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Helicomyxa everhartioides, a new helicosporous sporodochial hyphomycete from Taiwan with relationships to the *Hyaloriaceae* (*Auriculariales*, *Basidiomycota*)

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Abstract: A hyphomycete producing hemi-circinate, hyaline, aseptate conidia from clamped conidiophores in gelatinous sporodochia was discovered in Taiwan. In view of its unique combination of characteristics, the new genus *Helicomyxa* and the new species *H. everhartioides* are proposed. Morphological and ultrastructural studies as well as an analysis of partial nuclear large subunit ribosomal DNA sequences suggest a relationship to teleomorphs belonging to the *Hyaloriaceae* in the *Auriculariales*.

Taxonomic novelties: *Helicomyxa* R. Kirschner & Chee J. Chen gen. nov., *H. everhartioides* R. Kirschner & Chee J. Chen sp. nov.
Key words: clamps, heterobasidiomycetes, anamorphic fungi, nuc LSU rDNA.

INTRODUCTION

Hyphomycetes with coiled conidia are informally classified as "Helicosporae", a term introduced by Saccardo and still retained in a user-friendly coding system for anamorphic genera in the latest edition of the "Dictionary of the Fungi" (Kirk *et al*. 2001). Monographs on the helicosporous hyphomycetes are listed in the latest review of this group by Goos (1987). He also provided a terminology for different types of helicospores, e.g. hemi-circinate for conidia that are coiled 0.5–1 times in one plane.

While several genera of helicosporous hyphomycetes are connected to ascomycetous teleomorphs (Goos 1987), only three have hitherto been reported to have relationships with species of the *Basidiomycota*. By means of cultural experiments, *Nematoctonus campylosporus* Drechsler was recognized as the anamorph of *Hohenbuehelia portegna* (Speg.) Singer and *Pseudohelicomyces albus* Garnica & E. Valenz. as the anamorph of *Psilocybe merdaria* (Fr.) Ricken (Thorn & Barron 1986, Valenzuela & Garnica 2000). According to analyses of ribosomal small subunit (SSU) DNA sequences, the helicosporous hyphomycete *Hobsonia mirabilis* (Peck) Linder seems to be closely related to species of *Helicogloea* (Sikaroodi *et al*. 2001).

Studying microfungi of Taiwan, we discovered a sporodochial hyphomycete producing hemi-circinate conidia from conidiophores with clamp connections. Morphological, ultrastructural, and molecular biological investigations confirmed the basidiomycetous nature of this fungus.

MATERIALS AND METHODS

Sporodochia of the fungus were collected on a rotting branch on the ground on the Chu Yun Shan Lin Dao, 400–600 m, Kaohsiung, Taiwan, 28 Apr. 2001, R. Kirschner & C.-J. Chen 831. Dried material was deposited in TNM. After the material was kept in a moist chamber for 5 d, whole sporodochia were transferred to a medium composed of autoclaved *Fagus sylvatica* L. wood chips embedded in 1.5 % water agar. Conidia and sporodochia were transferred from the newly developed sporodochia to fresh media like the same natural one mentioned above, as well as to 2 % malt extract agar (LAB M^{TM} MC 23 malt extract, distributed by Roth, Karlsruhe, Germany) and other media. From the very beginning, the fungus was maintained by transferring it together with a contaminating hyphomycete (*Phaeoacremonium* sp.) onto new Petri dishes containing the natural medium. The mixed culture was sent to the Centraalbureau voor Schimmelcultures (CBS, Utrecht, the Netherlands). After several attempts to obtain a pure culture of the fungus on different media, a living mixed culture of both fungi was preserved at CBS.

Table 1. GenBank accession numbers of species used in the phylogenetic analyses.

