

Morphotaxonomy and phylogeny of *Paoayensis lignicola* gen. et sp. nov. (ascomycetes) from submerged wood in Paoay Lake, Ilocos Norte, the Philippines

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Abstract – A new genus of freshwater ascomycetes, *Paoayensis lignicola* collected from Paoay Lake in Ilocos Norte, in the Philippines is described and illustrated and compared with analogous taxa. *Paoayensis lignicola* is characterized by immersed, slightly erumpent ascomata with openings that fuse into a single ostiole. Asci are unitunicate, clavate and short pedicellate with a discoid refractive apical ring and ascospores are lemoniform, brown to dark brown and with an unusual germ slit. Characters suggest that the genus should be placed in the *Sordariales*, however, placement at the familial level is not clear. Molecular based phylogenies using 18S rDNA sequence data indicates a close relationship to *Xylomelasma sordida* and *Ceratostomella pyrena* whose taxonomic placement is still obscure. 28S rDNA based phylogenies, on the other hand, depicts a close affiliation with members of the *Annulatasceae* which are freshwater ascomycetes. An appropriate familial placement for *Paoayensis lignicola* is still unknown (*Sordariomycetes incertae sedis*).

freshwater fungi / new species / lignicolous / LSU / Sordariaceae / Sordariales / SSU

INTRODUCTION

Freshwater ascomycetes are defined as ascomycetes which have been recorded in freshwater habitats and which complete part, or the whole of their lifecycle within freshwater environments (Shearer, 1993; Thomas, 1996; Wong *et al.*, 1998) and include lignicolous ascomycetes and their anamorphs that grow on wood, leaves or foam (Ho *et al.*, 2001; Tsui *et al.*, 2001; Jeewon *et al.*, 2003a; Cai *et al.*, 2003a; Sakayaroj *et al.*, 2005; Pascoal *et al.*, 2005; Gönczöl & Révay, 2006; Vijaykrishna *et al.*, 2004, 2005; Vijaykrishna & Hyde, 2006). According to

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this definition, in addition to species that function in water, transient fungi present in water and terrestrial fungi that release spores and are dispersed in water are all regarded as freshwater ascomycetes (Luo *et al.*, 2004). Freshwater ascomycetes inhabit submerged woody material in lentic (lakes, ponds, swamps, pools) and lotic (rivers, streams, creeks, brooks) habitats (Vijaykrishna *et al.*, 2006), playing an important role in recycling organic matter in the ecosystem. Shearer (1993) listed 288 fungi that had been recorded from freshwater habitats; this number has grown to 511 (Shearer, 2001; Cai *et al.*, 2003a). The number increased dramatically during the last ten years because numerous new taxa have been recorded from submerged wood in the tropical streams (e.g. Cai *et al.*, 2005a; Raja & Shearer, 2006; Vijaykrishna *et al.*, 2006) and to a lesser extent temperate streams (Hyde & Goh, 1999).

In this paper we describe an interesting ascomycete isolated from submerged wood collected in Paoay Lake, Ilocos Norte Province, in the Philippines. Superficially it resembles some genera of *Xylariaceae* with brown to dark brown ascospores and a germ slit. Microscopic examination found that in *Paoayensis lignicola* is very different from all genera of *Xylariaceae* and probably belongs in the *Sordariales*. The taxon is different among other genera of *Sordariales* because of its unique combination of morphological characteristics and is therefore introduced as a new genus and species here.

MATERIALS AND METHODS

Microscopic examination

Submerged wood and bamboo samples were collected from Paoay Lake, Paoay, Ilocos Norte Province, in the Philippines on 6 January 2006 and 3 February 2007. Paoay Lake covers less than 5 sq. km and is 10 m deep in the centre. It is 3 km from the sea, yet the surface is below sea level, and a subterranean source keeps the water fresh (Exconde, 2006). Samples collected were placed in sterile plastic bags and returned to the laboratory in The University of Hong Kong. Samples were incubated in plastic boxes lined with moistened paper towels at room temperature (~25°C) under normal light conditions and examined after 1-2 weeks for the presence of fungi, and then periodically over 6 months (Shenoy *et al.*, 2005, 2006). The moistened paper towels were placed beneath the samples to maintain humidity in the box. Material supporting *Paoayensis lignicola* were air-dried and preserved as herbarium specimens.

