Neodactylariales, Neodactylariaceae (Dothideomycetes, Ascomycota): new order and family, with a new species from China

Min Qiao*, Hua Zheng*, Ruili Lv, Zefen Yu

Laboratory for Conservation and Utilization of Bio-resources, Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, Yunnan, 650091, China

Corresponding author: Zefen Yu (zfyuqm@hotmail.com)

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Abstract

During a mycological survey of aquatic hyphomycetes on submerged decaying leaves in southwest China, a distinct new fungus was collected. The collection was cultured and sequenced and a BLAST search of its ITS and LSU sequence against data in GenBank revealed a dothideomycetous affiliation, with the closest related taxa in the genus Neodactylaria. Phylogenetic analyses of a multigene matrix containing sequences from four genes (LSU, SSU, rpb2, and tef1), representing broad groups of Dothideomycetes, revealed its placement within Dothideomycetes, but without a supported familial or ordinal affiliation. Based on further phylogenetic analyses and morphological investigations, the new fungus is described here as a new species of Neodactylaria, *N. simaoensis* sp. nov., and placed in a new family Neodactylariaceae fam. nov. and a new order Neodactylariales ord. nov.

Keywords

Dothideomycetes, new family, new order, new species, phylogenetic analysis, taxonomy

Introduction

The kingdom Fungi contains an estimated 700,000 to over 5 million species, amongst which only about 120,000 have been described (Lynne 2016). Dothideomycetes is one

* Authors contributed equally to this work.
of the largest and most significant classes of fungi within Ascomycota (Kirk et al. 2008; Schoch et al. 2009a; Hyde et al. 2013). Thousands of species have been included in the class Dothideomycetes, and many of them are important plant pathogens (Cortinas et al. 2006; Crous et al. 2007; Wikee et al. 2011, 2013a, b; Manamgoda et al. 2012), human and animal pathogens (Siu and Lzumi 2004; da Cunha et al. 2012, 2013), or used in biotechnological applications (Verkley et al. 2004; Damm et al. 2008; de Wit et al. 2012; Ohm et al. 2012; Stergiopoulos et al. 2012; Hyde et al. 2014). The members of Dothideomycetes are still increasing with the discovery of many novel species and inclusion of DNA sequence data. In the past few years, molecular phylogenetic studies have advanced our understanding of the systematics of Dothideomycetes (Inderbitzin et al. 2001; Schoch et al. 2009b; Hirayama et al. 2010; Suetrong et al. 2011; Hyde et al. 2013; Wijayawardene et al. 2014; Liu et al. 2017; Jiang et al. 2020). Wijayawardene et al. (2014) recommended 23 orders and 110 families in Dothideomycetes based on culture characteristics and molecular phylogenetic analyses. More recently, Liu et al. (2017) provided an updated phylogenetic assessment of Dothideomycetes at the order level by using molecular clock methods and accepted 29 orders. However, the latest research by Wijayawardene et al. (2018) expanded this to 33. Despite the progress in our understanding of the systematics of Dothideomycetes, a number of newly described and/or previously reported taxa are currently incertae sedis and their family and order level positions within the Dothideomycetes remain obscure; many taxa lack sequencing data or appropriate classification rank to accommodate them (Hyde et al. 2013; Wijayawardene et al. 2018).

The genus *Neodactylaria* Guevara-Suarez et al., typified by *N. obpyriformis* Guevara-Suarez et al., was originally described from human bronchoalveolar lavage in the USA (Crous et al. 2017). The genus is characterized by having integrated, polyblastic and sympodial extended conidiogenous cells producing solitary, septate, obpyriform or rostrate conidia (Crous et al. 2017). Morphologically, *Neodactylaria* is similar to two *Dactylaria* species, *D. kumamotoensis* Matsush. and *D. madrasensis* Matsush., and several *Pyricularia* species, such as *P. grisea* Cooke ex Sacc. and *P. pennisetigena* Klaubauf, M.-H. Lebrun & Crous. However, in the phylogeny inferred from sequences of the large subunits of nuclear ribosomal DNA (LSU), *Neodactylaria* was placed within Dothideomycetes, but the ordinal and familial position was unresolved.

