
Systematic revision of *Tubeufiaceae* based on morphological and molecular data

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The family *Tubeufiaceae* is circumscribed for taxa in the *Pleosporales* that possess superficial, white, pallid to bright, ascomata, which may darken at maturity. The family currently includes 21 genera with varied taxonomic histories, a result of disparate opinions regarding the importance of several different morphological characters. In this study, nucleotide sequences from 28S rDNA from different taxa of the *Tubeufiaceae* and allied families were analysed under different optimality criteria (Maximum Parsimony, Likelihood and Bayesian) to assess phylogenetic relationships. Phylogenies obtained using different tree construction methods yielded essentially similar topologies. Results from the molecular data do not correspond to established morphological schemes. Characters such as colour of ascomata, shape of ascospores and anamorphic taxa do not appear to be significant in delineating several genera within the *Tubeufiaceae*, while at the familial level, *Tubeufiaceae* does not appear to be restricted to those bitunicate fungi characterised by superficial, white and pallid to bright ascomata and filiform ascospores. In addition, phylogenies also indicate that the *Tubeufiaceae* is more closely related to the *Venturiaceae*, and therefore its current taxonomic placement within the order *Pleosporales* is justified. Results also indicate that *Acanthostigma*, *Boerlagiomyces* and *Letendraea* are phylogenetically unrelated to other members of the *Tubeufiaceae* and therefore it is highly possible that they should be excluded from the *Tubeufiaceae*. In this study, the family *Tubeufiaceae* is redefined based on existing morphological information and phylogenies.

Key words: *Ascomycota*, molecular systematics, morphological systematics, *Tubeufiaceae*

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Introduction

The family *Tubeufiaceae* was introduced by Barr (1979) to accommodate a number of bitunicate taxa in the *Pleosporales* with superficial, solitary or grouped, pallid or yellowish ascomata. The surface of the ascomata is smooth or roughened by protruding cells, setae or hyphal appendages. The anamorphs of the *Tubeufiaceae* are mostly helicosporous, while some are staurosporous or dictyosporous i.e. *Asteromella*, *Monodictys*-like, *Pendulispora* and *Tetracrium* (Barr, 1980, 1987a,b; Sivanesan, 1984). The *Tubeufiaceae* comprises over 81 species in 21 genera (Kirk *et al.*, 2001). Members of this family are predominantly saprobic, occasionally parasitic on foliicolous fungi, scale insects and rarely on living leaves (Barr, 1980). Recently two new lichenised species have been described, having symbiotic relationships with algae (Etayo, 2002).

Taxonomic relationships at the familial level have rarely been questioned, and intergeneric and intrageneric classification based on morphology is controversial. Some species have been transferred to, or synonymised to other genera in the *Tubeufiaceae*, while other species have been transferred or synonymised to allied genera in other families such as *Leptosphaeriaceae* (*Ophiobolus trichellus* = *Tubeufia trichella*), *Letendraceae*** (Eriksson, 1982), *Lophiostomataceae* (*Herpotrichia pezizula* = *Thaxteriella pezizula*), *Pleosporaceae* (*Letendraea* sp.), *Nectriaceae* (*Calonectria aurantiella* = *Tubeufia aurantiella*) and *Melanommataceae* (*Melanomma helicophilum* = *Thaxteriella pezizula*) [Barr, 1980; Sivanesan 1984; Scheuer, 1991, 1993; Kodsueb *et al.*, 2004].

Letendraea, *Melioliphila*, *Podonectria*, *Rebentischia*, *Thaxteriella* and *Tubeufia* were thought to be representative genera of the *Tubeufiaceae* (Barr, 1979). Barr (1987a) did not consider including *Melioliphila* and *Thaxteriella*, presumably because the latter is a synonym of *Tubeufia*. Various workers have since included additional genera in the *Tubeufiaceae* (see Table 1), which now encompasses between 18-21 genera (Eriksson and Hawksworth, 1998; Kirk *et al.*, 2001). Kirk *et al.* (2001) listed 21 genera and 14 synonyms while Eriksson (2005) list 23 genera (Table 1).

In this paper we set out firstly to assess the monophyly of the family *Tubeufiaceae* utilising 28S rDNA sequence data, and secondly to assess the phylogenetic significance of morphological characters used in intergeneric and intrageneric delineation. Based on the phylogenetic results, we propose a revision of the *Tubeufiaceae* as well as point out its affinities with other members of the *Pleosporales*.

**illegitimate or not validly published name

Table 1. Various classifications of *Tubeufiaceae*.

| Barr, 1979 | Eriksson and Hawksworth, 1986 | Rossmann, 1987 | Barr, 1987b |
|---------------------|--------------------------------------|-----------------------|---------------------|
| <i>Letendraea</i> | <i>Allonectria</i> | <i>Allonectria</i> | <i>Letendraea</i> |
| <i>Melioliphila</i> | <i>Byssocallis</i> | <i>Boerlagiomyces</i> | <i>Podonectria</i> |
| <i>Podonectria</i> | <i>Hyalocrea</i> | <i>Byssocallis</i> | <i>Rebentischia</i> |
| <i>Rebentischia</i> | <i>Letendraea</i> | <i>Letendraea</i> | <i>Tubeufia</i> |
| <i>Thaxteriella</i> | <i>Melioliphila</i> | <i>Malacaria</i> | |
| <i>Tubeufia</i> | <i>Paranectriella</i> | <i>Melioliphila</i> | |
| | <i>Podonectria</i> | <i>Paranectriella</i> | |
| | <i>Puttemansia</i> | <i>Podonectria</i> | |
| | ? <i>Rebentischia</i> | <i>Puttemansia</i> | |
| | ? <i>Thaxteriellopsis</i> | <i>Rebentischia</i> | |
| | <i>Tubeufia</i> | <i>Tubeufia</i> | |
| | | <i>Uredinophila</i> | |

| Eriksson and Hawksworth, 1998 | Kirk et al., 2001 | Eriksson, 2005 | This study |
|--------------------------------------|--------------------------|---------------------------|-----------------------------|
| <i>Acanthostigma</i> | <i>Acanthophiobolus</i> | <i>Acanthophiobolus</i> | <i>Acanthophiobolus</i> * |
| <i>Allonecte</i> | <i>Acanthostigmella</i> | <i>Acanthostigma</i> | <i>Acanthostigmella</i> * |
| <i>Boerlagiomyces</i> | <i>Allonecte</i> | <i>Acanthostigmella</i> | <i>Byssocallis</i> * |
| <i>Byssocallis</i> | <i>Amphinectria</i> | <i>Allonecte</i> | <i>Chaetocrea</i> * |
| <i>Glaxoa</i> | <i>Boerlagiomyces</i> | ? <i>Amphinectria</i> | <i>Chaetosphaerulina</i> * |
| <i>Letendraea</i> | <i>Borinquenia</i> | <i>Boerlagiomyces</i> | <i>Glaxoa</i> * |
| <i>Letendraeopsis</i> | <i>Byssocallis</i> | <i>Byssocallis</i> | <i>Letendraeopsis</i> * |
| <i>Malacaria</i> | <i>Chaetocrea</i> | <i>Chaetocrea</i> | <i>Malacaria</i> * |
| <i>Melioliphila</i> | <i>Glaxoa</i> | <i>Chaetosphaerulina</i> | <i>Melioliphila</i> * |
| <i>Paranectriella</i> | <i>Letendraea</i> | <i>Glaxoa</i> | <i>Paranectriella</i> * |
| <i>Podonectria</i> | <i>Letendraeopsis</i> | <i>Letendraea</i> | <i>Podonectria</i> * |
| <i>Puttemansia</i> | <i>Malacaria</i> | <i>Letendraeopsis</i> | <i>Puttemansia</i> * |
| <i>Rebentischia</i> | <i>Melioliphila</i> | <i>Malacaria</i> | <i>Rebentischia</i> * |
| <i>Taphrophila</i> | <i>Paranectriella</i> | <i>Melioliphila</i> | <i>Taphrophila</i> * |
| ? <i>Thaxteriellopsis</i> | <i>Podonectria</i> | <i>Paranectriella</i> | ? <i>Thaxteriellopsis</i> * |
| <i>Thaxterina</i> | <i>Puttemansia</i> | <i>Podonectria</i> | <i>Thaxterina</i> * |
| <i>Tubeufia</i> | <i>Rebentischia</i> | <i>Puttemansia</i> | <i>Tubeufia</i> |
| <i>Uredinophila</i> | <i>Taphrophila</i> | <i>Rebentischia</i> | <i>Uredinophila</i> * |
| | <i>Thaxterina</i> | <i>Taphrophila</i> | |
| | <i>Tubeufia</i> | ? <i>Thaxteriellopsis</i> | |
| | <i>Uredinophila</i> | <i>Thaxterina</i> | |
| | | <i>Tubeufia</i> | |
| | | <i>Uredinophila</i> | |

