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 the investigation of the diversity of aquatic hyphomycetes from southern China, two interesting isolates were collected. Then, the two isolates were cultured and sequenced, and a BLAST search of its LSU sequences against data in GenBank revealed that the closest related taxa in the genus *Microthyrium*. Phylogenetic analyses, based on the combined sequence data from the small and large nuclear subunit ribosomal DNA (SSU and LSU), revealed that our isolates belong to the Microthyriaceae. Combined morphological characters, we finally described 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.

Introduction

Microthyriales was introduced by Arnaud in 1918, with type family Microthyriaceae (Arnaud 1918). Originally, Microthyriales included two families, Microthyriaceae and Micropeltidaceae, based on their flattened ascomata with 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 (Wijayawardene et al. 2018; Hongsanan and Hyde Kd 2017), and has 6 genera as family incertae sedis in this order (Wijayawardene et al. 2020).

Saccardo (1883) established the family Microthyriaceae in 1883, with sexual genus *Microthyrium* Desm. as type genus. In 1913, Theissen included Microthyriaceae in the order Hemisphaeriales (Theissen 1913). Subsequently, Arnaud established a new order Microthyriales to accommodate Microthyriaceae and Microthyriopsidaceae (Arnaud 1918). After that, the family has experienced a complicated taxonomic history, and various genera were included, such as foliar epiphytes or saprobes. Until 2011, Wu et al. (2011) reappraised the Microthyriaceae 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 outline of Ascomycota and accepted 9 genera in the family, including 8 sexual genera and one asexual genus. In recent study, Hongsanan et al. (Hongsanan et al. 2020) accepted 11 genera in this family based on morphology and phylogeny, including 8 sexual genera and three asexual genera, besides, they added definition of asexual morph in family level.

China has enormous fungal diversity, with the southern region in China 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 in soils, submerged leaves, and aquatic plants, and described many new taxa (Zheng et al. 2019; 2020a,b; 2021a,b; Qiao et al. 2017; 2018a,b; 2019; 2020). During our ongoing studies of freshwater hyphomycetes in Yunnan Province and Hainan Province, two interesting fungi were collected

on submerged leaves of an unidentified dicotyledonous plants. The two isolates were cultured and sequenced, and a BLAST search of its LSU sequences against data in GenBank revealed that the closest related taxa 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 nuclear large subunits ribosomal DNA (LSU) genes. Combined morphological characters, we finally described our isolates as two new genera in Microthyriaceae, named as: *Keqinzhangia* and *Pseudocoronospora*.

Materials And Methods

Isolation and morphological study of strain

Submerged dicotyledonous leaves were collected from Yunnan Province and Hainan Province. Samples were preserved in zip-lock plastic bags, labelled, and transported to the laboratory. The decomposed leaves were cut into several 2–4 × 2–4 cm sized fragments in the laboratory and then spread onto the surface of corn meal agar (CMA, 20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) medium for 10 days; single conidium was isolated with a sterilized needle and transferred to CMA plates while viewing with an Olympus BX51 microscope. Morphological observations were made from CMA after incubation at 25°C for one week, and photographs were taken with an Olympus BX51 microscope connected to a DP controller digital camera. Measurement data 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 at 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), 1µL 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.

Table 1 Sequence data used in this study. All new sequence data generated in this study are in bold.

Taxon	Strain	GenBank accession no.	
		LSU	ITS
Chaetothyriothecium elegans	CPC 21375T	KF268420	
Hamatispora phuquocensis	VICCF 1219	LC064073	LC064074
Heliocephala elegans	MUCL 39003	HQ333478	HQ333478
Heliocephala gracilis	MUCL 41200	HQ333479	HQ333479
Heliocephala natarajanii	MUCL 43745T	HQ333480	HQ333480
Heliocephala zimbabweensis	MUCL 40019	HQ333481	HQ333481
Keqinzhangia aquatica	YMF 1.04626	**MK577809**	**MK569507**
Kirschsteiniothelia lignicola	MFLUCC10-0036	HQ441568	HQ441567
Microthyrium buxicola	MFLUCC 15-0212	KT306551	–
Microthyrium buxicola	MFLUCC 15-0213	KT306552	–
Natipusilla decorospora	AF236-1	HM196369	–
Natipusilla naponense	AF217-1	HM196371	–
Neoanungitea eucalypti	CBS 143173	MG386031	MG386031
Ochroconis dracaenae	CPC 26115	KX228334	KX228283
Phaeotrichum benjaminii	CBS 541.72	AY004340	MH860561
Pseudocoronospora hainanensis	YMF 1.04517	**MK577807**	**MK569505**
Pseudomicrothyrium thailandicum	MFLU 14-0286	MT741680	–
Pseudopenidiella gallaica	CBS 121796	LT984843	LT984842
Sympoventuria capensis	CBS 120136	KF156104	DQ885906
Trichodelitschia bisporula	CBS 262.69	GU348996	MH859305
Tumidispora shoreae	MFLUCC 12-0409	KT314073	–
Tumidispora shoreae	MFLUCC 14-0574	KT314074	–
Venturia inaequalis	CBS 594.70	GU301879	KF156040
Zeloasperisporium ficusicola	MFLUCC 15-0221	KT387733	–
Zeloasperisporium hyphopodioides	CBS 218.95	EU035442	EU035442
Zeloasperisporium siamense	IFRDCC 2194	JQ036228	–

Sequence alignment and phylogenetic analysis

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 the genus *Microthyrium*. Based on this information, related sequences at the two marker loci, 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 Zeloasperisporiales, were downloaded according 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.