Southwestern China is one of the world’s 34 biodiversity hotspots (Myers et al. 2000; Zhang et al. 2020). During a survey of aquatic hyphomycetes on submerged decaying leaves from this area, several new species have been reported (Guo et al. 2019; Qiao et al. 2019a, b; Yu et al. 2019). In a further study, an unidentified fungus was collected, which had a similar morphology to *Heliocephala proliferans* V. Rao et al. (Pezizomycotina incertae sedis; Rao et al. 1984; Mel’nik et al. 2013), but detailed morphological examination showed that the conidiogenous cells were terminal or intercalary, with short-cylindrical denticles, and the conidia were 1- or 2-septate and constricted at the septum. Sequence data obtained from cultures of conidia confirmed that this species does not belong in *Heliocephala*. A BLAST search of its LSU gene sequences against the public sequence records in GenBank (Sayers et al. 2019) confirmed its dothideo-
mycetous affinity and that it was closely related to members of the genus *Neodactylaria*. Subsequently, we obtained the type species of *Neodactylaria*, *N. obpyriformis* Guevara-Suarez et al., from the CBS-KNAW Fungal Biodiversity Centre (Netherlands) and processed it with full morphological and phylogenetic analyses. Our new collection prompted the study of the molecular phylogenetic relationships of taxa within *Neodactylaria*, as well as the higher order phylogenetic relationship of *Neodactylaria* within the Dothideomycetes.

Our comparative analyses identified that the newly collected fungus is a species of *Neodactylaria*, *N. simaoensis*. However, due to their significant divergence, there was no apparent family or order for placement of *Neodactylaria*. We propose that the genus be placed in a new family and new order within Dothideomycetes.

**Materials and methods**

**Isolation and morphological study**

Submerged dicotyledonous leaves were collected from a stream in Simao, Yunnan Province, southern China. Samples were preserved in zip-lock plastic bags, labelled and transported to the laboratory. Each rotted leaf was cut into several 3–4 × 4–5 cm sized fragments, and these were incubated on CMA (20 g cornmeal, 18 g agar, 1000 ml distilled water), supplemented by two antibiotics (penicillin G, 0.04 g/l; and streptomycin, 0.03 g/l; Gams et al. 1998), for 5 days at room temperature. Individual conidia were isolated using a sterilised toothpick under a BX51 microscope and cultivated on CMA plates. Morphological observations were conducted on cultures growing on CMA after incubation at 25 °C for 1 week. Colony colour was based on the colour charts of Rayner (1970).

Pure cultures have been 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).

**DNA extraction, polymerase chain reaction (PCR) amplification and sequencing**

Pure cultures were grown on PDA for 5 days at 25 °C. Actively-growing mycelia were scraped off the surface of a culture and transferred to 2 ml Eppendorf micro-centrifuge tubes. Total genomic DNA was extracted according to the procedures in Turner et al. (1997). To determine the phylogenetic position of *Neodactylaria*, we amplified five nuclear genomic loci, including the internal transcribed spacer (ITS), the 28S large subunit ribosomal RNA (LSU), the 18S small subunit ribosomal RNA (SSU), the translation elongation factor1-alpha partial gene (*tef1*) and the RNA polymerase II subunit 2 (*rpb2*). The following primers were used: the ITS region was amplified us-
ing the primers ITS1 and ITS4 (White et al. 1990); the LSU nuc rDNA region was amplified with primers LROR and LR7 (Vilgalys and Hester 1990); the SSU nuc rDNA region was amplified with primers NS1 and NS4 (White et al. 1990); an approx. 1.1 kb fragment of the \textit{rpb2} gene was amplified using the primer pair fRPB2-5f and fRPB2-7cr (Liu et al. 1999); an approximately 1.0 kb fragment of the \textit{tef1} gene was amplified with the primers TEF983F and TEF2218R (initially obtained from S. Rehner; http://ocid.nacse.org/research/deephyphae/EF1primer.pdf).

