Gohteikhimyces, a novel hyphomycete genus from submerged wood, based on three collections in Taiwan

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Research Article

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Abstract

Gohteikhimyces, a new genus of dematiaceous hyphomycete occurring on decaying wood submerged in a freshwater stream of Taiwan, is proposed based on morphological and molecular data. It produces effuse, black, and somewhat glistening colonies on the surface of natural substratum, which mainly composed of solitary, dry, dark, cymbiform or ellipsoidal to obovoid, 3–4-euseptate conidia, and secede rhexolytically from semi-macronematous conidiophores with percurrent regeneration. The conidia resemble those of *Bactrodesmium* species but they are not produced from sporodochial conidiomata. Phylogenetically, this new genus is placed in the Savoryellomycetidae incertae sedis, closely related to *Flammispora*, but is distant from the Fucosporellales which contains the *Bactrodesmium* species. This new genus is proposed based on three collections from different localities in Taiwan, with descriptions of two novel species, *G. bactrodesmioides* and *G. taroides*, which differ in geographical conditions of collection sites and conidial morphology. The two species are supported by sufficient differences in their LSU, SSU, and the ITS sequences. In this paper, the genus *Gohteikhimyces* is illustrated with line diagrams, light micrographs, and scanning electron micrographs.

Introduction

During our continuing survey of freshwater fungi in Taiwan, we obtained three collections of an undescribed hyphomycete from decaying wood submerged in freshwater streams. These collections were from three different localities in Taiwan, namely Fuxing District (Taoyuan City), Lijia of Alishan Township (Chiayi County), and Taoyuan District (Kaohsiung City), representing arbitrarily three geographical areas of Taiwan (i.e., northern, middle, and southern, respectively). These hyphomycetes share similar morphological features and resemble species of *Bactrodesmium* Cooke (Seifert et al. 2011) in having dematiaceous phragmoconidia with accentuated septa and secede rhexolyticaly. In contrast to *Bactrodesmium*, these conidia are not produced from sporodochial conidiomata but from discrete micronematous or semi-macronematous conidiophores. We have successfully obtained pure cultures of the three collections from single-spore isolations, and their gene were sequenced. These sequences were subjected to several BLAST search but resulted in no closer matching of any known taxon. Preliminary sequence analysis revealed that these hyphomycetes were phylogenetically placed in the Savoryellomycetidae (Sordariomycetes) incertae sedis. Since there is no other known lineage with similar morphology or DNA sequences, the new genus *Gohteikhimyces* is proposed in this paper, with descriptions of two novel species. A synopsis for the comparison of the three strains is given in Table 2. The two *Gohteikhimyces* species are illustrated with light micrographs, scanning electron micrographs, and line diagrams.

Materials and methods

Sample collection and mycological procedures

Plant litter including wood was collected in plastic bags and returned to the laboratory where they were incubated at room temperature on moist filter paper in sterile plastic boxes. Materials were examined periodically for the presence of fungal sporulating structures and species were identified primarily based on morphology. Single-spore isolations were made by using a hand-made glass needle described in Goh (1999). Single conidia from natural substratum were cleaned employing a fundamental drag-and-roll method on the surface of 3% water agar examined using a stereo-microscope. Several small agar blocks, each containing a single conidium that was cleaned in this manner, were eventually picked up by a sterile mycological needle, and placed on the surface of potato dextrose agar (PDA) slants or plates. The agar slants and plates containing the single conidia were incubated at 20 C to obtain pure cultures. No antibiotic was needed for this method. Holotype specimens of fungal taxa were deposited in the Herbarium (Herbarium Code: TNM) at the National Museum of Natural Science (NMNS), Taichung, Taiwan. Ex-type cultures were deposited at the Bioresource Collection and Research Centre (BCRC), Food Industry Research and Development Institute, Hsinchu, Taiwan. Other dried specimens and cultures were deposited at the Department of Plant Medicine, National Chiayi University (NCYU), Chiayi, Taiwan.

Scanning electron microscopy (SEM)

Fungal material was cut from natural substratum 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 10–90% ethanol. The material was then washed in 95% ethanol followed by three 15-min changes of absolute ethanol. Ethanol was replaced with acetone in 2:1 and 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 using 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.

DNA Extraction, PCR Analysis, and Sequencing

Genomic DNA Genomic DNA was extracted using the commercial Plant Genomic DNA Purification Kit (Biokit Co., Taiwan) from fungal colonies cultured on PDA for over two weeks, following the manufacturer's instructions. Amplification of the small (SSU) and large subunit (LSU) ribosomal RNA genes (rDNA) as well as the internal transcribed spacer (ITS) region was performed using the following primers: SSU: NS1 (5’-GTAGTCATATGCTTGTCTC-3’) / ITS5R (5’-CCTTGTTACGACTTTTACTTCC-3’); ITS and LSU: ITS5 (5’-GGAAGTAAAAGTCGTAACAAGG-3’) / LR5 (5’-TCCTGAGGGAAACTTCG-3’) (White et al. 1990; Hopple and Vilgalys 1994). The PCR reactions were conducted with PowerPol 2X PCR Mix (ABclonal Inc., Massachusetts) under the following conditions: an initial denaturation at 98°C for 2 minutes, followed by 35 cycles of denaturation at 98°C for 20 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 2 minutes. A final extension was performed at 72°C for 5 minutes. Sequencing was carried out on an ABI PRISM 377 DNA sequencer located at the Biotechnology Center of National Chung Hsing University. For the sequencing, the same primers used in the PCR reaction were employed, along with additional primers: ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) for the ITS region, and LR0R (5’-

ACCCGCTGAACTTAAGC-3') for the LSU region (Vilgalys and Hester 1990; White et al. 1990). All the sequences that were derived have been deposited in DDBJ (Mashima et al. 2016).

Molecular Identification and Phylogenetic Analysis

For molecular identification and phylogenetic analysis, preliminary comparisons of the SSU, ITS, and LSU sequences of the strains NCYU-111LJ2-2A1 (BCRC FU31851), NCYU-111FX3-1D2, and NCYU-108L1-6 (BCRC FU31431) were conducted using NCBI's BLAST tool. Subsequently, sequences of related fungal strains were retrieved from GenBank (TABLE 1). These sequences were initially aligned using MAFFT. Poorly aligned regions were corrected through manual inspection or subsequently removed with ClipKit (Steenwyk et al. 2020). The SSU, ITS, and LSU sequences were then concatenated, with gaps indicating missing data. The construction of the maximum likelihood tree was performed using IQ-Tree 2.2.2.6 (Minh et al. 2020). During this process, the optimal DNA substitution model for each DNA regions was determined using the model selection feature within IQ-TREE. The aligned sequences underwent 100 rounds of bootstrap resampling, and Felsenstein's bootstrap support values were calculated for each node. Bayesian phylogenetic trees were generated using Mrbayes 3.2.7a (Ronquist et al. 2012), running 1,000,000 generations with tree sampling occurring every 100 generations. The initial 20% of sampled trees were discarded as burn-in, resulting in a consensus tree with posterior probabilities. All computations were performed on a virtual machine (Ubuntu 22.04.2 LTS) provided by Google Colaboratory (https://colab.research.google.com/). For reproducibility, a consistent random seed (56) was used throughout all necessary procedures.

