New *Arthrobotrys* Nematode-Trapping Species (Orbiliaceae) from Terrestrial Soils and Freshwater Sediments in China

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Abstract: *Arthrobotrys* is the most complex genus of Orbiliaceae nematode-trapping fungi. Its members are widely distributed in various habitats worldwide due to their unique nematode-trapping survival strategies. During a survey of nematophagous fungi in Yunnan Province, China, twelve taxa were isolated from terrestrial soil and freshwater sediment habitats and were identified as six new species in *Arthrobotrys* based on evidence from morphological and multigene (ITS, TEF, and RPB2) phylogenetic analyses. These new species i.e., *Arthrobotrys eryuanensis*, *A. jinpingensis*, *A. lanpingensis*, *A. luquanensis*, *A. shuifuensis*, and *A. zhaoyangensis* are named in recognition of their places of origin. Morphological descriptions, illustrations, taxonomic notes, and a multilocus phylogenetic analysis are provided for all new taxa. In addition, a key to known species in *Arthrobotrys* is provided, and the inadequacies in the taxonomic study of nematode-trapping fungi are also discussed.

Keywords: 6 new taxa; molecular phylogeny; morphological; nematode-trapping hyphomycetes; taxonomy

1. Introduction

Nematophagous fungi are a group of fungi that parasitize, capture, and poison nematodes and important balancing agents of the nematode population in nature [1–3]. They were divided into different groups according to their mode of action on nematodes: (1) nematode-trapping fungi capture nematodes with specialized hypha structure, (2) endoparasitic fungi infect nematodes with spores, (3) egg parasitic fungi invade nematode eggs and females with hypha tips, and (4) toxin-producing fungi produce toxins that paralyze and kill nematodes [3–5]. Among these, nematode-trapping fungi have been the focus of related studies due to their highly specialized, sophisticated, and diverse trapping structures. Since Corda described the first nematode-trapping species (*Arthrobotrys superba* Corda) [6], more than 120 species have been discovered in Zygomycota (Zoopagaceae), Basidiomycota (*Nematocotonus*), and Ascomycota (Orbiliomycetes) over the past 180 years [5,7,8]. Nematode-trapping fungi in Zygomycota (Zoopagaceae) are poorly understood due to their immature isolation and culture methods [8,9]. All nematode-trapping fungi in Basidiomycota catch nematodes with adhesive knobs or adhesive spores, and all of them belong to *Nematocotonus* [8,10–12]. All nematode-trapping fungi in the Ascomycota belong to Orbiliaceae (the only family of Orbiliomycetes), accounting for more than 80%
of all nematode-trapping fungi, which is a typical monophyletic group. They capture nematodes by producing constricting rings, adhesive networks, adhesive branches, adhesive knobs, and non-constricting rings [4,13].

Orbiliaceae nematode-trapping fungi have become the focus of studies on carnivorous fungi and also a focus group of fungal evolutionists due to their unique survival strategies, diverse and complex trapping structures, abundant species, and relatively mature research methods [13–16]. At present, 103 species have been discovered [4,17–19]. The history of its taxonomic research can be roughly divided into two periods: (1) from 1839 to about 1995, 26 genera were established to accommodate these species based on the morphological characteristics of conidia and conidiophores. With the subsequent discovery of more and more species, systematic comparative morphological studies were carried out, and the idea of dividing Orbiliaceae nematode-trapping fungi into Arthrobotrys, Dactylella, and Monacrosporium was proposed and widely accepted [19]. (2) Since 1995, with the development of molecular biology techniques, molecular phylogenetic studies based on DNA sequences, restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA (RAPD) indicate that species with the same trapping structure have closer phylogenetic relationships. Additionally, the idea that the types of trapping devices are more informative than conidia and conidiophores for the division of genera among Orbiliaceae nematode-trapping fungi was proposed. All Orbiliaceae nematode-trapping fungi are also classified into Arthrobotrys, Dactylellina, or Drechserella according to their types of trapping structure [4,8,14].

Arthrobotrys is the largest genus among Orbiliaceae nematode-trapping fungi. At present, 118 records of *Arthrobotrys* are listed in the Species Fungorum (http://www.speciesfungorum.org; (accessed on 6 March 2022)), which represent 59 accepted species [4,5,8,13,19]. It was established by Corda (1839), with *A. superba* Corda as the type species. These taxa are characterized by regularly 1-septate conidia growing on the nodes or short denticles of conidiophores [6]. At the time of its establishment, this genus was known for saprobic taxa [6,20]. Zopf (1888) provided a detailed description of a unique phenomenon in which *A. oligospora* produces adhesive networks to capture nematodes and clarified the relationship between *Arthrobotrys* and nematodes [21]. In the following decades, due to the limitations of the available research techniques, the understanding of this group remained relatively poor. It was not until Drechsler and Duddington (1933) improved the isolation method that an increasing number of species were discovered [22–33]. Because scholars attached different levels of importance to different morphological features, these species were parked in several genera such as *Didymozoophaga*, *Anilosporium*, and *Drechsleromyces* [34–36]. Subsequently, scholars redefined the characteristics of the genus *Arthrobotrys* by systematic comparative morphological studies as follows: branched or simple conidiophores; obovoid, elliptic, pyriform, 0–3-septate conidia, growing asynchronously on the nodes or on short denticles of conidiophores; and including species that capture nematodes with adhesive networks, constricting rings, and adhesive knobs [36–42]. Subsequently, modern molecular biology techniques have been used to explore the taxonomy of Orbiliaceae nematode-trapping fungi and indicate that species with adhesive networks usually have similar molecular characteristics. Therefore, the main characteristic of *Arthrobotrys* was correspondingly changed to producing an adhesive network to capture nematodes [4,8,14,15]. In addition, *Arthrobotrys* is the most widely distributed nematode-trapping fungi and the dominant group in most habitats. They mainly occur in the soil or sediment of various ecosystems such as farmland, forests, mangroves, and freshwater, and they are also recorded in hot springs, animal waste, and tree trunks [3,17,18,31,34,43–48]. Most *Arthrobotrys* species have strong saprophytic and reproductive capacity and can quickly colonize in soil [3,4,19], so they are ideal materials for the development of parasitic nematode biocontrol agents. At the same time, they are also a good group for the evolutionary studies of nematode-trapping fungi within the genus because of the abundant species and obvious morphological differentiation of conidia and conidiophores [4,19]. The six new species described in this study enhance the diversity of nematode-trapping fungi,
provide more materials for the biological control of parasitic nematodes, and add precious research objects for evolutionary studies of nematode-trapping fungi.

2. Materials and Methods

2.1. Sampling, Fungal Isolation and Morphological Observation

The strains included in this study were isolated from terrestrial soil and freshwater sediment collected in Yunnan Province, China. Terrestrial soil samples were collected from 0–10 cm depth using a 35 mm-diameter soil borer after removing fallen leaves from the soil surface [49–51]. Freshwater sediment samples were removed from the water with a Peterson bottom sampler (HL-CN, Wuhan Hengling Technology Company, Limited, Wuhan, China). The samples were placed into a zip-lock bag, and relevant site information were recorded. The samples were stored at 4 °C until processing.