PCR reactions were prepared in a 25 μl final volume as described by Zheng et al. (2019, 2020a). PCR amplifications were performed in an Eppendorf Mastercycler thermal cycler. PCR conditions were as follows: an initial 4 min denaturing step at 94 °C, followed by 35 cycles of 75 s at 94 °C, 90 s at 52 °C (for \textit{rpb2}, LSU, and SSU) and 100 s at 72 °C. After a final extension step of 7 min at 72 °C, the samples were stored at 4 °C. Conditions for amplification of the ITS and \textit{tef1} regions were an initial step of three cycles at an annealing temperature of 54 °C, followed by 30 cycles with the annealing temperature set at 48 °C. When needed, a ‘touchdown’ (Don et al. 1991) protocol preceded the PCR cycle. PCR products were then purified using a commercial kit (Bioteke Biotechnology Co. Ltd, China). Each fragment was sequenced from both directions using the forward and reverse primers in separate reactions using a LI-COR 4000L automatic sequencer as described by Kindermann et al. (1998). The sequences obtained have been submitted to GenBank at the National Center for Biotechnology Information (NCBI) and the accession numbers are listed in Table 1.

**Sequence alignment and phylogenetic analysis**

Preliminary BLAST searches with ITS, SSU, LSU, \textit{rpb2}, and \textit{tef1} gene sequences of the new isolate against GenBank and UNITE databases (Nilsson et al. 2019) identified sequences closely related to our isolates. However, we were only able to robustly determine their placements within the class Dothideomycetes. To infer a phylogenetic relationship for our strain, an initial alignment of the newly generated sequences (SSU, LSU, \textit{rpb2}, and \textit{tef1}) and 74 representatives belonging to 33 orders of the Dothideomycetes, extracted from recent studies (Mapook et al. 2016; Nieuwenhuijzen et al. 2016; Voglmayr et al. 2016; Hernandez-Restrepo et al. 2017; Liu et al. 2017; Wijayawardene et al. 2018) with a species from the sibling class, Arthoniomycetes, as the outgroup, was performed using the online MAFFT interface (Katoh and Standley 2013; http://mafft.cbrc.jp/alignment/server). This alignment was used to infer a preliminary phylogenetic relationship for the new sequences based on Bayesian inference (BI) analyses (data not shown).

Based on the initial analysis, a second alignment combined SSU, LSU, and \textit{tef1} sequence data were constructed from the closest relatives to our strain in Botryosphaeriales, Dothideales, Hystereales, Minutisphaeriales, Myriangiales, Patellariales, Phaeotrichales, Pleosporales, Tubeufiales, and Venturiales. In the second alignment, \textit{Schismatomma decolorans} (DUKE 47570) was used as an outgroup taxon. All sequence data were aligned using MAFFT (v. 7.110) online program (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley 2013). The alignments were checked and uninformative gaps minimized.
Table 1. Species, strains, and their corresponding GenBank accession numbers of sequences used for phylogenetic analyses.