Results

Sequencing and Phylogenetic Analysis

The SSU, ITS, and LSU regions of strains NCYU-111LJ2-2A1 (BCRC FU31851), NCYU-111FX3-1D2, and NCYU-108L1-6 (BCRC FU31431) were successfully sequenced in this study. The lengths of these sequences varied, with the SSU sequences ranging from 2429 to 2867 bp, ITS from 536 to 543 bp, and LSU from 853 to 916 bp. All sequences obtained in this study have been uploaded to DDBJ (TABLE 1, LC794304–LC794309). Phylogenetic analysis showed that these three strains formed a highly supported clade (100/1), clustering as sister groups with *Flammispora bioteca* (Savoryellomycetidae, incertae sedis), and were positioned at the basal node of Savoryellomycetidae (Fig. 1). Notably, the similarity of the SSU sequences of these strains to the type strain of *Flammispora bioteca*, BCC13367, which has only SSU sequences available, ranged from 56.5–73.1%. Additionally, the SSU, ITS, and LSU regions of NCYU-108L1-6 showed similarities of only 74.4–74.5%, 93.2–93.4%, and 92.7% to NCYU-111LJ2-2A1 and NCYU-111FX3-1D2, respectively. Therefore, based on DNA evidence, these three strains are recognized as a new genus, *Gohteikhimyces*, distinct from the genus *Flammispora*, and they include two new species, namely, *Gohteikhimyces bactrodesmioides* and *Gohteikhimyces taroides*.

Taxonomy

Gohteikhimyces J.H. Ou, S.Y. Hsieh & C.H. Kuo, gen. nov.

Index Fungorum number

IF901621

Type species: Gohteikhimyces bactrodesmioides J.H. Ou, S.Y. Hsieh & C.H. Kuo, described below.

Etymology

In honor to Dr. Teik-Khiang Goh, with his major contribution to the taxonomy of hyphomycetes.

Phylogenetic position

Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Savoryellomycetidae, incertae sedis; *Gohteikhimyces*.

Description: Species lignicolous and saprobic in freshwater habitats. Asexual morph hyphomycetous: Colonies on natural substratum effuse, black, somewhat glistening. Setae absent, Hyphopodia absent. Mycelium partly immersed, partly superficial, composed of septate, branched, hyaline to pale brown, smooth, moderately thick-walled hyphae. Conidiomata absent. Conidiophores absent or micronematous to semi-macronematous, mononematous, short, arising from superficial hyphae, subhyaline, simple, septate, smooth-walled. Conidiogenous cells borne directly on sub-immersed hyphae or on short conidiophores, terminal, integrated, holoblastic, monoblastic, thin-walled, subhyaline, determinate or sometimes percurrently regenerating. Conidia acrogenous, solitary, dry, dematiaceous, broadly cymbiform or ellipsoidal to obovoid, conical or rounded at the distal end, tapering to a distinctly paler, cuneiform basal cell, thick-walled, 3–4-euseptate, with thickening or banding at the septum and a conspicuous central septal pore connecting sac-like cytoplasm of adjacent cells, penultimate cell usually smaller than the distal and central cells. Conidial secession rhexolytic, usually with distinct frills of torn conidiogenous cells at the base.

Notes: Gohteikhimyces resembles *Endophragmiella* B. Sutton by its conidiophores that regenerate percurrently, producing dematiaceous euseptate conidia that secede rhexolytically. However, the latter differs in having macronematous conidiophores which are branched and bear conspicuous conidiogenous cells (Hughes 1979). Conidia of *Gohteikhimyces* resemble those of *Bactrodesmium* with accentuated septa, but the latter are produced in sporodochial conidiomata. In phylogenetic analysis, *Gohteikhimyces* forms a highly supported sister group with *Flammispora*, which is also found on plant substrates in aquatic environments. However, *Flammispora* does not undergo asexual reproduction either on natural substrates or in culture (Pinruan et al. 2004), distinguishing it from the genus *Gohteikhimyces*.

Gohteikhimyces bactrodesmioides J.H. Ou, S.Y. Hsieh & C.H. Kuo, sp. nov. (Figs. 2–5)

Index Fungorum number

IF901622

Etymology: *bactrodesmioides*, referring to the morphology of the conidia which resembles those of *Bactrodesmium* species.

Description

Asexual morph hyphomycetous. Colonies on natural substratum effuse, black, somewhat glistening. Mycelium partly immersed, partly superficial, composed of septate, branched, hyaline to pale brown, smooth-walled, moderately thick-walled hyphae. Conidiomata absent. Conidiophores absent or micronematous to semi-macronematous, mononematous, short, arising from sub-immersed hyphae, subhyaline, simple, sparsely and closely septate, smooth-walled. Conidiogenous cells discrete and borne directly from sub-immersed hyphae or on short hypha-like conidiophores, terminal, integrated, holoblastic, monoblastic, thin-walled, subhyaline, determinate or sometimes percurrently regenerating. Conidia 28–41(–46) µm × (17–)19–23.5 µm ($\bar{x}$ = 40.7 × 20.4 µm, n = 53), acrogenous, solitary, dry, dematiaceous, broadly cymbiform or ellipsoidal to obovoid, broadly conical or rounded at the distal end, tapering to a distinctly paler, cuneiform basal cell, straight or sometimes slightly curved, thick-walled, smooth, 3–4-euseptate, with thickening at the septum and a conspicuous central septal pore connecting sac-like cytoplasm of adjacent cells, penultimate cell usually smaller than the distal and central cells. Conidial secession rhexolytic, usually with distinct frills of torn conidiogenous cells at the base. Sexual morph unknown.

Typification: TAIWAN, Chiayi County: Lijia Village, Alishan Township (23.460–120.710, elevation 1084 m a.s.l.) on decorticated wood submerged in a freshwater stream, 2 May 2022, leg. Chang-Hsin Kuo, NCYU-111LJ2-2A1 (**holotypus**, TNM F0037702); ex-type living culture: BCRC FU31851; available gene sequences: ITS = LC794305, LSU = LC794305, SSU = LC794308. TAIWAN, Taoyuan City: Fuxing District, 56K on the Northern Cross-Island Highway (24.651–121.423, elevation 1118 m a.s.l.), on decorticated wood of *Acer serrulatum* Hayata (Aceraceae) submerged in a freshwater stream, 14 May 2022, leg. Chang-Hsin Kuo, NCYU-111FX3-1D2 (**paratypus**, TNM F0037703); available gene sequences: ITS = LC794304, LSU = LC794304, SSU = LC794307.

Known distribution

Known only from the type locality (Taiwan).

Notes

The species epithet for this fungus is proposed due to its resemblance to *Bactrodesmium*, and is based on the two strains collected from different localities in Taiwan, with a little intra-specific variation. Conidia from the holotype (NCYU-111LJ2-2A1) have a dimension of 27.5–46 × 19–23.5 µm ($\bar{x}$ = 41.8 × 21.6 µm, n = 24) and those from the paratype (NCYU-111FX3-1D2) measured 29–44.5 × 17–21 µm, $\bar{x}$ = 39.6 × 19.2 µm, n = 29). Under the SEM, conidial wall of strain NCYU-111FX3-1D2 appears slightly rough-walled (Fig. 5d), however, this feature is not visible under the light microscope. Both strains of *G.*

bactrodesmioides were collected at intermediate altitudes (*ca.* 1100 m a.s.l.) in the subtropical regions (northern and middle part) of Taiwan island.

