A morphological and molecular survey of *Neoconidiobolus* reveals a new species and two new combinations

Yong Nie

Anhui University of Technology

Zi-Min Wang

Anhui University of Technology

Xiao-Yong Liu

Chinese Academy of Sciences

Bo Huang (✉ bhuang@ahau.edu.cn)

Anhui Agricultural University

Research Article

Keywords: Ancylistaceae, Morphology, Taxonomy, Molecular Phylogeny

Posted Date: June 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-186207/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

The genus *Neoconidiobolus* was recently established to accommodate all members of the *Conidiobolus* subgenus *Conidiobolus*. Based on mitochondrial small subunit (mtSSU), nuclear large subunit (nuLSU) of rDNA and translation elongation factor 1-alpha (TEF1), this study further resolved the genus *Neoconidiobolus* into three clades, with three new taxa being added. They are *N. kunyushanensis* B. Huang & Y. Nie, **sp. nov.**, *N. lamprauges* (Drechsler) B. Huang & Y. Nie, **comb. nov.**, and *N. nanodes* (Drechsler) B. Huang & Y. Nie, **comb. nov.** Meanwhile, a morphological comparison among species in the three clades and a key to the species of the genus *Neoconidiobolus* are provided herein.

Introduction

Conidiobolus Brefeld (*Ancylistaceae*, *Entomophthorales*) was erected by Brefeld (1884). It is a large and worldwide distributed group containing 80 records in the Index Fungorum database (<http://www.indexfungorum.org/>). Most members of *Conidiobolus* occur saprophytically in soil and on decaying leaves, and few have been noted as pathogens of insects, animals and humans (Vilela et al. 2010; Gryganskyi et al. 2012; Mendoza et al. 2014). During 1950s–1960s, almost all the 40 *Conidiobolus* species have been reported in the North America and South Asia (Drechsler 1952; Srinivasan and Thirumalachar 1961; Nie et al. 2020a). Till up now, more than 15 novel species were added in *Conidiobolus* (Bałazy et al. 1987; Waters and Callaghan 1989; Bałazy 1993; Tosi et al. 2004; Keller 2007; Waingankar et al. 2008; Huang et al. 2007; Nie et al. 2012, 2016, 2017, 2018, 2020b; Goffre et al. 2020), most being found from East Asia. According to the numerical taxonomy (King 1976a, b, 1977) and molecular phylogeny (Chen and Huang 2019; Nie et al. 2020a), 41 species are currently accepted in this genus.

Although some morphological and phylogenetic analyses proved *Conidiobolus* to be polyphyletic (Ben-Ze'ev and Kenneth 1982; Jensen et al. 1998; Callaghan et al. 2000; Gryganskyi et al. 2013; Spatafora et al. 2016; Nie et al. 2018), there is a long lack of comprehensive taxonomic studies on this genus. Recently, we proposed a taxonomic revision of the well-known genus *Conidiobolus* s.l. (Nie et al. 2020a). Combining molecular phylogeny with morphology resulted in a more natural classification: the traditional *Conidiobolus* s.l. was divided into four genera, *Capillidium* B. Huang & Y. Nie 2020, *Conidiobolus* s.s. Brefeld 1884, *Microconidiobolus* B. Huang & Y. Nie 2020, and *Neoconidiobolus* B. Huang & Y. Nie 2020. Among these genera, the *Neoconidiobolus* accommodated all species in the traditional *Conidiobolus* subgenus *Conidiobolus*. It is mainly characterised by forcibly discharged and globose / pyriform / obovoid primary conidia, forcibly discharged and elongated secondary conidia, and two types of resting spores, zygospores and chlamydospores (King 1976a,b, 1977; Nie et al. 2020a). This taxonomic treatment was supported by the phylogenomic analyses of nuclear and mitochondrial genomes (Nie et al. 2019; Wang et al. 2020).

In all, the genus *Neoconidiobolus* currently includes 10 species: *N. couchii*, *N. lachnodes*, *N. mirabilis*, *N. osmodes*, *N. pachyzygosporus*, *N. sinensis*, *N. stilbeus*, *N. stromioideus*, *N. thromboides*, and *N. vermicola* (Chen and Huang 2018; Nie et al. 2012, 2016, 2018, 2020a). This study aims at characterizing a new species and two new combinations into the *Neoconidiobolus* on the basis of morphology. Their molecular phylogeny was also reconstructed by using the small subunit of the mitochondrial ribosomal DNA (mtSSU), the large subunit of nuclear ribosomal DNA (nuLSU), and the translation elongation factor 1-alpha (TEF1). Also, some new insights of *Neoconidiobolus* will be provided in this article.

