Nova Hedwigia, Vol. 108 (2019), Issue 1–2, 197–206 **Article**
Published online July 17, 2018; published in print February 2019

Michenera incrustata sp. nov. (Peniophoraceae, Russulales) from southern China

Shi-Liang Liu[1], Karen K. Nakasone[2], Shuang-Hui He[1*]

[1] Institute of Microbiology, Beijing Forestry University, Beijing 100083, China

[2] Center for Forest Mycology Research, Northern Research Station, U.S. Forest Service, One Gifford Pinchot Drive, Madison, WI 53726-2398, USA

* Corresponding author: shuanghuihe@yahoo.com

With 4 figures

Abstract: A new species, *Michenera incrustata* from southern China, is described and illustrated. It is the second species in *Michenera* and differs from the generic type, *M. atrocreas*, by its hyaline, thick-walled lamprocystidia and larger basidia and basidiospores. The 5.8S–ITS2 and nrLSU sequence similarities between *M. incrustata* and *M. atrocreas* are 83% of 437 base pairs and 95.5% of 870 base pairs, respectively.

Key words: anamorph, Basidiomycota, corticioid fungi, teleomorph, wood-inhabiting fungi

Introduction

Michenera Berk. & M.A. Curtis was created in 1868 for *M. artocreas* Berk. & M.A. Curtis from Cuba with cupulate-discoid fruitbodies producing thick-walled, reddish brown, globose to fusoid chlamydospores with a filamentous apical appendage. Berkeley & Curtis (1868) classified *Michenera* in the Hymenomycetes although Patouillard (1891) believed it was related to *Melampsora* Castagne, *Phragmidium* Link, *Puccinia* Pers. and related taxa in the Pucciniales. Later, Patouillard (1900, p. 67) recognized that *Michenera* was a conidial form of a white *Corticium* that produced basidia and smooth, globose basidiospores. Peirce (1890) accurately described and illustrated the conidia of *M. artocreas*; although he noticed a Corticium-like fruitbody associated with *Michenera*, he dismissed any connection between the two fungi. Lyman (1907) proved that *M. artocreas* and *Corticium subgiganteum* Berkeley were the same species when cultures obtained from spores and basidiospores, respectively, produced similar *Michenera* fruitbodies.

 DOI: 10.1127/nova_hedwigia/2018/0500 www.borntraeger-cramer.de 0029-5035/2018/0500 $ 2.50

In 1964, Lemke introduced the monotypic *Licrostroma* P.A. Lemke for the teleomorph of *M. artocreas*. *Licrostroma* is characterized by smooth, corticioid to stereoid basidiocarps, a dimitic hyphal system with simple-septate generative and non-dextrinoid binding hyphae, large gloeocystidia and basidia, and large, globose basidiospores with thick walls that are smooth, cyanophilous, and do not react in Melzer's reagent (Lemke 1964, Ryvarden 2010, Giraldo et al. 2017). Morphologically, *Gloiothele* Bres. species also develop large spherical basidiospores with smooth, thick, cyanophilous walls. Some *Scytinostroma* Donk species develop basidiocarps similar to *Licrostroma* that are composed predominantly of skeletal or binding hyphae.

Because of the one fungus, one name principle enacted on January 1 2013 (McNeill et al. 2012), *Michenera* has priority over *Licrostroma*. Phylogenetically, *Michenera* is in the Russulales and related to *Scytinostroma*, *Asterostroma* Massee and *Gloiothele* in the Peniophoraceae (Larsson 2007, Giraldo et al. 2017). The anamorphic *M. artocreas* is striking but not as common as the teleomorphic *L. subgiganteum* although the two forms can occur together on the same branch. This species is well-known in eastern North America and also reported from Bhutan, China, India, and Japan (Lemke 1964, Wu 2008, Dhingra et al. 2011). Recently, an undescribed species of *Michenera* was collected from southern China. Morphological evidence and DNA sequences analyses support the recognition of the new taxon, *M. incrustata*.

