, Alcalá de Henares, Madrid, Spain. Pp. 120, col. plates. ISBN not indicated (Dep. Legal: M-52838-2004). Price: Free while stocks lasts.

This amazing guide, completely financed by the local authority of Alcalá de Henares, the city where Cervantes lived part of his life in Spain, is an example of how local administrative bodies can promote interest in fungi, as a part of their broader concerns for nature and its conservation, and considering organisms as part of the area's natural heritage. It provides a concise history of mycological studies in the area, a catalogue that describes 65 species beautifully illustrated by coloured paintings rom the 200 known from Alcalá de Henares. It comprises seven chapters, some of which are brief introductions as to what a fungus is, habitats where they grow, fungal interactions with other organisms, the importance and applications of fungi, fungal classification, and characters of macromycete fruit bodies, as well as a key for the 65 species treated in the guide. Chapter 7 provides scientific and common names (where they exist), macroscopic descriptions, detailed drawings of the species (the second author, Michel's wife), and observations on their culinary or toxic properties, medicinal fungi, and fungi of industrial interest. A basic glossary of scientific terms is provided, as well as an index to the species. A three-week exhibition of the original paintings in a hall close to the town centre was also staged in April-May 2005. This is useful book for amateurs, and all interested in fungi from this area, combining identification with sound information on the broader relevance of fungi. The initiative in making such a work available free of charge is a model that I would like to see followed by many other local authorities worldwide.

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**Palearctic *Lyophyllaceae* (*Tricholomatales*) in Northern and Eastern Europe and Asia.** By Kuulo Kalamees. 2004. Estonian Academy Publishers, Tartu, Estonia. [Scripta Mycologica No. 18.] Pp. 135, figs 32, maps 3. ISBN 9985 50 356 2. Price: Not indicated.

This book presents a survey of the taxonomy, ecology and distribution for most of the genera in family *Lyophyllaceae sensu* Bon 1999 from the Palearctic regions of Europe and Asia: *Lyophyllum* s.str., *Hypsizygus*, *Gerhardtia*, *Calocybe s. str.*, *Tricholomella*, *Rugosomyces*, and *Asterophora* are treated in detail but the genus *Tephrocybe* is not considered. The book is divided in two parts: an introductory part (pp. 5-15) and a taxonomic part (pp. 16-105). The introductory part summarizes the actual state of knowledge on family *Lyophyllaceae*, presents the significant taxonomic features used for species determination, and the classification and the abbreviations used by the author.

The taxonomic part constitutes some kind of practical guide to the identification of the Palearctic *Lyophyllaceae*, which is certainly not one of the easy agaric families as pointed out by the author. The author provides a key to genera and species, species descriptions illustrated by some line drawings of microscopical features (spores, basidia and mycelium), clarifies differences in species concepts between mycologists, and adds also valuable information about the distribution and ecology of the species. Ecology is indeed very important but too often a neglected aspect in many revisions. Particularly in the case of *Lyophyllaceae*, ecology may very well be one of the most important factors underlying evolution since molecular studies have shown that all the classifications proposed for the tribe *Lyophylleae* are artificial and that species sharing a mode of nutrition cluster together.

The book is well edited and provides detailed macroscopic and microscopic descriptions and well referenced discussions and comments. It is therefore all the more deplorable that the quality of the illustrations is disappointing. The rather simplistic line drawings often lack sufficient precision or detail (e.g. scales are not always respected, as for the spores of *L. loricatum*), while in others being superfluous or not essential for understanding or identification or not representative of the text (e.g. *Rugosomyces obscurissimus* spores). The reader has the impression that the author considers microscopic illustrations of secondary or no importance (even when a lot of collections have been checked, as for example in the case of *L. decastes*, where one would expect mature basidia instead of basidioles). As already very few morphological characters are available to separate species in virtually all of the *Lyophyllaceae*, the illustration of taxonomically significant microscopic features such as size, shape and ornamentation of spores should be as detailed, precise and representative as possible. In my opinion, the book would also have benefited from coloured pictures illustrating the species (in the form of an annex for example) in addition to a list of often too inaccessible references to most of the users of this type of monograph.

I also regret that the discouraging nomenclatural chaos in *Tephrocybe* was apparently responsible for its exclusion from this revision of *Lyophyllaceae*. Indeed, molecular studies suggest that both *Tephrocybe* and *Lyophyllum* are polyphyletic. Exclusion of either one results automatically in partial revisions of the species in several of the natural groups within *Lyophyllaceae*. In that sense, the impact of this study is reduced, not only as a taxonomic guide to *Lyophyllaceae*, but also as a useful tool for future revisions of the more natural entities.

With the new insights of molecular systematics, a reappraisal of the morphology of *Lyophylleae* in the context of a more natural classification of this fungal group will hopefully soon emerge. It will facilitate the difficult task of the purely morphological approach in *Lyophylleae*. Taxonomic studies as this one are really needed to help clarify the nomenclature, morphology and ecology of this very difficult group of fungi, and this book can therefore be considered as an important contribution to attaining this goal.

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**Monograph of the genus *Hemileia* (*Uredinales*).** By Anja Ritschel. 2005. J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, D-14129 Berlin, Germany. [Bibliotheca Mycologica No. 200.] Pp. 132, pl. 8, figs 39. ISBN 2 443 59102 7. Price: 44 €.

The work on this tropical fungal genus, known essentially for its parasitism on coffee, but affecting at least ten families of angiosperms, has been conducted on herbarium material, mostly from European herbaria, but also from ones in the USA and South Africa. In many cases the descriptions rely on (very) old material, which is probably caused by the absence of collectors, providing fresh samples, in the tropics. For some species, no material was available to the writer. Descriptions were then gathered from previous publications.

There is a pertinent discussion of the validity of the genus name and the affiliation of *Hemileia* with the *Chaconiaceae*. The author keeps all species without a known teleomorph in the anamorph genus *Uredo*. This concerns 17 of the 43 species described. A new genus, *Desmosurus* is proposed. One new name, a new species, and some new combinations are introduced. Doubtful and excluded taxa are mentioned.

Full attention is given to the formation of the sori, and two types, suprastomatal or erumpent, are distinguished. The individual species descriptions are as complete as can be, and include all the references needed. Urediniospore characters are congruent with the host range and differentiate the species, and the author gives a key based on length and echinulation of the urediniospores. For the species of *Hemileia s. str.*, a description of the teliospores is provided. The drawings of spores are very good and clear, and the SEM photographs excellent.

The cited literature comprises 125 references. The last cytological study mentioned is dated 1968, and two references concern molecular techniques, which were probably not possible with the herbarium material available. Further work remains to be done in that field.

This book is a valuable contribution to our knowledge of the genus, and will be of great use for mycologists everywhere, and not only in the tropics. One species is described from Japan, and another reported from orchids in heated greenhouses in Europe.

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***Russulaceae*: *Lactarius*.** By R[onald] W. Rayner, assisted by R[oy] Watling & E[lizabeth] Turnbull. 2005. Royal Botanic Garden, 20A Inverleith Row, Edinburgh EH3 5LR, UK (e-mail: pps@rbge.org.uk). [British Fungus Flora Vol. 9.] Pp, 203, figs 200. ISBN 1 872291 34 1. Price: £12.50

This ninth number in the agarics and boleti series documenting the British fungi treats *Lactarius* in the Russulaceae. It is presumed that Roy Watling and Elizabeth Turnbull brought the compilation together for publication after the passing of "Ronnie" Rayner, although this is not mentioned in the publication. An item that appeared early on in the series and is included with this number is that of the trifold colour identification chart.

This chart will be repeatedly consulted for determination of spore colour in mass as well as other colours described for the basidiomes.

The overall treatment of *Lactarius* follows the same general scheme presented in previous issues of this series with introductory text, references, family description, a generic key to just *Russula* and *Lactarius* with a note that sequestrate representatives are to be keyed elsewhere. *Lactarius* is then treated in detail with a description and discussion of features (including useful macrochemical reagents) in the genus followed by a key to seven sections, diagnostic synopses of the sections, the systematic arrangement (essentially that of Singer's fourth edition with some minor rearrangement, and incorporating Hesler and Smith's treatment of sect. *Tristes*). This is followed by a complete dichotomous key to the 64 species. A handful of varieties are also treated. Although key to sections is provided, a key to species in each section (and subordinate subtaxa) is lacking.

