
Two new species of *Hyalorbilia* from Taiwan

Mei-Lee Wu^{1*}, Yu-Chih Su², Hans-Otto Baral³ and Shih-Hsiung Liang²

¹Graduate School of Environment Education, Taipei Municipal University of Education. No.1, Ai-Kuo West Rd., Taipei 100, Taiwan

²Department of Biotechnology, National Kaohsiung Normal University, No. 62, Shenjhong Rd., Yanchao Township, Kaohsiung County, Taiwan

³Blaihofstr. 42, D-72074. Tübingen, Germany

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During our study of orbiliaceous fungi from southern Taiwan, two uncommon taxa referable to the genus *Hyalorbilia* growing on the bark of decayed undetermined wet branches of broad-leaved trees were collected. They are here described as new species, *Hyalorbilia arcuata* and *H. biguttulata*. *Hyalorbilia arcuata* collected from 800-1500 m altitude is characterized by strongly curved ascospores, while *H. biguttulata* collected from 800 m altitude has two large globose spore bodies in broadly ellipsoid ascospores.

Key words: *Hyalorbilia*, orbiliaceous fungi, taxonomy

Introduction

The family *Orbiliaceae* has traditionally been placed in the *Helotiales* (Korf, 1973). However, recent phylogenetic analyses of molecular data concluded that *Orbiliaceae* should be separated from that order. Therefore, a new order *Orbiliales* and a new class *Orbiliomycetes* were recently proposed (Eriksson *et al.*, 2003). *Hyalorbilia* and *Orbilia* are the only genera presently accepted in the family (Eriksson *et al.*, 2003; Liu *et al.*, 2006) and some of which have nematophagous anamorphs (Mo *et al.*, 2005a). The main key distinguish the two genera are the asci of *Hyalorbilia* arising from croziers, while those of *Orbilia* arise from simple septa with mostly forked or inversely T- or L-shaped bases (Baral *et al.*, 2003).

The family *Orbiliaceae* has rarely been studied in Taiwan and only four taxa were previously recorded, including *Orbilia auricolor* (Bloxam ex Berk. & Broome) Sacc., *O. delicatula* (P. Karst.) P. Karst., *O. inflatula* (P. Karst.) P. Karst. (Wu, 1998) and *O. juruensis* Henn. (Wang, 2002). However, *Orbilia inflatula* was transferred to *Hyalorbilia* (Baral and Marson, 2001). The genus

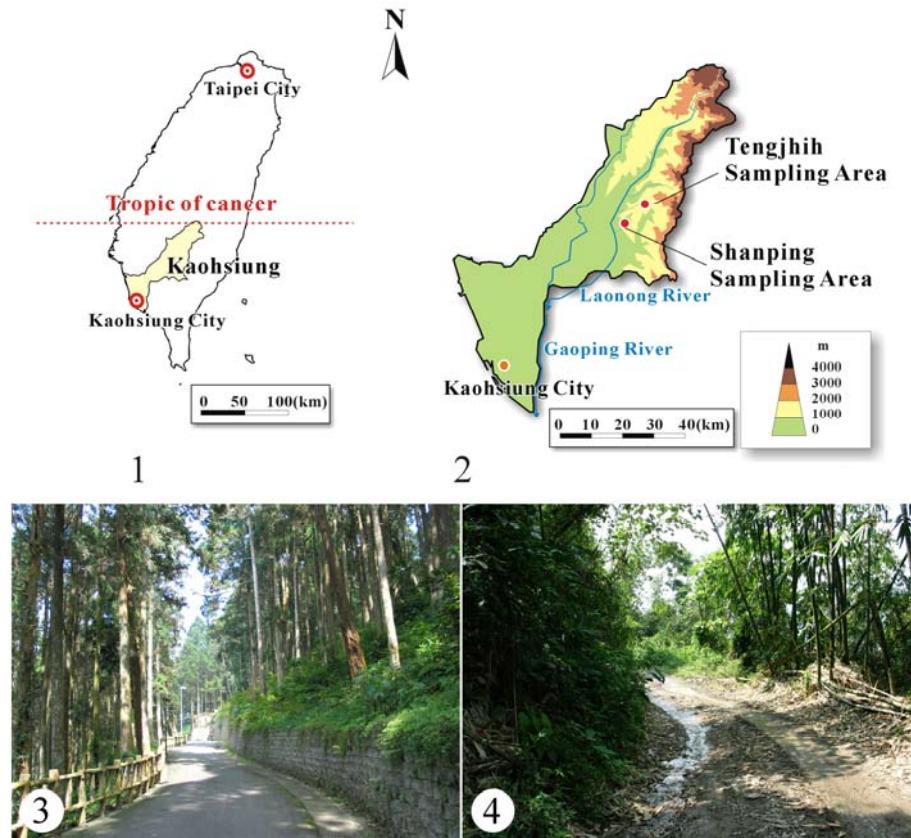
*Corresponding author: M.L. Wu; e-mail:wuml@tmue.edu.tw