| Species                                      | Strain\(^a\) | GenBank accession numbers\(^b\) |
|----------------------------------------------|--------------|---------------------------------|
|                                              | LSU          | SSU                             | ref/
| Acianthosigmaria tingzhangiae Boonmee & K.D. Hyde | MFLUCC 10-0125\(^c\) | JN865197 JN865185 | KF301506 |
| Allocybeula marinaeformis Aiyaw., Camporei & K.D. Hyde | MFLUCC 13-0349\(^d\) | KP765681 KP765682 | – |
| Bambusaria bambusar (J.N. Kapoor & H.S. Gill) Jaklitsch, D.Q. Dai, K.D. Hyde | CBS 139763 | KP687813 KP687972 | KF687983 |
| Bambusaria fuscocornis Phook., Jian K. Liu & K.D. Hyde | MFLUCC 11-0143\(^e\) | JX646808 JX646826 | – |
| Bambusaria agarita (Henn.) E.J. Butler | MFLUCC 11-0125\(^f\) | JX646808 JX646825 | – |
| Bambusaria disthala (Mong.) Ces. & De Not. | CBS 115476 | DQ67805 | DQ677998 DQ676737 |
| Caphispora uniloculata (Mehl & Slippers) A. Alves & A.J.L. Phillips | MFLUCC 11-0425\(^g\) | JX646817 JX646835 | – |
| Dematiopilepora mariae Wanata, Camporei, E.B.G. Jones & K.D. Hyde | MFLUCC 13-0612\(^h\) | KF749563 KF749565 | KF749565 |
| Doliolida biporphyraa Puckel | CBS 188 85 | DQ678048 U42475 | DQ677667 |
| Doliolida uniloculata Wals | CBS 189 58 | DQ247802 DQ247810 | DQ2471081 |
| Gloeotrichia psalma (Schwein.) Underw. & Earle | CBS 112415 | FJ161173 FI161134 | FI161090 |
| Helicangiospora lignicola Boonmee, Bhat & K.D. Hyde | MFLUCC 11-0378\(^i\) | KF301531 KF301539 | KF301552 |
| Helicoma chongpapatanee Boonmee & K.D. Hyde | MFLUCC 10-0115 | JN668518 JN685176 | KF301551 |
| Helicoma fagacearum Boonmee & K.D. Hyde | MFLUCC 11-0379 | KF301532 KF301540 | KF301553 |
| Hydromis rautagastian Alb. & Schwein. | CBS 236 34 | FJ161180 GU179359 | FI161096 |
| Hydromis rautagastian Alb. & Schwein. | CBS 114601 | FJ161174 FI161135 | FI161091 |
| Hydromis rautagastian Alb. & Schwein. | CBS 131716\(^j\) | JX444874 JX444902 | – |
| Hydromis rautagastian Alb. & Schwein. | CBS 131727 | JX444883 JX444908 | – |
| Minutisphaera arieta Raja, Obereg, Speaker & A.N. Mill. | DSM 29478 | KP999993 KP399999 | – |
| Minutisphaera fanerispiare Speaker, A.N. Mill. & A. Ferrer | A242 8a | HM196367 HM196367 | – |
| Minutisphaera japonica Kar, Tanaka, Raja & Speaker | JCM 18560\(^k\) | AB733420 AB733424 | AB733424 |
| Moriporia rubicunda (Nies) Y. Zhang ter, J. Fourn. & K.D. Hyde | FIRD 2017 | FJ799507 GU456308 GU456268 |
| Myriangium duriae Mont. & Berk. | CBS 260 36 | DQ678059 AY106347 | DQ677990 |
| Myriangium rubricolum (Aptroot, Aa & Petrin) Jaklitsch & Voglmayr | CBS 109505 | GU456324 GU456303 | GU456260 |
| Myriangium rubricolum (Aptroot, Aa & Petrin) Jaklitsch & Voglmayr | CBS 139605 | KP687686 KP687976 KP688030 |
| Myriangium rubricolum (Aptroot, Aa & Petrin) Jaklitsch & Voglmayr | CBS 139968 | KP687688 KP687979 KP688053 |
| Neodactylaria obpyriformis Guevara-Suarez, Deanna A. Sutton, Wieder & Gené | MFLUCC 142668 | MK562571 MK562578 | MK562748 |
| Neodactylaria obpyriformis Guevara-Suarez, Deanna A. Sutton, Wieder & Gené | MFLUCC 142668 | MK562571 MK562578 | MK562748 |
| Neodactylaria spongiiformis F.J. Zheng & Z.F. Yu | YMF 1 584 | MK877920 MK120837 | MK120847 |
| Oedohysterium setidens (Schwein.) Cooke & M.L. Schoch | CBS 238 34 | FJ161182 FJ161142 | FJ161097 |
| Tanasemieromyces pyggi Cerqueira & M.J. Wingf. | CBS 141353\(^m\) | KX228339 |
| Tuttelaria atrata (Hedw.) Fr. | CBS 958 97 | GU301855 GU296181 GU349038 |
| Thamnophila benjaminae Malloch & Cain | CBS 541 72 | AY016344 AT016344 DQ678072 |
| Phyllosticta amdaica (Engel.) Aa | CBS 237 48 | DQ678085 DQ678034 |
| Phyllosticta citriscarpa (McLinpen) Aa | CBS 102374 | GU301855 GU296159 GU349053 |
| Populocrescentia forlicesenensis (McLinpen) Aa | MFLUCC 14-0651\(^n\) | KT7300920 |
| Populocrescentia forlicesenensis (McLinpen) Aa | MFLUCC 14-0651\(^n\) | KT7300920 |
| Decuadlepistogenus indica Phadke & V.C. Rao | MTCUCC 11985\(^o\) | KM052821 KM052825 |
| Piloplumaria aranacanum (Speg.) E. Boehm, Marine, & C.L. Schoch | CBS 112412 | FJ161172 FI161133 FI161089 |
| Saccharota paleata (Wakef.) Deman & Crous | CBS 115206 | GU301869 GU296194 GU349030 |
| Schizomama decivora (Erichsen) Clauzade & Vézda | DUKE 47570 | AY548815 AY548809 DQ883723 |
| Speimis pedetaphora Tubaki | CBS 397 59 | KR678977 |
| Trematosphaeria pertula Fucel | CBS 122368 | FJ201990 FJ201991 GU456276 |
| Trematosphaeria pertula Fucel | CBS 122368 | FJ201990 FJ201991 GU456276 |
| Trichodelitschia biporula (P. Crouan & H. Crouan) Munk | CBS 262 60 | GU349896 GU349000 GU349020 |
| Trichodelitschia unicolor N. Lundq. | Knys 201 | D3480906 D3480470 |

