
***Pseudorbilia* gen. nov. (*Orbiliaceae*) from Yunnan, China**

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A new orbiliaceous fungus, *Pseudorbilia bipolaris* gen. et sp. nov., is described. This fungus was collected from decayed coniferous wood on the floor of a semi-tropical forest in YiLiang County, Yunnan Province, China. It is characterized by a special type of bacilliform ascospores: both ends of the spore contain a large lens-shaped refractive spore body visible only in living spores. This spore body and the other morphological characteristics indicate that this species belongs to the family *Orbiliaceae* where it appears to take a position intermediate between the two accepted genera, *Orbilia* and *Hyalorbilia*.

Keywords: *Orbiliaceae*, new genus, *Pseudorbilia bipolaris***Introduction**

Members of the family *Orbiliaceae* are among the globally distributed discomycete fungi. They are characterized by small, waxy, often translucent apothecia with an ectal excipulum composed of round to angular or prismatic, usually hyaline cells which are horizontally or vertically oriented; small asci intermixed with paraphyses that are typically swollen or encrusted at the apex; and by spore bodies inside living ascospores (Baral, 1994). The anamorphs in the family *Orbiliaceae* are distributed in about ten hyphomycetous genera (Mo *et al.*, 2005; Liu *et al.*, 2005a, b) and include both predacious and non-predacious species. Several species of orbiliaceous fungi has been described by many authors (Velenovský, 1934; Svrček, 1954; Jeng and Krug, 1977; Spooner, 1987; Korf, 1992). At this time, only two genera, *Orbilia* Fr. and *Hyalorbilia* Baral & G. Marson, were accepted based on morphological and molecular evidences (Baral, 1994; Eriksson *et al.*, 2003). Records of orbiliaceous fungi from China were reviewed by Liu *et al.* (2006), but in our

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survey of *Orbilia* species from China, we found a distinctive species in southwestern China in September 2005. Since its morphological characteristics do not fit either of the existing two genera of this family, we describe it here as a new genus.

Materials and methods

The fresh specimen was collected from XiaoBaiLong Forest Park, Yi Liang County, Yunnan Province, China in September, 2005. The living asci, paraphyses and ascospores were observed and measured directly in a tap water mount from the fresh collection. The size of asci, ascospores, spore bodies and paraphyses were measured for 50 random individuals. Vital staining of living elements was done using aqueous Toluidin blue (TB). Rehydrated specimens were sectioned longitudinally using a Leica freezing microtome for the observation of the structure of apothecia. An Olympus BX51 microscope and an Olympus zoom stereo microscope were used to take micrographs. Abbreviations: * = living hydrated state; † = dead hydrated state.

Taxonomic description

Pseudorbilia Y. Zhang, Z.F. Yu, H.O. Baral & K.Q. Zhang, **gen. nov.**

MycoBank: 510798.

Etymology: *Pseudorbilia* refers to the similar morphological characteristics to the genus

Apothecia superficialia, subhyalina vel pallide ochracea, translucientia. *Asci* 8-spori, cylindrico-clavati, apice rotundati vel valde truncati in statu emortuo, tenuiter tunicati, inamyloidea, breviter stipitati, e uncis nati. *Ascosporae* hyalinae, non-septatae, bacilliformes, cum duobus inclusionibus refringentis, lentiformes. *Paraphyses* cylindricae. *Excipulum* ectale textura angulare, cellulis tenuiter tunicatis.

Species typica: *Pseudorbilia bipolaris* Y. Zhang, Z.F. Yu, H.O. Baral & K.Q. Zhang, sp. nov.