Samples of 1–2 g of soil or sediment were spread on the surface of cornmeal agar (CMA) plates with sterile toothpicks. Approximately 5000 nematodes (*Panagrellus redivivus* Goodey, free-living nematodes) were added as bait to promote the germination of the nematode-trapping fungi [4,32,52,53]. The plates were incubated at 26 °C for three weeks and then observed under a stereomicroscope; the spores of nematode-trapping fungi were transferred to fresh CMA plates using a sterile needle. This step was repeated until a pure culture was obtained [4,5].

The pure cultures were transferred to fresh CMA plates with observation well (a square slot 2 × 2 cm created by removing agar in each plate) using a sterile needle and incubated at 26 °C until the mycelium spread beyond the well. Approximately 1000 living nematodes were placed in the well to induce the formation of the trapping device [4,5]. The types of trapping devices were checked using a stereomicroscope. All micromorphological features were photographed and measured with an Olympus BX53 microscope (Olympus Corporation, Tokyo, Japan).

2.2. DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from mycelium grown on potato dextrose agar (PDA) plates using a rapid fungal genomic DNA isolation kit (Sangon Biotech Company, Limited, Shanghai, China). The ITS, TEF, and RPB2 regions were amplified with the primer pairs ITS4-ITS5 [54], 526F-1567R [55], and 6F-7R [56], respectively. The PCR amplification was performed as follows: 4 min of pre-denaturation at 94 °C; followed by 35 cycles of 45 s denaturation at 94 °C; 1 min of annealing at 52 °C (ITS), 55 °C (TEF), or 54 °C (RPB2), and 1.5–2 min of extension at 72 °C; with a final extension of 10 min at 72 °C. The PCR products were purified with a DiaSpin PCR Product Purification Kit (Sangon Biotech Company, Limited, Shanghai, China). The purified PCR products of the ITS and RPB2 regions were sequenced in the forward and reverse directions using PCR primers, and the primer pair 247F–609R [57] was used to sequence the TEF genes (BioSune Biotech Company, Limited, Shanghai, China). SeqMan v. 7.0 (DNASTAR, Madison, WI, USA) [58] was used to check, edit, and assemble the sequences. The sequences generated in this study were deposited in the GenBank database at the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/, accessed on 26 February 2022), and the accession numbers are listed in Table 1.
Table 1. The GenBank accession numbers of the isolates included in this study. Ex-type strains are in bold. The newly generated sequences are indicated in blue.

| Taxon                      | Strain Number | GenBank Accession Number | Reference |
|----------------------------|---------------|--------------------------|-----------|
|                            |               | ITS                      | TEF       | RPB2     |          |
| Arthrobotrys amerospora    | CBS 268.83    | NR 159625                | —         | —        | [59]     |
| Arthrobotrys anomala       | YNW502-5-1   | AY773451                 | AY773393  | AY773422 | [57]     |
| Arthrobotrys arthrobotryoides | CBS 119.54 | MH857262                 | —         | —        | [59]     |
| Arthrobotrys arthrobotryoides | AOA5780   | —                        | —         | —        | Unpublished |
| Arthrobotrys botryospora   | CBS 321.83    | NR 159626                | —         | —        | [59]     |
| Arthrobotrys cladodes      | 1.03514       | MH179793                 | MH179616  | MH179893 | Unpublished |
| Arthrobotrys clavispora    | CBS 545.63    | MH883353                 | —         | —        | [59]     |
| Arthrobotrys conoides      | 670           | AY773455                 | AY773397  | AY773426 | [57]     |
| Arthrobotrys cookedickinson| YMF1.00024    | MF948393                 | MF948550  | MF948474 | [4]      |
| Arthrobotrys cystosporia   | CBS 439.54    | MH857364                 | —         | —        | [59]     |
| Arthrobotrys dianchiensis  | 1.00571       | MH179720                 | —         | —        | [60]     |
| Arthrobotrys elegans       | 1.00027       | MH179688                 | MH179797  | —        | —        | Unpublished |
| Arthrobotrys eryuanensis   | CGMCC3.197715 | MT612105                | OM850307  | OM850301 | This study |
| Arthrobotrys eryuanensis   | YXY145        | ON886616                 | ON890547  | ON890553 | This study |
| Arthrobotrys eudermata     | SDT24         | AY773407                 | —         | —        | Unpublished |
| Arthrobotrys flagrans      | 1.01471       | MH179741                 | MH179583  | MH179845 | Unpublished |
| Arthrobotrys globospora    | CBS 127.83    | U51960                   | —         | —        | [61]     |
| Arthrobotrys guizhouensis  | YMF1.00014    | MF948390                 | MF948547  | MF948471 | [4]      |
| Arthrobotrys indica        | YMF1.01845    | KT932086                 | —         | —        | Unpublished |
| Arthrobotrys jianus        | 521           | AY773452                 | AY773394  | AY773423 | [57]     |
| Arthrobotrys javanica      | 105           | EU977514                 | —         | —        | Unpublished |
| Arthrobotrys jinanensis    | CGMCC3.20896  | OM855569                 | OM850311  | OM850305 | This study |
| Arthrobotrys jinanensis    | YXY101        | ON886821                 | ON890552  | ON890558 | This study |
| Arthrobotrys koreensis     | CA5           | JP304780                 | —         | —        | [63]     |
| Arthrobotrys lanpingensis  | CGMCC3.20998  | OM855566                 | OM850308  | OM850302 | This study |
| Arthrobotrys lanpingensis  | YXY80         | ON886816                 | ON890549  | ON890555 | This study |
| Arthrobotrys latispora     | H.B. 8952     | MK943125                 | —         | —        | Unpublished |
| Arthrobotrys longiphora    | 1.00538       | MH179707                 | MH179815  | —        | —        | Unpublished |
| Arthrobotrys latispora     | CGMCC3.20894  | OM855567                 | OM850309  | OM850303 | This study |
| Arthrobotrys latispora     | YXY97         | ON886815                 | ON890550  | ON890556 | This study |
| Arthrobotrys megalospora   | MGDW17        | EU573354                 | —         | —        | Unpublished |
| Arthrobotrys microsphaeroides | YMF1.00028 | MF948395                 | MF948552  | MF948476 | [4]      |
| Arthrobotrys multiformis   | CBS 773.84    | MH861834                 | —         | —        | [59]     |
| Arthrobotrys multiformis   | SQ77-1        | AY773469                 | AY773411  | AY773447 | [57]     |
| Arthrobotrys multiformis   | 1.03481       | MH179783                 | MH179607  | MH179883 | Unpublished |
| Arthrobotrys multiformis   | YMF1.01852    | FJ185261                 | —         | —        | —        | Unpublished |
| Arthrobotrys obovata       | YMF1.00011    | MF948389                 | MF948546  | MF948470 | [4]      |
| Arthrobotrys oligospora    | 920           | AY773462                 | AY773404  | AY773433 | [57]     |
| Arthrobotrys paucispora    | ATCC 96704    | EF455991                 | —         | —        | [57]     |
| Arthrobotrys polycyphala   | 1.01588       | MH179760                 | MH179952  | MH179862 | Unpublished |
| Arthrobotrys pseudoclavata | 1130          | AY773446                 | AY773388  | AY773417 | [57]     |
| Arthrobotrys pschrophylla  | 1.01412       | MH179727                 | MH179578  | MH179832 | Unpublished |
| Arthrobotrys pschrophylla  | YNW502-3-1   | AY773450                 | AY773392  | AY773421 | [57]     |
| Arthrobotrys reticulata    | CBS 550.63    | MH883855                 | —         | —        | [59]     |
| Arthrobotrys robusta       | nefuA4        | MZ326655                 | —         | —        | Unpublished |
| Arthrobotrys salina        | SF 0459       | KP036623                 | —         | —        | Unpublished |
| Arthrobotrys schizanska    | 1.01442       | MH179732                 | MH179836  | —        | —        | Unpublished |
| Arthrobotrys shuifungensis | CGMCC3.19716  | MT612334                | OM850306  | OM850300 | This study |
| Arthrobotrys sinensis      | YXY48         | ON886817                 | ON890548  | ON890554 | This study |
| Arthrobotrys sinensis      | 105-1         | AY773445                 | AY773387  | AY773416 | [57]     |
| Arthrobotrys sphaeroides   | 1.0141        | MH179726                 | MH179577  | MH179831 | Unpublished |
| Arthrobotrys superba       | 127           | EU977538                 | —         | —        | Unpublished |
| Arthrobotrys thunemia      | 917           | AY773461                 | AY773403  | AY773432 | [57]     |
| Arthrobotrys vermicola     | 629           | AY773454                 | AY773396  | AY773425 | [57]     |
| Arthrobotrys xiangyanensis | YXY10-1       | MK537299                 | —         | —        | [17]     |
| Arthrobotrys yunnanensis   | AFTOL-1D 906  | DQ491512                 | —         | —        | Unpublished |
| Arthrobotrys zhaoyangensis | CGMCC3.20944  | OM855568                 | OM850310  | OM850304 | This study |
| Arthrobotrys zhaoyangensis | YXY86        | ON886620                 | ON890551  | ON890557 | This study |
Table 1. Cont.