Hyalorbilia is characterized by a mostly symmetrical guttulation of the living ascospores and a rather short and thick ascus stalk that arises from croziers. Paraphyses of *Hyalorbilia* species are mostly not or only slightly inflated at the apex and conglutinated with the asci by a gel that externally terminates by a very thin, mostly finely warted, pale chlorinaceous layer of exudate (Baral and Marson, 2001). Six species have been combined in *Hyalorbilia*: *H. berberidis* (Velen.) Baral (type of *Hyalorbilia*), *H. erythrostigma* Baral & G. Marson, *H. fusispora* (Velen.) Baral & G. Marson, *H. inflatula* (P. Karst.) Baral & G. Marson, *H. lunata* (Korf) Baral and *H. brevistipitata* B. Liu, X.Z. Liu & W.Y. Zhuang (Liu *et al.*, 2005).

During the fungal survey in southern mountain area of Taiwan, some members of *Orbiliaceae* were collected. Two taxa on decayed bark were identified as belonging to *Hyalorbilia*. Based on an unpublished key to the species of *Hyalorbilia* (Baral & Weber, ined.) they proved to be new to science. Specimens collected in this survey were deposited at the Herbarium, Department of Natural Science in Taipei Municipal University of Education (TMUE, the former Taipei Municipal Teachers' College, TMTC). Holotypes are deposited at National Museum of Natural Science, Taichung, Taiwan (TNM F20071 and TNM F20072).

Materials and methods

Southern mountain areas of Taiwan including Tengjhih and Shaping, Kaohsiung (Fig 1) had been selected for field expeditions since September 2004. The specimens were collected from Tengjhih Forestry Recreation Garden (abbreviated as Tengjhih) (Figs 2, 3) and Shaping Natural Education Center of the Forestry Bureau (abbreviated as Shaping) in Kaohsiung (Figs 2, 4). Tengjhih and Shaping are subtropical evergreen broad-leaved forests. The collection site of Tengjhih is located at 23°04' N and 120°44' E with 1450-1500 m alt., while Shaping is located at 22°58' N and 120°40' E with 800-900 m alt. Various fungi growing on fallen leaves of native deciduous trees and needles of cultivated gymnosperms, rotten wood, stem of grass and bamboo as well as rachis or petioles of ferns were collected. The weight of each substrate before drying and after drying was measured in order to determine the water content of the substrate when collected. The ecological data including temperature, humidity and precipitation were also collected. Detailed methods given by Wu *et al.* (1998) were followed. Special emphasis was placed on the vital microscopic characters of the fresh apothecia, while only dried material was available for one of us (H.O. Baral). Drawings were mainly made with a drawing tube (Baral free-hand). Terms used to describe the excipular type are those of Korf (1973).