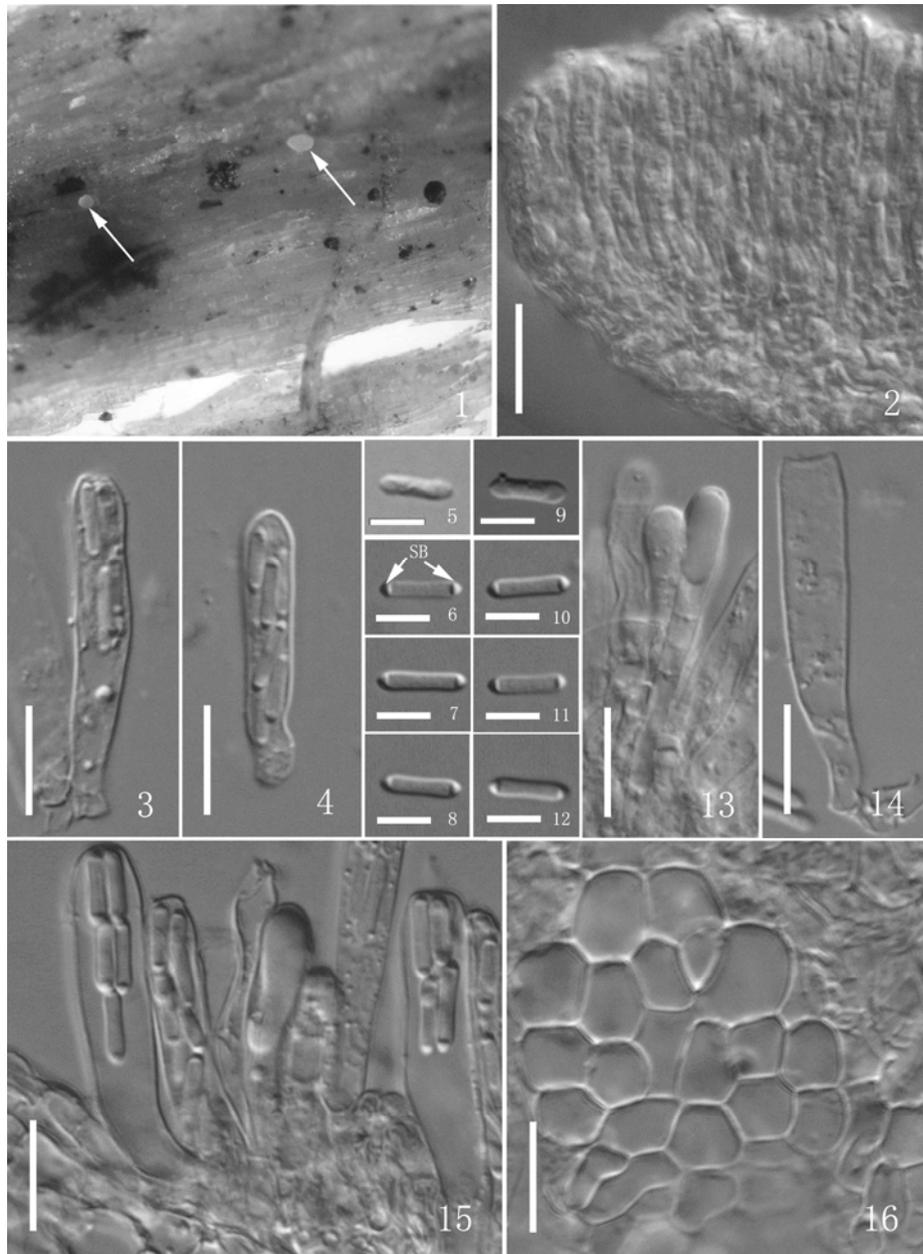
Apothecia superficial, whitish to pale cream-yellowish, often translucent when fresh. *Asci* with eight 2-4-seriate ascospores, cylindric-clavate, apices hemispherical to slightly conical, slightly (in living state) or strongly (in dead state) truncate, with short truncate stipes at the base, arising from croziers (without perforation). *Ascospores* aseptate, hyaline, bacilliform to very slightly dumbbell-shaped, containing two lens-shaped, refractive spore bodies (SB), one at each pole (bipolar symmetrically arranged, living state!). *Paraphyses* hyaline, not embedded in gel, cylindrical. *Ectal excipulum* of globose to subangular, isodiametrical, hyaline cells, near the margin composed of angular or slightly elongated cells.