* not examined

Materials and methods

DNA extraction

Thirteen isolates of *Tubeufiaceae* [*Acanthostigma* (1 isolate), *Boerlagiomyces* (1 isolate), *Helicomycetes* (2 isolates), *Letendraea* (2 isolates), *Thaxteriella* (2 isolates), *Tubeufia* (5 isolates)] were selected for this study. Species names and accession numbers of the isolates used in this study are listed in Table 2. For each isolate, hyphal tips were subcultured and plated on potato dextrose agar and incubated at 25°C for 10-20 days prior to DNA extraction. Genomic DNA was extracted from fresh fungal mycelia following a protocol outlined by Jeewon *et al.* (2002, 2003, 2004) and Cai *et al.* (2005). Briefly, mycelia were scraped off from the surface of the plate. The mycelia were ground with 200 mg of sterilised quartz sand and 600 µl of 2X CTAB extraction buffer (2% w/v CTAB, 100 mM Tris-HCL, 1.4 M NaCl, 20 mM EDTA, pH 8) in a 1.5 ml eppendorf tube. The whole contents were incubated at 60°C in a water bath for 30 min with gentle swirling every 10 min. The solution was then extracted three times with an equal volume of phenol:chloroform (1:1) at 13,000g for 30 min until no interface was visible. The upper aqueous phase containing the DNA was precipitated by addition of 2.5 volumes of absolute ethanol and kept at -20°C overnight. The precipitated DNA was washed two times with 70% ethanol, then dried under vacuum and suspended in TE buffer (1mM EDTA, 10 mM Tris-HCl, pH 8.0), and treated with RNase (1 mg/ml) prior to DNA amplification.

DNA amplification and sequencing of 28S rDNA

Approximately 800-900 nucleotides at the 5' end of the 28S rDNA region were amplified by primer pairs LROR/LRO5 (Vilgalys and Hester, 1990). PCR was carried out in 50 µl reaction volume containing 31.7 µl sterile water, 5 µl of 10X Mg free PCR buffer, 3 µl of 25 mM MgCl₂, 4 µl of 2.5 mM deoxyribonucleotide triphosphate (dNTPs), 1.5 µl of each 10 µM primers, 3 µl of DNA template, 0.3 µl of 2.5 units of Taq DNA polymerase (Promega, Madison, WI). Typical amplification parameters were as follows; initial denaturation of 95°C for 3 min; 35 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 50 sec and extension at 72°C for 1 min; and then a final extension of 72°C for 10 min. Double-stranded DNA products were purified using GFXTM PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Catalog no. 27-9602-01) following manufacturer's protocol. Sequencing reactions were performed and sequences determined

Table 2. Fungal isolates used in this study.

| Species | Isolates/sources | GenBank Accession number | Family, order |
|--|-------------------------------|--------------------------|--|
| Ingroups | | | |
| <i>Acanthostigma perpusillum</i> | MUCL41721 | DQ296556 | <i>Tubeufiaceae</i> |
| <i>Boerlagiomyces websteri</i> | BCC3834 | DQ296553 | <i>Tubeufiaceae</i> |
| <i>Bimuria novae-zelandiae</i> | CBS107.79 | AY016356 | <i>Melanommataceae</i> |
| <i>Byssothecium circinans</i> | CBS675.92 | AY016357 | <i>Dacampiaceae</i> |
| <i>Capronia coronata</i> | ATCC56201 | AF050242 | <i>Herpotrichiellaceae</i> , <i>Chaetothyriales</i> |
| <i>Capronia parasitica</i> | CBS123.88 | AF050252 | <i>Herpotrichiellaceae</i> , <i>Chaetothyriales</i> |
| <i>Cochliobolus heterostrophus</i> | CBS134.39, AFTOL-ID 54 | AY544645 | <i>Pleosporaceae</i> |
| <i>Cunodia circinans</i> | J. Platt 232 | AY533013 | <i>Cudoniaceae</i> , <i>Rhytismatales</i> |
| <i>Cudonia lutea</i> | wz225 | AF433140 | <i>Cudoniaceae</i> , <i>Rhytismatales</i> |
| <i>Curvularia oryzae</i> | MRL1089 | AF163991 | <i>Mitosporic Cochliobolus</i> |
| <i>Dothidea ribesia</i> | CBS195.58 | AY016360 | <i>Dothideaceae</i> , <i>Dothideales</i> |
| <i>Dothidea sambuci</i> | CBS198.58 | AF382387 | <i>Dothideaceae</i> , <i>Dothideales</i> |
| <i>Exophiala dermatidis</i> | CBS748.88 | AF050270 | <i>Mitosporic</i> <i>Herpotrichiellaceae</i> |
| <i>Fonsecaea pedrosoi</i> | CBS271.37 | AF050276 | <i>Mitosporic Ascomycota</i> |
| <i>Helicomycetes macrofilamentosus</i> | HKUCC10235 | AY849942 | <i>Mitosporic Tubeufiaceae</i> |
| <i>Helicomycetes roseus</i> | BCC3381 | AY787932 | <i>Mitosporic Tubeufiaceae</i> |
| <i>Karstenula rhodostoma</i> | CBS690.94 | AY787933 | <i>Melanommataceae</i> |
| <i>Leptosphaeria coniothyrium</i> | ICMP10654 | AY849945 | <i>Leptosphaeriaceae</i> |
| <i>Leptosphaeria maculans</i> | ICMP13554 | AY849946 | <i>Leptosphaeriaceae</i> |
| <i>Letendreaa eurotioides</i> | CBS212.31 | AY787935 | <i>Tubeufiaceae</i> |
| <i>Letendreaa helminthicola</i> | CBS884.85 | AY016362 | <i>Tubeufiaceae</i> |
| <i>Letendreaa padouk</i> | CBS485.70 | AY849951 | <i>Tubeufiaceae</i> |
| <i>Lophiostoma caulium</i> | N/A | AF383953 | <i>Lophiostomataceae</i> |
| <i>Lophiostoma fuckelii</i> | CBS 101952 | DQ399531 | <i>Lophiostomataceae</i> |
| <i>Neofabraea malicorticis</i> | CLS003, AFTOL-ID 149 | AY544662 | <i>Dermateaceae</i> , <i>Helotiales</i> |
| <i>Ostropa barbara</i> | CBS260.58, AFTOL-ID 77 | AY584642 | <i>Stictidaceae</i> , <i>Ostropales</i> |
| <i>Phaeosphaeria avenaria</i> | OSC100096, AFTOL-ID 280 | AY544684 | <i>Phaeosphaeriaceae</i> |
| <i>Phaeosphaeria vagans</i> | CBS604.86 | AY849953 | <i>Phaeosphaeriaceae</i> |
| <i>Pleospora herbarum</i> var. <i>herbarum</i> | CBS191.86 | AF382386 | <i>Pleosporaceae</i> |
| <i>Preussia terricola</i> | OSC100098, AFTOL-ID 282 | AY544686 | <i>Sporormiaceae</i> |
| <i>Rhytisma acerinum</i> | J. Platt (DUKE) | AF356696 | <i>Rhytismataceae</i> , <i>Rhytismatales</i> |
| <i>Sarea resiniae</i> | Miadlikowska Aug. 1999 (DUKE) | AY640965 | <i>Agyriaceae</i> , <i>Agyriales</i> |
| <i>Setomelanomma holmii</i> | CBS110217 | AF525678 | <i>Phaeosphaeriaceae</i> |
| <i>Setosphaeria monoceras</i> | CBS154.26 | AY016368 | <i>Pleosporaceae</i> |
| <i>Spathularia flavida</i> | wz214 | AF433141 | <i>Cudoniaceae</i> , <i>Rhytismatales</i> |
| <i>Strangospora pinicola</i> | Roux 2001/01/12 (DUKE) | AY640970 | <i>Lecanoromycetes incertae sedis</i> |

Table 2 continued. Fungal isolates used in this study.