Materials And Methods

Isolates and morphology

Plant detritus was collected from Kunyu Mountain, Shandong Province, China. Isolations were carried out using the canopy plating approach (Drechsler 1952, King 1976a). A Petri dish incubated at 21 °C with PDA (potato 200 g, dextrose 20 g, agar 20 g, H₂O 1000 ml) was inverted over the plant detritus. When entomophthoroid fungi were observed on the PDA canopy, they were transferred to a PDA plate. Morphological features were examined under an Olympus BX51 microscope (Olympus, Japan) with bright-field and differential interference contrast (DIC) equipments. Photomicrographs were taken with an Olympus DP25 microscope-camera system (Olympus, Japan). Different fungal structures were measured and described with the method of King (1976a). The living culture was deposited in the Research Center for Entomogenous Fungi of Anhui Agricultural University, Anhui Province, China (RCEF), and duplicated in the China General Microbiological Culture Collection Center, Beijing, China (CGMCC). The dried cultures were deposited in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS).

DNA extraction, amplification and sequencing

A portion of fungal mycelia was ground in liquid nitrogen with a pestle and mortar, and genomic DNA was extracted from the mycelia using the CTAB method (Watanabe et al. 2010). The extracted DNA was stored in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at -20 °C. Universal primer pairs used in the PCR amplification of nucLSU, mtSSU and TEF1 were LR0R (5'-ACC CGC TGA ACT TAA GC-3') / LR5 (5'-TCC TGA GGG AAA CTT CG-3') (Vilgalys and Hester 1990), mtSSU1 (5'-GCW GCA GTG RGG AAT NTT GGR CAA T-3') / mtSSU2R (5'-GTR GAC TAM TSR GGT ATC TAA TC-3') (Zoller et al. 1999), and EF983 (5'-GCY CCY GGH CAY CGT GAY TTY AT-3') / EF1aZ-1R (5'-ACA TCW CCG ACA CCC TTG ATC TTG - 3') (Nie et al. 2012), respectively. PCR reactions were performed according to Nie et al. (2020): denaturation at 95 °C for 5 min; 34 cycles of denaturation at 94°C for 1 min plus 55 / 54 / 57 °C (nucLSU / mtSSU / TEF1) for 2 min plus 72 °C for 2 min, and a final extra extension at 72 °C for 10 min. The PCR products were sequenced with respective primers by Shanghai Genecore Biotechnologies Company (Shanghai, China). Sequences were assembled with BioEdit (Hall 1999). The obtained sequences were deposited in GenBank under the accession numbers MN061286 (nucLSU), MN061289 (mtSSU) and MN061483 (TEF1).

Phylogenetic analyses

Other nucLSU, TEF1 and mtSSU sequences of *Neoconidiobolus* spp. were downloaded from GeneBank for phylogenetic reconstruction. Seven species of *Conidiobolus* s.l. were chosen as outgroups (Table 1). The nucLSU, TEF1 and mtSSU sequences were locally aligned with BioEdit (Hall 1999) and concatenated with SequenceMatrix (Vaidya et al. 2011). Maximum Parsimony (MP) analyses were conducted with PAUP* 4.0b10 (Swofford 2002) using 1000 heuristic search replicates. The best models for the Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were selected with Akaike Information Criterion (AIC) by using jModelTest 2.1.7 (Guindon and Gascuel 2003; Darriba et al. 2012). ML tree was constructed using RAxML 8.1.17 with 1000 bootstrap replicates (Stamatakis 2014). The BI tree was constructed using MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003). Markov Chain Monte Carlo (MCMC) chains ran until the convergences met and the standard deviation fell below 0.01. Phylogenetic trees were modified in FigTree 1.4 (Rambaut 2012). Sequence alignment and phylogenetic tree were deposited at TreeBase (submission ID S27217).

Table 1
The taxa used in phylogenetic analyses.

Species	Strains*	GenBank accession numbers			References
		nucLSU	TEF1	mtSSU	
<i>Capillidium adiaereturum</i>	CGMCC 3.15888	MN061284	MN061481	MN061287	Nie et al. 2020
<i>Ca. lobatum</i>	ATCC 18153 (T)	JF816218	JF816233	MK301187	Nie et al. 2012, 2020
<i>Ca. rhyosporum</i>	ATCC 12588 (T)	JN131540	JN131546	MK301195	Nie et al. 2016, 2020
<i>Conidiobolus coronatus</i>	NRRL 28638	AY546691	DQ275337	DQ364224	Lutzoni et al. 2004
<i>Co. lamprauges</i>	CBS 461.97	MH874268	–	–	Vu et al. 2019
<i>Co. lamprauges</i>	CBS 728.97	MH874275	–	–	Vu et al. 2019
<i>Co. nanodes</i>	CBS 154.56 (T)	MH869096	–	–	Vu et al. 2019
<i>Co. polytocus</i>	ATCC 12244 (T)	JF816213	JF816227	MK301194	Nie et al. 2012, 2020
<i>Microconidiobolus nodosus</i>	ATCC 16577 (T)	JF816217	JF816235	MK333388	Nie et al. 2012, 2020
<i>M. terrestris</i>	ATCC 16198 (T)	KX752050	KY402208	MK301199	Nie et al. 2016, 2020
<i>Neoconidiobolus antarcticus</i>	ARSEF 6913 (T)	DQ364207	–	DQ364227	Nie et al. 2018, 2020
<i>N. couchii</i>	ATCC 18152 (T)	JN131538	JN131544	MK301179	Nie et al. 2016, 2020
<i>N. kunyushanensis</i>	CGMCC 3.15890 (T)	MN061286	MN061483	MN061289	This article
<i>N. lachnodes</i>	ARSEF 700	KC788408	–	–	Nie et al. 2018, 2020
<i>N. mirabilis</i>	CGMCC 3.17763 (T)	MH282852	MH282853	MK333389	Nie et al. 2018, 2020
<i>N. osmodes</i>	ARSEF 79	EF392371	–	DQ364219	Chen and Huang 2018
<i>N. osmodes</i>	RCEF 4447	JN131539	JN131545	MK333392	Nie et al. 2016, 2020
<i>N. pachyzygosporus</i>	CGMCC 3.17764 (T)	KP218521	KP218524	MK333390	Nie et al. 2018, 2020