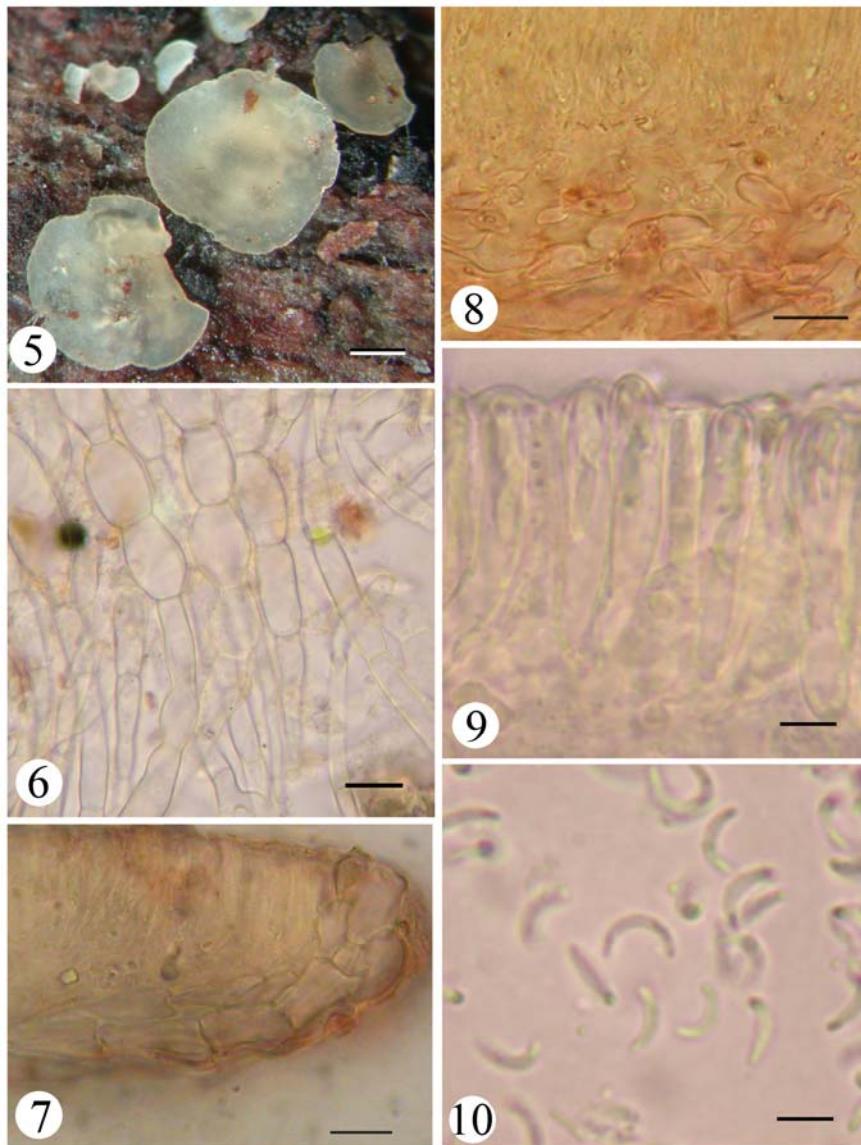
Figs. 1-4. Locations and habitat of sampling areas. **1.** Locations of collecting areas in Taiwan. **2.** Locations of Tengjih and Shaping in Kauoshing County. **3.** Sampling area in Tengjih Forestry Recreation Garden. Note the area is a mixed deciduous and gymnosperm forest. **4.** Path of a sampling area in Shaping Natural Education Center of Forestry Bureau.

Results

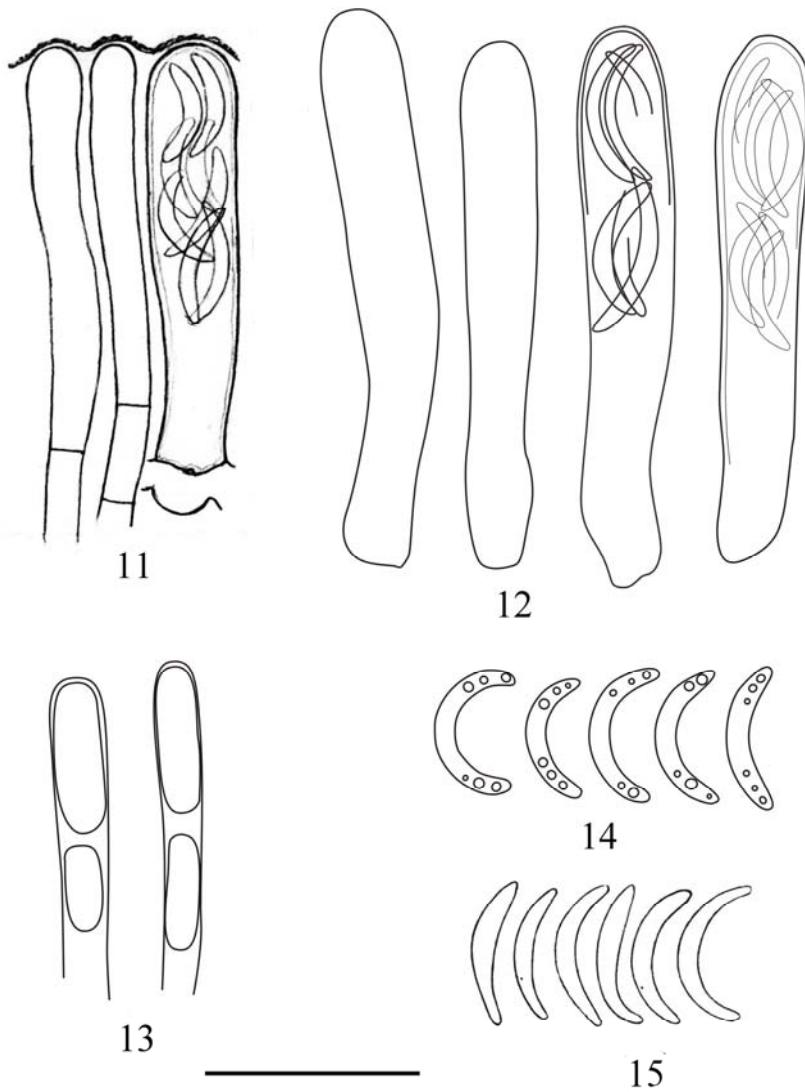
Hyalorbilia arcuata H.O. Baral, M.L. Wu & Y.C. Su, sp. nov. (Figs 5-15)