Materials and methods

Voucher specimens are deposited at the herbaria of Beijing Forestry University (BJFC), Beijing, China and Centre for Forest Mycology Research (CFMR), U.S. Forest Service, Madison, Wisconsin, U.S.A. Thin and freehand sections were made from basidiocarps and mounted in 2% (w/v) potassium hydroxide (KOH), 1% (w/v) phloxine, or Melzer's reagent. Cyanophily of basidiospore walls was determined by examination of spores in 1% cotton blue in 60% lactic acid. Microscopic examination was carried out with a Nikon Eclipse 80i microscope at magnifications up to 1000 ×. Drawings were made with the aid of a drawing tube. The following abbreviations are used: L = mean spore length, W = mean spore width, Q = L/W ratio, n (a/b) = number of spores (a) measured from number of specimens (b). Color codes and names follow Kornerup & Wanscher (1978). Herbarium code designations are from Index Herbariorum (Thiers, continuously updated).

The CTAB plant genome rapid extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing, China) was used for DNA extraction from dried specimens. ITS and nrLSU gene regions were amplified with the primer pairs ITS5/ITS4 (White et al. 1990) and LR0R/LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm), respectively. PCR procedures followed Liu et al. (2018). DNA sequencing was performed at Beijing Genomics Institute, and the sequences were deposited in GenBank (accession numbers: MH142900 – MH142910).

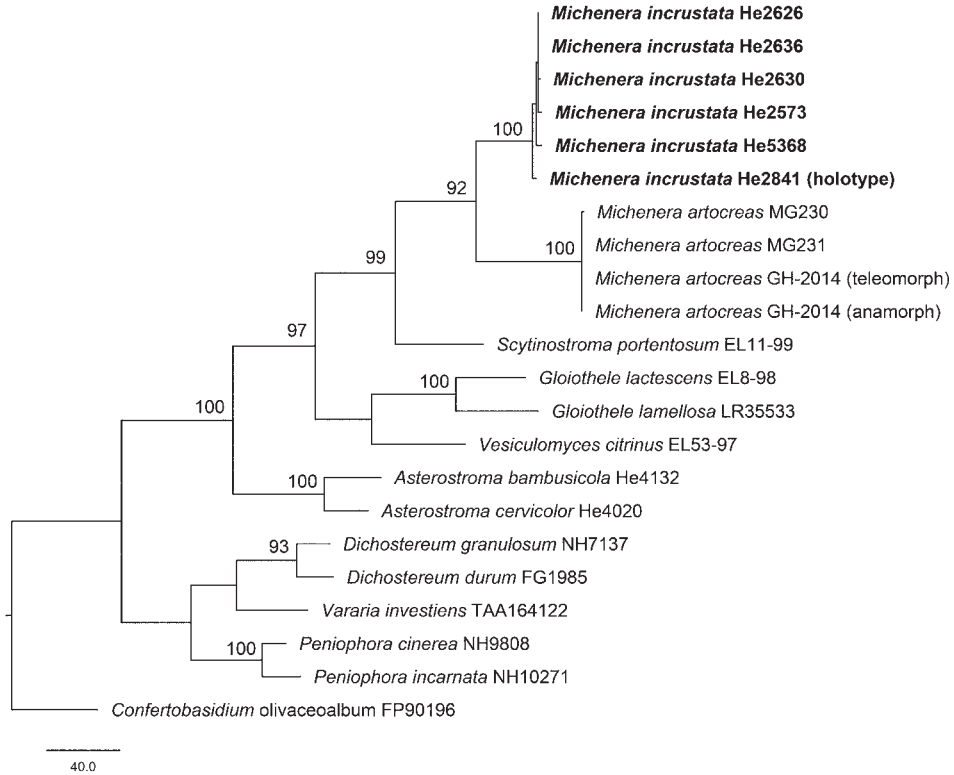

Fig. 1. Maximum parsimony phylogenetic tree inferred from combined 5.8S–ITS2 and nrLSU sequence data of taxa in Peniophoraceae. Branches are labeled with parsimony bootstrap values > 80%.