*ARSEF, ARS Entomopathogenic Fungus Collection (Ithaca, U.S.A.). ATCC, American Type Culture Collection (Manassas, U.S.A.). CBS, Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands). CGMCC, China General Microbiological Culture Collection Center (Beijing, China). FSU, Jena Microbial Resource Collection (Friedrich-Schiller-University of Jena, Germany). NRRL, ARS Culture Collection (Peoria, U.S.A.). RCEF, Research Center for Entomogenous Fungi (Hefei, China). T = ex-type.

Species	Strains*	GenBank accession numbers			References
		nucLSU	TEF1	mtSSU	
<i>N. sinensis</i>	RCEF 4952 (T)	JF816224	JF816238	MK301196	Nie et al. 2012, 2020
<i>N. stilbeus</i>	RCEF 5584 (T)	KP218522	KP218525	MK301197	Nie et al. 2016, 2020
<i>N. stromoideus</i>	ATCC 15430 (T)	JF816219	JF816229	MK301198	Nie et al. 2012, 2020
<i>N. thromboides</i>	ATCC 12587 (T)	JF816214	JF816230	MK301200	Nie et al. 2012, 2020
<i>N. thromboides</i>	FSU 785	JX242597	–	JX242637	Gryganskyi et al. 2012
<i>N. thromboides</i>	RCEF 4492	JF816223	JF816236	MK333393	Nie et al. 2012, 2020
*ARSEF, ARS Entomopathogenic Fungus Collection (Ithaca, U.S.A.). ATCC, American Type Culture Collection (Manassas, U.S.A.). CBS, Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands). CGMCC, China General Microbiological Culture Collection Center (Beijing, China). FSU, Jena Microbial Resource Collection (Friedrich-Schiller-University of Jena, Germany). NRRL, ARS Culture Collection (Peoria, U.S.A.). RCEF, Research Center for Entomogenous Fungi (Hefei, China). T = ex-type.					

Results

Phylogeny in *Neoconidiobolus*

The combined nucLSU + TEF1 + mtSSU datasets consisted of 24 taxa and 1880 characters. Except 931 constant characters, 787 characters were parsimony-informative and 162 parsimony-uninformative. The characters 1–1025, 1026–1526 and 1527–1880 were partitioned to nucLSU, TEF1 and mtSSU, respectively. A parsimonious tree was generated with a length of 2913 (CI = 0.5287, HI = 0.4713, RI = 0.6132, RC = 0.3242). Best model estimated for the matrix was GTR + I + G. For the ML analysis, the final ML Optimization Likelihood is –14492.684374. For the BI analysis, Markov chains ran from random starting trees for 0.5 million generations and trees were sampled every 100th generations. When convergent, and the average standard deviation of split frequencies was 0.002707. ML, MP and BI analyses of the combined dataset resulted in similar topologies and the ML tree was presented with MP/ML/BI robustness values along branches. The members of *Neoconidiobolus* (82/98/1.00) were grouped into three clades (FIG. 1). Clade I included five species *N. thromboides*, *N. mirabilis*, *N. sinensis*, *N. stromoideus* and *N. stilbeus* (96/99/1.00) Clade II included four species *N. lampauges* comb. nov., *N. kunyushanensis* sp. nov., *N. nanodes* comb. nov. and *N. lachnodes* (77/73/0.98). Clade III included four species *N. antarcticus*, *N. osmodes*, *N. couchii* and *N. pachyzygosporus* (100/100/1.00).

Taxonomy

New species

Neoconidiobolus kunyushanensis B. Huang & Y. Nie, sp. nov., Fig. 2

MycoBank no. MB831600

Etymology. *kunyushanensis* (Lat.), referring to the geographical location where the fungus was isolated.

Known distribution. Shandong Province, China.

Specimens examined. China, Shandong Province, Yantai City, Kunyu Mountain, 37°26' N, 121°74' E, from plant detritus, 20 Mar 2009, *C.F. Wang*, HMAS 248357, holotype, culture ex-holotype CGMCC 3.15890 (= *RCEF 4584*). GenBank: nucLSU = MN061286; TEF1 = MN061483; mtSSU = MN061289).

