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Taxonomic Paper

# *Neomonodictys aquatica* sp. nov. (Pleurotheciaceae) from a plateau lake in Yunnan Province, China

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## Abstract

### Background

In this study, a new species *Neomonodictys aquatica* was collected from submerged decaying wood in Erhai Lake, Yunnan Province, China.

### New information

*Neomonodictys aquatica* is characterised by acrogenous, solitary, oval, dictyospores (most are transverse septum, occasionally vertical septum, in immaturity the septum is clear, but when mature, the conidia becomes darker so the septum is not clear), smooth-walled conidia. The immature conidia are usually hyaline to olivaceous and mature conidia are usually darkened to black, sometimes with one pale basal cell. Phylogenetic analyses of combined ITS and LSU sequence data showed that the new collection is distinct from other *Neomonodictys* species. Description and illustration are provided as well.

## Keywords

new species, asexual morph, freshwater fungi, phylogeny, taxonomy

## Introduction

Pleurotheciales was introduced by Réblová et al. (2016), based on morphological characters and phylogenetic analyses. Members of Pleurotheciales are mostly saprobic on wood (Hyde et al. 2020) and some species have been identified as opportunistic human pathogens (Guarro et al. 2000, Chew et al. 2010, Réblová et al. 2020). Species of the order were collected on submerged decaying wood in lentic and lotic habitats in temperate, subtropical and tropical zones in Asia, Europe, Melanesia and North America (Matsushima 1971, Réblová et al. 2012, Réblová et al. 2016, Réblová et al. 2020, Hernández-Restrepo et al. 2017, Hyde et al. 2018, Hyde et al. 2020, Luo et al. 2018a).

Pleurotheciaceae is a single family of Pleurotheciales. It is typified by *Pleurothecium* with *P. recurvatum* as the type species (Morgan) Höhn (Réblová et al. 2016). Recently, Hyde et al. (2020) updated the phylogenetic tree for Pleurotheciales and introduced a new genus *Neomonodictys* Y.Z. Lu, C.G. Lin & K.D. Hyde. Currently, ten genera are accepted in this family (Réblová et al. 2016, Maharachchikumbura et al. 2016, Hernández-Restrepo et al. 2017, Hyde et al. 2020, Goh and Kuo 2021).

The monophyletic asexual genus *Neomonodictys* is established for a fungus (*Neomonodictys muriformis*) collected from a freshwater habitat in Thailand, which is morphologically similar to members of *Monodictys* S. Hughes ( Hyde et al. 2020). *Neomonodictys* is characterised by holoblastic, monoblastic, integrated, terminal, determinate conidiogenous cells and muriform, subglobose to globose, smooth-walled, pale brown to darkened to black conidia (Hyde et al. 2020).

In this study, the fungus was isolated from submerged decaying wood in Erhai Lake, Yunnan Province in China. The morphology and phylogeny show that our collection is distinct from related species. We provide detailed descriptions, illustrations for *Neomonodictys aquatica* and a synopsis table for the morphology comparison.

## Materials and methods

### Isolation and morphological examination

Submerged decaying wood was collected from Erhai Lake, Dali City, Yunnan, China. The coordinates of sampling sites are 25°44′29.65″N, 100°09′49.33″E and at an altitude of 1966 m. Samples were returned to the laboratory in plastic bags. The samples were incubated in aseptic plastic boxes, lined with moistened tissue paper at room temperature for one week. Specimen observations and morphological studies were conducted following the protocols provided by Luo et al. (2018b).

Morphological observations were made by using a SMZ760 series stereomicroscope and photographed using a Nikon-80i microscope. The fungal structures were measured with Tarosoft (R) Image Frame Work programme and images were processed using Adobe Photoshop CS6 extended version 13.0 (Adobe Systems, USA). Single spore isolation was carried out following the method described in Chomnunti et al. (2014). Germinating conidia were transferred aseptically to PDA plates with 0.5 mg/l of Amoxicillin and incubated at room temperature under dark conditions. The colonies were checked every three days. A herbarium was deposited in the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), Yunnan, China. Living cultures were deposited in Kunming Institute of Botany Culture Collection (KUNCC) and China General Microbiological Culture Collection Center (CGMCC). Facesoffungi numbers were registered as described in Jayasiri et al. (2015) and Index Fungorum numbers as in Index Fungorum(2021).

