Phylogenetic placement of new species and combinations of Neopodoconis within Torulaceae

Ling Qiu
Jiangxi Agricultural University
Ya-Fen Hu
Jiangxi Agricultural University
Jing-Wen Liu
Jiangxi Agricultural University
Ji-Wen Xia
Shandong Agricultural University
Rafael F. Castañeda-Ruíz
instituto de investigaciones fundamentales en agricultura tropical "Alejandro de Humboldt"
Zhao-Huan Xu
Jiangxi Agricultural University
Jian Ma (majian821210@163.com)
Jiangxi Agricultural University https://orcid.org/0000-0001-9783-1860

Research Article

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Abstract

Five new species of *Neopodoconis*, *N. jiangxiensis*, *N. meilingensis*, *N. obclavata*, *N. saprophyticus* and *N. sinensis* are described and illustrated from specimens collected on dead branches of unidentified plants in China. Phylogenetic analysis of partial DNA sequences of nuclear ribosomal small subunit (SSU) and nuclear ribosomal large subunit (LSU), using Maximum-Likelihood (ML) and Bayesian Inference (BI), supported the establishment of the five new species, and indicated a close relationship to Torulaceae in Pleosporales, Dothideomycetes. *Sporidesmioides* and *Rostriconidium* are synonymised with *Neopodoconis* based on morphological and molecular phylogenetic data, and the new combinations *Neopodoconis aquaticum*, *N. cangshanense*, *N. pandanicola* and *N. thailandica* are proposed. A synoptic table to *Neopodoconis* species are provided.

Introduction

The genus *Neopodoconis* Rifai was established by Rifai (2008) for two combinations, *N. ampullacea* (Petch) Rifai and *N. megasperma* (Boedijn) Rifai, derived from *Exosporium* Link (Link 1809), and regarding *N. ampullacea* as type species. The genus is a morphologically well-characterised by acropleurogenous, solitary, euseptate conidia seceding schizolytically from polytretic, integrated, terminal and later intercalary, elongated sympodially, blackly cicatricated conidiogenous cells on macronematous, mononematous, unbranched conidiophores. The conidia are obclavate to broadly obclavate, smooth walled or verruculous, brown but much paler towards the apex, with protruding truncate dark scar at the base and distinctly rostrate at the apex. To date, only *N. ampullacea* and *N. megasperma* are reported in *Neopodoconis*, which were collected from dead stem or dried stick in Java, Ceylon, Ghana, and Sierra Leone (Rifai 2008; Indexfungorum 2022). However, no DNA sequence exists for both species in GenBank, and so that *Neopodoconis* is even unclear of its taxonomic placement within *Ascomycota* (Wijayawardene et al. 2020).

Neopodoconis was segregated from *Exosporium* by Rifai (2008), both genera show similar conidial ontogeny, and the nature of conidial septation was regarded as the only character for separating *Neopodoconis* from *Exosporium*. Such an approach was also used as the fundamental criterion in distinguishing a number of hyphomycete genera (e.g. Subramanian 1992; Wu and Zhuang 2005; Seifert et al. 2011; Ma et al. 2016), but the phylogenetic significance of conidial septation in *Neopodoconis-Exosporium* taxonomy has not been undertaken by molecular methods.

Based on sequence data, two new genera *Sporidesmioides* Jun F. Li, Phook. & K.D. Hydeand and *Rostriconidium* Z.L. Luo, K.D. Hyde & H.Y. Su were respectively introduced by Li et al. (2016) and Su et al. (2018), but both genera were not compared morphologically with the closely related genus *Neopodoconis*, although no DNA sequence exists for *Neopodoconis* species to be used in their molecular phylogeny. Remarkably, *Sporidesmioides* and *Rostriconidium* exhibit the essential characteristics of *Neopodoconis*. These striking morphological consistency and the incomplete taxon and sequence sampling in the phylogenies of Li et al. (2016) and Su et al. (2018) call for a critical treatment of the status of *Sporidesmioides* and *Rostriconidium* as distinct genera. Thus, *Sporidesmioides* and *Rostriconidium* were proposed as synonyms of *Neopodoconis* based on their distinct morphological features and phylogenetic analyses of combined SSU and LSU sequence data, and the new combinations *Neopodoconis aquaticum*, *N. cangshanense*, *N. pandanicola* and *N. thailandica* are proposed.

Jiangxi Province is located in the southeast of China, and in the south bank of the middle and lower reaches of the Yangtze River. It covers 166, 900 km^2 and has a mountainous topography with remarkable subtropical monsoon climate. Its annual mean temperature is 16.3–19.5 ℃, and the mean annual precipitation is 1341–1943 mm, so it is particularly suitable for the growth of microscopic fungi in plant remains. During our mycological surveys in this Province, five specimens of *Neopodoconis* were collected on dead branches of unidentified plants. Based on

morphological and phylogenetic analyses of combined SSU and LSU sequence data, they were described as new to science in the present study.

Materials And Methods

Isolates and Morphological analysis

Samples of dead branches were collected from humid environments and river bank in the forest ecosystems of Jiangxi Province, China, and returned to the laboratory in Ziploc™ bags. Samples were processed and examined following the methods described in Ma et al. (2011). Fungi were mounted in a drop of lactic acid on microscope slides, and examined and photographed with an Olympus microscope (model BX 53), with a 100 × (oil immersion) objective at the same background and scale. Adobe Photoshop 7.0 was used for image processing to assemble photographs into images. Single-spore isolations were made on potato dextrose agar (PDA) following Goh (1999). All fungal strains were stored in 10% sterilised glycerine at 4°C for further studies. The studied specimens and cultures were deposited in the Herbarium of Jiangxi Agricultural University, Plant Pathology, Nanchang, China (HJAUP).

Dna Extraction, Pcr Amplification And Sequencing

Genomic DNA was extracted from fungal mycelia grown on PDA, using the Solarbio Fungi Genomic DNA Extraction Kit following the manufacturer's protocol (Solarbio, China). DNA sequence data was obtained from small and large subunits of the nuclear ribosomal RNA genes (SSU, LSU). Primer sets used for these genes were as follows: SSU: 18S-F/18S-R and LSU: 28S1-F/28S3-R (Xia et al. 2017). The final volume of the PCR reaction was 25 µl, containing 1 µl of DNA template, 1 µl of each forward and reward primer, 12.5 µl of 2 × Power Taq PCR MasterMix and 9.5 µl of ddH_2O. The PCR thermal cycling conditions of SSU and LSU were initialized at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 50 s, elongation at 72°C for 1 min, a final extension at 72°C for 10 min, and finally kept at 4°C. The PCR products were checked on 1% agarose gel electrophoresis stained with ethidium bromide. Purification and DNA sequencing were carried out at Beijing Tsingke Biotechnology Co., Ltd. China.