Molecular methods

DNA extraction was carried out using CTAB lysis buffer and phenol chloroform as outlined by Promputtha *et al.* (2005) and Kodsueb *et al.* (2006). Partial sequences from three different regions of the rDNA molecule (characterized by different rates of evolution) were amplified (further details in Cai *et al.*, 2006). Primers pairs NS1 (5'-GTA GTC ATA TGC TTG TCT C-3') and NS4 (5'-CTT CCG TCA ATT CCT TTA AG-3') were used to amplify a region spanning approximately 1200 nucleotides from the small subunit (18S) of the rDNA. LROR (5'- ACCCGCTGAACTTAAGC-3') and LR5 (5'-

TCCTGAGGGAAACTTCG-3') primer pairs were used to amplify a segment of the large 28S subunit (about 950 nucleotides) as outlined by Li *et al.* (2005). The amplification conditions were performed in a 50 µl reaction volume as outlined by Cai *et al.* (2005b) and Duong *et al.* (2006). DNA sequencing were performed using primers as mentioned above in an Applied Biosystem 3730 DNA Analyzer at the Genome Research Centre (University of Hong Kong).

Analytical methods

Partial sequences generated from the different primers were assembled using Bioedit (Hall, 1999). Once consensus DNA sequences were obtained from the different rDNA regions under investigation, a blast search was performed in GenBank to find the possible sister groups of the new taxa. DNA sequences were also submitted in GenBank. Based on the blast search results, putative taxa were selected as sister groups for further analyses. Further details on analyses are outlined in Jeewon *et al.* (2002, 2004). In addition, fungal members from *Halosphaeriales*, *Ophiostomatales*, *Sordariales* and *Xylariales* (*Amphisphaeriaceae*) were also included in the 18S and 28S datasets, while species from *Dothidea* and *Phaeosphaeria* were used as outgroups.

Phylogenetic analyses were conducted in PAUP* 4.0b10 (Swofford, 2004). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to maximize alignment. Analyses were done under different optimality criteria (Jeewon *et al.*, 2003b,c). Gaps were treated as missing in all analyses but the characters were also reweighted at different transitions transversion ratios to examine the effect weighting. Maximum Parsimony (MP) analyses were conducted using heuristic searches as implemented in PAUP, with the default options. One thousand pseudo-resamplings were performed, each with 10 replicates of random stepwise addition of taxa, to determine bootstrap support levels. Trees were figured in Treeview (Page, 1996).

RESULTS

Taxonomy

***Paoayensis* Cabanela, Jeewon & K.D. Hyde, gen nov.**

Mycobank 510824

Ascomata pseudostromata, immersa, globosa vel obpyriformes, ostiolata, papillata, paraphysaticum. Ostiola periphysata coadnata porum communem centram formantia. Asci 4-sporei, unitunicati, clavati, breves pedicellati, apparatus apicalis discoideus praediti, nonamyloidae. Ascosporeae uniseriatae, lemoniformae, (-0)1(-3) septatae, fissura germinativa brevis et recta praeditae".

Etymology: From "Paoay" referring to the lake where this fungus was collected and "ensis" meaning "pertaining to Paoay Lake".

Ascomata immersed, slightly erumpent through the host surface, although only papilla visible, globose to obpyriform, in small groups with a common ostiole, developing beneath a dark brown to black extensive pseudostroma. Ostiole central, short, brownish-black and periphysate. Peridium comprising several layers of brown cells, darker externally and lighter or hyaline

and compressed internally. Paraphyses tapering distally, septate, rarely branching and early deliquescing. Asci 4-spored, unitunicate, clavate, short pedicellate, with discoid refractive apical ring, very early deliquescing and rarely seen. Ascospores uniseriate or slightly overlapping, lemoniform, irregularly septate, brown, dark-brown at maturity, germ slit short and arising from the base perpendicular to the ascospores, smooth-walled and lacking a mucilaginous sheath.

