



Keqinzhangia aquatica gen. et sp. nov. and *Pseudocoronospora hainanense* gen. et sp. nov., isolated from freshwater in southern China

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Abstract During an investigation of the diversity of aquatic hyphomycetes from southern China, two interesting isolates were collected. These two isolates were cultured and sequenced, and a BLAST search of their LSU sequences against data in GenBank revealed that the closest related taxa were in the genus *Microthyrium*. Phylogenetic analyses, based on the combined sequence data from the internal transcribed spacer (ITS) and large nuclear subunit ribosomal DNA (LSU), revealed that our isolates belong to the Microthyriaceae. Combined morphological characters allowed us to describe our isolates as two new genera and species in Microthyriaceae, named as: *Keqinzhangia aquatica* and *Pseudocoronospora hainanense*. The full descriptions, illustrations, and a phylogenetic tree showing the position of the two new genera were provided in this paper.

Keywords Aquatic fungi · Ascomycota · Microthyriaceae · Phylogeny · Taxonomy

Introduction

Microthyriales was introduced by Arnaud in 1918, with the type family Microthyriaceae. Originally, Microthyriales included two families, Microthyriaceae and Micropeltidaceae, based on their flattened ascomata with a poorly developed base. However, Hongsanan and Hyde (2017) excluded Micropeltidaceae from Microthyriales based on their phylogenetic analyses and morphological characteristics. Moreover, their phylogenetic analyses showed that species of Microthyriales cluster together as a distinct clade within Dothideomycetes with high support. Currently, Microthyriales only contains a single family Microthyriaceae (Hongsanan and Hyde 2017; Wijayawardene et al. 2018, 2020).

Saccardo (1883) established the family Microthyriaceae, with the sexual genus *Microthyrium* Desm. as the type genus. Theissen (1913) included Microthyriaceae in the order Hemisphaeriales. Subsequently, Arnaud (1918) established a new order Microthyriales to accommodate Microthyriaceae and Microthyriopsisidaceae. After that, the family has experienced a complicated taxonomic history, and various genera were included, such as foliar epiphytes or saprobes. Wu et al. (2011) reappraised the Microthyriaceae

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based on examinations of generic types and provided sequence data of several species. They finally accepted seven sexual genera in the Microthyriaceae. In 2017, Wijayawardene et al. (2018) merged both asexual and sexual genera in the outline of Ascomycota and totally accepted nine genera in the family, including eight sexual genera and one asexual genus. In a recent study, Hongsanan et al. (2020) accepted eleven genera in this family based on morphology and phylogeny, including eight sexual genera and three asexual genera. At the same time, they added the definition of an asexual morph on family level.

China has an enormous fungal diversity, and the southwestern region in this country was assessed as one of the world's 34 biodiversity hotspots (Myers et al. 2000). In recent years, we have been investigating the fungal diversity in China, including soils, submerged leaves, and aquatic plants, and described many new taxa (Qiao et al. 2017, 2018a, b; 2019; 2020; Zheng et al. 2019, 2020a, b, 2021a, b, c). During our ongoing studies of freshwater hyphomycetes in the Yunnan and Hainan provinces, two interesting fungi were collected from submerged leaves of unidentified dicotyledonous plants. These two isolates were cultured and sequenced, and a BLAST search of their LSU sequences against data in GenBank revealed that the closest related taxa were in the genus *Microthyrium*. To further confirm the position of our isolates, phylogenetic analyses with related taxa within Microthyriaceae were carried out based on complete sequences of internal transcribed spacer (ITS) and partial sequences of the nuclear large subunit ribosomal DNA (LSU) genes. Combining morphological characters, we finally described our isolates as two new genera and species in Microthyriaceae, named as: *Keqinzhangia aquatica* gen. et sp. nov. and *Pseudocoronospora hainanense* gen. et sp. nov.

Materials and methods

Isolation and morphological study of strains

Submerged dicotyledonous leaves were collected from Yunnan and Hainan Provinces. Samples were preserved in zip-lock plastic bags, labeled, and transported to the laboratory. The decomposed leaves were cut into several 2–4 × 2–4 cm sized fragments

in the laboratory and then were incubated on corn meal agar (CMA, 20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) medium for 10 days at room temperature. Single conidia were isolated with a sterilized needle under an Olympus BX51 microscope and cultivated on. Morphological observations were made from CMA after incubation at 25 °C for 1 week, and microscopic photographs were taken on an Olympus BX51 microscope under differential interference contrast model and captured with an Olympus DP 10 digital camera using Olympus DP controller (V.3.1,1208) software. Measurements were based on 30 random conidia and 10 conidiophores.

Pure cultures were deposited in the Herbarium of the Laboratory for Conservation and Utilization of Bio-Resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF, formerly Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan), the China Center for type Culture Collection (CCTCC), and the China General Microbiological Culture Collection Center (CGMCC).