Etymology: After the strongly curved ascospores.

Apothecia 0.8-1.8 mm diam, superficia, translucentia, gregaria, plana vel concava, sessilia, cum subiculo sparso. Exipulum ectale textura prismatica. Ascii in statu vivo 20-27(32.5) × 4-4.5 µm, cylindrici, apice rotundati, tenuiter tunicati, octospori, breviter crasse stipitati, in basi non uncinati, non furcati, cum paraphysibus conglutinati. Ascosporae in statu vivo 5.8-7 × (0.8-)0.9-1.2(-1.4) µm, hyalinae, conspicue curvatae (arcuatae), non septatae, in duobus polis 2-4 guttulae globosae continentae. Paraphyses cylindricae, apice non vel vix inflatae, 2-3.5 µm in diametro, guttulae globosae vel cylindraceae, hyalinae continentae.



Figs 5-10. Photographs of *Hyalorbilia arcuata* (from holotype). **5.** Apothecia in fresh state. **6.** Anchoring hyphae of subiculum in living state. **7.** Median section of the marginal region of apothecium, mounted in KOH. **8.** Heavily gelatinized medullary excipulum (KOH). **9.** Hymenium with living mature asci. **10.** Ascospores (living state). Bars 5 = 0.5 mm; 6-8 = 10 µm.; 9-10 = 5 µm. (All the photographs were from Y.-C. Su except Figs. 7 and 8 which were from H.-O. Baral.)



Figs 11-15. Line drawings of *Hyalorbilia arcuata* (from holotype). **11.** Mature ascus and two paraphyses, covered by thin layer of exudate (in KOH). Note the biseriate ascospores lying on 2/3 top of the ascus and the crozier at the ascal base. **12.** Four ascii (in living state, the left three ones from Y.-C. Su and the ascus with ascospores in the right one from H.-O. Baral). **13.** Living paraphyses with cylindrical vacuolar bodies. **14.** Ascospores in living state, with globose spore bodies near both ends. **15.** Ascospores in dead state (spore bodies invisible). Bars 11-15 = 10 µm. (All drawings were from Y.-C. Su except Figs. 11 and 15 which were from H.-O. Baral.).

Apothecia moist 0.8-1.8 mm diam, 0.12 mm high, superficial, sessile on a broad base, gregarious, membranous, slightly gelatinous, smooth at the margin; disc plano-concave, fresh watery-whitish, translucent, dried light buff (rehydrated pale chlorinaceous), with whitish subiculum. Ascii in the living state 20-27(-32.5) × 4-4.5 µm, in KOH 18-22 × 3.2-4.3 µm, cylindrical, apex hemispherical, thin-walled, 8-spored, arising from croziers, IKI-. *Ascospores*, strongly curved with sickle-shaped in living state, 5.8-7 × 0.8-1.4 µm (in situ, upto 11 µm real length); in dead state less strongly curved, slightly to medium fusiform, 5.5-7 × 0.8-1.2 µm; never distinctly helicoidal, homopolar, both ends obtuse, sometimes subacute, without septa; spore bodies (SBs) globose, 0.2-0.4(0.5) µm diam, 2-3(-4) near each end, invisible in KOH. *Paraphyses* cylindrical, not or only slightly inflated at the apex, hyaline, terminal cell 2-3.5 µm wide, (13)-17-20 × 2-2.5 µm when mounted in KOH, lower cells in KOH 4-5 × 1.6-1.8 µm, hymenium strongly gelatinized. *Ectal excipulum* of hyaline, thin-walled (in KOH common walls 0.3-0.7 µm thick), textura prismatica of horizontally oriented cells; on lower flanks 50-60 µm thick, cells in KOH 18-40(-48) × (6-)8-15(-22) µm; at margin 15-20 µm thick; marginal cortical cells in KOH 11-15 × 7-10 µm, clavate, thin-walled. *Medullary excipulum* 10-15 µm thick, of medium to strongly gelatinized textura angularis-prismatica, sharply delimited from ectal excipulum. Anchoring hyphae abundant, projecting, in KOH (2.5-) 3-5(-6) µm wide, walls (0.2-)0.3-0.8(-1) µm thick. Exudate over paraphyses and marginal excipulum 0.2 µm thick, rough-warted. Vacuolar bodies (VBs) in terminal cells of living paraphyses globose to elongate, hyaline, slightly refractive.