Sequence Alignment And Phylogenetic Analyses

Phylogenetic analyses were performed by concatenated SSU and LSU sequence data. Sequences generated from this study were analyzed with other similar sequences obtained from GenBank, and those derived from recent studies in Li et al. (2016), Su et al. (2018) and Shen et al. (2021). The newly generated sequences together with other sequences obtained from GenBank and the recent studies (Table 1) were initially aligned using MAFFTv.7 (Katoh and Standley 2013) on the onlineserver (http://maffTh.cbrc.jp/alignment/server/), and optimized manually when needed. Phylosuite software v1.2.1 (Zhang et al. 2020) was used to construct the phylogenetic tree based on SSU and LSU sequence data. The concatenated aligned dataset was analyzed separately using Maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood phylogenies were inferred by using IQ-TREE (Anisimova et al. 2011; Nguyen et al. 2015) under Edge-linked partition model for 1000 standard bootstraps. The optimal ML tree search was conducted with 1000 separate runs using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing the likelihood scores using the K80 + G for SSU and TRN + I + G for LSU substitution model. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under partition model (2 parallel runs, 2000000 generations), in which the initial

25% of sampled data were discarded as burn-in. The best-fit model was K80 + I + G for SSU and SYM + I + G for LSU. The trees were viewed in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree) and the layout of the trees was done in Adobe Illustrator CS v. 5. Newly generated sequences were deposited in GenBank.

Table 1
Isolates and sequences used in this study and newly generated sequences are indicated in bold

Name	Voucher information	GenBank accession numbers		References
		LSU	SSU	
Arthopyrenia salicis	CBMAI 1330	JN903536	–	Passarini et al. (2013)
Biatriospora mackinnonii	CBS 110022	GQ387614	GQ387553	Ahmed et al. (2014)
Biatriospora mackinnonii	CBS 674.75	GQ387613	GQ387552	Ahmed et al. (2014)
Biatriospora marina	CY 1228	GQ925848	GQ925835	Suetrong et al. (2009)
Byssosphaeria salebrosa	SMH 2387	GU385162	–	Mugambi and Huhndorf (2009)
Dendryphion europaeum	CPC 23231	KJ869202	–	Crous et al. (2014)
Dendryphion europaeum	CPC 22943	KJ869203	–	Crous et al. (2014)
Elongatopedicellata lignicola	MFLUCC 15–0642	KX421368	KX421369	Ariyawansa et al. (2015)
Exosporium livistonae	CBS 131313	JQ044446	–	Crous et al. (2011)
Exosporium livistonicola	MUCC 190	MF951161	–	Videira et al. (2017)
Exosporium stylobatum	CBS 160.30	JQ044447	–	Crous et al. (2011)
Herpotrichia macrotricha	GKM 196N	GU385176	–	Mugambi and Huhndorf (2009)
Hysterium angustatum	CBS 236.34	FJ161180	GU397359	Boehm et al. (2009)
Melanomma pulvis-pyrius	CBS 124080	GU456323	GU456302	Zhang et al. (2009)
Monotosporella tuberculata	CBS 256.84	GU301851	–	Schoch et al. (2009)
Neooccultibambusa chiangraiensis	MFLUCC 12–0584	KU764699	KU712458	Doilom et al. (2017)
Neopodoconis aquaticum	MFLUCC 16-1113	MG208143	–	Su et al. (2018)
Neopodoconis aquaticum	KUMCC 15–0297	MG208144	–	Su et al. (2018)
Neopodoconis cangshanense	MFLUCC 20–0147	MW010285	–	Shen et al. (2021)
Neopodoconis jiangxiensis	**HJAUP C0947**	**ON693846**	**ON693847**	**This Study**
Neopodoconis meilingensis	**HJAUP C0905**	**ON693849**	**ON693843**	**This Study**
Neopodoconis obclavata	**HJAUP C0829**	**ON693848**	**ON693844**	**This Study**
Neopodoconis pandanicola	MFLUCC 20–0145	MW010280	MW010282	Shen et al. (2021)
Neopodoconis pandanicola	KUMCC 17–0176	MH260318	MH260358	Tibpromma et al. (2018)
Neopodoconis saprophyticus	**HJAUP C0830**	**ON693851**	**ON705129**	**This Study**

Name	Voucher information	GenBank accession numbers		References
		LSU	SSU	
Neopodoconis sinensis	**HJAUP C0909**	**ON693845**	**ON693850**	**This Study**
Neopodoconis thailandica	MFLUCC 13–0840	KX437757	KX437759	Li et al. (2016)
Neopodoconis thailandica	KUMCC 16 – 0012	KX437758	KX437760	Li et al. (2016)
Neoroussoella bambusae	MFLUCC 11–0124	KJ474839	–	Liu et al. (2014)
Occultibambusa bambusae	MFLUCC 13–0855	KU863112	KU872116	Dai et al. (2017)
Occultibambusa bambusae	MFLUCC 11–0394	KU863113	KU872117	Dai et al. (2017)
Occultibambusa fusispora	MFLUCC 11–0127	KU863114	–	Dai et al. (2017)
Occultibambusa pustule	MFLUCC 11–0502	KU863115	KU872118	Dai et al. (2017)
Paradictyoarthrinium diffractum	MFLUCC 12–0557	KP744497	–	Liu et al. (2015)
Paradictyoarthrinium diffractum	MFLUCC 13–0466	KP744498	KP753960	Liu et al. (2015)
Paradictyoarthrinium tectonicola	MFLUCC 12–0556	KP744499	–	Liu et al. (2015)
Paradictyoarthrinium tectonicola	MFLUCC 13–0465	KP744500	KP753961	Liu et al. (2015)
Pleomassaria siparia	CBS 279.74	DQ678078	DQ678027	Tanaka et al. (2010)
Prosthemium stellar	CBS 126964	AB553781	AB553650	Tanaka et al. (2010)
Roussoella angustior	MFLUCC 15–0186	KT281979	–	Ariyawansa et al. (2015)
Roussoella chiangraina	MFLUCC 10–0556	KJ474840	–	Liu et al. (2014)
Roussoella hysterioides	CBS 125434	AB524622	AB524481	Ahmed et al. (2014)
Roussoella intermedia	CBS 170.96	KF443382	KF443390	–
Roussoella magnatum	MFLUCC 15–0185	KT281980	–	Ariyawansa et al. (2015)
Roussoella nitidula	MFLUCC 11–0182	KJ474843	–	Liu et al. (2014)
Roussoella nitidula	MFLUCC 11–0634	KJ474842	–	Liu et al. (2014)
Roussoella scabrispora	MFLUCC 11–0624	KJ474844	–	Liu et al. (2014)

