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First documented occurrence of *Neoconidiobolus lachnodes* (Drechsler) B. Huang & Y. Nie (Zoopagomycota, Entomophthorales) in central China

Mohan Zhang[1], Yong Nie[2], Heng Zhao[3], Xiao-Yong Liu[4], Bo Huang[1]

1 *Anhui Provincial Key Laboratory for Microbial Pest Control, Anhui Agricultural University, Hefei, Anhui, China*
2 *School of Civil Engineering and Architecture, Anhui University of Technology, Ma'anshan, Anhui, China*
3 *State Key Laboratory of Efficient Production of Forest Resources, School of Ecology and Nature Conservation, Beijing Forestry University, Beijing, China*
4 *College of Life Sciences, Shandong Normal University, Jinan, Shandong, China*

Corresponding authors: *Bo Huang (bhuang@ahau.edu.cn); XiaoYong Liu (liuxy@sdnu.edu.cn)*

Abstract. *Neoconidiobolus lachnodes* (Drechsler) B. Huang & Y. Nie was originally isolated from plant debris in Florida, United States of America, in 1954. Here, we report the first documented occurrence of *N. lachnodes* in central China. Morphologically, this fungus is characterized by its abundant aerial hyphae, typically arranged closely, with hyphae aggregating into chlamydospores during the later stages of growth. Phylogenetic analysis revealed that the *N. lachnodes* isolate from China (RCEF 7519) is closely related to the reference strain *N. lachnodes* (ARSEF 700). To elucidate the phylogenetic position of *N. lachnodes* within the *Neoconidiobolus* lineage, we included two nucleotide sequences of *EFL* and mtSSU loci. This discovery significantly expands the known geographic distribution of *N. lachnodes*.

Key words. Neoconidiobolaceae, Morphology, Phylogeny, Saprophytic fungi

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INTRODUCTION

The genus *Conidiobolus* Brefeld is classified within the order Entomophthorales and encompasses over 80 taxa, as documented by the Index Fungorum database (Index Fungorum 2024). The majority of these taxa are saprotrophic, inhabiting plant debris and soil, and their instances of infection in insects and mammals are relatively rare (Vilela et al. 2010; Gryganskyi et al. 2012).

Established by Brefeld in 1884, the genus *Conidiobolus* s.l. was later subdivided into three subgenera based on variations in secondary conidia types (Ben-Ze'ev and Kenneth 1982). The emergence of molecular analysis in fungal taxonomy in the 1990s marked a pivotal moment (Bruns et al. 1991), leading to a reassessment of the genus *Conidiobolus* s.l. through combined molecular and morphological analyses. Subsequently, this re-evaluation resulted in the delineation of four new genera from *Conidiobolus* s.l., namely *Capillidium*, *Conidiobolus* s.s., *Microconidiobolus*, and *Neoconidiobolus* (Nie et al. 2020). *Neoconidiobolus* is distinguished from *Capillidium* and *Conidiobolus* s.s by the absence of capilliconidia and microconidia, respectively, and it differs from *Microconidiobolus* species by the larger size of primary conidia. As part of this taxonomic revision, *Conidiobolus lachnodes* Drechsler (Drechsler 1955) was reclassified as *Neoconidiobolus lachnodes* (Drechsler) B. Huang & Y. Nie.

Previous studies have extensively described eight *Neoconidiobolus* species in China, namely *N. kunyushanensis* B. Huang & Y. Nie, *N. mirabilis* (B. Huang & Y. Nie) Y. Nie & B. Huang, *N. pachyzygosporus* (B. Huang & Y. Nie) Y. Nie & B. Huang, *N. pseudothromboides* B. Huang & Y. Nie, *N. osmodes* Drechsler, *N. sinenses* (B. Huang & Y. Nie) Y. Nie, X.Y. Liu & B. Huang, *N. stilbeus* (B. Huang & Y. Nie) Y. Nie & B. Huang, and *N. thromboides* Drechsler (Drechsler 1953, 1954; Nie et al. 2016, 2018, 2020, 2021, 2022). Although *N. lachnodes* (ARSEF 700) was reportedly isolated by D.W. Roberts from *Nilaparvata lugens* in southern China and cataloged in the USDA-ARS Collection of Entomopathogenic Fungal Cultures, its official documentation and description are lacking. Hence, we deem it necessary to designate strain RCEF 7519, isolated from Anhui province, as a new record in central China in this study. Herein, we present the first detailed description and illustration of *N. lachnodes* collected from central China, substantiated by morphological and molecular evidence.