Anamorph: *Dactylella lignatilis* M.H. Mo & K.Q. Zhang, observed in pure culture by MingHe Mo (Mo *et al.*, 2005b).

Habitat: on outer bark surface (periderm) of a wet, partially decayed corticated branch of unidentified broad-leaved tree lying on the ground (about 1-2 cm thick and 40 cm long). The water content of the holotype substrate was 70.38%.

Known distribution: Yunnan Province of Mainland China and Taiwan.

Material examined: Taiwan, Kaohsiung: Tengjih Forestry Recreation Garden, 23°04' N and 120°44' E, 1500 m alt., 30 October 2005, Y. C. Su (TNM F20072; **holotype**); ibid., 24 January 2006, Y.C. Su (T060124T4). Kaohsiung: Shaping Natural Education Center of the Forestry Bureau, 22°58' N and 120°40' W, 800 m alt., 13 January 2005, 14 March 2005, and 10 October 2005, Y.C. Su (S050113T4, S050314T9, S051010T3 and S051010T5).

Notes: The new species is characterized by sickle-shaped ascospores with 2-4 spore bodies in each half in the living state. This fungus is similar to *H. berberidis* but it differs from that species in the ascospores being smaller (5.8-7 × 0.8-1.2 µm vs. 10-17.5 × 1.1-1.8 µm) and spore bodies being smaller and

fewer (2-3(-4) small SBs 0.2-0.5 μm diam vs. 3-6 large SBs 0.3-0.8 μm diam, Baral, ined.).