| Taxon                  | Strain Number | GenBank Accession Number | Reference       |
|------------------------|---------------|--------------------------|-----------------|
| Dactylaria higginsii   | CBS 121934    | KM009164                 | —               |
| Dactylellina appendiculata | CBS 206.64   | AF106531                  | —               |
| Dactylellina copepodii | CBS 487.90    | U51964                    | —               |
| Dactylellina mammillata| CBS229.54     | AY902794                  | —               |
| Dactylellina yushanensis| CGMC3.19713  | MK372061                  | —               |
| Drchslerella coelobrocha| FWY03-25-1   | AY773464                  | —               |
| Drchslerella dactyloides| expo-5       | AY773463                  | —               |
| Drchslerella stenobrocha| YNWS02-9-1  | AY773460                  | —               |
| Drchslerella brochopaga| 701          | AY773456                  | —               |
| Orbilia jesu-laurae    | 859a          | MN816816                  | —               |
| Vermispora fusarina    | YXJ02-13-5    | AY773447                  | —               |

2.3. Phylogenetic Analysis

The sequences generated in this study were compared against the NCBI GenBank database using BLASTn (https://blast.ncbi.nlm.nih.gov/, accessed on 11 February 2022). The BLASTn search results and the morphological features of these six species indicated that they belong to the genus Arthrobotrys. This genus was searched in the Species Fungorum (http://www.speciesfungorum.org, accessed on 13 February 2022), and all relevant records were checked individually according to the relevant documents to ensure that all Arthrobotrys taxa were considered in this study [4,5,8,13,19]. All reliable ITS, TEF, and RPB2 sequences of Arthrobotrys taxa were downloaded from the GenBank database (Table 1). Three genes were aligned using the online program MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/, accessed on 15 February 2022) and manually adjusted using BioEdit v7.2.3 [68]; they were then linked with MEGA6.0 [69]. Phylogenetic trees were inferred via maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) analyses.

The SYM+I+G, GTR+I+G, and GTR+I+G models were selected via jModelTest v2.1.10 [70] as the best-fit optimal substitution models for ITS, TEF, and RPB2, respectively, for maximum likelihood (ML) and Bayesian inference (BI) analysis.

Maximum likelihood (ML) analysis was implemented using IQ-Tree v1.6.5 [71]. The dataset was partitioned, and each gene was analysed with the corresponding model. The statistical bootstrap support values (BS) were computed using rapid bootstrapping with 1000 replicates [72].

PAUP 4. a168 on XSEDE [73] in the CIPRES Science Gateway v. 3.3 web resource was used to generate the maximum parsimony (MP) analysis. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set up at 5000 and no-increase. Clade stability was assessed via a bootstrap analysis with 1000 replicates [72]. Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) values were calculated for all trees generated under different optimality criteria. All of the above parameters were edited into the PAUP block in the NEX file.

Bayesian inference (BI) analysis was conducted with MrBayes v. 3.2.6. [74]. The multiple sequence alignment file was converted into a MrBayes-compatible NEXUS file using FastaConvert [75]. The dataset was partitioned, and the optimal substitution models of each gene were equivalently replaced to conform to the setting of MrBayes. Six simultaneous Markov chains were run for 10,000,000 generations, and trees were sampled every 100 generations. The first 25% of the trees were discarded, and the remaining trees were used to calculate the posterior probabilities (PP) in the majority rule consensus tree. All of the above parameters were edited in the MrBayes block in the NEX file.

The tree was visualized with FigTree v1.3.1 [76]. The backbone tree was edited and reorganized using Microsoft PowerPoint (2013) and Adobe Photoshop CS6 software (Adobe Systems, San Jose, CA, USA).
3. Results

3.1. Phylogenetic Analysis

A total of 118 *Arthrobotry* related taxa were listed in the Species Fungorum (http://www.speciesfungorum.org/ (accessed on 6 March 2022)), representing 59 valid *Arthrobotrys* species. Among them, 51 species had confirmed molecular data. Therefore, the combined ITS, TEF, and RPB2 alignment dataset contained 64 *Arthrobotrys* isolates representing 57 *Arthrobotrys* species (plus our 12 isolates and 6 new species) and other related species in Orbiliaceae (*Dactylellina*: 4 species and *Drechslerella*: 4 species). The final dataset comprised 1918 characters (551 for ITS, 547 for TEF, and 820 for RPB2), among which 872 bp were constant, 1004 bp were variable, and 748 bp were parsimony informative. The maximum likelihood analysis of a best-scoring tree was performed with a final ML optimization likelihood value of \(-6304.618465\). Within the MP analysis, a strict consensus MP tree was obtained from the three most equally parsimonious trees (TL = 3443, CI = 0.546, RI = 0.510, RC = 0.298, HI = 0.419). For the Bayesian analysis (BI), the consensus tree was calculated with the remaining 75% of trees, and the Bayesian posterior probabilities were evaluated with a final average standard deviation of the split frequency of 0.009254. Although the trees inferred by ML, MP, and BI showed slightly different topologies in some clusters, all trees showed that all six species clustered together with known *Arthrobotrys* species, with distinct divergence from other species. The best-scoring ML tree was selected for presentation (Figure 1).

