Azygosporus gen. nov., a synapomorphic clade in the family Ancylistaceae

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Academic editor: Kerstin Voigt | Received 24 August 2021 | Accepted 30 October 2021 | Published 31 December 2021

Citation: Cai Y, Nie Y, Zhao H, Wang Z, Zhou Z, Liu X, Huang B (2021) Azygosporus gen. nov., a synapomorphic clade in the family Ancylistaceae. MycoKeys 85:161–172. https://doi.org/10.3897/mycokeys.85.73405

Abstract
The fungal genus Conidiobolus sensu lato was delimited into four genera based on morphology and phylogeny. However, the taxonomic placement of C. parvus has not been determined until now. Here, we show that C. parvus belongs to a distinct lineage based on mitochondrial (mtSSU) and nuclear (TEF1 and nrLSU) phylogenetic analyses. Phylogenetic analyses further revealed a new species as sister to C. parvus. We identified a synapomorphy uniting these lineages (azygospore production) that was not observed in other allied genera of the family Ancylistaceae, and erected a new genus Azygosporus gen. nov. for this monophyletic group, with a new combination, A. parvus comb. nov. as the type species. Within Azygosporus, the novel species A. macropapillatus sp. nov. was introduced from China based on morphological characteristics and molecular evidence, which is characterized by its prominent basal papilla, in comparison to other closely related species, measuring 7.5–10.0×5.0–10.0 µm. Our study resolved the phylogenetic placement of C. parvus and improved the taxonomic system of the Ancylistaceae family.

Keywords
Entomophthorales, resting spores, saprophytic fungi, taxonomy

* These authors contribute equally to this study and share the first author.

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Introduction

*Conidiobolus* is the largest genus within the family Ancylistaceae, and includes mainly saprotrophs occurring in soil and plant debris, but also parasites of insects and animals (Vilela et al. 2010; Gryganskyi et al. 2012). After decades of study on more than 35 American and Indian *Conidiobolus* taxa (Drechsler 1952, 1953, 1954, 1955a, b, 1957, 1960, 1962; Srinivasan and Thirumalachar 1961, 1962a, b, 1967, 1968b), a numerical taxonomy was proposed that included 27 distinct species (King 1976a, b, 1977). Subsequently, the genus *Conidiobolus* was divided into three subgenera according to secondary conidia types (Ben-Ze’ev and Kenneth 1982). However, these morphologies can be difficult to distinguish and possess limited phylogenetic information that has limited our understanding of the evolution of *Conidiobolus* (Humber 1989). Since the division of *Conidiobolus*, several single- and multi-locus phylogenetic analyses of the genus have shown that the proposed groups are polyphyletic (Jensen et al. 1998; Gryganskyi et al. 2013; Nie et al. 2018). The latest taxonomic revision of *Conidiobolus*, based on morphology and four genetic loci, revealed four lineages, and four genera (*Capillidium*, *Conidiobolus* sensu stricto, *Microconidiobolus* and *Neoconidiobolus*) were established (Nie et al. 2020a).

In addition to the size of primary conidia and the type of secondary conidia, resting spores are another character with taxonomic importance for recognizing *Conidiobolus* species (Humber 1997). Until now, four styles of resting spores have been reported: villose spores in *C. coronatus* and *C. lunulus* (Nie et al. 2020a; Goffre et al. 2020), zygospores and chlamydosporus in most members (King 1977; Nie et al. 2020a), and azygospores found only in *C. parvus* (Drechsler 1962; King 1977). Consequently, the taxonomic status of *C. parvus* remained uncertain, as a monotypic lineage in the most recent phylogeny analysis (Nie et al. 2020a, b).

Previous phylogenetic analyses have shown that it is not only *Conidiobolus parvus* that has questionable taxonomic placement. Our recent research has indicated that *C. lampragues* and *C. nanodes* should be assigned into the genus *Neoconidiobolus* (Nie et al. 2021). In this article, we describe a new genus, *Azygosporus* gen. nov., and a new species, *A. macropapillatus* sp. nov., and compare them to other allied taxa. We construct a multilocus (nrLSU, mtSSU, and *TEF1*) phylogeny that supports morphological results and confirm the treatment of ex-type cultures of *C. parvus* as a new combination in *Azygosporus* gen. nov., named *A. parvus* (Drechsler) B. Huang & Y. Nie, comb. nov.

