Cryptogamie, Mycologie, 2006, 27 (4): 1-•••

A new species of *Orbilia* from China

*Y. ZHANG, Z.F. YU, M. QIAO & K.Q. ZHANG**

Laboratory for Conservation and Utilization of Bio-resources
Yunnan University, Kunming 650091, P.R. China

Abstract – A collection of *Orbilia* was found on rotten wood in Xishuangbanna County, Yunnan province, China. It is characterized by cylindrical-clavate ascospores, slightly twist, tapered and rounded to the apex, with ellipsoid to rod-shaped spore bodies, which was not described within the known *Orbilia* species. Furthermore, the ITS sequences of ribosomal DNA of related *Orbilia* species were compared. We report it as a new *Orbilia* species according to combination of morphology and rDNA sequence analysis.

***Orbilia* / new species / ITS sequence**

INTRODUCTION

Orbilia Fr. was established for *Peziza leucostigma* by Fries (1849). The *Orbiliaceae* that was originally placed into the *Helotiales* includes three genera: *Orbilia* Fr, *Hyalinia* Boad, and *Patinella* Sacc (Nannfeldt, 1932), where the marginal hairs of apothecia and the shape of paraphyses apices were the basis of genus classification. Besides, the color of apothecia, as well as the size and shape of ascospores and paraphyses were combined to species identification (Seaver, 1951; Haines & Egger, 1982; Spooner, 1987; Korf, 1992). Then, a new concept of living fungal classification was advanced by Baral (1992). Subsequently, Baral (1994) divided *Orbilia* into two subgenus: *Hemiorbilia* and *Orbilia*. Moreover a small different group around *Orbilia inflatula* described that time was then classified to a new genus *Hyalorbilia*, the genera *Orbilia* and *Hyalorbilia* were only accepted within *Orbiliaceae* (Baral & Marson, 2001). According to Baral's unpublished work "World monograph of Obiliomycetes (Ascomycota)", the characters such as shape of ascospores, apical wall structure of asci, type and arrangement of spore body (SB)'s within living ascospores and type of ectal excipulum were thought to be more reliable to species delimitation (Baral pers.comm). In our research, we found a species of *Orbilia* in the southwest of China in July, 2004. Since it is an undescribed species, here we describe it as a new species of *Orbilia.*

MATERIALS AND METHODS

The fresh specimen of *Orbilia* was collected from Xishuangbanna County, Yunnan Province, China in July, 2004. The living asci, paraphyses and ascospores were observed and measured directly from the tap water mount of

* Corresponding author: K.Q. Zhang. Tel.: 86 871 5034878. E-mail address: kqzhang111@yahoo.com.cn

fresh collections. The size of asci, ascospores, spore bodies and paraphyses were obtained after measuring 50 randomly. Rehydrated specimens were sectioned longitudinally using a Leica freezing microtome. An Olympus BX51 microscope and an Olympus zoom stereo microscope were used to take micrographs.

DNA was extracted from hydrated apothecia according to the method of Walsh *et al.* (1991) with modification, which allows for the extraction of DNA for polymerase chain reaction (PCR). For each individual specimen, 200 µL of a 5% weight-tovolume mixture of Chelex-100 ion-exchange resin and ddH_2O were placed in a microcentrifuge tube with 5-8 apothecia. The specimen was ground into the mixture and incubated for two hours at 56°C then 12 min at 99.9°C. The samples were then centrifuged for 5 min at 14,000 rpm. The top 50 µL of the supernatant was drawn off and aliquots used directly for PCR.

Primer pairs ITS5 & ITS4 (White *et al.,* 1990) were used to amplify the complete ITS (including 5.8S). The parameters for PCR amplifications are as follows: 1 min initial denaturation at 94°C, followed by 30 cycles of 1 min denaturation at 94°C, 1 min primer annealing at 50°C, 90 s extension at 72°C, and a final extension period of 10 min at 72°C. The purified PCR products were directly sequenced on both strands with the same primers that were used for amplification.

