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

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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 (PSRFs) 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, $15–25 \times 6–12$ μm; cystidia absent; fusoid cystidioles occasionally present; basidia more or less barrel-shaped, with four sterigmata and a simple septum at the base, $7–12 \times 4–5$ 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) × (2.5–)2.7–3.0(–3.1) μm, L = 3.79 μm, W = 2.79 μm, Q = 1.30–1.42 (n = 60/2).
     Material examined – CHINA. Hunan, Yizhang County, Mangshan Natural Reserve, on dead angiosperm tree, 16 August 2014, *Dai 15136* (Holotype in BJFC). CHINA. HUNAN: Yizhang County, Mangshan Natural Reserve, on fallen angiosperm tree, 16 August 2014, *Dai 15129* (BJFC) (paratype).

Discussion
Neomensularia rectiseta has pileate basidiocarps with a duplex context, a dimitic hyphal system,

Arambarria destruens KP347520 100/1
Arambarria destruens KP347521
Arambarria destruens KP347522 100/1
Arambarria destruens KP347523
Fomitiporella cavicola AY059052 80/0.98
Fomitiporella umbrinella AY059036 100/1
Fomitiporella caviphila KF729937
76/0.97
Inocutis ludoviciana AY059044
Inocutis dryophila AF311012 100/1
Inocutis tamaricis AF311021 83/1
Inocutis rheades AF311019 95/1
61/0.98
Phylloporia spathulata AF411822 78/1
Phylloporia pectinata AF411823
Phylloporia bibulosa AF411824 100/1
Phylloporia ephedrae AF411826
Phylloporia ribis AF311040
Fulvifomes indicus KC879259
Fulvifomes robiniae AF411825
99/1
Fulvifomes fastuosus AY059057
Fulvifomes kawakamii AY059028 95/1
Fulvifomes nilgheniensis AY059023 100/1
Inonotus hispidus AF311014 81/1
Inonotus andersonii AY059041 72/0.89
Inonotus cuticularis AF311010 95/1
Inonotus ulmicola AY059042 100/1
Inonotus obliquus AF311017
88/1
Tropicoporus guanacastensis KP030778 100/1
Tropicoporus cubensis KP030776 86/1
Tropicoporus tropicalis AY059037 72/0.99
Tropicoporus linteus JX467701
Sanghuangporus baumii AY059058
Sanghuangporus sanghuang KP030773 100/1
Sanghuangporus vaninii AY059056
Sanghuangporus weirianus AY059035
62/1
Mensularia nodulosa AF311013 68/0.97
Mensularia rhododendri KP420016 89/1
Mensularia hastifera AF311013 70/0.95
Mensularia lithocarpi KF684968 100/1
Mensularia radiata AF311018
Neomensularia rectiseta Dai 15129 100/1