Type species: Paoayensis lignicola Cabanela, Jeewon & K.D. Hyde

***Paoayensis lignicola* Cabanela, Jeewon & K.D. Hyde, sp. nov.**

Mycobank 510825.

(Figs 1-10)

Ascomata 546-626 μm *alta*, 520-586 μm *diametro*, *immersa*, *erumpentes*, *globosa* to *obpyriformes*, *ostiolata*, *papillata*, *paraphysata*, *pseudostromaticum*. *Ostiola periphysata coadnata porum communem centralem formantia*. *Asci* 45-130 \times 13-35 μm , *4-sporei*, *unitunicati*, *clavati*, *breve pedicellati*, *apparatu apicali discoideus praediti*, *nonamyloidae*. *Ascosporeae* 53-90 \times 30-58 μm , *lemoniformes*, *(-0)1(-3)-septatae*, *brunneae*, *fissura germinativa breve et recta praeditae*.

Etymology: “*lignicola*” in reference to the occurrence on wood.

Ascomata 546-626 μm *high*, 520-586 μm *diam*, *immersed*, slightly *erumpent* through the host surface, *papilla* only visible, *globose* to *obpyriform*, in small groups with common *ostiole*, developing beneath a dark brown to black extensive *pseudostroma* (Figs 1, 10). *Ostiole* central, short, brownish-black and *periphysate*. *Peridium* comprising several layers of light brown to brown cells which are darker externally and lighter to hyaline and compressed internally (Fig. 2). *Paraphyses* 3-10 μm in *diam.*, tapering distally, septate, rarely branching and early deliquescing (Fig. 5). *Asci* 45-130 \times 13-35 μm (\bar{x} = 90 \times 23 μm , *n* = 30), 4 spored, unitunicate, clavate, short pedicellate, with discoid refractive apical ring, very early deliquescing and rarely seen (Fig. 6). *Ascospores* 53-90 \times 30-58 (\bar{x} = 77 \times 42 μm , *n* = 60), uniseriate or slightly overlapping, lemoniform, (-0)1(-3)-septate, first septum formed near the base, second septum central, third septum near the rounded apex, brown, dark-brown at maturity, germ slit 10-12 μm long, not full length, arising from the base, perpendicular to the ascospore, smooth-walled, and lacking a mucilaginous sheath (Figs 7-9).

Distribution: PHILIPPINES.

Anamorph: Unknown.

Habitat: on submerged wood.