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METHODS

Plant debris was collected from Manshuihe Town, Huoshan County, Anhui Province, China, on 29 November 2015 (Figure 1). *Conidiobolus*-like fungi were isolated following the methods described by Drechsler (1952) and King (1976), with modifications as outlined by Nie et al. (2012). In brief, samples were fragmented into small pieces and evenly distributed on the covers of Petri dishes, which were then inverted and incubated on Potato Dextrose Agar (PDA) culture medium at 21 °C for 7 days. Colonies were monitored daily using a stereomicroscope (SMZ1500, Nikon Corporation, Japan). Upon the appearance of a *Conidiobolus*-like fungus during this time, it was transferred to a fresh PDA plate to establish a pure culture for morphological studies. Microscopic observation of mycelial structures, primary conidia, conidiophores, secondary conidia, and resting spores was examined using a BX51 (Olympus Corporation, Tokyo, Japan) microscope. Digital images captured using an attached digital camera. All isolated strains were deposited at the Research Center for Entomogenous Fungi, Anhui Agricultural University, Anhui Province, China (RCEF).

Genomic DNA was extracted from fresh fungal mycelia using a modified CTAB method based on Watanabe et al. (2010). PCR amplification reactions and programs were conducted as previously described by Nie et al. (2020). The large subunit of nuclear ribosomal DNA (nucLSU) region was amplified using LR0R/LR5 primers (Vilgalys and Hester 1990), the small subunit of mitochondrial ribosomal DNA (mtSSU) region was amplified using mtSSU1/mtSSU2R primers (Zoller et al. 1999), and the elongation-factor-like (*EFL*) region was amplified using EF983/EF1aZ-1R primers (Nie et al. 2018). Subsequently, PCR products were purified and sequenced by Shanghai Genecore Biotechnologies Company (Shanghai, China) using the same primers. Sequences were deposited in GenBank (Table 1).

For phylogenetic analysis, sequences of three loci (nucLSU, mtSSU, and *EFL*) from *Neoconidiobolus* species and related genera were retrieved from GenBank. These sequences were aligned using MAFFT (Katoh et al. 2019) and subsequently corrected with BioEdit (Hall 1999). Gaps or missing sequences were treated as partial deletions, and the final alignments for each locus were concatenated using SequenceMatrix (Vaidya et al. 2011). Maximum-likelihood (ML) and Bayesian-inference (BI) methods were employed for phylogenetic analyses. The best-fit substitution models were determined using the Akaike Information Criterion (AIC) as implemented in Modeltest 3.7, with the GTR + I + G model selected for both ML and BI analyses (Posada and Crandall 1998). ML phylogenetic analysis was conducted using RAxML 8.1.17 with 1000 bootstrap replicates (Stamatakis 2014), while BI phylogenetic analyses were performed with four Markov Chain Monte Carlo (MCMC) chains in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), running for 1.0 million generations until the average standard deviation of split frequencies reached 0.0032. Phylogenetic trees were visualized using FigTree 1.4 (Rambaut 2012) and further refined using Adobe Illustrator CS 6.0 and Adobe Photoshop CS 3.0.