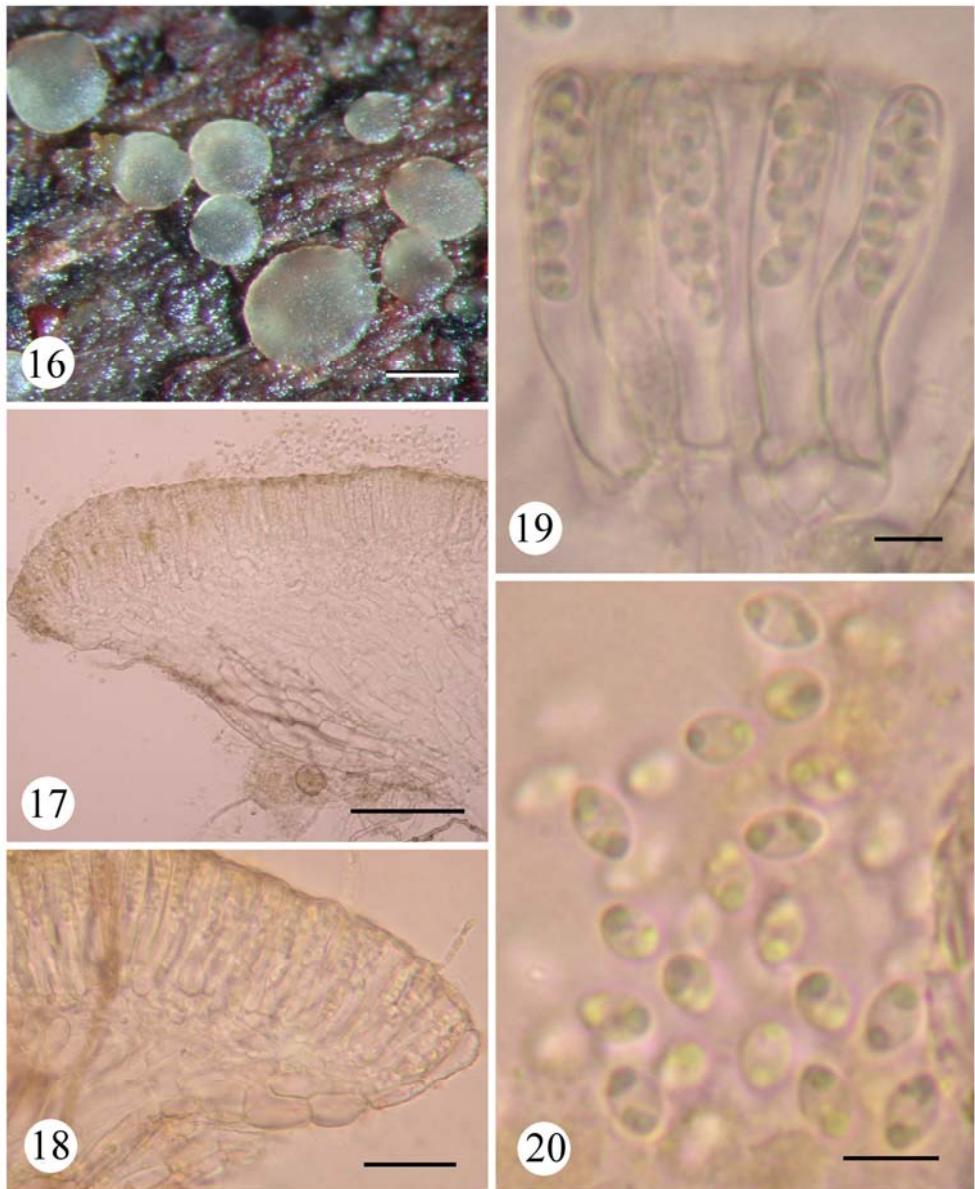
Mo *et al.* (2005b) reported a collection from Xiaobailong Mountain, Kunming City, Yunnan Province, P. R. China by MingHe Mo on 10 Sep 2003, which they referred to as *Hyalorbilia* sp., with a *Dactylella* anamorph obtained in pure culture. The microscopic characteristics of this Chinese *Hyalorbilia* sample described in the dead state fit rather well the *H. arcuata* holotype from Taiwan. However, the substrate of the Chinese sample was from the periderm of a decayed branch of *Pinus* submerged into the moist soil while the substrate of Taiwan sample was from the wet periderm of a broad-leaved tree. Although *H. arcuata* had not been cultured from the Taiwan sample, therefore no data of its anamorph are available, we believe that the Chinese sample represents the same species.

***Hyalorbilia biguttulata* H.O. Baral, M.L. Wu & Y.C. Su, sp. nov.** (Figs 16-26)

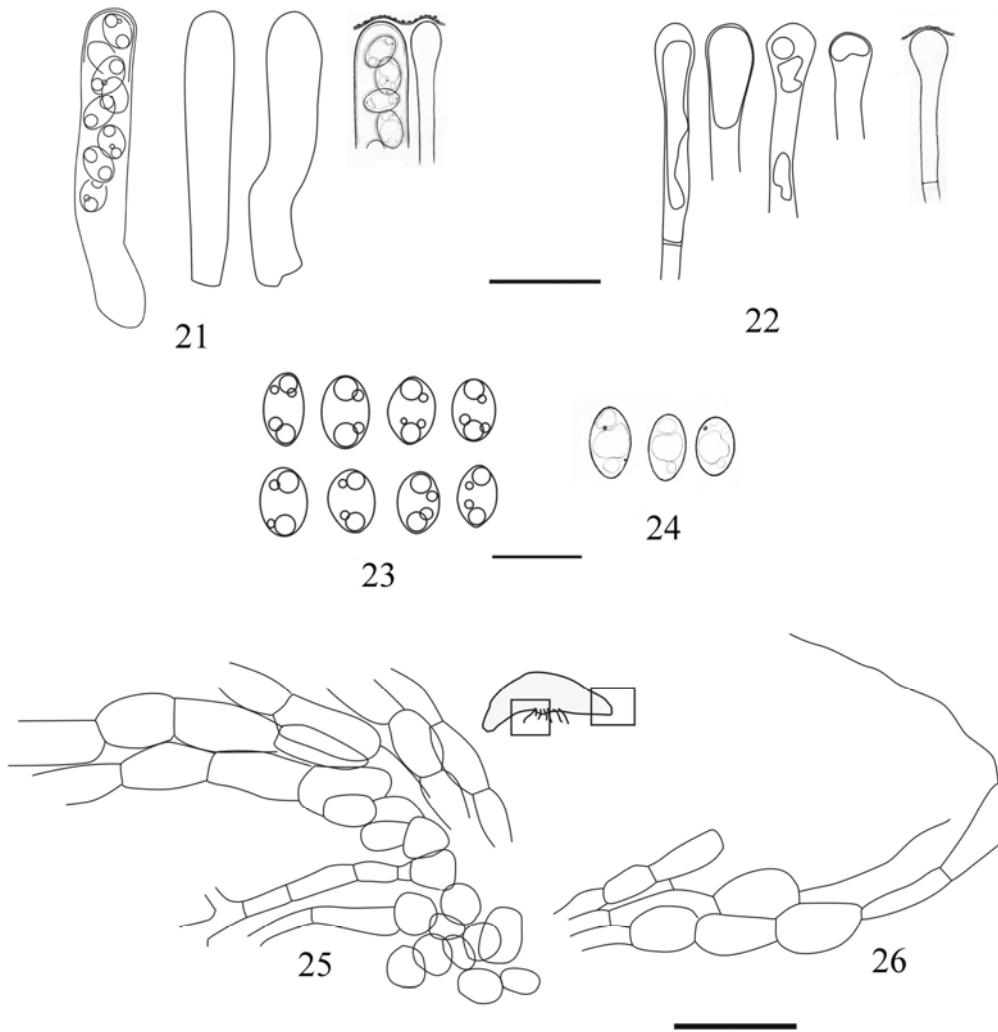
Etymology: From the two large spore bodies in the ascospores.