Name	Voucher information	GenBank accession numbers		References
		LSU	SSU	
Roussoella siamensis	MFLUCC 11–0149	KJ474845	KU872125	Liu et al. (2014)
Roussoella thailandica	MFLUCC 11–0621	KJ474846	–	Liu et al. (2014)
Roussoella pustulans	KT 1709	AB524623	AB524482	Tanaka et al. (2009)
Roussoellopsis macrospora	MFLUCC 12 –0005	KJ474847	KJ739608	Liu et al. (2014)
Roussoellopsis tosaensis	KT 1659	AB524625	AB524484	Tanaka et al. (2009)
Seriascoma didymospora	MFLUCC 11–0179	KU863116	KU872119	Dai et al. (2016)
Seriascoma didymospora	MFLUCC 11–0194	KU863117	KU872120	Dai et al. (2017)
Sporidesmium australiense	HKUCC 10833	DQ408554	–	Shenoy et al. (2006)
Torula herbarum	CBS 111855	KF443386	KF443391	Crous et al. (2015)
Torula herbarum	CBS 379.58	KF443383	KF443388	Crous et al. (2015)
Torula hollandica	CBS 220.69	KF443384	KF443389	Crous et al. (2015)
Versicolorisporium triseptatum	JCM 14775	AB330081	–	Hatakeyama et al. (2008)

Results

Molecular Phylogeny

The phylogenetic analyses based on SSU and LSU sequences was used to infer the relationships of the new taxon and its morphologically similar species (Fig. 1). A combined dataset of 59 characters (SSU and LSU sequence data) with 48 taxa analyzed either under ML or Bayesian criteria resulted in trees which were topologically congruent with respect to the position of the new taxa investigated herein including *Hysterium angustatum* (CBS 236.34) as the outgroup. Maximum likelihood and Bayesian Inference analyses of the combined dataset resulted in phylogenetic reconstructions with largely similar topologies, and the best scoring RAxML tree is shown in Fig. 1. Bootstrap support values for maximum likelihood (MLBS) higher than 85% and Bayesian posterior probabilities (BPP) greater than 0.95 are given above the nodes. Most of the core genera of *Torulaceae* (Crous et al. 2015) and *Roussoellaceae* (Liu et al. 2014) in Pleosporales (Wijayawardene et al. 2014) are included in our phylogenetic analysis (Fig. 1), and the two families *Torulaceae* and *Roussoellaceae* are represented with well-supported clades. All species collected in this study have a close phylogenetic relationship with *Sporidesmioides* and *Rostriconidium*, and well-supported in the phylogenetic analyses as members of the Torulaceae. In the phylogenetic reconstructions based on analysis of the combined SSU and LSU dataset, all the new taxa are well-separated with high bootstrap support as shown in Fig. 1. The newly collected *Neopodoconis meilingensis* and *N. saprophyticus* composed a clade is sister to the new combinations *N. cangshanense* and *N. pandanicola* with high statistical support (MLBS = 96%, BPP = 1.00). The

newly collected *N. obclavata*, *N. jiangxiensis* and *N. sinensis* formed a well-supported monophyletic clade, and was placed sister to the new combination *N. thailandica* with strong support (MLBS = 98%, BPP = 1.00).

Taxonomy

Neopodoconis jiangxiensis Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, **sp. nov.** (Fig. 2)

Index Fungorum number: IF559728

Etymology

In reference to the province where the type specimen was found.

Holotypus

HJAUP M0947

Colonies on natural subatrate effuse, scattered, hairy, brown to black. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight to curved, cylindrical, brown to pale brown, smooth, thick-walled, septate, up to 185 µm long, 10–12 µm thick. Conidiogenous cells polytretic, integrated, terminal, later becoming intercalary, cylindrical, brown, smooth, elongated sympodially, blackly cicatricated, with thickened and blackened scars. Conidial secession schizolytic. Conidia solitary, acropleurogenous, simple, brown to pale brown, obclavate, rostrate, sometimes smooth walled, but usually verruculose, 6–16-septate, with a thick, black truncate scar at base and pale pigment cell above the scar, 80–170 µm long, 14–18 µm thick in the broadest part, tapering to 2.5–5 µm near the apex.

Material examined China, Jiangxi Province, Nanchang, Meiling Scenic Spot, on dead branches of an unidentified broadleaf tree, 10 July 2020, L. Qiu, HJAUP M0947 (**Holotype**), ex-type living culture HJAUP C0947.

Notes Phylogenetic analysis showed that our isolate clustered together and formed a sister clade with the isolate of *N. obclavata*, but is well-separated with strong statistical bootstrap value support (MLBS = 95%; BPP = 1.00). *Neopodoconis jiangxiensis* is morphologically distinguished from *N. obclavata* which has longer conidiophores (up to 400 µm vs. up to 185 µm) and smooth conidia mostly with 8-septate. *Neopodoconis jiangxiensis* is also morphologically most similar to *N. ampullacea*, but clearly differs in the size of conidiophores (up to 185 × 10–12 µm vs. up to 300 × 10–13 µm) and conidia (80–170 × 14–18 µm vs. 80–220 × 16–22), and in its conidia with fewer septa (6–16-septate vs. 5–20-septate) and narrower apex (2.5–5 µm vs. 4–7 µm).

Neopodoconis meilingensis Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, **sp. nov.** (Fig. 3)

Index Fungorum number: IF559729

Etymology

In reference to the locality where the type specimen was found.

Holotypus

HJAUP M0905

Colonies on natural subatrate effuse, scattered, hairy, brown to black. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight to curved, cylindrical, brown to dark brown, smooth, thick-walled, septate, up to 400 µm long, 7.5–10 µm thick. Conidiogenous cells polytretic, integrated, terminal, later becoming intercalary, cylindrical, dark brown, smooth, elongated sympodially, blackly cicatricated, with thickened and blackened scars. Conidial secession schizolytic. Conidia solitary, rarely catenate, acropleurogenous, simple, obclavate, rostrate, brown, smooth, 7–20-septate, slightly constricted at some septa, with a thick, black truncate scar at base and pale pigment cell above the scar, 90–230 µm long, 12–17 µm thick in the broadest part, tapering to 2.5–6 µm near the apex.

Material examined China, Jiangxi Province, Nanchang, Meiling Scenic Spot, on dead branches of an unidentified broadleaf tree, 10 July 2020, L. Qiu, HJAUP M0905 (**Holotype)**, ex-type living culture HJAUP C0905.