\(^a\) ex-type strains are indicated with * after the strain number. \(^b\) Abbreviations of culture collections (where known): CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; G, University of North Carolina, Greensboro, Department of Chemistry and Biochemistry Fungal Culture Collection; DUKE, Duke University Herbarium, Durham, North Carolina; IFRDCC, International Fungal Research and Development Culture Collection; JCM, Japan Collection of Microorganisms, BIKEN BioResource Center, Japan; MFLUCC, Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; MUF: The Herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, China. \(^c\) Sequences obtained in this study are shown in bold.
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manually where necessary in BioEdit 7.0.1 (Hall 1999). Maximum likelihood (ML) and BI were used in the analyses following the methodology as described in Mapook et al. (2016). The nucleotide substitution models use for analyses was determined using jModelTest 2.0 (Posada 2008). The GTR+I+G model with inverse gamma rate were selected for individual data from each partition with the combined aligned dataset. The phylogenetic tree was visualized in FigTree v. 1.4 (Rambaut 2012) and the layout of the tree was done in Adobe Illustrator v. CS5.1. The alignment of phylogenetic analyses was deposited in TreeBASE (https://www.treebase.org, submission number 24051).

Results

Molecular phylogeny

Following the results of preliminary phylogenetic analysis of the initial alignment (data not shown), the phylogenetic reconstruction of the second alignment was performed including SSU, LSU, and tef1 sequences from 53 strains representing 10 different orders in the Dothideomycetes and one order in the Arthoniomycetes (Table 1). The three-gene dataset comprised of LSU sequences for all 52 ingroup sequences, 50 SSU sequences, and 36 tef1 sequences. After exclusion of ambiguous regions and introns, the combined dataset included 2555 characters (826 for LSU, 1012 for SSU, and 717 for tef1). In the BI analysis, the alignment has 952 distinct patterns, 600 parsimony-informative, 205 singleton sites, and 1750 constant sites.