Gohteikhimyces taroides J.H. Ou, S.Y. Hsieh & C.H. Kuo, sp. nov. (Figs. 6, 7)

Index Fungorum number

IF901623

Etymology: taroides, referring to the morphology of the conidia which resembles the rhizomes of taro (*Colocasia esculenta*).

Description

Asexual morph hyphomycetous. Colonies on natural substratum effuse, black, somewhat glistening. Mycelium partly immersed, partly superficial, composed of septate, branched, hyaline to pale brown, smooth-walled, moderately thick-walled hyphae. Conidiomata absent. Conidiophores absent or micronematous to semi-macronematous, mononematous, short, arising from sub-immersed hyphae, subhyaline, simple, sparsely and closely septate, smooth-walled. Conidiogenous cells discrete and borne directly from sub-immersed hyphae or on short, hypha-like conidiophores, integrated, terminal, holoblastic, monoblastic, thin-walled, subhyaline, sometimes percurrently regenerating. Conidia 42–50.5 × 18.5–23 µm ($\bar{x}$ = 47 × 20.8 µm, n = 22), acrogenous, solitary, dry, dematiaceous, ellipsoidal, broadly cymbiform or mitriform, rarely obovoid, usually conical at the distal end, tapering to a distinctly paler, cuneiform basal cell, usually slightly curved or sometimes slightly sigmoid, thick-walled, smooth, 3–4-euseptate, with thickening at the septum and a conspicuous central septal pore connecting sac-like cytoplasm of adjacent cells, penultimate cell usually smaller than the distal and central cells. Conidial secession rhexolytic, usually with distinct frills of torn conidiogenous cells at the base. Sexual morph unknown.

Typification: TAIWAN. Kaohsiung City: Taoyuan District, 20 Provincial Highway (on the Southern Cross-Island Highway), Jianshan Village (23.102–120.688, elevation 454 m a.s.l.), on decaying wood submerged in a freshwater stream, 17 November 2019, leg. Chang-Hsin Kuo, NCYU-108L1-6 (**holotypus**, TNM F0037704); ex-type living culture: BCRC FU31431; available gene sequences: ITS = LC794306, LSU = LC794306, SSU = LC794309.

Known distribution

Known only from the type locality (Taiwan).

Notes

The species epithet for this fungus is proposed due to its resemblance to taro. It is proposed as a second species in the genus based on a single specimen collected from the southern, tropical region of Taiwan (Kaohsiung City) at a lower altitude (454 m a.s.l.). It differs from *G. bactrodesmioides* in conidial

morphology. The two species are also distinct due to the low similarity in their sequences of LSU, SSU and ITS segments. Conidia of *G. taroides* ($\bar{x}$ = 47 × 20.8 µm) are distinctly larger than those of *G. bactrodesmioides* ($\bar{x}$ = 40.7 × 20.4 µm). Moreover, although conidia of all the three strains are similar in shape, being more or less ellipsoidal or broadly cymbiform, the conidia of *G. taroides* are more curved or sometimes sigmoid in contour, more attenuated at the apex, relatively more elongated in shape and hence appear mitriform. The differences in conidial shape between *G. taroides* and *G. bactrodesmioides* are also apparent under the SEM (Figs. 5, 7). Comparable to that of *G. bactrodesmioides*, the conidial wall of *G. taroides* is also slightly rough-walled but this feature is not visible under the light microscope. A diagrammatic representation for comparison of the two *Gohteikhimyces* is given in Fig. 8.

Discussion

Phylogenetic analysis and multiple insertions in SSU

In this study, phylogenetic analyses of the SSU, ITS, and LSU regions revealed that the genus *Gohteikhimyces* is situated at the basal node of the Savoryellomycetidae (Fig. 1) and is closely related to *Flammispora* Pinruan, Sakay., K.D. Hyde & E.B.G. Jones, a freshwater ascomycete described from decaying palm leaves (Pinruan et al. 2004). Notably, the SSU sequences of *Gohteikhimyces* show low similarity (56.5–73.1%) with those of its closest relative, *Flammispora bioteca* (strains BCC13367 and BCC13368). This low similarity can be attributed to the presence of multiple insertions in the SSU sequences of *Gohteikhimyces* compared to *Flammispora*: *Gohteikhimyces bactrodesmioides* NCYU-111LJ2-2A1 and NCYU-111FX3-1D2 exhibit up to six insertions, and *Gohteikhimyces taroides* NCYU-108L1-6 contains as many as ten. This significant structural difference indicates a clear genetic distance between *Gohteikhimyces* and *Flammispora*, as well as between *Gohteikhimyces bactrodesmioides* and *Gohteikhimyces taroides*. Multiple insertions in the SSU have been reported in various fungal taxa and are believed to be related to group I introns, as noted in species like *Verticillium* and *Cordyceps militaris* (Lian et al. 2014; Papaioannou et al. 2014). In our analysis, only two of these introns were found in the SSU sequences of *Melanotrigonum ovale* (CBS 138744 and CBS 138815) and *Conioscypha nakagirii* (BCC 77658), both of which are members of Savoryellomycetidae, while similar introns were not observed in the SSU sequences of other members of Savoryellomycetidae. Considering that *Gohteikhimyces* occupies the basal node in the phylogenetic analysis, this suggests that the occurrence of multiple intron insertions may be an ancestral characteristic within Savoryellomycetidae, which has been progressively lost through evolutionary processes.

Generic comparison

Gohteikhimyces resembles *Endophragmiella* by its conidiophores that regenerate percurrently due to rhexolytic conidial secession. In *Endophragmiella*, however, the conidiophores are normally distinct, sparingly to highly branched, with conspicuous conidiogenous cells, and produce euseptate conidia that vary from amerosporous, didymosporous to phargmosporous (Seifert et al. 2011). The genus *Endophragmiella* is a heterogeneous assemblage of similar hyphomycetes (Hughes 1979; Wu and

Zhuang 2005) and awaits further taxonomic treatments based on phylogeny. Only two *Endophragmiella* species, namely *E. dimorphospora* (Strain FMR 12150; Hernández-Restrepo et al. 2017)d *taxi* (Strain CBS 614.84; Vu et al. 2019), have sequence data available in the GenBank, and currently they are placed in the Helminthosphaeriaceae (Sordariales) which are phylogenetically distant from *Gohteikhimyces*.

The genus *Bactrodesmium* includes over 60 species of dematiaceous hyphomycetes forming sporodochia in the substrata and produce clavate, transversely septate conidia (Seifert et al. 2011). The conidial secession of *Bactrodesmium* has been addressed several times and according to various authors it was considered either rhexolytic or schizolytic (Ellis 1976; Hughes 1983; Hughes and White 1983; Hernández-Restrepo et al. 2013; Réblová et al. 2020). The genus is obviously heterogeneous (Hernández-Restrepo et al. 2017) and needs further taxonomic studies based on phylogeny and other scientific evidences. Réblová et al. (2020) reviewed the genus *Bactrodesmium* and its allied genera based on morphological and molecular data, and found that the representative species of the genus are phylogenetically placed in the Savoryellales. *Gohteikhimyces* resembles *Bactrodesmium* in producing conidia with accentuated septa, but these conidia are not produced in sporodochial conidiomata. Based on current study (FIG. 1), *Gohteikhimyces* is phylogenetically distant from representative species of *Bactrodesmium* (Réblová et al. 2020).