The 5.8S–ITS2–nrLSU sequence alignment was constructed in MAFFT v.6 (Katoh & Toh 2008, http://mafft.cbrc.jp/alignment/server/) and maximum parsimony phylogenetic analysis was conducted in PAUP* 4.0b10 (Swofford 2002) with default parameters. Larsson & Larsson (2003) was consulted for the ingroup taxa sampling and outgroup selection. The phylogenetic tree is shown in Fig. 1.

Species description

Michenera incrustata S.H. He, S.L. Liu & Nakasone, **sp. nov.** (Figs 2–4)

MycoBank: MB 824981

GenBank rDNA sequences ex-holotype: MH142906 (ITS); MH142910 (nrLSU).

Diagnosis: Differs from *Michenera artocreas* by its thick-walled lamprocystidia and larger basidia and basidiospores.

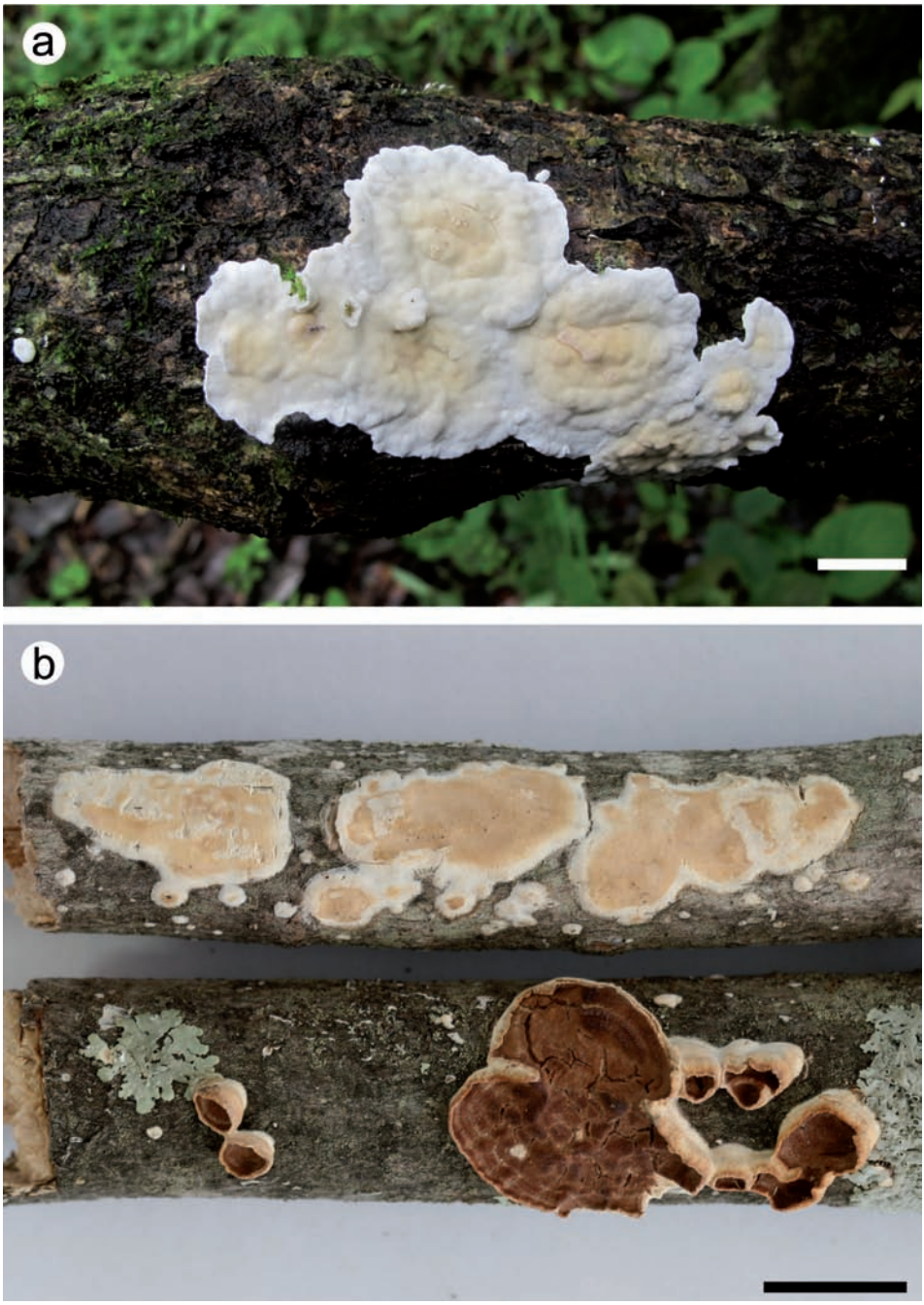

Fig. 2. a. Basidiocarps of *Michenera incrustata* (He 2841, holotype). b. Basidocarps (upper branch) and conidiomata (lower branch) of *M. artocreas* (GH-2014). Scale bars = 1 cm.

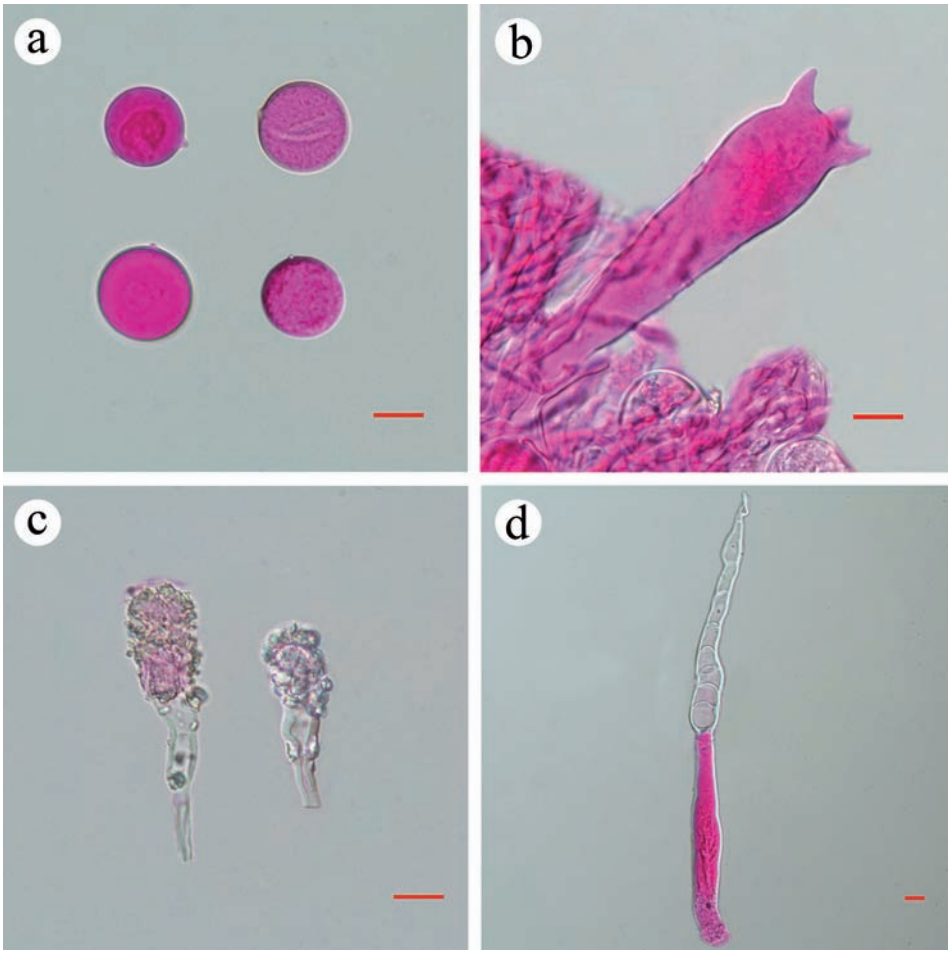

Fig. 3. Micrographs of microscopic structures of *Michenera incrustata* (He 2841, holotype) in phloxine. a. Basidiospores. b. Basidium. c. Encrusted cystidium. d. Gloeocystidium. Scale bars = 10 μm.