DNA extraction, PCR amplification, and sequencing

Pure cultures were grown on potato dextrose agar (PDA, 200 g potato, 20 g dextrose, 18 g agar, 1000 ml distilled water) medium for 7 days at 25 °C. Actively growing mycelium was scraped off from the surface of the culture and transferred to 2 ml Eppendorf micro-centrifuge tubes. Total genomic DNA was extracted according to the procedures in Turner et al. (1997). Primers used for PCR amplification and sequencing of the nuclear large subunits ribosomal DNA (LSU) and the internal transcribed spacer (ITS) were LROR/LR7 (White et al. 1990) and ITS1/ITS4 (Vilgalys and Hester 1990), respectively. Each 25 µL PCR reaction volume consisted of 12.5 µL T5 Super PCR Mix (containing Taq polymerase, dNTP and Mg²⁺, Beijing TsingKe Biotech Co., Ltd., Beijing, China), 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM), 1 µL DNA template, 5 µL of PCR buffer, and 4.5 µL sterile water. PCR reactions were run in an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) following the PCR thermal cycle programs described by Qiao et al. (2020). PCR products were purified by using the PCR product purification kit (Biocolor BioScience and Technology

Co., Shanghai, China), and forward and reverse sequenced on an ABI 3730 XL DNA sequencer (Applied Biosystems, Foster City, CA, USA) with the same primers, using a Thermo Sequenase Kit as described by Kindermann et al. (1998). These sequences were deposited in the GenBank database at the National Center for Biotechnology Information (NCBI) and the accession numbers are listed in Table 1.

Sequence alignment and phylogenetic analyses

Preliminary BLAST searches with the LSU sequences of our isolates against the GenBank nucleotide database determined the closely related species, it showed that their closest related taxon is in the genus *Microthyrium*. Based on this information, related sequences at the two marker loci of ITS and LSU, which include 13 representatives belonging to Microthyriaceae, two representatives belonging to Natipusillales, two representatives belonging to Phaeotrichales, three representatives belonging to Venturiales, and three representatives belonging to Zeloasporisporiales, were downloaded according to recent studies (Crous et al. 2019; Gonzalez et al. 2020; Hongsanan et al. 2020). *Kirschsteiniothelia lignicola* Boonmee & K.D. Hyde was used as the outgroup.

The sequences, together with the newly generated sequences, were manually aligned with ClustalX 1.83 (Thompson et al. 1997). The resulting alignments were subsequently checked and refined using BioEdit version v. 7.0.4.1 (Hall 1999). The two alignments were combined with BioEdit and then converted to a NEXUS file using the programme MEGA6 (Tamura et al. 2013). The resulting combined sequence matrix contained 1,215 nucleotide positions from two loci (855 from LSU, 360 from ITS), and it was uploaded to TreeBASE (www.treebase.org; accession number: S28478).

Bayesian inference (BI) and maximum likelihood (ML) were used in this study for phylogenetic analyses. BI analysis was conducted with MrBayes v3.2.2 (Ronquist et al. 2012) with NEXUS files. The Akaike information criterion (AIC) implemented in jModelTest 2.0 (Posada 2008) was used to select the best fit models after likelihood score calculations were done. GTR + F + I + G4 was estimated as the best-fit model under the output strategy of AIC. Metropolis-coupled Markov chain Monte Carlo

(MCMCMC) searches were run for 1,000,000 generations sampling every 500th generation. Two independent analyses with four chains each (one cold and three heated) were run until stationary distribution was achieved. The initial 25% of the generations of CMC sampling were excluded as burn-in. The refinement of the phylogenetic tree was used for estimating Bayesian inference posterior probability (BIPP) values. ML analysis was computed by RAxML (Stamatakis 2006) with the PHY files generated with ClustalX 1.83, using the GTR-GAMMA model. Maximum likelihood bootstrap proportions (MLBP) were computed with 1000 replicates. Trees were visualized in FigTree 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>, July 2021). Bayesian inference posterior probabilities (BIPP) ≥ 0.95 and maximum likelihood bootstrap proportions (MLBP) $\geq 75\%$ are indicated at nodes.

Results

Phylogenetic analyses

BLAST analyses with LSU sequences revealed *Microthyrium* as closely related taxon to our isolates but with relatively low percentage of identities. The combined analysis of the two loci (ITS and LSU), which was analyzed by BI and ML approaches, confirmed the status of our isolates in the family Microthyriaceae. In this tree, YMF 1.04626 and YMF 1.04517 grouped into the Microthyriaceae with good support. YMF 1.04626 was clustered together with the sexual genus *Microthyrium* with good support (MLBP/BIPP = 92%/1.0), and the clade was close to the asexual genus *Neoanungitea* Crous. YMF 1.04517 formed an isolated clade, close to *Hamatispora* L.T.H. Yen, K. Yamag. & K. Ando, *Neoanungitea*, and *Microthyrium* with good support (MLBP/BIPP = 80%/0.98). Combined with morphological differences, we described YMF 1.04626 and YMF 1.04517 as two new asexual genera and species in Microthyriaceae, named as *Keqinzhangia aquatica* and *Pseudocoronospora hainanense* (Fig. 1).