Bactrodesmiastrum Hol.-Jech. (Holubová-Jechová 1984) is a small lignicolous genus currently comprising of five species which resembles *Bactrodesmium* (Seifert et al. 2011) in producing phragmosporous conidia with accentuated eusepta from sporodochial conidiomata. Recent phylogenetic studies (Hernández-Restrepo et al. 2013, 2015, 2017) have shown that *Bactrodesmiastrum* is a member of the Fuscosporellales and not congeneric with *Bactrodesmium* (Savoryellales). In the present study (Fig. 1), we have shown that *Gohteikhimyces* is phylogenetically distant from representative species of *Bactrodesmiastrum*.

Another genus similar to *Gohteikhimyces* is *Hadrosporium* currently comprises two species. The ellipsoidal conidia of *Gohteikhimyce*s are reminiscent of those produced by *H. fraserianum* Syd. (Hughes 1979). However, *Hadrosporium* differs in being sporodochial, with conidiophores bearing percurrent collarettes, and producing conidia that secede schizolytically (Seifert et al. 2011). Molecular data for *Hadrosporium* is current lacking.

Comparison of collection sites

Gohteikhimyces bactrodesmioides and *G. taroides* are proposed in the present paper as two distinct species based on differences in the conidial morphology and the sequences of three segments of their rDNA. However, the localities where these two species were collected are worthy of remarking. Taiwan lies across the temperate zone and tropical zone. The Tropic of Cancer passes through the middle of the island, giving the northern regions a subtropical climate and the south a tropical climate. Fuxing District of Taoyuan City (northern Taiwan) and Chiayi County (nearly at the middle region of Taiwan), where the two strains of *G. bactrodesmioides* were collected, are located in the temperate zone and experience subtropical climate, with mean annual temperature (MAT) of 20–22 °C and mean annual rainfall (MAR)

of 2000–2500 mm (Central Weather Administration, https://www.cwa.gov./eng/). In contrast, Kaohsiung City (southern Taiwan), where *G. taroides* was collected, has a warmer (MAT = 24–25 °C), drier (MAR = 1500–2000 mm) tropical climate. Both strains of *G. bactrodesmioides* were collected at intermediate altitudes (around 1100 m a.s.l.), whereas *G. taroides* was collected at a lower altitude (around 450 m a.s.l.). Based on the availability of only three strains, the significance of the geographical differences (e.g. climates, altitudes, etc.) at these collection sites relating to the occurrence of two *Gohteikhimyces* species cannot be over-emphasized for the time being.

Septa pores

A peculiar feature of *Gohteikhimyces* is the distinct septal pores found in its conidia which connect sac-like cytoplasm of adjacent cells. In a revision of *Bactrodesmium* species, Réblová et al. (2020) also discussed similar septal structure in some of the species, which appeared as a conspicuous thickening in the middle of each septum surrounding the pore. This feature is well visible especially in species with more or less evenly spaced septa such as *B. diversum*, *B. leptopus*, *B. pallidum* and *B. spilomeum*. In the side view, it is barrel-shaped but in surface view, the thickening has a circular outline. These structures resemble a dolipore septum occurring in basidiomycete hyphae. Ho and Hyde (2004) had looked into the details of similar septal pore structures in 46 species of fungi under the light and transmission electron microscopes, which they termed as the “doliiform infrastructure”. These include 33 species of hyphomycetes and 13 species of coelomycetes. Fungi with such dolliform infrastructure in their euseptate conidia include hyphomycetes such as *Bactrodesmium* spp., *Cancellidium applantum*, *Canalisporium* spp., *Junwangia globulosa*, and *Pithomyces obscuriseptatus*, and coelomycetes such as *Sarcostroma grevilleae* and *Stegonsporium pyriforme* (Tubaki 1975; Hughes and White 1983; Nag Raj 1994; Ho and Hyde 2004; Goh and Kuo 2021). Fungi with such structure in the distoseptate species are more diverse, including hyphomycetes such as *Acarocybellina arengae*, *Annellophora mussaendae*, *Cheiromyces recurvus*, *Cordana abramovii*, *Drechslera iridis*, *Ellisembia aquirostrata*, *Helminthosporium* spp., *Janetia curviapicis*, *Matsushimiella* spp., *Pseudospiropes josserandii*, and *Sporidesmium brachypus*, and coelomycetes such as *Coryneum* spp. (Goh and Hyde 1996; Castañeda Ruiz et al. 2001; Ho and Hyde 2004; Monteiro et al. 2015; Luo et al. 2019; Yang et al. 2023). Although such septal pore structures resemble dolipores of basidiomycetes, the presence of these structures in these fungi does not imply their close affinity to the basidiomycetes. In fact, there have been many phylogenetic studies of these fungi throughout the decades which have proven that they are anamorphic states of ascomycetes, either in the form of hyphomycetes or coelomycetes. So far, there is no report of such distinct barrel-shaped septal pore occurring in any known septate ascospores; seemingly this structure only occurs in the mitospores (conidia) of their asexual morphs. The presence of such septal pores in conidia probably is an important means for survivor, which may be involved in nutrient or cytoplasm translocation from non-germinating cells to germinating cells during spore germination (Cannon 1995; Ho and Hyde 2004). This is merely a speculation regarding the functional role of such septal pore structures which awaits verification in future studies.

Conclusions

In the present paper, the genus *Gohteikhimyces* is proposed based on three collections from different localities in Taiwan (arbitrarily representing the northern, middle, and southern geographical areas of the island), with descriptions of *G. bactrodesmioides* and *G. taroides*. The two species differ in conidial morphology and are supported by sufficient differences in their rDNA sequences. As a remark, the two strains of *G. bactrodesmioides* were found at intermediate altitudes in the subtropical regions of Taiwan, whereas *G. taroides* was collected from the tropical region at a lower altitude. This paper confirms the phylogenetic placement of *Gohteikhimyces* in the Savoryellomycetidae, however, its ordinal and familiar lineages remain unknown and await future discoveries of more relevant taxa for analyses.

Declarations

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Authors' contributions

All authors contributed to the study's conception and design. Funding was acquired by Chang-Hsin Kuo and Sung-Yuan Hsieh. Material preparation, data collection, and analysis were performed by Jie-Hao Ou, Chang-Hsin Kuo, and Sung-Yuan Hsieh. The first draft of the manuscript was written by Jie-Hao Ou and Chang-Hsin Kuo. All authors commented on previous versions of the manuscript and approved the final manuscript.

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Data availability

The authors declare that all data and materials as well as software application or custom code support their published claims and comply with field standards. The sequence data generated in this study are deposited in the DNA Data Bank of Japan (DDBJ).

Ethics approval and Consent to participate

The collections of plant and fungal materials were approved by the National Science and Technology Council of Taiwan and the Ministry of Economic Affairs of Taiwan. The authors declare that the data and materials included in the present publication were authentical and ethical. The entire manuscript was written by the authors and submitted only to Mycological Progress for consideration of publication. All technical staff and involved personnel have the consent to participate in the present research.