The phylogram inferred from the ITS+TEF+RPB2 dataset showed these six species clustered in *Arthrobotrys*. Among these species, *Arthrobotrys eryuanensis* clustered together with *A. musiformis* and *A. shizishanna* with 98% MPBS, 99% MLBS, and 0.98 BYPP support. *Arthrobotrys jinpingensis* and *A. shuifuensis* were sisters to *Orbilia jesu-laurae* and *A. arthrobotryoides*, respectively, with high support values (95% MPBS, 95% MLBS, 0.95 BYPP). *Arthrobotrys luquanensis* formed a basal lineage with *A. iridis* and *A. multiformis* with 87% MPBS and 90% MLBS support. *Arthrobotrys lanpingensis* clustered together with *A. psychrophila*, *A. salinum*, and *A. gampsospora* with 91% MPBS, 90% MLBS, and 0.90 BYPP support. The phylogenetic position of *Arthrobotrys zhaoyangensis* was uncertain, but this species showed significant divergence from known species.

3.2. Taxonomy

*Arthrobotrys eryuanensis* F. Zhang & X.Y. Yang sp. nov. (Figure 2).

Index Fungorum number: IF556938; Facesoffungi number: FoF 10760

Etymology: The species name “eryuanensis” refers to the name of the sample collection site: Eryuan County, Dali City, Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Dali City, Eryuan County, Xihu Lake, 26°9'8.77" N, 99°57'17.03" E, from freshwater sediment, 20 June 2014, F. Zhang. Holotype CGMCC3.19715, preserved in the China General Microbiological Culture Collection Center. Ex-type culture DLUCC 14-1, preserved in the Dali University Culture Collection.

Colonies on PDA white, cottony, growing rapidly, reaching 50 mm diameter after 7 days in the incubator at 26 °C. *Mycelium* partly superficial, partly immersed, composed of septate, branched, smooth hyphae. *Conidiophores* 110-308 µm (\(\bar{x} = 213.5 \, \mu m, n = 50\)) long, 2.5–4.5 µm (\(\bar{x} = 3.2 \, \mu m, n = 50\)) wide at base, gradually tapering upwards to apex, 1.5–3 µm (\(\bar{x} = 2.2 \, \mu m, n = 50\)) wide at apex, erect, septate, branched, hyaline, producing 2–10 short polylastic denticles at apex, with each denticle bearing a single holoblastic conidium. *Conidia* two types: *Macroconidia* 18–44.5 × 5–11.5 µm (\(\bar{x} = 28.4 \times 8.7 \, \mu m, n = 50\)) long, 2.5–4.5 µm (\(\bar{x} = 3.2 \, \mu m, n = 50\)) wide at base, gradually tapering upwards to apex, 1.5–3 µm (\(\bar{x} = 2.2 \, \mu m, n = 50\)) wide at apex, erect, septate, branched, hyaline, producing 2–10 short polylastic denticles at apex, with each denticle bearing a single holoblastic conidium. *Conidia* two types: *Microconidia* 7.5–28 × 4–11 µm (\(\bar{x} = 17.6 \times 8.6 \, \mu m, n = 50\)), subglobose to clavate, obovoid, wider rounded at apex, truncate at papillate bulged base, aseptate, hyaline, guttulate. *Chlamydospores* 7–18.5 × 3.5–8 µm (\(\bar{x} = 10.7 \times 5.8 \, \mu m, n = 50\)), cylindrical, hyaline, in chains when present, sometimes guttulate, slightly verruculose-walled. Captures nematodes with adhesive networks.
Figure 1. Maximum likelihood tree based on a combined ITS, TEF, and RPB2 sequence from 65 species of Orbiliaceae nematode-trapping fungi. Bootstrap support values for maximum likelihood (red) and maximum parsimony (black) greater than 50% and Bayesian posterior probabilities values (green) greater than 0.90 are indicated above the nodes. The new isolates are in blue; type strains are in bold. The tree is rooted by *Vermispora fusarina* YXJ13-5 and *Dactylaria higginsii* CBS 121934.
Figure 2. *Arthrobotrys eryuanensis* (CGMCC3.19715). (a) Colony. (b,d) Macroconidia. (e,g) Microconidia. (c,f,j) Conidiophores. (h) Chlamydospores. (i) Trapping device: adhesive networks. Scale bars: (a) = 1 cm, (b,d,e,g–i) = 10 μm, (c,f,j) = 20 μm.
Additional specimen examined: CHINA, Yunnan Province, Dali City, Eryuan County, Xihu Lake, 26°9‘8.77’’ N, 99°57‘17.03’’ E, from freshwater sediment, 20 June 2014, F. Zhang. Living culture YXY45.

Notes: Phylogenetically, *Arthrobotrys eryuanensis* clusters together with *A. shizishanna* and *A. musiformis* with high support values (98% MLBS, 99% MPBS, 0.99 BYPP). *A. eryuanensis* was 6.7% (39/586 bp) and 5.3% (26/486 bp) different from *A. shizishanna* and *A. musiformis* in ITS sequence. Morphologically, *A. eryuanensis* can be easily distinguished from *A. shizishanna* in shape, size, septation, and numbers of conidia and conidiophores [77]. It is more similar to *A. musiformis* in the morphology of its macroconidia [4, 19]. Their differences are as follows: (1) *A. musiformis* produces one type of conidia, most of which are curved, while *A. eryuanensis* produces two types of conidia. Macroconidia is 1-septate, partly curved and partly symmetrical, and microconidia is aseptate and truncate at the base with a papillate bulge. (2) The conidiophores of *A. musiformis* are unbranched, while most of those in *A. eryuanensis* are branched.

*Arthrobotrys jinpingensis* F. Zhang & X.Y. Yang sp. nov. (Figure 3).

![Figure 3](image-url)
Index Fungorum number: IF 556018; Facesoffungi number: FoF 10761.

Etymology: The species name “jinpingensis” refers to the name of the sample collection site: Jinping County, Gejiu City, Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Gejiu City, Jinping County, 23° 4′ 54.80″ N, 103° 12′ 40.80″ E, from terrestrial soil, 19 April 2017, F. Zhang. Holotype CGMCC3.20896, preserved in the China General Microbiological Culture Collection Center. Ex-type culture DLUCC 21-1, preserved in the Dali University Culture Collection.

Colonies on PDA white, cottony, growing rapidly, reaching 60 mm diameter after 10 days in the incubator at 27 °C. Mycelium partly superficial, partly immersed, composed of septate, branched, smooth hyphae. Conidiophores 225–509 μm (x = 348.2 μm, n = 50) long, 3–8.5 μm (x = 4.9 μm, n = 50) wide at base, gradually tapering upwards to apex, 1.5–3 μm (x = 2.1 μm, n = 50) wide at apex, erect, septate, unbranched, hyaline, producing several separate nodes by the repeated elongation of conidiophores, with each node bearing 2–11 polyblastic conidia. Conidia 11–26.5 × 6.5–14.5 μm (x = 18.6 × 10.8 μm, n = 50), subglobose, oval to ovoid, obovoid, wider rounded at apex, narrow towards with truncate at base, sometimes with a bud-like projection at base, 0 or 1-septate, hyaline, rough to smooth-walled. Chlamydospores 7–18.5 × 5.5–9.5 μm (x = 13.3 × 7.4 μm, n = 50), cylindrical, ellipsoidal, in chains, hyaline, guttulate, rough-walled. Captures nematodes with adhesive networks.

