Arthrobotrys Sinensis (*Orbiliaceae Orbiliales*), a New Record of Nematode-Trapping Fungal Species for Sumatra, Indonesia

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Abstract. Nematode-trapping fungi (NTF) are a unique group of predacious fungi which produce various type of trapping devices to prey on free-living and parasitic nematodes. These fungi are potential to be utilized in agricultural fields to control the infestation of root-knot nematodes into the horticultural plants. Exploration of indigenous NTF from Indonesia, especially North Sumatra region is still limited and worth investigated for the field application. A fungus was isolated from an urban organic waste in Deli Serdang Regency, North Sumatra, being able to entrap nematodes (*Caenorhabditis elegans*) by using three dimensional adhesive networks. The fungus was morphologically described as an unidentified *Arthrobotrys* species with hyaline conidiophores and 1–3 septated subsphaerical to obovoid-shaped conidia. PCR-amplified DNA encoding Internal Transcribed Spacer (ITS) region displayed 99% similarity to *Arthrobotrys sinensis.* Based on these morphological and molecular features, the fungus was then identified as *A. sinensis,* strain from North Sumatra and was regarded as a new record for Indonesia.

Keywords: Nematode-trapping fungi; Arthrobotrys sinensis; North Sumatra

1 Introduction

Nematode-trapping fungi (NTF) are a specialized group of predacious fungi which have been extensively studied for the past decades regarding its distribution, ecology, systematics, and utilization as biocontrol agents of animal- and plant-parasitic nematodes [1-3]. The group is distinctive for the unique vegetative hyphal modification as trapping devices with features of adhesive or mechanical traps [4]. Asexual hyphomycetous NTF were grouped into the family of *Orbiliaceae* (*Orbiliales, Orbiliomycetes*) as monophyletic lineage based on their morphologies and molecular evidences [5-7]. The first finding of *Arthrobotrys superba* Corda, an asexual state of *Orbilia fimicola* Jeng & Krug, initiated the study of predacious fungi with more discovery of novel species and newly described strains in the following years [8,9].

Arthrobotrys is one of the genus in family *Orbiliaceae* (*Orbiliales*), which capture and prey on nematodes using three dimensional adhesive networks (nets) differentiated from unstalked adhesive knobs [10]. There are currently 129 accepted species of *Arthrobotrys* [11] with some recently documented or renamed species in 2014, such as *A. cookedickinsonianus* Z.F. Yu [12], *A. dianchiensis* (Y. Hao & K.Q. Zhang) Z.F. Yu [13], *A. huaxiensis* Z.F. Yu

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[13], *A. janus* (S.D. Li & Xing Z. Liu) Z.F. Yu [12], *A. rutgeriensis* (R.C. Cooke & Pramer) Z.F. Yu [13], *A. sphaeroides* (Castaner) Z.F. Yu [13], and *A. xiangyunensis* [14].

To date, new records of NTF have been reported exclusively from aquatic to terrestrial habitats and extreme environment with the potential of finding new records in any unexplored sites or regions [14–16]. There are presently limited information of Indonesian NTF, especially within Sumatra region. Preliminary studies have reported three species of NTF identified as *Arthrobotrys oligospora, Candelabrella musiformis,* and *Dactylella eudermata,* isolated from agricultural soil samples in North Sumatra [17,18]. Following a survey of nematophagous fungi in 2019, we collected some moist soil samples near urban organic waste. A previously undescribed NTF isolate was isolated and morphologically identified in *Arthrobotrys* while molecular identification using ITS-rDNA region revealed its identity as *A. sinensis*, as a new record for Sumatra and Indonesia.

2 Materials and Method

2.1. Fungus isolation

Soil samples were collected from a dumping site of residential organic wastes in an urban area of North Sumatra, Indonesia in 2019. The soil samples were placed in Ziplock plastic bags using a sterile scoop and delivered to the laboratory. Isolation of the nematode-trapping fungus was based on a modified soil sprinkle method [19,20]. Two grams of soil samples were spread in the edge of plate containing 0.1% (w/v) chloramphenicol and 2% (w/v) water-agar medium (CWA) in triplicate. Then, 1 mL of suspended *Caenorhabditis elegans* (± 500 larvae) was poured then cultures were incubated at 25–27 ºC temperature for 30 days. Daily microscopic observation was carried out to monitor the presence of entrapped nematodes within the fungal structures. Suspected NTF isolate DS0818 was picked from the PDA medium fragments and transferred into a new Potato Dextrose Agar (PDA) medium and sub-cultured until pure fungal culture was obtained. Micro- and macro-morphological characteristics of the fungal colony was observed in a Corn Meal Agar medium (CMA) by using available references [13,21].