Species	GenBank accession no.	Reference
Amanita citrina (Schaeff.) Pers.	AF041547	Hopple & Vilgalys (1999)
Auricularia auricula-judae (Fr.) J. Schröt.	AF291289	Weiß & Oberwinkler (2001)
Basidiodendron rimosum (H.S. Jacks. & G.W. Martin) Luck-Allen	AF291298	Weiß & Oberwinkler (2001)
Boletus edulis Bull. : Fr.	AF291300	Weiß & Oberwinkler (2001)
Calocera cornea (Batsch) Fr.	AF291302	Weiß & Oberwinkler (2001)
Chionosphaera cuniculicola R. Kirschner, Begerow & Oberw.	AF393473	Kirschner *et al.* (2001b)
Craterocolla cerasi (Schumach.) Bref.	AF291308	Weiß & Oberwinkler (2001)
Cuniculitrema polymorpha R. Kirschner & J.P. Samp.	AY032662	Kirschner *et al.* (2001c)
Dacrymyces stillatus Nees	AF291309	Weiß & Oberwinkler (2001)
Exidia japonica Lloyd	AF291320	Weiß & Oberwinkler (2001)
Exidia saccharina Fr.	AF291323	Weiß & Oberwinkler (2001)
Exidiopsis grisea (Pers.) Bourdot & Maire	AF291328	Weiß & Oberwinkler (2001)
Ductifera sucina (A. Møller) K. Wells	AF291316	Weiß & Oberwinkler (2001)
Helicomyxa everhartioides R. Kirschner & Chee J. Chen	AY640107	
Heterochaetella dubia (Bourdot & Galzin) Bourdot & Galzin	AF291337	Weiß & Oberwinkler (2001)
Hyaloria pilacre A. Møller	AF291338	Weiß & Oberwinkler (2001)
Myxarium granulum Hauerslev	AF291348	Weiß & Oberwinkler (2001)
Myxarium grilletii (Boud.) D.A. Reid	AF291349	Weiß & Oberwinkler (2001)
Myxarium mesonucleatum Kisim.-Hor., Oberw. & L.D. Gómez	AF291350	Weiß & Oberwinkler (2001)
Myxarium nucleatum (Schwein.) Wallr.	AF291351	Weiß & Oberwinkler (2001)
Myxarium sp.	AF291353	Weiß & Oberwinkler (2001)
Polyporus varius (Pers.) Fr.	AF291356	Weiß & Oberwinkler (2001)
Protodontia piceicola (Kühner ex Bourdot) G.W. Martin	AF291266	Weiß & Oberwinkler (2001)
Pseudohydnum gelatinosum (Scop.) P. Karst.	AF291360	Weiß & Oberwinkler (2001)
Tremella flava Chee J. Chen	AF042238	Chen (1998)

For light microscopy, material was mounted in 5–10 % KOH (Fluka Chemie GmbH, Buchs, Switzerland) with or without staining with 1 % aqueous phloxine. Measurements of 30 conidiogenous cells are given as extreme values in brackets and means ± SD.

The ultrastructure of the hyphal septa of the new anamorphic fungus was investigated by transmission electron microscopy (TEM) as described in Kirschner *et al*. (2001a).

A sporodochium of the anamorphic fungus grown in culture was used for isolating DNA using the PEQLAB E.Z.N.A.® Fungal DNA Kit (PEQLAB Biotechnologie GmbH, Erlangen, Germany), and for PCR as mentioned in Kirschner *et al*. (2001c). PCR products were purified using the PEQLAB E.Z.N.A.® Cycle-Pure Kit. Sequencing of dsDNA was done by Scientific Research and Development GmbH (Oberursel, Germany). An alignment was produced with MEGALIGN of the Lasergene package (DNASTAR, Inc. 1997) without manual manipulations within the alignment using partial DNA sequences of the nuclear gene coding for the ribosomal large subunit RNA deposited in GenBank (accession numbers listed in Table 1). The PHYLIP package, version 3.5c (Felsenstein 1993), was used to perform a neighbour-joining analysis (Kimura two-parameter distances, transition/transversion ratio 2.0), followed by a bootstrap analysis with 1000 replicates. *Chionosphaera cuniculicola* R. Kirschner, Begerow & Oberw. was chosen as outgroup.