Additional specimen examined: CHINA, Yunnan Province, Gejiu City, Jinping County, 23° 4′ 54.80″ N, 103° 12′ 40.80″ E, from terrestrial soil, 19 April 2017, F. Zhang. Living culture YXY101.

Notes: Phylogenetically, Arthrobotrys jinpingensis forms a sister lineage to Orbilia jesu-laurae with 97% MLBS, 97% MPBS, 0.99 BYPP support. There is 2.5% (15/600 bp) difference in their ITS sequences. However, the conidiophores of A. jinpingensis are unbranched, producing several separate nodes by repeated elongation, while the conidiophores of O. jesu-laurae are branched and produce only one node at apex. In addition, some conidia of A. jinpingensis have a bud-like projection at base, while the conidia of O. jesu-laurae do not.[76]

Arthrobotrys lanpingensis F. Zhang & X.Y. Yang sp. nov. (Figure 4).

Index Fungorum number: IF559021; Facesoffungi number: FoF 10762.

Etymology: The species name “lanpingensis” refers to the name of the sample collection site: Lanping County, Nuijiang City, Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Nuijiang City, Lanping County, 26° 22′ 13.50″ N, 99° 23′ 0.20″ E, from freshwater sediment, 16 May 2015, F. Zhang. Holotype CGMCC3.20998, preserved in the China General Microbiological Culture Collection Center. Ex-type culture DLUCC 18-1, preserved in the Dali University Culture Collection.

Colonies on PDA white, cottony, growing rapidly, reaching 50 mm diameter after 10 days in the incubator at 27 °C. Mycelium partly superficial, partly immersed, composed of septate, branched, smooth hyphae. Conidiophores 241–503 μm (x = 307.5 μm, n = 50) long, 3.5–7 μm (x = 4.7 μm, n = 50) wide at base, gradually tapering upwards to apex, 2–3.5 μm (x = 2.4 μm, n = 50) wide at apex, erect, septate, unbranched, hyaline, bearing a single holoblastic conidium at apex. Conidia 31–55 × 13.5–24.5 μm (x = 45.4 × 19.7 μm, n = 50), obovoid, cuneiform to slightly pyriform, upper cell wider than lower cell, apex rounded, widest at median cell, tapering towards the narrow and subacute with truncate base, 1-septate when immature, becoming 3-septate at maturity (2 at base and 1 at apex), hyaline, minutely guttulate, smooth-walled. Chlamydospores 8–27 × 8–25 μm (x = 17.4 × 14.5 μm, n = 50), globose to subglobose or ellipsoidal, growing in chains, hyaline, guttulate, rough-walled. Capturing nematodes with adhesive networks.

Additional specimen examined: CHINA, Yunnan Province, Nuijiang City, Lanping County, 26° 22′ 13.50″ N, 99° 23′ 0.20″ E, from freshwater sediment, 16 May 2015, F. Zhang. Living culture YXY80.
Figure 4. Arthrobotrys lanpingensis (CGMCC3.20998). (a) Colony. (b,c) Conidia. (d) Chlamydospores. (e) Trapping device: adhesive networks. (f) Conidiophores. Scale bars: (a) = 1 cm, (b–f) = 10 µm.

Notes: Phylogenetically, Arthrobotrys lanpingensis formed a sister lineage to A. psychrophila, A. salinum and A. gampospora with 91% MLBS, 90% MPBS, and 0.90 BYPP support. A. lanpingensis was 9.3% (56/602 bp), 6.4% (32/503 bp), and 8.7% (50/576 bp) different from A. gampospora, A. psychrophile, and A. salinum in ITS sequences, respectively. Morphologically, A. lanpingensis is most similar to A. guizhouensis in their subfusiform conidia. However, A. guizhouensis produces two types of conidia, while A. lanpingensis produces only one type of conidia. In addition, most conidia of A. lanpingensis are 3-septate, whereas the conidia of A. guizhouensis are 2-septate, and the conidia of A. lanpingensis are significantly...
smaller than those of *A. guizhouensis* [A. lanpingensis, 31.1–55.2 (45.4) × 13.5–24.3 (19.7) µm versus *A. guizhouensis*, 30.5–71.5 (52.7) × 18.5–28.5 (23.9) µm] [4,19].

*Arthrobotrys luquanensis* F. Zhang & X.Y. Yang sp. nov. (Figure 5).

**Figure 5.** *Arthrobotrys luquanensis* (CGMCC3.20894). (a) Colony. (b,c) Conidia. (d) Chlamydospores. (e) Trapping device: adhesive networks. (f) Conidiophore. Scale bars: (a) = 1 cm, (b–f) = 10 µm.

Index Fungorum number: IF 557884; Facesoffungi number: FoF 10763.

Etymology: The species name “luquanensis” refers to the name of the sample collection site: Luquan County, Kunming City, Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Kunming City, Luquan County, 26°10'33.20” N, 102°45'43.50” E, from terrestrial soil, 24 May 2017, F. Zhang. Holotype CGMCC3.20894, deposited in the China General Microbiological Culture Collection Center. Ex-type culture DLUCC 19-1, deposited in the Dali University Culture Collection.

Colonies on PDA white, cottony, growing rapidly, reaching 55 mm diameter after 10 days in the incubator at 27 °C. Mycelium partly superficial, partly immersed, composed
of septate, branched, smooth hyphae. **Conidiophores** 216–522 µm (x = 346.5 µm, n = 50) long, 2.5–6.5 µm (x = 4.3 µm, n = 150) wide at base, gradually tapering upwards to apex, 1.5–3.5 (2.3) µm (x = 2.3 µm, n = 50) wide at apex, erect, septate, unbranched, hyaline, bearing a single holoblastic conidium at apex. **Conidia** 28–53.5 × 17–2.5 µm (x = 40.9 × 26.3 µm, n = 50), subglobose to widely ovate, with largest cell located at supramedian towards and rounded apex, tapering towards the subacute with truncate at base, 1–2-septate, mostly located at base, sometimes 3-septate (with 2 septa located at basal part and 1 at apex), hyaline, smooth-walled. **Chlamydospores** 6.5–17.5 × 6–14 µm (x = 11.2 × 9.1 µm, n = 50), globose to subglobose, ellipsoidal, in chains, hyaline, guttulate, rough-walled. Captures nematodes with adhesive networks.

Additional specimen examined: CHINA, Yunnan Province, Kunming City, Luquan County, 26°10′33.20″ N, 102°45′43.50″ E, from terrestrial soil, 24 May 2017, F. Zhang. Living culture YXY87.

Notes: The phylogenetic analyses revealed that *Arthrobotrys luquanensis* is related to *A. multiformis* and *A. iridis*. *A. luquanensis* was 9.5% (56/590 bp) and 8% (47/589 bp) different from *A. multiformis* and *A. iridis* in ITS sequences, respectively. In morphology, *A. luquanensis* is similar to *A. cookedickinson* and *A. sphaerooides* in simple conidiophores and subfusiform or obovate conidia [4,19,39,40], whereas the conidia of *A. luquanensis* are wider than those of *A. cookedickinson* [**A. luquanensis**, 28.1–53.3 (40.9) × 17–32.4 (26.3) µm versus *A. cookedickinson*, 30–52.5 (42) × 15–22.5 (17.5) µm] and bigger than those of *A. sphaerooides* [**A. luquanensis**, 28.1–53.3 (40.9) × 17–32.4 (26.3) µm versus *A. sphaerooides*, 20–44 (32) × 17–25 (20.4) µm].