2.2. DNA extraction and sequencing

Extraction of fungal genomic DNA was following the protocol of a kit (Promega Corp., USA). Mycelial samples were suspended into a mixture of Phenol : Chloroform (600 µL) and SDS Tris-HCl buffer pH 8.0 (600 µL). DNA concentration and purity of the samples were estimated based on $A_{260/280}$ using NanoPhotometer P-Class® (Implen, US).

Amplification of fungal genomic DNA in the ITS-rDNA region used a pair of universal primers, ITS-1F (5'-CTTGGTCATTTAGAGGAAGTAA-3) and ITS-4R (5'-TCCTCCGCTTATTGATATGC-3') [22]. PCR was programmed for 35 cycles in a thermal cycler (SensoQuest GmbH, Germany) with following specification: 95°C (3 min), 95°C (45 sec), 55°C, (45 sec), 72°C, (45 sec), and 72°C (7 min). The ITS-rDNA amplicons was visualized on 1% agarose gel electrophoresis prior sequencing by Macrogen, Inc. (Singapore).

2.3. Bioinformatics study

Complete ITS1, 5.8S, and ITS2 rDNA sequences were aligned with 14 sequences retrieved from GenBank from standard database and reference sequences (RefSeq) from targeted loci project information using megablast (Table 1) [23,24]. Multiple sequences were aligned using

MUSCLE feature [25] provided in MEGA-X [26]. The DNA matrix of 15 taxa and 626 nucleotides were adjusted manually and were used to construct a maximum likelihood (ML) phylogenetic tree based on Kimura's two-parameter model with bootstrap of 1000 replicates [27,28]. Alignment gaps and indels were treated as data; the transitions and transversions model was selected for the substitution model; branch swap filter was performed very weak. The nucleotide sequence obtained in this study was submitted to GenBank and was provided with an accession number MT_448860.

Table 1. List of Arthrobotrys species used in this study as references retrieved from GenBank

Taxon	GenBank accession number (ITS)	Geographical origin	Reference
Arthrobotrys amerospora	NR_159625	USA	[27]
Arthrobotrys botryospora	NR_159626	Canada	[27]
Arthrobotrys dendroides	NR_159642	Malaysia	[27]
Arthrobotrys elegans	MH_179688	China	Unpublished
Arthrobotrys iridis	NR_159630	Japan	[27]
Arthrobotrys javanica	NR_159640	Indonesia	[27]
Arthrobotrys microscaphoides	MF_948395	China	Unpublished
Arthrobotrys multiformis	NR_164434	Canada	[27]
Arthrobotrys multisecundaria	EF_059815	China	Unpublished
Arthrobotrys polycephala	NR_160072	USA	[27]
Arthrobotrys scaphoides	NR_145361	China	Unpublished
Arthrobotrys sinensis	AY_773445	China	[8]
Arthrobotrys thaumasia	AF_106526	Germany	[10]
Arthrobotrys vermicola	NR_144911	China	Unpublished
DS0818 (*Arthrobotrys sinensis*)	MT_448860	Indonesia	Current study

3 Results and Discussion

2.4. Species description

Orbiliaceae Nannf. (1932)

Arthrobotrys sinensis Xing Z. Liu & K.Q. Zhang (1999)

Syn: *Monacrosporium sinense* Xing Z. Liu & K.Q. Zhang (1994)

Description: Colonies growing rapidly on CMA, ⌀ 9 cm in 7 days at 25–27 ºC, sparse and cottony colony. Vegetative hyphae hyaline, septate, branched, mostly 2.2–9.0 µm wide. Conidiophores hyaline, simple, erect, septate, rarely branched, mostly 90–500 µm, 5–6 µm wide both at the base and apex, bearing a single conidium. Conidia hyaline, subsphaerical to subglobose, 1–3, mostly 3-septate (55 %), 30 % 2-septate, and 15 % 1-septate, 20.4–30 (25.5) × 18–22 (20) µm. Chlamydospores rarely present. Specialized trapping hypha present in the form of three dimensional adhesive networks (Figure 1).