These, together with these 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 1215 nucleotide positions from two genes (855 from LSU, 360 from ITS), and the matrix 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. Me-tropolis-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 MCMC 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) ≥ 75% are indicated at nodes.

Results

Phylogenetic analysis

BLAST analyses with LSU sequences revealed that *Microthyrium* is the closest 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, which close to *Hamatispora* L.T.H. Yen, K. Yamag. & K. Ando, *Neoanungitea*, *Microthyrium*, and YMF 1.04517 with well 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*.

Taxonomy

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

Etymology: Name in honors to Prof. Keqing Zhang of the Yunnan University for his contribution to the biological sciences.

MycoBank number: MB 840430

Asexual morph hyphomycetous. Vegetative hyphae cylindrical, branched, microguttulate, septate, hyaline, smooth-walled. Fertile hyphae cylindrical-obclavate, extended inflated subulate to the tip grow, 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-oblecythiform 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.** (Fig. 2–4).

Etymology: Epithet refers to it growing in water.

MycoBank number: MB 840432

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, extended inflated subulate to the tip grow, 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, suboblecythiform 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, arise after random disarticulation of fertile hyphae at the darker septa. Clamydospores 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, preserved in a metabolically inactive state (deep freezing) in the Conservation and Utilization of Bio-Resources in Yunnan. Ex-type culture CCTCC AF 2021070.

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 it is similar to the genus *coronospora* in morphology.

MycoBank number: MB 840431

Asexual morph hyphomycetous. Conidiophores macronematous, mononematous, erect, septate, unbranched, brown. Conidiogenous cells polyblastic, denticulate, integrated, sympodial extended, terminal, indeterminate. 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.

Notes: The genus *Coronospora* was established by Ellis with *C. dendrocalami* M. B. Ellis as 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* in culture of *Ascoronospora* Matsush., so he thought that *Coronospora* is asexual state of *Ascoronospora*. Then Asthton et al. (2009) and Wijayawardene et al. (2018) accepted the link between two genera. So far, molecular sequences of two genera were not obtainable, so the connection between two genera was not confirmed by molecular data. However, *Ascoronospora* was treated as Pleosporales genera incertae sedis (Wijayawardene et al. 2018), which is morphologically different from members of Microthyriaceae.

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

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

MycoBank number: MB 840433

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, mid brown or pale brown below, pale brown to subhyaline towards the apex, 16.5–49 µm long, 3.5–5.0 µm wide. Conidiogenous cells polyblastic, denticulate, denticle conspicuous, narrowly cylindrical, integrated, sympodial extended, terminal, sometimes intercalary, indeterminate, pale brown to subhyaline. 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, 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., Apr. 2014, Zefen Yu, preserved in a metabolically inactive state (deep freezing) in the Conservation and Utilization of Bio-Resources in Yunnan. Ex-type culture CGMCC 3.18823.

Discussion

In recent years, more and more molecular data of species in Microthyriaceae were available. Hongsanan et al. (2020) accepted 11 genera, which include three asexual genera *Hamatispora*, *Neoanungitea*, and *Pseudopenidiella* Crous & Koukol, in Microthyriaceae based on morphological characteristics and sequence analysis of the ITS and LSU barcodes. In this study, our phylogenetic analysis determined the two isolates 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* was phylogenetically close to the sexual genus *Microthyrium* and the asexual genus *Neoanungitea*. *Microthyrium* is the type genus of Microthyriaceae in Microthyriales (Saccardo 1883). Although we observed cultures for 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. *Neoanungitea* was introduced by Crous in 2017, with *N. eucalypti* as type species (Crous et al. 2017). Although *Neoanungitea* was asexual genus, *Keqinzhangia* was obviously different from *Neoanungitea* in morphology.

Our established another new genus *Pseudocoronospora* was phylogenetically close to the asexual genus *Hamatispora* and *Neoanungitea. 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 was easily distinguished from *Hamatispora* and *Neoanungitea* in morphology.

Mycrothyriales is a poorly known order. Previously accepted species in this order was almost based on morphological characters; little molecular data were available. For the past few years, more and more molecular data were available. Recent study showed that species of Microthyriales cluster together as a distinct clade within Dothideomycetes with high support based on sequence analysis of LSU and ITS (Hongsanan et al., 2020). It revealed the importance of obtaining pure cultures and gene sequences in order to identify the origins and phylogenetic positions of fungal species.

Declarations

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Conflicts of interest/Competing interests

The authors declare that have no conflict of interest.

Availability of data and material

The relevant data is uploaded to the public database, and is available.

Code availability

Not applicable

Authors' contributions

ZY conceived and designed the study. HZ and MQ wrote the manuscript. JG and JP conducted the experiments. R.F.C contributed actively in the identification and the taxonomy of the fungal strains.