Taxonomy

Keqinzhangia Z.F. Yu, M. Qiao & R.F. Castañeda, **gen. nov.**

Table 1 Species, strains, and their corresponding GenBank accession numbers of sequences used for phylogenetic analyses

| Taxon | Strain ^{a,b} | Substrate | Country | GenBank accession no. ^c | | References |
|--|-----------------------------|---|--------------|------------------------------------|-----------------|--------------------------|
| | | | | LSU | ITS | |
| <i>Chaetothyriothecium elegans</i> | CPC 21375 ^T | Leaves of <i>Castanopsis</i> sp. | Thailand | KF268420 | – | Hongsanan et al. (2014) |
| <i>Hamatispora phuquocensis</i> | VICCF 1219 ^T | Unidentified fallen leaves | Vietnam | LC064073 | LC064074 | Yen et al. (2018) |
| <i>Heliocephala elegans</i> | MUCL 39,003 | Fallen leaf of <i>Andira inermis</i> | Cuba | HQ333478 | HQ333478 | Abarca et al. (2011) |
| <i>Heliocephala gracilis</i> | MUCL 41,200 | Fallen leaf of <i>Matayba oppositifolia</i> | Cuba | HQ333479 | HQ333479 | Abarca et al. (2011) |
| <i>Heliocephala natarajanii</i> | MUCL 43745 ^T | Basideocarp of <i>Pisolithus tinctorius</i> | India | HQ333480 | HQ333480 | Abarca et al. (2011) |
| <i>Heliocephala zimbabweensis</i> | MUCL 40019 ^T | Unidentified leaf litter | Zimbabwe | HQ333481 | HQ333481 | Abarca et al. (2011) |
| <i>Keqinzhangia aquatica</i> | YMF 1.04262 | Unidentified submerged leaves | China | MK577809 | MK569507 | This paper |
| <i>Kirschsteiniothelia lignicola</i> | MFLUCC10-0036 ^T | Unidentified decaying wood | Thailand | HQ441568 | HQ441567 | Boonmee et al. (2012) |
| <i>Microthyrium buxicola</i> | MFLUCC 15-0212 ^T | Leaves of <i>Buxus</i> sp. | Italy | KT306551 | – | Ariyawansa et al. (2015) |
| <i>Microthyrium buxicola</i> | MFLUCC 15-0213 ^T | Leaves of <i>Buxus</i> sp. | Italy | KT306552 | – | Ariyawansa et al. (2015) |
| <i>Natipusilla decorospora</i> | AF236-1 | Unidentified submerged wood | Ecuador | HM196369 | – | Ferrer et al. (2011) |
| <i>Natipusilla naponensis</i> | AF217-1 | Unidentified submerged wood | Ecuador | HM196371 | – | Ferrer et al. (2011) |
| <i>Neoanungitea eucalypti</i> | CBS 143173 ^T | Leaves of <i>Eucalyptus obliqua</i> | Australia | MG386031 | MG386031 | Crous et al. (2017) |
| <i>Ochroconis dracaenae</i> | CPC 26115 ^T | Leaf spots of <i>Dracaena reflexa</i> | American | KX228334 | KX228283 | Crous et al. (2016) |
| <i>Phaeotrichum benjaminii</i> | CBS 541.72 | Dung of Rodentia | American | AY004340 | MH860561 | Lumbsch et al. (2000) |
| <i>Pseudocoronospora hainanensis</i> | YMF 1.04517 | Unidentified submerged leaves | China | MK577807 | MK569505 | This paper |
| <i>Pseudomicrothyrium thailandicum</i> | MFLU 14-0286 ^T | Unidentified dead leaves | Thailand | MT741680 | – | Hongsanan et al. (2020) |
| <i>Pseudopenidiella gallaica</i> | CBS 121796 ^T | Unidentified dead leaves | Spain | LT984843 | LT984842 | Crous et al. (2018) |
| <i>Sympoventuria capensis</i> | CBS 120,136 | Fallen leaf of <i>Eucalyptus</i> sp. | South Africa | KF156104 | DQ885906 | Samerpitak et al. (2014) |
| <i>Trichodelitschia bisporula</i> | CBS 262.69 | Unkown | Unkown | GU348996 | MH859305 | Schoch et al. (2009) |
| <i>Tumidisporea shoreae</i> | MFLUCC 12-0409 ^T | Dead leaves of <i>Shorea</i> sp. | Thailand | KT314073 | – | Ariyawansa et al. (2015) |
| <i>Tumidisporea shoreae</i> | MFLUCC 14-0574 | Dead leaves of <i>Shorea</i> sp. | Thailand | KT314074 | – | Ariyawansa et al. (2015) |
| <i>Venturia inaequalis</i> | CBS 594.70 | Dead leaves of <i>Pyrus aria</i> | Britain | GU301879 | KF156040 | Schoch et al. (2009) |
| <i>Zeloasperisporium ficicola</i> | MFLUCC 15-0221 ^T | Leaves of <i>Ficus benjamina</i> | Thailand | KT387733 | – | Hongsanan et al. (2015) |