RESULTS

***Neoconidiobolus lachnodes* (Drechsler) B. Huang & Y. Nie, Mycokeys 66: 73 (2020)**
≡ *Conidiobolus lachnodes* Drechsler, Am. J. Bot. 42: 442 (1955)
Figure 2

New record. China – ANHUI PROVINCE • Huoshan County, Manshuihe Town; 31°19′21″N, 116°01′22″E; 29.XI.2015; Y. Nie leg.; isolated from plant debris in a broad-leaved forest after rainfall (at the bottom in

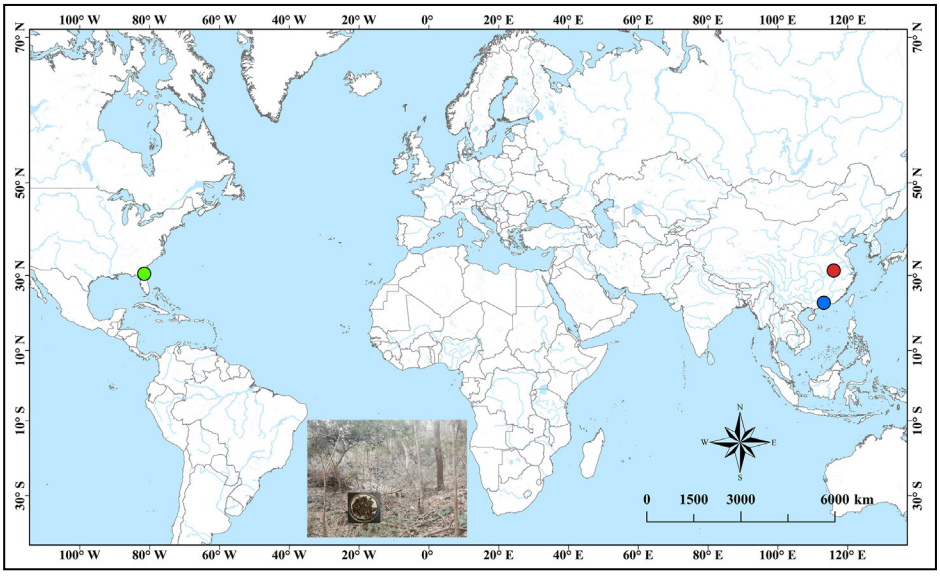

Figure 1. Location map showing the collection localities. The red circle denotes the new record in China, the blue one is site recorded of ARSEF 700, and the green one is site recorded by Drechsler (1955).

Table 1. The species used in the phylogenetic analyses.

Species	Strains*	GenBank accession numbers		
		nucLSU	**EFL**	**mtSSU**
Azygosporus macropapillatus	CGMCC 3.16068 (T)	MZ542006	MZ555650	MZ542279
A. parvus	ATCC 14634 (T)	KX752051	KY402207	MK301192A
Conidiobolus coronatus	NRRL 28638	AY546691	DQ275337	–
C. lichenicolus	ATCC 16200 (T)	JF816216	JF816232	MK301186
Microconidiobolus nodosus	ATCC 16577 (T)	JF816217	JF816235	MK333388
M. paulus	ARSEF 450 (T)	KC788409	–	–
M. terrestris	ATCC 16198 (T)	KX752050	KY402208	MK301199
M. undulatus	ATCC 12943 (T)	JX946693	JX946699	MK301201
Neoconidiobolus antarcticus	ARSEF 6913 (T)	DQ364207	–	DQ364227
N. couchii	ATCC 18152 (T)	JN131538	JN131544	MK301179
N. kunyushanensis	CGMCC 3.15890 (T)	MN061286	MN061483	MN061289
N. lachnodes	ARSEF 700	KC788408	–	–
N. lachnodes	RCEF 7519	PP716879	PP716880	PP721186
N. lamprauges	CBS 461.97	MH874268	–	–
N. mirabilis	CGMCC 3.17763 (T)	MH282852	MH282853	MK333389
N. osmodes	ARSEF 79	EF392371	–	DQ364219
N. osmodes	RCEF4447	JN131539	JN131545	MK333392
N. pachyzygosporus	CGMCC 3.17764 (T)	KP218521	KP218524	MK333390
N. sinensis	RCEF 4952 (T)	JF816224	JF816238	MK301196
N. stilbeus	RCEF 5584 (T)	KP218522	KP218525	MK301197
N. stromoideus	ATCC 15430 (T)	JF816219	JF816229	MK301198
N. thromboides	ATCC 12587 (T)	JF816214	JF816230	MK301200
N. thromboides	RCEF 4492	JF816223	JF816236	MK333393