Holotypus: China, Yunnan Province, Binchuan County, Jizhushan Forest Park, on fallen angiosperm branch, 30 Aug 2015, He 2841 (holotype, BJFC 021276).

Etymology: "*incrustata*" (Latin), refers to the encrusted cystidia.

Fruitbody: Annual, discoid or resupinate, effused-reflexed, adnate with slightly detached margins, membranaceous, soft, flexible when fresh, becoming coriaceous, more or less brittle upon drying, beginning as small discoid colonies that become confluent, up to 15 × 3.5 cm, up to 1 mm thick, pileus narrow, up to 0.4 cm wide, glabrous, light orange [6A(4–5)] to greyish orange [6B(3–6)]; margin thinning out, incurved upon drying. Hymenophore smooth to tuberculate or undulating, pale orange (5A3), light orange

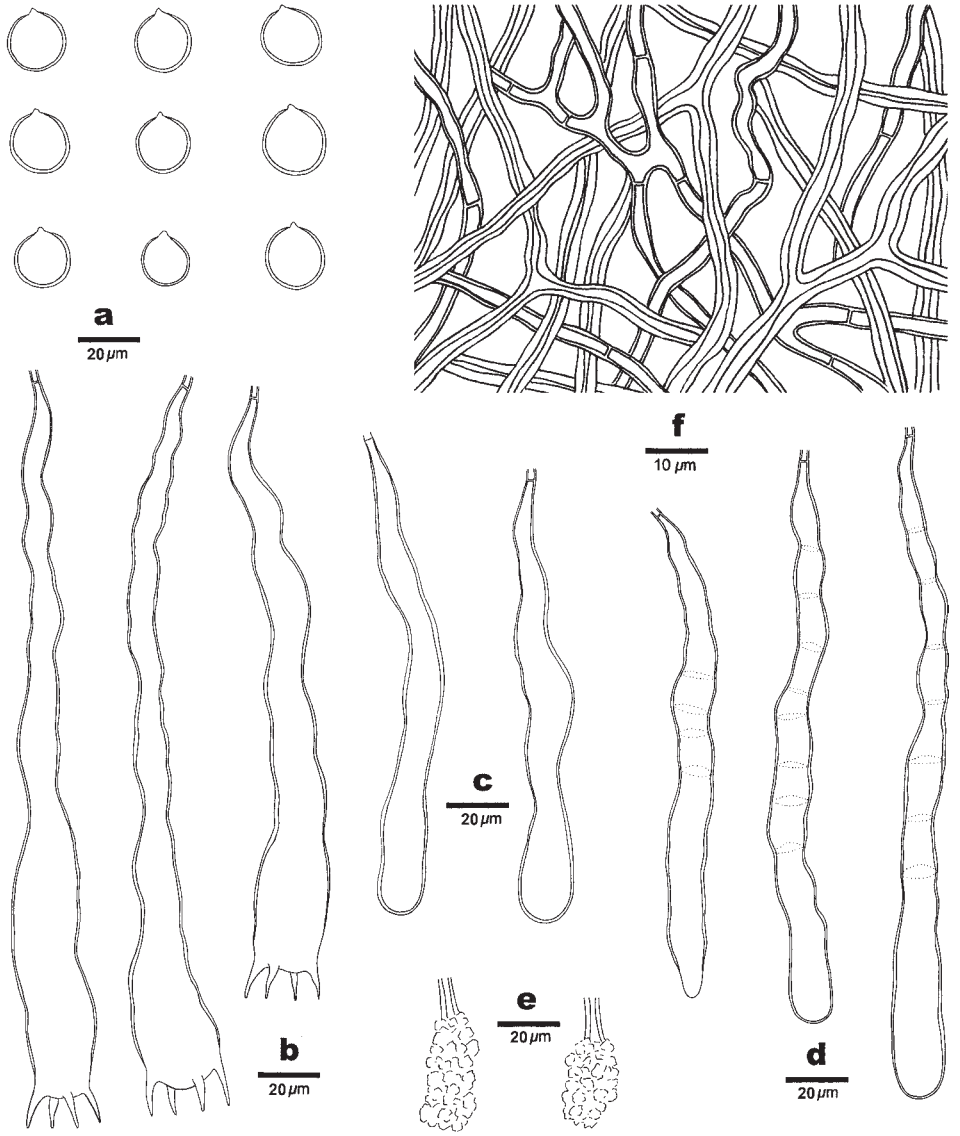

Fig. 4. Line drawings of microscopic structures of *Michenera incrustata* (He 2841, holotype). a. Basidiospores. b. Basidia. c. Basidioles. d. Gloeocystidia. e. Encrusted cystidia. f. Hyphae from subiculum.

[5A(4–5)], greyish orange [5B(3–6)] to orange [5B(7–8)], not cracking; margin thinning out, slightly paler or concolorous with hymenophore. Context white.

Microscopic structure: Hyphal system dimitic. Subiculum thick, composed of loosely interwoven, non-agglutinated generative and binding hyphae. Binding hyphae dominant, hyaline, distinctly thick-walled, aseptate, frequently branched, 2–5 µm in diam., not reacting in Melzer's reagent. Generative hyphae abundant, hyaline, thin- to slightly thick-walled, simple-septate, moderately branched, 2–4 µm in diam. Cystidia of two types (1) lamprocystidia abundant, embedded, clavate, simple-septate at base, thick-walled, hyaline, 35–70 × 10–15 µm (crystals included); (2) gloeocystidia numerous, embedded, cylindrical to subclavate, with a rounded or slightly tapered apex, simple-septate at base, usually stalked, slightly thick-walled, hyaline, smooth, empty or contents