**Molecular Phylogenetic Analyses**

**DNA Sequencing and Sequence Alignment**

The appropriate fungal mycelium was scraped from the surface of colonies on Potato Dextrose Agar (PDA) plates with a scalpel into a 1.5 ml EP tube (Bao et al. 2018). Genomic DNA was extracted using the Trelief$^{TM}$ Plant Genomic DNA Kit (Beijing TsingKe Biological Technology and Services Co. Ltd, China) according to the manufacturer's protocols.

The primers ITS4/ITS5 for Internal transcribed spacer (ITS) and LR0R/LR5 for Large subunit ribosomal ribonucleic acid (LSU rRNA) were selected for PCR amplification (Vilgalys and Hester 1990). Polymerase Chain Reaction (PCR) mixture was performed in a 25 µl system reaction containing 9.5 µl dd$H_2O$, 12.5 µl of 2 × Power Taq PCR Master Mix, 1 µl of DNA template and 1 µl of each primer (10 µM) (Wang et al. 2019). The PCR thermal cycles for amplification of the ITS gene region were as per Su et al. (2015) and the LSU gene followed Sun et al. (2020). PCR amplifications were confirmed on 1% agarose electrophoresis gels stained with ethidium bromide.

Sequences were assembled with BioEdit. Sequences with high similarity indices were determined from a BLAST search to find the closest matches with taxa in *Neomonodictys* and from recently published data (Ariyawansa et al. 2015, Wanasinghe et al. 2015, Hyde et al. 2019, Hyde et al. 2020). All consensus sequences and the reference sequences were aligned in MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh and Standley 2013). Aligned sequences of each gene region (ITS and LSU) were combined and manually improved using BioEdit v. 7.0.5.2 (Hall 1999). Ambiguous regions were excluded from the analyses and gaps were treated as missing data.

**Phylogenetic Analyses**

Maximum Likelihood analysis was performed in the CIPRES Science Gateway v.3.3 (Miller et al. 2010) using RAxML v. 8.2.8 as part of the "RAxML-HPC2 on XSEDE" tool (Stamatakis 2006, Stamatakis et al. 2008). The final ML search was conducted using the

GTRGAMMA + I model estimated using MrModeltest 2.2 (Nylander 2004), with ML bootstrap support being calculated from 1000 bootstrap replicates.

Bayesian analysis was performed using MrBayes v. 3.1.2. (Ronquist and Huelsenbeck 2003). The model of each gene was estimated using MrModeltest 2.2 (Nylander 2004), with GTR + I + G model being the best-fit model of ITS and LSU for Bayesian analysis. Posterior Probabilities (PP) (Rannala and Yang 1996) were performed by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v.3.1.2 (Liu et al. 2012). Six simultaneous Markov chains were run for 50 million generations and trees were sampled every 5000$^{th}$ generation (resulting in 10,000 trees). The first 2000 trees, representing the burn-in phase of the analyses, were discarded and the remaining 8000 (post burning) trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai et al. 2006, Liu et al. 2012).

Phylogenetic trees were visualised by FigTree v. 1.4.4 (Rambaut 2014) and edited in Microsoft Office PowerPoint 2016 (MicrosoftInc. United States). Newly-produced sequences in this study were submitted to GenBank (Table 1).

Table 1.