The individual descriptions of species occupies the bulk of the publication and these are quite complete with attention to the details of fresh macroscopic appearance, macrochemical reactions, and important microscopical features, describing spores, hymenial cystidia and the pileus surface. The ecological habitat, phenology and distribution in Britain follow the description along with a commentary on distinctive or odd features and comparisons to like taxa. On a rare occasion, mention is made of the taxon as it might occur outside of Britain. In addition, some general synonymy is given along with citation of illustrations (icones) representing well the British species.

The descriptions are followed by a listing of species according to ecological preference such as: in oak woods, with ash, with *Betula nana*, on calcareous soils to name just a few. Some species are mentioned more than once in this list. There are further lists of synonyms and misidentifications, rejected names (and why rejected), an index to epithets mentioned in the commentaries, and lastly the index to described species. The last 65 pages of 203 are given over to line illustrations of habit sketches and microscopic features of spores, cystidia, and hyphal configurations making up the pileus surface. These latter are relevant in a diagnostic sense as examples of taxa in particular sections.

It is noteworthy to mention that the descriptions are almost entirely based on observation of fresh material, either collected by the author or given to him by several individuals who are dutifully acknowledged.

For those who need to identify *Lactarius* species among the macromycetes in Britain, users will find this treatment comfortably familiar to past treatments in the series as well as thorough and well documented. The addition of the colour chart will be necessary for colour determination but is also an added bonus and can be used with the previous publications.

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## Ascomycetes

**Discomycetes. Fasc. 1. Familiae *Ascobolaceae* (species coprotrophicae), *Iodophanaceae* (species coprotrophicae), *Ascodesmidaceae* (species coprotrophicae), *Pezizaceae* (species coprotrophicae), *Pyrenomycetaceae* (species coprotrophicae), *Thelebolaceae* (species coprotrophicae).** By V. P. Prochorov. 2004. Oficina Editoria KMK, 123100 Moscow a/r 16, Russia (e-mail: kmk2000@online.ru). Pp. 256, figs 148. [Definitorium Fungorum Rossiae.] ISBN 5 87317 136 X. [In Russian.] Price: not indicated.

This volume aims to provide a detailed account of the coprophilous pezizalean discomycetes known from Russia. It treats 194 species disposed through 23 genera: *Ascobolus, Ascodesmis, Ascozonus, Caccobius, Cheilymenia, Coprobia, Coprobolus, Coprotus, Dictyocoprotus, Dictyosporus, Fimaria, Hapsidomyces, Iodophanus, Lasiobolus, Ochotrichobolus, Peziza, Pseudacozonus, Ramgea, Saccobolus, Thecotheus, Thelebolus, Trichobolus,* and *Trichophaeopsis.* These are all listed here as only 11 of the genera are named on the contents page. For each species, the place of publication of the accepted name and any synonyms is provided (sometimes with a page-range not the actual page). Descriptions of genera and species are detailed, keys are provided, there are references to published illustrations, and especially welcome fine original line illustrations of microscopic features -- covering three quarters of the included species. Notes on habitat and frequency are very brief, and regions in Russia the species occur are sometimes indicated, but not consistently; extra-Russian distributions are also summarized. The work has evidently been in press for some years, as the latest reference cited is one of the author's from 1996 and so seems a little dated in part, for example in not taking up *Pseudombrophila* for the later synonym *Fimaria.* While this is no rival to Doveri's *Fungi Fimicoli Italici* (2004; see *Mycotaxon* 90(1): 220-221, 2004), it is a major achievement for a mycologist who has only been publishing on these fungi since 1991, and does include some species not treated in the Italian account. I will certainly find the illustrations of value in trying to name minute coprophilous discomycetes.

## Lichen-forming fungi

**Lichens: An Illustrated Guide to the British and Irish Species.** By Frank S. Dobson. 2005. 5th edn. Richmond Publishing, P.O. Box 963, Slough SL2 3RS, UK (e-mail: rpc@richmond.co.uk). Pp. 480, col. pls, maps. ISBN 0 85546 095 4 (hardback), 0 85546 096 2 (paperback). Price: £ 45 (hardback), £ 35 (paperback).

Each fresh edition of this book, the first of which was published in 1979, goes from strength to strength. The number of species treated has increased from about 450 in 1979, to about 700 in the fourth edition of 2000 (reviewed in *Mycotaxon* 78: 510, 2001), to about 850 in the fifth – approaching half of all the 1800 or so lichen-fungi known from Great Britain and Ireland. Further, an increasing number are illustrated in colour and crisper and more true-to-life than ever thanks to the use of digital photography. The tried and tested format of keys that has been used so successfully by almost a new

generation of amateur lichenologists has been maintained, as has the basic layout of the entries with the neat and now familiar distribution maps based on data in the British Lichen Society's Mapping Scheme. Unfortunately, the nomenclature follows the last British checklist too slavishly, for example in the adoption of some of the segregates proposed for parmelioid lichens for the first time after papers synonymizing several had appeared, retaining *Usnea subfloridana* as distinct from *U. florida*, and not taking up *Xanthoria aureola* for *X. ectaneoides*. In consequence there will be some discrepancies from the new edition of the multi-authored *The Lichen Flora of Great Britain and Ireland* currently in preparation. But these are small points. To produce one identification guide is a major achievement for an amateur mycologist, but to be able to produce one in five continuously improving editions over a period of 26 years is an unparalleled event and a 'first' for lichenology. The impact that this title has had on developing expertise in lichenology in Great Britain and Ireland over this extended period of time has been immense, especially through its use on several field courses each year by its' author and other tutors. Lichenology owes Frank an immense debt.

**Opredelitel' Lishainikov Rossii.** Vol. 8. ***Bacidiaceae, Catillariaceae, Lecanoraceae, Mycobilimbiaceae, Rhizocarpaceae, Trapeliaceae.*** By M. P. Andreev, L. I. Bredkina, N. S. Golubkova, A. A. Dobrysh, Y. V. Kotlov, I. I. Makarova, I. N. Urbanavichene & G. P. Urbanavichus. 2003. Nauka, St Petersburg, Russia. Pp. 278, figs 93. ISBN 5 02 026044 4. Price: Not indicated.

**Opredelitel' Lishainikov Rossii.** Vol. 9. ***Fuscideacae, Teloschistaceae.*** By A. Y. Khodovtsev, S. Y. Kondratyuk, I. I. Makarova, & A. N. Oxner. 2004. Nauka, St Petersburg, Russia. Pp. 340, figs 133. ISBN 5 02 026207 2. Price: Not indicated.

This series started publication in 1971 as *Opredelitel' Lishainikov SSSR*, with 'SSR' being replaced by 'Rossii' from volume six which appeared in 1996. I was pleased to see these two recent volumes as no additions to the series had appeared since volume seven in 1998, and it would have been unfortunate if this ambitious project joined the ranks of unfinished multi-volume projects. The style follows that of the previous volumes with keys and detailed descriptions, but with more line and half-tone illustrations.

Volume Eight tackles a series of difficult families in which concepts are still being worked out and in many cases await testing by molecular phylogenetic approaches. But, at least the authors have endeavoured to follow the latest proposals, for example in accepting *Calvitimela* (into which three new combinations are made). In some cases all the species in a genus are not treated, for instance under *Lecanora* only the *L. marginata/ L. sulphurea* complex is covered. Volume Nine has been particularly awaited for its treatment of *Caloplaca* and especially *Xanthoria* on which Sergey Kondratyuk and the late A. N. Oxner worked for many years. The treatment of *Caloplaca* follows traditional lines, and the account is particularly well-illustrated by line drawings. The authors' approach to *Teloschistes* and *Xanthoria*, however is much more controversial with the introduction of three new genera, none validly published here: *Oxneria* for the *X. fallax* group (18 spp.), *Rusavskia* for the *X. elegans* group (11), and *Xanthoanaptychia* for the *Teloschistes chrysophthalmus* group (5). Further, many more species are being accepted in these segregate genera than are currently in use. There may be good molecular evidence

for such changes, but it would have been preferable to see the new evidence first and for the scientific names and combinations to have been validly published elsewhere before being utilized in an essentially floristic study.