DNA sequences were aligned with additional sequences obtained from GenBank using ClustalX 1.83 (Thompson *et al.,* 1997) and adjusted manually using BioEdit sequence alignment editor. Parsimony analysis was run in PAUP* 4.0b10 (Swofford, 2002), with the following settings: gaps treated as missing characters, all characters equally weighted, using heuristic searches with TBR (tree-bisection-reconnection) as branch-swapping algorithm, initial "MaxTrees" setting at 100; bootstrap values were generated using the settings 1000 replications.

TAXONOMY

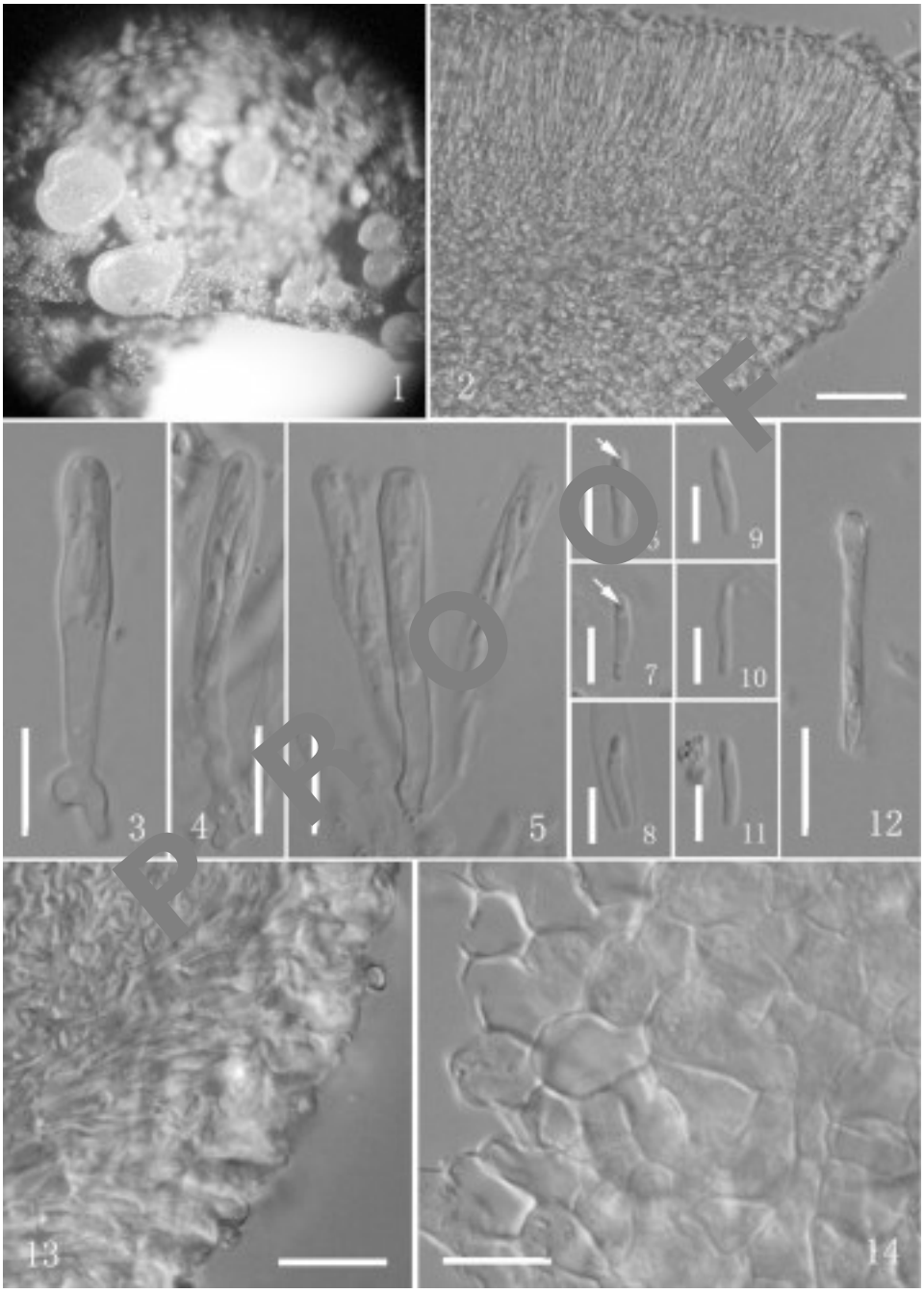
Orbilia bannaensis Y. Zhang, Z.F. Yu et K.Q. Zhang sp.nov. Figs 1-14