Isolates and sequences used in this study (newly-generated sequences are indicated in bold and with "*" after species name, the type strains are in bold).

| Taxon | Strain | GenBank Accession No. | |
|---|---|---|---|
| | | ITS | LSU |
| ***Adelosphaeria catenata*** | **CBS 138679** | **NR_145396** | **MH877664** |
| ***Anapleurothecium botulisporum*** | **FMR 11490** | **NR_153582** | KY853483 |
| *Ascotaiwania mitriformis* | HKUCC3706 | – | AF132324 |
| *Ascotaiwania sawadae* | SS00051 | HQ446340 | HQ446363 |
| ***Bactrodesmiastrum obovatum*** | **FMR 6482** | **NR_152537** | FR870266 |
| *Bactrodesmiastrum pyriforme* | FMR 11931 | HE646636 | HE646637 |
| *Brachysporiella setasa* | HKUCC 3713 | – | AF132334 |
| *Canalisporium caribense* | SS03683 | GQ390284 | GQ390269 |
| *Canalisporium elegans* | SS00895 | GQ390286 | GQ390271 |
| *Canalisporium exiguum* | SS00809 | GQ390296 | GQ390281 |
| *Canalisporium pulchrum* | SS03982 | GQ390292 | GQ390277 |
| *Conioscypha japonica* | CBS 387.84 | – | AY484514 |
| *Conioscypha lignicola* | CBS 335.93 | – | AY484513 |
| ***Conioscypha minutispora*** | **CBS 137253** | **NR_137847** | **NG_066275** |
| *Conioscypha peruviana* | ILL 41202 | – | **NG_058867** |
| *Conioscypha varia* | CBS 113653 | – | AY484512 |
| ***Fuscosporella pyriformis*** | **MFLUCC 16-0570** | **NR_152555** | **NG_059711** |

| Taxon | Strain | GenBank Accession No. | |
|---|---|---|---|
| | | ITS | LSU |
| *Helicoascotaiwania farinosa* | DAOM 241947 | JQ429145 | JQ429230 |
| *Melanotrigonum ovale* | MR 3685 | KT278726 | KT278712 |
| *Melanotrigonum ovale* | CBS 138744 | KT278725 | KT278710 |
| *Melanotrigonum ovale* | CBS 138815 | KT278722 | KT278711 |
| ***Melanotrigonum ovale*** | **CBS 138743** | **NR_145397** | **NG_058197** |
| *Melanotrigonum ovale* | CBS 138742 | KT278723 | KT278708 |
| ***Mucispora obscuriseptata*** | **MFLUCC 15-0618** | **NR_152556** | **NG_059709** |
| ***Neomondictys aquatica*** * | **KUNCC21-10708** | **MZ686200** | **OK245417** |
| ***Neomonodictys muriformis*** | **MFLUCC 16-1136** | **NR_168231** | **NG_068916** |
| ***Parafuscosporella moniliformis*** | **MFLUCC 15-0626** | **NR_152557** | **NG_059710** |
| ***Phaeoisaria aquatica*** | **MFLUCC 16-1298** | **NR_160592** | **NG_066194** |
| *Phaeoisaria clematidis* | MFLUCC 16-1273 | MF399229 | MF399246 |
| *Phaeoisaria clematidis* | DAOM 226789 | JQ429155 | JQ429231 |
| *Phaeoisaria clematidis* | MFLUCC 17-1968 | MG837022 | MG837017 |
| *Phaeoisaria clematidis* | MFLUCC 17-1341 | MF399230 | MF399247 |
| *Phaeoisaria fasciculata* | DAOM 230055 | KT278720 | KT278706 |
| ***Phaeoisaria fasciculata*** | **CBS 127885** | **NR_145395** | **NG_064241** |
| *Phaeoisaria guttulata* | MFLUCC 17-1965 | MG837021 | MG837016 |
| ***Phaeoisaria loranthacearum*** | **CBS 140009** | **NR_56593** | **NG_064294** |
| ***Phaeoisaria pseudoclematidis*** | **MFLUCC 11-0393** | **NR_155648** | **NG_059559** |
| *Phaeoisaria sedimenticol* | CGMCC 3.14949 | MK878380 | MK835851 |
| *Phaeoisaria sparsa* | FMR11939 | HF677179 | HF677185 |
| *Phaeoisaria microspora* | MFLUCC 16-0033 | MF671987 | – |
| ***Pleurotheciella aquatica*** | **MFLUCC 17-0464** | **NR_160591** | **NG_066193** |
| ***Pleurotheciella centenaria*** | **DAOM 229631** | **NR_111709** | **NG_060098** |
| ***Pleurotheciella lunata*** | **MFLUCC 17-0111** | **NR_160593** | **NG_066195** |
| ***Pleurotheciella rivularia*** | **CBS 125238** | **NR_111711** | **NG_057950** |
| *Pleurotheciella rivularia* | CBS 125237 | JQ429161 | JQ429233 |
| *Pleurotheciella fusiformis* | KUMCC 15-0192 | MF399234 | MF399251 |
| *Pleurotheciella fusiformis* | MFLUCC 17-0113 | MF399233 | MF399250 |
| *Pleurotheciella fusiformis* | MFLUCC 17-0115 | MF399232 | MF399249 |
| *Pleurotheciella fusiformis* | MFLUCC 16-1356 | MF399235 | MF399252 |
| *Pleurotheciella guttulata* | KUMCC 15-0442 | MF399239 | MF399256 |
| ***Pleurotheciella guttulata*** | **KUMCC 15-0296** | **NR_160594** | **NG_066399** |
| *Pleurotheciella krabiensis* | MFLUCC 16-0852 | MG837018 | MG837013 |