As the series grows, it becomes increasingly difficult to check in which volume a particular taxon is treated, and it would be great if in future that each volume had a complete list of genera so far treated in the series with an indication in which volume they were to be found. Treated families were listed in each part up to and including volume six, which was a gesture in this direction, but even that was dropped in subsequent volumes.

**The Lichens, Lichenicolous and allied Fungi of Poland: An annotated checklist.** By Wiesław Fałtynowicz. 2003. W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland (e-mail: ed-office@ib-pan.krakow.pl). Pp. 435, figs 1. [Biodiversity of Poland Vol. 6.] ISBN 83 89648 06 7. Price: Not indicated.

The tradition in which Polish records are synthesized is not only maintained but taken to new heights in this new checklist. The previous checklist (Fałtynowicz 1993) included 1500 lichenized and 119 lichenicolous fungi, and these totals have now swelled to 1554 and 214 respectively. But the 1993 list consisted only of the scientific names, with no references as to sources let alone information on habitats and distribution. This one has synonyms listed under the names, information on ecology and localities (down to province), and literature references from the mid-nineteenth century on. Each genus and species is also given a name in Polish, most coined here for the first time. But I have to wonder if this is really worthwhile – especially in the case of the lichenicolous fungi so named! This is a landmark publication of synthesis on the Polish lichens and lichenicolous fungi, on which the author has worked for over 25 years, and is the most detailed checklist I have seen for some years for any country.

Fałtynowicz, W. (1993) A checklist of Polish lichen forming and lichenicolous fungi including parasitic and saprophytic fungi occurring on lichens. *Polish Botanical Studies* 6: 1-65.

**Flora Liquenológica Ibérica: *Ostropales*: *Graphidaceae, Solorinellaceae; Gyalectales: Gyalectaceae.*** 2004. Sociedad Española de Liquenología, Murcia, Spain. Pp. 48, figs 9, map 1. ISSN 1696-0521. Price: 10 €.

The first volume in this series dealt with the *Peltigerales* (see *Mycotaxon* 92: 492-493, 2004). This second volume covers somewhat disparate groups by different authors, but is presented to the same high standards in both the descriptions and drawings. The plate of ascospores in *Gyalecta* is especially fine. As the series builds it will become of increasing value as a major work of reference.

**Catalogue of the lichenized and lichenicolous fungi in Bulgaria.** By Helmut Mayrhofer, Cvetomir M. Denchev, Dimitar Y. Stoykov & Siyka O. Nikolova. 2005. Bulgarian Mycological Society, c/o Institute of Botany, Bulgarian Academy of Sciences, 23 Acad. G. Bonchev Street, 1113 Sofia, Bulgaria (e-mail: denchev@bio.bas.bg). Pp. 59. [Mycologica Balcanica 2: 3-61.] ISSN 1312-3300. Price: 19 €.

This paper is mentioned here as it was specifically submitted to *Mycotaxon* for review. It is the first synthesis of information on the lichens of Bulgaria to have been published since the flora of Popnikolov & Zhelezova (1964). That work accepted 636 species and included keys, descriptions, and many illustrations. This new checklist has almost 50% more species, 893, of which a mere nine are lichenicolous and 14 non-lichenized fungi traditionally studied by lichenologists. The list is based on 216 publications supplemented by previously unpublished reports backed by herbarium material. Eighteen species are reported from Bulgaria for the first time; some of the latter are rather widespread in Europe (e.g. *Lecanora saligna, Phylctis argena, Tuckermannopsis chlorophylla*) and their inclusion at such a late date is a witness to the scant attention lichenology in the country has received. Species are arranged alphabetically and their sources are given, but without any information on substratum or locality except in the case of new records for the country. An impressive list of synonyms is included, with 1625 infrageneric epithets. Hopefully, the availability of this new work will stimulate interest in lichenology in Bulgaria.

Popnikolov, A. & Zhelezova, B. (1964) *Lishei.* [Flora na B"lgariya.] Sofia: Narodna Prosveta.

**101 Common Mosses, Liverworts & Lichens of the Olympic Peninsula.** By Martin Hutten, Karen Hutten & Andrea Woodward. [2001]. [US Government Printing Office.] Pp. xi + 109, col. ISBN none. Price: US $ 9 [now out of print].

This delightful little spiral bound book, only 14.5 x 11.5 cm so it easily slips into a pocket, seems to have escaped other listings of lichen literature. Bryophytes and lichens are conspicuous even to the casual tourist in the high-rainfall parts of the Olympic Peninsula in Washington State, USA. The annual rainfall is said to be higher in some parts of the Peninsula than anywhere else in North America (600 cm p.a. plus), and pendent lichens to over 2 m in length can be encountered. After the introductory matter, each species has a full page almost half of which is a high-quality colour photograph, and the remainder presents descriptive notes, information on size, and separation from similar species. Altitudinal ranges are indicated by a vertical scale bar, and the species are grouped by habitat (e.g. forest floor, log, conifer). In many case there are small inset photographs of allied species that might be confused with those featured. The lichen pages have been prepared to the highest standards, with critical material checked by Bruce McCune. The production of the book was funded by several organizations including Cannon, The Green Earth Campaign, National Park Service, US Geological Survey, National Park Foundation, and the Northwest Interpretive Association; their vision in supporting such an initiative to generate a low-price product is to be commended. I found the little book very valuable in the field when encountering some of the endemic *Pseudocyphellaria*'s for my first time on the Peninsula in October 2005, but after praising, recommending it, and being presented with a copy by Maggie Rogers (Portland, Oregon), it emerged that this little gem was already out of print. If you see one available second-hand do secure it, even if just to sell one at a society auction.

## Conidial fungi

***Sporidesmium, Endophragmiella* and related Genera from China.** By Wenping Wu & Wenying Zhuang. 2005. Fungal Diversity Press, Centre for Research in Fungal Diversity, Department of Ecology and Biodiversity, University of Hong Kong, Pokfulham Road, Hong Kong SAR, People's Republic of China. Pp. x + 351, figs 152. [Fungal Diversity Research Series no. 15.] ISBN 962 86765 8 X. Price: US $ 80.

The fungi formerly referred to *Sporidesmium* have been segregated into an increasing number of genera over the course of the last three decades. The generic separations have been based on methods of conidiogenesis and conidial secession, schizolytic (*Sporidesmium*-type) or rhexolytic (*Endophragmiella*-type), and then by the septation in the conidia, which can be eu- or distoseptate, or the form of the conidia. These fungi had been little studied in China, indeed only one species of *Sporidesmium* is mentioned in Teng (1996), *S. polymorphum* (a record apparently overlooked by the authors), but Wu made around 300 collections in 'the last few years'. This book is a report of those collections and does not include studies of material in HMAS (Beijing) or other Chinese collections. This makes the results all the more impressive, as out of those collections emerged 143 species in 25 genera, including one genus and 43 species new to science (i.e. 30 % of those collected), and 16 new combinations. Further, 23 of the genera and 99 species proved to be first records for China.

The introduction includes comparative illustrations of the differences between the genera, as well as a key, which is sure to be of value to all starting to get to grips with the current generic concepts in the group. Very full descriptions are provided and there are clear line drawings, mostly full-page, but no photographs. Photographs would have been a valuable addition as it is not always clear what artistic licence has been taken in adapting complex wall structures to drawings. In particular, incomplete septa are in some cases illustrated between thickened distoseptate cells, with structures indicated between some and not others. That may well be so, but photographs would have made the situation incontrovertible. Also, there are strong similarities between the conidia of some species placed in different genera, for instance the *S. novozymium* group and *Ellisembia bambusae,* which even have similar gelatinous sheaths at their apices, and I wonder how significant a complete as opposed to an incomplete septum really is. It would be interesting to see a molecular phylogenetic study carried out on these collections to address such issues.

Original places of publication are always given, but there is no indication as to whether the type collections of already described species were examined. At least in some cases the concepts seem to have been taken from publications of other authors. With respect to the type specimens and ex-type cultures of the newly described species, I was somewhat concerned to read that all the specimens studied including the types "are preserved in Wu's mycological herbarium in Novozymes, China", with living cultures in the Novozymes collection. This is hardly in the spirit of the *Code,* and at least the holotypes would have been better deposited in HMAS (Beijing).