*ARSEF, ARS Entomopathogenic Fungus Collection (Ithaca, USA). ATCC, American Type Culture Collection (Manassas, USA). CBS, Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands). CGMCC, China General Microbiological Culture Collection Center (Beijing, China). NRRL, ARS Culture Collection (Peoria, USA). NRRL, ARS Culture Collection (Peoria, USA). RCEF, Research Center for Entomogenous Fungi (Hefei, China). T = ex-type. The new record reported in this study is indicated in bold.

Figure 1); subtropical monsoon climate; GenBank nucLSU PP716879; *EFL* PP716880; mtSSU PP721186. AHAU RCEF 7519.

Identification. Colonies on PDA at 21 °C after 3 d, white, reaching ca. 10 mm in diameter. Mycelia colorless, 4.0–9.0 µm wide (*n* = 30), usually unbranched at the edge of colony. Aerial hypha formed after 15 d, 2.5–4.5 µm wide (*n* = 30). Primary conidiophores colorless, 40.0–70.0 × 6.0–9.0 µm (n=30), arising from hyphal segments, sometimes widening upward at the tip, producing a single primary conidium. Primary conidia forcibly discharged, colorless, globose, 18.0–23.0 × 14.0–19.0 µm (*n* = 30), with a basal papilla 5.0–9.0 µm wide and 2.0–4.0 µm long. Secondary conidia arising from primary conidia, similar to but smaller than primary conidia. 15.0–19.0 × 11.0–14.0 µm (n=30). Chlamydospores colorless, globose, usually formed terminally on procumbent hyphae after 20 d, 12.0–17.0 µm (*n* = 30).

Phylogenetic analyses were conducted using nucLSU, *EFL*, and mtSSU revealed a close association between the sequence of *N. lachnodes* RCEF 7519 and that of *N. lachnodes* (ARSEF 700), supported by a combination of high bootstrap value (100/1.00) and significant bayesian posterior probabilities (Figure 3). Additionally, BLASTn analysis of the nucLSU gene sequences also demonstrated a significant identity (98%) between RCEF 7519 and ARSEF 700.

DISCUSSION

Conidiobolus lachnodes was initially isolated by Drechsler from the United States of America in 1954 (Drechsler 1955). Notably, King (1977) later consolidated *C. nodosus* and *C. terrestris* with *C. lachnodes* based on numerical taxonomy. However, our recent studies have distinguished *C. nodosus* and *C. terrestris* from *C. lachnodes* based on their distinct phylogenetic relationships and morphological characteristics (Nie et al. 2020). Consequently, *C. nodosus* and *C. terrestris* were reinstated as separate species within the genus *Microconidiobolus*, while *C. lachnodes* was transferred to the genus *Neoconidiobolus*.

