A new species of *Neomensularia* (Hymenochaetales, Basidiomycota) from China

Ji XH  Wu F*

Institute of Microbiology, PO Box 61, Beijing Forestry University, Beijing 100083, China

*Corresponding authors. E-mail: fangwubjfu2014@yahoo.com Tel. & fax number: +86-10-62336309


Abstract

*Neomensularia rectiseta* sp. nov. is described and illustrated from China based on morphological characters and molecular evidence. It produces perennial, pileate basidiocarps with concentrically sulcate and zonate pileal surface, a duplex context, subulate and straight hymenial setae, and yellowish basidiospores. The new species macroscopically resembles *N. duplicata*, which differs in having annual fruiting bodies, ventricose and hooked hymenial setae, and occurs in tropical forests. In LSU and ITS based phylogenies, the new species formed a distinct lineage in the *Neomensularia* clade.

Key words – phylogenetic analysis – polypore – taxonomy

Introduction

Hymenochaetaceae is one of the most important families of Basidiomycota because it accommodates medicinal species (Dai et al. 2009) and forest pathogens (Dai et al. 2007) and its functioning in forest ecosystems (Zhou et al. 2011, Zhou & Dai 2012, Dai et al. 2015). Therefore, the taxonomy of this family is important. Molecular phylogenetic studies in last two years have revealed some new genera (Rajchenberg et al. 2015, Zhou 2015a, Wu et al. 2016, Zhou et al. 2016a, b) and many new species (Cui et al. 2015, Wu et al. 2015a, b, Zhou 2015b, c, d, e, 2016, Zhou et al. 2016c, etc.) in Hymenochaetaceae around the world. Of the newly erected genera in this family, *Neomensularia* is the latest one, which was typified with *N. duplicata* (Wu et al. 2016). Two additional species, viz. *N. crocitincta* and *N. kanehirae*, were also referred to this genus (Wu et al. 2016). *Neomensularia* is characterized by annual, pileate, brown to fuscous fruiting bodies, a duplex context, a dimitic hyphal structure, ventricose and hooked hymenial setae, and golden yellow, thick-walled, smooth basidiospores (Wu et al. 2016).

Hunan, surrounded by mountains on three sides, is located in southern China and has a typical subtropical humid climate with mostly evergreen broad-leaved forests (Dai et al. 2003). When reexamining the polypore specimens collected from Hunan in 2014, two specimens, close to *Neomensularia*, were found. Based on both morphological and phylogenetic analyses, they are described as a new member of *Neomensularia* in the present paper. Accordingly, the concept of *Neomensularia* is emended.

Materials & Methods
Morphological studies

Studied specimens are deposited in the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC). The microscopic procedure follows He and Li (2011, 2013). Special colour terms followed Petersen (1996). Spores were measured from sections cut from the tubes. 5% of measurements were excluded from each end of the range, and were given in parentheses. The following abbreviations were used: KOH = 5% potassium hydroxide; IKI = Melzer’s reagent; IKI− = neither amyloid nor dextrinoid; CB = Cotton Blue; CB+ = cyanophilous; L = mean spore length (arithmetic average of all spores); W = mean spore width (arithmetic average of all spores); Q = the ratios of L/W; n = number of spores measured from given number of specimens. Specimens are deposited in the herbaria of the Institute of Microbiology, Beijing Forestry University (BJFC).

Molecular study

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain PCR products from dried specimens. ITS4 and ITS5 were used as primers for ITS. LR0R and LR7 were used as primers for nLSU. The PCR procedure for ITS was as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min, and a final extension of 72°C for 10 min. The PCR products were purified and sequenced in Beijing Genomics Institute, China, with the same primers.

Phylogenetic analysis

To explore the phylogenetic position of the two newly sequenced specimens, their LSU sequences were compiled to the LSU dataset in Wu et al. (2016). In addition, the phylogenetic results from Rajchenberg et al. (2015) and Zhou et al. (2016a) are also used in the current analysis. Hyphodontia pallidula (Bres.) J. Erikss. was selected as the outgroup. The phylogenetic affinity of the two newly sequenced specimens was further evaluated by adding their ITS sequences and those of the Fulvifomes clade in Zhou (2015c) and Neomensularia in Wu et al. (2016) into a single dataset.