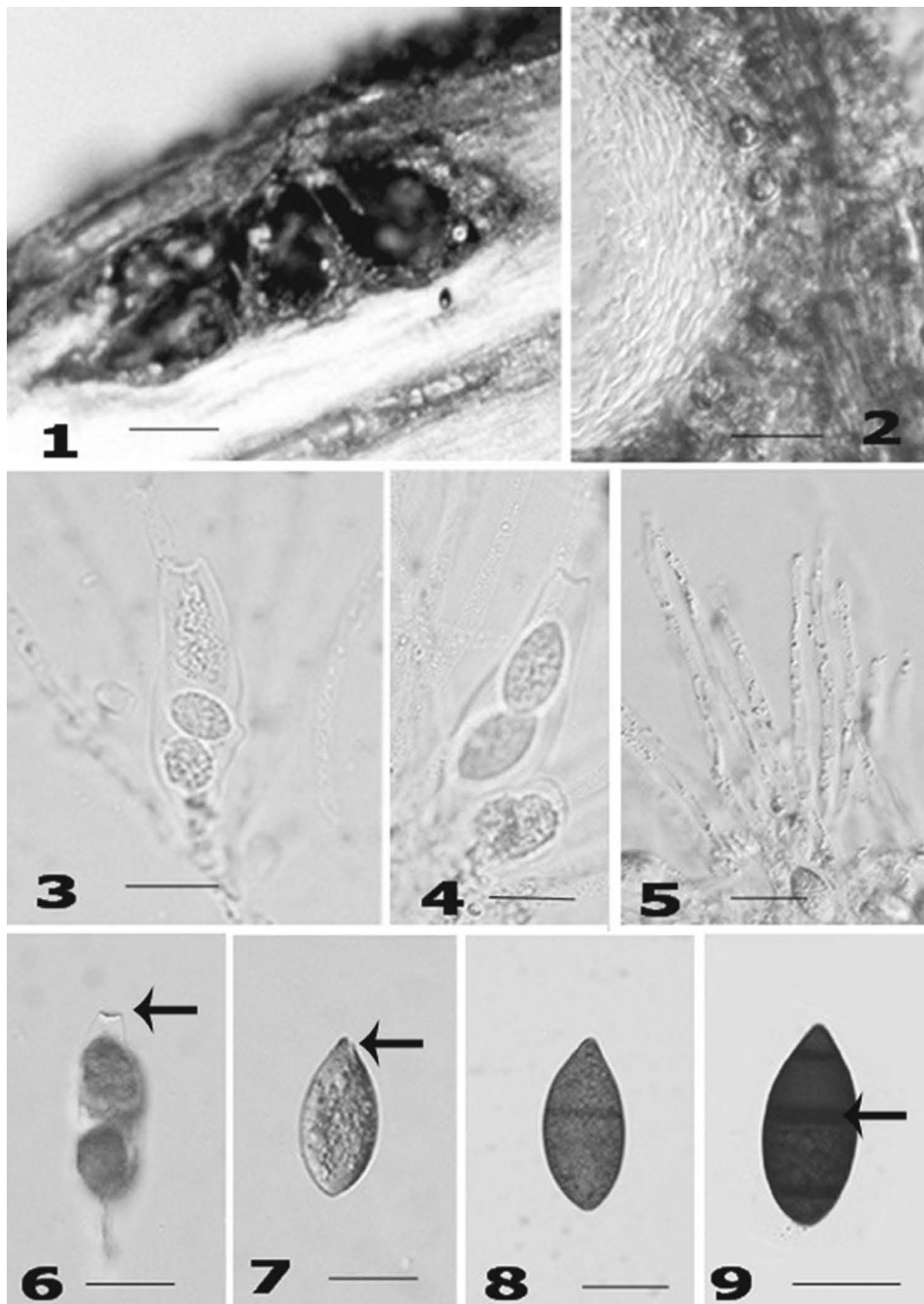
Material examined: Philippines, Ilocos Norte Province, Paoay, Paoay Lake (latitude 18.12.00; longitude 120.53.00), on submerged wood, 6 January 2006, M.V. Cabanela (HKU(M) 17516; **holotype**); extype living culture HKUCC 10927.

Isotypes: Philippines, Ilocos Norte Province, Paoay, Paoay Lake (latitude 18.12.00; longitude 120.53.00), on submerged wood, 31 January 2007, M.V. Cabanela MRC 00801, MRC 00802.

GenBank accession Numbers are EF622536 and EF622535 (28S and 18S rDNA respectively).

DISCUSSION

The aim of this paper is to describe *Paoayensis lignicola* as a new taxon, discuss its affinities with morphologically similar fungi and its phylogenetic relatedness based on rDNA sequence data.



Figs 1-9. *Paoayensis lignicola* (from holotype). **1.** Section of the ascomata. **2.** Peridium. **3-4.** Asci and paraphyses. **5.** Paraphyses. **6.** Asci apical ring. Note arrow showing apical ring. **7-9.** Ascospores. Note arrows pointing to germ slit (7) septa (9). Bars: 1 = 400 μm , 3-9 = 40 μm ; 2 = 200 μm .