| Species | Isolates/sources | GenBank Accession number | Family |
|-------------------------------------|---------------------------|-----------------------------|---|
| Ingroups | | | |
| <i>Stylodothis puccinioides</i> | CBS193.58 | AY004342 | <i>Dothideaceae</i> , <i>Dothideales</i> |
| <i>Thaxteriella amazonensis</i> | ATCC42524 | AY787938 | <i>Tubeufiaceae</i> |
| <i>Thaxteriella helicoma</i> | JCM2739 | AY787939 | <i>Tubeufiaceae</i> |
| <i>Trematosphaeria heterospora</i> | CBS644.86 | AY016369 | <i>Melanommataceae</i> |
| <i>Tubeufia</i> sp. BCC3580 | BCC3580 | DQ296554 | <i>Tubeufiaceae</i> |
| <i>Tubeufia</i> sp. MLTS119 | MLTS119 | DQ296555 | <i>Tubeufiaceae</i> |
| <i>Tubeufia cerea</i> | IFO9014 | AY849964 | <i>Tubeufiaceae</i> |
| <i>Tubeufia cylindrothecia</i> | BCC3559 | AY849965 | <i>Tubeufiaceae</i> |
| <i>Tubeufia paludosa</i> | HKUCC9118 | AY849966 | <i>Tubeufiaceae</i> |
| <i>Venturia carpophila</i> | ICMP5402 | AY849967 | <i>Venturiaceae</i> |
| <i>Venturia hanliniana</i> | ATCC96019 | AF050290 | <i>Venturiaceae</i> |
| <i>Westerdykella cylindrica</i> | CBS454.72 | AY004343 | <i>Sporormiaceae</i> |
| Outgroups | | | |
| <i>Dyrithiopsis lakefuxianensis</i> | HKUCC7303 | AF452047 | <i>Amphisphaeriaceae</i> , <i>Xylariales</i> |
| <i>Xylaria acuta</i> | ATCC56487, AFTOL-ID 63 | AY544676 | <i>Xylariaceae</i> , <i>Xylariales</i> |

automatically in an Applied Biosystem 3730 Genetic Analyzer/Sequencer (Genome Research Centre, The University of Hong Kong) using PCR primers mentioned above.

Phylogenetic analyses

28S rDNA sequences from fifty taxa were aligned with the computer program Bioedit (Hall, 1999) and Clustal X (Thompson *et al.*, 1997) with default parameter settings and alignments. Gaps were inserted manually to optimise alignment in Se-AI (Rambaut, 1996). Genbank accession numbers of the nucleotide sequences used in this study is given in Table 2. Phylogenetic analyses of LSU rDNA were performed using maximum parsimony employing a heuristic search (1000 random replicates) in PAUP* v4.0b10 (Swofford, 2002). Ambiguously aligned regions were excluded from the phylogenetic analyses.

Maximum parsimony (MP) analyses were carried out treating gaps as missing and as fifth character (newstate) with the exclusion of ambiguously aligned portions. A third parsimony analyses was carried out under Gapcoder Method (Young and Healy, 2003), using simple indel coding (Simmons and Ochoterena, 2000), to automate incorporation of recoded indels into the maximum parsimony and Bayesian analyses. To evaluate the support for the

observed branching topologies for maximum parsimony, we performed bootstrap analysis (Felsenstein, 1985), with 1000 bootstrap. The model of substitution used for Bayesian and Maximum Likelihood (ML) was chosen using the program Mrmodeltest 2.2 (Nylander, 2004). Independent Bayesian phylogenetic analysis was performed in MrBayes 3.0 (Huelsenbeck and Ronquist, 2001) using a uniform GTR+I+G model, as selected by hLRT in Mrmodeltest 2.2: [GTR+I+G] Lset Base = (0.2404 0.2165 0.2949), Nst = 6, Rmat = (0.8944 3.0518 1.1695 0.7638 7.0842), Rates=gamma, Shape=0.5985 and Pinvar = 0.451. The Metropolis-coupled Markov chain Monte Carlo (MCMC) sampling approach was used to calculate posterior probabilities. Four simultaneous Markov chains, three heated and one cold, were run under a General Time Reversible (GTR) model of sequence evolution and gamma approximation for rate variation among sites. Chains were analysed with random starting trees for 10⁶ generations. Trees collected before the stable likelihood value point was discarded as “burnin” (Huelsenbeck and Ronquist, 2001). The remaining trees were used to build a majority rule consensus tree where the percentage of the remained ‘best’ trees supporting a branch represents the Bayesian posterior probabilities. For ML analyses, tree was inferred with PAUP* using the heuristic search option starting with a parsimony tree with random sequence addition. The Kishino-Hasegawa and Templeton tests were performed in order to determine whether the trees inferred under different optimality criteria were significantly different. Trees were viewed in Treeview (Page, 1996).

Results

28S rDNA phylogenies

The data set consisted of 50 taxa (Outgroup: *Dyrithiopsis lakefuxianensis* and *Xylaria acuta*) and 872 aligned nucleotide characters (951 characters for Gapcoder, including 79 additional regions), totally 48 characters were excluded, of which 243, 251 were phylogenetically informative. In the dataset, 541, 518 characters are constant and 40, 55 variable characters are parsimony-uninformative (missing, newstate). All characters were weighted equally. This data matrix has been deposited in treebase (Accession Number = SN2564). A heuristic search for 1000 replicates most-parsimonious trees with random addition of taxa that treated gaps as missing and as fifth character (newstate), both generated 4 equally parsimonious trees, which were similar in topology and not significantly different. Inclusion of 79 ambiguous regions from Gapcoder resulted in only 2 trees one of which is shown in Fig. 1. This gap

treatment resulted in better bootstrap support for some of the clades. This tree was, however, not different in topology as in previous analysis.

Fig. 1 can be divided into 7 distinct clades (Clades A-G). Clade A comprises two families; *Phaeosphaeriaceae* (*Phaeosphaeria vagans*, *P. avenaria* and *Setomelanomma holmii*) and *Leptosphaeriaceae* (*Leptosphaeria maculans*). Clade B comprises members of the *Pleosporaceae*. In Clade C, three species of *Letendraea* (*Tubeufiaceae*) cluster with *Bimuria* and *Karstenula* (*Melanommataceae*), *Byssothecium* (*Dacampiaceae*) and *Leptosphaeria* (*Leptosphaeriaceae*) with high bootstrap support. Clade D comprises members of *Lophiostomataceae* and *Sporormiaceae*.

Clade E consists of representatives of *Tubeufiaceae* and forms a monophyletic clade with high bootstrap support. This clade comprises 9 species from *Acanthostigma*, *Tubeufia* and *Thaxteriella*, as well as anamorphic representatives from *Helicomycetes*. Clade F is represented by *Boerlagiomyces websteri*, which clusters with apothecial fungi (*Rhytisma acerinum*, *Ostropa barbara*, *Neofabraea malicorticis*, *Cudonia circinans*, *C. lutea* and *Spathularia flavida*). Clade G consists of *Acanthostigma perpusillum* grouping with species in *Herpotrichiellaceae* (*Capronia parasitica*, *C. coronata*, *Fonsecaea pedrosoi* and *Exophiala dermatidis*) with very high bootstrap support.

Maximum-likelihood analyses yielded a single tree of log likelihood – 6485.26804 (Fig. 2). Estimated nucleotide frequencies were equal as follows: (A = 0.24040, C = 0.21650, G = 0.29490 and T = 0.24820, shape parameter (alpha) was 0.5985). Maximum Likelihood and Bayesian analyses yielded trees that were slightly different in topology from those derived in maximum parsimony. However Bayesian support was better than Bootstrap support (Fig. 3).

Discussion

Phylogenetic relationships of Tubeufiaceae and taxonomic implications

Although the ordinal placement of *Tubeufiaceae* is still controversial most mycologists placed it in the order *Pleosporales* (Barr, 1980; Sivanesan, 1984; Rossman, 1987; Kirk *et al.*, 2001). However, some authors prefer to accommodate it within the *Dothideales* (Eriksson and Winka, 1998; Eriksson, 1999; Eriksson, 2001). Eriksson (2005) referred it to *Dothideomycetes* et *Chaetothyriomycetes incertae sedis*.