The best tree (RAxML) obtained using the ML analysis is shown as Fig. 1, with the support values from the ML and BI analyses plotted at the nodes. In this tree, our newly proposed species and *N. obpyriformis* formed a distinct clade within Dothideomycetes with significant ML bootstrap support (100%) and Bayesian posterior probability (1.0). Moreover, the *Neodactylaria* clade is sister to the Pleosporales clade, but only with low bootstrap support values (51%) and Bayesian posterior probabilities (0.72). The results suggested that our strain belongs to the genus *Neodactylaria*. The order Pleosporales has characters that are very different from those of species of *Neodactylaria* and, therefore, we introduce a new order and new family, Neodactylariales and Neodactylariaceae, respectively, for this group of fungi. In addition, combined with morphological differences, our strain was described and illustrated herein as a new species of *Neodactylaria*.

Taxonomy

**Neodactylariales** H. Zheng & Z.F. Yu, ord. nov.
MycoBank No: 830161

**Type family.** Neodactylariaceae H. Zheng & Z.F. Yu.

**Description.** Asexual morph from human-associated organs or saprobic on plant debris. Conidiophores acroauxic, macronematous, mononematous, branched...
**Figure 1.** Maximum likelihood (RAxML) tree obtained by phylogenetic analyses of the combined LSU, SSU, and tefl sequence alignment of 53 taxa belonging to the 11 orders shown to the right of the tree. The numbers of nodes in clades represent Maximum likelihood bootstrap support values (ML-BS, 0–100) and Bayesian posterior probabilities (BPP, 0–1.0). ML-BS greater than 50% and BPP above 0.5 are indicated at the nodes (ML-BS/BPP). The scalebar represents the number of changes. *Schizomatomma decorans* DUKE 47570 was used as outgroup. The strain numbers are noted after the species names with ex-type strains indicated with 1. The proposed new order is in boldface.

or unbranched. Conidiogenous cells mono- and polyblastic, sympodially extended. Conidia solitary, hyaline or pale pigmented, smooth, verrucous or echinulate. Sexual morph not observed.

**Neodactylariaceae H. Zheng & Z.F. Yu, fam. nov.**
MycoBank No: 830162

**Type genus.** *Neodactylaria* Guevara-Suarez, Deanna A. Sutton, Wiederh. & Gené.

**Description.** Mycelium superficial or immersed, composed of branched, septate, hyaline to subhyaline hyphae. Conidiophores macronematous, mononematous, straight or flexuous, septate, unbranched. Conidiogenous cells terminal or intercalary, polyblastic, sympodial, with short-cylindrical denticles. Conidial secession schizolytic. Conidia solitary, smooth or finely echinulate. Sexual morph not observed.
Neodactylaria Guevara-Suarez, Deanna A. Sutton, Wiederh. & Gené, in Crous et al. Persoonia 38: 345 (2017)

**Type species.** Neodactylaria obpyriformis Guevara-Suarez, Deanna A. Sutton, Wiederh. & Gené.

**Description.** Mycelium superficial or immersed, composed of branched, septate, smooth-walled, hyaline to subhyaline hyphae. Conidiophores macronematous, mononematous, straight or flexuous, septate, unbranched, smooth-walled, pale to mid-brown. Conidiogenous cells polyblastic, sympodial extended, integrated, terminal or intercalary, denticulate, with short cylindrical denticles, pale to medium-brown. Conidial secession schizolytic. Conidia obpyriform to obclavate, unicellular or septate, attenuate, subulate or rostrate toward the obtuse apex, with a tiny, protuberant basal hilum, smooth or finely echinulate, subhyaline or pale brown. Sexual morph not observed.