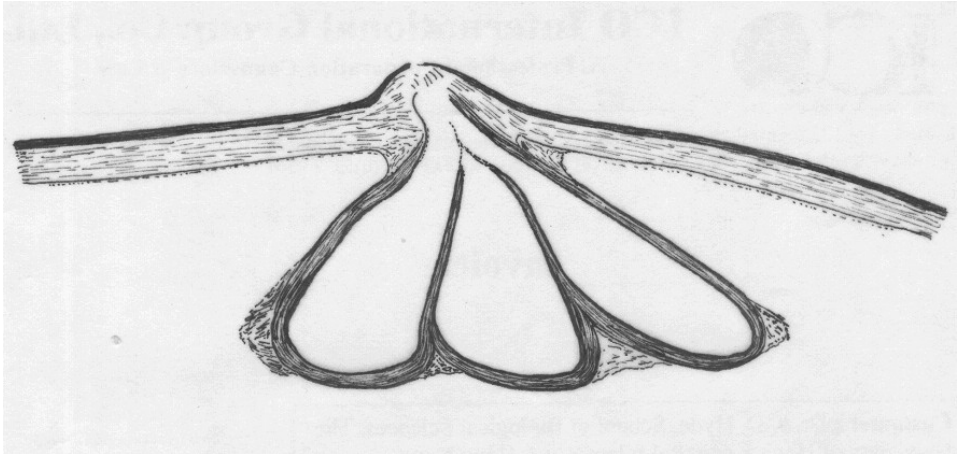


Fig. 10. *Paoayensis lignicola* (drawn from holotype) showing groups of ascomata in a common ostiole.

Paoayensis lignicola differs among other genera of ascomycetes because of its unique combination of morphological characteristics. These include: (i) a common neck for a few grouped ascomata; (ii) early deliquescing unitunicate asci but with a refractive apical ring; (iii) relatively massive brown irregularly septate ascospores; and (iv) ascospores with a unique basal germ slit.

Genera of freshwater ascomycetes with species producing brown septate ascospores in unitunicate asci that could vaguely be confused with *Paoayensis* include *Ascomauritiana*, *Ascotaiwania*, *Jobellisia*, *Phaeonectriella*, *Submersisphaeria* and *Savoryella* (Ranghoo & Hyde, 1999; Cai *et al.*, 2006). *Ascomauritiana* has similar relatively massive ascospores and early deliquescing asci, but differ as the ascomata are relatively small and individual, asci lack an apical ring and ascospores lack a germ slit and are regularly septate (Ranghoo & Hyde, 1999). *Ascotaiwania*, *Jobellisia*, *Savoryella* and *Submersisphaeria* differ in having ascospores which lack a germ slit, cylindrical asci with a relatively massive refractive apical ring as well as individual, usually superficial ascomata (Cai *et al.*, 2006). The asci of *Phaeonectriella* are somewhat similar to those of *Paoayensis lignicola*, but in *Phaeonectriella* the ascospores are regularly septate, relatively small and lack a germ slit, and ascomata are individual and usually superficial (Cai *et al.*, 2006).

Because of the presence of a germ slit *Paoayensis lignicola* should also be compared to other genera of *Xylariaceae*. However, *Paoayensis lignicola* asci lack a J+ ring, instead have a refractive J- discoid ring which excludes it from the *Xylariaceae*.

Ribosomal DNA nuclear gene phylogenies clearly indicate that there is a close relationship between *Paoayensis lignicola* and other members of ascomycetes whose taxonomic position within the Sordariomycetes is still obscure. 18S rDNA phylogeny generated from Maximum Parsimony (Fig. 12) show that *Paoayensis lignicola* is closely related to *Xylomelasma sordida* and *Ceratostomella pyrena* but this relationship did not receive any statistical support. In addition these three taxa are nested in between the *Magnaporthaceae* and *Coniochaetaceae*