Pseudorbilia bipolaris Y. Zhang, Z.F. Yu, H.O. Baral & K.Q. Zhang, **sp. nov.**
 MycoBank: 510799. (Figs 1-21)

Etymology: *bipolaris* refers to the bipolar arrangement of the spore bodies within ascospores.

Apothecia 0.15-0.3 mm diam., superficialia, subhyalina vel pallide ochracea, translucientia. *Asci* 25.0-33.8 × 4.5-6.8 μm in statu vivo, 8-spori, cylindrico-clavati, apice rotundati vel valde truncati in statu emortuo, tenuiter tunicati, inamyloidea, breviter stipitati, e uncis nati. *Ascosporae* hyalinae, non-septatae, 6.8-8.5 × 1.5-1.8 μm, bacilliformes, cum duobus inclusionibus refringentis, lentiformes 1.3-1.8 μm diam. *Paraphyses* cylindricae, 2-3 μm diam., apice usque 2-5 μm diam. *Excipulum* ectale textura angulare, cellulis tenuiter tunicatis.

Apothecia gregarious in a small group, 0.15-0.3 mm diam., superficial or with an immersed, shortly obconical stipe, whitish to pale cream-yellowish and often translucent when fresh, light ochraceous when dry, disc pulvinate, plane or convex, margin even. *Asci* *25-33.8 × 4.5-6.8 μm, †21-28 × 4.2-5.5(-6) μm, cylindric-clavate, with eight 2-4-seriate ascospores, apices hemispherical to slightly conical or, after a turn for 90° along its longitudinal axis, slightly (in living state) or strongly (in dead state) truncate, without apical thickening, rupturing by a pore near one of the "shoulders", inamyloid (in IKI, KOH-pretreated), slightly tapered but not forked at the base, with short truncate stipes, arising from croziers (without perforation). *Ascospores* *6.8-8.5 × 1.5-1.8 μm, †6.4-7.5 × 1.4-1.5(-1.6) μm, aseptate, hyaline, bacilliform to very slightly dumbbell-shaped, rounded at both poles, straight, sometimes very slightly curved, containing two lens-shaped, refractive spore bodies (SB) affixed to the wall of spore, one at each pole (bipolar symmetrically arranged, living state!), 0.8-1.0 μm thick, 1.3-1.8 μm wide, inner face of SB flattened, SBs irreversibly losing refractiveness in herbarium material or when treated by KOH, original shape of SB not further clearly visible in Congo Red or IKI, living spores containing in each half one large, ellipsoid, non-refractive vacuole that stains violet in TB, in dead spores still visible when stained with IKI. *Paraphyses* hyaline, not embedded in gel, cylindrical, apically usually cylindrical or only slightly enlarged, moderately capitate in KOH, apex hemispherical, about *2-5 μm in diam., terminal cell *12.5-19 × 3-5 μm, containing one large, very elongate, hyaline refractive vacuole (living state), septate only in the lower part, lower cells *4-7 × 2-2.2(-3.2) μm wide. *Ectal excipulum* of globose to subangular, isodiametrical, hyaline cells, with thin or slightly thickened walls, 6.8-10.5 μm diam., near the margin composed of angular or slightly elongated cells (*textura angularis-globulosa*), marginal cortical cells 5-9 × 4-6 μm, without glassy caps. Exudate over paraphyses and margin scattered, granular 0.2-0.5 μm thick. *Medullary excipulum* of hyaline, gelatinous textura intricata. Anchoring hyphae sparse, 1.2-2.2 μm wide, thin-walled, very sparsely septate.