References

1. Hall TA (1999) BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nuclc Acids Symposium Series 41(41): 95–98. https://doi.org/10.1021/bk-1999-0734.ch008

2. Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21):2688–2690. https://doi.org/10.1093/bioinformatics/btl446
3. Posada D (2008) jModelTest: Phylogenetic model averaging. Mol Biol Evol 25(7):1253–1256. https://doi.org/10.1093/molbev/msn083
4. Turner D, Kovacs W, Kuhls K, Lieckfeldt E, Peter B et al (1997) Biogeography and phenotypic variation in Trichoderma sect Longibrachiatum and associated Hypocrea species. Mycol Res 101:449–459. https://doi.org/10.1017/S0953756296002845
5. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A et al (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Syst Biol 61(3):539–542. https://doi.org/10.1093/sysbio/sys029
6. Theissen F (1913) Hemisphaeriales. Annales Mycologici 11(5): 425–467
7. Arnaud G (1918) Lés Asterinées. Ann Éc Natl Agric Montp 2(16):1–288
8. Cole GT (1986) Models of Cell-Differentiation in Conidial Fungi. Microbiol Rev 50(2):95–132
9. Ashton H (2009) Ainsworth and Bisby's Dictionary of the Fungi (10th edition). Reference Reviews 23(5): 42–42. https://doi.org/10.1108/09504120910969104
10. Zheng H, Li J, Gou JS, Qiao M, Yu ZF (2021a) Anacraspedodidymum submersum sp. nov. (Chaetosphaeriaceae, Chaetosphaeriales), a new species of freshwater hyphomycetes from southwest China. Int J Syst Evol Microbiol 71(2). https://doi.org/10.1099/ijsem.0.004650
11. Zheng H, Qiao M, Lv Y, Du X, Zhang KQ et al (2021b) New Species of Trichoderma Isolated as Endophytes and Saprobes from Southwest China. Journal of Fungi 7(6):467. https://doi.org/10.3390/jof7060467
12. Zheng H, Wan Y, Li J, Castaeda-Ruiz RF, Yu ZF (2020a) Phialolunulospora vermispora (Chaetosphaeriaceae, Sordariomycetes), a novel asexual genus and species from freshwater in southern China. Mycokeys 76:17–30. https://doi.org/10.3897/mycokeys.76.57410
13. Zheng H, Yu Z, Xu J, Castaeda-Ruiz RF, Qiao M (2020b) Ramichloridium endophyticum sp. nov., a novel species of endophytic fungus from Potamogeton pectinatus. Int J Syst Evol Microbiol 70(5). https://doi.org/10.1099/ijsem.0.004190
14. Zheng H, Zhang Z, Liu DZ, Yu ZF (2019) Memnoniella sinensis sp. nov., a new species from China and a key to species of the genus. Int J Syst Evol Microbiol 69(10). https://doi.org/10.1099/ijsem.0.003605
15. Wu HX, Schoch CL, Boonmee S, Bahkali AH, Chomnunti P et al (2011) A reappraisal of Microthyriaceae. Fungal diversity 51(1):189–248. https://doi.org/10.1007/s13225-011-0143-8
16. Gonzalez II, Garcia D, Guarro J, Gene J (2020) Heliocephala variabilis and Pseudopenidiella vietnamensis: Two New Hyphomycetous Species in the Microthyriaceae (Dothideomycetes) from Vietnam. Microorganisms 8(4):478. https://doi.org/10.3390/microorganisms8040478

17. Kindermann J, El-Ayouti Y, Samuels GJ, Kubicek CP (1998) Phylogeny of the genus Trichoderma based on sequence analysis of the internal transcribed spacer region 1 of the rDNA cluster. Fungal Genet Biol 24(3):298–309. https://doi.org/10.1006/fgbi.1998.1049
18. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25(24):4876–4882. https://doi.org/10.1093/nar/25.24.4876
19. Seifert K, Morgan-Jones G, Gams W, Kendrick B (2011) The genera of hyphomycetes. CBS Biodiversity Series 9:997 p
20. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30(12):2725–2729
21. Yen LTH, Yamaguchi K, Tsurumi Y, Hop DV, Ando K (2018) Hamatispora, a new genus of aquatic fungi in Microthyriales isolated from fallen leaves in Vietnam. Mycoscience 59(6):467–472. https://doi.org/10.1016/j.myc.2018.04.004
22. Qiao M, Zheng H, Lv R, Yu Z (2020) Neodactylariales (Dothideomycetes, Ascomycota) Neodactylariales, Neodactylariaceae (Dothideomycetes, Ascomycota): new order and family, with a new species from China. Mycokeys 73(6):69–85. https://doi.org/10.3897/mycokeys.73.54054
23. Qiao M, Zheng H, Zhang Z, Yu ZF (2019) Seychellomyces sinensis sp. nov. from China. Mycotaxon 134(2):391–398. https://doi.org/10.5248/134.391
24. Qiao M, Guo JS, Tian WG, Yu ZF (2018a) Ellisembia hainanensis sp. nov. from Hainan, China. Mycotaxon 133(1):97–10. https://doi.org/10.5248/133.97
25. Qiao M, Du X, Bian ZH, Peng J, Yu ZF (2018b) Ellisembia pseudokaradkensis sp. nov. from Hainan, China. Mycotaxon 132(4):813–817. https://doi.org/10.5248/132.813
26. Qiao M, Huang Y, Deng C, Yu ZF (2017) Tripospermum sinense sp. nov. from China. Mycotaxon 132(3):513–517. https://doi.org/10.5248/132.513
27. Zhang M, Zhang TY (2004) A new secies of Coronospora from China. Mycosystema 23:331–332. https://doi.org/10.3969/j.issn.1672-6472.2004.03.004
28. Ellis MB (1971) Dematiaceous Hyphmycetes X. Mycological Paper 125: 1–30
29. Myers N, Mittermeier RA, Mittermeier CG, Fonseca G, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772):853–858. https://doi.org/10.1038/35002501
30. Wijayawardene N, Hyde KD, Al-Ani L, Tedersoo L, Haelewaters D et al (2020) Outline of Fungi and fungi-like taxa. mycosphere 11(1): 1160–1456. https://doi.org/10.5943/mycosphere/11/1/8
31. Wijayawardene NN, Hyde KD, Lumbsch HT, Jian KL, Maharachchikumbura SSN et al (2018) Outline of Ascomycota: 2017. Fungal diversity 88(2):167–263. https://doi.org/10.1007/s13225-018-0394-8
32. Saccardo PA (1883) Sylloge Pyrenomycetum. Sylloge Fungorum 2 II:1–815
33. Crous PW, Wingfield MJ, Lombard L, Roets F, Swart WJ et al (2019) Fungal Planet description sheets: 951–1041. Persoonia 43:223–425. https://doi.org/10.3767/persoonia.2019.43.06