| Taxon | Strain | GenBank Accession No. | |
|---|---|---|---|
| | | ITS | LSU |
| *Pleurotheciella krabiensis* | MFLUCC 16-0858 | MG837019 | MG837014 |
| ***Pleurotheciella saprophytica*** | **MFLUCC 16-1251** | **NR_160595** | **NG_066196** |
| ***Pleurotheciella submersa*** | **MFLUCC 17-1709** | **NR_160596** | MF399260 |
| *Pleurotheciella submersa* | DLUCC 0739 | MF399242 | MF399259 |
| *Pleurotheciella submersa* | MFLUCC 17-0456 | MF399244 | MF399261 |
| *Pleurotheciella tropica* | MFLUCC 16-0867 | MG837020 | MG837015 |
| *Pleurotheciella uniseptata* | KUMCC 15-0407 | MF399231 | MF399248 |
| ***Pleurothecium aquaticum*** | **MFLUCC 17-1331** | **NR_160597** | **NG_066197** |
| ***Pleurothecium floriforme*** | **MFLUCC 15-0628** | **NR_156614** | **NG_059791** |
| *Pleurothecium obovoideum* | CBS 209.95 | EU041784 | EU041841 |
| *Pleurothecium pulneyense* | MFLUCC 16-1293 | – | MF399262 |
| *Pleurothecium recurvatum* | CBS 138686 | KT278727 | KT278715 |
| *Pleurothecium recurvatum* | CBS 138747 | KT278728 | KT278714 |
| *Pleurothecium recurvatum* | CBS 131646 | JQ429150 | JQ429236 |
| *Pleurothecium recurvatum* | CBS 131272 | JQ429149 | JQ429237 |
| *Pleurothecium recurvatum* | CBS 101581 | JQ429148 | – |
| *Pleurothecium semifecundum* | CBS 131482 | JQ429158 | JQ429239 |
| ***Pleurothecium semifecundum*** | **CBS 131271** | **NR_111710** | **NG_057951** |
| *Savoryella aquatica* | SS 03801 | HQ446349 | HQ446372 |
| *Savoryella lignicola* | NF00204 | HQ446357 | HQ446378 |
| *Savoryella longispora* | SAT00322 | HQ446359 | HQ446380 |
| *Savoryella paucispora* | SAT00866 | – | – |
| *Savoryella verrucosa* | SS 00052 | HQ446353 | HQ446374 |
| *Sterigmatobotrys macrocarpa* | PRM 915682 | JQ429153 | _ |
| *Sterigmatobotrys macrocarpa* | DAOM 230059 | – | GU017316 |
| *Sterigmatobotrys rudis* | DAOM 229838 | JQ429152 | JQ429241 |
| *Triadelphia uniseptata* | DAOMC 250376 | – | KT278718 |