Description. Colonies white, reaching ca 25–30 mm diameter on PDA for 3 d at 21 °C. Mycelia colorless, 3.5–9.0 µm wide, usually branched at the colony edge. Primary conidiophores colorless, often widening upward near the tip, mostly unbranched and producing a single conidium, 62.0–121.0 × 7.0–12.0 µm, in some instances 2 primary conidiophores without widening arising from hyphal bodies at the same point. Primary conidia forcibly discharged, colorless, globose to subglobose, 13.0–17.0 µm wide and 15.0–21.0 µm long, with a basal papilla 4.0–8.0 µm wide and 1.0–4.0 µm long. Secondary conidia arising from the primary ones with a similar shape and a smaller size. Zygosporangia formed between segments of separate hyphae, smooth, mostly globose, sometimes ellipsoidal, 12.0–25.0 µm in diameter with a wall 1.0–2.8 µm thick.

Notes. *Neoconidiobolus kunyushanensis* sp. nov. is grouped with other three *Neoconidiobolus* species, i.e. *N. lamprauges* comb. nov., *N. nanodes* comb. nov. and *N. lachnodes* (MP / ML / BI = 77 / 73 / 0.98; Clade II in Fig. 1). It is phylogenetically closely related to *N. lamprauges* (MP / ML / BI = 87 / 91 / 1.00; Fig. 1) and morphologically most allied to *N. lamprauges* (Table 2). This new species differs from *N. lamprauges* by two primary conidiophores without widening upward near the tip arising from hyphal body at the same point, larger zygosporangia, and branched mycelia at the colony edge. In addition, the conidia usually filled with a large mass of conglutinated protoplasmic lumps in *N. kunyushanensis*, but with densely granular protoplasm in *N. lamprauges* (Drechsler 1953).

Table 2
Morphological comparison of *Neoconidiobolus* species in three Clades in Fig. 1.

Clades	Species	Growth rate (mm/d) at 21°C on PDA	Diameter of mycelia (µm)	Primary conidiophores (µm)	Primary conidia (µm)	Resting spores (µm)	References
Clade I	<i>N. mirabilis</i>	3.3–5.0	6.0–12.0	40.0–145.0 × 6.0–9.5	20.5–27.5 × 16.5–21.0	13.5–21.0 × 1.5–2.3	Nie et al. 2018
	<i>N. sinensis</i>	7.0	5.0–10.0	32.5–110.0 × 10.0–15.0	22.5–32.5 × 17.5–25.0	25.0–31.0 × 1.0–2.0	Nie et al. 2012
	<i>N. stilbeus</i>	7.3–8.3	5.0–10.0	68.0–133.0 × 10.0–12.0	21.0–25.0 × 17.0–21.0	15.0–19.0 × 1.0–2.5	Nie et al. 2016
	<i>N. stromoideus</i>	3.8–10.0	7.0–9.0	12.0–40.0	25.0–33.0 × 22.0–26.0	23.0–32.0 × 1.0–2.0	Srinivasan and Thirumalachar 1962
	<i>N. thromboides</i>	10.0–15.0	3.0–10.5	35.0–150.0 × 10.0–15.0	24.0–32.0 × 19.0–26.5	17.5–27.0 × 2.0–2.5	Drechsler 1953
Clade II	<i>N. lachnodes</i>	2.6–7.1	2.0–8.0	15.0–40.0 × 5.0–10.0	10.0–27.0 × 9.0–25.0	11.0–16.0	Drechsler 1955b
	<i>N. lamprauges</i>	less than 5.0	3.0–8.0 (4.0–7.0)	25.0–100.0 (25.0–50.0) × 4.0–8.0 (5.0–15.0)	15.0–22.0 × 12.5–20.0	12.0–18.0 × 1.3–2.2	Drechsler 1953
	<i>N. kunyushanensis</i>	8.3–10.0	3.5–9.0	62.0–121.0 × 7.0–12.0	15.0–21.0 × 13.0–17.0	12.0–25.0 × 1.0–2.8	This article
	<i>N. nanodes</i>	–	2.0–7.0	10.0–25.0 × 3.5–6.0	10.0–17.0 × 8.0–14.0	7.0–14.0 × 0.8–2.0	Drechsler 1955b
Clade III	<i>N. couchii</i>	3.0	3.5–6.0	30.0–50.0 × 4.0–7.0	12.5–27.0 × 10.0–16.0	12.0–16.0 × 0.8–1.2	Srinivasan and Thirumalachar 1968
	<i>N. osmodes</i>	7.5	4.0–12.0	30.0–60.0 × 9.0–15.0	25.0–37.0 × 22.0–30.0	13.0–37.0 × 2.0–6.5	Drechsler 1954

Clades	Species	Growth rate (mm/d) at 21°C on PDA	Diameter of mycelia (μm)	Primary conidiophores (μm)	Primary conidia (μm)	Resting spores (μm)	References
	<i>N. pachyzygosporus</i>	12.0	3.0–14.0	34.0–156.0 × 6.0–12.0	15.5–23.0 × 11.0–18.0	15.0–25.0 × 2.0–3.2	Nie et al. 2018

New combinations

***Neoconidiobolus lamprauges* (Drechsler) B. Huang & Y. Nie, comb. nov.**

MycoBank no. MB837759

Conidiobolus lamprauges Drechsler, J. Wash. Acad. Sci. 43: 35 (1953). Basionym.