Notwithstanding these concerns, there is no question that this work represents a substantial contribution to our knowledge of these fascinating fungi, and that it has

been carried out with care and patience that is in the best traditions of studies of dematiaceous hyphomycetes. With so many taxa covered, this work is set to become the major international reference work on *Sporidesmium* and similar genera for many years to come.

Teng, S. C. (1996) *Fungi of China*. Ithaca, NY: Mycotaxon.

**Morphotaxonomic Revision of Fungicolous *Cladosporium* Species.** By Bettina Heuchert, Uwe Braun & Konstanze Schubert. 2005. Druckerei der Martin-Luther-Universität Halle-Wittenberg, Kröllwitzer Straße 44, D-06120 Halle/Salle, Germany. Pp. 78, figs 26, plates 2. [Schlechtendalia Vol. 13.] ISSN 1436-2317. Price: 5 €.

With the nature of *Cladosporium s. str.* now clarified, the numerous species described but whose affinities have not been reassessed in the light of current concepts need to be revisited. Here, this task is undertaken for the fungicolous (including the lichenicolous) species. Most fungicolous species prove to belong to *Cladosporium s. str.* Eleven obligately fungicolous species are accepted and described in detail, and illustrated by line drawings; information on hosts, geographical occurrences, and lists of specimens examined is also provided. One of these species is newly described (*C. gerwasiae* on a *Gerwasia* sp. rust on *Rubus*), and a new variety of *C. exobasidiae* occurring on *Exobasidium vaccinii* is also recognized . In addition, descriptions of six saprobic species which can also occur on decaying fungi are treated in similar detail. Twelve species are regarded as dubious or doubtful due to insufficient data or ambiguous information as to their substrate, and seven are excluded from the genus. New generic names are introduced for two of the excluded species, *Digitopodium* (for *C. hemileiae*) and *Parapericoniella* (for *C. asterinae*), and there are suggestions that two more may eventually also require different generic names.

The authors place square brackets ([. . .]) around author citations for infraspecific taxa other than the type variety, i.e. *C. exobasidii* Japp var. *exobasidii* but *C. exobasidii* [Jaap] var. *verruculosum* Heuckert *et al.* Under the *Code*, the citation of the author of the species name is not appropriate in the last and similar cases. However, if it is considered desirable to mention the author of the species name, their placement between squared brackets is a novel approach, though potentially ambiguous, as the device has a long informal use for the indication of pre-Linnean names.

This is a morphologically based study in which especial attention has been paid to the nature of the conidial scars and conidiophore branching, and there are coloured plates of the type species of the new genera and scanning electron micrographs of scars. Keys by the features of the fungi alone, and also by host, are provided. This is a carefully executed study in the best traditions of hyphomycete taxonomy and will facilitate and encourage future critical identifications in this ecological group of the genus.

## Miscellaneous

**Species Plantarum 250 Years: Proceedings of the Species Plantarum Symposium held in Uppsala August 22-24, 2003.** Edited by Inga Hedberg. 2005. Uppsala University Library, Box 510, SE-751 20 Uppsala, Sweden (e-mail: acta@ub.uu.se). Pp. 219. [Symbolae Botanicae Upsalienses Vol. 33 (3).] ISBN 91 554 6192 1. Price: 215 SEK.

Although only one of 20 contributions in this work has a fungal (actually lichen) flavour through its examples, attention is drawn to it here as these include important background information to the Linnaean literature and Linnaeus' way of working -- as well as some thought-provoking contributions on the future of botanical nomenclature. The contributors include the leading authorities on Linnaeus (e.g. Charlie Jarvis, Bengt Jonsell, Per Magnus Jørgensen, Walter Lack) and others at the cusp of heated debates on aspects of the future of biological nomenclature (e.g. Werner Greuter, Kevin de Queiroz). A final tranch of seven papers deals with inventorying the world's flora, and occasions reflection on the huge gap between the state of plant and fungal inventories.

**Biological Resource Centres and the Use of Microbes.** Edited by Nelson Lima & David Smith. 2003. Micoteca da Universidade do Minho, Centro de Engenharia Biológica, Campus de Gualtar, 4710-057 Braga, Portugal. Pp. 422. ISBN 972 97916 3 5. Price: 30 €.

This volume represents the proceedings of the 22nd European Culture Collections' Organization (ECCO) Meeting held in Braga, Portugal, on 17-19 September 2003. It is important that those involved in the curation and maintenance of genetic resource centres of fungus cultures keep abreast of both technical advances in preservation science and the increasingly complex worlds of databasing and legal or ethical issues. Such occasions facilitate such exchanges of views with those working with other organisms, from bacteria to human cell lines. Starting with a powerful opening contribution on the importance of *ex situ* collections by Manuel Mota, the papers presented in four symposia follow. These concern: The use of microbes, Microbial identification techniques, Bioactive molecules, and Biological resource centres. There are also reports from a round-table discussion covering the Global Biodiversity Information Facility (GBIF), activities of the World Federation for Culture Collections (WFCC), Accredited Biological Resource Centres, and the realm of acronyms. If you curate a collection of fungal cultures and would like to obtain a copy, prompt action is recommended as only 300 copies of the proceedings were printed.

## Nomenclatural novelties proposed in Mycotaxon 94

*Anthracoidea setosae* L. Guo, p. 47
*Anthracoidea xizangensis* L. Guo, p. 48
*Blumenavia toribiotalpaensis* Vargas-Rodriguez, p. 8
*Clavulinopsis antillarum* (Pat.) García-Sandoval & Cifuentes, p. 277
*Coltricia tsugicola* Y.C. Dai & B.K. Cui, p. 342
*Cordyceps spegazzinii* M.S. Torres, J.F. White & J.F. Bischoff, p. 257
*Dactylellina illaqueata*, D.S. Yang & M.H. Mo, p. 215
*Fomitiporia torreyae* Y.C. Dai & B.K. Cui, p. 344
*Hirsutella crinita* Z.Q. Liang, p. 352
*Hirsutella hunanensis* Z.Q. Liang, p. 351
*Hirsutella zhangjiajiensis* Z.Q. Liang & A.Y. Liu, p. 350
*Hymenochaete flexosetosa* Parmasto, p. 199
*Hymenochaete geniculata* Parmasto, p. 201
*Hymenochaete legeri* Parmasto, p. 203
*Hymenochaete nothofagicola* Parmasto, p. 212
*Hypocenomyce isidiosa* Elix, p. 219
*Hypoxylon kanchanapisekii* Suwannasai, Rodtong, Thienhirun & Whalley, p. 307
*Hypoxylon sublenormandii* Suwannasai, Rodtong, Thienhirun & Whalley, p. 307
*Hypoxylon suranareei* Suwannasai, Rodtong, Thienhirun & Whalley, p. 308
*Lecanicillium pissodis* Kope & Leal, p. 334
*Lepiota catenariocystidiata* Han C. Wang & Zhu L. Yang, p. 52
*Lepiota trichroma* Montoya & Bandala, p. 112
*Lepraria lobata* Elix & Kalb, p. 220
*Leprocaulon australasicum* Elix, p. 221
*Lewia chlamidosporiformans* B.S. Vieira & R.W. Barreto, p. 246
*Otidea crassa* W.Y. Zhuang, p. 366
*Otidea onotica var. brevispora* W.Y. Zhuang, p. 368
*Paecilomyces parvosporus* Y.F. Han & Z.Q. Liang, p. 360
*Pertusaria cyathicola* Elix, p. 223
*Pertusaria subarida* A.W. Archer & Elix, p. 134
*Ramaria luteoaeruginea* P. Zhang & Zhu L. Yang, p. 236
*Ramaria pallidolilacina* P. Zhang & Z.W. Ge, p. 238
*Russula mukteshwarica* K. Das, S.L. Mill., J.R. Sharma & R.P. Bhatt, p. 86
*Streptopodium passiflorae* (Syd.) Liberato & R.W. Barreto, p. 90
*Thyrostoma negundinis* (Berk. & M.A. Curtis) A.W. Ramaley, p. 131
*Tuber umbilicatum* Juan Chen & P.G. Liu, p. 2
*Vamsapriya* Gawas & Bhat, p. 150
*Vamsapriya indica* Gawas & Bhat, p. 150
*Weddellomyces turcicus* Halici & Orange, p. 249

# Author Index—Volume 94

## Reviewers, Volume Ninety-four

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers appearing in this volume.