Distribution: China (Anhui, Jiangxi, Xizang, Yunnan) [13], Indonesia (Sumatra) [Current study]

Notes: Generally, the conidial shapes were suspected to resemble between *A. sinensis*, *A. thaumasia*, and *D. gephyropaga*, however the conidia of DS0818 tend to be subglobose

similar to *A. sinensis* in contrary to the broad-shaped, and somewhat clavate conidia as in *D. gephyropaga*, with a single conidium-bearing conidiophore differing from *A. thaumasia* which commonly bear more than 3 conidia.

Specimen examined: INDONESIA—Bangun Purba: Deli Serdang Regency, moist soils near a dumping site of urban organic wastes, 157 m alt., 3°18'42.0"N, 98°48'47.9"E, 05.13.2018, DS0818.

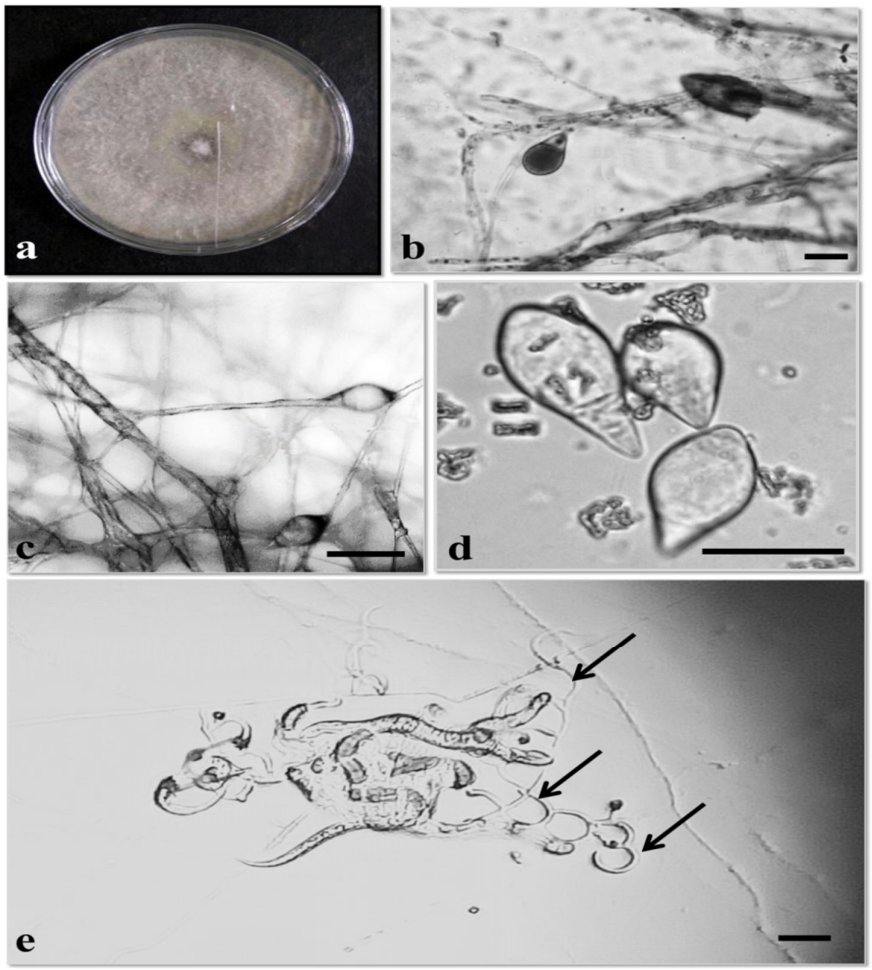

Figure 1. Arthrobotrys sinensis. Macro- and micro-morphological characters. **a.** colony surface grown on CMA of the isolate incubated for 7 d at 25 °C. **b.** young conidia. **c.** conidiophore. **d.** conidia. **e.** adhesive network (arrow). Bar = 30 µm. Isolate number: DS0818

2.5. DNA sequence analysis

Confirmation of DS0818 identity was further investigated through molecular analysis in the region of ITS-rDNA. The precise identification of fungi, has been informatively facilitated by the use of ITS as the common DNA barcoding marker for fungal genomic DNA

comparison. However, a consensus has been made in specific to the members of *Orbiliales* which suggested the use of other supporting molecular markers, such as LSU, SSU, *rpb1, rpb2,* and *tef1* since the some experts have doubted on the use of single marker to differentiate or identify novel species [28]. In this study, we found that the use of ITS was still sufficient in discriminate even in the level of species by optimizing the statistical option and model to describe the genetic relationship as revealed from the dendrogram result (Figure 2).