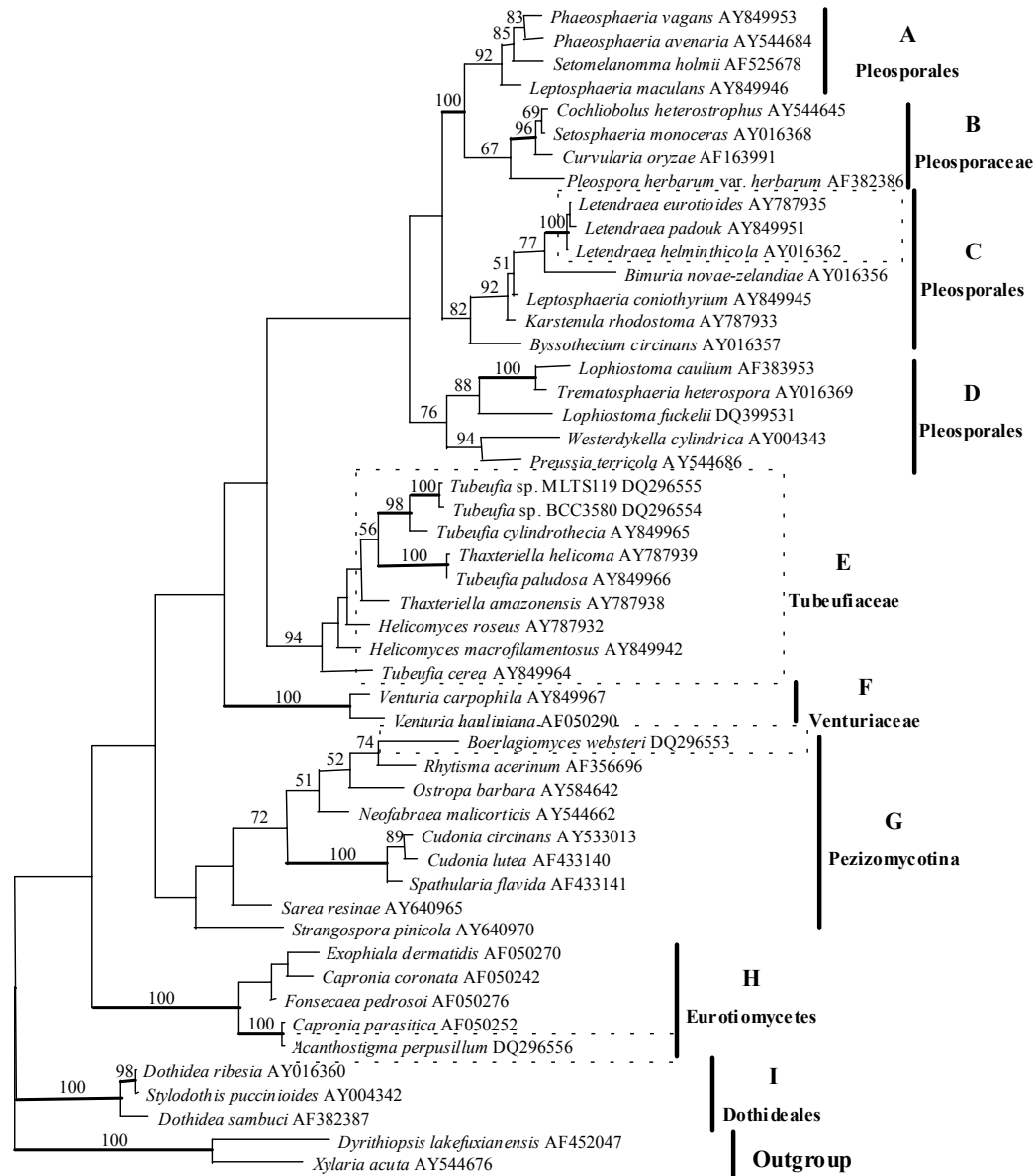


Fig. 1. The 28S rDNA maximum parsimony tree generated under Gapcoder and treating gap as missing (Total Length = 1249, CI = 0.445, RI = 0.771, RC = 0.343, HI = 0.555). Designated outgroup are *Dyrithiopsis lakefuxianensis* and *Xylaria acuta*. Bootstrap support values above 50% shown at nodes are based on 1000 replicates. Clades A-G represent members from different genera forming distinct monophyletic groups.

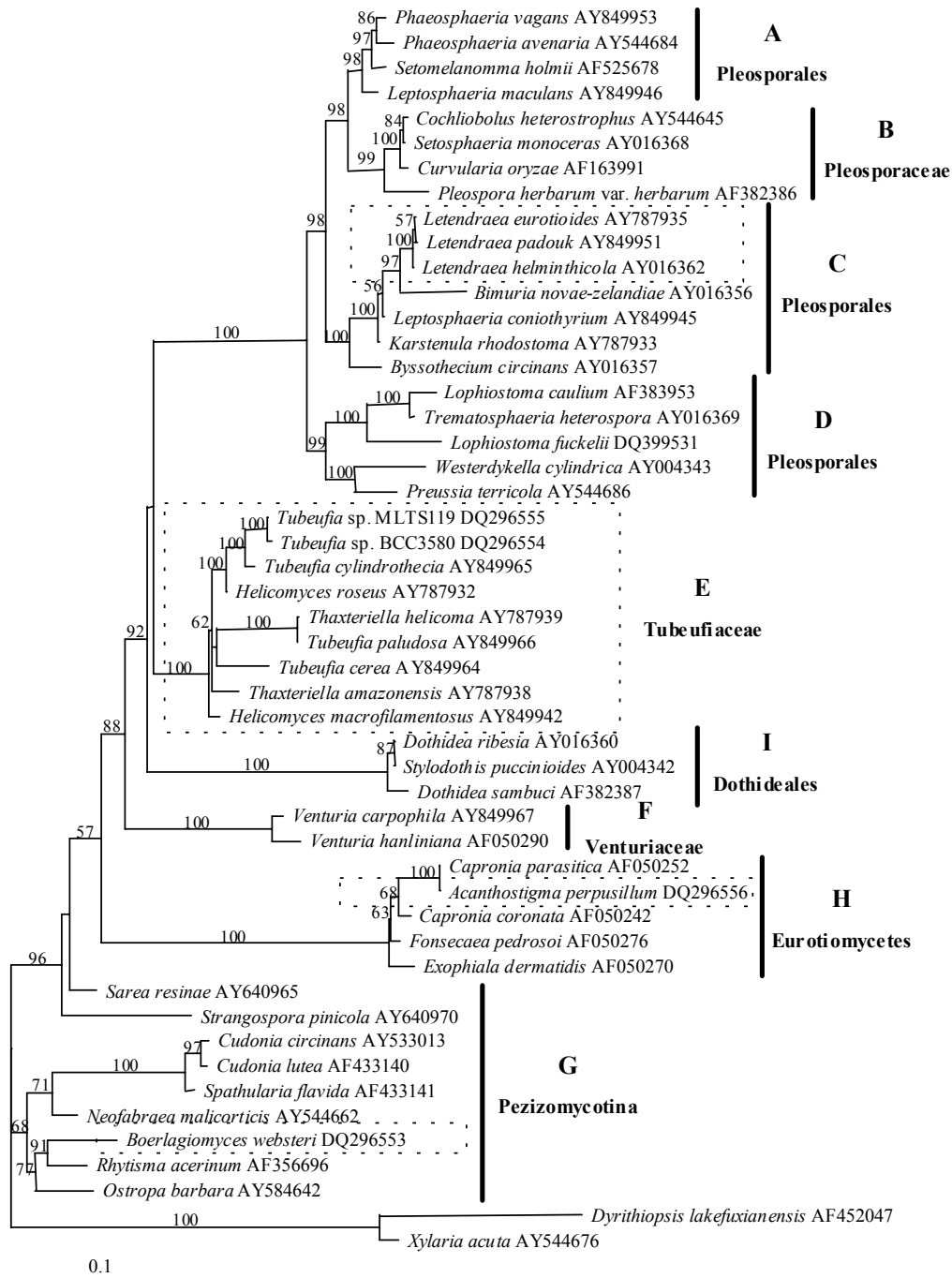


Fig. 2. Phylogeny of the *Tubeufiaceae* within *Pleosporales* estimated under Maximum likelihood (TL = 1117, CI = 0.4288, RI = 0.7686, RC = 0.3296, HI = 0.5712). Outgroup taxa are *Dyrithiopsis lakefuxianensis* and *Xylaria acuta*.