Table 1 continued

| Taxon | Strain ^{a,b} | Substrate | Country | GenBank accession no. ^c | | References |
|---|--------------------------|--------------------------|----------|------------------------------------|----------|-------------------------|
| | | | | LSU | ITS | |
| <i>Zeloasperisporium hyphopodioides</i> | CBS 218.95 ^T | Air | Cuba | EU035442 | EU035442 | Hongsanan et al. (2015) |
| <i>Zeloasperisporium siamense</i> | IFRDCC 2194 ^T | Unidentified dead leaves | Thailand | JQ036228 | – | Wu et al. (2011) |

^aEx-type strains are indicated with ^T

^bAbbreviations of culture collections (where known): *CBS* Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; *CPC* Culture collection of Pedro Crous housed at the CBS; *IFRDCC* International Fungal Research and Development Centre Research Institute of Resource Insects, Kunming, China; *MFLUCC* Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; *MUCL* Mycothèque de l'Université catholique de Louvain, Louvain-la-Neuve, Belgium; *VICCF* Vietnam Type Culture Collection, Institute of Microbiology and Biotechnology, Vietnam National University, Ha Noi, Vietnam; *YMF* Herbarium of the Laboratory for Conservation and Utilization of Bio-Resources, Yunnan University, Kunming, Yunnan, P.R. China

^cNew sequences generated in this study are in bold

Etymology: Named in honor of Prof. Keqing Zhang of Yunnan University for his contribution on biological control of pathogenic nematodes.

MycoBank number: MB 840,430.

Asexual morph hyphomycetous. Vegetative hyphae cylindrical, branched, microguttulate, septate, hyaline, smooth-walled. Fertile hyphae cylindrical-obclavate, inflated and subulate at the tip, macroguttulate, dark septate, hyaline, smooth-walled. Conidiophores prostrate, not differentiated. Conidiogenous cells holothallic, narrow cylindrical to cylindrical, discrete, indeterminate, forming conidia by random thallic-arthric conidial ontogeny. Conidial secession schizolytic. Conidia thallic-arthric, solitary, polymorphic, cylindrical, cylindrical-obclavate, obclavate, bacilliform, fusiform, sub-obolecythiform or cuneiform, unicellular to septate, hyaline. Chlamydospores globose, terminal, solitary or short catenulate, subhyaline. Sexual state: Unknown.

Type species: *Keqinzhangia aquatica* Z.F. Yu, M. Qiao & R.F. Castañeda.

Keqinzhangia aquatica Z.F. Yu, M. Qiao & R.F. Castañeda, **sp. nov.** (Figs. 2, 3, 4).

Etymology: Epithet refers to the collection from stream water.

MycoBank number: MB 840,432.

Asexual morph hyphomycetous. Colonies flat, growing slowly on CMA, attaining about 2.4 cm diam. after 20 days at 25 °C. Pale mouse grey, reverse

mouse grey. Mycelium mostly immersed, composed of cylindrical, branched, densely micro-guttulate, septate, subhyaline to hyaline vegetative hyphae and cylindrical-obclavate, inflated and subulate at the tip, macroguttulate, dark septate, hyaline, smooth-walled fertile hyphae. Conidiophores prostrate, undifferentiated. Conidiogenous cells holothallic, narrowly cylindrical, frequently undifferentiated, hyaline, forming conidia by random thallic-arthric disarticulation. Conidia thallic-arthric, solitary, polymorphic, cylindrical-obclavate, long obclavate, cylindrical, bacilliform, fusiform, narrow doliiform, subdolabriform, subobolecythiform or cuneiform, truncate at the ends or truncate at the base and obtuse or rounded at the apex, 0–6(-7)-septate, slightly or strongly constricted at the dark septa, sinuate, macroguttulate, smooth, hyaline, 12–76.5 × 3–6.2 μm, arising after random disarticulation of fertile hyphae at the darker septa. Chlamydospores solitary or catenate, broad globose, subglobose to ellipsoidal, terminal, slightly or densely guttulate, smooth, subhyaline, 8–12.6 × 4.1–5.4 μm. Sexual state: Unknown.