Notes Phylogenetic analysis showed that our isolate clustered together and formed a sister clade with the isolate of *N. saprophyticus*, but is well-separated with strong statistical bootstrap value support (MLBS = 95%; BPP = 1.00). *Neopodoconis meilingensis* is morphologically distinguished from *N. saprophyticus* in its longer conidiophores (up to 400 × 7.5–10 µm vs. up to 325 × 8.5–10 µm) and solitary or rarely catenate, larger conidia (90–230 × 12–17 µm vs. 70–150 × 10–15 µm) with more septa (7–20-septate vs. 5–13-septate) and wider apex (2.5–6 µm vs. 1.5–2.5 µm). *Neopodoconis meilingensis* also differs from *N. ampullacea* in its longer and narrower conidiophores (up to 400 × 7.5–10 µm vs. up to 300 × 10–13 µm) and solitary or rarely catenate, narrower conidia (90–230 × 12–17 µm vs. 80–220 × 16–22 µm).

Neopodoconis obclavata Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, **sp. nov.** (Fig. 4)

Index Fungorum number: IF559730

Etymology

In reference to the obclavate conidia.

Holotypus

HJAUP M0829

Colonies on natural subatrate effuse, scattered, hairy, brown to black. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to dark brown, smooth hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight to curved, cylindrical, brown to dark black, paler towards the apex, smooth, thick-walled, septate, up to 400 µm long, 10–12.5 µm thick. Conidiogenous cells polytretic, integrated, terminal, later becoming intercalary, cylindrical, brown to dark brown, smooth, elongated sympodially, blackly cicatricated, with thickened and blackened scars. Conidial secession schizolytic. Conidia solitary, acropleurogenous, simple, obclavate, brown, rostrate, smooth, 7–11-septate (mostly 8), with a thick, black truncate scar at base and pale pigment cell above the scar, 90–170 µm long, 10–20 µm thick in the broadest part, tapering to 3.5–6.5 µm near the apex.

Material examined China, Jiangxi Province, Yichun, Guanshan Mountain, on dead branches of an unidentified broadleaf tree, 10 May 2020, L. Qiu, HJAUP M0829 (Holotype), ex-type living culture HJAUP C0829.

Notes Our molecular data confirmed a clear separation with strong statistical support as shown in Fig. 1. *Neopodoconis obclavata* shares similar characters with *N. ampullacea* in having macronematous, unbranched conidiophores, and polytretic, integrated, terminal and intercalary, elongated sympodially, blackly cicatricated conidiogenous cells with thickened and blackened scars and solitary, acropleurogenous, euseptate conidia. however, *N. obclavata* differs from *N. ampullacea* in the size of conidiophores (up to 400 × 10–12.5 µm vs. up to 300 × 10–13 µm) and conidia (90–170 × 15–18 µm vs. 80–220 × 16–22), and in its conidia usually with 8-euseptate.

Neopodoconis saprophyticus Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, **sp. nov.** (Fig. 5)

Index Fungorum number: IF559731

Etymology

In reference to the saprophytic habit on dead branches.

Holotypus

HJAUP M0830

Colonies on natural subatrate effuse, scattered, hairy, brown to black. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight to curved, cylindrical, brown to dark brown, smooth, thick-walled, septate, up to 325 µm long, 8.5–10 µm thick. Conidiogenous cells polytretic, integrated, terminal, later becoming intercalary, cylindrical, dark brown to brown, smooth, elongated sympodially, blackly cicatricated, with thickened and blackened scars. Conidial secession schizolytic. Conidia solitary, acropleurogenous, simple, brown to pale brown, obclavate, rostrate, smooth, 5–13-septate (mostly 8), slightly constricted at some septa, with a thick, black truncate scar at base and pale pigment cell above the scar, 70–150 µm long, 10–15 µm thick in the broadest part, tapering to 1.5–2.5 µm near the apex.

Material examined China, Jiangxi Province, Yichun, Guanshan Mountain, on dead branches of an unidentified broadleaf tree, 10 May 2020, L. Qiu, HJAUP M0830, (**Holotype)**, ex-type living culture HJAUP C0830.

Notes *Neopodoconis saprophyticus* clusters with *N. meilingensis*, but is well-separated with high bootstrap support as shown in Fig. 1. Moreover, *N. saprophyticus* morphologically differs from *Neopodoconis meilingensis* in the size of conidiophores (up to 325 × 8.5–10 µm vs. up to 400 × 7.5–10 µm) and conidia (70–150 × 10–15 µm vs. 90–230 × 12–17 µm), and in its conidia with fewer septa (5–13-septate vs. 7–20-septate) and narrower apex (1.5–2.5 µm vs. 2.5–6 µm). *Neopodoconis saprophyticus* also superficially resembles *N. ampullacea*, but differs in its narrower conidiophores (8.5–10 µm vs. 10–13 µm wide) and smaller conidia (70–150 × 10–15 µm vs. 80–220 × 16–22 µm) with fewer septa (5–13-septate vs. 5–20-septate) and narrower apex (1.5–2.5 µm vs. 4–7 µm).

Neopodoconis sinensis Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, sp. nov. (Fig. 6)

Index Fungorum number: IF559732

Etymology

In reference to the country in which the fungus was collected.

Holotypus

HJAUP M0909

Colonies on natural subatrate effuse, scattered, hairy, brown to dark brown. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, smooth, pale brown to brown, smooth hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight to curved, cylindrical, brown to dark brown, slightly paler at the apex, smooth, thick-walled, 8–12-septate, up to 310 µm long, 8–10 µm thick. Conidiogenous cells polytretic, integrated, terminal, later becoming intercalary, cylindrical, brown to dark brown, smooth, elongated sympodially, blackly cicatricated, with thickened and blackened scars. Conidial secession schizolytic. Conidia solitary, acropleurogenous, simple, obclavate, rostrate, smooth, 7–10-septate, slightly constricted at some septa, brown to dark brown, with a thick, black truncate scar at base and pale pigment cell above the scar, 90–150 µm long, 13–15 µm thick in the broadest part, tapering to 3–5 µm near the apex.

Material examined China, Jiangxi Province, Nanchang, Meiling Scenic Spot, on dead branches of an unidentified broadleaf tree, 10 July 2020, L. Qiu, HJAUP M0909 (**Holotype**), ex-type living culture HJAUP C0909.

Notes Our phylogenetic analyses showed that *N. sinensis* forms an independent clade (MLBS = 96%, BPP = 1.00), and is phylogenetically related to *N. obclavata* and *N. jiangxiensis*. *Neopodoconis sinensis* morphologically shares similarities with *N. ampullacea*, but is clearly different in the size of the conidiophores (up to 310×8–10 µm vs. up to 300 × 10–13 µm), conidia (90–150 × 13–15 µm vs. 80–220 × 16–22) and the number of conidial septa (7–10 vs. 5–20). *Neopodoconis sinensis* is also differs from *N. megasperma* which has shorter and wider conidia (60–90 × 20–28.5 µm, 4–7-septate) with fewer eusepta.

