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***Mazosia hainanensis* sp. nov. from tropical China**

ZONG-TING YAO^{1,2}, ZE-FENG JIA^{1* A}, SHU-HUA JIANG^{2* B}

¹ College of Life Sciences, Liaocheng University, Liaocheng, P. R. China

² State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, P. R. China

* CORRESPONDENCE TO: ^Azjfia2008@163.com ^Bjiangsh@im.ac.cn

ABSTRACT—A new species, *Mazosia hainanensis*, is described from Hainan, China. It is most similar to *M. pseudobambusae* but differs by its yellowish green thallus and smaller ascospores (14–18.5 × 3–4 μm). An analysis of its relationships based on molecular phylogeny is given. A key to the *Mazosia* species recorded in China is also presented.

KEY WORDS—*Arthoniales*, lichenized fungi, *Roccellaceae*, taxonomy

Introduction

Mazosia A. Massal. is a genus of lichenized fungi typified by *M. rotula* (Mont.) A. Massal. (Massalongo 1854), belonging to *Roccellaceae*, *Arthoniales*, *Arthoniomycetes* (Lücking 2008, Aptroot & al. 2014, Lücking & al. 2016). The status of this genus was in disarray until 1996, when Lücking & Matzer determined its position in *Roccellaceae* (Lücking & Matzer 1996). *Mazosia* is characterized by the rounded ascomata with three layers: excipulum layer with dark brown hyphae, a layer of crystals, and a thin thallus layer dominated by the photobiont. There are 8 ascospores per ascus that are hyaline, fusiform, and transversely septate. Conidia of two types are sometimes present (Santesson 1952, Lücking 2008, Lücking & al. 2016). According to Index Fungorum, *Mazosia* includes c. 34 species, most of which occur on leaves, except for four found on bark (Sparrius 2004, Aptroot & al. 2014).

Only six *Mazosia* species have been found in China, distributed in Guangdong, Hongkong, Sichuan, Taiwan, and Yunnan (Zahlbrückner 1930, Santesson 1952, Thrower 1988, Wei & Jiang 1991, Aptroot & Seaward 1999, Aptroot & Sipman 2001, Aptroot & al. 2003, Aptroot & Sparrius 2003).

TABLE 1. Specimens of *Mazosia* and related species and their LSU sequences used in the phylogenetic analysis.

| SPECIES | VOUCHER SPECIMEN | GENBANK No. |
|----------------------------------|---------------------------|-----------------|
| <i>Dendrographa alectoroides</i> | Ertz 12418 (BR) | HQ454527 |
| | Robertson 8970 (S-L63276) | EF081384 |
| <i>Dendrographa decolorans</i> | DUKE:0047570 | NG027622 |
| | UPS: Frisch 11/Se28 | KJ851054 |
| <i>Mazosia bambusae</i> | UPS: Joensson Guyana 3c | KJ851057 |
| <i>Mazosia carnea</i> | Ertz 15686 (BR) | KJ524309 |
| | Ertz 15684 (BR) | KJ524308 |
| <i>Mazosia melanophthalma</i> | UPS: Joensson Guyana 3b2 | KJ851063 |
| <i>Mazosia paupercula</i> | Ertz 9264 (BR) | KJ524310 |
| <i>Roccella fuciformis</i> | S-F206191 | KF036035 |
| | Tehler 8171 (L12540) | FJ638979 |
| <i>Tylophoron hibernicum</i> | Diederich 16335 | JF295084 |
| | Ertz 11546 (BR) | JF295083 |
| <i>Mazosia hainanensis</i> | HMAS-L 0145281 [T] | MT683119 |
| | HMAS-L 0145282 | MT683120 |

Note: Newly generated sequences are set in bold font.

Materials & methods

SPECIMENS. All materials were collected in Hainan Province, China, and are deposited in the Fungarium-Lichenes of the Institute of Microbiology, Chinese Academy of Sciences (HMAS-L). Morphological characters were examined and photographed under a LEICA M125 dissecting microscope. The anatomical characters were examined and photographed under Zeiss Axioscope2 compound microscope with a Zeiss Axio Imager A2 and an AxioCam MRC5 camera. Ascospores were tested using I (1% iodine solution). The lichen substances were detected using standardized thin layer chromatography (TLC) using solvent systems C and A (Orange & al. 2010).

DNA EXTRACTION, AMPLIFICATION, & SEQUENCING. Genomic DNA was extracted from ascomata of the specimens followed a modified CTAB method (Rogers & Bendich 1988). The nuclear ribosomal RNA gene region, large subunit rDNA (LSU) was amplified using the LIC24R with LR7 primers (Frisch & al. 2014, Ertz & al. 2009). Reactions were carried out in 25 µL reaction system containing 1 µL each primer solution (10 µM), 2 µL genomic DNA, 8 µL ddH₂O, and 13 µL 2×Taq PCR

MasterMix®. Thermocycling conditions comprised: initial denaturation for 15 min at 95 °C; followed by 45 cycles of 45 s at 95 °C, 45 s at 53 °C, 1 min at 72 °C, and a final extension for 7 min at 72 °C. The target product of PCR was affirmed by electrophoresis on 1% agarose gels and sequenced by Majorbio (Shanghai).