RESULTS

One month after inoculation on the natural medium mentioned above, sporodochia developed at room temperature (Fig. 1) that were morphologically identical to those found *in situ* in the field. They grew, however, only in the presence of a *Phaeoacremonium* species that had been present since the initial isolation as a contaminant. In spite of several attempts by us and L. Mostert (CBS), the fungus could not be isolated in pure culture. Morphological structures indicating parasitic interactions between both fungi were not found.

Microscopic characteristics are described below and shown in Fig. 2. The septal pore seen by TEM was a dolipore with associated continuous parenthesomes (Fig. 3). In the neighbour-joining analysis, the fungus was placed within a cluster including species of *Hyaloria* and *Myxarium*. This cluster was supported by a bootstrap value of 100 % (Fig. 4).

Helicomyxa R. Kirschner & Chee J. Chen, **gen. nov.** MycoBank MB500087.

Etymology: *Helico-* – referring to the strongly curved conidia, *myxa* – referring to the slimy sporodochia.

Sporodochia superficialia, gelatinosa, cupulata, pulvinata vel discoidea. Stromata absentia. Conidiophora stratum ad centrum sporodochiorum formantia, irregulariter ramosa, fibulata, hyalina.

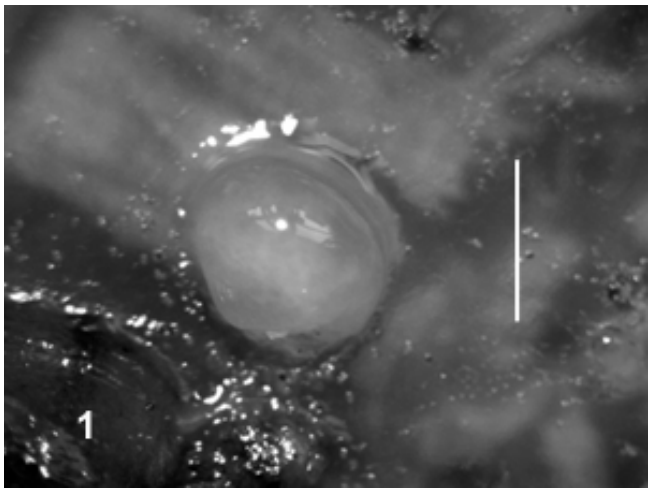

Fig. 1. *Helicomyxa everhartioides*. Mature sporodochium in culture on *Fagus sylvatica* wood chips embedded in water agar. Scale bar = 1 mm.

Cellulae conidiogenae terminales vel laterales, hyalinae. Conidia a fibulis oriunda, curvata, dikaryotica, hyalina, in massa mucosa aggregata. Septa hypharum doliporis et parenthesomatibus continuis.
Typus generis: *Helicomyxa everhartioides* R. Kirschner & Chee J. Chen, in opere ipso descripta.

Sporodochia superficial, gelatinous, cupulate, pulvinate, or discoid. Stromatic elements absent. *Conidiophores* forming a central layer in the sporodochium, irregularly branched, with clamps at the septa, hyaline. *Conidiogenous cells* terminal and lateral, hyaline. *Conidia* developing at the apex of conidiogenous cells from clamps that arise from the base of the previously developed conidium, curved, dikaryotic, hyaline, forming a slimy mass covering the sporodochium. Hyphal septa with dolipores and continuous parenthesomes.

Helicomyxa everhartioides R. Kirschner & Chee J. Chen, **sp. nov.** MycoBank MB500088. Figs 1–2.

Etymology: The epitheton refers to *Everhartia* Sacc. & Ellis, a genus with species similar to *H. everhartioides*.

Sporodochia superficialia, gelatinosa, juvenilia cupulata, matura pulvinata, 140–600 µm diam *ca.* 300 µm alta. Stromata absentia. Hyphae marginales 1–2 µm latae, in textura gelatinosa evanescente. Conidiophora irregulariter ramosa, fibulata, hyalina, laevia. Cellulae conidiogenae terminales vel laterales, cylindricae, leniter undulatae, hyalinae, laeves, (15–)22–38(–45) × 1.5–2 µm. Conidia e fibulis oriunda, unicellularia, hemi-circinata, aliqua reniformia vel irregulariter curvata, dikaryotica, hyalina, 5.5–7 × 2–3 µm, in massa mucosa hyalina aggregata.