The two datasets were aligned by MAFFT 7.110 with the G-ini-i option (Katoh & Standley 2013). Sequence alignments were deposited at TreeBase. The best-fit evolutionary models of the two resulted alignments were estimated using jModelTest 2.1.4 (Guindon & Gascuel 2003, Darriba et al. 2012). According to the estimated models, the two alignments were phylogenetically analyzed using maximum likelihood (ML) and Bayesian inference (BI) algorithms. The ML trees were constructed using raxmlGUI 1.2 (Stamatakis 2006, Silvestro & Michalak 2012) with GTR + I + G and GTR + G models for the nLSU and ITS alignments, respectively. Bootstrap (BS) values were calculated under the auto FC option (Pattengale et al. 2010). BI algorithm was conducted using MrBayes 3.2 (Ronquist et al. 2012) with two independent runs, each with four chains for 10 million generations and starting from random trees. Trees were sampled every 1000th generation. The first 25% of sampled trees were discarded as burn-in, and other trees were used to construct a 50% majority consensus tree and for calculating Bayesian posterior probabilities (BPPs). Chain convergence was determined using Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer/).

Results

Molecular phylogeny

Two LSU and ITS sequences were newly generated for this study and their accession numbers in GenBank are labeled in the phylogenetic trees (Figs. 1, 2). The LSU dataset of 106 sequences resulted in an alignment of 925 characters with GTR+ I + G as the best-fit evolutionary model. The ML search stopped after 200 BS replicates. All chains in BI converged after 10 million generations, which was indicated by the effective sample sizes (ESSs) of more than 2000 for all parameters and the potential scale reduction factors (PSRPs) close to 1.000. The ITS dataset has 35
taxa with an alignment of 841 characters, and the HKY + G was estimated as the best-fit evolutionary model. The ML search stopped after 250 BS replicates. All chains in BI converged after 10 million generations, where the ESSs of all parameters were more than 6000 and the PSRFs were close to 1.000. The two algorithms generated nearly congruent topologies in major lineages for both datasets. Therefore, only the topologies from the ML algorithm are presented along with BS values from the ML algorithm and BPPs from the BI algorithm, if simultaneously above 50% and 0.8, respectively, at the nodes (Figs. 1, 2).

The phylogeny inferred from the LSU dataset shows that the two newly sequenced specimens Dai 15129 and Dai 15136 formed a terminal lineage with full statistical supports within the Neomensularia clade (97% BS, 1 BPP), and this lineage occupied a separate position from known species of Neomensularia (Fig. 1). The ITS-based tree rooted at midpoint indicates the fully supported lineage of Dai 15129 and Dai 15136 had a closer relationship with Neomensularia than with Fulvifomes, and occupied a separate position within the Neomensularia clade (Fig. 2). A combination of the phylogenetic topologies and morphological characters indicate Dai 15129 and Dai 15136 should be described as a new species of Neomensularia.

**Taxonomy**

*Neomensularia rectiseta* X.H. Ji, L.W. Zhou & F. Wu, sp. nov.  
Figs 3–4  
MycoBank 818173; Facesoffungi number: 02937

Etymology – Rectiseta (Lat.): referring to the rectus setae of the species.

Basidiocarps perennial, pileate, corky and without odour or taste when fresh, hard corky when dry. Pilei fan-shaped, projecting up to 12 cm, 5 cm wide and 2 cm thick at center. Pileal surface dark grey and margin buff-yellow, with distinctly concentric zones in different shades; margin acute. Pore surface dark brown to fuscous; sterile margin very narrow to almost lacking; pores circular, 8–9 per mm; dissepiments thick, entire. Context dark brown, up to 3 mm thick, duplex, separated by one black line. Tubes yellowish brown, distinctly paler than pores and context, up to 17 mm thick, tube layers distinctly stratified with intermittent context layers, individual tube layer up to 4 mm long.

Hyphal structure – Hyphal system dimitic; generative hyphae simple septate; skeletal hyphae dominant; tissue darkening but otherwise unchanged in KOH.

Context – Generative hyphae pale yellowish, slightly thick-walled, rarely branched, occasionally simple septate, 3–4 mm in diameter; skeletal hyphae dominant, yellowish to golden brown, thick-walled with a narrow to wide lumen, unbranched, interwoven, 3–5 μm diameter.

Tubes – Generative hyphae hyaline to pale yellow, thin- to slightly thick-walled, rarely branched, frequently simple septate, 2–4 mm in diameter; skeletal hyphae dominant, pale yellow, thick-walled with a wide to narrow lumen, unbranched, aseptate, loosely interwoven, 2.5–4 mm in diam. Setae frequent, subulate with a straight tip, arising from hymenium, dark brown, thick-walled, 15–25 × 6–12 μm; cystidia absent; fusoid cystidioles occasionally present; basidia more or less barrel-shaped, with four sterigmata and a simple septum at the base, 7–12 × 4–5 μm; basidioles in shape similar to basidia, but slightly smaller.