34. Crous PW, Wingfield MJ, Burgess TI, Carnegie AJ, Hardy GESJ et al (2017) Fungal Planet description sheets: 625–715. Persoonia 39:270–467. https://doi.org/10.3767/persoonia.2017.39.11
35. Vilgalys R, Hester M (1990) Rapid Genetic Identification and Mapping of Enzymatically Amplified Ribosomal DNA from Several Cryptococcus Species. J Bacteriol 172(8):4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990
36. Hongsanan S, Hyde KD (2017) Phylogenetic placement of Micropeltidaceae. mycosphere 8(10): 1930–1942. https://doi.org/10.5943/mycosphere/8/10/15
37. Hongsanan S, Hyde KD, Phookamsak R, Wanasinghe DN, McKenzie EHC et al (2020) Refined families of Dothideomycetes: orders and families incertae sedis in Dothideomycetes. Fungal diversity 105(1):17–318. https://doi.org/10.1007/s13225-020-00462-6
38. Matsushima T (2001) Matsushima Mycological Memoirs No. 10. Matsushima Fungus Collection, Published by author, Kobe, Japan
39. White T, Bruns T, Lee S, Taylor F, White T et al (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications 18: 315–322

Figures

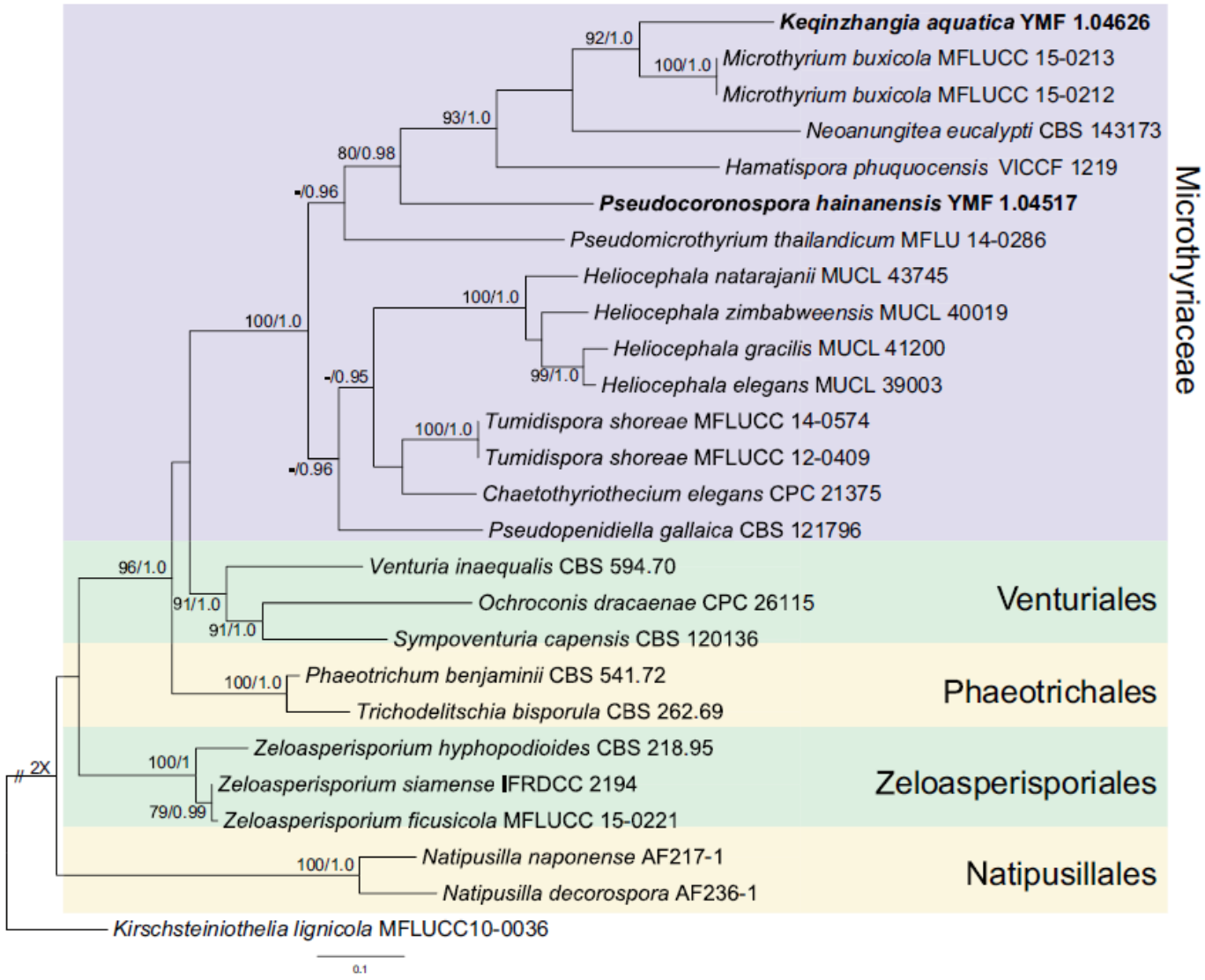

Figure 1

Phylogenetic tree generated by the maximum likelihood analysis using combined sequences of the nuclear large subunit (LSU) and the internal transcribed spacers (ITS) gene. 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. Novel species are indicated in bold.

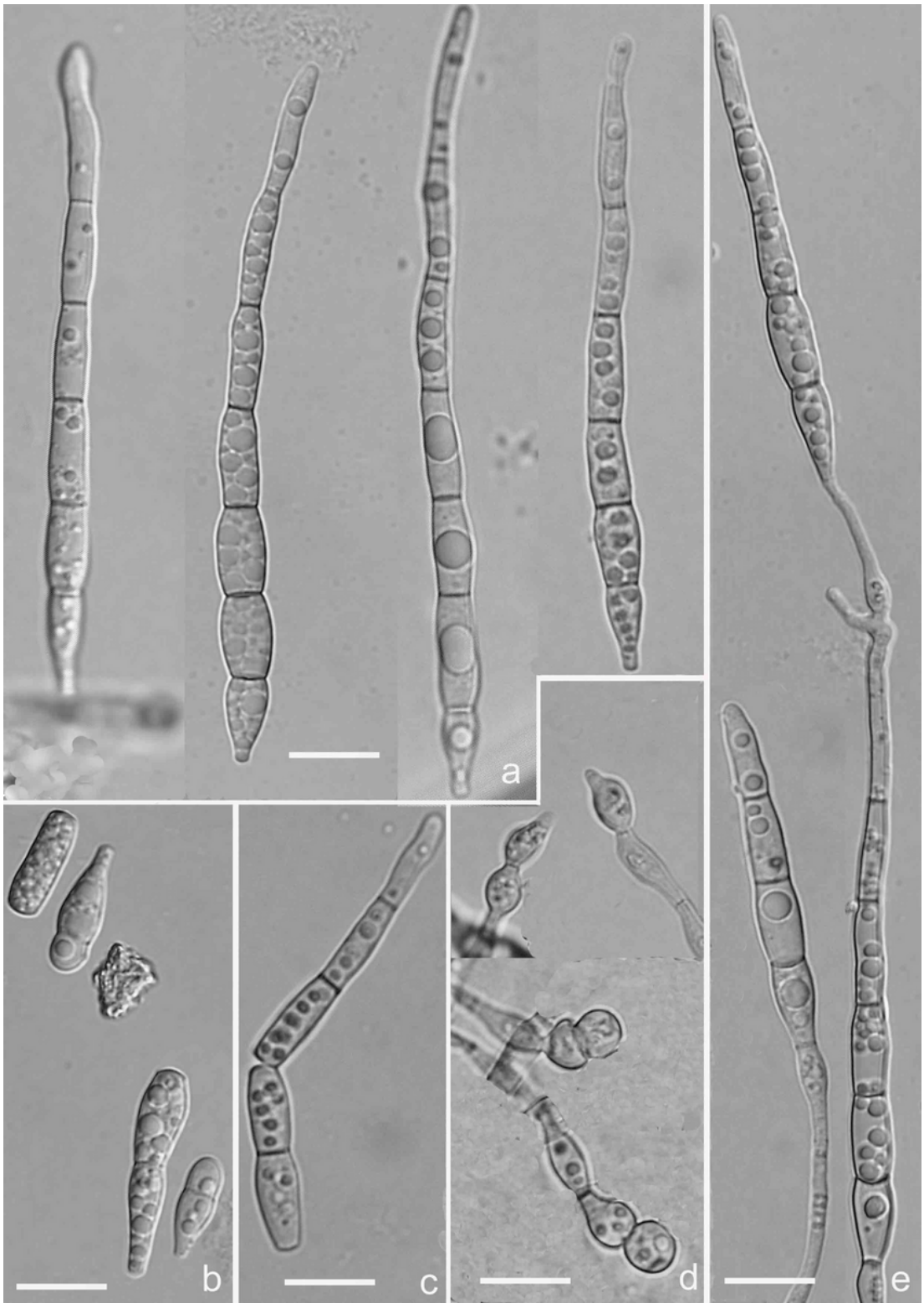

Figure 2

Keqinzhangia aquatica (YMF 1.04262). (a–c) conidia. (d) clamydospores. (e) Sections of the fertile hyphae. Scale: (a–e) = 10 μm.