PHYLOGENETIC ANALYSIS. Due to lack of *Mazosia* ITS sequences, we chose LSU for sequence analysis. Our two newly generated LSU sequences were submitted to GenBank and aligned using MEGA (Kumar & al. 2016) with seventeen other sequences representing similar taxa in *Arthoniales* (TABLE 1), selected based on the results of LSU sequence Blast searches. *Tylophoron hibernicum* (D. Hawksw. & al.) Ertz & al., was chosen as outgroup (Urbanavichus & Urbanavichene 2017). The ML tree involving 1000 pseudoreplicates was generated by MEGA (Kumar & al. 2016), and the evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). The alignment was also subjected to a Neighbor-Joining analysis involving 1000 replicates with MEGA7 (Kumar & al. 2016), and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura & al. 2004).

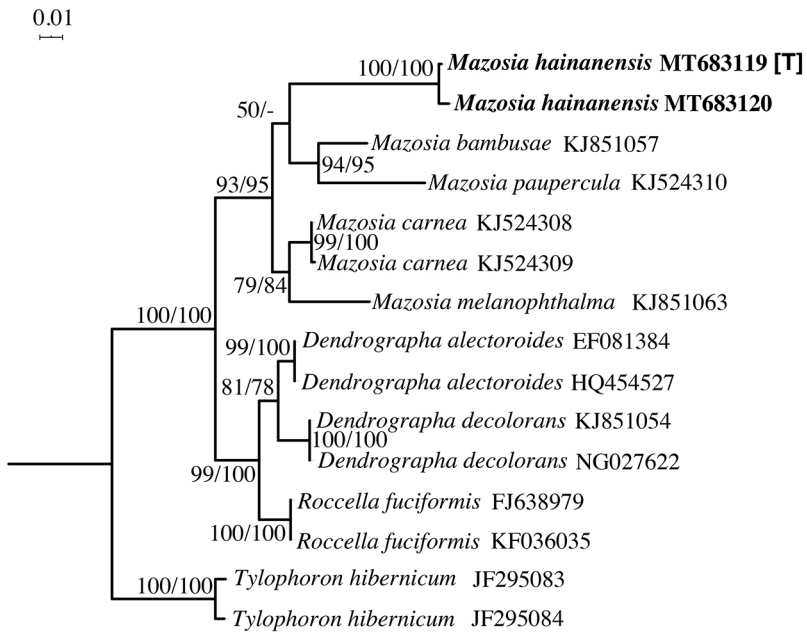


FIG. 1. Phylogenetic tree constructed from ML based on nrLSU sequences of *Mazosia* and related species. Maximum likelihood and Neighbor-Joining bootstrap support >50% are shown at nodes (ML/NJ). New sequences are set in bold font; other sequences were downloaded from GenBank. Genetic distance scale = 0.01 changes per site.

Phylogeny

The phylogenetic tree constructed from ML based on the LSU sequences (1200 bp) is shown in FIG. 1. Other genera in *Roccellaceae*—*Dendrographa* Darb. and *Rocella* DC.—were included in the phylogenetic analysis. Within the well-supported (93/95) monophyletic five-species clade of *Mazosia*, the new species *M. hainanensis* formed an independent clade. *Mazosia bambusae* (Vain.) R. Sant. is placed as sister to *M. paupercula* (Müll. Arg.) R. Sant. with *M. carnea* (Eckfeldt) Aptroot & M. Cáceres shown as sister to *M. melanophthalma* (Müll. Arg.) R. Sant.

Taxonomy

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FIG. 2

MB 836195

Differs from *Mazosia pseudobambusae* by its yellowish green thallus and smaller ascospores.

TYPE: China, Hainan, Baoting County, Mount Qixian, on unidentified angiosperm leaf, 7 Sept. 2017, S.H. Jiang HN20171277 (**Holotype**, HMAS-L 0145281; GenBank MT683119).

ETYMOLOGY: The epithet refers to the type locality province.