Consent for publication

The authors have agreed to publish the data included in the present manuscript and confirmed the co-authorship of the present publication.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Tables are available in the Supplementary Files section.

Figures

Fig. 1 Phylogenetic tree

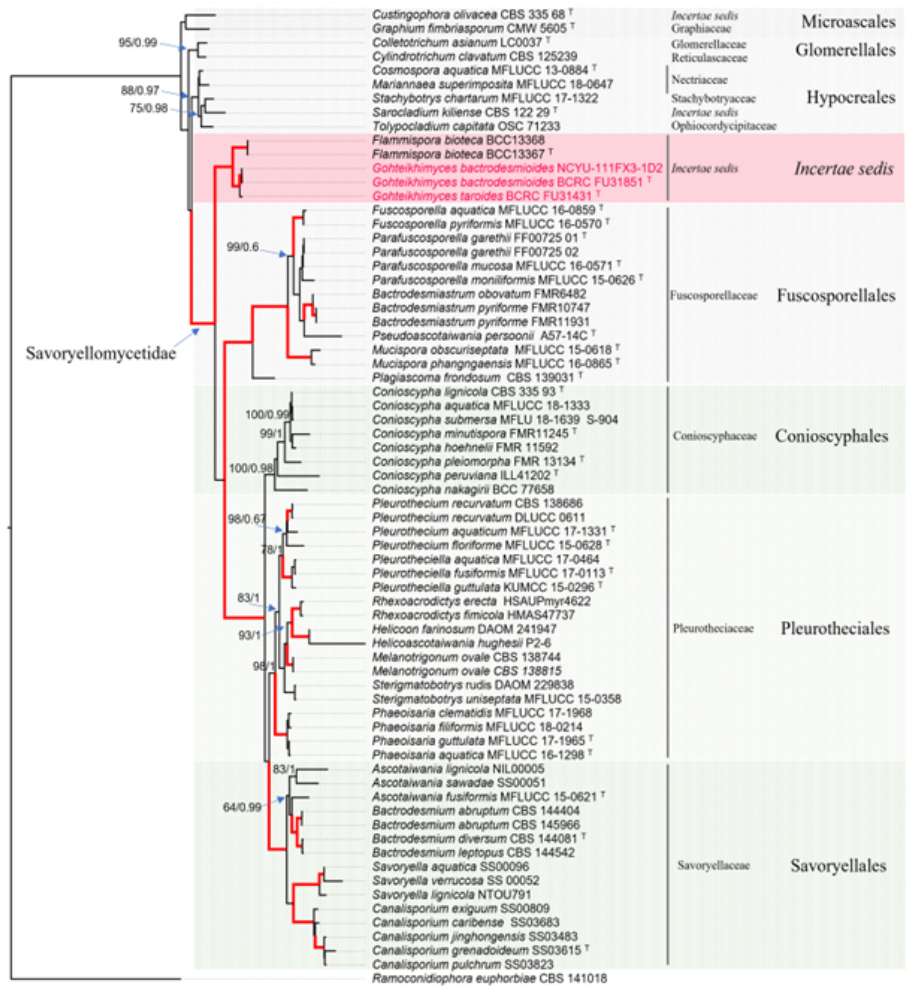

Figure 1

Maximum Likelihood tree, based on the SSU, ITS, and LSU regions of strains examined in this and related studies, was constructed using IQ-Tree. Bootstrap (BS) values and posterior probabilities (PP) derived from IQ-Treeand Mrbayes are indicated next to the nodes as BS/PP. Nodes are labeled with their statistical support only when the bootstrap value exceeds 50. For nodes with a BS/PP of 100/1.0, the numerical value is omitted and the node is highlighted with a bold red line.

Fig. 2. *Gohteikhimyces bactrodesmioides* (NCYU-111LJ2-2A1, TNM: F0037702)

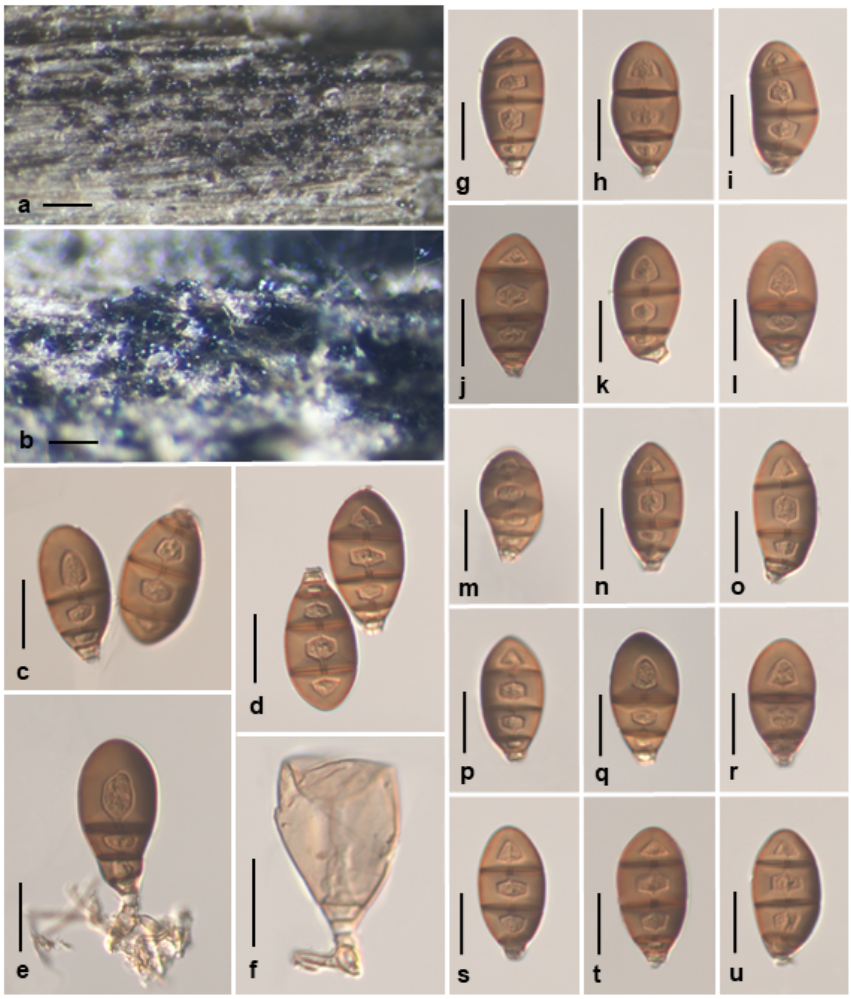

Figure 2

Gohteikhimyces bactrodesmioides (TNM: F0037702, holotype, NCYU-111LJ2-2A1). **a**, **b** Colonies on natural substratum. **c**, **d** Conidia. **e** A conidium borne on conidiogenous cell attaching to hyphae. **f** Conidiogenous cell and developing conidium. **g–u** Conidia. Scale bars: a = 200 mm, b = 100 mm, c-u = 20 mm.