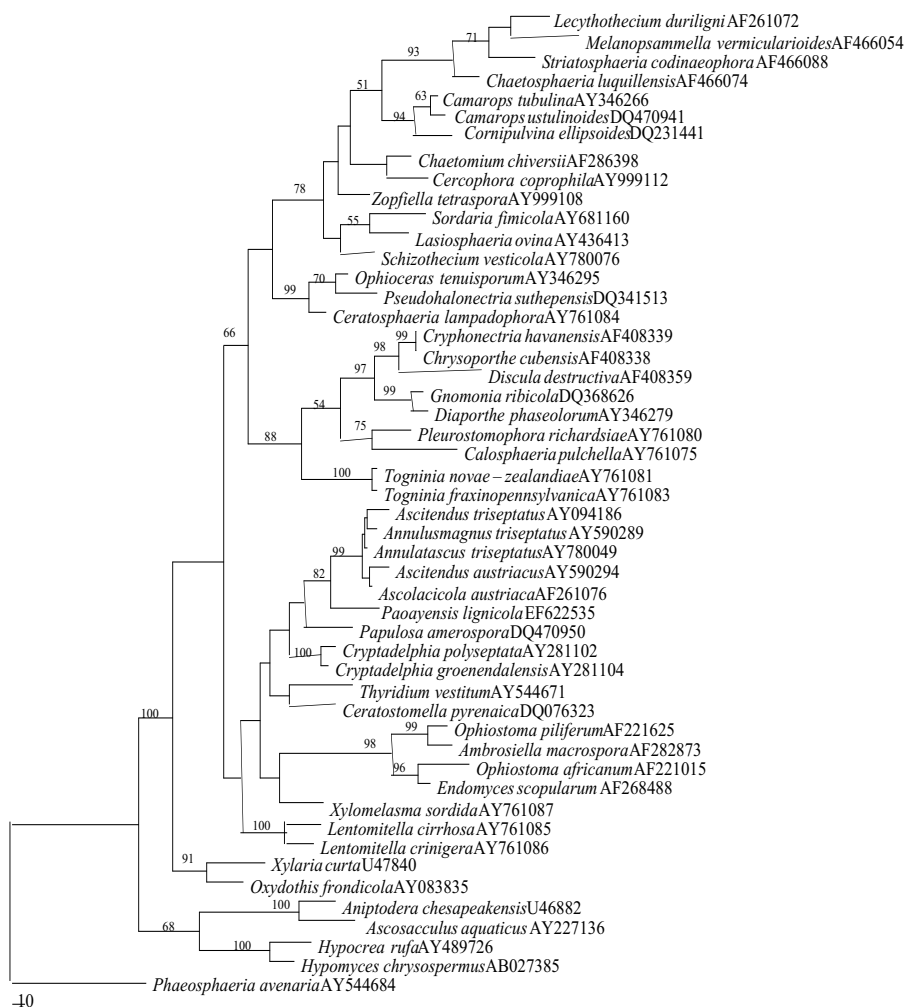


Fig. 11. 28S rDNA maximum parsimony analysis showing phylogenetic relatedness of *Paoayensis lignicola* to the other members of the Sordariomycetes. Outgroup is *Phaeosphaeria avenaria*.

but all these clades are weakly supported or did not receive any support and therefore any conclusive phylogenetic inference is not appropriate with this molecular dataset (Fig. 11). Morphologically, *Paoayensis lignicola*, *Xylomelasma sordida* and *Ceratostomella pyrena* are different from members of the *Magnaporthaceae* and *Coniochaetaceae*. Relationships based on rDNA sequence data of *Ceratostomella* and phenotypically similar fungi have already been detailed by Réblová (2006) and are not detailed here.

