Gastrodia sikkimensis (Orchidaceae: Gastrodiaceae), a new holomycotrophic species from Sikkim, India

MADHUSUDHAN KHANAL, SHUVADIP SARKAR, SANTOSH KUMAR RAI, DEVENDRA KUMAR, SANDEEP RAWAT, PRAMOD RAI, DINESH KUMAR AGRAWAL

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FLAVIO MACEDO ALVES, JULIANA LOVO, PEDRO LAGE VIANA, DANIELA CRISTINA ZAPPI

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HONG-MIN ZHOU, ZI-RUI GU, CHANG-LIN ZHAO

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Molecular phylogeny and morphology reveal a new species of *Asterostroma* from Guizhou Province, China

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Abstract

A new corticioid fungus from Guizhou Province in southwest China, *Asterostroma rhizomorpharum*, is described and illustrated based on morphological and molecular evidence. It is characterized by producing annual, resupinate basidiomata with long rhizomorphs, a dimitic hyphal structure with simple septa on generative hyphae, asterosetae frequently present in subiculum, asterohyphidia present in hymenium, ellipsoid to subglobose, and echinulate and amyloid basidiospores (5.5–6.8 × 4.6–5.9 μm). The phylogenetic analysis based on ITS+nLSU rDNA sequences shows that the new species belongs to the section *Asterostroma*.

Key words: Corticioid fungi, Phylogenetic analysis, Taxonomy, Wood-decaying fungi

Introduction

The corticioid genus *Asterostroma* Massee (1889: 154), belonging to the order Russulales, was established by Massee with *A. apalum* (Berk. & Broome) Massee (1889: 154) as its type species. The genus is characterized by resupinate and felted-membranous basidiomata, gloeocystidia, and dextrinoid astrosetae (Hallenberg & Eriksson 1985, Bernicchia & Gorjón 2010), and based on the astrosetae, *Asterostroma* was placed in the family Lachnocladiaceae (Reid 1965, Parmasto 1971, Hallenberg & Eriksson 1985). *Asterostroma* belongs to Peniophoraceae of russuloid lineage in their phylogenetic analysis of 5.8S+nLSU (Larsson & Larsson 2003, Miller et al. 2006, Larsson 2007, Liu et al. 2017).


During an examination of the wood-decaying fungi in Guizhou Province, an unknown species was found. Both the morphological and phylogenetic analyses indicate that the species is distinguished from all the known species of *Asterostroma*. Thus, we describe and illustrate it as *A. rhizomorpharum* in the present paper.
Materials and methods

Morphological studies

Samples were collected from Guizhou Province of China and deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. The macro-morphology was based on fresh and dried specimens. The color terms in the description followed Anonymous (1969) and Petersen (1996). Micro-morphology was studied at magnifications 1000 ×, using a Nikon Eclipse 80i microscope with phase contrast illumination. Melzer’s reagent (IKI), Cotton Blue (CB) and 5% potassium hydroxide (KOH) were used. Drawings were made with the aid of a drawing tube. In the text further abbreviations were used: IKI− = non-dextrinoid and non-amyloid, IKI+ = amyloid, CB− = acyanophilous, L = mean basidiospore length (arithmetic average of all basidiospores), W = mean basidiospore width (arithmetic average of all basidiospores), Q = variation in the L/W ratios, n = number of basidiospores measured.

DNA extraction, Polymerase Chain Reaction, and Sequencing

Genomic DNA was extracted from dried basidiomata using a cetyltrimethylammonium bromide rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing) according to the manufacturer’s instructions with some modifications (Liu et al. 2017, Zhao et al. 2023). ITS and LSU gene fragments were analyzed to determine the taxonomic status of Asterostroma. PCR was performed following Zhou et al. (2023). The newly generated ITS and LSU sequences were deposited in GenBank.