Materials and methods

Isolates and morphology

Plant debris was collected from Tiantangzhai National Forest Parks (31°17’48" N, 115°78’18") and Fangtang (30°30’57" N, 118°42’17" E), Anhui Province, China. Isolations were carried out using the canopy-plating approach (King 1976a). A Petri dish
with potato dextrose agar (PDA; potato 200 g, dextrose 20 g, agar 20 g, H₂O 1000 ml) was inverted over the plant debris and incubated at 21 °C. We surveyed the PDA canopy daily for entomophthoroid fungi, which were transferred to new PDA for purification when detected. Morphological characters of mycelia, primary conidiophores, primary and secondary conidia, and resting spores were described with the method of King (1976a). The length and width of 35 primary conidia, 35 conidiophores and 50 azygospores were measured using an Olympus BX50 research microscope, and then photographed by an Olympus DP25 microscope-camera. Meanwhile, we observed the morphology of secondary conidia grown on 2% agar plates (agar 20 g, H₂O 1000 ml) under a light microscope (Olympus BX50, Japan). 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, PCR amplification and sequencing

Fungal mycelia were incubated on PDA for 7 d at 21 °C. Total genomic DNA was extracted from fresh fungal mycelia by using a CTAB method followed Watanabe et al. (2010). We targeted three genetic loci for phylogenetic analyses: the large subunit of the nuclear ribosomal RNA (nrLSU), the small ribosomal subunit of the mitochondria (mtSSU), and translation elongation factor 1-alpha gene 1 (TEF1) were used for phylogenetic analysis. Details of the PCR primers and reactions can be found in Nie et al. (2020b). PCR products were purified according to the manufacturer protocol of Biotek's Purification Kit (Biotek Corporation, Beijing, China). The sequences of the PCR products were determined on both strands by using dideoxy-nucleotide chain termination on an ABI 3700 automated sequencer at Shanghai Genecore Biotechnologies Company (Shanghai, China). Sequence chromatograms were proofread and assembled with Geneious 9.0.2 (http://www.geneious.com) and the nine new nucleotide sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

We downloaded nrLSU, mtSSU, and TEF1 sequences of 5 Capillidium species, 19 Conidiobolus s.s. strains, four Microconidiobolus strains, 12 Neoconidiobolus species, C. parvus, and two outgroup taxa (Entomophthora muscae and Erynia conica) from GenBank. Individual sequences of each locus were aligned using MUSCLE 3.8.31 (Edgar 2004) and concatenated matrices were assembled by SequenceMatrix 1.7.8 (Vaidya et al. 2011). We partitioned the concatenated matrix by selecting the best model of sequence evolution for each gene according to the Akaike Information Criterion (AIC) using Modeltest 3.7 (Posada and Crandall 1998). We then conducted a Maximum Likelihood (ML) phylogenetic analysis using the best model using RAxML 8.1.17 with 1000 bootstrap replicates (Stamatakis 2014). We
also built a phylogeny using Bayesian Inference (BI) with MrBayes 3.2.2 (Ronquist and Huelsenbeck 2003). We ran four Markov chains for 400,000 generations, sampling every 100th generation, and chains were run until the standard