Teuvo Ahti
T.V. Andrianova
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# Errata

## Volume 3

The new generic name *Burenia* is misspelled on pp. 1, 9-12, 46-47, and 570. It is an orthographic error, correctable under the International Code of Botanical Nomenclature. The name honors G. von Büren, misspelled Buren on pg. 11, but spelled correctly on pg. 48. All the Latin names cited should thus be corrected to *Buerenia*. We are advised that the correct spelling will appear in the 10th edition of *Dictionary of the Fungi* (CMI).

## Volume 91

| | | |
|---|---|---|
| pg. 28, ln. 11 | for: *Thecispora* | read: *Trechispora* |
| pg. 36, f. 5 caption | for: *Coltricella* | read: *Coltriciella* |
| pg. 129, ln. 4 | for: *P. redivivus* | read: *Panagrellus redivivus* |
| pg. 130, ln. 11 | for: *tenius* | read: *tenuis* |
| pg. 137, abstr. ln. 8 | for: *fomitiporoides* | read: *fomitoporoides* |
| pg. 144, ln. 8 | for: *fomitiporoides* | read: *fomitoporoides* |
| pg. 154, ln. 17 | for: *fomitiporoides* | read: *fomitoporoides* |
| pg. 224, ln. 18 | for: *segetum* | read: *segetum* ∂ |
| pg. 224, ln. 18 | for: *carbo* | read: *carbo* ∂ |
| pg. 224, ln. 19 | for: Wallr. | read: Wallr. ß |
| pg. 226, par. 3, ln. 2 | for: *versatilis* | read: *versatile* |
| pg. 250, ln. 5 | for: *tanganykeanum* | read: *tanganyikeanum* |
| pg. 362, ln.15 | for: *griseiviridis* | read: *griseoviridis* |
| pg. 363, ln. 2 | for: *griseiviridis* | read: *griseoviridis* |
| pg. 526, ln, 12 | delete *Pertusaria hueangensis* Jariangprasert, p. 283 | |
| pg. 526, after ln. 21 | add: PHAEOTREMATACEAE Popoff ex Piatek, p. 181 | |
| pg. 526, ln. 21 | for: *parvicarpum* ... 352 | read: *parvicarpum* ... 351 |
| pg. 527, ln. 2 | for: *namaqualandensis* | read: *namaqualandus* |

## Volume 93

| | | |
|---|---|---|
| p. 379, line 4 | for: *aurelio@castillo@uah.es* | read: *aurelio.castillo.uah.es* |

# Instructions for Mycotaxon Authors

*(Updated December 2005)*

Mycotaxon is an international mycological journal devoted to research on the taxonomy and nomenclature of fungi. Publication is open to everyone. Authors are responsible for obtaining *peer reviews* from experts in the field prior to presubmission of their manuscripts to the *Nomenclature* and *French Language Editors*. The *Editor-in-Chief* reviews all expert peer reviews, presubmission recommendations, and author submissions and prepares all files and hard copy for printing by *Sheridan Press*, which also handles reprints. Subscriptions, author invoices, and payments are handled by the *Business Manager*. Mycotaxon is indexed by the *Index Editor* and the *Webmaster* is responsible for maintaining the journal website on *www.mycotaxon.com*.

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Hard-copy submission is still accepted, but authors are asked to ask the Editor-in-Chief for additional guidance before submission. MYCOTAXON now charges a $5 per page/illustration scanning fee to digitize hard copy and is unable to scan artwork larger than 23 × 31cm (9 × 12").

### Clones: What are they and how are they used?

MYCOTAXON asks authors to submit text clones for nomenclatural and final editorial reviews. These 'clones' contain all formatted text that will appear in the published paper, but no illustrations, which should be submitted separately. The four text clone types are *master* (for review & final submission) and *body*, *legend*, and *table* (for final submission). Authors submitting preflighted PDFs approved by Sheridan Press for publication need not submit clones.

The **master** text clone contains ONLY text (*no* graphics) used during peer and nomenclatural review where MYCOTAXON masthead mockup, tables, legends, footnotes, breaks, and empty lines indicating graphics placement are all present. The **body** text clone is the master clone with masthead, empty paragraph lines, tables, footnotes, legends, and line, section & page breaks *removed*. The **legend** text clone contains all footnotes & legends with explanatory text for placing each footnote and illustration. The **table** text clone contains all formatted title+table+footnote text. (Two table text clones are submitted when tables have differing (i.e., landscape vs. portrait) orientations.) **Text clones must be correct!** The complete manuscript is used *only* as a placement guide for tables and figures. The clones are combined with illustrations to generate the final PDF press file.

> Pages 7–8 in the 'MYCOTAXON Instructions to Authors' PDF (which can be downloaded for free from www-mycotaxon.com website) explain in detail how to prepare clones. Authors are also encouraged to write the *Editor-in-Chief* for additional assistance if needed.

### Text must conform to the following specifications

**Print area size** is 11 × 17.5 cm (4.33 × 6.89"). The MYCOTAXON Word shell has been formatted for a US letter (A3) paper size with 5.25 cm top/bottom & 5.3 cm side margins. It also contains a mock-up of the MYCOTAXON logo and header for the first page. Those using other word-processing applications should follow the above guidelines, allowing 2.5 cm (1") for the title page masthead on the first page.

**Fonts & Paragraph formatting**—Authors are asked to use two basic font families: serif TIMES or TIMES NEW ROMAN (TNR)—and sans serif ARIAL or HELVETICA (HELV). Characters not displayed on keyboards (α, β, μ, ×) should be selected from the SYMBOLS menu if not available in the regular fonts. COURIER is used only when comparative columnar arrangement is essential (e.g., for DNA sequences). (All other fonts require editorial permission.) Lines *must* be single-spaced, and text formatted with 1 1/2 spaces between lines is *not* acceptable. Authors should use *paragraph format menu* options rather than hit the return key twice to separate paragraphs or stand-alone subheadings or use the tab key to indent paragraph first lines by 0.5 cm.

> Note: Standard elements of an article are the title, author address information, abstract, key words, main text, figure legends, acknowledgments, and references. The MYCOTAXON instruction PDF offers a properly formatted sample manuscript and includes other helpful manuscript preparation suggestions.

## Required formats

• **Title**—*Font:* Arial, 11-pt, Bold, sentence case (*never* upper or title); *Paragraph:* no full stop (dot) at the end (unless ending with an abbreviation), no indent, center aligned.

*Special:* Set Latin scientific names in ***bold italic.*** Titles should not exceed three lines. Author citations are not acceptable unless authors demonstrate why an authority is necessary to the title. Abbreviate genus names after the first use, but otherwise avoid abbreviations. Use arabic (not roman) numerals. *Example:*

**Studies in *Agaricales* 3:**
***Phaeocollybia phaeogaleroides* and *P. rifflipes*,**
**new western North American species**

• **Author names**—*Font:* Times, 10-pt, Roman (regular), 'SMALL CAPS'; *Paragraph:* no indent, center aligned.

*Special:* Given names and initials always stand before surnames. Separate authors with commas, but use '&' before the last author's name.

• **Address information**—*Font:* Times, 9-pt, Italic; *Paragraph:* no indent, center aligned, no periods at line ends; *Placement:* E-mail address (required*) on top line; Institution/Street on middle line; City, Code, Country on bottom line.

*Special:* *Junior author E-mail addresses recommended, but optional. Do not end lines with commas or periods (except for abbreviations).

• **Abstract**—*Font:* Times, 8-pt; set '**Abstract**' and '**Key words**' in bold and precede em-dash and remaining paragraph/list formatted in regular or italic as needed. *Paragraphs:* 1 cm right & left margins, no indent, fully justified.