Holotype: YMF 1.04262, isolated from leaves of an unidentified dicotyledonous plant submerged in a stream, E'mei National Conservation Area, Sichuan Province, China, 29°35'1"N, 103°17'3"E, ca. 1750 m elev., Jun 2014, Zefen Yu, permanently preserved in a metabolically inactive state (deep freezing) in the Conservation and Utilization of Bio-Resources in

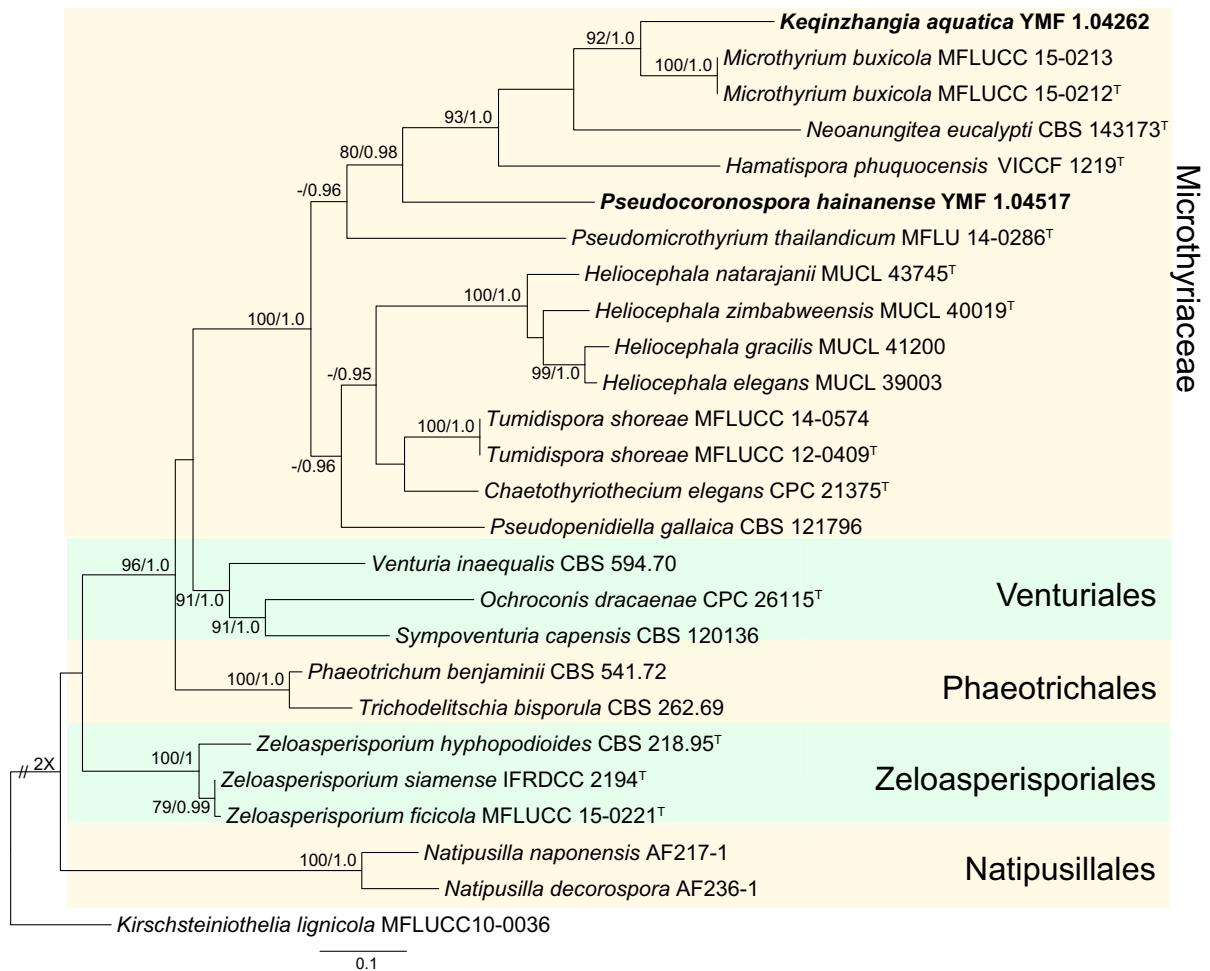


Fig. 1 Phylogenetic tree generated by maximum likelihood analyses using combined sequences of the nuclear large subunit (LSU) and the internal transcribed spacers (ITS) loci. Bootstrap support values for maximum likelihood (ML) over 75% and Bayesian posterior probabilities greater than 0.95 are indicated

above or below the nodes as MLBP/BIPP. *Kirschsteiniothelia lignicola* strain MFLUCC10-0036 was used as the outgroup. The strain numbers are noted after the species names with ex-type strains indicated by ^T. Novel species are indicated in bold

Yunnan. Ex-type culture CCTCC AF 2,021,070 = CGMCC 3.16100.

Notes: In *Keqinzhangia aquatica*, the fertile hyphae are located at the margin of the colony arise laterally from vegetative hyphae forming aerial mycelium with narrow cylindrical, cylindrical, long cylindrical-obclavate, obclavate, inflated or globose, subulate cellular structures, that include the tip growth. The thallic-arthric conidia are formed by random fission at the darker septa of preexisting cells of the fertile hyphae in a similar holothallic mode described by Cole (1986) and Seifert et al. (2011).

Pseudocoronospora Z.F. Yu, M. Qiao & R.F. Castañeda, **gen. nov.**

Etymology: Name refers to the morphological similarity to the genus *coronospora*.