THALLUS yellowish green, continuous, smooth, slightly shiny, often irregular in outline, finely verrucose, verrucae 20–30 µm tall. Photobiont cells rectangular, 6–15 × 2–4 µm, in radiate plates or slightly irregular. Ascomata rounded, 50–170 µm wide and 59–76 µm tall; disc black, translucent when moistened; margin slightly higher than disc and the disc is slightly lower than or as high as the thallus. Excipulum brown, 8–16 µm thick. Hypothecium 5–15 µm high, pale brown. Hymenium 42–50 µm high, colorless. Asci clavate, 34–45 × 10–12.5 µm. Ascospores fusiform, 3-septate, 14–18.5 × 3–4 µm, the second cell is slightly enlarged. Pycnidia low conical, 10–50 µm, Microconidia fusiform-ellipsoid, non-septate, 2–3.5 × 1–1.5 µm. Macroconidia not seen.

CHEMISTRY: unidentified red fluorescent substance in the fourth and sixth zones in solvent system C and in the fourth and seventh zones in solvent system A.

ECOLOGY & DISTRIBUTION: On unidentified angiosperm leaves in tropics; known only from the type locality, Hainan.

ADDITIONAL SPECIMEN EXAMINED: CHINA, HAINAN, Baoting County, Mount Qixian, on unidentified angiosperm leaf, 7 Sept. 2017, S.H. Jiang HN20171184 (HMAS-L 0145282; GenBank MT683120).

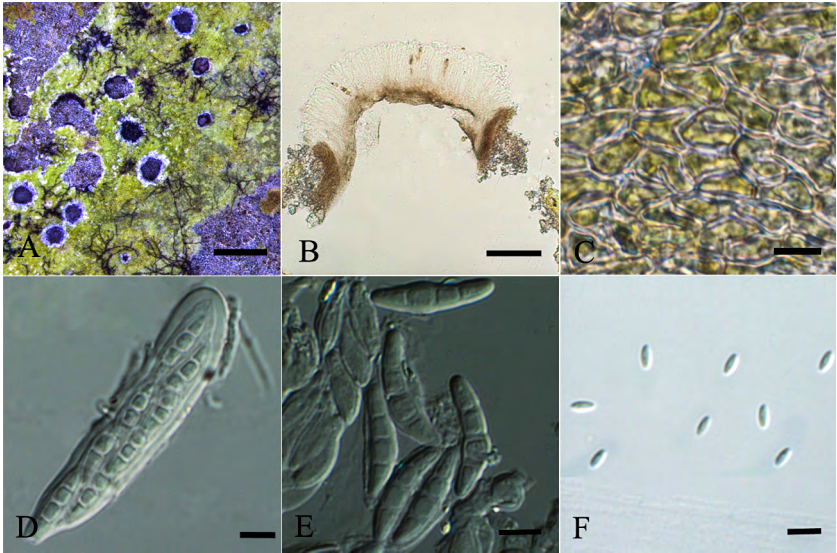


FIG. 2. *Mazosia hainanensis* (holotype, HMAS-L 0145281). A. Thallus with ascomata; B. Apothecium section; C. Photobiont; D. Ascus with ascospores; E. Ascospores; F. Microconidia. Scale bars: A = 0.2 mm; B = 50 µm; C–F = 5 µm.

REMARKS: This species is characterized by its yellowish green, finely verrucose thallus, verrucae 20–30 µm, transversely septate ascospores 14–18.5 × 3–4 µm. *Mazosia pseudobambusae* Kalb & Vězda differs from the new species by its very pale brownish grey thallus, dark brown prothallus, and larger (20–28 × 4–5 µm) ascospores (Lücking 2008). The new species is also similar to *M. melanophthalma*, which differs by its larger (50–100 µm) verrucae, longer (15–22 µm) ascospores, and an ascomatal margin that always slopes outwards (Lücking 2008; Conran & Rogers 1983). Another related species is *M. dispersa* (J. Hedrick) R. Sant., distinguished by its less finely verrucose thallus and longer (26–39 µm) ascospores (Lücking 2008).

Key to species of *Mazosia* known from China

- 1. Thallus glabrous 2
- 1. Thallus with radiate ridges or finely to coarsely verrucose 3
- 2. Ascospores 15–25 µm long *M. phyllosema*
- 2. Ascospores 25–35 µm long, irregularly 3–7-septate, *M. paupercula*

- 3. Thallus with radiate ridges, ascospores 3-septate, 14–22 × 4–6 μm *M. rotula*
- 3. Thallus lacking radiate ridges, thallus verrucose 4
- 4. Ascospores 5(–7)-septate, 26–39 μm long *M. dispersa*
- 4. Ascospores 3-septate 5
- 5. Verrucae 50–100 μm tall, ascospores 15–22 × 3–4 μm *M. melanophthalma*
- 5. Verrucae 20–30 μm tall, ascospores 14–18.5 × 3–4 μm *M. hainanensis*

Conclusion

The morphological and molecular data demonstrate that the material from Hainan province represents a previously unknown foliicolous lichen that merits the status of a new species in *Mazosia*. The preliminary key is provided to the species of *Mazosia* currently known from China.

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