**Arthrobotrys shuifuensis** F. Zhang & X.Y. Yang sp. nov. (Figure 6).
Index Fungorum number: IF556937;Facesoffungi number: FoF 10764.

Etymology: The species name “shuifuensis” refers to the name of the sample collection site: Shuifu County, Zhaotong City, Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Zhaotong City, Shuifu county, 28°32′31.80″ N, 104°19′9.50″ E, from terrestrial soil, 16 June 2017, F. Zhang. Holotype CGMCC3.19716, deposited in the China General Microbiological Culture Collection Center. Ex-type culture DLUCC 15-1, deposited in the Dali University Culture Collection.

Colonies on PDA initially white and turned to pink tinged after 2 weeks, cottony, rapidly growing, reaching 50 mm diameter after 9 days in the incubator at 26 °C. Mycelium partly superficial, partly immersed, composed of septate, branched, smooth hyphae. Conidiophores 105–305 µm (X = 218.2 µm, n = 50) long, 3–5 µm (X = 3.8 µm, n = 50) wide at base, gradually tapering upwards to apex, 1.5–3.5 µm (X = 2.5 µm, n = 50) wide at apex, erect, septate, unbranched or rarely branched, hyaline, producing several separate nodes by repeated elongation of conidiophores, with each node consisting of 2–8 papilliform bulges and bearing polyblastic conidia. Conidia 17–36 × 5–12.5 µm (X = 27.2 × 8.2 µm, n = 50), oblong or capsule-shaped, narrower towards the lower and pointed base, 1-septate, median septum, hyaline, rough-walled. Chlamydospores 6–18 × 3–7.5 µm (X = 9.7 × 8.2 µm, n = 50), cylindrical, in chains, hyaline, rough-walled. Capturing nematodes with adhesive networks.

Additional specimen examined: CHINA, Yunnan Province, Zhaotong City, Zhaoyang County, 28°32′31.80″ N, 104°19′9.50″ E, from terrestrial soil, 16 June 2017, F. Zhang. YXY48.

Notes: Phylogenetic analysis showed that Arthrobotrys shuifuensis is the closest species to A. arthrobotryoides, there are 9.6% (57/596 bp) differences in ITS sequence between them. Morphologically, this species is similar to A. arthrobotryoides in their capsule-shaped, 1-septate conidia, whereas the conidia of A. shuifuensis are significantly longer than those of A. arthrobotryoides [A. shuifuensis, 17–36 (27.2) µm versus A. arthrobotryoides 20–22 µm]. In addition, the conidiophores of A. arthrobotryoides are unbranched and produces a continuous irregularly swollen node at apex, whereas the conidiophores of A. shuifuensis are branched, producing several separate nodes with the repeated elongation of the conidiophores [19,78].

Arthrobotrys zhaoyangensis F. Zhang & X.Y. Yang sp. nov. (Figure 7).

Index Fungorum number: IF 556055; Facesoffungi number: FoF 10765.

Etymology: The species name “zhaoyangensis” refers to the name of the sample collection site: Zhaoyang County, Zhaotong City, Yunnan Province, China.

Material examined: CHINA, Yunnan Province, Zhaotong City, Zhaoyang County, 28°32′31.80″ N, 104°19′9.50″ E, from freshwater sediment, 14 April 2015, F. Zhang. Ex-type culture DLUCC 20-1, deposited in the Dali University Culture Collection.

Colonies on PDA white, cottony, growing rapidly, reaching 48 mm diameter after 10 days in the incubator at 27 °C. Mycelium partly superficial, partly immersed, composed of septate, branched, smooth hyphae. Conidiophores 207–498 µm (X = 316.5 µm, n = 50) long, 3–9.5 µm (X = 5.9 µm, n = 50) wide at base, gradually tapering upwards to apex 2–4 µm (X = 2.6 µm, n = 50) wide at apex, erect, septate, unbranched, hyaline, bearing a single holoblastic conidium at apex. Conidia 25.5–52 × 14–32 µm (X = 35.4 × 22.9 µm, n = 50), subglobose, obovoid to obpyriform, wider at median towards supramedian, rounded at apex, tapering towards narrow with subacute and truncate base, 1–3-septate, mostly 3-septate (2 septa at base and 1 at apex), hyaline, rough to smooth-walled. Chlamydospores 12.5–31.5 × 6.6–12.5 µm (X = 19.2 × 9.4 µm, n = 50) cylindrical, globose or ellipsoidal, in chains, hyaline, guttulate. Captures nematodes with adhesive network.

Additional specimen examined: CHINA, Yunnan Province, Zhaotong City, Zhaoyang County, 28°32′31.80″ N, 104°19′9.50″ E, from freshwater sediment, 14 April 2015, F. Zhang. Living culture YXY86.
Notes: Phylogenetic analysis revealed that the systematic position of *Arthrobotrys zhaoyangensis* is uncertain but showed significant distinction from known species. *A. zhaoyangensis* is most similar to *A. sinensis* and *A. sphaeroides*. *A. zhaoyangensis* can be distinguished from *A. sinensis* and *A. sphaeroides* by bigger conidia [39,79].