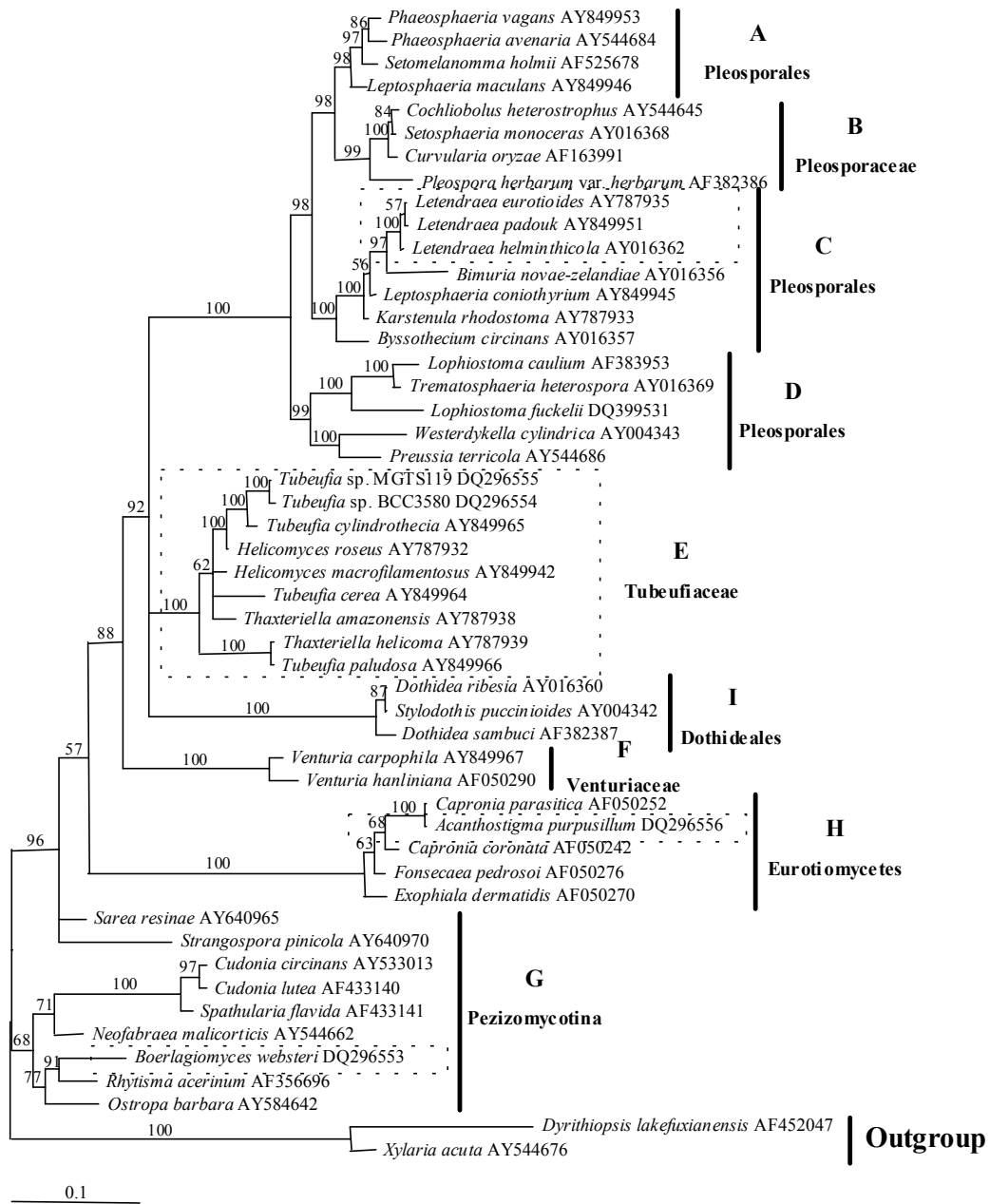


Fig. 3. Phylogeny of *Tubeufiaceae* based on 28S sequence data estimated under Bayesian analysis (TL = 1135, CI = 0.422, RI = 0.762, RC = 0.322 and HI = 0.578)

The *Pleosporales* comprises 19 families characterised by the lack of, or a poorly developed stroma, immersed to erumpent perithecial ascomata, which are sometimes clypeate and sometimes hairy or setose, pseudoparaphyses, and cylindrical asci which contain hyaline to brown, septate ascospores (Barr, 1980; Rossman, 1987; Kirk *et al.*, 2001). The family *Pleosporaceae* is characterised by perithecial ascomata that are immersed and become erumpent, are usually black and sometimes hairy or setose, cylindrical with cellular pseudoparaphyses, fissitunicate asci and brown phragmosporous or dictyosporous ascospores (Dong *et al.*, 1998; Kirk *et al.*, 2001). Fungi that possess bright-coloured superficial ascomata and long-multiseptate ascospores were originally placed in the family *Pleosporaceae*, but were then transferred to a new family, *Tubeufiaceae* by Barr (1979). The remaining pleosporaceous taxa were restricted to species with brown, fusiform to muriform ascospores (with the exception of *Cochliobolus*). The taxonomic status of *Tubeufiaceae* and related genera has undergone several changes during the past few decades (e.g. Barr, 1980; Rossman, 1987; Rossman *et al.*, 1999; Sivanesan, 1984; Réblová and Barr, 2000; Crane *et al.*, 1998). Many problematic fungi were transferred to several other genera, either in the same family or different families, and sometimes were reinstated to the former genera. Sequence analyses indicate that *Tubeufiaceae* is monophyletic and does not cluster within *Phaeosphaeriaceae*, *Leptosphaeriaceae* and *Pleosporaceae* (Clade E; Fig. 1). In the MP tree, the *Tubeufiaceae* clade, is close to *Venturiaceae*, while in the ML and Bayesian trees it seems closer to the *Dothideaceae* (Figs. 2, 3).

Recently, Eriksson (2005) placed the *Tubeufiaceae* in Dothideomycetes or Chaetothyriomycetes *incertae sedis*. However, our molecular data show that this family clusters within the *Pleosporales*. *Tubeufiaceae* does possess morphological characters that are typical of the *Pleosporales*. This includes absence of stroma, hairy or setose ascomata with cellular pseudoparaphyses, cylindrical asci and hyaline to brown-septate ascospores. Therefore, we consider that the ordinal circumscription as proposed by Barr (1980), Sivanesan (1984), Rossman (1987) and Kirk *et al.* (2001) should be maintained.

Several studies have reported anamorph-teleomorph relationships between helicosporous fungi (i.e. *Helicoma* sp., *Helicomycetes* sp. and *Helicosporium* sp.) and teleomorphic fungi in *Tubeufiaceae* (Samuels and Müller, 1978; Barr, 1980; Sivanesan, 1984; Rossman, 1987; Réblová and Barr, 2000; Kodsueb *et al.*, 2004). From our molecular data, two *Helicomycetes*, *H. macrofilamentosus* and *H. roseus* cluster within the *Tubeufiaceae* with high bootstrap support (85%), thus confirming that these two *Helicomycetes* species are anamorphic *Tubeufiaceae*.

Accepted genera from molecular study***Tubeufiaceae***

The most recent investigation of *Tubeufiaceae* is that of Crane *et al.* (1998) who revised and made notes on *Boerlagiomyces* and discussed some related genera in the family. They accepted 10 genera in the family, i.e. *Acanthophiobolus*, *Acanthostigmina*, *Boerlagiomyces*, *Chaetosphaerulina*, *Letendraeopsis*, *Rebentischia*, *Taphrophila*, *Thaxteriella*, *Thaxterina* and *Tubeufia*, but did not mention characters that they used in their revision. Unlike many other authors they accepted *Acanthostigmella*, *Acanthostigmina*, *Chaetosphaerulina* and *Thaxteriella* in the family. The current investigation supports the inclusion of *Tubeufia* and *Thaxteriella* in the family *Tubeufiaceae*. Based on 28S rDNA sequences data, nine species in this family clustered together, forming a monophyletic clade with 94% bootstrap support (Fig. 1). Similar phylogenies were obtained from Bayesian and maximum-likelihood analysis. We did not include representatives from *Acanthophiobolus*, *Acanthostigmina*, *Chaetosphaerulina*, *Letendraeopsis*, *Rebentischia*, *Taphrophila* and *Thaxterina* because they were not available in culture collections.

