Mycoscience xxx (2018) 1–8

Contents lists available at ScienceDirect

Mycoscience

journal homepage: www.elsevier.com/locate/myc

Full paper

A new species and a new record of *Helicomyces* from Taiwan

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ARTICLE INFO

Article history:
Received 29 November 2017
Received in revised form
14 March 2018
Accepted 10 April 2018
Available online xxx

Keywords:
Aquatic hyphomycetes
Freshwater fungi
Phylogenetics
Taxonomy
Tubeufiaceae

ABSTRACT

Helicomyces geniculatus sp. nov. from decaying wood submerged in a freshwater stream at Juchi Town of Alishan area, Chiayi County, Taiwan, is described and illustrated. It differs from other *Helicomyces* species in having geniculate conidiophores arising from repent hyphae and also borne on erect setae. Abundant stalked sclerotia were also found in this species. *Helicomyces torquatus* is described in this paper as a new record for Taiwan, with new observation. The phylogenetic relationship of *H. geniculatus*, *H. torquatus*, and related taxa were sought by comparing the sequences of their ITS barcode of the nuc rDNA. A synopsis of the 13 accepted *Helicomyces* species is given.

1. Introduction

Helicosporous fungi have been the subject of systematic studies because they are morphologically diverse and produce unusual but elegant conidia for reproduction (Goos, 1987). They are mostly saprobes on plant litter, rotten wood, decaying twigs in moist places or around water (Tsui, Baschien, & Goh, 2016; Wong et al., 1998). *Helicoma* Corda, *Helicomyces* Link, and *Helicosporium* Nees are the three earliest erected helicosporous genera. They are anamorphs of ascomycetes, and their teleomorphs are known to be members of the Tubeufiaceae (Kodsueb et al., 2006; Tsui & Berbee, 2006; Tsui, Sivichai, & Berbee, 2006; Boonmee et al., 2011, 2014). The taxonomy of species in these three genera has been traditionally morphological (Morgan, 1892; Linder, 1929, 1931; Moore, 1953, 1954, 1955, 1957). According to original generic circumscriptions, distinctions between *Helicoma*, *Helicomyces* and *Helicosporium* were often vague due to similarities in coiling of their conidia. Pirozynski (1972) suggested that the taxonomy of these three genera could emphasize on the characters of conidiogenous cells, conidial attachment position, presence of "*conidiola*" (secondary conidia), and presence of "*sclerotes pedicelees*" (stalked sclerotia). To date, more than 200 species names have been assigned into these three genera. Currently taxonomists of these groups of fungi generally distinguish the three genera as follows: in *Helicomyces* and *Helicosporium*, conidial filaments are relatively thin in proportion to their length and hygroscopic (they uncoil in water; Morgan, 1892). Conidiophores are well-developed in *Helicosporium* (Goos, 1989) whereas in *Helicomyces* they are much reduced or lacking (Goos, 1985). In *Helicoma*, conidia are non-hygroscopic, and the conidial filaments are relatively thick in proportion to their length (Goos, 1986).

To date, there are 40 names in *Helicomyces* (Index Fungorum, 2017), but many of these names have been synonymised, excluded or transferred to more appropriate genera. Currently there are 13 accepted names in *Helicomyces* (Boonmee et al., 2014; Goos, 1985; Zhao, Liu, & Wu, 2007). During a survey of microfungi occurring on plant litter submerged in a stream of Alishan area, Chiayi County, Taiwan, we found several helicosporous fungi, among them are *Helicomyces torquatus* L.C. Lane & Shearer (Lane & Shearer, 1984) and an undescribed *Helicomyces* species, both growing on decaying wood. The identities of these two *Helicomyces* species were sought by seeking phylogenetic relationships among similar taxa using available ITS sequences from Genbank, and morphologically comparing with previously described species using a number of references (Linder, 1929, 1931; Goos, 1985, 1986, 1987, 1989; Zhao et al., 2007; and; Cruz, Gusmão, Leão-Ferreira, & Castañeda-Ruiz, 2009). In this paper, we describe and illustrate *H. torquatus* as a new record for Taiwan, with new observation, and *H. geniculatus* as a new species. Scanning electron micrographs of *H. torquatus* are provided. A morphological comparison of accepted *Helicomyces* species is given in Table 1.

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https://doi.org/10.1016/j.myc.2018.04.002
1340-3540/

Table 1
Morphological comparison accepted *Helicomyces* species[a].