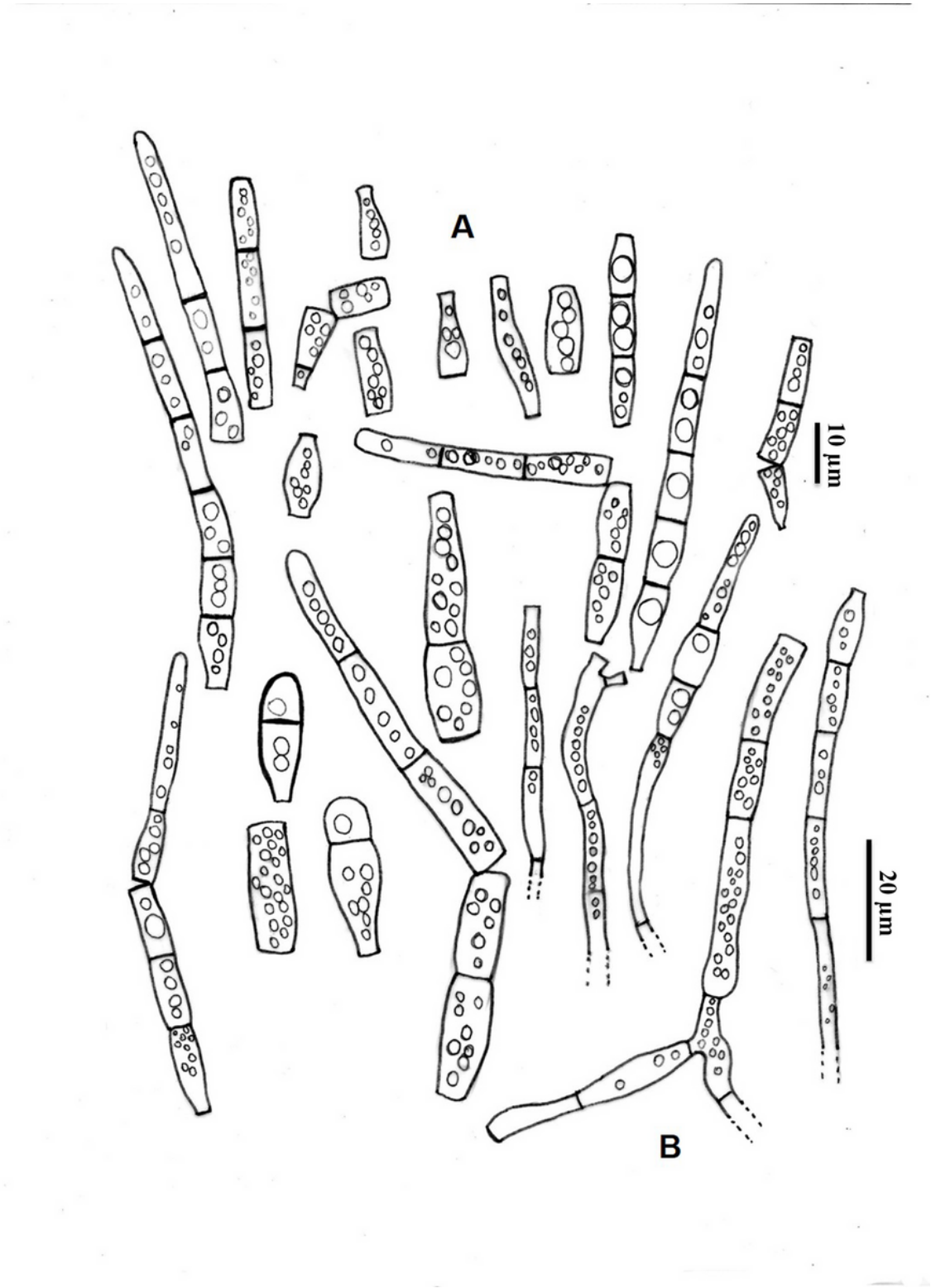

Figure 3

Drawing of Keqinzhangia aquatica made by Rafael. (A) Conidia. (B) Conidiogenous cells with attached and 387 detached conidia after the thallic-arthric disarticulation.