Neomensularia rectiseta Dai 15136
Neomensularia crocitincta AY059043 97/1
Neomensularia kanehirae KX708223 85/1
Neomensularia duplicata KU573971
Neomensularia duplicata KU573972 100/1
Neomensularia duplicata KX708221
Phellinus laevigatus AF311034
Phellinus tremelae AF311042 99/1
Phellinus lundellii AF311035
Phellinus populicola AF311038 86/0.98
Phellinus igniarius AF311033 53/0.84
Fomitiporia punctata AF311007
Fomitiporia robusta AF311008 100/1
Fomitiporia hartigii AF311005
63/0.84
Pseudoinonotus chondromyelus AF311009
Pseudoinonotus dryadeus AF311011
Hymenochaete denticulata AF385155
Hymenochaete duportii AF323740 100/1
Hymenochaete rubiginosa AF323741
Hymenochaete cyclolamellata AF385163 86/1
Hymenochaete adusta AF385161 72/0.88
Hymenochaete porioides AF385164
67/0.99
Phellinopsis conchata AF311028 100/1
Phellinopsis overholtsii AY059019
Porodaedalea pini AF311037 85/0.96
Porodaedalea chrysoloma AF311026
Porodaedalea niemelaei AY059054 100/1
Porodaedalea cancriformans AY059029
Onnia tomentosa AF311023
Onnia triquetra AF311024
Onnia leporina AF311022
Hymenochaetopsis lamellata JQ279617
Hymenochaetopsis rigidula JQ279619 100/1
Hymenochaetopsis corrugata JQ279621
Hymenochaetopsis yasudai JQ279627
Hymenochaetopsis tabacina AF385145
Nothophellinus andinopatagonicus KP347530
Nothophellinus andinopatagonicus KP347529 100/1
Nothophellinus andinopatagonicus KP347531
Phellinidium noxium HQ328532 100/1
Pyrrhoderma adamantinum AY059031
Cylindrosporus flavidus KP875560 100/1
Cylindrosporus flavidus KP875561
Coltricia cinnamomea AF311003 87/0.99
Coltricia montagnei AY039683 71/0.94
Coltricia perennis AF287854
Coltriciella navisporus AY059062 100/1
Coltriciella dependens AY059059
Coltriciella oblectabilis AY059061 52/0.85
Coltriciella pusilla AY059060 89/1
Phellinidium fragrans AY059027 100/1
Phellinidium pouzarii AF311039 91/0.99
Phellinidium ferrugineofusca AF311031
Pyrrhoderma scaurum AY059030
Coniferiporia weirii AY059040 100/1
Coniferiporia sulphurascens AY059016 52/0.95
Fuscoporia ferrea AF311030
Fuscoporia viticola AF311044 97/1
Fuscoporia contigua AF311029 69/1
Fuscoporia ferruginosa AF311032
96/1
Asterodon ferruginosus AF323735
Phellopilus nigrolimitatus AF311036
Inonotopsis subiculosa AF311020
Oxyporus populinus AJ406467
Oxyporus corticola DQ873641 96/1
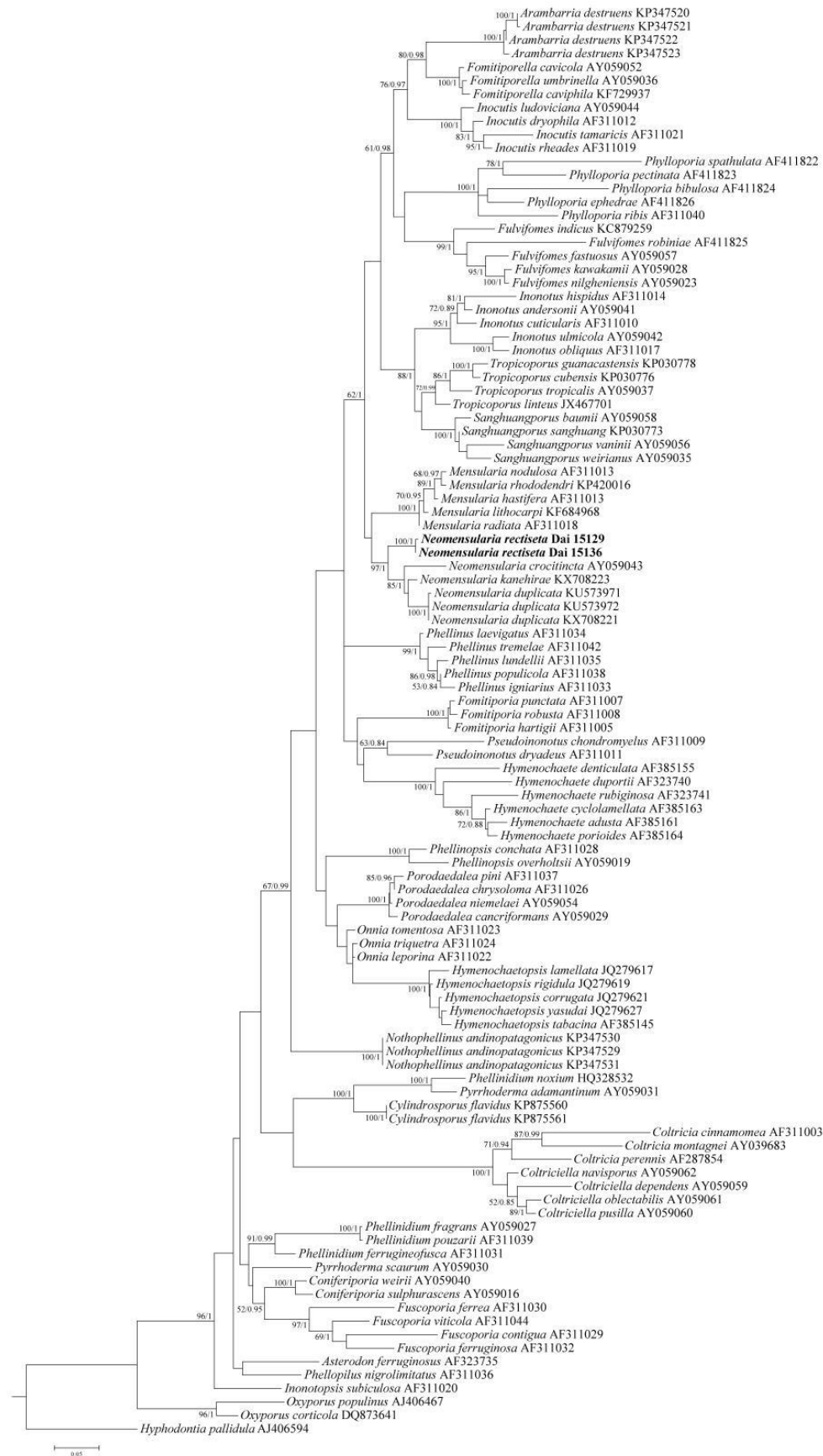*Hyphodontia pallidula* AJ406594