MycoBank number: MB 840,431.

Asexual morph hyphomycetous. Conidiophores macronematous, mononematous, erect, straight, septate, unbranched, smooth, brown. Conidiogenous cells polyblastic, sympodial extended, integrated, terminal, indeterminate, denticulate. Conidial secession rhexolytic. Conidia solitary, acropleurogenous, obclavate, crowned, with mammiform protuberances arranged near the apex; septate, smooth or verruculose, hyaline, fringed at the base. Sexual state: Unknown.

Type species: *Pseudocoronospora hainanense* Z.F. Yu, M. Qiao & R.F. Castañeda.

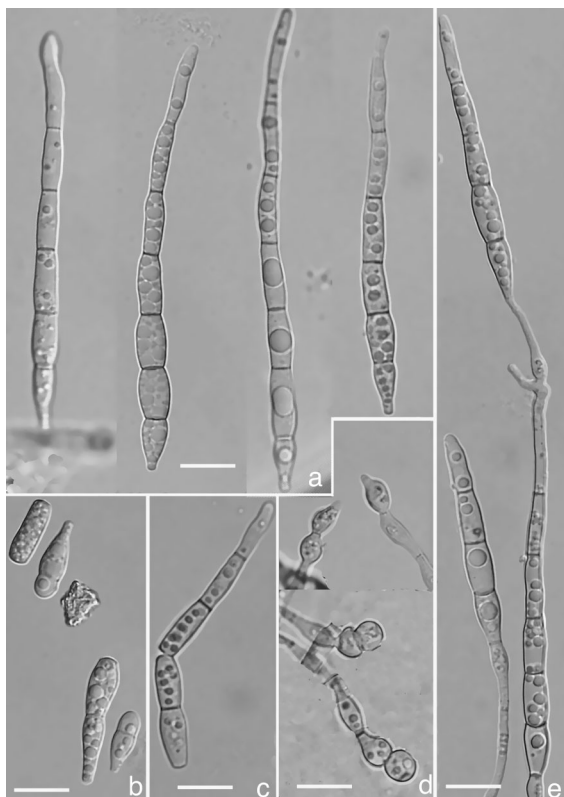


Fig. 2 *Keqinzhangia aquatica* (YMF 1.04262). **a–c** Conidia. **d** chamydospores. **e** Sections of the fertile hyphae. Scale: (a–e) = 10 μm

Notes: The genus *Coronospora* was established by Ellis (1971) with *C. dendrocalami* M.B. Ellis as the type species, in which after the conidiogenous events the cicatrized loci are produced following sympodial extensions of the polyblastic conidiogenous cells disposed in geniculate conidiophores and the conidia are liberated via schizolytic conidial secession (Seifert et al. 2011; Zhang and Zhang 2004; Ellis 1971), but in *Pseudocoronospora hainanense* the conidiogenous loci are tiny or conspicuous denticles and the conidial basal cells are fringed after the rhexolytic conidial secession. Matsushima (2001) observed the *Coronospora* morph in the culture of *Ascoronospora* Matsush., so he thought that *Coronospora* is the asexual state of *Ascoronospora*. Then Kirk et al. (2008) and Wijayawardene et al. (2018) accepted the link between two genera.

Pseudocoronospora hainanense Z.F. Yu, M. Qiao & R.F. Castañeda, **sp. nov.** (Figs. 5 and 6).

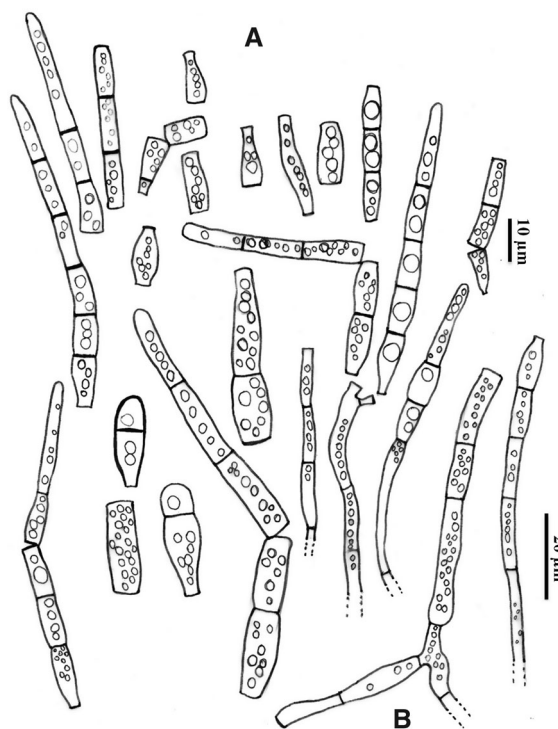


Fig. 3 Drawing of *Keqinzhangia aquatica* made by R.F. Castañeda-Ruiz. **(a)** Conidia. **(b)** Conidiogenous cells with attached and detached conidia after the thallic-arthric disarticulation

Etymology: Epithet refers to the region Hainan where the type strain was collected.