concentrated in upper part, usually with adventitious septa, 100–200 × 12–18 µm. Hyphidia in hymenium numerous, embedded, filamentous, straight to slightly sinuous, unbranched or occasionally branched, thin-walled, hyaline, 40–90 × 2.5–3.5 µm. Basidia clavate, elongate, somewhat sinuous, with a simple septum at base, slightly thick-walled, hyaline, with four sterigmata, 170–230 × 18–26 µm; basidioles similar but smaller. Basidiospores subglobose to globose with a distinct apiculus, thick-walled, hyaline, smooth, cyanophilous, not reacting in Melzer's reagent, (15–)17–22(–24) × (15–)16–21(–24) µm, L = 19.4 µm, W =18.6 µm, Q = 1–1.1 (n = 90/3).

Habitat: On dead but still attached or fallen, corticated branches of *Quercus* or other angiosperms; associated with a white rot.

Additional specimens examined:

Michenera incrustata: China, Fujian Province, Wuyishan County, Wuyishan Nature Reserve, on dead but still attached branch of *Quercus*, 6 Apr 2018, He 5368 (BJFC; ITS: MH204689, nrLSU: MH204690). Yunnan Province, Baoshan County, Gaoligongshan Nature Reserve, Baihualing, on fallen angiosperm branch, 30 Nov 2015, He 3370 (BJFC 021765); Binchuan County, Jizushan Forest Park, on dead *Quercus* branch, 30 Aug 2015, He 2815 (BJFC 021252); Jingdong County, Ailaoshan Nature Reserve, on fallen angiosperm branch, 24 Aug 2015, He 2573 (CFMR, BJFC 021026; ITS: MH142901); 25 Aug 2015, He 2616 (BJFC 021063; ITS: MH142902), He 2626 (CFMR, BJFC 021072; ITS: MH142903, nrLSU MH142908), He 2630 (BJFC 021075; ITS: MH142904, nrLSU: MH142907) & He 2636 (BJFC 021081; ITS: MH142905; nrLSU: MH142909); Mouding County, Huafoshan Nature Reserve, on bark of fallen *Quercus* branch, 25 Nov 2015, He 3146 (BJFC 021541); on fallen corticated angiosperm branch, 29 Oct 2017, KKN2017-58 (CFMR); Yongde County, Daxueshan Nature Reserve, Qingrengu, on fallen angiosperm branch, 28 Aug 2015, He 2784 (BJFC 021222).