Description. Refer to Drechsler (1953).

Notes. The ex-type living culture is ATCC 12592 (United States, Virginia, 22 January 1952, Drechsler). According to the original morphological description and illustration (Drechsler 1953), *Conidiobolus lamprauges* should be allied to the *Neoconidiobolus* or *Microconidiobolus* lineages. In this phylogenetic analysis, two authentic strains CBS 154.56 and CBS 728.97 of the species *C. lamprauges* (Vu et al. 2019) were located in the Clade II of the *Neoconidiobolus* lineage, and therefore it was reclassified herein as a new combination in the genus *Neoconidiobolus*.

***Neoconidiobolus nanodes* (Drechsler) B. Huang & Y. Nie, comb. nov.**

MycoBank no. MB837760

Conidiobolus nanodes Drechsler, Am. J. Bot. 42: 439 (1955). Basionym.

Description. Refer to Drechsler (1955b).

Notes. The ex-type living culture is ATCC 12585 (United States, New Jersey, 25 February 1954, Drechsler). King (1977) synonymized *Conidiobolus nanodes* with *C. lamprauges* on the basis of their similar size and components of primary conidia. However, the primary conidia and zygospores of *C. nanodes* are smaller than *C. lamprauges* (Table 2); they were grouped into two distinct branches in the *Neoconidiobolus* phylogram (Fig. 1). Consequently, *C. nanodes* recovered its specific status and be accepted as a new combination in the genus *Neoconidiobolus*.

Discussion

The 12 *Neoconidiobolus* species in the three Clades within *Neoconidiobolus* (Fig. 1) were morphologically compared (Table 2). In general, we found species in Clade II share smaller primary conidia than members in Clade I (except *N. stilbeus*) and Clade III (except *N. pachyzygosporus*). This morphological characteristic might separate Clade II as a monophyletic taxa from *Neoconidiobolus* in the future upon the availability of more types.

Remarkably, the *Microconidiobolus*, an allied genus of *Neoconidiobolus*, is also characterized by small primary conidia resembling those of the Clade II within the *Neoconidiobolus* in size and shape. Besides, *Capillidium pumilum*, a species of another allied genus, also produces small primary conidia (7.3–14.0 × 9.0–18.0 µm; Drechsler 1955a). These exceptions indicated that the relationships within the *Conidiobolus* s.l. need further studies, including more ex-type materials, new phenotypes such as the maximum growth temperature and the nuclear number in primary conidia, and phylogenomics for a insightful understanding.

Besides the species recognized in this article, three other species should also be treated in *Neoconidiobolus* morphologically. They are *Conidiobolus eurymitus* Drechsler producing elongate secondary conidia and allied to *N. couchii* (Drechsler 1965; Srinivasan MC and Thirumalachar MJ 1968), *C. multivagus* Drechsler in which resting spores has not been observed (Drechsler 1960), and *C. thermophilus* Waingankar, Singh & Srinivasan which is a thermophilic fungus. Unfortunately, there was no molecular evidence to support the morphological affinity, so we classified them into *Conidiobolus* s.l. herein and their taxonomic status should be clarified in the future study. Actually, two more issues make it difficult: 1) The type species *C. utriculosus* Brefeld and some other ex-types are missing; 2) As environmental species, it is often not possible to obtain multiple strains per species except a few common species.

We noted that recent phylogenetic analyses showed that *N. antarcticus* and *N. osmodes* did not group together (Goffre et al. 2020). However, our previous phylogeny showed that these two species grouped in one clade with a robust support (Chen and Huang 2018; Nie et al. 2016, 2017, 2018, 2020a). Also, the phylogenetic tree in this article supports *N. antarcticus* synonymized with *N. osmodes*. In addition, molecular data of *N. lachnodes* and *N. osmodes* were not type-derived sequences, but the morphological characteristic confirmed their taxonomic placement in *Neoconidiobolus*.

Until now, 13 species have been accepted in the genus *Neoconidiobolus* based on the combined morphology and phylogeny: *N. couchii*, *N. kunyushanensis*, *N. lachnodes*, *N. lamprauges*, *N. mirabilis*, *N. nanodes*, *N. osmodes*, *N. pachyzygosporus*, *N. sinensis*, *N. stilbeus*, *N. stromoideus*, *N. thromboides* and *N. vermicola*. More than half are distributed in China, including two pandemic species (*N. osmodes* and *N. thromboides*) and five China-endemic species (*N. kunyushanensis*, *N. mirabilis*, *N. pachyzygosporus*, *N. sinensis* and *N. stilbeus*) (Chen and Huang 2018, Nie et al. 2012, 2016, 2018, 2020a).