*Abstract:* Abstracts briefly summarize the content & conclusions and list all new taxa (but not authorities unless differentiating homonyms). English abstracts are required; 1–2 abstracts in other languages are permitted for longer articles. Abstracts should not exceed 15 printed lines.

*Key words:* Up to five key words or phrases are permitted. Do not repeat terms already used in the title or abstract. Separate list items with commas. Capitalize only proper nouns.

• **Subheadings: Primary (stand-alone)**—*Font:* Arial 10-pt, Bold/Bold Italic; *Paragraph:* no indent, center aligned. **Secondary (stand-alone)**—*Font:* Times 10-pt, Bold/Bold Italic; *Paragraph:* no indent, left aligned. Arial 10-pt regular (roman) and Arial 9-pt, Bold/Bold Italic are optional secondary subheadings.

**Taxonomic (special)**—*Font:* Times; *Size & Style:* **Latin name**—10-pt Bold Italic, **Author citation**—9-pt regular; **'nom. nov.' & 'Fig. #'**—9-pt bold. *Paragraph:* hanging indent, left aligned; set right tab to 11 cm and use tab to justify Fig. # at right.

• **Basic text**—*Font:* Times 9-pt; *Paragraphs:* fully justified. Select 6-pt gaps or 0.5 cm first line indents to separate paragraphs (*not* both).

Introduce your subject briefly by citing significant background literature. Provide necessary abbreviations and other data in a brief Materials & Methods section (8-pt font permitted here). Document your own observations concisely, stressing new discoveries. Minimize confusion between generic epithets beginning with the same initial letter by abbreviating one using the first two letters of the generic name. Italics are required for Latin scientific names of taxa up through and including order or reserved for emphasis; common Latin abbreviations (e.g., i.e., inter al., etc.) and references are **not** to be italicized. List author citations *once* only: in the taxonomic heading where a new taxon is proposed *or* where first mentioned in the text. Authors should list previously misidentified taxa as 'misapplied' *not* 'sensu + author + reference.'

**Required references:** Consult the current INTERNATIONAL CODE OF BOTANICAL NOMENCLATURE <http://www.bgbm.fu-berlin.de/iapt/nomenclature/...> when describing new taxa or proposing new combinations. Author citations *must* follow either the updated Kirk & Ansell's AUTHORS OF

FUNGAL NAMES <http://www.indexfungorum.org/Names/Names.asp> or INTERNATIONAL PLANT NAMES INDEX <http://www.ipni.org/ipni/query_author.html>. Consult INDEX HERBARIORUM <http://www.nybg.org/bsci/ih/searchih.html> for herbarium/collection acronyms.

Authors describing new taxa *must* cite relevant acronyms and numbers to facilitate retrieval by readers *and* deposit new names/data in **MycoBank** <http://www.mycobank.org>, type specimens in an official public **herbarium**, ex-type strains in a public **culture collection**, and/or sequence data in **GenBank** <http://www.ncbi.nih.gov/Genbank/submit.html>.

• **Latin diagnoses, nomenclators, and 'Specimens examined'**—*Font:* Times, 8-pt; *Paragraph:* margins indented ~1cm, fully justified with first line flush. *Special:* Italicize all Latin text in diagnoses but only Latin scientific names in nomenclators & 'Specimens examined.'

• **Acknowledgments**—*Font:* Times, 8-pt. *Paragraph:* no indent, left aligned, fully justified. *Special:* Acknowledge or thank pre-publication peer reviewers here.

• **Literature cited**—*Font:* Times, 8-pt; *Paragraph:* Hanging indent, no line spaces between entries. **Required:** All references must be cited in the main text. Follow a consistent citation style throughout. Never use more than one space between entries. *Author names:* substitute initials (no periods after initials) for given names, order surnames before initials and do not separate with commas, separate author entries only by commas and never by "&" or "and", and place a period (dot) after the last author entry. Standardize journal abbreviations.

*Examples* (consult also MYCOTAXON sample manuscript in Instructions PDF):

Kirk PM, Cannon PF, David JC, Stalpers JA. 2001. Ainsworth & Bisby's dictionary of the fungi. CAB International, Surrey UK.

Senior AA, Junior BB. 1997. Title with *Latin name* here. MYCOTAXON 56: 254–272.

**Useful references:** See BOTANICO-PERIODICO-HUNTIANUM (BPH: Lawrence & al. 1968), BOTANICO-PERIODICO-HUNTIANUM/SUPPLEMENTUM (BPH/S: Bridson 1991), TAXONOMIC LITERATURE, (TL: Stafleu & Cowan 1976-1988), and SUPPLEMENTS TO TAXONOMIC LITERATURE (TL2: Stafleu & Mennenga 1992-1995) for recommended abbreviations.

## Pay particular attention to the following

**Hyperlinks**—*Disable* hyperlinks in all clones. Clones with active hyperlinks will be returned to the author for correction. Be aware that text imported from PDFs or websites frequently retains the original hyperlinks or appear in foreign [unrecognizable] fonts during the press conversion process. When in doubt, authors should retype the text themselves.

**Italics & underlines**—Reserve *italics* only for Latin taxonomic names or emphasis. Names of all taxa from subspecific to ordinal levels *must* be italicized; using italics for higher-level names is at the author's discretion. Do not italicize common 'Latin' abbreviations (e.g., et al., etc., inter al.) or reference titles. Never underline text intended for publication.

**Symbols**—Generate diacritical marks and symbols (e.g., ã, ñ, µ, ×) by using special keystrokes (e.g., "option+m" for µ) or by inserting from the 'symbols' menu. When possible, select symbols from the corresponding text font; in Word insert 'µ' via the Insert > Symbol > Times pathway. Some symbols (e.g., '×') exist only in the 'Symbols' *font*. Authors should list symbols inserted from the Symbols (instead of the Times/TNR menu) on the submission form.

**Punctuation**—Place commas, periods, and other punctuation in the same font style as the word directly preceding them. For instance, commas stand in italics after words in *italics*, in bold after words in **bold**, and in bold italic after words in ***bold italic***. However, the closing mark of paired marks such as parentheses, square brackets, quotation marks, single quotation marks, and long dashes exhibits the same font style as its companion open mark, *even* when preceded by a word in a different style. With the exception of the **sanctioning colon** (e.g., Fr. : Fr.), no space stands between

a punctuation mark and the preceding text. Likewise, no space stands between an initial paired mark (e.g., open parenthesis) and the first word. In computer-formatted manuscripts, only one space follows a full stop at the end of a sentence.

**The *hyphen*, *en-dash*, and *em-dash* have different uses**—The *hyphen* separates word elements and breaks at line end. Do not introduce hyphens to long words in text clones as they may appear in midline after PDF conversion. The *en-dash* (keyed on a 'Mac' using 'option+dash') is longer, and does not break at line end. Use an en-dash with spaces for 'minus' in mathematical notation and without spaces in range expressions: the en-dash in '8–10' keeps the '8' and '10' together on one line. The *em-dash* ('shift+option+dash' on a Mac) is the longest and—generally—is reserved for emphatic clauses that are otherwise enclosed in parentheses. No spaces are used.

**Spelling and grammatical errors**—*All* co-authors should proofread text for spelling, typographic, and grammatical errors; grammar and spell checkers can also be helpful. The two most common spelling errors are still (i) misspelling authors' own names & addresses (!) and (ii) spelling names of taxa one way in the text and another way in figure captions.

Non-native English speakers *must* ask someone fluent in English or French to proofread the paper before *each* review (peer and nomenclatural) and again before final submission. Additional attention should be given to Latin diagnoses, which should be brief. Diagnoses that list only the key characters separating a proposed new taxon from closely related ones generally contain fewer errors than more complicated descriptions. Save details for the technical description.

## 4. Preparing illustrations

*Color option:* Those wishing to publish color illustrations should correspond with Sheridan Press before final submission. Current press charges are $475 per color page.
*Returns:* MYCOTAXON will return original illustrations to authors who include a self-addressed envelope that is *stamped* or submitted with sufficient *International Postal Reply Coupons*, with their final submission.

### Size & placement

All articles published in MYCOTAXON begin on an odd-numbered (right-hand) page. It is preferable to place a figure on a right-hand page facing the text first referring to the illustration. Group figures to facilitate species comparisons and keep page numbers to a minimum. Do not border or edge photographs and drawings with a line. Illustrations are best formatted at publication size. Plan to place figure legends below figures (preferred) or at the bottom of pages *facing* illustrations.