Species	Setae	Conidiophore color	Conidiophore size (μm)	Conidial diam (μm)	Conidial filament width (μm)	Conidial coiling (no. of times)	Sclerotia	Conidiola	Outstanding feature	Reference
H. ambiguus (Morgan) Linder	nil	hyaline	1–30 × 2.5–3.5	30–40	5–7	3–5	nil	nil	Conidial base not swollen and not eccentrically attached	Linder, 1929
H. bellus Morgan	nil	(N.A.)	absent	15–25	1.5–3	2.5–3.5	nil	nil	Teeth on repent mycelium	Morgan, 1892
H. colligatus	nil	hyaline	18–45 × 2.5–4	(32–) 50–65	3.5–8	1.5–3	present	present	Robust conidiophores, conidia brittle; conidial filament thick (8 μm) but tapering at both ends (3.5 μm)	Moore, 1954
H. denticulatus G.Z. Zhao, Xing Z. Liu & W.P. Wu	nil	hyaline	5–13 × 3–4.5	20–26	2–3.5	3.5–3.75	nil	nil	Short stout conidiophores with denticles; conidia many coils	Zhao et al., 2007
H. geniculatus	present	pale brown	23– × 4–6	29–36	3–4.5	2.5–3.5	present	nil	Presence of mononematous setae bearing geniculate conidiophores; abundant stalked sclerotia	This paper
H. hyderbadensis P. Rag. Rao & D. Rao	nil	pale yellowish	10–28 × 3–7	19–25	1.8–2.8	1.5–3.5	nil	nil	Small conidia; generally similar to *H. lilliputeus*	Rao & Rao, 1964
H. lilliputeus R.T. Moore	nil	hyaline	28–65 × 2–6	11–28	1.5–2.5	1.5–3	nil	nil	Small conidia; distinctive branching conidiophores	Moore, 1957
H. louisianensis Gooss	nil	pale brown	25–75 × 2–4	40–60	5–7	5–10	nil	nil	Conidia tightly coiled 5–7 times	Goos, 1985
H. macrofilamentosus Matsush.	nil	pale brown	7–20 × 3–4	35–55	5–12	2–3	nil	nil	Wide conidial filament (up to 12 μm)	Matsushima, 1983
H. roseus	nil	pale brown	10–50 × 3.5–5	25–60	4–6(–6.5)	2.25–3	present	present	Conidia borne on teeth from repent mycelium or on short erect branches; pinkish in mass	Linder, 1929
H. scandens	present	pale brown	7.5–40 × 3.5–5	15–35	1.5–3	2–3	nil	nil	Distinct synnema-like dark setae	Morgan, 1892
H. tenuis	nil	hyaline	micronematous	15–20	1.5–2	3–4	present	present	Small sized species producing slim conidia from repent mycelium	Linder, 1929
H. torquatus[b]	nil	hyaline	19–56 × 3.5–5	50–130	4.8–7.2	1.5–3	nil	present	Thick conidial filament filled with abundant oil globules, tapering to a swollen base, and becoming torque-shaped in water.	Lane & Shearer, 1984
H. torquatus[c]	nil	hyaline	21–49 × 3.5–5	76–80	6.3–7.7	1.5–1.75	present	present	Conidial filament bearing multiple conidiola; presence of stalked sclerotia	This paper

[a] *Helicomyces paludosa* (P. Crouan & H. Crouan) Boonmee & K.D. Hyde is not included in this table because this taxon was proposed based on phylogenetic data of its teleomorphic state only (Boonmee et al., 2014).
[b] Data were based on the Panama collection (Lane & Shearer, 1984).
[c] Data were based on the present Taiwanese collection.

2. Materials and methods

2.1. Sample collecting and mycological procedures

Sample collecting and mycological procedures were similar to methodology described in Goh, Hyde, and Ho (1998). Plant litter including wood were collected in plastic bags, and returned to the laboratory where they were incubated at room temperature under a humid condition in sterile plastic boxes. Materials were examined periodically for the presence of fungal fruiting bodies and species were identified. Single-spore isolation (Goh, 1999) of fungi were made and grown in potato dextrose agar (PDA) slants and malt-extract agar (MEA) plates at 20 °C. The pure cultures were the source of DNA for phylogenetic analysis.

2.2. Scanning electron microscopy

Fungal material was cut from natural substratum (decaying wood submerged in freshwater) and then fixed by immersion in 2% (W/V) aqueous osmium tetroxide (OsO_4) for 12 h at 4 °C in the dark. Fixed material was washed in distilled water for 15 min to remove excess osmium tetroxide, and then dehydrated in a 10% graded ethanol series, 15-min steps from 10% to 90% ethanol. Material was then washed in 95% ethanol followed by three 15-min changes of absolute ethanol. Ethanol was replaced with acetone in 2:1, 1:2 (ethanol: acetone) steps followed by three changes of absolute acetone (15 min each change). Dehydrated material was critical-point dried, and then coated with platinum by a Hitachi E-1045 ion sputter coater at 15 mA and 7 Pa condition for 90 s, acquiring ca. 10 nm in thickness, and then examined in a Hitachi S-4700 Field Emission Scanning Electron Microscope at 1 kV.

2.3. Fungal DNA extraction, rDNA barcoding polymerase chain reaction (PCR) and DNA sequencing

Fungal isolates grown on PDA plates for 60 d were prepared for DNA extraction. The protocol of DNA extraction was carried out following Sambrook and Russell (2001). PCR amplification was performed by using experimental sample cocktail, consisting of 2–8 ng DNA template, 0.4 ng upstream primer and downstream primer, PCR Master Mix II (5 ×) (GeneMark Technology Co., Ltd., Taichung, Taiwan). Each of the PCR reactions in 25 μL (1 ×) total volume contained 0.75 U of Taq DNA polymerase, 2 nM $MgCl_2$, 250 μM dNTPs, reaction buffer, and enzyme stabilizer. For the rDNA barcoding, the primer set used to amplify the internal transcribed spacer (ITS) region was ITS5 and ITS4. The amplification protocol was: initial denaturation at 94 °C for 2 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 50 °C, and 30 s at 72 °C, and concluded with a final extension of 10 min at 72 °C. The sequencing of the ITS sequences was achieved by using the same primer set used in PCR

Please cite this article in press as: Kuo, C.-H., & Goh, T.-K., A new species and a new record of *Helicomyces* from Taiwan, Mycoscience (2018), https://doi.org/10.1016/j.myc.2018.04.002