| Species                        | Strains*          | GenBank accession numbers | Table 1. Accession information for samples used in phylogenetic analyses. |
|--------------------------------|-------------------|---------------------------|--------------------------------------------------------------------------|
| Azygosporus macropapillatus    | RCEF 4444         | MZ542004 MZ555648 MZ542277 |                                                                             |
| A. macropapillatus             | RCEF 6334         | MZ542005 MZ555649 MZ542278 |                                                                             |
| A. macropapillatus             | CGMCC 3.16068 (T) | MZ542006 MZ555650 MZ542279 |                                                                             |
| A. parus                       | ATCC 14634 (T)    | KX752051 KY402207 MK301192 |                                                                             |
| Capillidium adiaeretum         | CGMCC 3.15888     | MN061284 MN061481 MN061287 |                                                                             |
| Ca. bangolenerae               | ARSEF 449 (T)     | DQ364204 – DQ364225       |                                                                             |
| Ca. betropororum               | RCEF 4430         | JF816225 JF816239 MK301183 |                                                                             |
| Ca. lobatum                    | ATCC 18153 (T)    | JF816218 JF816233 MK301187 |                                                                             |
| Ca. ryphosphorum               | ATCC 12588 (T)    | JN131540 JN131546 MK301195 |                                                                             |
| Conidiobolus bifurcatu         | CGMCC 3.15889 (T) | MN061285 MN061482 MN061288 |                                                                             |
| C. breidlinites                | ARSEF 452 (T)     | EF392832 – EF392495       |                                                                             |
| C. chlamydopus                 | ATCC 12242 (T)    | JF816212 JF816234 MK301184 |                                                                             |
| C. coronatus                   | NRRL 28638        | AY546901 DQ275337 –       |                                                                             |
| C. dabieshanensis              | CGMCC 3.15763 (T)| KY398125 KY402206 MK301180|                                                                             |
| C. fermipilus                  | ARSEF 6384        | JX242592 – JX24632       |                                                                             |
| C. goniomodes                  | ATCC 14445 (T)    | JF816221 JF816226 MK301182|                                                                             |
| C. humicola                    | ATCC 28849 (T)    | JF816220 JF816231 MK301184|                                                                             |
| C. iuxtagenitus                | ARSEF 6378 (T)    | KC788410 – –             |                                                                             |
| C. khandalensis                | ATCC 15162 (T)    | KX686994 KY402204 MK301185|                                                                             |
| C. lichenicolus                | ATCC 16200 (T)    | JF816216 JF816232 MK301186|                                                                             |
| C. maronpora                   | ATCC 16578 (T)    | KY398124 KY402209 MK301188|                                                                             |
| C. megathecus                  | ATCC 28854 (T)    | MF616383 MF616385 MK301189|                                                                             |
| C. myophagus                   | ATCC 16201 (T)    | JX946694 JX946698 MK301190|                                                                             |
| C. myophilus                   | ATCC 16199 (T)    | KX686995 KY402205 MK301191|                                                                             |
| C. polypermus                  | ATCC 14444 (T)    | MF616382 MF616384 MK301193|                                                                             |
| C. polytrichus                 | ATCC 12244 (T)    | JF816213 JF816227 MK301194|                                                                             |
| C. taihushanensis              | CGMCC 3.16016 (T)| MT250088 MT274290 MT250086|                                                                             |
| C. variabilis                  | CGMCC 3.16015 (T)| MT250087 MT274289 MT250085|                                                                             |
| Erysia conica                  | ARSEF 439         | EF392396 – EF392506      |                                                                             |
| Entomophthora muscae           | ARSEF 3074        | DQ273772 DQ275343 –      |                                                                             |
| Microconidiobolus nodosus      | ATCC 16577 (T)    | JF816217 JF816235 MK333391|                                                                             |
| M. paulus                      | ARSEF 450 (T)     | KC788409 – –             |                                                                             |
| M. terrestris                  | ATCC 16198 (T)    | KX752050 KY402208 MK301199|                                                                             |
| M. wendlatus                   | ATCC 12943 (T)    | JX946693 JX946699 MK301201|                                                                             |
| Neoconidiobolus couchii        | ATCC 18152 (T)    | JN131538 JN131544 MK301179|                                                                             |
| N. kunyushanensis              | CGMCC 3.15890 (T)| MN061286 MN061483 MN061289|                                                                             |
| N. lanumata                   | CBS 461.97        | MH874268 – –             |                                                                             |
| N. lachnodes                   | ARSEF 700         | KC788408 – –             |                                                                             |
| N. marilabils                  | CGMCC 3.17763 (T)| MH282852 MH282853 MK333392|                                                                             |
| N. nanodes                     | CBS 154.56 (T)    | MH869096 – –             |                                                                             |
| N. osmodes                     | ARSEF 79          | EF392371 – DQ364219      |                                                                             |
| N. pachyzygosporus             | CGMCC 3.17764 (T)| KP218521 KP218522 MK333393|                                                                             |
| N. sinensis                    | RCEF 4952 (T)     | JF816224 JF816238 MK301196|                                                                             |
| N. stelkeus                    | RCEF 5584 (T)     | KP218522 KP218525 MK301197|                                                                             |
| N. stromoides                  | ATCC 15430 (T)    | JF816219 JF816229 MK301198|                                                                             |
| N. thromboidei                 | ATCC 12587 (T)    | JF816214 JF816230 MK301200|                                                                             |

*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.
deviation of split frequencies fell below 0.01. Maximum Parsimony (MP) analyses were performed with PAUP* 4.0b10 (Swofford 2002) using the heuristic research option with random stepwise addition, random taxon addition of sequences, tree bisection and reconnection (TBR) as the branch swapping algorithm, and 1000 replicates. All characters were weighted equally and character state transitions were treated as unordered. Parameters measured for parsimony included tree length (TL), consistency index (CI), rescaled consistency index (RC), retention index (RI), and homoplasy index (HI). The sequence matrix was deposited at TreeBase (No. S28467). Phylogenetic trees were viewed in TreeView (Page 1996) and edited in FigTree 1.4 (Rambaut 2012).