Apothecia 0.2-0.6 mm in diam, superficialia, sessilia, gregaria, translucentia. Asci 8-spori, 31.2-46.0 × 3.0-5.0 µm, cylindrico-clavati, apice truncati vel rotndati, otospori ad basin angustati. Ascosporae hyalinae, non-septatae, 7.4-9.5 × 1.3-1.8 µm, cylindri-coclavatae. Incliusione ellipsoideae vel ovata, 1.3-2.3 × 0.5-1.0 µm. Paraphyses filiformes, apicaler vix inflatae, 2.5-4.3 µm in diam. Excipula ectale textura prismatica vel textura angulari.

Etym.: The species *Orbilia bannaensis* was named after Xishuangbanna county, Yunnan Province, China, where it was first collected.

Holotype: OT003, collected from Xishuangbanna County, Yunnan Province, China in July, 2004, deposited in the Laboratory for Conservation and Utilization of Bio-resources of Yunnan University.

Apothecia often gregarious, sessile, waxy, superficial on rotten cortex of *Broussonetia*, usually 0.2-0.6 mm in diam (Fig. 1). Disc reddish throughout when dry, margin slightly inrolled, but transluant and orange when rehydrated, smooth, plane or undulating, margin even (Fig. 2). Asci 8-spored, 31.2-46.0 × 3.0-5.0 µm, cylindrical-clavate, sometimes slightly curved at the middle part, tapered and sometimes forked at the base, apex truncature to rounded (Figs 3-5). Ascospore



Figs 1-14. ***Orbilia bannaensis***, holotype. (OT003) 1. Fresh apothecia. 2. Vertical section of an apothecium. 3-5. Living asci. 6-11. Ascospores with SBs. 12. Paraphyse. 13. Margin of ectal exvipulum. 14. Ectal excipulum cells. Bars: 2 = 20 µm, 3-5, 12-14 = 10 µm, 6-11 = 5 µm.