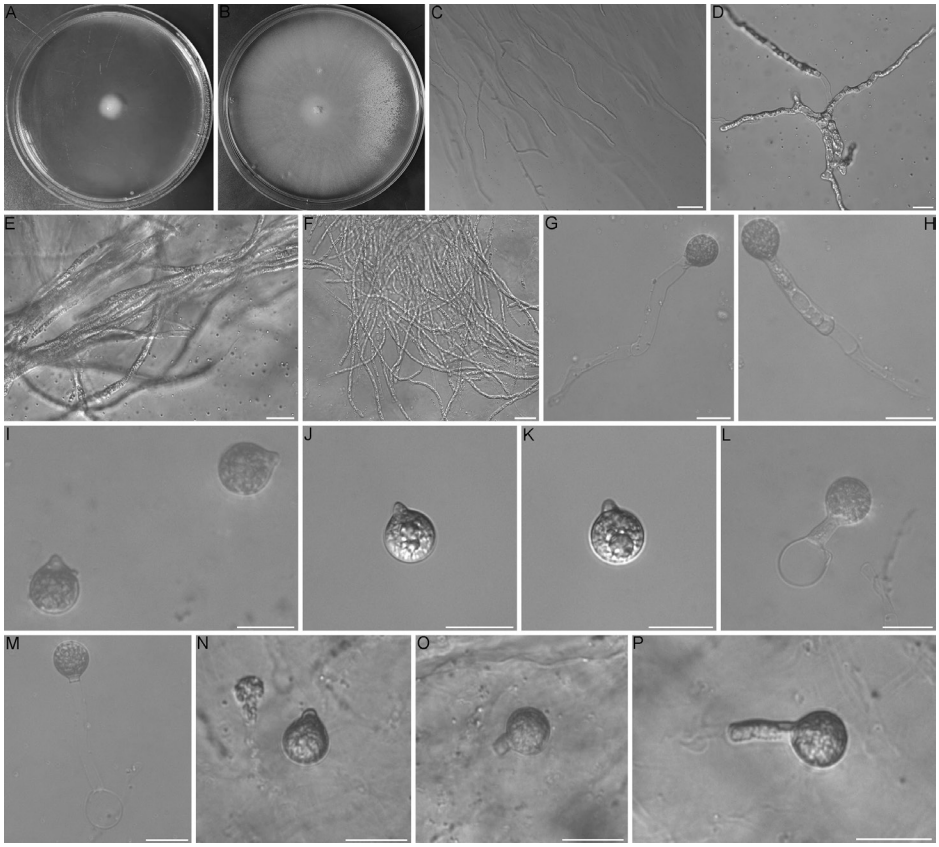

Figure 2. *Neoconidiobolus lachnodes* RCEF 7519. **A.** Colony on PDA after 3 d at 21 °C. **B.** Colony on PDA after 30 d at 21 °C. **C.** Mycelia unbranched at the edge of the colony. **D.** Mycelium. **E, F.** Aerial hypha. **G, H.** Primary conidiophores bearing primary conidia. **I–K.** Globose primary conidia. **L, M.** Primary conidia bearing a single secondary conidium. **N–P.** Chlamydospores. Scale bars: C = 100 µm; D–P = 20 µm.

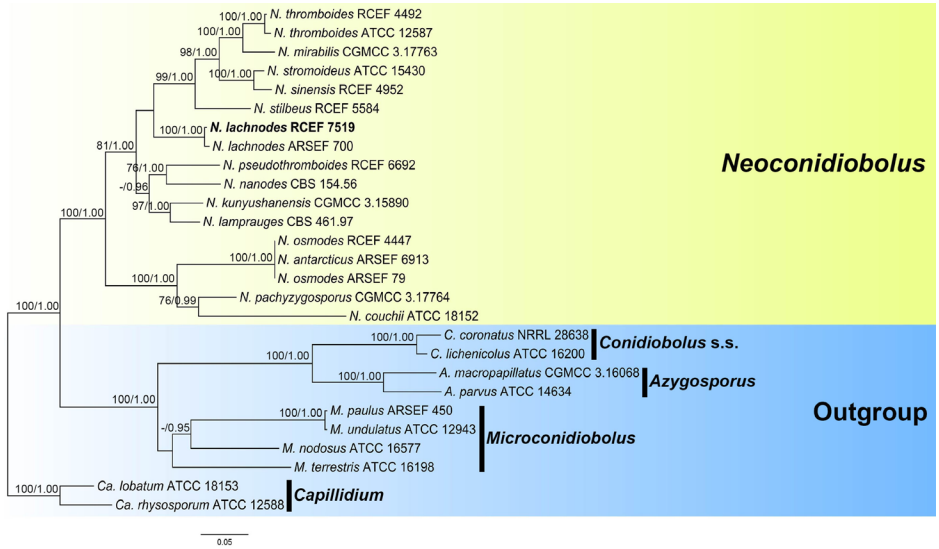

Figure 3. The phylogenetic tree of *Neoconidiobolus* constructed based on combined nucLSU, *EFL*, and mtSSU sequences. *Azygosporus*, *Capillidium*, *Conidiobolus* s.s., and *Microconidiobolus* species were chosen as outgroups. New record is shown in bold. Maximum-likelihood bootstrap values (≥70%) / Bayesian posterior probabilities (≥0.95) of clades are provided alongside the branches. The scale bar at the bottom indicates substitutions per site.