Results

Phylogenetic analyses

The total alignment length of the 46 taxa was 2,002: nrLSU, 1–1,095; TEF1, 1,096–1,597; and mtSSU, 1,598–2,002. The concatenated matrix contained 957 parsimony-informative and 225 parsimony-uninformative sites. The MP tree had a length of 5,463 with CI = 0.3815, RC = 0.2467, RI = 0.6404, and HI = 0.6471. We found that the optimal model of sequence evolution for nrLSU and TEF1 were GTR+I+G4, while TVM+I+G4 was selected for mtSSU, and the resulting BI, ML, and MP trees had similar topologies; the ML tree was selected to represent the phylogeny with MP/ML/BI support values (Fig. 1). Samples from Azygosporus macropapillatus sp. nov. were sister to C. parvus (= A. parvus) in a single clade mostly related to the genus Conidiobolus s.s. in the phylogenetic tree. Both the clades of Azygosporus gen. nov. and A. macropapillatus sp. nov. were monophyletic with strong support (100/100/1.00).

Taxonomy

Azygosporus B. Huang & Y. Nie, gen. nov.
MycoBank No: 840849

Etymology. Referring to produce azygospores.

Type species. Azygosporus parvus (Drechsler) B. Huang & Y. Nie.

Description. Mycelia colorless. Primary conidiophores simple, bearing single primary conidia. Primary conidia forcibly discharged multinucleate, colourless, globose to subglobose, small, less than 22.5 µm. Producing only globose or subglobose replicative conidia, similar to and smaller than primary conidia. Azygospores formed in the middle region of the old hyphal segments. Mature azygospores colourless or yellowish, smooth, without thickening or less thickening (0.5–1.2 µm).

Notes. Azygosporus is strongly supported as monophyletic and is distinguished from other Ancylistaceae lineages by the synapomorphy of azygospore production.
Therefore, we classify this lineage as a new genus, named *Azygosporus* gen. nov. *Azygosporus* currently contains only two members: *C. parvus* (= *A. parvus*) and *A. macropapillatus* sp. nov. (Fig. 1). Morphologically, *Azygosporus* is most similar to *Microconidiobolus*, which forms small primary conidia (less than 22.5 µm) (Table 2). However, the synapomorphy of azygospore production clearly distinguishes *Azygosporus* from *Microconidiobolus* and other allied genera of the family.

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**Figure 1.** ML tree of *Coniobolus* s.l. using nrLSU + *TEF1* + mtSSU sequences. *Entomophthora muscae* and *Erynia conica* are selected as outgroups. Support for each node is shown as MP bootstrap support/ML bootstrap support/Bayesian posterior probability (MPBS/MLBS/BPP) for nodes with MPBS ≥ 70%, MLBS ≥ 70%) and BPP ≥ 0.95. The new genus, *Azygosporus*, and new species, *A. macropapillatus*, are shown in red, and the new combination is shown in blue.
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**Azygosporus parvus** (Drechsler) B. Huang & Y. Nie, comb. nov.
MycoBank No: 840850

*Conidiobolus parvus* Drechsler, Bull. Torrey bot. Club 89: 233 (1962) Basionym.

**Description.** Refer to Drechsler (1962).

**Host and distribution.** Isolated from decaying leaves in Maryland, United States.

**Notes.** The ex-type living culture is ATCC 14634 (United States, Maryland, Cumberland, 4 November 1962, Drechsler). It was reported to produce azygospores in *Conidiobolus* (King 1977); therefore, we recognize it as the type species of the genus *Azygosporus* gen. nov.