### Line drawings

Authors may submit originals, digital images prepared from scanned originals, or high quality reduced photocopies of original artwork. Authors with access to computer scanners and photographic software are encouraged to digitize original drawings themselves.

**Digital files**— Digital files that incorporate fonts or vector information should be submitted as 1200dpi TIF (in bitmap mode) or 900dpi EPS files. Simple line drawings can be scanned using software such as ADOBE PHOTOSHOP®, but more sophisticated illustration software (e.g., ADOBE ILLUSTRATOR®) should be used to convert phylotrees and other drawings containing vector information to EPS format. Image adjustments — e.g., insertion of scales, numbers, and arrows — should be handled in such applications. Remember that digital figures should *not* include legends, which belong in the legend text clone.

**Hard copy**— Authors who submit digital text with hard copy artwork may include photocopies within a manuscript printout to indicate figure placement; however, they should also submit line

drawings separately *without* legends. Authors who submit exclusively hard copy materials should position line drawings onto existing text pages *with* captions printed directly below or to the side of each drawing. With care, authors generally scale down oversized drawings using the reduction feature on photocopiers without decreasing image quality and assemble an attractively arranged plate from the cut & paste illustrations and captions.

### Photographic 'halftones'

MYCOTAXON prints only grayscale ("black & white") photographs, but authors who submit film-based (not digital) prints may submit color photos for conversion to grayscale if necessary. Both grayscale and color photographs have a "continuous tone" requiring transformation into a separate *halftone image* before they can be printed for publication. (A halftone image consists of many variously sized and spaced dots, which are easily seen using a hand lens.).

**Digital grayscale halftones**—Photographic software such as ADOBE PHOTOSHOP® allows preparation of compressed JPG files (useful for peer review) and TIF files (non-compressed files used in journal publications). TIF or JPG halftones must be set in *grayscale* mode with a minimum resolution of *300-600 dpi* (dots per inch) matching the target width, generally 4.33" (11 cm). Authors who group photographs into plates should ensure that the contrast among individual photographs is not too great; individual photographs within plates may be set flush or separated by a 1-2mm white or black band. Authors who prepare their own PDFS for printing may insert both line and photographic art using publishing software (e.g. INDESIGN®).

**Negative-based photographs**—To prepare negative-based photographic plates, crop individual photographs so that edges touch and the plate is exactly 4.33" (11 cm) page wide. Mount photographs on separate sheets of heavy white paper or matte board. Apply annotations (arrows, numbers, scales, etc.) and band strips after the photographs are positioned and be certain to allow space around the photograph for editorial markings. NOTE: Authors must **not** submit halftone prints prepared from digital files as hard copy intended for publication; they should submit instead grayscale computer files formatted as described above.

### Labeling

Title all graphic files (JPG, TIF, EPS) with the *first* author's name and *figure* number (e.g., *name_fig#.tif*). Label all original artwork hard copy with the first author's name and figure number on the **reverse** side.

### Final note about digital images

The Editor-in-Chief is able to convert a good many different file formats quickly to TIF or EPS files in ADOBE PHOTOSHOP®. Therefore, there are no fees for converting PHOTOSHOP, BMP, JPG, PCX, PICT, PIXAR, PNG, SCITEX CT, or TARGA files, provided they open in PHOTOSHOP in proper grayscale/bitmap mode and with the required resolution. A $5 fee is charged for processing each MSWORD embedded mega- or picture file through ILLUSTRATOR® before press.

The Editor-in-Chief cannot improve the quality of a bad image. Authors should submit as clear and clean an image as possible but must remember that, in science, reality supersedes beauty. Do **not** 'over-photoshop' or otherwise distort images.

Authors who do use artwork to enhance certain features within a photograph must explain what has been added in the accompanying figure legend.

## 5. Peer review

MYCOTAXON is unusual among scientific journals in that authors are expected to obtain their own peer reviews *before* submission by contacting two scientists in their field but *outside* the senior author's home institution. When authors are uncertain whom to approach for manuscript review, they may send their title and abstracts to the Editor-in-Chief for the names of suitable reviewers. Although both MYCOTAXON Nomenclature Editor and Editor-in-Chief will review each manuscript and may solicit outside reviews when necessary, English grammar, nomenclature, and author citations must have first been thoroughly checked by at least one of the two peers. Authors whose first language is not English are urged to contact a native English-speaking expert to serve as one peer-reviewer.

The corresponding author sends each reviewer the Reviewer Guidelines & Checklist document file with one of the following: a master text clone with individual JPG files, a MSWORD manuscript with embedded figures, or a printed manuscript with photocopied figures. (Guidelines and checklist can be downloaded from <http//:www.mycotaxon.com> or E-mailed by the Editor-in-Chief). After review, each expert returns annotated text to the corresponding author and sends the formal checklist and brief comments to the corresponding author and the Editor-in-Chief.

Authors should revise manuscripts following reviewer suggestions and must explain during final submission which recommendations were not followed and why. They may also ask experts for additional guidance during the post-nomenclatural review revision and may add reviewers who make major improvements as co-authors. The Editor-in-Chief acknowledges all peer reviewers in the closing pages of each volume, but authors should also thank peer reviewers in their own acknowledgements.

## 6. Nomenclatural and French Language Review

After peer review, all authors must E-mail their master text clone file to the MYCOTAXON *Nomenclature Editor,* who reviews text for adherence to the International Code of Botanical Nomenclature and standardization of author citations. Manuscripts written in French should be sent to the *French Language Editor* at the same time. These editors determine whether a manuscript is ready for final submission and return annotated clones with a list of needed corrections to the authors, peer-reviewers, and Editor-in-Chief. Graphics files are to be sent only by editorial request. Be certain to write 'MYCOTAXON review: [Senior author surname] [genus/order name] [date]" in E-mail subject headers.

## 7. MYCOTAXON final submission

Final submission materials should be sent to the Editor-in-Chief. Materials may be **E-mailed** or sent by **registered airmail** (*signature required*). (Ask permission before E-mailing messages & attachments exceeding 10 mgb.) Authors who intend to submit print-ready manuscripts for scanning should contact the Editor-in-Chief before preparing their final submission. The following items must be received before final manuscript review is begun by the Editor-in-Chief:

**Two expert peer reviews**—*[sent by reviewers to the Editor-in-Chief]*

**Presubmission reviews from the Nomenclature Editor** (& **French Language Editor**, when the submission is in French)—*[sent by Editors to Editor-in-Chief]*

**Manuscript Text**—[a] One PDF/document file *OR* a manuscript printed to show approximate figure placement. Print hard copy on only one side; pages may be stapled and have author + page number headers *unless* intended for scanning. [b] **Master text clone and body, legend, & text clone** document files as needed. (Consult 'Text & text clones' above and the posted MYCOTAXON author instruction PDF for more information.)

**Illustrations**—***Digital:*** E-mail TIF or EPS files (preferred) or airmail on CDs. Other graphic formats are also accepted, but fees are accessed for time-consuming file conversions. ***Hard copy:*** See instructions above. There is a $5 per page or figure to be scanned.

**Brief cover letter accompanied by MYCOTAXON submission form**—The submission form can be downloaded from the Mycotaxon webite.)

Consult the **Author's Checklist** below before sending the final submission. Remember to prepare back-ups to safeguard against loss or damage to files in transit.

## 8. Author Checklist

All authors are responsible for providing error-free, properly formatted text and will benefit by downloading the MYCOTAXON Word shell and the expanded Instructions for Authors PDF files from <http//:www.mycotaxon.com/instructions.html >. Those submitting press-PDFs and photo-ready hard copy are also both *designer* and *printer* of their paper and acknowledge that the Editor **cannot** change submitted text. Authors who do not understand instructions are urged to ask their peer reviewers or the Editor-in-Chief for help!

**• Text format (very important)**

— Fonts and paragraphs all conform to *MYCOTAXON* requirements (see above).

— The backs of camera-ready manuscript pages are numbered and labeled with a soft pencil.