Figs 1-16. *Pseudorbilia bipolaris*. (from holotype). **1.** Fresh apothecia. **2.** Vertical section of an apothecium. **3, 4.** Living asci. **5, 9.** Ascospores treated with KOH: SBs disappeared. **6-8, 10-12.** Living ascospores with SBs, **13.** Paraphysis or Paraphyses. **14.** Dead ascus. **15.** Cluster of asci and paraphyses. **16.** Ectal excipulum cells. Bars: 2-4, 13-16 = 10 μ m, 5-12 = 5 μ m.

Anamorph: not seen.

Habitat: On decorticated decayed branch of unidentified coniferous tree lying on the moist ground in a shady subtropical forest

Known distribution: China.

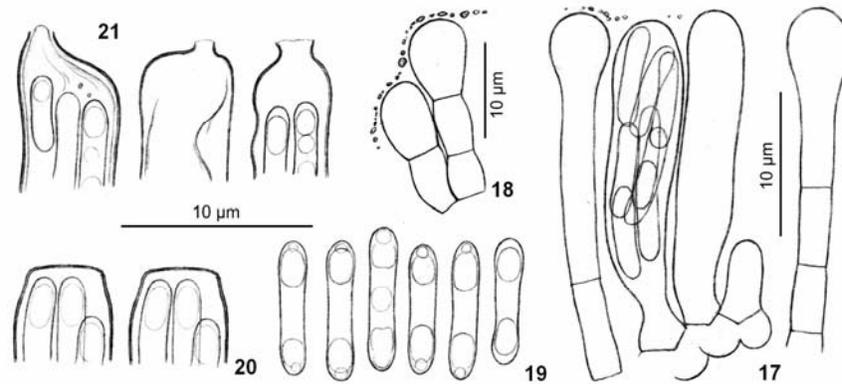
Material examined: PR China, Yunnan Province, Yi Liang County, Xiao Bai Long Forest Park, alt.1843 m, in September 2005, Ying Zhang (**holotype**: H.B. 8310 (slide and wood fragment), deposited in M (Botanische Staatssammlung München, Germany).

Discussion

The family *Orbiliaceae* was established by Nannfeldt in 1932 and was first viewed as one of the families in the *Helotiales* with the smallest number of species. At that time, *Orbiliaceae* included three genera: *Orbilium* Fr., *Hyalinia* Boud., and *Patinella* Sacc. Macroscopical characters (e.g. the colour, shape and translucence of the apothecia) and microscopical characters (e.g. the shape of the paraphyses, the presence of marginal hairs, and to a less extent, the size and shape of the ascospores) played an important role in the generic concepts. The genus *Patinella* was excluded from *Orbiliaceae* by Spooner (1987). Baral (1994) found that fresh collections of the type species of *Hyalinia* (*H. crystallina* (Quél.) Boud.) and *Habrostictis* (*H. rubra* Fuckel) and a species of *Orbiliaster*, *O. paradoxa* Huht., were very similar to the typical members of *Orbilium* grouped around the most often collected *Orbilium delicatula* (P. Karst.) P. Karst. He suggested dividing *Orbilium* into two subgenera, *Hemiorbilium* and *Orbilium*, the former being characterized by an apical thickening of the ascus wall of the hemispherical apex, the latter by a thin-walled truncate apex (often with "shoulders"). The genera *Habrostictis*, *Hyalinia* and *Orbiliaster* were viewed as belonging to the subgenus *Orbilium*. Meanwhile, six species around *Orbilium inflatula* (P. Karst.) P. Karst. were separated from *Orbilium* to form a new genus, *Hyalorbilia* (Baral & Marson, 2001). The new genus is distinguished from *Orbilium* by its unstalked asci arising from croziers, a hemispherical to broadly conical, thin-walled ascus apex, asci and paraphyses embedded in a gelatinous matrix, and an ectal excipulum of horizontal textura prismatica (Baral, 1994; Wu *et al.*, 2007).