Sporodochia superficial, gelatinous, cupulate when young and pulvinate when mature, with base broadly attached to the substratum (Figs 1, 2A), hyaline or white, 140–600 µm diam, *ca.* 300 µm high, with a sterile margin of branched hyphae of 1–2 µm wide possessing clamps at some but not all septa and initially embedded in *textura gelatinosa* that breaks down during maturation (Fig. 2B). *Hyphae* non-stromatic, with clamps at the septa; clamps in some cases with a posterior appendage that in some cases is delimited by a retraction septum (Fig. 2C, D). *Conidiophores* forming a layer in the centre of the sporodochia, irregularly branched, with clamps at the septa, hyaline, smooth (Fig. 2E). *Conidiogenous cells* terminal and lateral, hyaline, smooth, with basal clamp, straight or slightly undulate, apical part in some cases slightly geniculate, (15–)22–38(–45) × 1.5–2 µm. *Conidia* develop from a clamp at the apex of the conidiogenous cell, then form a new clamp basally that becomes delimited by a septum and fuses with the conidiogenous cell.

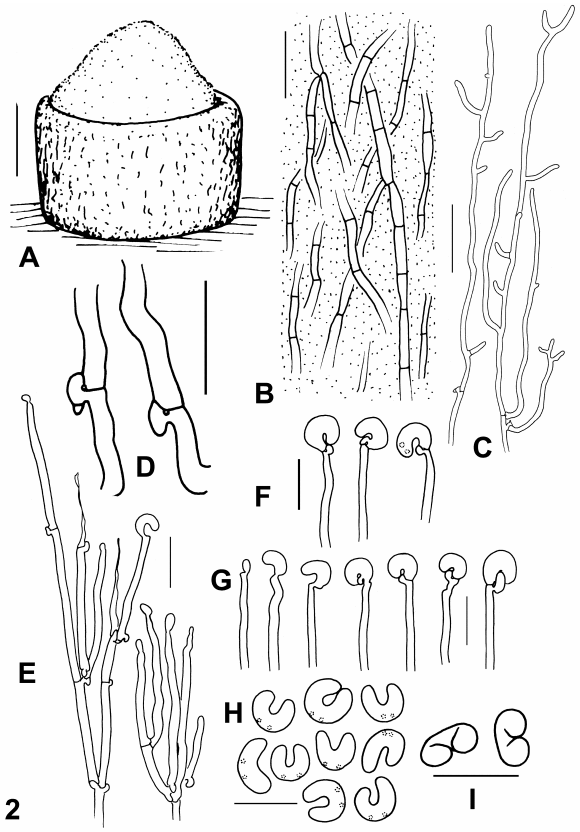

Fig. 2. *Helicomyxa everhartioides*, drawings of characteristics seen in light microscopy. Scale bar = 10 µm except where otherwise noted. A. Habit sketch of a young sporodochium *in situ*. Scale bar = 300 µm. B. Surface of the margin of a sporodochium *in situ* with marginal hyphae embedded in *textura gelatinosa*. C. Marginal hyphae in a squash-mounting from a sporodochium in culture. Scale bar = 20 µm. D. Two clamps with appendages. E. Conidiophores *in situ*. F. Conidia attached to the apex of conidiogenous cells from culture. G. Apical parts of conidiogenous cells from culture arranged from left to right according to the hypothetical sequence of conidial development. H. Hemi-circinate dikaryotic conidia *in situ*. Nuclei indicated by dotted lines. I. Two conidia from culture seen in an inclined position.

During conidial secession, the clamp remains attached to the apex of the conidiogenous cell and grows out to form the new conidium (Fig. 2F, G). Conidia (Fig. 2H, I) one-celled, strongly curved, mostly hemi-circinate, in some cases reniform or irregularly curved, dikaryotic, hyaline, smooth; diameter of the curved conidium as a whole 5.5–7 µm; cell diameter 2–3 µm; conidia forming a hyaline, slimy mass covering the sporodochium.