Spores – Basidiospores ellipsoid, yellowish brown, thick-walled, smooth, IKI–, CB+, (3.4–)3.5–4.0(–4.2) × (2.7–)3.0(–3.1) μm, L = 3.79 μm, W = 2.79 μm, Q = 1.30–1.42 (n = 60/2).


**Discussion**

*Neomensularia rectiseta* has pileate basidiocarps with a duplex context, a dimitic hyphal system,
Fig. 1 Phylogeny of *Neomensularia* inferred from LSU dataset. The topology is from the maximum likelihood method with bootstrap values from the maximum likelihood method and Bayesian posterior probabilities from Bayesian inference method, if simultaneously above 50% and 0.8, respectively, at the nodes. The newly sequenced specimens from species of *Neomensularia* are in boldface.
coloured and thick-walled basidiospores, which fit well with the concept of *Neomensularia sensu Wu et al. (2016). However, the perennial basidiocarps and straight hymenial setae make *N. rectiseta* different from other species of *Neomensularia*. Moreover, both LSU- and ITS-based phylogenies support *N. rectiseta* as a separated lineage from other species within the *Neomensularia* clade (Figs 1, 2). Here, we emend the concept of *Neomensularia* to accommodate *N. rectiseta* as a member of *Neomensularia* rather than introduce a new genus. Further phylogenetic
analyses referring to more samples related to Neomensularia might provide a clue to whether *N. rectiseta* belongs to Neomensularia or not.

*Fulvifomes* was previously considered to be a genus accommodating species both with and without setae (Dai 2010). Its circumscription was emended to include species without setae (Zhou 2014). This emendation was accepted by later study (Hattori et al. 2014); however, the phylogenetic positions of the excluded species bearing setae from *Fulvifomes* are uncertain. Recently, one of the excluded species *Fulvifomes kanehirae* was combined to *Neomensularia* (Wu et al. 2016). Because the phylogeny inferred from the LSU dataset places *N. rectiseta* in a separated position within the *Neomensularia* clade, the ITS dataset was used to further check whether the new species belongs to *Neomensularia* or *Fulvifomes*. The phylogeny inferred from the ITS dataset was rooted at midpoint and confirmed *N. rectiseta* had a closer relationship with *Neomensularia*, but distant from *Fulvifoems* (Fig. 2).

*Neomensularia rectiseta* is distinct from other species of *Neomensularia*. It differs mainly from *N. duplicata* by its perennial basidiocarps, and subulate and straight hymenial setae. *Neomensularia kanehirae* can be distinguished from *N. rectiseta* by the presence of ventricose and hooked hymenial setae, and larger pores (6–7 per mm; Dai 2010).
Fig. 4 Microscopic structures of Neomensularia rectiseta (drawn from the holotype). a: Basidiospores; b: Basidioles; c: Basidia; d: Cystidioles; e: Setae; f: Hyphae from trama; g: Hyphae from context.
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References
Dai YC, Cui BK, Yuan HS, Li BD. 2007 – Pathogenic wood-decaying fungi in China. Forest Pathology 37, 105–120.
He SH, Li HJ. 2013 – Pseudochaete latesetosa and P. subrigidula spp. nov (Hymenochaetales, Basidiomycota) from China based on morphological and molecular characters. Mycological Progress 12, 331–339.
Silvestro D, Michalak I. 2012 – raxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12, 335–337.
Zhou LW. 2014 – Fulvifomes hainanensis sp. nov. and F. indicus comb. nov. (Hymenochaetales, Basidiomycota) evidenced by a combination of morphology and phylogeny. Mycoscience 55, 70–77.
Zhou LW. 2015b – Four new species of Phylloporia (Hymenochaetales, Basidiomycota) from tropical China with a key to Phylloporia species worldwide. Mycologia 107, 1184–1192.
Zhou LW. 2015c – Fulvifomes imbricatus and F. thailandicus (Hymenochaetales, Basidiomycota): two new species from Thailand based on morphological and molecular evidence. Mycological Progress 14, 89.
Zhou LW. 2015d – Phellinopsis asetosa sp. nov. (Hymenochaetales, Basidiomycota) and an emended circumscription of Phellinopsis with a key to accepted species. Mycoscience 56, 237–242.
Zhou LW. 2015e – Phylloporia osmanthi and P. terrestris spp. nov. (Hymenochaetales, Basidiomycota) from Guangxi, South China. Nova Hedwigia 100, 239–249.
Zhou LW. 2016 – Phylloporia minutipora and P. radiata spp. nov. (Hymenochaetales, Basidiomycota) from China and a key to worldwide species of Phylloporia. Mycological Progress 15, 57.

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