Based on combined analysis of morphological characters and existing molecular data, Eriksson *et al.* (2003) established a new order *Orbiliales* and a new class *Orbiliomycetes* including only one family *Orbiliaceae* with two genera, *Orbilium* and *Hyalorbilia*. At present, according to Baral's unpublished "World monograph of *Orbiliomycetes*", additional characters of orbiliaceous fungi which are only visible in the living state were found to be of similar importance for species delimitation than current characters like shape of spores and paraphysis apices. The genus *Orbilium* includes species in which the asci

and paraphyses are rather easily separable because the wall is covered only by a very thin gel layer, with the asci arising from an often long-stalked furcate base without croziers, and the thin-walled excipulum on the flanks consisting of globose to angular cells. The ascospores of almost all of the species possess a single, very striking, usually apical spore body (SB) which is often impossible to observe in herbarium materials.



Figs 17-21. *Pseudorbilia bipolaris*. (from holotype) (all dead state, in KOH + CR). **17.** Asci and paraphyses. **18.** Marginal ectal excipulum in median section. **19.** Free ascospores. **20.** Two apices of mature, unopened asci. **21.** Three apices of mature, opened asci.

The new genus *Pseudorbilia* we propose here was collected a single time from rotten coniferous wood, its morphological characteristics include minute translucent apothecia, an ectal excipulum of globose to angular cells, asci and paraphyses not embedded in gel, short-stipitate but not forked asci arising from croziers, and with a hemispherical or truncate apex, depending on the side of view. This bilateral-symmetrical ascus apex together with characteristic SBs in the living ascospores are the reason for including this specimen in the family *Orbiliaceae*. The non-gelatinized, easily separable asci and paraphyses, the truncate ascus apex, and the globose to angular cells of the ectal excipulum are similar to *Orbilium*. However, the asci arising from croziers, as well as the symmetrically arranged SBs within the ascospores are same as *Hyalorbilia*. We therefore describe a new genus because the new species combines some of the characteristics of the two existing genera (*Orbilium* and *Hyalorbilia*) within the *Orbiliaceae*. The distinguishing characters of the three genera were given in Table 1.

Table 1. Synoptic table of known genera of *Orbiliomycetes*.

Genera of Orbiliomycetes	Ascus apex		Ascus base	Ectal excipulum (flanks)	Glassy processes	SBs in spores		Paraphyses	Hymenial gelatine	Pigment
<i>Pseudorbilia</i>	trc	thin	crz	angularis	-	2	l	1	-	hy
<i>Hyalorbilia</i>	hem	thin	crz	prismatica	-	2/1	g	0-2	+	hy
<i>Orbilia</i>	hem/trc	thin/ thick	frc	angularis	-/+	1	glt v	0-4	-	hy/rd/bl

Explanation: ascus apex: hem = hemispherical, trc = truncate, thn = thin-walled, thk = thick-walled; ascus base: crz = croziers, frc = simple septate (furcate). Ascospores: 1 = one SB per spore (usually apically), 2 = two or more SBs, one at each end; g = globose, l = lens-shaped, t = tear-shaped, v = vermiform/filiform. Paraphyses: apex: 0-4 = not to very strongly inflated. Pigment: hy = ± hyaline, rd = ± red or yellow, bl = black.

So far, several anamorphs of the species in the two existing genera (*Orbilia* and *Hyalorbilia*) have been reported (Mo *et al.*, 2005; Liu *et al.*, 2005a, b). Unfortunately, the anamorph was not obtained from our new taxon through the method described by Yu *et al.* (2006). Moreover, it is difficult to isolate DNA from the tiny and exiguous apothecia rather than from the pure culture of its anamorph, so there is insufficient molecular evidence herein to provide a comprehensive phylogenetic analysis of the three genera in the family *Orbiliaceae*. More specimens and further studies are needed.

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