Fig. 3. *Gohteikhimyces bactrodesmioides* (NCYU-111FX3-1D2, TNM: F0037703)

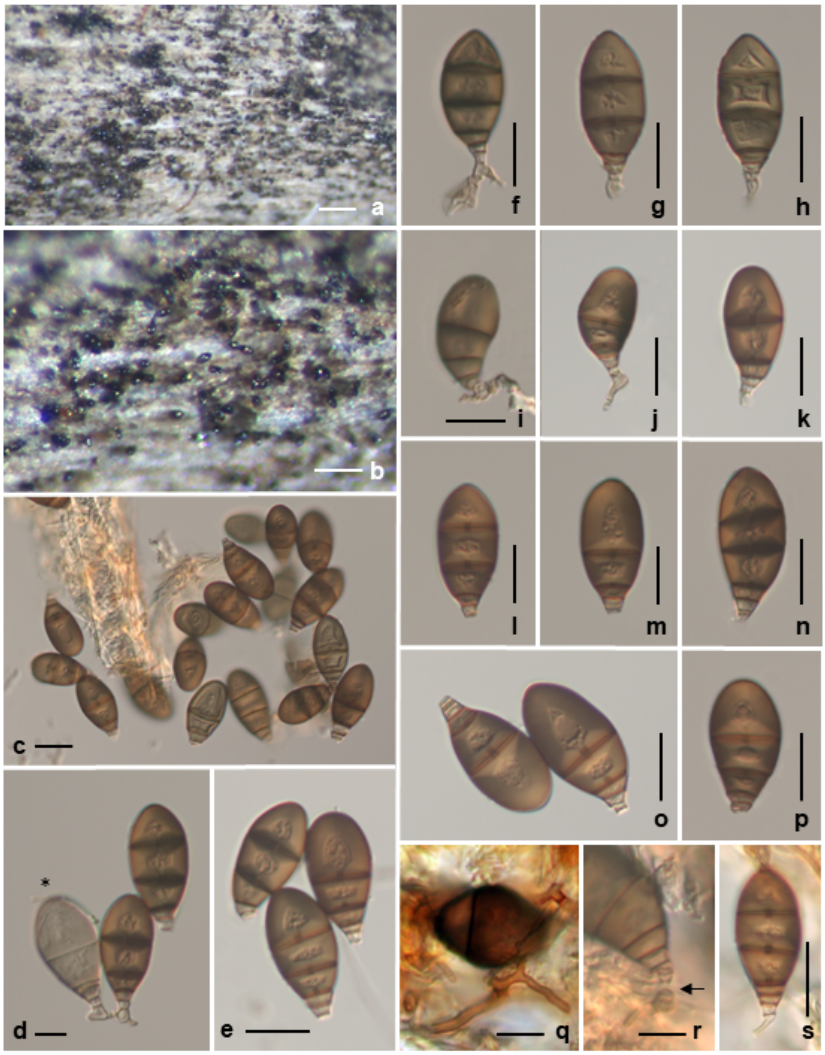

Figure 3

Gohteikhimyces bactrodesmioides (TNM: F0037703, paratype, NCYU-111FX3-1D2). **a**, **b** Colonies on natural substratum. **c** Squash mount showing a number of conidia. **d, e** Conidia. asterisk (*) denotes an immature conidium attached to conidiogenous cell. **f–k**Individual conidia, each showing part of conidiogenous hyphae or remnants of conidiogenous cells at the base resulting from rhexolytic conidial secession. **i–p** Conidia. Note the conspicuous septal pores and the remnant of conidiogenous cells

resulting from rhexolytic conidial secession. **q**Conidium and septate hyphae in natural substratum. **r** Proximal end of a conidium showing rhexolytic detachment (arrowed) from the conidiogenous cell. **s**Conidium focusing at the septal pores. Scale bars: a = 200 mm, b = 100 mm, c, e-p, s = 20 mm, d, q, r = 10 mm.

Fig. 4. ***Gohteikhimyces bactrodesmioides*** **(NCYU-111FX3-1D2, TNM: F0037703)**

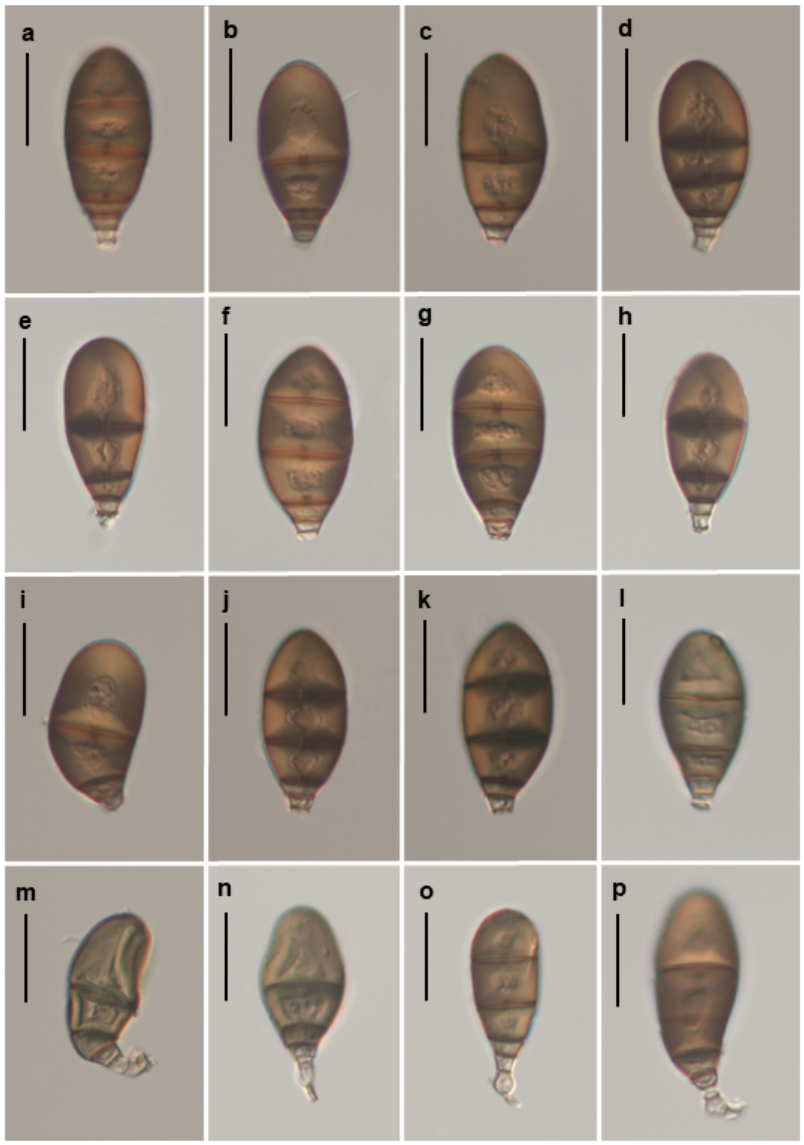

Figure 4

Gohteikhimyces bactrodesmioides (TNM: F0037703, paratype, NCYU-111FX3-1D2). **a–l** Conidia that seceded rhexolytically. **m–p** Conidia with the conidiogenous cells still attached to the base. Scale bars: a-p = 20 mm