Phylogenies based on 28S rDNA sequence data, however suggest a slightly different evolutionary scenario. *Paoayensis lignicola* forms a sister taxon

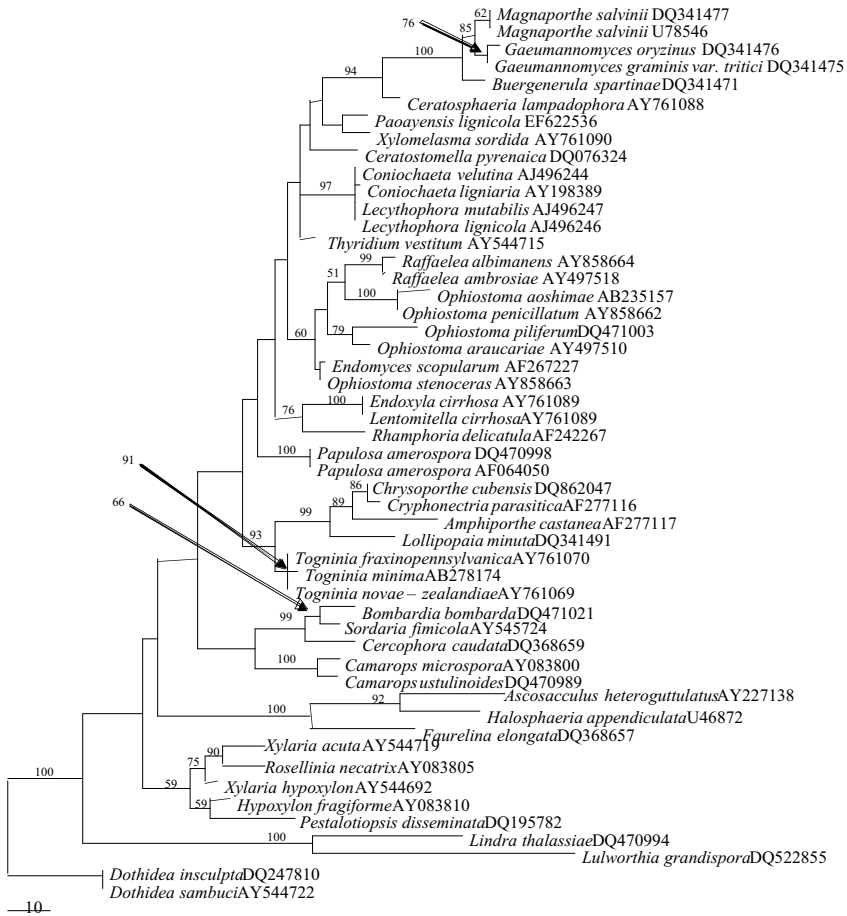


Fig. 12. Maximum parsimony tree based on partial 18S rDNA of *Paoayensis lignicola*. *Dothidea* species are the outgroups.

relationship with other members of freshwater fungi (*Annulatasceae*) with 82% bootstrap support (Fig. 11). Other related taxa include *Ceratostomella*, *Cryptodelphia*, *Papulosa* and *Thyridium* but the affinities of these taxa to *Paoayensis lignicola* is not statistically supported (similar results were obtained from the 18S rDNA dataset). It might be possible that phylogeny recovered from the 28S rDNA sequence data reflects true relationships and is more appropriate to discuss any evolutionary relationships. The *Annulatasceae* are mainly freshwater fungi with a wide range of distribution. Vijaykrishna *et al.* (2006) reviewed the evolutionary origin and molecular taxonomy of freshwater fungi and reported that freshwater ascomycetes have evolved separately through different lineages (not a monophyletic group) and that there are no exclusive morphologies to explain why some of these fungi are restricted and adapted to aquatic environments. In this study, *Paoayensis lignicola* has been collected from an

aquatic habitat and phylogenetically it appears to be related to other freshwater fungi but at present it is unknown whether it should be strictly considered as an aquatic fungus.

Although morphological examination suggests that *Paoayensis lignicola* is possibly linked to the *Sordariales*, molecular based relationships indicate that there is a close affinity to members of the *Annulatasceae* (freshwater fungi) and other fungi that have been not been accommodated in any families within the *Sordariomycetes*. Therefore, we would prefer to refer to *Paoayensis lignicola* as *Sordariomycetes incertae sedis*.

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