Phylogenetic position of the genera Nadvornikia and Pyrgillus (Ascomycota) based on molecular data

H. Thorsten Lumbsch, Armin Mangold, Robert Lücking, Miguel A. García and María P. Martín

Lumbsch, H. T., Mangold, A., Lücking, R., García, M. A. & Martín, M. P. 2004. Phylogenetic position of the genera *Nadvornikia* and *Pyrgillus* (Ascomycota) based on molecular data. – Acta Univ. Ups. *Symb. Bot. Ups.* 34:1, 9–17. Uppsala. ISBN 91-554-6025-9.

The phylogeny and systematic placement of the two mazaediate genera *Nadvornikia* and *Pyrgillus* was reconstructed using a Bayesian analysis of nuclear LSU rDNA sequences. Partial sequences of 19 species were generated and aligned with 31 sequences retrieved from GenBank. Our results confirm with strong support that *Nadvornikia* belongs to the Thelotremataceae (Ostropales). The placement of *Pyrgillus* in the Pyrenulaceae (Pyrenulales) is also strongly supported. The Pyrenulaceae form a strongly supported monophyletic group that is the sister group to the non-lichenized Chaetothyriales. The Thelotremataceae are a significantly supported monophyletic group with Graphidaceae nested within. The phylogeny within Thelotremataceae is only partially resolved with the single data set.

Key words: Caliciales, mazaedia, Lecanoromycetes, large subunit rDNA, phylogeny.

H. Thorsten Lumbsch, Armin Mangold and Robert Lücking, Department of Botany, The Field Museum, 1400 South Lake Shore Drive, Chicago, IL 60605-2496, USA. E-mails: tlumbsch@fieldmuseum.org, amangold@filedmuseum.org, rlucking@fieldmuseum.org
Miguel A. García and María P. Martín, Real Jardín Botánico, C.S.I.C., Plaza de Murillo 2, 28014 Madrid, Spain. E-mails: mgarcia@ma-rjb.csic.es, maripaz@ma-rjb.csic.es

Introduction

Calicioid lichens and fungi are characterized by passive spore dispersal and the development of a thick layer of mature spores covering the ascoma surface, usually referred to as the mazaedium. In connection with the passive spore dispersal, these fungi usually have prototunicate asci. In traditional classifications, these fungi were generally believed to form a natural group (e.g. Zahlbruckner 1926). Also, most of the latest classifications of the pre-molecular era regarded the calicioid lichens and fungi as a natural group that was classified into a single order Caliciales (e.g. Poelt 1973).

Henssen & Jahns (1974) expressed some doubts on the placement of the unitunicate Mycocaliciaceae that were placed into an appendix of Caliciales, but considered the typical prototunicate calicioid lichens and fungi as monophyletic. Tehler (1996) put much emphasis on the ascus type and regarded Caliciales and Lichinales, which are also characterized by predominantly prototunicate asci, as sister groups to the unitunicate and bitunicate ascomycetes. An exception was the opinion of von Höhnel (1910), who believed the calicioid lichens and fungi to be of polyphyletic origin.

However, it was not until the seminal work of Tibell (1984) that the Caliciales were recognized as a heterogeneous assemblage, having clear connections between aberrant taxa within the Caliciales and other groups of lichen-forming fungi. Tibell's views were based on detailed morphological, anatomical, and chemotaxonomic examinations. Molecular studies supported Tibell's (1984, in Hawksworth 1994: 393) hypothesis on the polyphyly of Caliciales and the parallel evolution of passive spore dispersal in different clades of Ascomycota (Gargas et al. 1995; Wedin & Tibell

1997; Wedin et al. 1998, 2000, 2002; Wedin & Döring 1999). These studies also showed that some calicioid lichens and fungi belong to the Lecanorales (e.g. Caliciaceae, Sphaerophoraceae), while others cluster outside the Lecanoromycetes, such as the Mycocaliciaceae, which were subsequently placed in a new order Mycocaliciales (Tibell & Wedin 2000).