## Taxon treatment

### *Neomonodictys aquatica* D.F. Bao, S.P. Huang & Z.L. Luo, sp. nov.

- IndexFungorum 558842
- Species-ID Facesofungi number: FOF 10537

## Material

*Holotype:*

a. scientificName: ***Neomonodictys aquatica***; kingdom: Fungi; phylum: Ascomycota; class: Sordariomycetes; order: Pleurotheciales; family: Pleurotheciaceae; genus: ***Neomonodictys***; waterBody: Erhai Lake; locality: Baitaiyi; verbatimElevation: 1966 m; locationRemarks: China, Yunnan Province, Dali, saprobic on submerged decaying wood in Erhai Lake; verbatimLatitude: 25 44 29.65N; verbatimLongitude: 100d 09' 49.33" E; year: 2020; habitat: freshwater, submerged decaying wood; recordedBy: Longli Li; Siping Huang; collectionID: 2EH 3-17-1 H; collectionCode: L127.

## Description

**Sexual morph** Undetermined. **Asexual morph** Hyphomycetous (Fig. 1) sporodochia. Colonies on natural substratum superficial, scattered, black, glistening. Mycelium immersed in the substrate, composed of septate, smooth, thin-walled, light to dark brown, 2–3 µm wide hyphae. Conidiophores lacking. Conidiogenous cells short or occasionally missing, suborbicular, holoblastic, monoblastic, integrated, terminal, determinate, hyaline to pale brown. 3.7–6.4 × 2.9–4.7 µm ($\bar{x}$ = 5.1 × 3.8 µm, n = 10). Conidia 23.1–29.5 × 8.5–11.5 µm ($\bar{x}$ = 26 × 10 µm, n = 30), acrogenous, acrospore, oval, ellipsoidal to obovoid, muriform, smooth-walled, hyaline when young, becoming dark brown at maturity sometimes with one pale basal cell.

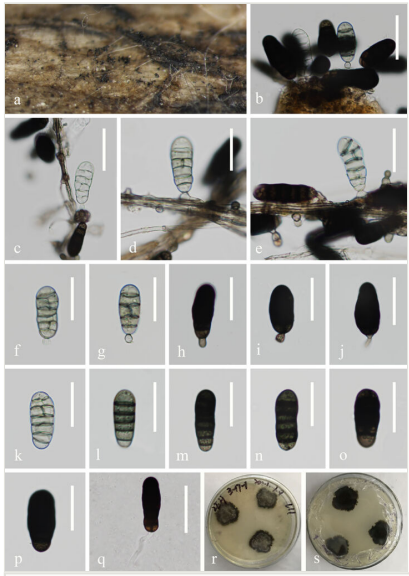


Figure 1. doi

***Neomonodictys aquatica*** (KUN-HKAS 115806, holotype). **a** Colonies on submerged wood; **b-e** Conidiophores with conidia; **f-j** Conidiogenous cells with conidia; **k-p** Conidia; **q** Germinating conidium; **r, s** Colony on PDA. Scale bars: **b-c, e, k** = 25 µm; **d, f-i, l-p** = 20 µm; **j, q** = 30 µm.

**Culture characteristics**: Conidia germinate on PDA in 36 h. Colonies growing on PDA, subglobose, with flat surface, edge jagged, reaching 3 cm long and 2.5 cm wide in 12 weeks at 28°C, dark grey in PDA medium. Mycelium superficial and partially immersed, branched, septate, hyaline to pale brown, smooth.

**Material examined**: China, Yunnan Province, Dali, sprobic on submerged decaying wood in Erhai Lake, September 2020, S. P. Huang, L-127 (KUN-HKAS 115806, holotype), ex-type living culture, KUNCC 21-10708 = CGMCC3.20681.

### Etymology

Name reflects the aquatic habitat of this fungus

### Notes

Morphologically, *Neomonodictys aquatica* is easily distinguished from *N. muriformis*. *Neomonodictys muriformis* has wider conidia than *N. aquatica* (15–25 vs. 8–12.2 µm). In addition, conidia of *N. aquatica* are oval or ellipsoidal to obovoid, while *N. muriformis* has subglobose to globose conidia. In the phylogenetic analysis, *N. aquatica* clustered with *N. muriformis* with strong support (99% ML and 1.00 PP) (Fig. 2). ITS comparison between our strain and MFLUCC 16-1136 revealed 57 bp difference in a total of 539 bp. LSU comparison between our strain and MFLUCC 16-1136 revealed 13 bp difference in total of 829 bp (Jeewon and Hyde 2016). Therefore, we introduce our new isolate as a new species.