New combinations from Rostriconidium **and** Sporidesmioides

Neopodoconis aquaticum (Z.L. Luo, K.D. Hyde & H.Y. Su) Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, comb. nov.

Index Fungorum number: IF559733

Holotypus

MFLU 17–1415

Basionym: *Rostriconidium aquaticum* Z.L. Luo, K.D. Hyde & H.Y. Su, Mycol. Progress 17(5): 536 (2018).

Notes For a detailed description of the species, see Su et al. (2018). *Rostriconidium aquaticum* is the generic type of *Rostriconidium*, which was established as a distinct genus based on a phylogenetic placement between *Sporidesmioides* and *Neotorula*. However, Su et al. (2018) didn't morphologically compare *Rostriconidium aquaticum* with the type species of *Neopodoconis*, although no DNA sequence exists for *Neopodoconis* species to be used in the molecular phylogeny. *Rostriconidium aquaticum* exhibits the key characters of *Neopodoconis*, acropleurogenous, solitary, euseptate conidia seceding schizolytically from polytretic, integrated, terminal and intercalary, elongated sympodially, blackly cicatricated conidiogenous cells with thickened and blackened scars. Therefore, *R. aquaticum* is clear congeneric with *Neopodoconis*, but not conspecific with the previously described species of *Neopodoconis*. *Rostriconidium aquaticum* morphologically shares similarities with *N. ampullacea*, but is clearly different in the size of the conidiophores (370–590 × 13–17 µm vs. up to 300 × 10–13 µm), conidia (134–180 × 22–26 µm vs. 80–220 × 16–22), and the number of conidial septa (8–9 vs. 5–20).

Neopodoconis cangshanense (H.W. Shen, Z.L. Luo & H.Y. Su) Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, comb. nov.

Index Fungorum number: IF559734

Holotypus: MFLU: 20–0572

Basionym: *Rostriconidium cangshanense* H.W. Shen, Z.L. Luo & H.Y. Su, Mycosystema 40(6): 1267 (2021).

Notes For a detailed description of the species, see Shen et al. (2021). *Neopodoconis cangshanense* was originally assigned to *Rostriconidium* by Shen et al. (2021) based on the particular morphological characters and the support of phylogeny. However, the genus *Rostriconidium* was proposed as a synonym of the earlier described *Neopodoconis* based on their distinct morphological features in the present study. Thus, *Rostriconidium cangshanense* is here transferred to *Neopodoconis*, but differs from *N. ampullacea* which has smooth or verruculose, longer and narrower conidia with more septa, and from *N. megasperma* which has shorter and wider conidia with the second cell from below largest (Table 2). In our phylogenetic analyses, the new combination, *Neopodoconis cangshanense* clusters with *N. pandanicola*, but is well-separated with high bootstrap support.

Table 2
Synoptic table for comparision of *Neopodoconis* species

Species	Conidiophores	Conidia					References
	Size (µm)	Shape	Size (µm)	Septation	Verrucose	Apical width (µm)	
N. ampullacea	Up to 300 × 10–13	Obclavate	80–150(–220) × 16–22	5–20	Yes	4–7	Rifai (2008)
N. aquaticum	370–590 × 13–17	Fusiform to pyriform	134–180 × 22–26	8–9	No	–	Su et al. (2018)
N. cangshanense	279–528 × 12–14	Pyriform, fusiform to obclavate, with a sheath at the tip	94–109 × 21–24	6–8	No	–	Shen et al. (2021)
N. jiangxiensis	Up to 185 × 10–12	Obclavate	80–170 × 14–18	6–16	Yes	2.5–5	
N. megasperma	Up to 480 × 8–11.5	Broadly obclavate, occasionally almost turbinate or subfusoidal	60–90 × 20–28.5	4–7	No	3–4.5	Rifai (2008)
N. meilingensis	Up to 400 × 7.5–10	Obclavate	90–230 × 12–17	7–20	No	2.5–6	This study
N. obclavata	Up to 400 × 10–12.5	Obclavate	90–170 × 10–20	7–11 (mostly 8)	No	3.5–6.5	This study
N. pandanicola	360–485 × 11–13	Obclavate, with a sheath at the tip	55–110 × 18–26	4–7	No	–	Tibpromma et al. (2018)
N. saprophyticus	Up to 325 × 8.5–10	Obclavate	70–150 × 10–15	5–13 (mostly 8)	No	1.5–2.5	This study
N. sinensis	Up to 310 × 8–10	Obclavate	90–150 × 13–15	7–10	No	3–5	This study
N. thailandica	(100–)120–200 × 7–9 (–9.5)	Ampulliform, with a sheath at the tip	62.5–80(– 97) × (16–)20–25	6–7	Yes	–	Li et al. (2016)

Neopodoconis pandanicola (Tibpromma & K.D. Hyde) Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, comb. nov.

Index Fungorum number: IF559735

Holotypus

HKAS 99620

Basionym: *Rostriconidium pandanicola* Tibpromma & K.D. Hyde, Fungal Diversity 93:45 (2018).

Notes For a detailed description of the species, see Tibpromma et al. (2018). Tibpromma et al. (2018) assigned *R. pandanicola* to the genus *Rostriconidium* based on morphological and molecular phylogenetic analyses. *Rostriconidium* is clear congeneric with *Neopodoconis* based on their distinct morphological features, and was combined into *Neopodoconis* in the present study. The description and illustrations of *R. pandanicola* fully match *Neopodoconis*. It differs from *N. ampullacea* and *N. megasperma* in the size of the conidiophores and conidia, and further from *N. ampullacea* in its smooth conidia with fewer septa (Table 2), from *N. megasperma* which has conidia with the second cell from below largest. Combined SSU and LSU phylogenetic analyses also confirm it as distinct taxa.

Neopodoconis thailandica (Jun F. Li, Phook. & K.D. Hyde) Y.F. Hu, L. Qiu, R.F. Castañeda & Jian Ma, comb. nov.

Index Fungorum number: IF559736

Holotypus

MFLU14-0827

Basionym: *Sporidesmioides thailandica* Jun F. Li, Phook. & K.D. Hyde, Mycol. Progress 15(10): 1171 (2016).