Phylogenetic Analyses

To determine the phylogeny, we compiled an ITS+nLSU dataset. In the combined dataset, Confertobasidium olivaceoalbum (Bourdot & Galzin) Jülich was used as outgroup for the Asterostroma analysis; the sequences were aligned initially by using MAFFT (https://mafft.cbrc.jp/alignment/server/) using the “G-INS-I” strategy and then manually optimized in BioEdit (Hall 1999). Finally, the two gene fragments were concatenated with Mesquite v3.70 (Maddison & Maddison 2021; https://www.mesquiteproject.org/) for the further phylogenetic analyses. ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit model using AIC criterion. Best-fit model according to AIC: GTR+I+G4. Sequence alignments were deposited at TreeBase (Submission ID 30866; www.treebase.org).

Maximum Likelihood (ML) analysis was conducted through the Cipres Science Gateway (https://www.phylo.org/portal2/login!input.action). BI was performed using MrBayes (Ronquist & Huelsenbeck 2003) with two independent runs, performing 4000000 replicates each for the dataset, sampling one tree every 1000 generations. The first 25% of the sampled trees discarded as burn-in, and the remaining trees were used to reconstruct a majority rule consensus and calculate BPP of the clades.

Branches of the consensus tree that received bootstrap support for ML greater than or equal to 75%, Bayesian posterior probabilities more than 0.9, respectively.

Results

The combined ITS+nLSU dataset included sequences from 31 specimens representing 23 species (Table 1). Bayesian analysis and ML analysis resulted in a similar topology, with an average standard deviation of split frequencies of 0.007910 (BI).

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Voucher</th>
<th>Locality</th>
<th>ITS</th>
<th>nLSU</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Asterostroma andinum</em></td>
<td>He 20,120,921-17</td>
<td>China</td>
<td>-</td>
<td>KY263874</td>
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<tr>
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<td>HHB-8546-sp</td>
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<td><em>Asterostroma bambusicola</em></td>
<td>He 4128</td>
<td>Thailand</td>
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</table>

......continued on the next page
The phylogeny (Fig. 1) inferred from ITS and nLSU sequences included 9 species. In the tree, species of *Asterostroma* clustered together with a low support value. Seven species including the new species *A. rhizomorpharum*, and other six species *A. bambusicola, A. cervicolor, A. macrosporum, A. medium, A. muscicola* and *A. ochroleucum*, are nested in the sect. *Asterostroma* clade with high supporting values. *Asterostroma vararioides* and *A. laxum* form the sect. *Laevispora* clade, and *A. andinum* forms its own clade.

Accessions in bold are those generated in this work.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Voucher</th>
<th>Locality</th>
<th>ITS</th>
<th>nLSU</th>
</tr>
</thead>
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<td>KY263871</td>
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<td>AB439560</td>
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<tr>
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<td>OR879302</td>
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<td>Spain</td>
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<tr>
<td><em>Peniophora incarnata</em></td>
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<td>Denmark</td>
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<td><em>Scytinostroma portentosum</em></td>
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</table>

Accessions in bold are those generated in this work.
Phylogeny of *Asterostroma rhizomorpharum* (in bold) and related species by ML analysis of combined ITS and nLSU rDNA sequences. Branches are labeled with maximum likelihood bootstrap higher than 75%, and Bayesian posterior probabilities more than 0.90, respectively.

**Taxonomy**

*Asterostroma rhizomorpharum* H.M. Zhou & C.L. Zhao, *sp. nov.* Figs. 2–4. MycoBank: MB 850568

**Diagnosis:** — The species is distinguished from all other *Asterostroma* species by its ellipsoid to subglobose, echinulate and amyloid basidiospores, and long rhizomorphs.

**Holotype:** — CHINA. Guizhou Province, Guiyang, Qianling Village, N 26.59976, E 106.69982, elev. 1100 m, on fallen angiosperm branch, 25 August 2023, CLZhao 31216 (SWFC F0031216).

**Etymology:** — *Rhizomorpharum* (Lat.) refers to the species having long rhizomorphs.

**Basidiomata:** — Annual, resupinate, felted-membranous, soft, up to 17 cm long, 500 μm thick. Hymenophore smooth, cream [4A2/3] to buff [4A4], some with bluish gray tint [20/21C4], cracked; margin thinning out, olivaceous buff [4C4], fimbriate with effusing generative hyphae, becoming indistinct when mature.