0.05

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.
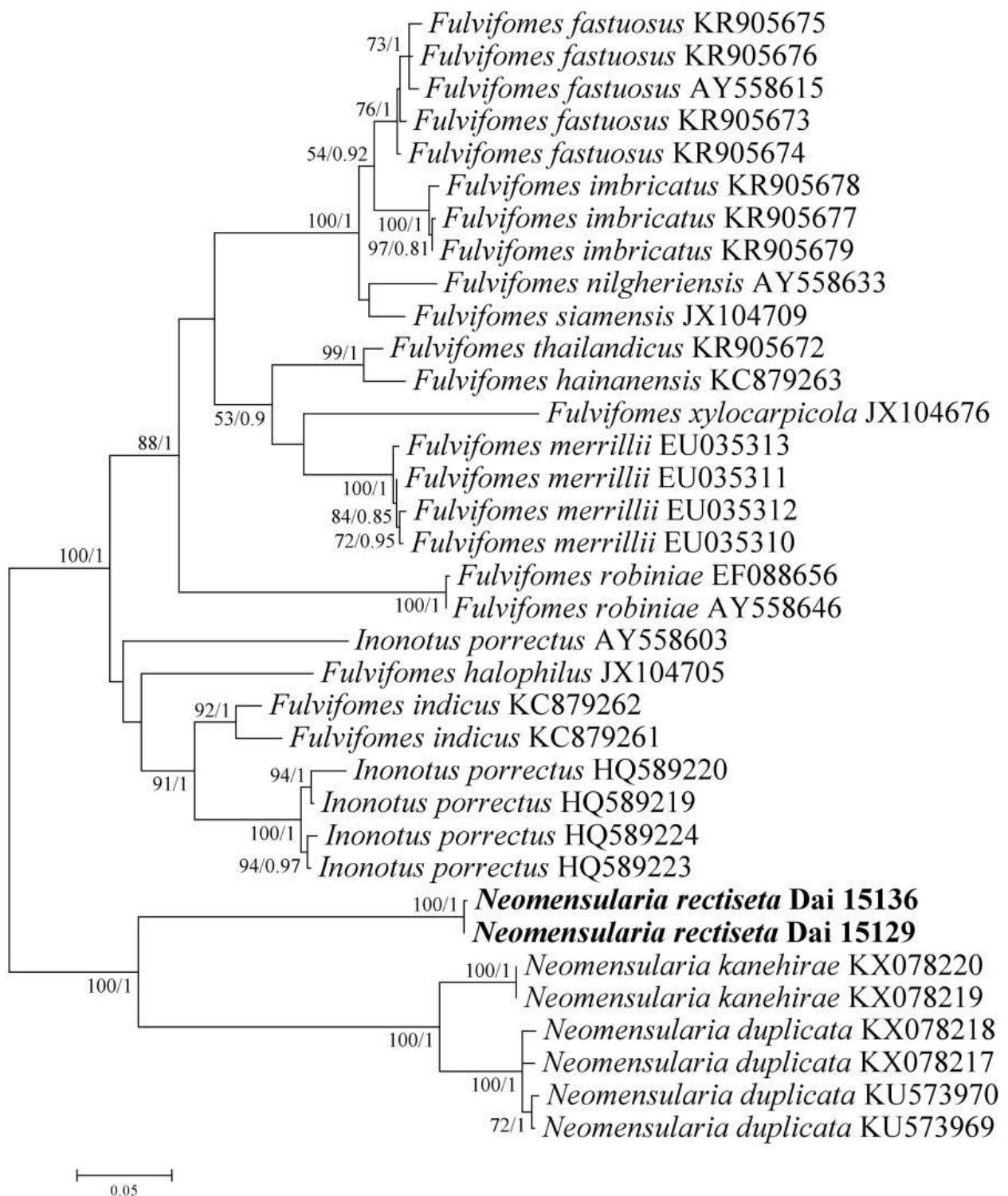
1045