The type strain of *N. lachnodes* is currently unavailable in culture. However, the nucLSU sequence of the authentic strain *N. lachnodes* (ARSEF 700) is documented in GenBank. The nucLSU sequence of our strain, RCEF 7519, shows 98% similarity to *N. lachnodes* (ARSEF 700). Morphologically, RCEF 7519 closely resembles the original description of *N. lachnodes*, characterized by abundant aerial hyphae and chlamydospores (Drechsler 1955). Specifically, the width of the mycelia is comparable between RCEF 7519 (4.0–9.0 µm) and *N. lachnodes* (2.0–8.0 µm), particularly in terms of aerial hyphae width (2.5–4.5 µm for both RCEF 7519 and *N. lachnodes*). Additionally, the chlamydospore size is similar (12.0–17.0 µm for RCEF 7519 vs. 11.0–16.0 µm for *N. lachnodes*) as noted by Drechsler (1955).

This study marks the first official report of *N. lachnodes* in central China, providing a detailed morphological description and illustration. Multi-gene locus analyses are widely employed to unravel the phylogenetic relationships of fungi (James et al. 2006). Accordingly, we conducted a comprehensive phylogenetic analysis of *Conidiobolus*-like fungi using nucLSU, nucSSU, EFL, and mtSSU sequences (Nie et al. 2020). To

better understand the phylogenetic position of *N. lachnodes* within the *Neoconidiobolus* lineage, we added sequences of the *EFL* and mtSSU loci. The phylogenetic analysis confirmed the morphological findings, grouping RCEF 7519 with *N. lachnodes* (ARSEF 700), thus confirming the identification of RCEF 7519 as *N. lachnodes*.

Despite the overall similarity, we observed some morphological and molecular differences between RCEF 7519 and *N. lachnodes*. For instance, the primary conidiophores of RCEF 7519 (40.0–70.0 µm) are longer than those of *N. lachnodes* (15.0–40.0 µm), and the primary conidia of RCEF 7519 (18.0–23.0 × 14.0–19.0 µm) are slightly smaller than those of *N. lachnodes* (10.0–27.0 × 9.0–25.0 µm). Additionally, there is a 2% genetic divergence in the nucLSU locus between RCEF 7519 and *N. lachnodes* (ARSEF 700). These differences may be attributed to geographic variation between populations in North America and central China. Overall, this finding expands the geographic distribution of *N. lachnodes* from North America to central China (Figure 1).

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ADDITIONAL INFORMATION

Conflict of interest
The authors declare that no competing interests exist.

Ethical statement
No ethical statement is reported.

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Author contributions
Conceptualization: LX, HB. Data curation: ZM, NY, ZH. Formal analysis: NY, ZH. Investigation: NY, LX, HB. Methodology: ZM, NY, ZH. Project administration: NY. Resources: ZM, NY. Software: NY, ZH. Supervision: LX, HB. Validation: NY, LX, HB. Visualization: LX, HB. Writing – original draft: ZM, NY. Writing – review and editing: LX, HB.

Author ORCID iDs
Mohan Zhang https://orcid.org/0009-0004-5297-690X
Yong Nie https://orcid.org/0000-0001-8964-1661
Heng Zhao https://orcid.org/0000-0003-2938-5613
Xiao-Yong Liu https://orcid.org/0000-0002-8808-010X
Bo Huang https://orcid.org/0000-0001-6032-7396

Data availability
All data that support the findings of this study are available in the main text.

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