Notes For a detailed description of the species, see Li et al. (2016). *Sporidesmioides thailandica* is the generic type of *Sporidesmioides*, which was established as a distinct monotypic genus based on a phylogenetic placement of forming a distinct, monotypic clade clustering with Torulaceae. The morphological features of this fungus fully match the existing genus *Neopodoconis*, but Li et al. (2016) didn't morphologically compare it with *Neopodoconis* species. Although no sequence data of *Neopodoconis* species are available, there is no doubt that the species belongs to *Neopodoconis*. Therefore, *Sporidesmioides thailandica* is here transferred to *Neopodoconis*. It differs from *N. ampullacea* and *N. megasperma* in the size of the conidiophores and conidia, and further from them by its conidial apex with a flap-like, hyaline sheath (Table 2). Our molecular data also confirmed a clear separation with strong statistical support.

Discussion

Rifai (2008) proposed the genus *Neopodoconis* to accommodate *Exosporium*-like species that have solitary or rarely catenate, euseptate conidia, and so far only two species have been reported (Rifai 2008). *Neopodoconis* and its closely related genus *Exosporium* show similar conidial ontogeny, and conidial euseptation was regarded as the only character for separating *Neopodoconis* from *Exosporium*, and their phylogenetic placement is well-separated as shown in Fig. 1.

Sporidesmioides and *Rostriconidium* are two aquatic genera within the family Torulaceae (Pleosporales, Dothideomycetes) (Li et al. 2016; Su et al. 2018). Both genera are clear congeneric with *Neopodoconis* based on their distinct morphological features, and were here synonymised with *Neopodoconis* under the current code (Turland & al. 2018: Art. 11.3). Our phylogenetic analyses of SSU and LSU sequence data also resolved a relationship of *Sporidesmioides* and *Rostriconidium* with *Neopodoconis*. The results show that the genus *Sporidesmioides* is closely related to *N. jiangxiensis*, *N. obclavata*, *N. sinensis*, and formed a distinct phylogenetic clade with high bootstrap values (MLBS = 98%; BPP = 1.00). The genus *Rostriconidium* is closely related to *N. saprophyticus* and *N. sinensis*,

and formed a distinct clade with strong support (MLBS = 97%; BPP = 1.00). All the taxa are well-separated with high bootstrap support as shown in Fig. 1. Based on morphology and sequence data, we introduced five new species, *Neopodoconis jiangxiensis*, *N. meilingensis*, *N. obclavata*, *N. saprophyticus* and *N. sinensis*, and four new combinations, *N. aquaticum*, *N. cangshanense*, *N. pandanicola* and *N. thailandica*, and treated *Neopodoconis* in Torulaceae, Pleosporales, Dothideomycetes. A synopsis of the morphological characters of these currently accepted *Neopodoconis* species is presented in Table 2.

Declarations

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Competing Interests The authors declare no competing interests.

Author contribution Conceived and designed the experiments: J Ma. Performed the experiments: L Qiu, YF HU, JW Liu and ZH Xu. Analyzed the data: L Qiu and YF Hu. Wrote the paper: L Qiu, JW Xia, RF. Castañeda-Ruíz and J Ma. All authors read and approved the final manuscript.

Data availability The data, including the sequences on GenBank and specimen data on Index Fungorum will be available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

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Figures

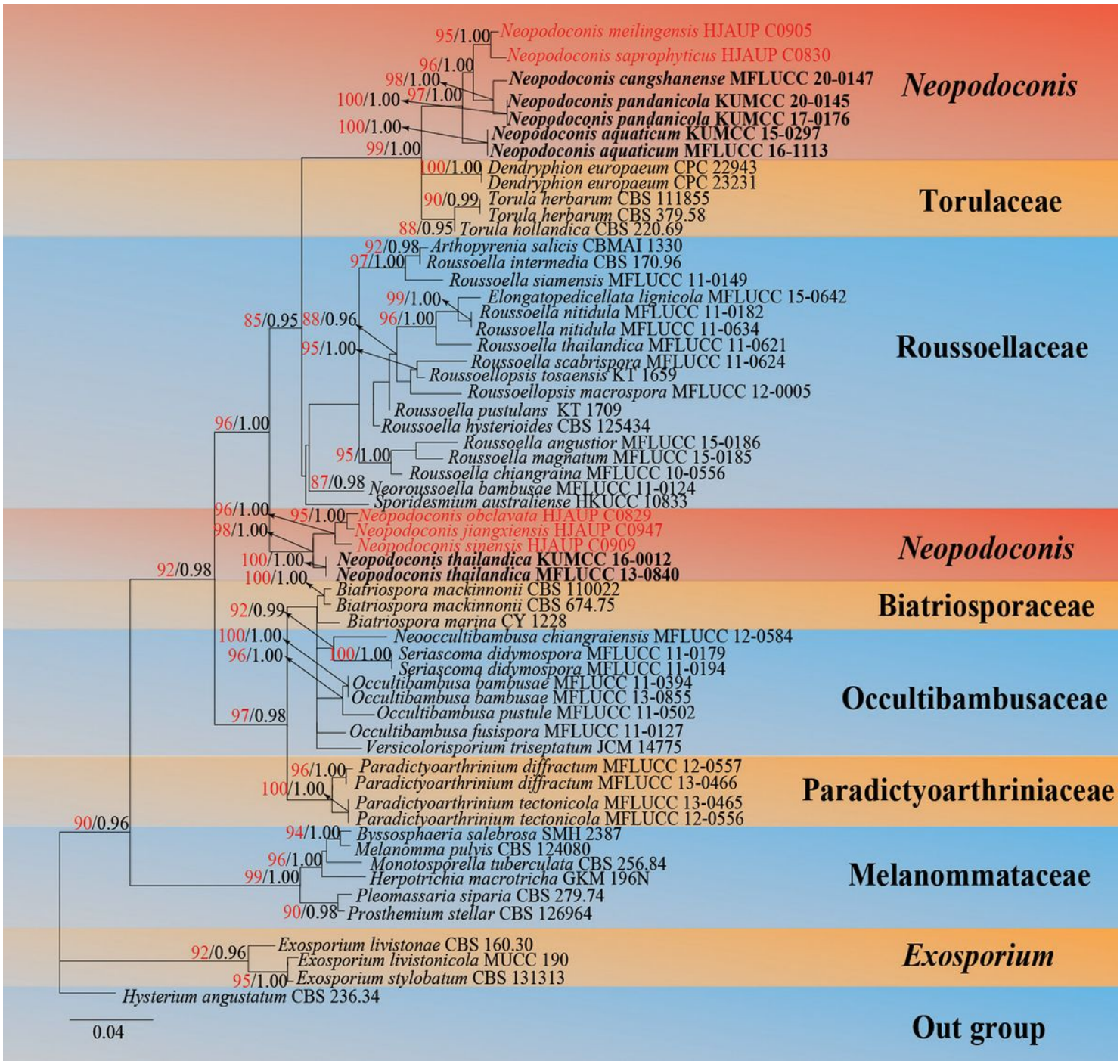

Figure 1

Phylogenetic tree inferred from maximum likelihood and Bayesian inference analysis based on a concatenated alignment of SSU and LSU sequences. Bootstrap support values for maximum likelihood (MLBS) equal to or greater than 85% and Bayesian posterior probabilities (BPP) equal to or greater than 0.95 are shown above the nodes at the first and second position, respectively. Ex-type isolates are in red, and new combinations are indicated in bold. The tree is rooted to *Hysterium angustatum* (CBS 236.34).