Key to the species of *Neoconidiobolus*

- 1. Forming mycelial strand with 2–6 aerial phototropic hyphae*N. stilbeus*
- 1'. Not forming mycelial strand 2
- 2. Primary conidiophores produced from cushion mycelia..... 3
- 2'. Primary conidiophores not produced from cushion mycelia..... 4
- 3. Usually branched at edge mycelia, much shorter conidiophores (12–40 µm) produced*N. stromoideus*

3'. Rarely branched at edge mycelia, much longer conidiophores (32.5–110 µm) produced	<i>N. sinensis</i>
4. Sepcially forming zygosporos among two to four adjacent hyphal segments.....	<i>N. mirabilis</i>
4'. Forming zygosporos among two adjacent hyphal segments.....	5
5. Zygosporos with a thick double wall, more than 3.2 µm.....	6
5'. Zygosporos with a thin single wall, less than 3.0 µm.....	7
6. Primary conidia larger, up to 37 µm.....	<i>N. osmodes</i>
6'. Primary conidia smaller, less than 23 µm.....	<i>N. pachyzygosporus</i>
7. Elongate secondary conidia produced.....	<i>N. couchii</i>
7'. Elongate secondary conidia not produced.....	8
8. Primary conidiophores in pairs arising from hyphal bodies.....	<i>N. kunyushanensis</i> sp. nov.
8'. Primary conidiophore solely arising from hyphal bodies.....	9
9. Primary conidia larger, up to 32 µm.....	<i>N. thromboides</i>
9'. Primary conidia smaller, less than 27 µm.....	10
10. Forming azygosporus.....	<i>N. vermicola</i>
10'. Not forming azygosporus.....	11
11. Primary conidiophores longer, up to 130 µm.....	<i>N. lamprauges</i> comb. nov.
11'. Primary conidiophores shorter, less than 40 µm.....	12
12. Primary conidia larger, up to 27 µm.....	<i>N. lachnodes</i>
12'. Primary conidia smaller, less than 17 µm.....	<i>N. nanode</i> comb. nov.

Declarations

Acknowledgements

We thank Dr. Shuang-hui He (Beijing Forestry University) for improving the manuscript. Mrs. C.F. Wang and Dr. De-shui Yu (Anhui Agricultural University) are acknowledged for helping with specimen collection and molecular work.

Funding

This work was supported by National Natural Science Foundation of China (30770008, 31900008, and 31970009).

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or vertebrates performed by any of the authors.

Authors' contributions

Conceived and designed the experiments: BH. Performed the experiments: YN. Analyzed the data: YN, ZW. Wrote the paper: YN, XL. All authors read and approved the final manuscript.

Data availability

The datasets including the sequences on GenBank, the specimen data on MycoBank and the phylogenetic data on TreeBase will be available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

References

- Bałazy S, Wiśniewski J, Kaczmarek S (1987). Some noteworthy fungi occurring on mites. *Bulletin of the Polish Academy of Sciences, Biological Sciences* 35:199–224.
- Balazy S (1993). *Entomophthorales*. Flora of Poland (Flora Polska), Fungi (Mycota) 24:1–356. Polish Academy of Sciences, W. Szafer Institute of Botany, Kraków, Poland.
- Ben-Ze'ev IS, Kenneth RG (1982). Features-criteria of taxonomic value in the *Entomophthorales*. I. A revision of the Batkoan classification. *Mycotaxon* 14:393–455.
- Brefeld O (1884). *Conidiobolus utriculosus* und *minor*. *Untersuchungen aus der Gesamtgebiete der Mykologie* 6(2):35–78.
- Callaghan AA, Waters SD, Manning RJ (2000). Alternative repetitional conidia in *Conidiobolus adiaeretus*: development and germination. *Mycological Research* 104: 1270–1275.
<https://doi.org/10.1017/S0953756200003063>
- Chen MJ, Huang B (2018). *Conidiobolus antarcticus*, a synonym of *C. osmodes*. *Mycotaxon* 133(4):635–641.
<https://doi.org/10.5248/133.635>.

- Darriba D, Taboada GL, Doallo R, Posada D (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8):772. <https://doi.org/10.1038/nmeth.2109>.
- Drechsler C (1952). Widespread distribution of *Delacroixia coronata* and other saprophytic *Entomophthoraceae* in plant detritus. *Science* 115:575–576. <https://doi.org/10.1126/science.115.2995.575>.
- Drechsler C (1953). Three new species of *Conidiobolus* isolated from leaf mold. *Journal of the Washington Academy of Science* 43(2):29–34.
- Drechsler C (1954). Two species of *Conidiobolus* with minutely ridged zygospores. *American Journal of Botany* 41: 567–575. <https://doi.org/10.1002/j.1537-2197.1954.tb14380.x>.
- Drechsler C (1955a). A small *Conidiobolus* with globose and with elongated secondary conidia. *Journal of Washington Academy of Sciences* 45(4):114–117.
- Drechsler C (1955b). Three new species of *Conidiobolus* isolated from decaying plant detritus. *American Journal of Botany* 42(5):437–443. <https://doi.org/10.1002/j.1537-2197.1955.tb11144.x>.
- Drechsler C (1960) Two new species of *Conidiobolus* found in plant detritus. *American Journal of Botany* 47: 368–377. <https://doi.org/10.1002/j.1537-2197.1960.tb07138.x>.
- Drechsler C (1965) A robust *Conidiobolus* with zygospores containing granular parietal proto[1]plasm. *Mycologia* 57(6): 913–926. <https://doi.org/10.2307/3756891>.
- Goffre D, Jensen AB, Lopez Lastra CC, Humber RA Folgarait PJ (2020). *Conidiobolus lunulus*, a new entomophthoralean species isolated from leafcutter ants. *Mycologia* (Online). <https://doi.org/10.1080/00275514.2020.1816387>.
- Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J, Wu S, Voigt K, Walther G, Anishchenko IM, Vilgalys R (2012). Molecular phylogeny of the *Entomophthoromycota*. *Molecular Phylogenetics and Evolution* 65: 682–694. <https://doi.org/10.1016/j.ympev.2012.07.026>.
- Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, Voigt K, Vilgalys R (2013). Phylogenetic lineages in *Entomophthoromycota*. *Persoonia* 30: 94–105. <https://doi.org/10.3767/003158513X666330>.
- Guindon S, Gascuel O (2003). A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52:696–704. <https://doi.org/10.1080/10635150390235520>.
- Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.
- Huang B, Humber RA, Hodge KT (2007). A new species of *Conidiobolus* from Great Smoky Mountains National Park. *Mycotaxon* 100:227–233.
- Jensen AB, Gargas A, Eilenberg J, Rosendahl S (1998). Relationships of the insect-pathogenic order *Entomophthorales* (*Zygomycota*, *Fungi*) based on phylogenetic analyses of nucleus small subunit ribosomal DNA sequences (SSU rDNA). *Fungal Genetics and Biology* 24(3):325–334. <https://doi.org/10.1006/fgbi.1998.1063>.