Fig. 2 Phylogeny of *Neomensularia* inferred from ITS dataset. The topology is from the maximum likelihood method with bootstrap values from 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
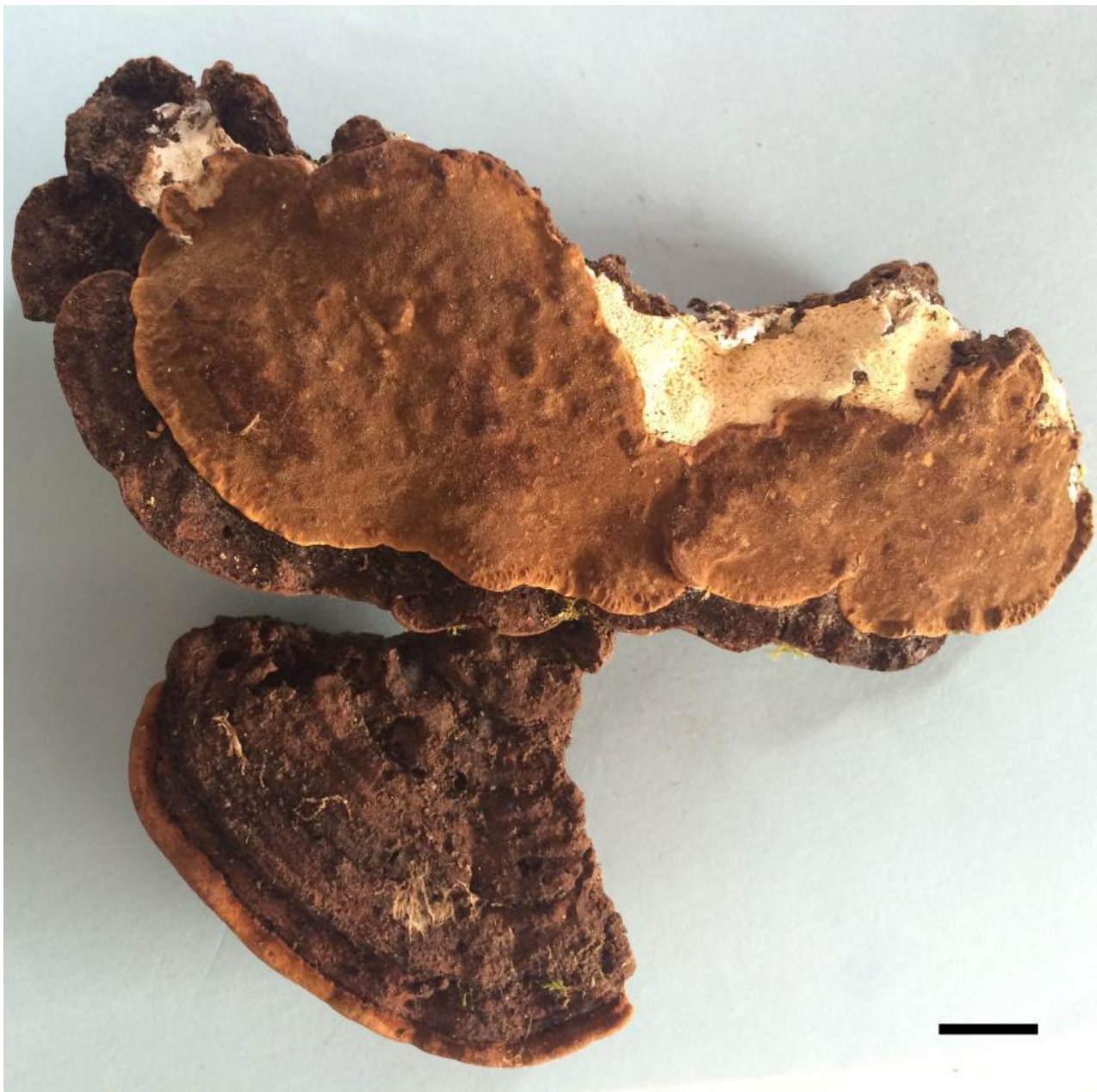
Fig. 3 Basidiocarps of *Neomensularia rectiseta* (holotype). Bar: 10 mm.