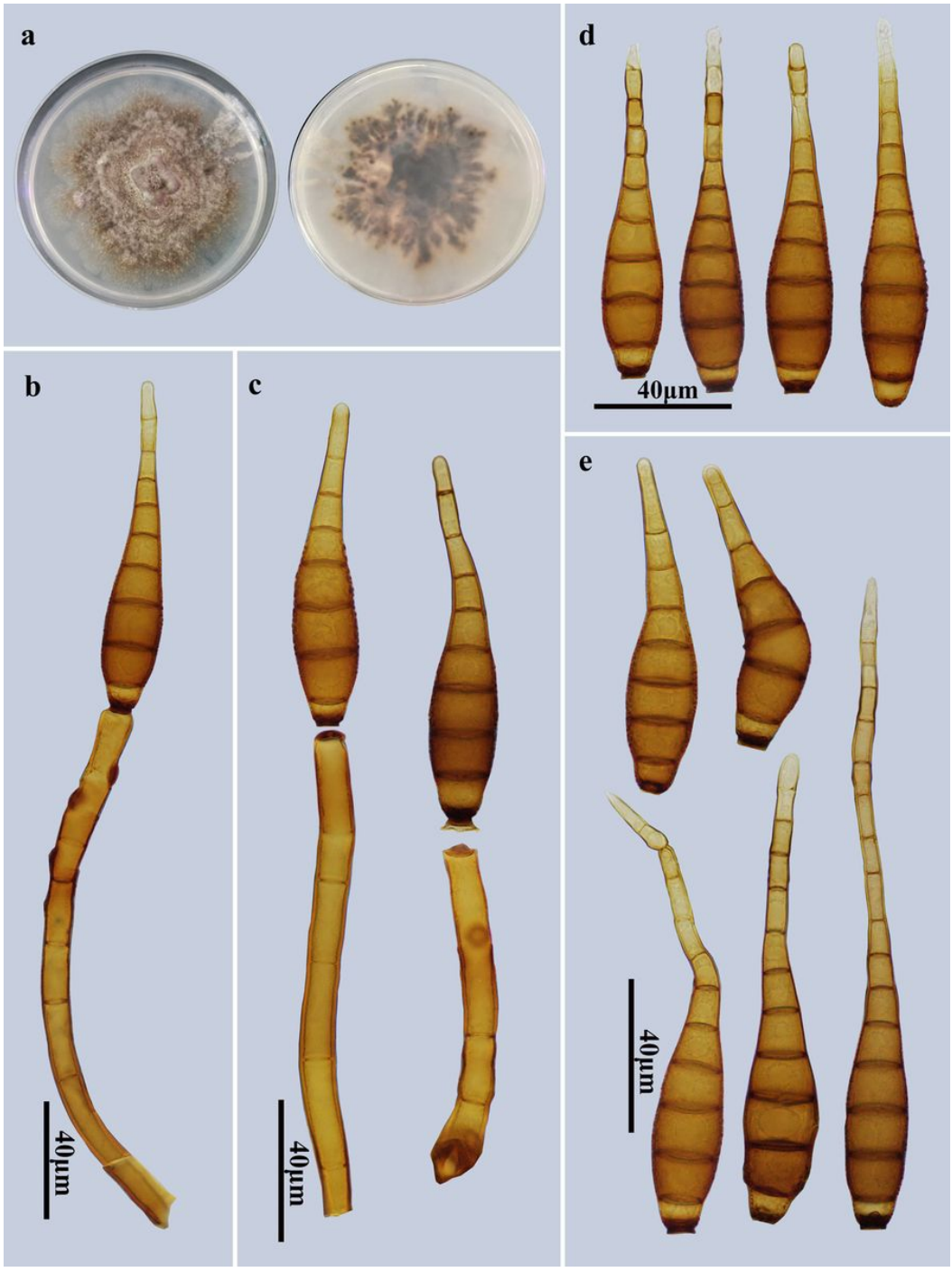

Figure 2

Neopodoconis jiangxiensis (HJAUP M0947). a Colony on PDA (surface and reverse). b, c Conidiophores, conidiogenous cells, and conidia. d, e Conidia.

Figure 3

Neopodoconis meilingensis (HJAUP M0905). a Colony on PDA (surface and reverse). b Conidiophores, conidiogenous cells, and conidia. c Conidiophores and conidiogenous cells. d, e Conidia.