**Azygosporus macropapillatus** B. Huang & Y. Nie, sp. nov.
MycoBank No: 840548
Fig. 2

**Etymology.** *macropapillatus* (Lat.), named by its prominent basal papilla.

**Host and known distribution.** Isolated from plant debris and mosses in Anhui Province, China.

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Table 2. Morphological measurements of *A. macropapillatus* and other related species.

| Species                 | Growth rate (mm/d at 21°C on PDA) | Diameter of mycelia (µm) | Primary conidiophores (µm) | Primary conidia (µm) | Basal papilla (µm) | Resting spores (µm) | References               |
|-------------------------|-----------------------------------|--------------------------|---------------------------|---------------------|--------------------|---------------------|-------------------------|
| *A. macropapillatus*    | 5.7–7.7                           | 3.0–7.5                  | 37.0–150.0×5.0–8.5         | 16.5–22.5×12.0–19.0 | 7.5–10.0×5.0–10.0  | azygosporus, 25.0–30.0×27.0–34.0 | This article            |
| *A. parvus*             | 1.5                               | 1.4–8.0 (3.5–5)          | 15.0–30.0×3.0–8.0          | 6.0–20.0×4.5–17.0   | 1.5–6.0×1.5–4.5    | azygosporus, 20.0–25.0×8.0–20.0 | Drechsler 1962         |
| *M. nodosus*            | 7.1                               | 3.5–6.5                  | 30.0–50.0                 | 17.0–22.0×13.0–16.0 | 2.5–5.0×1.5–2.5    | chlamydosporus         | Srinivasan and Thirumalachar 1967; King 1977 |
| *M. paulus*             | 1.3–3.3                           | 1.5–7.0 (4.0–5.0)        | 15.0–30.0×3.5–7.0          | 5.0–19.0×4.0–14.0   | 2.0–7.0×1.0–5.0    | azygosporus, 10.0–15.0 | Drechsler 1957         |
| *M. terrestris*         | 2.6                               | 2.8–4.5                  | 15.0–80.0×3.0–5.0          | 8.0–12.0            | 2.0–4.0×1.5–2.0    | chlamydosporus         | Srinivasan and Thirumalachar 1968a; King 1977 |
| *N. lampragues*         | less than 5.0                     | 3.0–8.0 (4.0–7.0)        | 25.0–100.0×5.0–8.0         | 15.0–22.0×12.5–20.0 | 2.5–7.0×1.5–4.0    | azygosporus, 12.0–18.0 | Drechsler 1953         |
| *N. kunyushanensis*     | 8.3–10.0                          | 3.5–9.0                  | 62.0–121.0×7.0–12.0        | 15.0–21.0×13.0–17.0 | 4.0–8.0×1.0–4.0    | azygosporus, 12.0–25.0 | Nie et al. 2021        |
| *N. pachyzigosporus*    | 12.0                              | 3.0–14.0                 | 34.0–156.0×6.0–12.0        | 15.5–23.0×11.0–18.0 | 3.0–5.0×1.0–4.0    | azygosporus, 15.0–25.0 | Nie et al. 2018        |
Figure 2. Morphological characters of *Azygosporus macropapillatus*: a) colony on PDA after 3 d at 21 °C, b) mycelia rarely branched at the colony edge, c-f) primary conidiophores bearing primary conidia, g-h) primary conidia with prominent basal papillum, j-k) secondary conidia arising from primary conidia, i-m) azygosporos formed in the middle region of the old hyphal segment, n) immature azygospore, and o-q) mature azygosporos. Scale bars: a) 10 mm, b) 100 µm, and c-q) 20 µm.
Type specimens examined. China, Anhui Province, Ningguo City, Fangtang Town, 30°30'57" N, 118°42'17" E, from plant debris, 12 Nov 2020, Y. Nie, HMAS 350621, holotype, culture ex-holotype CGMCC 3.16068 (= RCEF 6680). GenBank: nrLSU = MZ542006; TEF1 = MZ555650; mtSSU = MZ542279.

Additional specimens examined. China, Anhui Province, Jinzhai County, Tian-tangzhai National Forest Park, 31°20'68" N, 115°81'25" E, from mosses, 6 Nov 2008, C.F. Wang, culture RCEF 4444. GenBank: nrLSU = MZ542004; TEF1 = MZ555648; mtSSU = MZ542277. China, Anhui Province, Jinzhai County, Tian-tangzhai National Forest Park, 31°17'34" N, 115°78'13" E, from plant debris, 3 Dec 2015, Y. Nie and X.X. Tang, culture RCEF 6334. GenBank: nrLSU = MZ542005; TEF1 = MZ555649; mtSSU = MZ542278.