Michenera artocreas: USA, Connecticut, on fallen branch of *Acer*?, 2014, GH-2014 (CFMR, BJFC; rDNA sequences from basidiocarp: ITS: MH142900, nrLSU: MH204691; rDNA sequences from conidiomata: ITS: MH204688, nrLSU: MH204692).

Aleurodiscus reflexus: Japan, Bungo Province (Oita Prefecture), on fallen branch of *Quercus*, 30 Oct 1920, A. Yasuda 650 (isotype, BPI 331243).

Aleurodiscus orientalis: Japan, Yetchu Province (Toyama Prefecture), on fallen branch of *Quercus glauca* Thunb., 10 Jan 1918, A. Yasuda 519 (holotype, BPI 331237).

Discussion

Morphologically, *M. artocreas* is distinguished from *M. incrustata* by its smaller basidia (70–100 × 13–15 µm) and basidiospores (16–19 × 14–16 µm) and lack of lamprocystidia (Lemke 1964). In addition, *M. artocreas* produces an imperfect stage that is absent in *M. incrustata*. Surprisingly, the ITS1–5.8S–ITS2 sequence of *M. incrustata* is up to 820 base pairs long; the ITS1 region accounts for most of the extra length that is significantly different from *M. artocreas* and other species in Peniophoraceae. The 5.8S–ITS2 sequence similarity between the two species (He 2841, holotype of *M. incrustata* and MG 230, *M. artocreas*) is 83% (of 437 base pairs) whereas nrLSU sequence similarity is 95.5% (of 870 base pairs). The two species formed a well-supported lineage sister to *Scytinostroma* in the maximum parsimony phylogenetic tree based on a combined dataset of 5.8S–ITS2 and nrLSU sequences (Fig. 1). The sequences obtained from conidiomata and basidiocarps of the same specimen (GH-2014) of *M. artocreas* are identical.

Aleurodiscus orientalis Lloyd and *A. reflexus* Yasuda were described from Japan on *Quercus* and considered synonyms of *M. artocreas* (as *L. subgiganteum*) by Lemke (1964) and Maekawa (1993) after studying type specimens. One of us (KKN) examined types of the two species deposited in BPI; no lamprocystidia were observed, therefore, we concur with Lemke's and Maekawa's synonymy. Because of the limitations of morphology, confirmation of the occurrence of *M. artocreas* in Japan by DNA sequence is desirable.

Species of *Aleurocystis* Lloyd ex G. Cunn. also have lamprocystidia and large non-amyloid basidiospores, but differ from *M. incrustata* by having a monomitic hyphal system and clamped generative hyphae (Cunningham 1956, Ryvarden 2010, Giraldo et al. 2017). Moreover, ITS and nrLSU sequences in MegaBLAST searches in the GenBank database show that *Aleurocystis* belong in the Agaricales (Giraldo et al. 2017).

Acknowledgments

The authors would like to express their deep thanks to the two anonymous reviewers for providing many suggestions to improve the manuscript. This study was supported by the National Natural Science Foundation of China (Nos. 31670013 & 31470144).

References

Berkeley, M.J. & Curtis, M.A. (1868): Fungi Cubenses (Hymenomycetes). – Journal of the Linnean Society. Botany 10: 280–392.