Thaxteriella

This genus is characterised by black ascomata which are solitary or densely aggregated and have a short acute papilla at the apex. Ascomata collapse and become cupulate when dry and are partly embedded in a superficial subiculum. Asci are cylindrical to broadly clavate or subclavate, thickened at the apex and short stalked. Ascospores are cylindrical to fusiform and hyaline, becoming pale yellow-brown after liberation (Sivanesan, 1984). Petrak (1924) erected *Thaxteriella* for *Th. corticola*. It has been separated from *Tubeufia* on the basis of dark-coloured, globose, collabent ascomata seated in a well-developed subiculum, versus light-coloured, cylindrical, ovoid or globose ascomata in *Tubeufia* seated in a slight subiculum, and on variations in the conidial states. Samuels *et al.* (1979) argued that one problem concerning *Tubeufia* is its relationship to *Thaxteriella* and the similarity in morphological characters. Samuels *et al.* (1979) have discussed the relationship of this genus to some species of *Tubeufia* and indicated the close similarity in both the teleomorph and the anamorph. Barr (1980) treated *Thaxteriella* as a synonym of *Tubeufia*, while Crane *et al.* (1998) mentioned that *Thaxteriella* differs from *Tubeufia* in pigmentation and structure of the peridium and he preferred to maintain them as distinct genera. Crane *et al.* (1998) have transferred *Tubeufia helicoma* and *T. amazonensis* to *Thaxteriella* based on morphological

characters. However, in our molecular data, *Thaxteriella helicoma* appears to cluster with *Tubeufia paludosa*, the type species of the genus, with strong bootstrap support (100%). *Thaxteriella amazonensis* also clusters within the *Tubeufiaceae* Clade. This conflicting result suggests that the taxonomic scheme proposed by Crane *et al.* (1998) is doubtful and a revision is necessary. With evidence from phylogenies herein, we prefer to transfer these two *Thaxteriella* species to *Tubeufia*.

Relevance of morphological characters

The classification of *Tubeufia* and allied genera within the *Tubeufiaceae* have been resolved differently by several authors (von Höhnel, 1910; Pirozynski, 1972; von Arx and Müller, 1975; Barr, 1979, 1980; Rossman, 1978, 1987; Scheuer, 1991, 1993; Crane *et al.*, 1998). Barr (1980) divided the genus *Tubeufia* into four sections. *Tubeufia* for ascomata ellipsoid or cylindrical, hyaline, white, yellowish pinkish or becoming darkened or black at maturity and on drying; peridium smooth or ornamented at apex with protuding cells or short to elongate thick-walled hyphal appendages, rarely with setae, inner region never pigmented, surface cells horizontally elongate. *Nectrioidea* is characterised by ascomata globose or ovoid, pigmented vivid, peridium with hyphal or cellular protuding cells, ornamented. Species of *Thaxteriella* possess ascomata which are globose or ovoid, vinaceous, greyish or blackish brown pigmented; peridium smooth or ornamented with protuding cells or short thick-walled hyphae while *Acanthostigmina* possesses ascomata that are globose or ovoid, vinaceous or dark brown pigmented; peridium ornamented with dark brownish-black setae. However, according to the compilation of the saprobic *Tubeufiaceae* by Crane *et al.* (1998) the family encompassed ten saprobic or mycosaprobic genera including *Acanthostigmina*, *Thaxteriella* and *Tubeufia* (Sect. *Nectrioidea* and *Tubeufia*) as separate genera (Reblóvá and Barr, 2000). Species of *Tubeufia* differ from other members of the *Tubeufiaceae* in their occurrence on non foliicolous fungi or on old, rotten wood or herbaceous debris and *Tubeufia* species are often hypersaprobic occurring on over mature stromata of ascomycetes (Rossman, 1987).

From molecular results, *Tubeufia* and *Thaxteriella* which differ in colour of ascomata and ascospores cluster together in the same clade. This suggests that these two genera have similar ancestral origin but the colour of ascomata and spores are not necessarily significant characters to distinguish them.

On the other hand, *Boerlagiomyces*, which differ from *Tubeufia* and *Thaxteriella* in having muriform ascospores and dark, thick-walled setae on ascomata, respectively, are phylogenetically unrelated to other members of the *Tubeufiaceae*.

Systematics of Acanthostigma, Boerlagiomyces and Letendraea

Acanthostigma De Not., Sfer. Ital.: 85 (1863)

Acanthostigma was reinstated by Réblová and Barr (2000) following examination of the type material of *A. perpusillum* De Not. They considered it to be a well-characterised genus with minute, setose, dark ascomata, dark, thick-walled setae, bitunicate asci, narrow cellular pseudoparaphyses and hyaline cylindrical-fusiform transversely multiseptate ascospores, with *Helicosporium* and *Helicomycetes* anamorphs. Réblová and Barr (2000) accepted six species and considered *Acanthostigmina* as a synonym of the genus (Réblová and Barr, 2000).

The basic differences between *Acanthostigma* and *Tubeufia* lie in features of ascomata. They are vinaceous, reddish-brown, brown or dark brown, covered with dark brownish-black, often opaque, acute or obtuse setae in *Acanthostigma*. *Tubeufia* is characterised by ascomata that are hyaline, white, yellowish, becoming darkened when mature or on drying, thick-walled hyphal appendages or short dark setae (Réblová and Barr, 2000).

Müller (1965) and Barr (1990, 1997) accepted this genus in the *Trichosphaeriaceae*. Recent examination of newly discovered type material of *Acanthostigma perpusillum* revealed characters that fit in *Acanthostigmina* Höhn., the genus that was considered closely related to *Acanthostigmella* Höhn. (Réblová and Barr, 2000). In this study *Acanthostigma perpusillum* grouped with species in the *Herpotrichiellaceae* with 100% bootstrap support (Figs. 1, 2). Concordance with our result, Untereiner *et al.* (1995) used a molecular-morphotaxonomic approach to investigate the systematics of *Herpotrichiellaceae* and allied black yeasts. Their results showed *Tubeufia helicomycetes* nesting with *Capronia pinicola* while *Acanthostigmella brevispina* was basal to species of *Leptosphaeria* and *Pleospora rudis*. They mentioned that additional sequence data from the 18S rDNA gene may better resolve the position of *Capronia pinicola* and *Acanthostigmella brevispina* within the *Dothideales-Pleosporales* sub-clade.

Boerlagiomyces Butzin, Willdenowia 8: 39 (1977)

Boerlagiomyces is typified by *B. velutinus* (Penz. and Sacc.) Butzin which was described from decaying palm petioles in Cibodas, Indonesia (Greuter *et al.*, 1993; Crane *et al.*, 1998). *Boerlagella* was the earlier name of Penzig and Saccardo (1897) but cannot be used as it was predated by *Boerlagella* Cogn. According to Barr (1980), the dark, fleshy ascocarps on a well-developed, dark subiculum and elongate, muriform ascospores suggest that *Boerlagiomyces* species are similar to *Tubeufia* sect. *Thaxteriella*. The presence of longitudinal septa in cells of the ascospores is considered a

character important enough to distinguish the genus from *Tubeufia* sect. *Thaxteriella* (Rossman, 1987). Crane *et al.* (1998) revised the genus with a key and accepted six species in *Boerlagiomyces*. Species in this genus are distinguished by their large, mostly hyaline, muriform ascospores. Ascomata are membranous and superficial, with setose and hyphal appendages. Several species are known from submerged wood in freshwater and other rotting plant debris (Stanley and Hyde, 1997; Crane *et al.*, 1998).

The explanation about *Boerlagiomyces websteri* has not been discussed here since its morphology is quite different from all other fungi that nest together in the same clade. It is a member of *Tubeufiaceae* that possesses muriform ascospores and very different from other fungi that possesses filiform to fusiform ascospores.

Letendraea Sacc., *Michelia* 2: 73 (1880)

The genus *Letendraea* was originally placed in the *Hypocreales* and then transferred in *Pleosporaceae* by Wehmeyer (1975). Barr (1979) erected the new family, *Tubeufiaceae* and placed *Letendraea* in the family. Lumbsch and Lindemuth (2001) sequenced SSU rDNA from *Letendraea helminthicola* and included three additional sequences from *Tubeufia* and *Acanthostigmella* in the analysis. Their result showed that the position of these taxa is uncertain and further studies are required (Untereiner *et al.*, 1995). In this study, *Letendraea* species are distant from *Tubeufiaceae*. *Letendraea* species group with *Leptosphaeria coniothyrium* (*Leptosphaeriaceae*), *Byssothecium circinans* (*Dacampiaceae*), *Bimuria novae-zelandiae* and *Karstenula rhodostoma* (*Melanommataceae*) (Figs. 1, 2.). *Letendraea* species include nonstromatic, brown-spored species originally in *Nectria* and is typified *L. eurotioides* (= *Nectria helminthicola*) (Samuels, 1973). Samuels (1973) found that the ascomata of *L. helminthicola* to be associated with a *Helminthosporium* imperfect state. Richon (1881) first discussed that *Helminthosporium* was the anamorph of *Letendraea*. However, Samuels (1973) believed that the mycelia of the two fungi are distinct and the mycelium of *Letendraea* grow over and may be parasitising *Helminthosporium*. We believe that *Letendraea* should not have *Helminthosporium* anamorphs. Furthermore, it is phylogenetically distinct from other members of the *Tubeufiaceae* since this genus has brown, two-celled ascospores as compared to filiform, multi-septate and almost hyaline ascospores in other species of *Tubeufiaceae*. Morphological and molecular data indicate that *Letendraea* is more closely related to *Pleosporaceae* than *Tubeufiaceae* where it had formally been placed by Barr (1979). However, it does not cluster with pleosporaceous taxa (Clade B) but aligns with taxa in Clade C which include members of *Dacampiaceae*, *Leptosphaeriaceae* and

Melanommataceae. This grouping is not resolved and requires more sequence data.