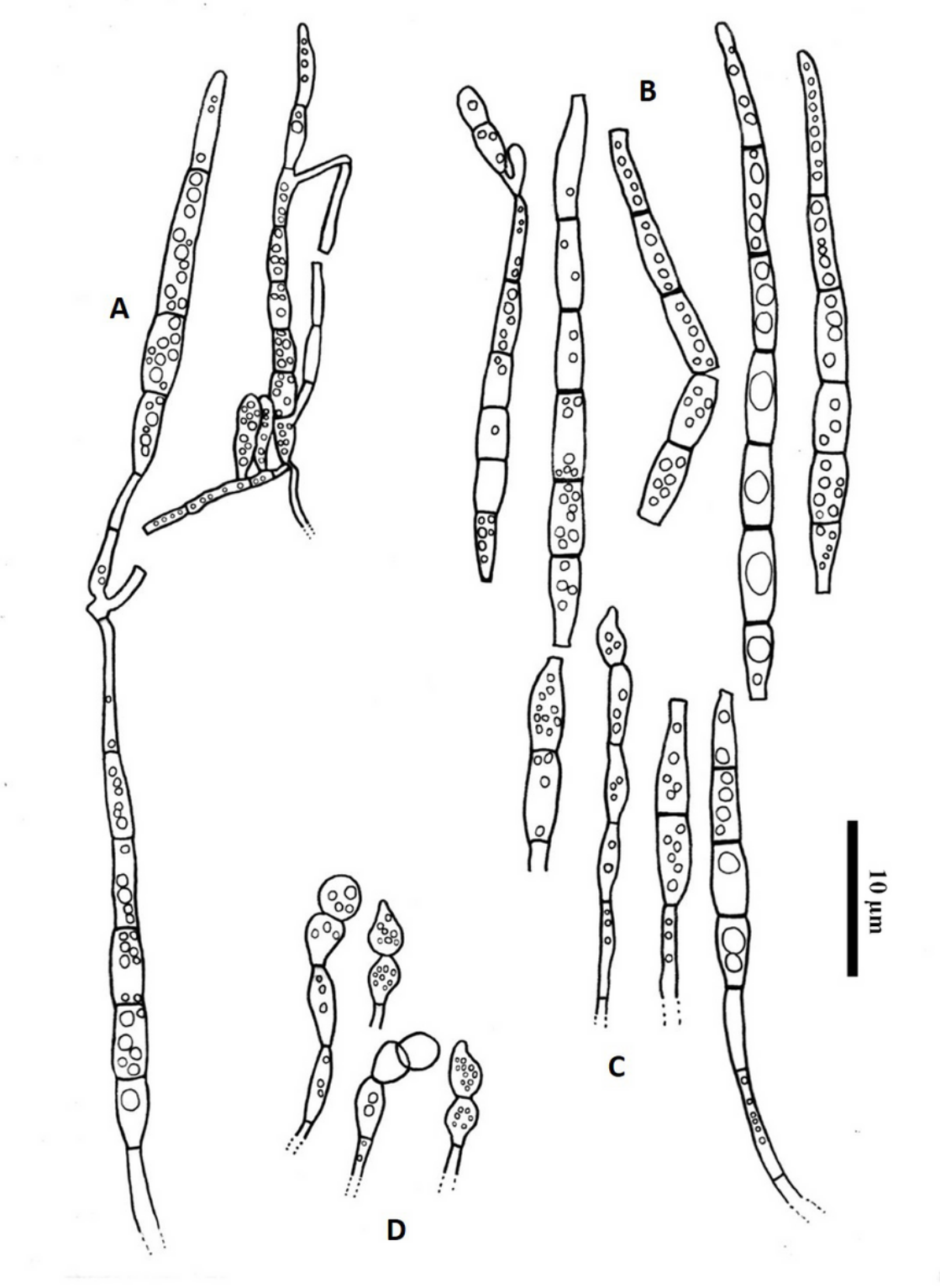

Figure 4

Drawing of Keqinzhangia aquatica made by Rafael. (A) Sections of the fertile hyphae. (B) Conidia. (C) Conidiogenous cells and attached “conidia”. (D) Chlamydospores.