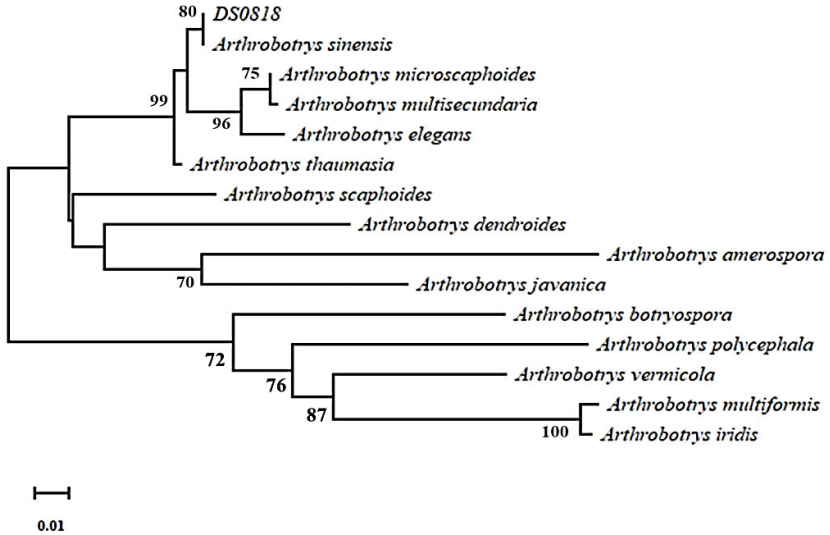

Figure 2. Dendrogram showing phylogenetic inference of aligned ITS-rDNA sequences based on ML statistical method. The nodes on the branches indicates the bootstrap values (BV) of 1000 replicates using Mega X. Sequences were retrieved from GenBank.

Based on the dendrogram analysis, we can observe three major clades within the species of *Arthrobotrys* with bootstrap values ranged from 70 to 100%. Clade 1 consisted of DS0818, *A. sinensis, A. multisecundaria, A. microscaphoides, A. elegans,* and *A. thaumasia*. Clade II consisted of *A. scaphoides, A. dendroides, A. amerospora,* and *A. javanica.* Clade III consisted of *A. botryospora, A. polycephala, A. vermicola, A. multiformis,* and *A. iridis.* In previous efforts, distance-based phylogenetic method of dendrogram construction was unable to discriminate the isolate DS0818, *A. sinensis,* and *A. thaumasia* indicating a relatively close similarity genetic distance among them (data not shown).

The character-based method by utilizing all nucleotide sites have produced a more distinctive genetic dissimilarities within members of clade I as revealed from this study, yet confirmed the identity of DS0818 as *Arthrobotrys sinensis*. The utilization of this species has been reported to control the animal-parasitic nematodes although the records still followed the use of older name, *Monacrosporium sinense*. The formulation of NTF consortium were effective to control the infestation and viability of *Ascaris suum* eggs [29], *Angiostrongylus vasorum* first-stage larvae [30], *Ancylostoma ceylanicum* third-stage larvae [31], and producing nematicidal compounds to digest *Angiostrongylus vasorum* larvae [32]. Moreover, *Monacrosporium sinense* showed good tolerances to temperature and pH condition, explaining its future potential to control parasitic nematodes in the rumen environments [32].

4 Conclusion

The finding of *Arthrobotrys sinensis* DS0818 originating from Sumatra, Indonesia may give us an insight for the possibility in exploring other sources, either accidental or suspected unique habitats and discovery of new strains or species as a collection of Indonesian nematode-trapping fungi. In addition, the biological characteristics of *A. sinensis* DS0818 may be investigated further to assign its potential as biocontrol agent of nematodes in the field trial, in specific against *Meloidogyne* spp.

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