Neodactylaria simaoensis, H. Zheng & Z.F. Yu, sp. nov.
MycoBank No: 830160
Fig. 2

**Diagnosis.** It is characterised by straight or flexuous, 2–4-septate, unbranched conidiophores, with denticulate conidiogenous cells and obclavate to long obpyriform, subulate or slightly rostrate towards the obtuse or rounded apex and 1–2 (–3)-septate conidia. Differs from *N. obpyriformis* by longer and slightly wider conidia and more septa.

**Type.** China, Yunnan Province, Simao country, 100°59'19"N, 22°46'38"E, ca 1330 m alt., from submerged unidentified dicotyledonous leaves, 28 Oct 2013, Z.F. Yu, live culture YMF 1.03984 – *holotype*, dried slide YMFT 1.03984.

**Description.** Mycelium partly superficial or partly immersed, composed of branched, septate, hyaline to subhyaline, creeping, 1.0–2.0 μm wide hyphae. Conidiophores macronematous, mononematous, straight or flexuous, slightly geniculate towards the apex, 2–4-septate, unbranched, hyaline or pale brown, 38–86 (–129) × 3–4 μm, arising from the creeping hyphae pale brown. Conidiogenous cells polyblastic, indeterminate, sympodial extended, integrated, terminal or intercalary, denticulate with protuberant cylindrical denticles. Conidia solitary, obclavate to long obpyriform, subulate or slightly rostrate towards the obtuse or rounded apex, lumina micro-guttulate, 1–2 (–3)-septate, constricted at the septa, pale to mid brown, 15–40 × 3.6–6.5 μm, with a subhyaline, protuberant basal hilum up to 1 μm long.

**Culture characteristics.** Colonies attaining 1 cm in diameter on CMA after 7 days at 25 °C. On CMA, colonies flat, floccose at the centre, lacking aerial mycelium towards periphery, white to cream-coloured, reverse same colour, sporulation abundant. On PDA, colonies flat, white to cream-coloured, margin entire; sporulation sparse.

**Habitat and distribution.** In submerged dicotyledonous leaves from southwestern China.
**Figure 2.** Culture and anamorph of *Neodactylaria simaoensis* (YMF 1.03984) A culture on CMA B–D conidia E conidia and conidiophores F immature conidium and conidiogenous cells G conidiophores and conidia under low power microscope. Scale bars: 1 cm (A); 10 μm (B–F); 50 μm (G).
Teleomorph. Not known.

Etymology. The species epithet indicates its occurrence in the county of Simao, China.

Notes. Based on a Blast search of NCBI’s GenBank nucleotide database, the closest hits using the ITS sequences of *N. simaoensis* (GenBank MH379209) is *N. obpyriformis* (GenBank NR_154267, Identities = 545/569(96%), Gaps = 4/569(0%)). Morphologically, the new species, *N. simaoensis*, shares several characters with *N. obpyriformis* (type species): both have white to cream-coloured colonies, with short-cylindrical denticles as conidiogenous cells and obpyriform to slightly rostrate conidia (Crous et al. 2017). However, *N. simaoensis* differs from *N. obpyriformis* by having obviously longer and slightly wider conidia (15–40 × 3.6–6.5 μm vs 10–14 × 3–5 μm) and more septa.

Discussion

Aquatic hyphomycetes, which have always been important members of the Dothideomycetes, play critical roles in the decomposition of organic compounds and nutrient cycling in aquatic habitats. Since Ingold (1942, 1943) first reported aquatic hyphomycetes in the 1940s, research on this group have been steadily increasing throughout the world. It was estimated that over 300 species of over 80 genera of aquatic hyphomycetes are reported worldwide (Kirk et al. 2008; Guo et al. 2015). Studies of aquatic hyphomycetes have revealed a huge fungal diversity. Our study again underlined the importance of these microorganisms for fungal taxonomic discovery.