Mycobank number: MB 840,433.

Asexual morph hyphomycetous. Colonies on CMA attaining 3 cm diam. after 20 days at 25 °C, effuse, white to pale flesh, reverse buff. Hyphae thin-walled, septate, hyaline, smooth. Conidiophores macronematous, mononematous, straight or slightly flexuous, somewhat geniculate toward the apex, septate, unbranched, smooth, mid brown or pale brown below, pale brown to subhyaline towards the apex, 16.5–49 × 3.5–5.0 μm. Conidiogenous cells polyblastic, sympodial extended, integrated, terminal, sometimes intercalary, indeterminate, pale brown to subhyaline, denticulate, denticle conspicuous, narrowly cylindrical. Conidial secession rhexolytic. Conidia solitary, acropleurogenous, obclavate, crowned with 2–3 broadly mammiform protuberances, radially arranged near the rounded to obtuse apex; 2-septate, smooth or slightly verruculose at the basal and central cells, hyaline,

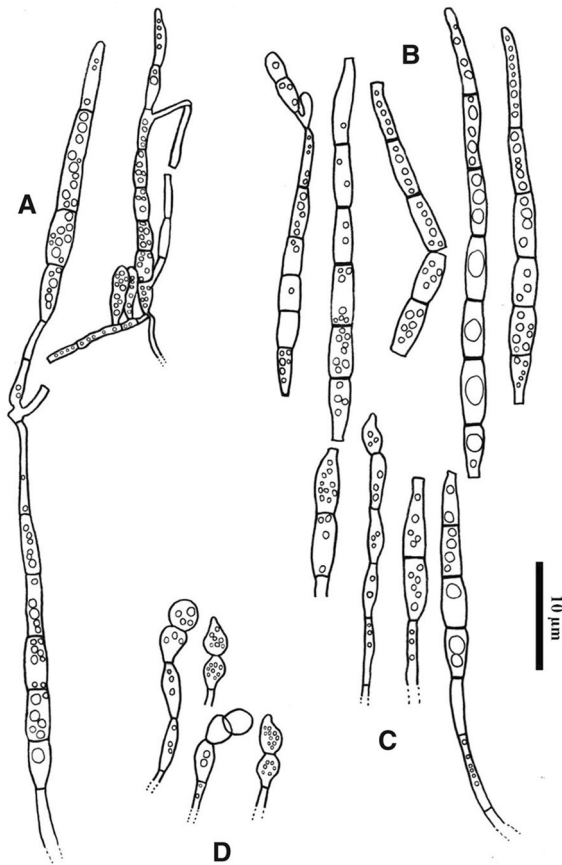


Fig. 4 Drawing of *Keqinzhangia aquatica* made by R.F. Castañeda-Ruiz. **a** Sections of fertile hyphae. **b** Conidia. **c** Conidiogenous cells and attached “conidia”. **d** Chlamydospores

27.2–33 × 3.7–8.0 μm, with a minute basal frill. Sexual state: Unknown.

Holotype: YMF 1.04517, isolated from leaves of an unidentified dicotyledonous plant submerged in a stream, Diaoluoshan National Forest Park, Hainan Province, China, 18°42′11″N, 109°53′16″E, ca. 1124 m elev., April 2014, Zefen Yu, permanently preserved in a metabolically inactive state (deep freezing) in the Conservation and Utilization of Bio-Resources in Yunnan. Ex-type culture CCTCC AF 2,021,129 = CGMCC 3.18823.

Discussion

In recent years, more and more molecular data of species in Microthyriaceae has become available. Hongsanan et al. (2020) accepted 11 genera, which

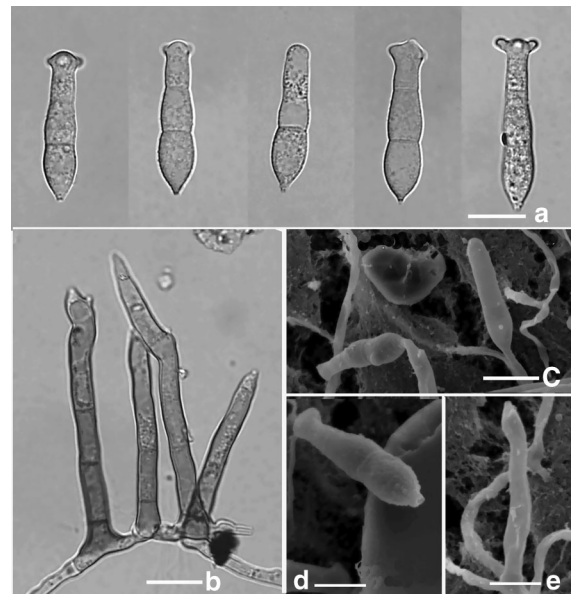


Fig. 5 *Pseudocoronospora hainanense* (YMF 1.04517). **a, d** Conidia. **b, e** Conidiophores and conidiogenous cells. **c** Conidia with conidiogenous cells. **c, d, e** were taken with SEM. Scale bars: (**a–e**) = 10 μm

include three asexual genera *Hamatispora*, *Neoanungitea*, and *Pseudopenidiella* Crous & Koukol, in Microthyriaceae based on morphological characteristics and sequence analyses of the ITS and LSU barcodes. In this study, our phylogenetic analyses determined two isolates to belong to the Microthyriaceae. Combined with morphological characteristics, we finally described them as two new asexual genera and species in Microthyriaceae, named as *Keqinzhangia aquatica* and *Pseudocoronospora hainanense*.