Fig. 5. SEM of *Gohteikhimyces bactrodesmioides* (NCYU-111FX3-1D2, TNM: F0037703)

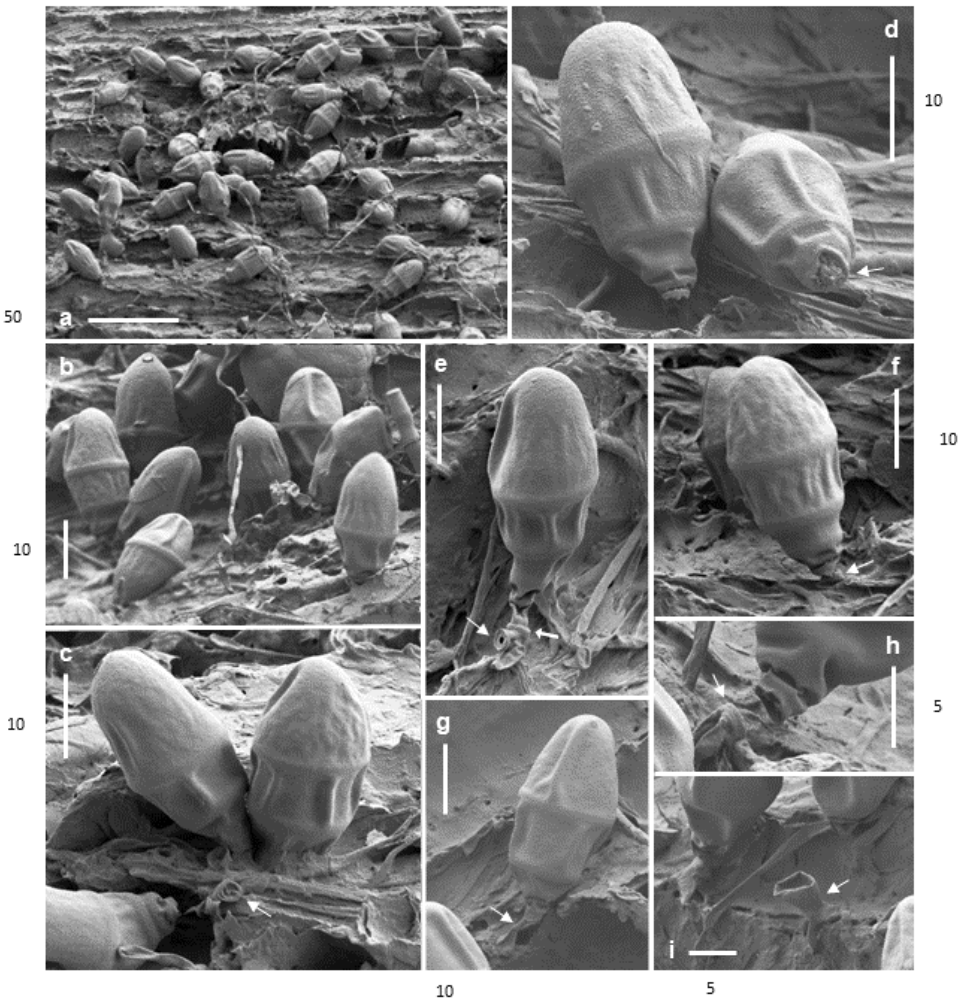

Figure 5

Gohteikhimyces bactrodesmioides (TNM: F0037703, paratype, NCYU-111FX3-1D2). Scanning electron micrographs. **a**Colonies on natural substratum. **b** Several conidia arising more or less upright from the surface of natural substratum. **c** Conidia and conidiogenous cells. Arrow points to one discrete conidiogenous cell. **d**Two detached conidia. Arrow points to the base of a conidium after rhexolytic secession. Note the slightly rough surface of conidial wall, which is not visible under the microscope. **e** a conidium attached to conidiogenous cell. Thinner arrow points to a conidiogenous cell with a central hole after conidial detachment. Thicker arrow points to an apparent percurrent regeneration of conidiogenous cell. **f** A conidium attached to a conidiogenous cell (arrowed). **g** A conidium attached to a conidiophore (arrowed). **h** Proximal end of a conidium detached from a conidiogenous cell (arrowed). **i** Two conidia showing the proximal end. Arrows points to a conidiogenous cell arising from sub-immersed hypha. Scale bars: a = 50 mm, b-g = 10 mm., h, i = 5 mm

Fig. 6. *Gohteikhimyces taroides* (NCYU-108L1-6, TNM: F0037704)

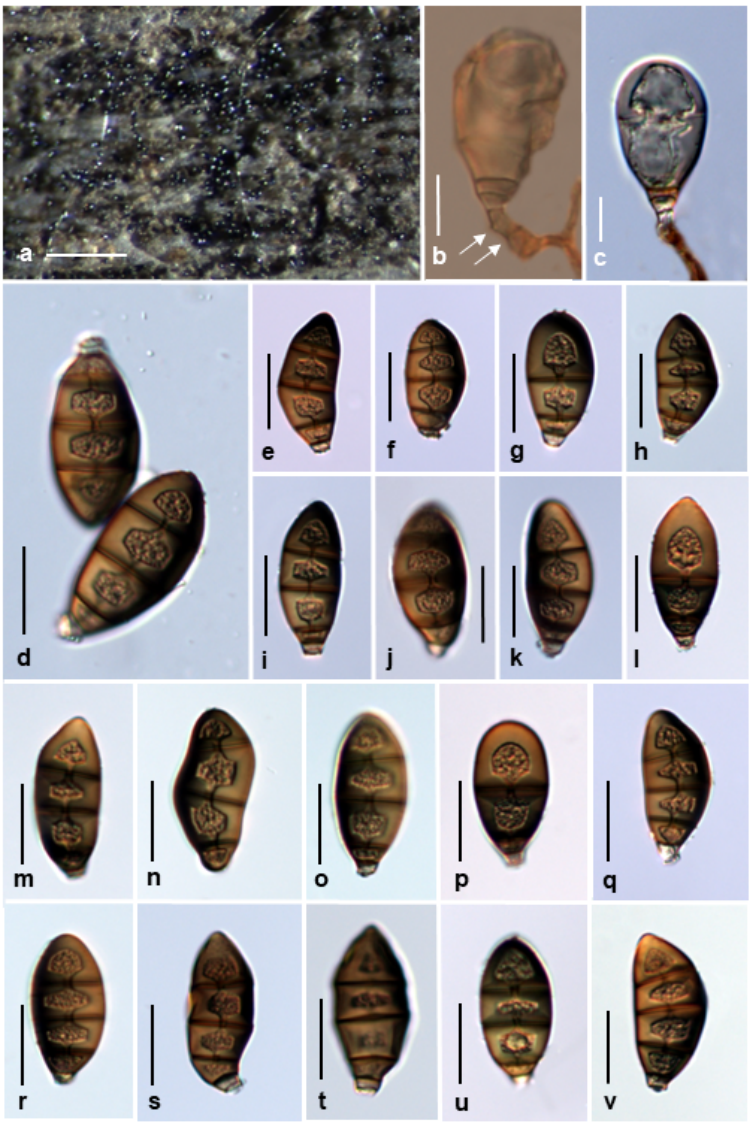

Figure 6

Gohteikhimyces taroides (TNM: F0037704, holotype, NCYU-108L1-6). **a** Colonies on natural substratum. **b** Conidiophore showing percurrent regenerations (arrowed) and developing conidium. **c** Conidiophore and developing conidium. **d–v**Conidia. Scale bars: a = 200 mm, b, c = 10 mm, d-v = 20 mm.