Excluded genera of Tubeufiaceae

In this study, we have examined the fungi in *Tubeufiaceae* based on the taxonomic characters, mostly from literature. Seventeen genera of *Tubeufiaceae* were included, with the exception of four genera; *Allonecte*, *Amphinectria*, *Borinquenia* and *Letendraea*, that should be excluded from the family due to lack of typical characters (it has two-celled, fusiform-ellipsoidal and brown ascospores, while other fungi in family mostly have hyaline and multi-celled ascospores).

Allonecte Syd., Ann. Mycol. 37: 378 (1939).

Allonecte is monotypic represented by *A. lagerheimii* (Pat.) Syd. This taxon is biotrophic on leaves of *Chusquea*, and has superficial ascomata that develop from a intramatrical hypostroma and cylindrical asci that contain wide, hyaline, uniseptate ascospores (Müller and Arx, 1962; Crane *et al.*, 1998). Wehmeyer (1975) used to place *Allonecte* in family *Pleosporaceae* as fungi who possess grey-brown, 2-celled ascospores, superficial, fleshy and smooth ascomata, light coloured on a short stalk or hypostroma. The genus was excluded from *Tubeufiaceae* by Crane *et al.* (1998) which produced a coelomycetous anamorph with pycnidia developing from a stroma and containing relatively large, aseptate, hyaline conidia. The genus probably belongs in the *Parodiellaceae* according to Crane *et al.* (1998).

Amphinectria Speg., Bol. Acad. Nac. Cienc. Córdoba 26: 346 (1924).

This monotypic genus is typified by *A. portoricensis* Speg. and was discussed recently by Rossman *et al.* (1999). The original specimen was depauperate and therefore Rossman *et al.* (1999) concluded that “the genus remains an ambiguous member of the *Tubeufiaceae*”.

Borinquenia F. Stevens, Trans. Illinois State Acad. Sci. 10: 173 (1917).

Borinquenia miconiae is the type and only one species of this genus. Pirozynski (1977) suggested that *Borinquenia* is a synonym of *Nematothecium*. The material is depauperate and this may be a species of *Malacaria* (Rossman *et al.*, 1999). *Borinquenia* was previously included in the *Hypocreales*, however, later was transferred to *Tubeufiaceae* because it possesses persistent paraphyses, bitunicate asci and smoky-brown ascospores (Rossman *et al.*, 1999).

Nematostoma H. Sydow, *Annals Mycol.* 12: 161 (1914)

= *Acanthostigmella* Höhn.

= *Acanthostigmia* Höhn.

The type species is *Nematostoma artemisiae*. The genus includes species in the *Dimeriaceae* that are associated with leaf hairs and have hyaline, multiseptate ascospores (Rossman, 1987). The most additional species have not been described in the recent literature.

Letendraea Sacc., *Michelia* 2: 73 (1880)

Letendraea was originally placed in the *Hypocreales* because of its light coloured ascocarps. Müller and von Arx state that they have bitunicate asci (Wehmeyer, 1975).

Wehmeyer (1975) placed *Letendraea* in family *Pleosporaceae* as fungi that possess hyaline, 2-celled ascospores, superficial, fleshy and tomentose ascomata, light coloured on a short stalk or hypostroma.

Morphological characteristics of accepted genera of Tubeufiaceae

Acanthophiobolus Berl., *Atti del Congresso Botanico Internazionale*, Genova 1892: 571 (1893).

The genus *Acanthophiobolus*, comprises 5 species (<http://www.indexfungorum.org/Names/Names.asp>), is typified by *A. helminthosporus* (Rehm) Berl. (Greuter *et al.*, 1993) and was originally described on rotting cloth from Augsburg, Germany (Walker, 1980). This taxon is common on dead plant parts, especially monocotyledons and should be compared with *Acanthostigma*. Ascomata are superficial, with rigid dark brown to black setae, especially on their upper half. There are numerous pseudoparaphyses, asci are bitunicate, and ascospores are filiform and tightly coiled within the asci (Walker, 1980). This is a pleosporaceous genus according to Barr (1997) and was determined to belong to *Tubeufiaceae*.

Acanthostigmella Höhn., *Ann. Mycol.* 3: 327 (1905).

Anamorph: *Xenosporium*

Crane *et al.* (1998) returned *Acanthostigmella* to generic rank, reinstated the name of the type species, *A. minuta* (Fuckel) Höhn. and accepted six species. In this genus ascomata are pallid or brown and covered in dark setae that taper to an apical point, while asci contain eight ascospores that is fusoid to elongate-fusoid with only transverse septa.

Byssocallis Syd., *Ann. Mycol.* 25: 14 (1927).

The genus is typified by *Byssocallis phoebes* (Sydow), characterised by superficial ascocarps with a thin hyphal stroma covering the host hyphae,

branch, anastomosing pseudoparaphyses and having cylindrical ascus. Ascospores fusiform to narrowly clavate, hyaline and multiseptate (Rossman, 1987). This genus is morphologically similar to species of *Melioliphila*. It was synonymised with *Puttemansia* by Petrak (1931) and Pirozynski (1977) based on the presence of apiculate ascospores (Rossman, 1987).

Chaetocrea Syd., Ann. Mycol. 25: 19 (1927).

Chaetocrea was firstly found as parasites on stroma of *Cyclostomella disciformis* on leaves of *Nectandrasanguinae*. The genus have only one species and the type specimen was apparently lost or destroyed (Rossman, 1987).

Ascomata superficially, white, hairy, found on the stroma of other fungi. Thin-walled, septate pseudoparaphyses, asci bitunicate, cylindrical with hyaline ascospores, multiseptate (Rossman *et al.*, 1999). The genus was originally in *Hypocreaceae* then transferred to *Tubeufiaceae* based on superficial ascomata, soft texture, light-coloured, bitunicate asci and presence of pseudoparaphyses. It is similar to several genera in *Tubeufiaceae* based on its occurrence on leaf. The thin-walled and white ascomata in *Chaetocrea* is similar to some *Paranectriella* (appendaged and black ascospores, hyphal, leaf-inhabiting ascomycetes) and some of *Uredinophila* (non-appendaged ascospores, occur on fern rusts).

Chaetosphaerulina I. Hino, Bull. Miyazaki Coll. Agric. Forestry 10: 62 (1938).

Chaetosphaerulina was reinstated by Crane *et al.* (1998) to include *C. yasudae* I. Hino the type species, *C. bambusicola* (Sivan. and N.D. Sharma) Crane, Shearer and M.E. Barr and *C. lignicola* (K.S. Panwar and S.J. Kaur) Crane, Shearer and M.E. Barr, the latter two taxa transferred from *Thaxteriellipsoidis*. The genus is most similar to *Tubeufia* but can be separated on account of the brownish pigmentation of the ascomata and hyphal appendages that may become agglutinated as stiff setae in *Chaetosphaerulina*. Ascospores are elongate and multiseptate, often with one to a few cells containing vertical septa (Crane *et al.*, 1998). Species occur on decaying wood, bamboo or palms (Hino, 1938).

Glaxoa P.F. Cannon, Systema Ascomycetum 15: 121-138 (1997)

The genus was typified by *G. pellucida*, found on dead cone scale of *Cupressus sempervirens* in United Kingdom (Cannon, 1997). Since it is most similar to *Letendraea* as Rossman (1987) and Samuels (1973) did stated, Cannon (1997) agreed to refer this fungus to the *Tubeufiaceae*. Differences between *Glaxoa* and *Letendraea* are size of ascomata (*Letendraea* is larger),

colour of ascospores (brown in *Letendraea* but hyaline in *Glaxoa*) and type of habit (*Letendraea* is closely associated with other fungi). Cannon (1997) made notes on comparison between *Glaxoa* to similar fungi. Even some are similar or the same species, there are, however, many characters that are present in *Glaxoa* but absent in other fungi (such as *Acanthostigmella* and *Letendraeopsis*).