Specimen examined: **Taiwan**, Kaohsiung, Chu Yun Shan Lin Dao, 400–600 m, on dead rotting branch on ground, 28 Apr. 2001, R. Kirschner & C.-J. Chen 831, (TNM, **holotype**). *Living culture*: CBS 116693, ex-type culture, grown together with *Phaeoacremonium* sp., cult. 2 May 2001, R. Kirschner.

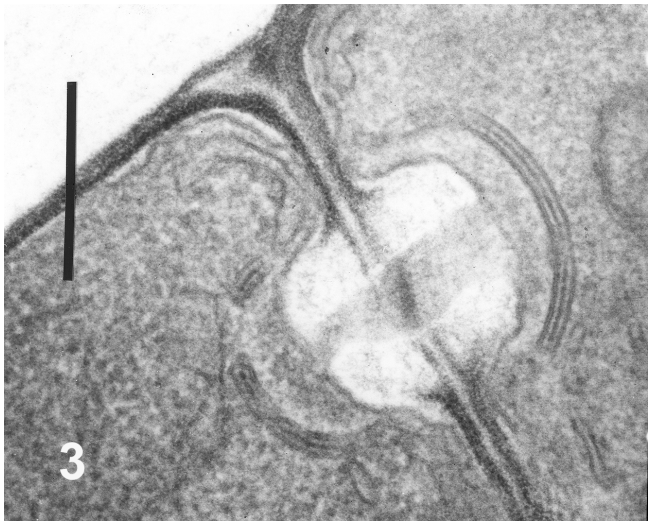

Fig. 3. *Helicomyxa everhartioides*, transmission electron micrograph of a nearly median section through a hyphal septum showing a dolipore with continuous parenthesomes. The large pore in the left parenthesome is apparently an artefact. Scale bar = 0.25 µm.

DISCUSSION

The new taxon *Helicomyxa everhartioides* is characterised by hyaline conidia that are hemi-circinate in the sense of Goos (1987) and are produced from a layer of conidiophores within mucous sporodochia. With these characteristics, it is morphologically similar to species of *Delortia* Pat. and *Everhartia* (Linder 1929, Goh & Hyde 1997, Yanna *et al*. 2000), but differs by the presence of clamp connections. Among the species of *Delortia* and *Everhartia*, only *E. phoenicis* Yanna, W.H. Ho, Goh & K.D. Hyde produces aseptate conidia (Yanna *et al*. 2000).

Another similar genus is *Hobsonia*. The type species *H. mirabilis* (Peck) Linder produces unclamped conidiophores irregularly embedded in gelatinous sporodochia and helicoidal, septate conidia (Linder 1929). This species was shown to be related to species of *Helicogloea* (*Atractiellales*, *Basidiomycota*) by DNA sequence analyses (Sikaroodi *et al*. 2001).

Ditangifibula dikaryotae G.C. Adams forms sporodochia producing allantoid conidia from clamped conidiophores. Conidiogenesis itself, however, is not connected with clamp development, sporodochia are not gelatinous, and the septal pore seen by TEM is associated with unique reticulated parenthesomes (Adams *et al*. 1995). This taxon is, therefore, probably not closely related to *H. everhartioides*.