— The ***body clone*** file contains no empty paragraph lines, initial tab settings, line breaks (main title excepted), or graphics files. All footnotes, legends, and tables are removed.

— The ***legend clone*** file contains only footnotes & figure legends, each accompanied by instructions noting on which page to place the item in the final manuscript.

— All tables are placed in one ***table clone.*** If both 'landscape' and 'portrait' tables are present, they may be placed in two clones according to orientation.

— The **title** is in Arial 11-pt **bold** (Latin names in ***bold italics***), sentence-case, and does not end in a full stop (abbreviations excepted). Taxonomic authorities are not included.

— Manuscript author **names** are centered & in Times, 10-pt roman (regular), 'SMALL CAPS.'

— **Addresses** are centered, in Times, 9-pt *italic*, with **E-mails** at top with hyperlinks disabled, **institutional** information in the middle, and **Street -> Country** information at bottom.

— The **Abstract** and **Key words** are centered with margins indented by 1-cm and in Times, 8-pt font. '**Abstract**' and '**Key words**' are in bold and other text is in regular except for italicized *Latin names*. The key words list contains only five terms separated by commas, does not repeat words in title or abstract, and does not end with a full stop.

— ALL text is single-spaced. Leading is 10-pt for paragraphs containing 8-pt font.

— **Latin scientific names** for all taxa from subspecies through order are *italicized.*

— **Author citations** conform to INSI or Kirk & Ansell, occur only once for each taxon, and have been checked by the Nomenclature Editor and at least one peer reviewer.

— No commas stand between author names and (first) dates in **text references.**

— **Literature cited** references follow a consistent order, conform to MYCOTAXON guidelines, have hanging indents, and are in 8-pt Time/TNR.

**• Illustrations**

— **Graphics** are ≤ 11 × 17.5 cm ***or*** authors have editorial permission to send oversized plates.

— **Photographs or composite plates** fill the entire width of the page (except where text or legend takes part of the width)

— **Digital halftones** are in TIF format, grayscale mode, and 300-600 dpi per 4.33" width.

— **Figures** submitted for scanning are mounted separately from the text on heavy stock and are clearly numbered & labeled with author name & brief title on the reverse side.
— **Legends** are separate from artwork. They do not contain taxonomic author citations.
— The corresponding author has **dated backup copies** of all materials submitted.

**• Final submission items**

— *Peer review checklists* & comments have been sent by two expert reviewers to MYCOTAXON.
— *Master text clone* has been reviewed by the Nomenclature Editor.
— *Master text clone* written in French has been reviewed by the French Language Editor.
— Final *master text and body clones* (with *legend & table clones* as needed)
— *Illustrations* digital files of proper resolution or hard copy
— PDF / MSWord file or printed *manuscript* showing figure placement
— Brief cover letter and completed MYCOTAXON *submission form*

## 9. MYCOTAXON corrections policy

MYCOTAXON has always expected authors to submit manuscripts that are free of errors and ready to publish when they arrive on the editorial desk. *All* coauthors are assumed to have viewed and approved text prior to submission. Immediately after final editorial approval, all must again double-check all files to return error-free clones to the Editor-in-Chief for processing.

The Editor-in-Chief processes clone and graphics files using ADOBE INDESIGN®. Text may shift during this process, but InDesign generally distributes text evenly and displays few unsightly gaps in justified lines. Authors receive a pre-publication PDF for final approval and so will have a chance to correct errors introduced by PDF conversion.

Correction of author errors after PDF conversion is costly and time-consuming: MYCOTAXON now charges a *minimum* of **$10** *per error* to correct mistakes present in approved clones and **$60** per error to correct mistakes discovered after the files have been sent to press. There is **no** charge for correction of author errors when authors send corrected clones to the Editor-in-Chief before the PDF-conversion process is begun.

MYCOTAXON corrects errors resulting from computer or editorial error free of charge. [Errors present in approved clones are considered author and *not* conversion errors.] MYCOTAXON also lists, free of charge, corrections of all published errors in the Errata.

## 10. Ordering reprints or downloadable PDFs

MYCOTAXON sends all authors a print-quality PDF file before publication and one copy of the printed article to the corresponding author immediately after publication; a local printer can make high quality reprints from either PDF or print. A PDF explaining how to order 100+ reprints or arrange for Sheridan Press to host the author's PDF file on its website can be downloaded from <http://www.mycotaxon.com/authors/reprints.html>.

## Still have questions?

Contact the *Editor-in-Chief* at **editor@Mycotaxon.com**
OR
Download the 'MYCOTAXON Instructions' to Authors PDF and
check the FAQ on our website at
<http://www.mycotaxon.com>

# EDITORS OF MYCOTAXON




MYCOTAXON is a quarterly, peer-reviewed journal devoted to mycological taxonomy and nomenclature. All articles are reviewed by specialists prior to submission. Publication is open to everyone. Papers may be in English or in French. Summaries in those or any additional languages desired by the authors are given for longer articles. Printing is on high quality, acid-free book paper. Authors prepare their own *text-ready* files after having received critical comments from pre-submission reviewers before undergoing additional presubmission review by the Nomenclature Editor. *Instructions to Authors* (Mycotaxon **90:** 495–507, 2004) and *Guidelines for Reviewers* (Mycotaxon **78:** 539–540, 2001) are updated on our website at <www.mycotaxon.com>.

***SUBSCRIPTION INFORMATION***

Current volumes contain at least 400 pages. All subscriptions are on an annual basis, with four quarterly volumes each year. Personal subscriptions are available at a substantially reduced rate for those who agree not to deposit their copies in a library other than their personal one within 3 years of receipt. Address all orders to the MYCOTAXON ORDER DEPARTMENT, P.O. Box 264, Ithaca, NY 14851-0264 USA, or your agent. Prices and availability of back volumes are posted on-line at <www.mycotaxon.com> or can be provided on request by Email from <info@mycotaxon.com>. Mycotaxon may be obtained on a journal-exchange basis. Proposals for such exchanges from journals, institutions, or individuals with difficulty in obtaining foreign currency should be addressed to the Order Department.

The annual subscription rates for 2005 & 2006 (four volumes annually) are

| | USA | Foreign (IMEX air assist) |
|---|---|---|
| REGULAR (multi-user) | $330 | $370 |
| PERSONAL (individual) | $150 | $190 |

*All back volumes [except volumes 22, 24, 34(2), 35, 41, 54, and 64, currently out of print] are available at $35 each by surface mail, $55 each by air mail. Volumes 1, 38, 39, 43 and 46 were reprinted in 2001; the original printing of volume 1, part 1 is provided, with parts 2 and 3 reprinted.*

***CUMULATIVE INDICES***

Printed versions of the *Mycotaxon Cumulative Indices* for Volumes 1-20 (ISBN 0-930845-00-5), Volumes 21-40 (ISBN 0-930845-01-03), and Volumes 41-60 (ISBN 0-930845-07-02) are available (see descriptions at <www.mycotaxon.com> and order there or through your local bookstore's listings under "Books in Print"). An online *Cumulative Index to Taxa in Volumes 61-ff.* and the *Cumulative Author Index to Volumes 61-ff.* are posted at <www.mycotaxon.com>.

***AVAILABILITY IN MICROFILM, PHOTOCOPY, & ELECTRONIC VERSIONS***

MYCOTAXON is also partially available in *microfilm* from National Archive Publishing Company <www.napubco.com>. *Tear sheets* or *photocopies* of individual articles may be obtained from ISI Document Solution <www.isidoc.com>. Many back volumes are available as free downloads through Cyberliber <www.cybertruffle.org.uk/cyberliber/index.htm> under 'Catalogues & Journals.'

***CONTACTING MYCOTAXON'S EDITOR-IN-CHIEF BY E-MAIL OR FAX***

To reach the Editor-in-Chief regarding manuscripts, E-mail to <editor@mycotaxon.com> or Fax to Lorelei L. Norvell at +503.297.3296.

***CONTACTING MYCOTAXON'S ORDER DEPARTMENT BY E-MAIL OR FAX***

To reach the Order Department for information or placing orders, you may E-mail to <info@mycotaxon.com> or you may Fax to Orders, Mycotaxon, Ltd., May–October at +607.273.4357, and November–April at +727.321.1460.