3.3. Key to Known Species of *Arthrobotrys*

1. Conidia 0–1-septate ................................................................. 2
2. Conidia multi-septate ................................................................. 30
3. Conidia mostly aseptate ............................................................. 3
2. Conidia mostly 1-septate .......................................................... 6
3. Conidiophores branched near apex, producing a node at each branch, or producing
several separate nodes by repeated elongation; conidia ovate, with a papilliform bulge
at the base ................................................................. A. botryospora
3. Conidiophores unbranched ......................................................... 4
4. Conidiophores with a cluster short denticles at apex; conidia obovoid,
15–31 (23.5) × 10–20 (15.9) µm ............................................... A. amerospora
4. Conidiophores producing several clusters of short denticles by repeated elongation. . . . . 5
5. Conidia elongated, ellipsoidal–cylindrical, 0–1-septate, mostly non-septate, 17.5–32.5
(22.6) × 2.75–7.5 (5.5) µm ................................................ A. yunnanensis
5. Conidia elongated, ellipsoidal, non-septate, constricted at the base, 11–16.8 × 5–6.6 µm
................................................................. A. nonseptata
6. Conidia develop on short denticles .................................................. 7
6. Conidia develop on nodes ............................................................. 13
7. Conidia curved .......................................................................... 8
7. Conidia straight .......................................................................... 10
8. Conidiophores unbranched, conidia in loose capitulate arrangement at apex; conidia
ellipsoid, mostly curved, 20–47.5 (30.9) × 7–12.5 (10.3) µm ............... A. musiformis
8. Conidiophores branched, producing several clusters short denticles by repeated
elongation ............................................................................. 9
9. Conidiophores simple or occasionally branched; conidia elongate–obovoid or elongate-
ellipsoidal, 1-septate, straight or curved, 33.5–57 × 11–15.5 µm ............ A. shahriari
9. Conidiophores branched; macroconidia 1-septate, straight or slightly curved, 18–44.5
(28.4) × 5–11.5 (8.7) µm, microconidia aseptate .............................. A. eryuanensis
10. Conidiophores producing short denticles by repeated elongation; conidia 1-septate near
the base, obpyriform, sometimes constricted at the septum, 24–32.5 × 12.5–20 µm ...
................................................................. A. perpasta
10. Conidiophores with clustered short denticles at apex; conidia in loose capitulate arrangement
at apex .................................................................................. 11
11. Conidia clavate, 1-septate at median or submedian, slightly constricted at the septum,
20–37.5 (27,9) × 7.5–10 (8.8) µm ............................................ A. javanica
11. Conidia obovoid or obpyriform ..................................................... 12
12. Conidia obovoid, 1-septate near the base, apical cell much larger, smaller at basal cell,
28.5–32 (30) × 18–20.5 (20) µm .......................................... A. obovata
12. Conidia obpyriform, 1-septate at submedian, slightly constricted at the septum,
21.4–26.9 × 11.6–15.6 µm .................................................... A. koreensis
13. Conidia develop on short denticles or obscure nodes of conidiophores ..................... 14
13. Conidia develop in clusters on swollen nodes of conidiophores ............................... 17
14. Conidiophores branched, producing short denticles by repeated elongation; conidia
obovate, elongate–obovate, 22.5–32 × 11–22.5 µm .......................... A. chazarica
14. Conidiophores unbranched; conidia clavate or pyriform ........................................ 15
15. Conidia develop on apical conidiophores, conidia clavate, 0 or 1-septate, constricted at
the base, 30–45 × 8–11 µm ................................................ A. pseudooclavata
15. Conidia pyriform, 1-septate near the basal, apical cell much larger, smaller at basal cell;
conidiophores producing several short denticles by repeated elongation ................. 16
16. Conidia perceptibly constricted at the septum, 25–33.8 × 12.5–16.3 µm .... A. paucispora
16. Conidia non-constricted, 25–35 × 18–24 µm ............................... A. cy stosporia
17. Conidiophores branched ............................................................. 18
17. Conidiophores unbranched .................................................................. 24
18. Conidia 1-septate at median ................................................................ 19
18. Conidia 1-septate at submedian ........................................................ 21
19. Conidia elongate–elliptical or cylindrical, 7.5–27.5 (15.8) × 5–10.5 (6.6) µm ... A. superba
19. Conidia short elliptical to oblong or capsule-shaped ................................. 20
20. Conidiophores occasionally branched, with distinct continuous swollen apical nodes; conidia ellipsoidal, 20–22 × 9–10 μm. 
   A. arthrobotryoides

20. Conidiophores usually branched, bearing conidia on slightly swollen nodes; conidia capsule-shaped, 17–36 (27.2) × 5–12.5 (8.2) μm. 
   A. shufusinensis

21. Conidiophores bearing conidia on apical nodes; conidia oblong–pyriform, 20–27.5 (24.4) × 7.5–12.5 (10.8) μm. 
   A. robusta

21. Conidiophores producing several separate nodes by repeated elongation. 

22. Conidia ellipsoid, elongate–ovovate, 10–20 (17.5) × 5–8 (6.2) μm. 
   A. cladodes

22. Conidia obvoid, obpyriform, or ovoid. 

23. Conidia subglobose or elliptical, 14.8–21.5 (18.3) × 10.1–16.3 (13.5) μm. 
   A. latipesora

23. Conidia obvoid or obpyriform, 1 septum at submedian, slightly constricted at the septum, 14–26 × 7.5–13 μm. 
   Orbilia jesu-laurea

24. Conidia develop on apical node of conidiophores. 

24. Conidiophores producing several separate nodes by repeated elongation. 

25. Conidia non-constricted at the septum, obconical or ellipsoidal, 25–50 × 10–15 μm. 
   A. flagrans

25. Conidia obconical or pyriform, constricted at the septum. 

26. Conidia larger size, constricted at septum, 21–42 (30.5) × 8–15 (12.7) μm. 
   A. apscheronica

26. Conidia small size, perceptibly constricted at the septum, 15–37.5 (28.4) × 7.5–14.5 (11.8) μm. 
   A. conoides

27. Conidia 1-septate at median. 

27. Conidia 1-septate at submedian. 

28. Conidiophores producing several slightly swollen nodes by repeated elongation; conidia cylindric, long ellipsoid, larger size, 13–22 × 3–7 μm. 
   A. anomala

28. Conidiophores producing several separate nodes by repeated elongation; conidia ovate, oblong, cylindric, smaller size, 10–20 (14.6) × 2.5–5 (4) μm. 
   A. dendroides

29. Conidia obpyriform or drop-shaped, some with a bud-like projection at the base, smaller size, 11.2–26.4(18.6) × 6.6–14.4(10.8) μm. 
   A. jinpingensis

29. Conidia pyriform or obovoid, slightly constricted at the septum, larger size, 17–35 (23) × 8.5–16 (12) μm. 
   A. oligospora

30. Conidia without largest cell, with several septa, uniformly distributed among conidial cells. 

30. Conidia with largest cell. 

31. Y-shaped conidia develop on conidiophores. 
   A. iridis

31. Conidia other type, never Y-shaped. 

32. Conidiophores branched. 

32. Conidiophores unbranched. 

33. Macroconidia spindle-shape or clavate, with 1–7-septate, mostly 2–5, 37.5–100 (70) × 10–17.5 (14.3) μm, microconidia spindle-shape, 0 or 1-septate. 
   A. dianchiensis

33. Conidia elongate–ovate to elongate–doliform or ellipsoidal, with 1–3-septate, 28.5–56 × 11.5–22.5 μm. 
   A. tabrizica

34. Conidia bearing on apical conidiophores. 

34. Conidiophores producing several cluster conidia by repeated elongation. 

35. Several conidia develop on apical conidiophores, macroconidia elongate-fusiform, clavate, 4–12-septate; microconidia clavate, cylindric-clavate, 0 or 1-septate. 
   A. multiformis

35. Conidiophores bearing single conidium; conidia clavate, sometimes slightly curved, 2–9-septate, 22.5–73.8 (50.6) × 5–10 (6.6) μm. 
   A. shizishanna