- Keller S. 2007. Arthropod-pathogenic *Entomophthorales* from Switzerland. III. First additions. *Sydowia* 59:75–113. <https://doi.org/10.1002/yea.1500>.
- King DS (1976a). Systematics of *Conidiobolus* (*Entomophthorales*) using numerical taxonomy I. Taxonomic considerations. *Canadian Journal of Botany* 54:45–65. <https://doi.org/10.1139/b76-008>.
- King DS (1976b). Systematics of *Conidiobolus* (*Entomophthorales*) using numerical taxonomy II. Taxonomic considerations. *Canadian Journal of Botany* 54:1285–1296. <https://doi.org/10.1139/b76-141>.
- King DS (1977). Systematics of *Conidiobolus* (*Entomophthorales*) using numerical taxonomy III. Descriptions of recognized species. *Canadian Journal of Botany* 55:718–729. <https://doi.org/10.1139/b77-086>.
- Lutzoni F, Kauff F, Cox CJ, McLaughlin D, Celio G, Dentinger B, Padamsee M, Hibbett DS, James TY, Baloch E, Grube M, Reeb V, Hofstetter V, Schoch C, Arnold AE, Miadlikowska J, Spatafora J, Johnson D, Hambleton S, Crockett M, Schoemaker R, Sun GH, Lücking R, Lumbsch HT, O'Donnell K, Binder M, Diederich P, Ertz D, Gueidan C, Hall B, Hansen K, Harris RC, Hosaka K, Lim YW, Liu Y, Matheny B, Nishida H, Pfister D, Rogers J, Rossman A, Schmitt I, Sipman H, Stone J, Sugiyama J, Yahr R, Vilgalys R (2004). Where are we in assembling the Fungal Tree of Life, classifying the fungi and understanding the evolution of their subcellular traits? *American Journal of Botany* 91(10):1446–1480.
- Mendoza L, Vilela R, Voelz K, Ibrahim AS, Voigt K, Lee SC (2014). Human fungal pathogens of *Mucorales* and *Entomophthorales*. *Cold Spring Harb Perspect Med* 5(4):1–33. <https://doi.org/10.1101/cshperspect.a019562>.
- Nie Y, Yu CZ, Liu XY, Huang B (2012). A new species of *Conidiobolus* (*Ancylistaceae*) from Anhui, China. *Mycotaxon* 120:427–435. <https://doi.org/10.5248/120.427>.
- Nie Y, Tang XX, Liu XY, Huang B (2016). *Conidiobolus stilbeus*, a new species with mycelial strand and two types of primary conidiophores. *Mycosphere* 7(6):801–809. <https://doi.org/10.5943/mycosphere/7/6/11>.
- Nie Y, Tang XX, Liu XY, Huang B (2017). A new species of *Conidiobolus* with chlamydospores from Dabie Mountains, eastern China. *Mycosphere* 8(7):809–816. <https://doi.org/10.5943/mycosphere/8/7/1>.
- Nie Y, Qin L, Yu DS, Liu XY, Huang B (2018). Two new species of *Conidiobolus* occurring in Anhui, China. *Mycological Progress* 17(10):1203–1211. <https://doi.org/10.1007/s11557-018-1436-z>.
- Nie Y, Wang L, Cai Y, Tao W, Zhang YJ, Huang B (2019). Mitochondrial genome of the entomophthoroid fungus *Conidiobolus heterosporus* provides insights into evolution of basal fungi. *Applied Microbiology and Biotechnology* 103(3):1379–1391. <https://doi.org/10.1007/s00253-018-9549-5>.
- Nie Y, Yu DS, Wang CF, Liu XY, Huang B (2020a). A taxonomic revision of the genus *Conidiobolus* (*Ancylistaceae*, *Entomophthorales*): four clades including three new genera. *Myckeys* 66: 55–81. <https://doi.org/10.3897/mycokeys.66.46575>.
- Nie Y, Cai Y, Gao Y, Yu DS, Wang ZM, Liu XY, Huang B (2020b). Three new species of *Conidiobolus* sensu stricto from plant debris in eastern China. *MycKeys* 73: 133–149. <https://doi.org/10.3897/mycokeys.73.56905>.
- Rambaut A (2012). FigTree version 1.4.0. Available at <http://tree.bio.ed.ac.uk/software/figtree/>