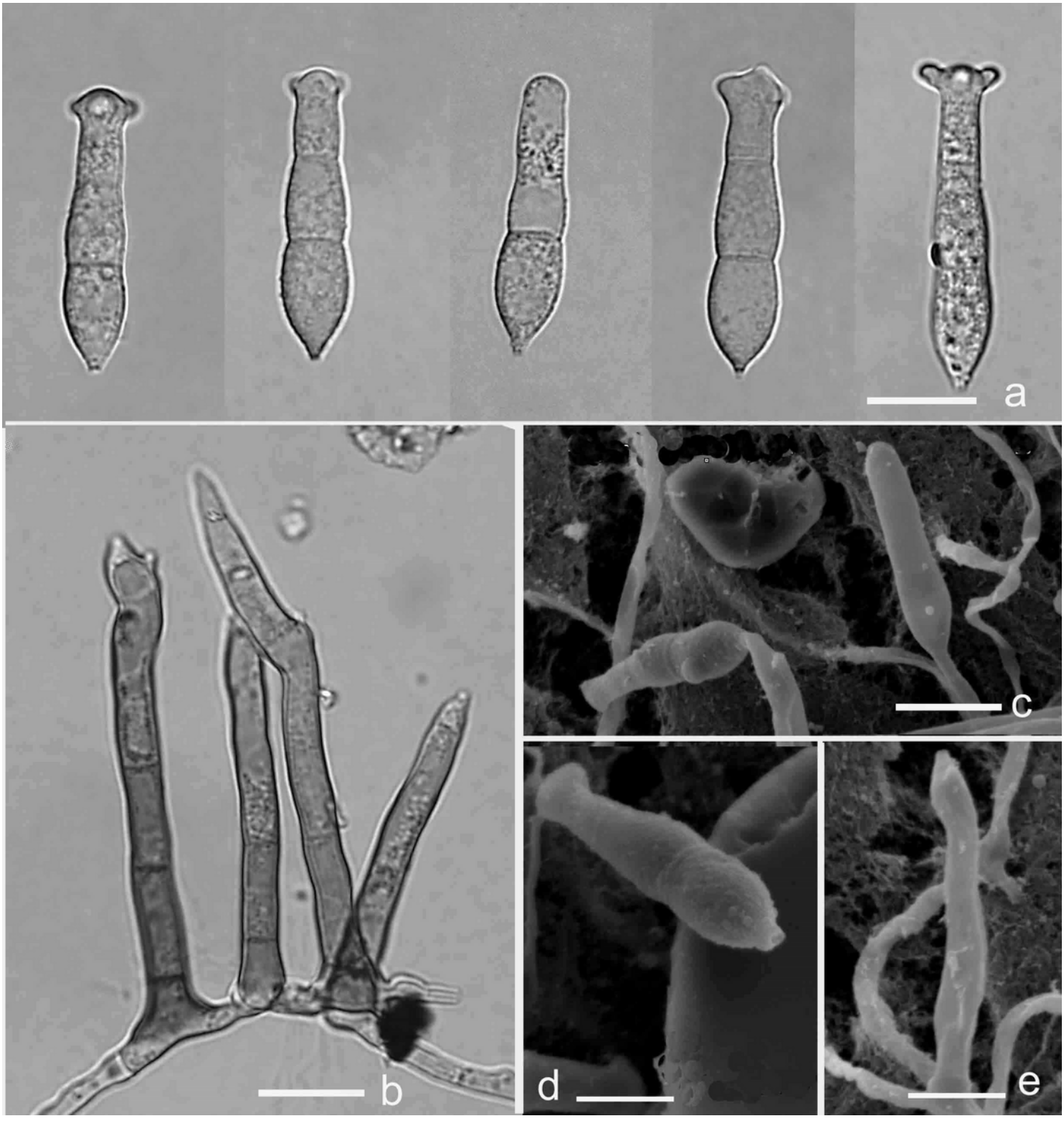

Figure 5

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

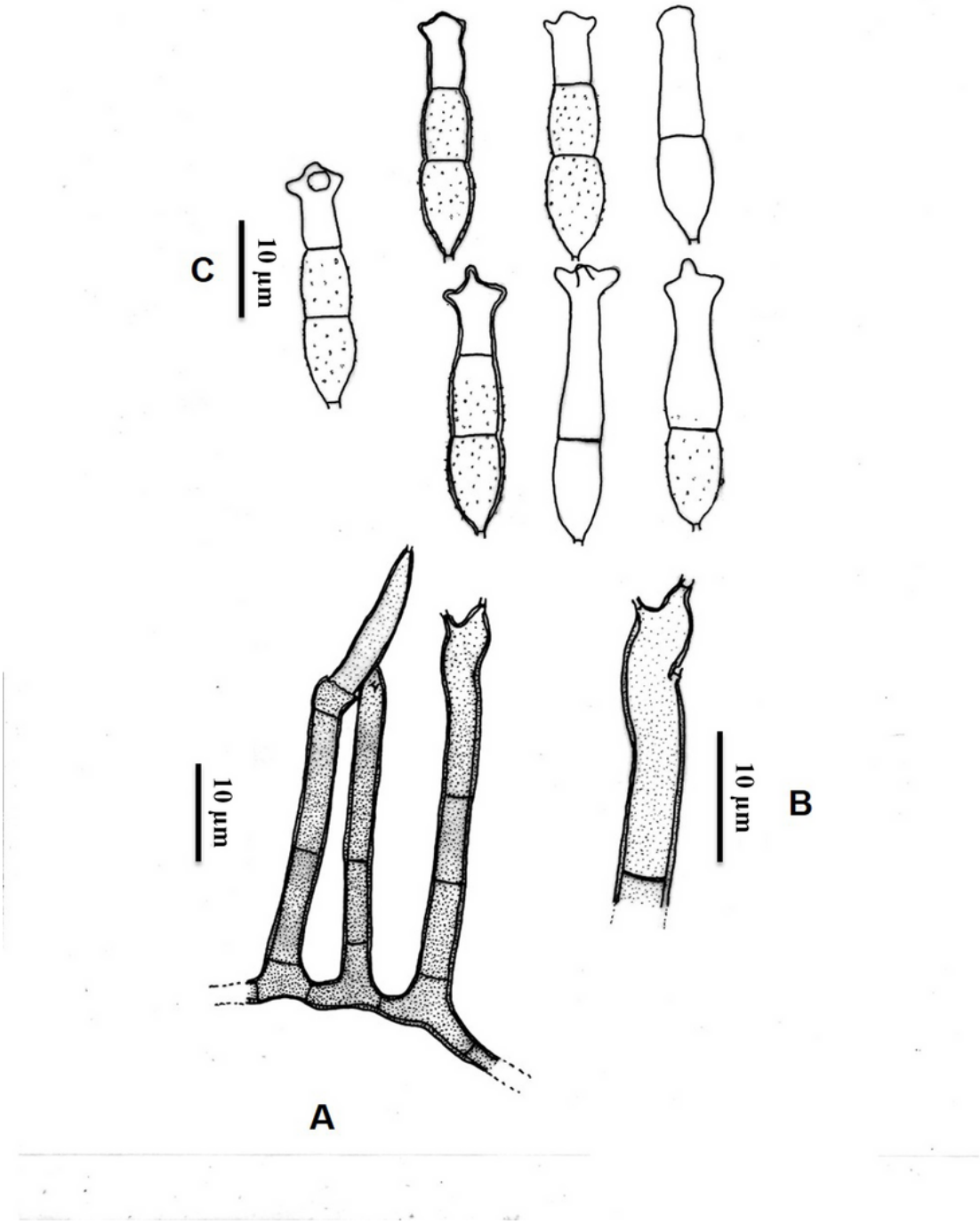

Figure 6

Drawing of Pseudocoronospora hainanensis made by Rafael. (A) Conidiophores and conidiogenous cells. (B) Conidiogenous cells. (C) Conidia