In this study, a preliminary phylogenetic analysis combined SSU, LSU, *rpb2*, and *tef1* sequences from 74 representative taxa of Dothideomycetes and Arthoniomycetes revealed the *Neodactylaria* as a unique clade within Dothideomycetes (data not shown). The second phylogenetic analyses using three loci (SSU, LSU, *tef1*) also showed our new collected strain and *N. obpyriformis* form a strongly supported monophyletic and distinct clade (ML-BS = 100%, BPP = 1.0) within the Dothideomycetes (Fig. 1). In this tree, the *Neodactylaria* clade is close to the *Pleosporales* but with low support (ML-BS = 51%, BPP = 0.72). The original study on *N. obpyriformis*, which conducted a phylogenetic analysis of the LSU sequence, also showed that *Neodactylaria* is related to Dothideomycetes, but with an uncertain taxonomic position at the ordinal level and family level (Crous et al. 2017). Thus, we establish a new order (*Neodactylariales*) and family (*Neodactylariaceae*) within the Dothideomycetes for this unique clade.

The genus *Neodactylaria* is morphologically similar to two species of the genus *Dactylaria*, *D. kumamotoensis* and *D. madresensis*, which were described by Matsushima from soil and plant debris in Japan and India, respectively (Matsushima 1981, 1984). Although these two fungi in *Dactylaria* could be congeneric with *N. simaoensis*, they are only known from the type collection and no living cultures are available for molecular comparison. Morphologically, the conidia of *N. simaoensis* are smaller than *D. kumamotoensis* and are distinguished from *D. madresensis* by their size and the number of septa. In addition, the genus *Dactylaria* is heterogeneous. Related information showed that the classification position of *D. kumamotoensis* was in the order Helotiales, the class Leotiomyces (http://www.indexfungorum.org/Names/NamesRecord.
Neodactylariales (Dothideomycetes, Ascomycota) 79

asp?RecordID=111390), but most Dactylaria species were placed in the Sordariomycetes (Crous et al. 2017). Thus, although the genus Neodactylaria shares some morphological characters with the genus Dactylaria, Neodactylaria was placed in the Dothideomycetes by phylogenetical analysis and was phylogenetically distant from Dactylaria.

In the Dothideomycetes, many orders show various morphological characteristics and lifestyles, such as the order Pleosporales. In our new order, the two species within genus Neodactylaria also have different habitats: N. obpyriformis was found from human bronchoalveolar lavage in the USA, but N. simaoensis was found from submerged decaying leaves in China. Therefore, it seems fungi in this genus may be broadly distributed in different habitats.

The class Dothideomycetes is one of the most important and diverse classes in the phylum Ascomycota. It comprises pathogenic fungi, aquatic hyphomycetes, fungi with different life cycles and habitats, and also fungi with biotechnological potential (Wijayawardene et al. 2014; Santos et al. 2015; Woudenberg et al. 2015; Zheng et al. 2020b). In recent years, this class has received significant attention, and several papers have highlighted its importance to fungal taxonomy, based on its fungal diversity and on new studies performed to improve the classification of dothideomycetous fungi (Schoch et al. 2009a; Hyde et al. 2013; Wijayawardene et al. 2014). In Dothideomycetes, most families comprise both sexual genera and asexual genera and only a few families are totally comprised of asexual genera, such as Cladosporiaceae Nann., which contains seven asexual hyphromycetous genera and Neodevriesiaceae Quaedvlieg & Crous, which contains one asexual hyphomycetous genus (Wijayawardene et al. 2014). However, the order Lichenoconiales, only comprising one family, was also established with an asexual genus (Hyde et al. 2013). Here, we added a new order containing only an asexual genus to Dothideomycetes. These results show asexual genera have equal status to sexual genera at various taxon ranks. In addition, the description of Neodactylariales, as a new order in this study, highlights the need to collect fungal biodiversity from a range of diverse environments and substrates, as these diverse niches frequently harbour fungal lineages that are still missing in current phylogenetic studies.

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