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).
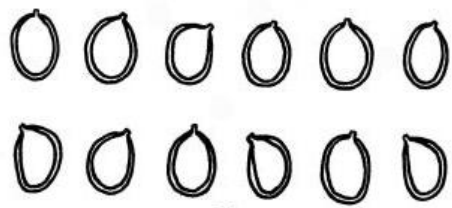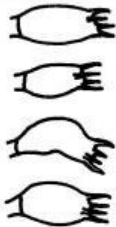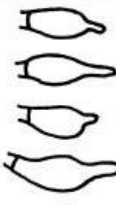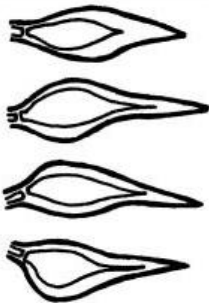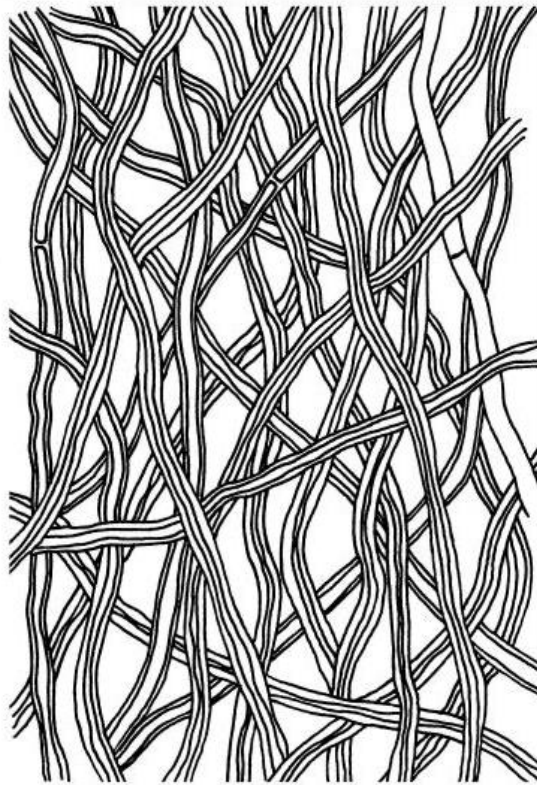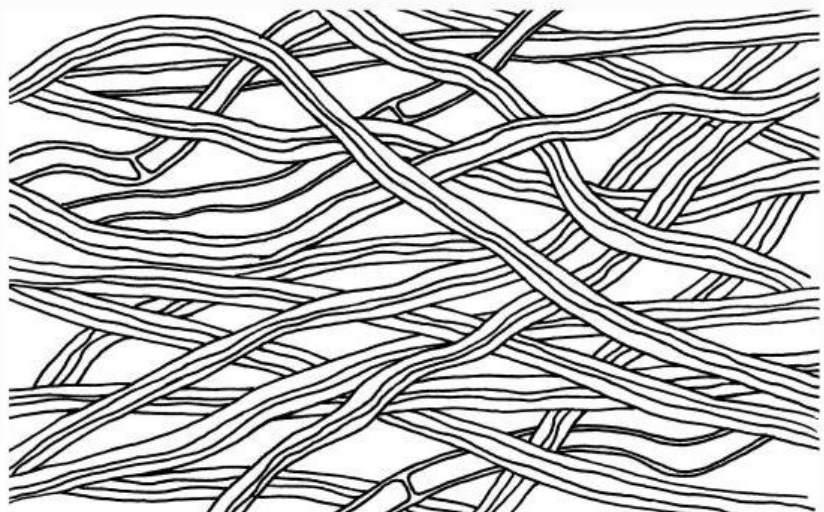
Fig. 4 Microscopic structures of *Neomensularia rectiseta* (drawn from the holotype). a: Basidiospores; b: Basidioles; c: Basidia; d: Cystidioles; e: Setae; f: Hyphae from trama; g: Hyphae from context.

Acknowledgements

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