Apothecia 0.3-0.6 mm diam, superficialia, translucentia, gregaria, plana vel convexa, subsessilia. *Excipulum ectale textura prismatica*. *Asci* in statu vivo 26-31 \times 5-5.8 μm , cylindrici, apice subconica vel subtruncata, tenuiter tunicati, octospori, breviter crasse stipitati, in basi non uncinati, non furcati, cum paraphysibus conglutinati. *Ascosporae* in statu vivo (3.3-)3.6-4.3(-4.5) \times (2.2-)2.3-2.7(-2.9) μm , unicellulares, subglobosae vel ellipsoideae, hyalinæ, duabus polis una guttula globosa continentæ. *Paraphyses* apice modice inflatae, 3.5-4.5 μm lata, guttulae globosae vel elongatae, luteolæ continentæ.

Apothecia moist (0.25-)0.3-0.6 mm diam, 0.12-0.15 mm high, subsessile, gregarious, superficial, with a very short, broad obconical base, disc flat to slightly convex, pale yellow-clorinaceous, translucent, round, non-gelatinous, margin indistinct, smooth. *Asci* in living state 26-31 \times 5-5.8 μm , in KOH 21-25 \times (3.5-)4.5(-5.7) μm , 8-spored, spores occupying upper 2/3 of living ascii, biserrate in upper part but nearly uniseriate in lower part; apex hemispherical (subconical to subtruncate), thin-walled; base without or with short and thick stalk, arising from croziers. *Ascospores* in living state (3.3-)3.6-4.3(-4.5) \times (2.2-)2.3-2.7(-2.9) μm , in KOH 3.3-4.2 \times 2-2.6 μm , broadly ellipsoid, homopolar, both ends obtuse; SBs 0.8-1.5 μm diam, one at each end, globose, strongly refractive, broadly attached to apical wall, in visible in KOH, with 1(-2) further smaller SBs and 0-1 minute lipid bodies (LBs) in each half. *Paraphyses* cylindrical, terminal cells apically slightly to medium clavate, in living state 3.5-4.5(-5.3) μm wide, in KOH 20-25 \times 2.5-5.3 μm ; lower cells in KOH 5-7 \times 2-3 μm . *Ectal excipulum* of thin-walled horizontally oriented textura prismatica from base to margin, 50-70 μm thick near base, cells 14-27 \times 9-13 μm in dead state, thin-walled; 10 μm thick near margin, marginal cortical cells 13-18 \times 5.5-7.5 μm in dead state, clavate, thin-walled. *Medullary excipulum* 30-50 μm thick, composed of dense, non-gelatinized textura



Figs 16-20. Photographs of *Hyalorbilia biguttulata* (from holotype). **16.** Apothecia in fresh state. **17-18.** Median section of an apothecium. Note the thin-walled marginal cortical cells in Fig 18. **19.** Hymenium, note the asci arising from croziers. **20.** Living ascospores with spore bodies. Bars 16 = 0.3 mm; 17 = 50 μm ; 18 = 20 μm ; 19-20 = 5 μm . (All photographs were from Y.-C. Su).