Cunningham, G.H. (1956): Thelephoraceae of New Zealand. Part IX. The genus *Stereum*. Part X. The genera *Cytidia* and *Aleurocystis*. Part XI. The genus *Aleurodiscus*. – Transactions and Proceedings of the Royal Society of New Zealand 84: 201–268.

Dhingra, G.S., Priyankda & Kaur, J. (2011): A checklist of resupinate, non-poroid agaricomycetous fungi from north-east India and Bhutan. – Synopsis Fungorum 29: 22–70.

Giraldo, A., Crous, P.W., Schumacher, R.K., Cheewangkoon, R., Ghobad-Nejhad, M. & Langer, E. (2017): The Genera of Fungi – G3: *Aleurocystis*, Blastacervulus, *Clypeophysalospora*, *Licrostroma*, *Neohendersonia* and *Spumatoria*. – Mycological Progress 16: 325–348.

Katoh, K. & Toh, H. (2008): Recent developments in the MAFFT multiple sequence alignment program. – Briefings in Bioinformatics 9: 286–298.

Kornerup, A. & Wanscher, J.H. (1978): Methuen Handbook of Colour. 3rd ed. – E. Methuen and Co., Ltd., London.

Larsson, K.H. (2007): Re-thinking the classification of corticioid fungi. – Mycol. Res. 111: 1040–1063. doi: 10.1016/j.mycres.2007.08.001

Larsson, E. & Larsson K.H. (2003): Phylogenetic relationships of russuloid basidiomycetes with emphasis on aphyllophoralean taxa. – Mycologia 95: 1037–1065.

Lemke, P.A. (1964): The genus *Aleurodiscus* (sensu lato) in North America. – Canadian Journal of Botany 42: 723–768.

Liu, S.L., Nakasone, K.K., Wu, S.H., He, S.H. & Dai, Y.C. (2018): Taxonomy and phylogeny of *Lopharia* s.s., *Dendrodontia*, *Dentocorticium* and *Fuscocerrena* (Basidiomycota, Polyporales). – MycoKeys 32: 25–48.

Lyman, G.R. (1907): Culture studies on polymorphism of Hymenomycetes. – Proceeding of the Boston Society of Natural History 33: 125–209 + plates 118–126.

Maekawa, N. (1993): Taxonomic study of Japanese Corticiaceae (Aphyllophoraceae) I. – Reports of the Tottori Mycological Institute 31: 1–149.

McNeill, J., Barrie, F.R., Buck, W.R., Demoulin, V., Greuter, W. et al. (eds.) (2012): The International Code of Nomenclature for algae, fungi, and plants ("Melbourne Code"). [Regnum Vegetabile 154: 1–208]. – Königstein, Koeltz Scientific Books. http://www.iapt-taxon.org/nomen/main.php

Patouillard, N. (1891): Remarques sur l'organisation de quelques champignons exotiques. – Bulletin de la Société mycologique de France 7: 42–49.

Patouillard, N. (1900): Essai taxonomique sur les familles et les genres des Hyménomycètes. – Lons-le-Saunier, Lucien Declume, 184 p.

Peirce, G.J. (189)0: Notes on *Corticium oakesii*, B. & C., and *Michenera artocreas*, B. & C. – Bulletin of the Torrey Botanical Club 17: 301–310.

Ryvarden, L. (2010): Stereoid fungi of America. – Synopsis Fungorum 28: 1–206.

Swofford, D.L. (2002): PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. – Sinauer Associates, Sunderland, Massachusetts.

Thiers, B., continuously updated – Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/

White, T.J., Bruns, T.D., Lee, S. & Taylor J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.) PCR protocols, a guide to methods and applications. Academic Press, Inc., San Diego, pp. 315–322.

Wu, S.H. (2008): Twenty species of corticioid fungi newly recorded from China. – Mycotaxon 104: 79–88.

Manuscript received: April 13, 2018
Revisions required: May 17, 2018
Revised version received: May 18, 2018
Accepted: June 4, 2018