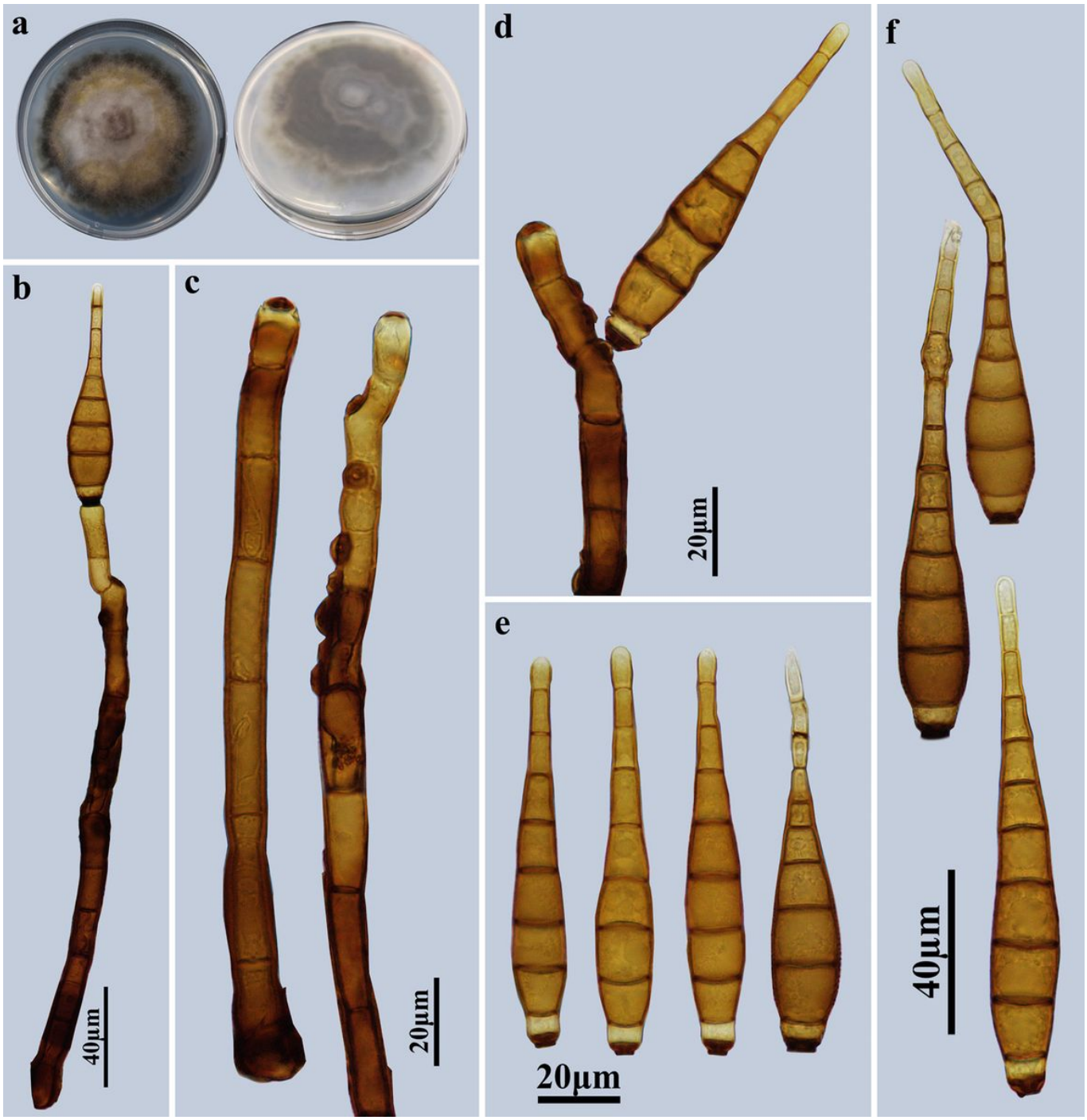

Figure 4

Neopodoconis obclavata (HJAUP M0829). a Colony on PDA (surface and reverse). b Conidiophores, conidiogenous cells, and conidia. c Conidiophores and conidiogenous cells. d Conidiogenous cells and conidia. e, f Conidia.

Figure 5

Neopodoconis saprophyticus (HJAUP M0830). a Colony on PDA (surface and reverse). b Conidiophores, conidiogenous cells, and conidia. c Conidiophores and conidiogenous cells. d Conidiogenous cells and conidia. e, f Conidia.

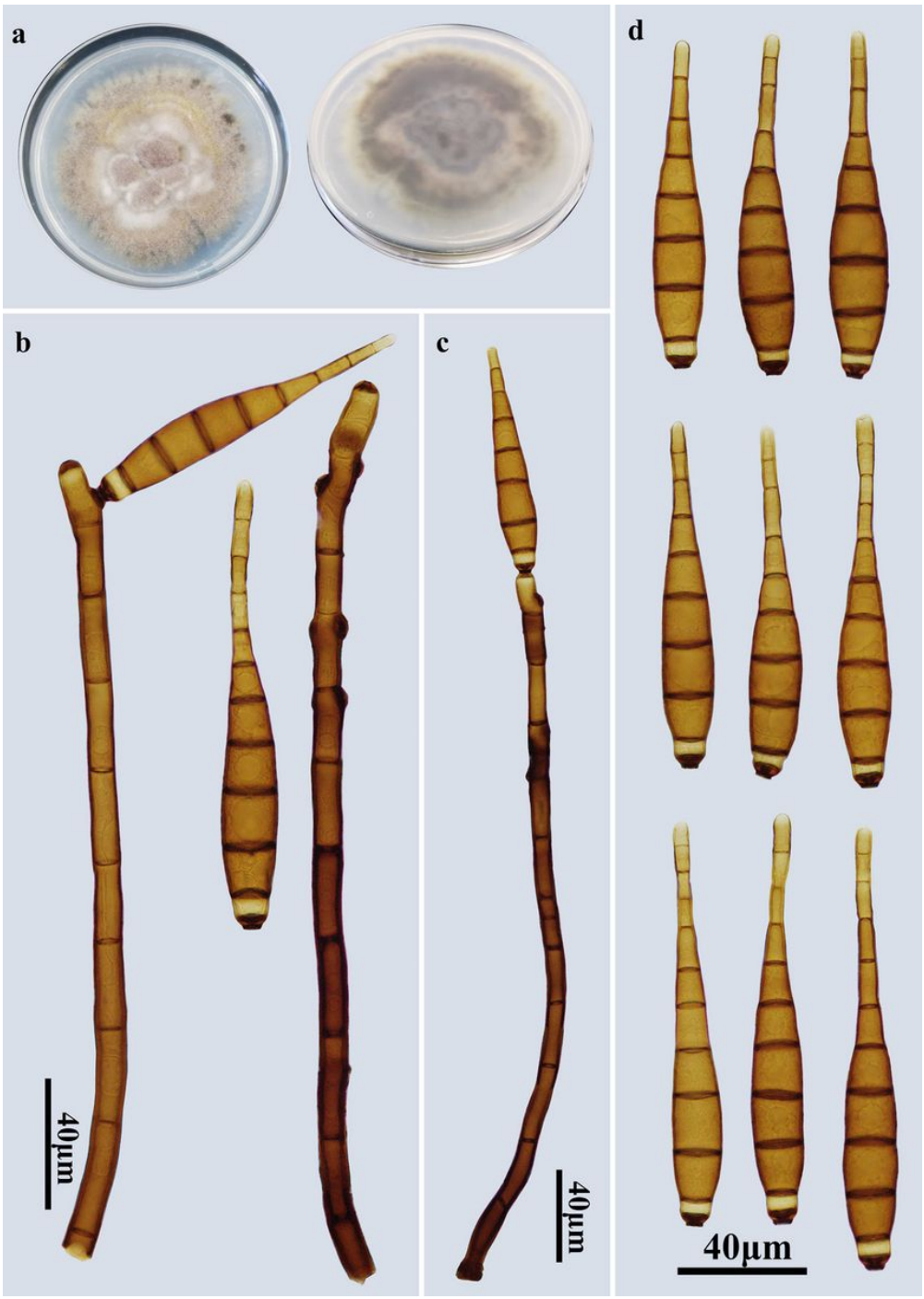

Figure 6

Neopodoconis sinensis (HJAUP M0909). a Colony on PDA (surface and reverse). b, c Conidiophores, conidiogenous cells, and conidia. d Conidia.