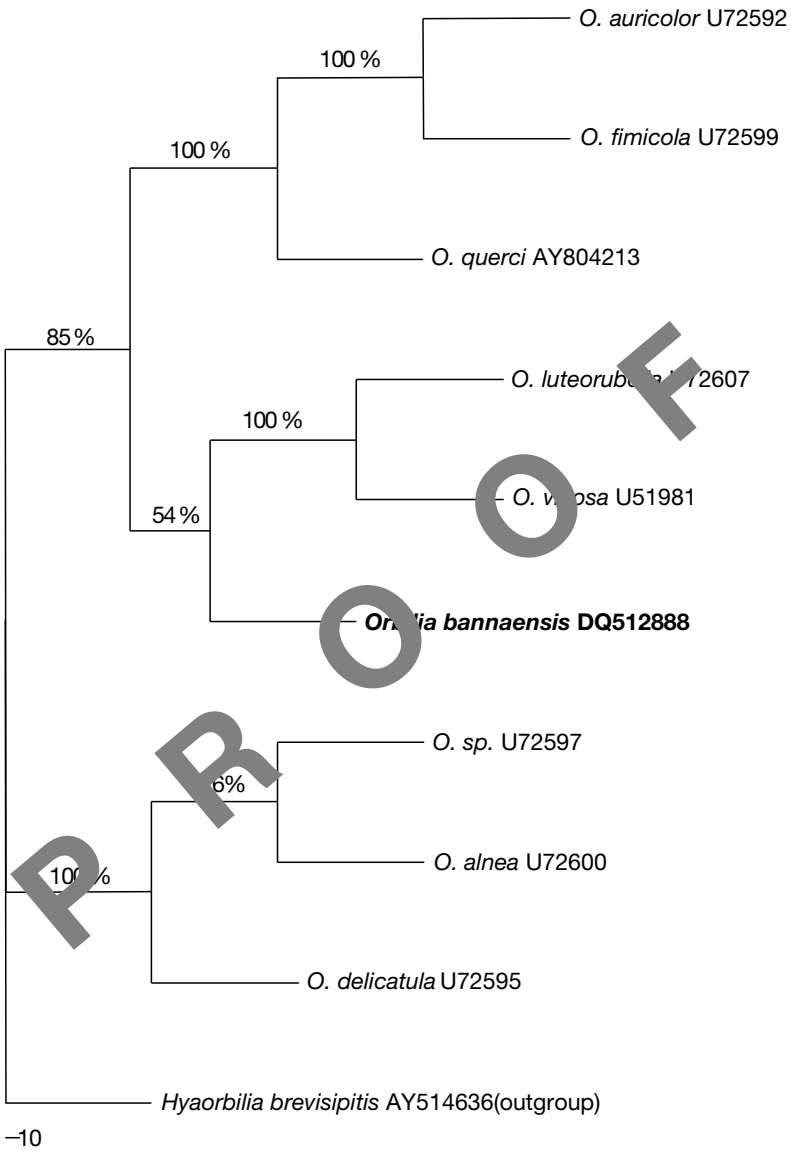


Fig. 15. Most parsimonious phylogenetic tree generated from a heuristic search based on the alignment of the ITS region sequences of *Orbilia* spp. Numbers above lines represent bootstrap values from 1000 replicates on all parsimony-informative characters, with only bootstrap > 50% shown. Tree length = 580, consistency index (CI) = 0.8121, homoplasy index (HI) = 0.1879, retention index (RI) = 0.7630, rescaled consistency index (RC) = 0.6196. Of 539 total characters: all characters have equal weight, 241 characters are constant, 94 variable characters are parsimony-uninformative, number of parsimony-informative characters = 204. The accession number of sequences gained from GeneBank are demonstrated.

hyaline, non-septate, 7.4-9.5 × 1.3-1.8 μm, cylindrical-clavate, slightly twist, slightly tapered and rounded at both ends (Figs 6-11), sometimes with 1-2 lipid bodies (LB), 0.5 μm in diam, bearing a refractive SB at proximal end (Figs 6, 7), ellipsoid to rod-shaped, 1.3-2.3 × 0.5-1.0 μm, fully fill the apex of ascospore at one end. Paraphyses hyaline, septated, not or slightly enlarged at the apex to 2.5-4.3 μm diam, and filliform with a clavate which is 2.0 μm wide (Fig. 12). Ectal excipulum composed of globose to subangular isodiametric cells, with thin or only slightly thickened walls, often containing orange pigment, 5.8-15.8 μm in diam (Fig. 14), the margin of ectal excipulum of rows of elongated subglobose cells, with swollen and rounded apex. (Figs 2, 13). Medullary excipulum composed of subglobose cells, approximately 15.0-32.0 μm thick.

DISCUSSION

So far, although more than two hundred species of *Orbilia* have been recorded, many species were not reviewed and the classification was still difficult because comprehensive monographic treatments based on combination of morphology and DNA sequence analysis were not produced. To get rational identification of our species, we performed the studies of both.

The ITS1-5.8s-ITS2 region of *O. bannaensis* was approximately 579 bp in length. The maximum parsimony analysis demonstrated that *O.sp*, *O. delicatula* and *O. alnea* formed a clade with 100% bootstrap support, and *O. vinosa* and *O. luteorubella* formed another 100% bootstrap valued clade, *O. auricolor* and *O. fimicolor* claded together with 100% bootstrap value. The result was identified with Hagedorn and Schooller's (1999). Our fungi formed a sister clade with the group comprising *O. vinosa* and *O. luteorubella* with 54% bootstrap value and nested in the monophyletic group containing species all of which were now assigned to the genus *Orbilia*, which supports our morphological classification that it belongs to the genus *Orbilia*.