The new genus *Keqinzhangia* is phylogenetically close to the sexual genus *Microthyrium* and the asexual genus *Neoanungitea*. Although we observed cultures for a long time, we did not see any sexual reproductive structures in *K. aquatica*. Besides, their LSU sequence similarity is relatively low (90%). Therefore, we cannot determine the connection between them. Although *Neoanungitea* is an asexual genus, *Keqinzhangia* is obviously different from *Neoanungitea* in conidiogenesis (holothallic vs. holoblastic) and the shape of conidia (cylindrical-obclavate, bacilliform, fusiform vs. fusoid-ellipsoid) (Crous et al. 2019).

Our other new genus, *Pseudocoronospora*, is phylogenetically close to the asexual genus

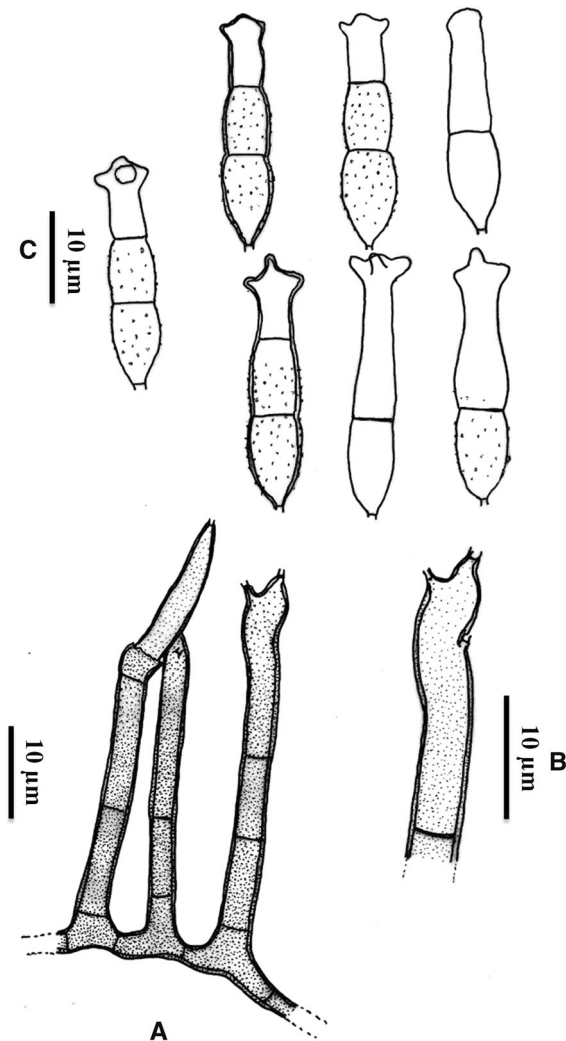


Fig. 6 Drawing of *Pseudocoronospora hainanense* made by R.F. Castañeda-Ruiz. **a** Conidiophores and conidiogenous cells. **b** Conidiogenous cells. **c** Conidia

Hamatispora and *Neonanungitea*. *Hamatispora* is a hyphomycetous genus with staurospores that are question-mark-shaped or hook-shaped with 3 arms developing from each cell on the helicoid part (Yen et al. 2018). Therefore, *Pseudocoronospora* species is easily distinguished from *Hamatispora* and *Neonanungitea* by morphology.

Microthyriales is a poorly known order. Previously accepted species in this order were based mostly on morphological characters; little molecular data were available. For the past few years, new molecular data are available (Crous et al. 2016, 2017, 2019; Hongsanan et al. 2020; Wu et al. 2011, 2014). A recent study

showed that species of Microthyriales cluster together as a distinct clade within Dothideomycetes with high support based on sequence analyses of LSU and ITS (Hongsanan et al. 2020). This study shows the importance of obtaining pure cultures and gene sequences in order to identify the origins and phylogenetic positions of fungal species.

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Authors' contributions ZY conceived and designed the study. HZ and MQ wrote the manuscript. JG and JP conducted the experiments. RFC contributed actively in the identification and the taxonomy of the fungal strains.

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Availability of data and material These new generated sequences were uploaded to the GenBank database at the National Center for Biotechnology Information (NCBI), and are available.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare to have no conflict of interest.

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