Description. Colonies white, reaching ca 17.0–23.0 mm diameter on PDA after 3 d at 21°C. Mycelia colorless, 3.0–7.5 µm wide, usually unbranched at the colony edge. Primary conidiophores colorless, without widening upward near the tip, unbranched and producing a single conidium, 37.0–150.0 × 5.0–8.5 µm. Primary conidia forcibly discharged, colorless, subglobose, 12.0–19.0 µm wide and 16.5–22.5 µm long, most primary conidia possessed a prominent basal papilla 5.0–10.0 µm wide and 7.5–10.0 µm long. Secondary conidia arising from the primary ones with a similar shape and a smaller size. Resting spores (azygospores) observed after 10 d, and the young spores formed in the middle region of the old hyphal segments. The young spores enlarge gradually to form mature azygospores with less thickening. Mature azygospores colorless, subglobose 25.0–30.0 × 27.0–34.0 µm with a wall 0.5–1.0 µm thick.

Notes. Morphologically, Azygosporus macropapillatus sp. nov. has conidial dimensions similar to six Conidiobolus s.l. species without capilliconidia and microconidia: C. parvus, M. nodosus, M. paulus, N. kunyushanensis, N. lamprauges, and N. pachyzygosporus (Drechsler 1953, 1957, 1962; Srinivasan and Thirumalachar 1967; Nie et al. 2018, 2021). However, A. macropapillatus sp. nov. produces a prominent basal papilla of primary conidia that differs from other related species (see detailed morphological comparisons in Table 2). A. macropapillatus sp. nov. forms azygospores most closely resembling those of C. parvus (= A. parvus), which is its closest known relative with robust support (100/100/1.00). A. macropapillatus sp. nov. is distinguished from C. parvus (= A. parvus) by its longer primary conidiophore and its prominent basal papilla.

Discussion

The genus Microconiobiolus, typified by M. paulus, was recently established as a monotypic genus based on its small discharged primary conidia (less than 20 µm) (Nie et al. 2020a). Besides the species shown in Table 2, we note that overlapping small primary conidial dimensions occur in other related genera such as, Capillidiurn pumilum (7.3–14 × 9–18 µm) (Drechsler 1955a) and Conidiobolus khandelensis (15–18 × 17–21 µm) (Srinivasan and Thirumalachar 1962b). Therefore, taxonomic definitions in this fungal group, as in all life, should be revised to follow phylogenetic relationships. Phylogenetically, Ca. pumilum and
C. khandalensis were distinct from Microconidiobolus spp. and C. parvus (= A. parvus) (Nie et al. 2018; 2020a). However, our previous phylogeny recovered C. parvus (= A. parvus) as distinct lineage within Conidiobolus s.s. (Nie et al. 2018; 2020b), but its taxonomic placement was uncertain due to its affinity with members of Microconidiobolus.

The phylogeny presented here (Fig. 1) is congruent with previous studies (Nie et al. 2018; 2020b) that investigated the placement of C. parvus (= A. parvus). Here, we demonstrate that C. parvus (= A. parvus) is sister to a new taxon, A. macropapillatus sp. nov., in a clade most closely related to Conidiobolus s.s., which we name Azygosporus. The primary character distinguishing Azygosporus from Conidiobolus s.s. is the production of microspores. Furthermore, Azygosporus can be distinguished from the related genus Microconidiobolus, by the production of azygospores, while members of Microconidiobolus form zygospores or chlamydospores. Azygospores were not observed in other related genera of the family Ancylistaceae. Consequently, we proposed a new genus, Azygosporus gen. nov., based on morphology and phylogeny. In addition, C. parvus (= A. parvus) was recognized as a new combination in the genus Azygosporus gen. nov. and introduced A. macropapillatus sp. nov. by its prominent basal papilla.

For decades, most published Conidiobolus species had been described by only one strain, with the exception of some pandemic species (e.g., C. coronatus, N. osmodes, and N. thromboides) (Nie et al. 2021). Unfortunately, the type species C. utriculosus Brefeld, along with other important ex-types are missing, which makes it difficult to determine the exact taxonomic placement of some questionable Conidiobolus spp. Expanding our descriptions of fungal diversity and improving the taxonomic system of this fungal group are continuing goals. Herein, we introduced a new genus and a new species, which are contributions to fungal taxonomy.

Acknowledgements

The authors are grateful to Dr. Ian Gilman (Yale University) for improving the manuscript. Mrs. C.F. Wang and Mrs. X.X. Tang (Anhui Agricultural University) are acknowledged for helping with specimen collection and molecular work.

This work was supported by the National Natural Science Foundation of China (Nos. 31900008, 30770008 and 31970009) and the Natural Science Foundation of Anhui Province (No. 2108085MH318).

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