Figs 21-26. Line drawings of *Hyalorbilia biguttulata* (from holotype). **21.** Three living asci on the left (from Y.-C. Su), dead ascus and paraphysis covered by thin exudate (from H.-O. Baral). **22.** Four living paraphyses with refractive vacuolar bodies (by Y.-C. Su) and one dead paraphysis where these bodies are invisible (by H.-O. Baral). **23.** Ascospores in living state, note one large spore body (SB) at each end (by Y.-C. Su). **24.** Ascospores in dead state (SBs invisible, from H.-O. Baral). **25.** Basal part of ectal excipulum with two anchoring hyphae (by Y.-C. Su). **26.** Ectal excipulum near margin (by Y.-C. Su). Bars 21-22 = 10 μm ; 23-24 = 5 μm ; 25-26 = 20 μm .

prismatica to textura angularis, medium sharply delimited from ectal excipulum. *Anchoring hyphae* only present at base, projecting, 2-4.5(-5.7) μm wide in dead state, walls 0.2-0.5 μm thick. *Exudate* over paraphyses and marginal excipulum 0.2 μm thick, more or less granular to warted. *Vacuolar bodies (VBs)* in terminal cells of living paraphyses yellow to pale orange, globose to elongate, slightly refractive.

Anamorph: unknown.

Habitat: on surface of periderm of a wet, decayed, branch of undetermined broad-leaved tree in a subtropical broad-leaved forest. The holotype's substrate was lying on the ground and about 0.5-1 cm thick and 6 cm long. The water content was 82.8%.

Known distribution: Taiwan.

Material examined: Taiwan, Kaohsiung: Shaping Natural Education Center of the Forestry Bureau, 22°58' N and 120°40' W, 800 m alt., 27 August 2005, Y.C. Su, (TNM F20071; **holotype**).

Notes: *Hyalorbilia biguttulata* resembles *H. erythrostigma* in spore shape. The main difference between the two species lies in the content of the living ascospores. Whilst the SBs in the spores of *H. biguttulata* are symmetrically arranged, the spores of *H. erythrostigma* contain only one strongly eccentric SB per spore. This feature is very difficult or even impossible to discern from herbarium material since SBs are hardly visible in dead spores, but in the type material of *H. erythrostigma* the eccentric SB could clearly be seen as a faint transparent region (Baral ined.).

Ecology

Baral and Marson (ined.) found that about 80% of the species of *Orbiliomyctetes* grow on xeric (periodically dry) plant material. All parts of the ascocarp survive complete drying down of their cells in such fungi being xerotolerant as a whole. The ascocarps may survive several months up to 2-3 years depending on the species adapted either to temperate or semi-desert climate.

The ecological environments in Tengjhih and Shaping, for *H. arcuata* and *H. biguttulata* respectively, are a subtropical evergreen broad-leaved forest. In addition to some cultivated gymnosperms, such as *Cryptomeria japonica*, *Chamaecyparis formosensis* and *Taiwania cryptomerioides*, the dominant native broad-leaved trees in Tehjghih and Shaping are *Lauraceae* and *Fagaceae*. Besides, there are tree ferns, such as *Cyathea spinulosa* and *Cyathea lepifera* and small ferns in addition to dominant herbaceous *Misanthus floridulus* on the ground level in the collecting areas. There is also a lot of cultivated bamboo in Shaping.

The average precipitation/year of Tengjih from 1979-2005 was 3090 mm while that of Shaping from 1980 to 2005 was 3303 mm. The sum of precipitation in Tengjih was 5950 mm and in Shaping was 6145 mm on the collecting year of 2005 although there is usually dry season in winter. The high precipitation of year 2005 is because of typhoon occurred in southern Taiwan. The average temperature/month of Tengjih and Shaping on 2005 was respectively 15.04°C and 19.08°C. The temperature of collecting date for *H. arcuata* was 11.83°C-20.63°C and for *H. biguttulata* was 22.63°C. The humidity/month of Tengjih and Shaping on 2005 was 93.64% and 93.89% respectively. Substrates of *H. arcuata* and *H. biguttulata* collected were from wet branches lying on ground with water content 70.38% and 82.8% respectively. The collecting areas are usually covered in mist all over the year. According to the ecological conditions for these two new species, there is little doubt that they are xerointolerant.

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