**Hyphal structure:** — Dimitic. Generative hyphae scattered, simple-septate, hyaline, thin-walled, moderately branched, 2–6 μm in diam. Asterosetae in subiculum abundant, predominant, yellowish brown, thick-walled, regularly star-shaped, weakly dextrinoid, rays up to 150 μm long, with acute tips. Asterohyphidia in hymenium similar to asterosetae in subiculum, but smaller and less regularly shaped, usually bifurcated at tips. Gloeocystidia subulate, thin-walled, with a basal simple septum, 13–26 × 4–7 μm. Basidia subcylindrical, hyaline, with four sterigmata and a basal simple septum, 30–45 × 5.5–8 μm. Basidiospores ellipsoid to subglobose, with a distinct apiculus, hyaline, thin-walled, echinulate, amyloid, (5.3–)5.5–6.8(–6.9) × (4.5–)4.6–5.9(–6) μm, L = 6.16 μm, W = 5.29 μm in diam. (n = 60/2); spines conical, 1.5–2 μm long.

**Additional specimen examined (paratype):** — CHINA. Guizhou Province, Guiyang, Qianling Village, N 26.59976, E 106.69941, elev. 1123 m, on fallen angiosperm branch, 25 August 2023, CLZhao 31212 (SWFC F0031212).
**Discussion**

*Asterostroma* is a monophyletic genus in our phylogenetic analysis with low statistical support, in contrast to Liu *et al.* (2017). Seven species with ornamented basidiospores formed the sec. *Asterostroma*, *A. bambusicola*, *A. cervicolor*, *A. macrosporum*, *A. medium*, *A. muscicola*, *A. ochroleucum*, and *A. rhizomorpharum*, while three species with smooth basidiospores formed two clades: *A. andinum* clade (only *A. andinum*) and sect. *Laevispora* (*A. laxum* and *A. vararioides*).

*Asterostroma rhizomorpharum* groups with *A. cervicolor*, but the latter has slightly smaller basidiospores (5–6 × 4–5.5 μm vs. 5.5–6.8 × 4.6–5.9 μm, Liu 2017), larger gloeocystidia (40–50 × 4–6 μm vs. 13–26 × 4–7 μm, Boidin 1997), and ITS sequences show a consistent difference of more than 3%.

*Asterostroma rhizomorpharum* and *A. bambusicola* both are described from China, but the latter has globose basidiospores and only grows on bamboo (Liu *et al.* 2017).

Morphologically, *Asterostroma rhizomorpharum* is similar to *Asterostroma macrosporum* by buff hymenophore and tuberculous basidiospores, but the latter has globose and larger basidiospores (8.5–11 × 7.5–9 μm, Suhara 2010). *Asterostroma medium* forms smaller basidiospores (4.2–5.5 × 3.8–4.6 μm in *A. medium*, 5.5–6.5 μm in *A. ochroleucum*, Boidin 1997). *Asterostroma muscicola* differs from *A. rhizomorpharum* by the larger basidiospores (6–8 × 5.5–7.5 μm vs. 5–6.8 × 4.6–5.9 μm, Boidin 1997).

Many wood-decaying fungi recently were being described from China (Krah *et al.* 2018, Gafforov *et al.* 2020, Wang *et al.* 2021, Liu *et al.* 2022, Dong *et al.* 2023, Duan *et al.* 2023, He *et al.* 2023, Yang *et al.* 2023, Yuan *et al.* 2023), and *A. rhizomorpharum* described from southwestern China represents the fourth species in the genus in China.
FIGURE 3. Microscopic structures of Asterostroma rhizomorpharum (holotype, CLZhao 31216). (A) Basidiospores; (B) Gloeocystidia; (C) Asterohyphidia from hymenium; (D) Basidia and Basidioles; (E) Generative hyphae; (F) Asterosetae from subiculum. Scale bars: A = 5 μm; B–F = 10 μm.
A NEW SPECIES OF ASTEROSTROMA FROM CHINA


Acknowledgements

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