Fig. 7. SEM of *Gohteikhimyces taroides* (NCYU-108L1-6, TNM: F0037704)

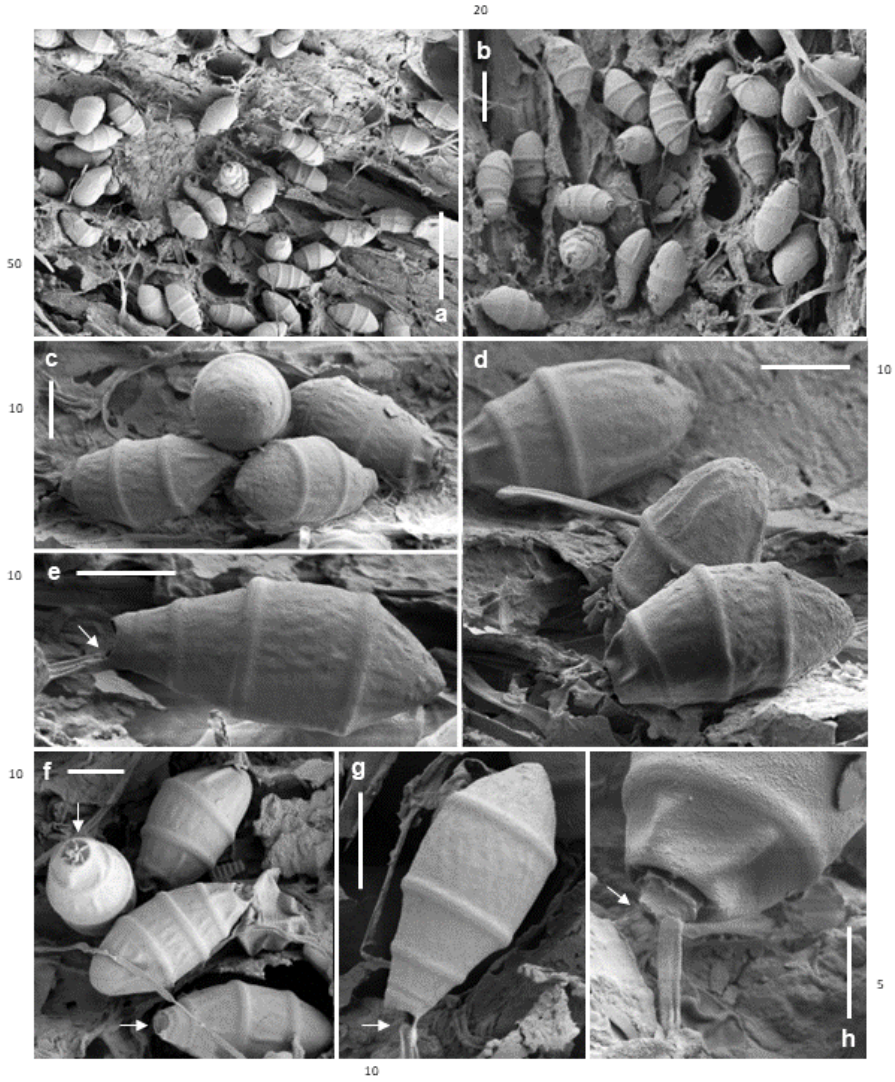

Figure 7

Gohteikhimyces taroides (TNM: F0037704, holotype, NCYU-108L1-6). Scanning electron micrographs. **a**Colonies on natural substratum. **b** Closer view of colonies on natural substratum. **c**, **d** Several detached conidia lying on the surface of natural substratum. **e** A conidium which has undergone rhexolytic secession, still attached to remnant of the conidiogenous cell (arrowed). **f** Several detached conidia. Arrow points to the base of two conidia after rhexolytic secession. **g** A conidium which has undergone

rhexolytic secession, still attached to remnant of the conidiogenous cell (arrowed). **h** Proximal end of a conidium detaching from a conidiogenous cell (arrowed). Note the slightly rough surface of conidial wall, which is not visible under the microscope. Scale bars: a = 50 mm, b = 20 mm, c-g = 10 mm, h = 5 mm.

Fig. 8. ***Gohteikhimyces*** **spp.**

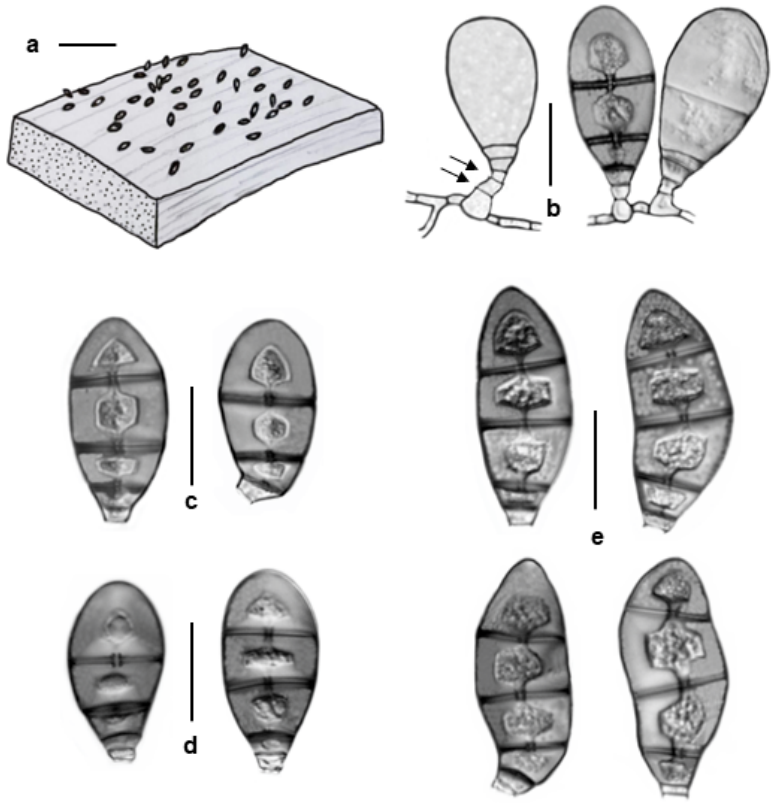

Figure 8

Gohteikhimyces spp., diagrammatical representation. **a** Colonies on natural substratum. **b**Conidiophores arising from hyphae bearing developing conidia. Arrows point to percurrent regenerations. **c** Two conidia of *Gohteikhimyces bactrodesmioides* from strain NCYU-111LJ2-2A1. **d** Two conidia of *Gohteikhimyces bactrodesmioides* from strain NCYU-111FX3-1D2. **e** Four conidia of *Gohteikhimyces taroides*. Scale bars: a = 200 mm, b-e = 20 mm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Ouetal2024GohteikhimycesTable1Seq.xlsx
- Ouetal2024GohteikhimycesTable2Synopsis.xlsx
- Gohteikhimycesdimensionscompared.xlsx