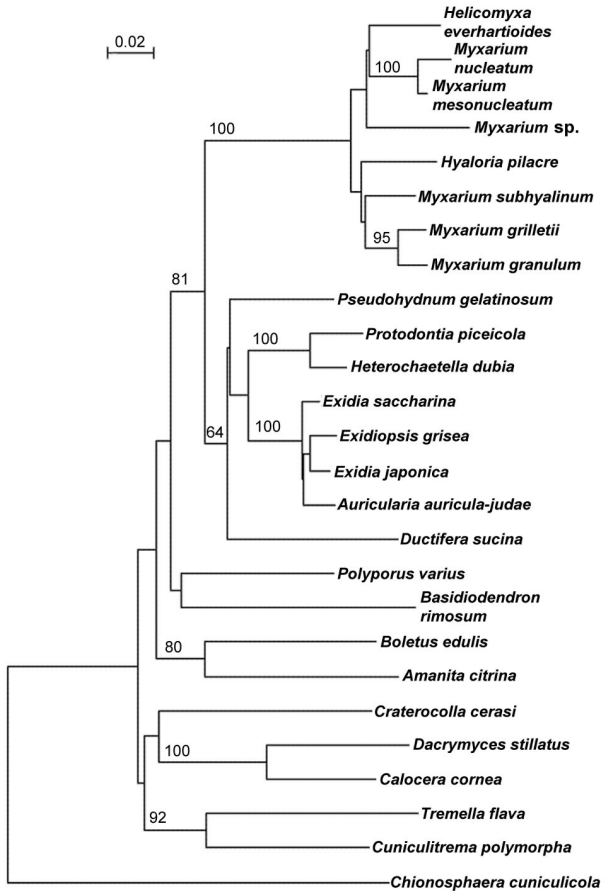

Fig. 4. Phylogenetic hypothesis derived from a neighbour-joining analysis of partial nuclear large subunit ribosomal DNA sequences of *Helicomyxa everhartioides* and selected *Basidiomycota*. The topology is rooted with *Chionosphaera cuniculicola*. Bootstrap values are given as numbers (in percentages) on branches, and are based on 1000 replicates. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site.

Ditangium cerasi (Tul.) Cost. & L. Duf., the anamorph of *Craterocolla cerasi* (Tul.) Bref., produces hyaline, one-celled, curved conidia in conspicuous conidiomata. DNA sequence analyses and the absence of clamps, however, indicate that this species is not closely related to the *Auriculariales* (Weiß & Oberwinkler 2001), nor to *H. everhartioides*.

Clamps on hyphae of *H. everhartioides* often develop a posterior protrusion that in some cases is delimited by a retraction septum (Fig. 2D). This kind of clamp was described in detail and designated as "spurred" clamp, considered typical of members of the *Auriculariales*, by Bandoni & Wells (1992). The appendaged clamps and continuous parenthesomes of *H. everhartioides* indicate a relationship with the

Auriculariales (Bandoni & Wells 1992, Wells 1994). This hypothesis is supported by DNA sequence analysis, which places the new taxon in a well supported cluster of *Myxarium* and *Hyaloria* species. This cluster was seen in previous analyses by Weiß & Oberwinkler (2001) and Wells *et al*. (2004). Wells *et al*. (2004) applied the family name *Hyaloriaceae* to it.

The anamorph of *Myxarium nucleatum* (Schwein.) Wallr. was studied in pure culture by Ingold (1984) and found to closely resemble anamorphs of *Auricularia* species. Conidiophores mostly developed as short side branches of hyphae and successively produced crescent-shaped conidia. Sporodochia and clamp connections were not mentioned. Though conidial shapes are similar in *M. nucleatum* and *H. everhartioides*, conidiophore characteristics are clearly distinct. It remains an open question whether *H. everhartioides* is the anamorph of a species of *Hyaloria* or of *Myxarium* or even of another, as yet undiscovered genus.

In contrast to the anamorph of *M. nucleatum*, *H. everhartioides* could not be cultivated in pure culture, but only in the presence of a species of *Phaeoacremonium*. In young sporodochia, a conidiomatal wall is formed by sterile hyphae appearing to lack clamps in surface view, but occasionally evincing clamps in the proximal region in squash mounts. This margin disappears during maturation of the sporodochium. It is not clear whether these unclamped hyphae are formed by *H. everhartioides* or by the accompanying *Phaeoacremonium* sp. or both. Mycoparasitic interactions could not be confirmed in light microscopy. Among the *Auriculariales*, mycoparasitic members have hitherto not been reported. It might, therefore, be more probable that *H. everhartioides* depends on a growth factor produced by the *Phaeoacremonium* species. Further studies are needed to clarify these interactions.

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