According to Baral's viewpoint, the SBs within living ascospores became the key character of taxonomy of Orbiliomycetes (Baral pers.comm). In our morphological study of *O. bannaensis*, we found the cylindrical-clavate shaped ascospores appears to be similar to *O. regalis aff* (Baral pers.comm) and *O. queri* B. Liu, X.Z. Liu & W.Y. Zhuang 2005 (Liu *et al.,* 2005), while the shape of SBs within it are much more different with *O. regalis aff*. For *O. bannaensis*, the SBs are ellipsoid to rod-shaped, but those of *O. regalis aff* are tear-shaped. Although the SBs of *O. queri* are short – rod shaped also, ITS region of rDNA comparison indicated that there was only 39.6% similarity between them and they were monophyletically distant in the phylogenetic tree, thus they were distinguishable species. Moreover, the ascospores of *O. bannaensis* are slightly twist, which is not found within *O. regalis aff* and *O. queri*. Because of these features, *O. bannaensis* can fully be combined to distinguish it from other species within the genus *Orbilia*.

Acknowledgments. We sincerely thank Prof. HO Baral for his unpublished work "World Monograph of the Orbiliaceae (Ascomycota)" and for his help to identify this species. This research was supported by the Science and Technology Department of Yunnan Province, China (2005NG05, 2005NG03).

REFERENCE

BARAL H.O., 1992 — Vital versus herbarium taxomony: morphological differences between living and dead cells of Ascomycetes, and their taxonomic implications. *Mycotaxon* 44: 333-390.

BARAL H.O., 1994 — Comments on "Outline of the ascomycetes – 1993". *Systema Ascomycetum* 13(1): 113-128.

BARAL H.O. & MARSON G., 2001 — Monographic revision of *Gelatinopsis* and Calloriopsis (Calloriopsideae, Leotiales). In: *Mycologia* 2000, pp. 23-46. Associazione Micologica Bresadola, Trento.

FRIES E.M., 1849 — *Summa vegetabilium Scandinaviae* Vol. 2. Stockholm, Leipzig. 261-572.

HAGEDORN G. & SCHOLLER M., 1999 — A reevaluation of predatory Orbiliaceous fungi. Phylogenetic analysis using rDNA sequence data. *Sydowia* 51(1): 27-48.

HAINES J.H. & EGGER K.N., 1982 — A new species of *Orbilia* from Canada. *Mycotaxon.* 16(1): 107-113

KORF R.P., 1992 — A preliminary discomycete flora of Macaronesia. *Mycotaxon* 45: 503-510.

LIU B., LIU X.Z. & ZHUANG W.Y., 2005 — *Orbilia querci* sp. nov. and its knob-forming nematophagous anamorph. *FEMS Microbiology Letters* 245: 99-105.

NANNFELDT J.A., 1932 — Studien über die morphologic und systematik der nichtlichenisierten inooperculaten Discomyceten. *Nova Acta Reg. Soc. Sci. Uppsaliensis ser.* 48(2): 1-368.

SEAVER F.J., 1951 — The North American Cup-fungi (Inoperculates). Seaver, New York.

SPOONER B.M., 1987 — *Helotiales* if Australasia: *Geoglossaceae*, *Orbiliacease*, *Scelrotiniaceae*, *Hyaloscyphaceae*. *Bibliotheca Mycologica* 116: 1-711.

SWOFFORD D.L., 2002 — *PAUP. Phylogenetic analysis using parsimony, Version 4.0.* Sinauer Associates, Sunderland, Massachusetts, USA.

THOMPSON J.D., GIBSON T.J., PLEWNIAK F., JEANMOUGIN F. & HIGGINS D.G., 1997 — The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research* 24: 4876-4882.

WALSH P.S., METZGER D.A. & HIGUCHI R., 1991 — Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biological Techniques* 10(4): 506-513.

WHITE T.J., BRUNS T., LEE S. & TAYLOR J., 1990 — *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics.* Pp. 315-322. Academic Press. San Diego, California.