- Ronquist F, Huelsenbeck JP (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>.
- Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A, James TY, O'Donnell K, Roberson RW, Taylor TN, Uehling J, Vilgalys R, White MM, Stajich JE (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* 108(5): 1028–1046. <https://doi.org/10.3852/16-042>.
- Srinivasan MC, Thirumalachar MJ (1962). Studies on species of *Conidiobolus* from India-II. *Sydowia, Annales Mycologici* 16: 60–66.
- Srinivasan MC, Thirumalachar MJ (1968). Two new species of *Conidiobolus* from India. *Journal of the Mitchell Society* 84: 211–212.
- Stamatakis A (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9):1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>.
- Swofford DL (2002). PAUP*: Phylogenetic analysis using parsimony (*and other methods), Version 4.0b10. Sinauer Associates, Sunderland.
- Tosi S, Caretta G, Humber RA. 2004. *Conidiobolus antarcticus*, a new species from continental Antarctica. *Mycotaxon* 90:343–347. <https://doi.org/10.1111/j.1439-0507.2004.01029.x>.
- Vaidya G, Lohman DJ, Meier R (2011). SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27(2): 171–180. <https://doi.org/10.1111/j.1096-0031.2010.00329.x>.
- Vilela R, Silva SMS, Correa FR, Dominguez E, Mendoza L (2010). Morphologic and phylogenetic characterization of *Conidiobolus lamprauges* recovered from infected sheep. *J Clin Microbiol* 48:427–432. <https://doi.org/10.1128/JCM.01589-09>.
- Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172:4238–4246. <https://doi.org/10.1128/jb.172.8.4238-4246.1990>.
- Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Studies in Mycology* 92: 135–154. <https://doi.org/10.1016/j.simyco.2018.05.001>.
- Waingankar VM, Singh SK, Srinivasan MC (2008). A new thermophilic species of *Conidiobolus* from India. *Mycopathologia* 165:173–177. <https://doi.org/10.1007/s11046-007-9088-6>.
- Wang YL, Nie Y, Yu DS, Xie XY, Qin L, Yang Y, Huang B (2020). Genome-wide study of saprotrophy-related genes in the basal fungus *Conidiobolus heterosporus*. *Applied Microbiology and Biotechnology* 104(14):6261–6272. <https://doi.org/10.1007/s00253-020-10698-6>.

Watanabe M, Lee K, Goto K, Kumagai S, Sugita-Konishi Y, Hara-Kudo Y (2010). Rapid and effective DNA extraction method with bead grinding for a large amount of fungal DNA. *Journal of Food Protection* 73(6):1077–1084. <https://doi.org/10.4315/0362-028X-73.6.1077>.

Waters SD, Callaghan AA (1989). *Conidiobolus iuxtagenitus*, a new species with discharge elongate repetitional conidia and conjugation tubes. *Mycological Research* 93:223–226. [https://doi.org/10.1016/S0953-7562\(89\)80121-2](https://doi.org/10.1016/S0953-7562(89)80121-2).

Zollera S, Scheidegger C, Sperisena C (1999). PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. *The Lichenologist* 31(5):511–516. <https://doi.org/10.1006/lich.1999.0220>.

Figures

Figure 1

The phylogenetic tree of *Neoconidiobolus* constructed by Maximum Likelihood analyses of nucLSU, TEF1 and mtSSU sequences, with seven strains in *Conidiobolus* s.l. as outgroups. *N. kunyushanensis* sp. nov. is shown in red. *N. lampauges* comb. nov. and *N. nanodes* comb. nov. are shown in blue. Maximum Parsimony bootstrap values ($\geq 70\%$) / Maximum Likelihood bootstrap values ($\geq 70\%$) / Bayesian Inference posterior probabilities

(≥ 0.95) of each clade are indicated along branches. Scale bar indicates substitutions per site. "T" indicates the ex-type cultures.

Figure 2

A–O. *Neoconidiobolus kunyushanensis* CGMCC 3.15890. A. Colony on PDA after 3 d at 21 °C; B, C Mycelia branched at the colony edge; D, E. Single primary conidiophores with widening upward near the tip and bearing a primary conidium; F – I. Two primary conidiophores without widening upward near the tip arising from hyphal body at the same point and bearing two primary conidia; J. Primary conidia; K, L. Producing secondary conidia; M, N. Zygospores formed between segments of separate hyphae; O. Zygospores. — Scale bars: A = 10 mm; B – C = 100 μ m; D – O = 20 μ m.