## Analysis

### Phylogenetic analyses

The phylogram generated from Maximum Likelihood analysis, based on combined ITS and LSU sequence data, represents Pleurotheciales and the closely related orders. Seventy-nine strains are included in the combined analyses, which comprise 2039 characters (ITS: 849 bp, LSU: 1190 bp) after aligning. *Leotia tubrica* (AFTOL-1) is the outgroup taxon in this phylogentic tree. The best RAxML tree with a final likelihood value of -12803.740107 is presented. The matrix had 698 distinct alignment patterns with 34.21% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.222096, C =0.295691, G = 0.272214, T = 0.209999; substitution rates AC = 1.588217, AG = 2.820721, AT = 2.535737, CG = 1.003016, CT = 5.905028, GT = 1.000000; gamma distribution shape parameter α = 0.570011.

In the phylogenetic analysis, our new isolate *Neomonodictys aquatica* clustered as a sister taxon with *N. muriformis* with strong bootstrap support (99 ML/1.00 PP, Fig. 2).

## Discussion

Up to now, two species are accepted in *Neomonodictys*, including the newly-introduced species. Both of them are collected from submerged wood in freshwater habitats (Hyde et al. 2020) and only asexual morphs are reported. Morphologically, *Neomonodictys* is similar to *Monodictys* in having solitary, dictyospores conidia and monoblastic, hyaline to brown conidiogenous cells (Ellis 1971, Seifert et al. 2011). Compared with the diaphragms of them, *Neomonodictys aquatica* have a mostly transverse septum, less of the vertical septum, but the transerve and vertical septa of *N. muriformis* are evenly distributed. The significant difference between *Neomonodictys* and *Monodictys* is conidiophores, which are shorter than in the former (Kukwa and Diederich 2005). Phylogenetically, they are distinct ( Hyde et al. 2020). In the phylogenetic analysis, *Monodictys* was placed in Dothideomycetes (Day et al. 2006, Seifert et al. 2011, Wijayawardene et al. 2020), while *Neomonodictys* was placed in Sordariomycetes (Hyde et al. 2020).

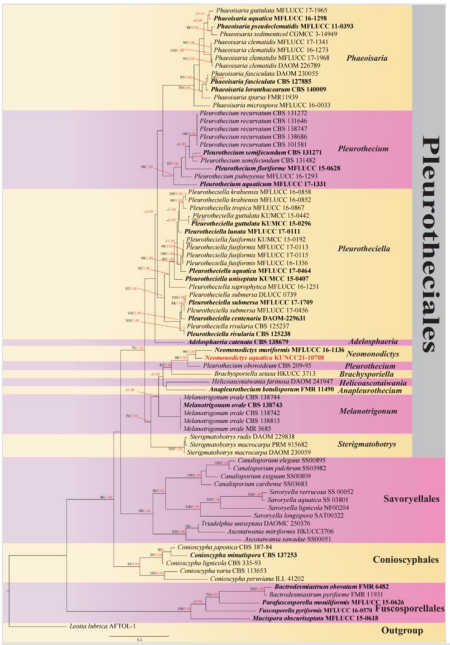


Figure 2. doi

Phylogenetic tree based on RAxML, generated from a combined ITS and LSU dataset. Bootstrap support values for Maximum Likelihood (ML, black) higher than 75% and Bayesian posterior probabilities (BYPP, red) greater than 0.95 are indicated above the nodes as ML/PP. The tree is rooted to *Leotia lubrica*. The type-derived sequences are indicated in bold and new isolates are in red. Bootstrap values for Maximum Likelihood (ML) equal to or greater than 75% and clade credibility values greater than 0.90 from Bayesian-inference analysis labelled on the nodes. Ex-type strains are in bold and black, the new isolate is indicated in bold and red. (Fig. 2).

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