Letendraeopsis K.F. Rodrigues & Samuels, Mycologia 86: 255 (1994)

This is a monotypic genus with *L. palmarum* K.F. Rodrigues and Samuels as the type species that was described as an endophyte from a palm. Ascomata lack a preformed opening and the few asci contain yellowish-brown, 1-septate ascospores (Rodrigues and Samuels, 1994). The superficial, light coloured ascomata, clavate to broadly cylindrical bitunicate asci and cellular pseudoparaphyses are indicative of *Pleosporales*, possibly *Tubeufiaceae*. However, the fact that ascomata are smooth-walled and lack an ostiole and ascospores are 1-septate with a larger apical cell is, atypical of other members of *Tubeufiaceae*.

Malacaria Sydow, Ann. Mol. 28: 69 (1930)

Malacaria is typified by *M. flagellata* (Hansf.) Hansf. (= *M. meliolicola*). The genus *Malacaria* was described for *M. meliolicola* occurring on *Meliola* in Venezuela (Rossman, 1987). Based on similarities in several unique features, *M. flagellata* was considered to be a synonym of *M. meliolicola* by Sydow (1930a). Rossman (1987) suggested that the presence of pale smoke-grey ascospores and also unbranched and septate pseudoparaphyses are atypical of genera within *Tubeufiaceae*.

Melioliphila Speg. (1924)

Anamorphs: *Chionomyces*, *Eriomycopsis*.

Type: *Melioliphila volutella* (Berk. and Broome) Rossman [= *M. graminicola* (F. Stev.) Speg., ≡ *Calonectria graminicola* F. Stev.).

The genus *Melioliphila* was included in the “hypocreoid *Dothideales*” by Pirozynski (1977) and later in *Tubeufiaceae* by Barr in 1980. Spegazzini (1924) described *Melioliphila* as a parasite on plant-associated fungi. This genus was initially included in *Tubeufiaceae* based on the presence of bitunicate asci and pseudoparaphyses possessed (Rossman *et al.*, 1999). Pirozynski (1977) have included *Subiculicola* as a synonym of *Melioliphila* following Höhnel’s method.

Paranectriella (Henn. Ex Sacc & D. Sacc) Höhn. (1910).

Type: *Paranectriella juranna* (Henn.) Henn. ex Piroz. (= *Nectria juranna* Henn.).

Anamorphs: *Araneomyces*, *Titaea*.

The genus consists of 9 species (Indexfungorum) and was included in the *Tubeufiaceae* based on the hyaline to pale luteous or pale peach, relatively thin-walled ascocarps, abundant pseudoparaphyses and a fungicolous habit. *Paranectriella* species were distinguished from other members of *Tubeufiaceae* by distinct apicule or cellular appendages at each end of the ascospores (Rossman, 1987). The nomenclature and taxonomic revision of fungi in this genus was well described in Rossman (1987). Rossman (1987) mentioned the similarity on macroscopic character of *Paranectriella hemileiae* to species of *Uredinophila* in having small, pale luteous, translucent ascocarps occurring superficially on rust pustules. However, the character used to separate these two genera is the difference in ascospore. Ascospores of *Uredinophila* are narrowly cylindrical and lack any kind of appendages, while ascospores of *Paranectriella hemileiae* have an apiculus at each end.

Podonectria Petch, Trans. Br. mycol. Soc. 7: 146 (1921)

Anamorphs: *Peziotrichum*, *Tetracrium*

This genus was typified by *Podonectria coccicola* (Ellis and Everh.) Petch. Eleven species have been described in Indexfungorum. They are parasitic on scale insects, having pale to bright-coloured ascocarps that sometimes appear dark due to a granular coating, bitunicate asci and elongate, multiseptate ascospores (Rossman, 1987). Rossman (1987) produced a monograph of the genus, which included a key plus descriptions and illustrations of the eight accepted species. Pirozynski (1977) included one additional species, *Podonectria bambusicola*, based on *Trichonectria bambusicola* Rehm. Rossman (1987) has examined the type specimen of this *Podonectria* and revealed that it was a synonym of *Uredinophila erinacea* (Rehm).

Puttemansia Henn., Hedwigia 41: 113 (1902).

Anamorphs: *Guelichia*, *Tetracrium*.

The genus was described as member of the *Pezizaceae* (Rossman *et al.*, 1999) and was included in the *Hypocreaceae* by Clements and Shear (1931) and by Rogerson (1970). Rossman (1987) examined the available type and non-type specimens and have discussed the disposition of all names in *Puttemansia*. The type species of *Annajenkinsia*, *A. fungicola*, is a synonym of *Puttemansia stromaticola*. Based on centrum and other characters, the only other species in *Annajenkinsia*, *A. hyperparasitica* was transferred to *Puttemansia* (Rossman, 1987).

Rossman (1987) have transferred *Tubeufia stromaticola* to *Puttemansia* based on its occurrence on a non-meliolaceous host and mentioned that this

species resembles *Tubeufia* species in having pallid, translucent ascocarps and narrowly clavate to cylindrical ascospores. The presence of short, blunt hairs and long ascospores differentiates *P. stromaticola* from other species.

Rebentischia P. Karst., Fungi Fennicae Exsiccati, no. 881 (1869).

The genus was typified by *R. massalongii* (Mont.) Sacc (= *R. pomiformis* P. Karst.). The two species of *Rebentischia* accepted by Barr (1980) have pale to dark-vinaceous ascocarps and ascospores hyaline at first, becoming pale fawn to pale brown-vinaceous at maturity. A key, descriptions, and illustrations of both accepted species, *R. massalongii* and *R. unicaudata* (Berk. and Broome) Sacc., are provided by Barr (1980) along with an account of excluded species (Rossman, 1987).

Taphrophila Scheuer, Bibliotheca Mycologica 123: 171 (1988)

Anamorph: *Mirandina* G. Arnaud ex Matsush., Icones microfungorum a Matsushima lectorum (Kobe): 96 (1975)

Taphrophila is typified by *T. cornu-capreoli* Scheuer which was described from *Carex* (*Gramineae*) in Austria. Scheuer (1988) tentatively placed the genus in the *Herpotrichiellaceae*, a second species was added later by Scheuer (1991) and the anamorph is *Mirandina*. Members in the genus are distinguished by dark dichotomously branching setae which form apically on the thin-walled ascomata (Scheuer, 1988; Crane *et al.*, 1998). Scheuer (1991) also described two *Tubeufia* species with dark setae and in *T. trichella* (Sacc., Bomm. and Rouss) Scheuer, the setae were simple or \pm irregularly branched, almost resembling those of *Taphrophila* species. Except for the distinguishing feature of straight conidia, the *Mirandina* anamorph is similar to *Helicoma*. It may be considered that this genus is a synonym of *Tubeufia*.

Thaxterina Sivan., R.C. Rajak & R.C. Gupta, Transactions of the British Mycological Society 90: 662 (1988).

This monotypic genus is represented by *T. multispora* Sivan., R.C. Rajak and R.C. Gupta and differs from other genera in *Tubeufiaceae* in having polysporous asci. Ascomata are also strongly setose in a hyphal subiculum (Sivanesan *et al.*, 1988; Crane *et al.*, 1998).

Tubeufia Penz. & Sacc., Malpighia 11: 517 (1898)

Anamorphs: *Helicoma*, *Helicosporium*, *Monodictys*-like.

Tubeufia is typified by *T. paludosa* which was described from *Bambusae emarcidis* in Java, Indonesia. Kodsueb *et al.* (2004) described a new species and provided a key to 16 accepted species in the genus. *Tubeufia* species are usually reported from rotting vegetation, but are also known from freshwater.

The ascomata are initially pale and have a glabrous surface or short setae, while ascospores are transeptate (Kodsueb *et al.*, 2004).

Barr (1980) argued that *Thaxteriella* was a synonym of *Tubeufia* because she considered that differences between these two genera were not significant. Crane *et al.* (1998) however, disagreed and maintained *Thaxteriella* as a separate genus because of pigmentation and structure of the peridium. *Thaxteriella* is typified by *Thaxteriella corticola* Petr. (Petrak, 1924). In this paper we prefer to treat *Thaxteriella* as a synonym of *Tubeufia* as although ascomata may be pallid or brightly pigmented when young, they become brown when mature or dry and both of them have similar anamorphs (Arx and Müller, 1975).

Uredinophila Rossman, Mycol. Pap. 157: 1-71 (1987).

The genus was typified by *Uredinophila tropicalis* (Speg.) Rossman. Rossman (1987) established the genus *Uredinophila*, from *Ophionectria tropicalis* (Speg.), for those members of the *Tubeufiaceae* that occur as rusts on living leaves, have translucent ascocarps with walls less than 20 µm wide, and have narrowly fusiform to cylindrical ascospores.

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