36. Conidiophores with inconspicuous short denticles; macroconidia fusoid-shaped, curved, 2–4-septate, mostly 3–4, 30–50 (45.1) × 8–16.5 (12.2) μm, microconidia ellipsoid, slightly curved, 1 or 2-septate. 
   A. polyccephala
36. Conidiophores producing several short denticles by repeated elongation; conidia ellagote–pyriform, 1–3-septate, mostly 2 or 3, 17–38 × 6.5–11.5 µm . . . A. pyriformi
37. Conidiophores branched ................................................................. 38
38. Conidiophores unbranched ............................................................ 43
39. Conidiophores bear a single conidium ........................................... 39
40. Conidiophores bear several conidia ................................................. 40
41. Conidia globose or oboviform, 1–2-septate, 25–37.5 × 15–22.5 µm . . . A. globospora
42. Conidia subspherical or ovoid or subfusiform, 1–3-septate, 23.5–30 (27.6) × 17–25 (20) µm ................................................................. A. sinensis
43. Conidia in capitate arrangement at apex of conidiophores ............ 41
44. Conidia in non-capitate arrangement on conidiophores ............... 42
45. Conidia fusiform, 2–4-septate, mostly 3 or 4, 46–70 (62.3) µm ....... A. azerbaijanica
46. Conidia fusiform, 1–4-septate, mostly 3 or 4 . . . . . . . . . . . . . . . . . 50
47. Conidia fusiform, sometimes slightly curved, 1–6-septate, mostly 2–3, 36.6–79.3 (57) × 11–17.5 (14) µm .................. A. scaphoides
48. Conidia fusiform, clavate, 1–3-septate, mostly 1 or 2, 25–50 × 17.5–25 µm ................................................................. A. vermicolua
49. Conidia fusiform, 1–4-septate, mostly 3 or 4 .................................. 46
50. Conidia fusiform, 1–2-septate, 7–16 (16.3) µm . . . . . . . . . . . . . . . 48
51. Conidia fusiform, clavate, 1–3-septate, mostly 1 or 2, 25–50 × 17.5–25 µm ................................................................. A. vermicolua
52. Conidia fusiform, 1–2-septate, mostly 1 or 2, 25–50 × 17.5–25 µm .... A. vermicolua
53. Conidia fusiform, 1–2-septate, mostly 1 or 2, 25–50 × 17.5–25 µm .... A. vermicolua
54. Conidia fusiform, 1–2-septate, mostly 1 or 2, 25–50 × 17.5–25 µm .... A. vermicolua
55. Conidia fusiform, 1–2-septate, mostly 1 or 2, 25–50 × 17.5–25 µm .... A. vermicolua
56. Conidia fusiform, 1–2-septate, mostly 1 or 2, 25–50 × 17.5–25 µm .... A. vermicolua
57. Conidia fusiform, 1–2-septate, mostly 1 or 2, 25–50 × 17.5–25 µm .... A. vermicolua
58. Conidia fusiform, 1–2-septate, mostly 1 or 2, 25–50 × 17.5–25 µm .... A. vermicolua
we should use more comprehensive molecular data in future studies. Clade II contained species that catch nematodes with active placement. Therefore, to thoroughly analyse the taxonomy of nematode-trapping fungi, Arthrobotrys position of some previous studies [8,15,57,80] and again emphasized the importance of different types of trapping devices in the division of genera among nematode-trapping fungi. At the genus level, the taxonomy of Orbiliaceae nematode-trapping fungi remains an open question, especially in Arthrobotrys, which contains the greatest number of species. Morphologically, 61 species of Arthrobotrys can be divided into different groups according to the morphologies of their conidiophores and conidia [19]; however, phylogenetic studies have not supported this division; many phylogenetic clades show low support values, and the phylogenetic position of some Arthrobotrys species are unclear. The reason for this dilemma is the lack of molecular data for many species, and the existing data cannot provide a stable phylogenetic placement. Therefore, to thoroughly analyse the taxonomy of nematode-trapping fungi, we should use more comprehensive molecular data in future studies.

The emergence of molecular phylogenetic methods has led to unprecedented breakthroughs in the study of fungal taxonomy. Phylogenetic studies based on only a few molecular barcodes cannot provide sufficient and reliable information for the definition of fungal species; therefore, morphological descriptions of each species are still extremely important [81,82]. However, a significant problem facing fungal taxonomy studies is that the description of species is too shallow [83]. This problem is particularly prominent in

### 4. Discussion

In this phylogenetic analysis, 65 species of nematode-trapping fungi used in this study were clustered into two large clades according to their mechanisms of catching nematodes. Clade I contained species that catch nematodes with adhesive trapping devices (adhesive nets and knobs). Clade II contained species that catch nematodes with active traps (constricting rings). Within clade I, species were clustered into two clades according to their trap types: one clade contained all species that produce adhesive nets, and the other contained those species that produce adhesive knobs. The results were consistent with previous studies [8,15,57,80] and again emphasized the importance of different types of trapping devices in the division of genera among nematode-trapping fungi. At the genus level, the taxonomy of Orbiliaceae nematode-trapping fungi remains an open question, especially in Arthrobotrys, which contains the greatest number of species. Morphologically, 61 species of Arthrobotrys can be divided into different groups according to the morphologies of their conidiophores and conidia [19]; however, phylogenetic studies have not supported this division; many phylogenetic clades show low support values, and the phylogenetic position of some Arthrobotrys species are unclear. The reason for this dilemma is the lack of molecular data for many species, and the existing data cannot provide a stable phylogenetic placement. Therefore, to thoroughly analyse the taxonomy of nematode-trapping fungi, we should use more comprehensive molecular data in future studies.

The emergence of molecular phylogenetic methods has led to unprecedented breakthroughs in the study of fungal taxonomy. Phylogenetic studies based on only a few molecular barcodes cannot provide sufficient and reliable information for the definition of fungal species; therefore, morphological descriptions of each species are still extremely important [81,82]. However, a significant problem facing fungal taxonomy studies is that the description of species is too shallow [83]. This problem is particularly prominent in
Orbiliaceae nematode-trapping fungi and is mainly reflected in two aspects. (1) The descriptions of some morphological characteristics are too indistinct. Among six described species in this study, only *A. eryuanensis* and *A. shuifuensis* could be easily distinguished from known species based on their distinct morphological characteristics. The remaining four species required more detailed characteristics (such as the size of conidia) to be identified from known species. When mycologists measure the size of conidia, they are accustomed to uniformly calculating the size data of conidia with different shapes and septate numbers, and the sizes of these conidia usually show significant differences. This causes the size range of conidia to be too extensive for effective comparisons of different species [4,19]. (2) There are too few morphological features that can be used for species identification; although the description of a species includes many features, such as its trap type, conidia, chlamydospores, and hyphae, only the trap type, conidia, and conidiophores can be used for species identification [4,19]. As an increasing number of new species are established, it is difficult to distinguish some similar species based on these three characteristics only. In conclusion, we should screen all potential morphological features in future studies to identify more features with significance for species identification. On the other hand, we should establish a unified standard morphological feature description model to facilitate comparisons between different species.

After the first nematode-trapping fungus was established in 1839 [6], the history of studies on the diversity of nematode-trapping fungi can be divided into three periods. In the nursery period, from 1839 to 1929, due to the limitation of separation methods, only five species were discovered over 90 years. In the rapid development period, from 1931 to 2009, the separation method improved gradually with the contributions of Drechsler et al. [24,25], and nearly 90 species were described over 80 years. From 2010 to 2019, only three species were discovered over 10 years (http://www.speciesfungorum.org (accessed on 6 March 2022)). These data indicated that the excavation of nematode-trapping fungi seems to have reached a plateau, and over time, it is unlikely that many new species will be discovered. However, in recent years, we have investigated nematode-trapping fungi in Yunnan Province and collected 10 new species (four previously published and six reported in this study) [18], which indicates that there are still many nematode-trapping fungi in nature that have not been discovered. Previous studies on the diversity of nematode-trapping fungi have mainly focused on soil habitat, whereas there have been considerably fewer investigations of aquatic nematode-trapping fungi [48,84,85]. However, three of the six new species described in this paper are from freshwater sediment, suggesting that aquatic habitats may also be important sources of nematode-trapping fungi and should not be ignored in future studies.

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