While most calicioid lichens and fungi appear to belong either in the Lecanorales or Mycocaliciales, there are a few calicioid taxa that show morphological similarities to other groups and are consequently placed outside the Caliciales (Tibell 1996). These include the two small genera *Nadvornikia* and *Pyrgillus*. *Nadvornikia* was established by Tibell (1984) as a monotypic genus to accommodate an unusual crustose species with a corona-like excipular veil surrounding the mazaedium (Fig. 1A). In the meantime, two additional species were described within *Nadvornikia* (Pant & Awasthi 1989, Harris 1990). In the protologue of the type species of the genus, *Acolium hawaiiense*, Tuckerman (1867: 233) pointed out morphological similarities to Thelotremataceae (Ostropales). Tibell (1984) made reference to Tuckerman's observations, but also emphasized the differences to Thelotremataceae, such as spore morphology. Subsequently, Harris (1990) proposed to classify *Nadvornikia* in the Thelotremataceae, which was accepted by Tibell (1996).

The genus *Pyrgillus* (Fig. 1B) was described by Nylander (1857), who recognized its similarities with certain pyrenocarpous lichens (Nylander 1860: 168). Tibell (1984) raised the issue of urgently needed additional studies to clarify the systematic position of the genus. Harris (1989) included the genus *Pyrgillus* in the Pyrenulaceae, a proposal followed by Aptroot (1991) and Tibell (1996).

To date, no molecular data have been available to evaluate the phylogenetic position of these two enigmatic genera. On a recent field trip to subtropical and tropical Queensland (Australia), two of us (HTL & AM) collected fresh material of *Nadvornikia hawaiiensis* and *Pyrgillus javanicus* suitable for molecular studies. We gathered molecular data of representatives of these genera and the presumably related groups. For this purpose, we targeted the nuclear LSU (nuLSU) region of the ribosomal DNA. We chose a Bayesian approach that allows efficient analysis of data sets while employing complex nucleotide substitution models in a parametric statistical framework (Larget & Simon 1999). Bayesian phylogenetics also allows simultaneous estimation of uncertainty in the phylogenetic topography, as well as hypothesis testing of alternative topographies, since posterior probabilities of alternative trees can be calculated (Huelsenbeck et al. 2000).

Materials and methods

DNA extraction, amplification, and sequencing. Sequence data of the nuLSU rDNA were collected from a total of 50 euascomycetes. New sequences of 19 species were obtained as listed in Table 1. Total DNA was extracted using the Qiagen Plant Mini Kit (Qiagen) or using E.Z.N.A. Fungal MiniPrep Kit (Omega-Biotech, Doraville, USA) following the instructions of the manufacturer. The nuclear LSU rDNA was amplified with the primers pairs nu-LSU-155-5' (Döring et al. 2000) and LR6 (Vilgalys homepage: http://www.biology.duke.edu/fungi/mycolab/primers.htm). The 25 µl PCR reactions contained 2.5 µl buffer, 2.5 µl dNTP mix, 1 µl of each primer (10 µM), 5 µl BSA, 2 µl Taq, 2 µl genomic DNA extract and 9 µl water. Thermal cycling parameters were: initial denaturation for 3 min. at 95°C, followed by 30 cycles of 1 min. at 95°C, 1 min. at 53°C, 1 min. at 73°C, and a final elongation for 7 min. at 73°C. Some amplifications were done using Ready-to-Go® PCR Beads (Amersham-Pharmacia Biotech) as mentioned in Winka et al. (1998) with the cycling parameters given in Martín & Winka (2000). Amplification products were viewed on 1% agarose gels stained with ethidium bromide and subsequently purified using the Nucleo Spin DNA purification kit (Macherey-Nagel). When light PCR were visualized on agarose gels, cloning was conducted using pGEM T easy-vector cloning kit (Promega). Fragments were sequenced using the Big Dye Terminator reaction kit (ABI PRISM, Applied Biosystems). Se-

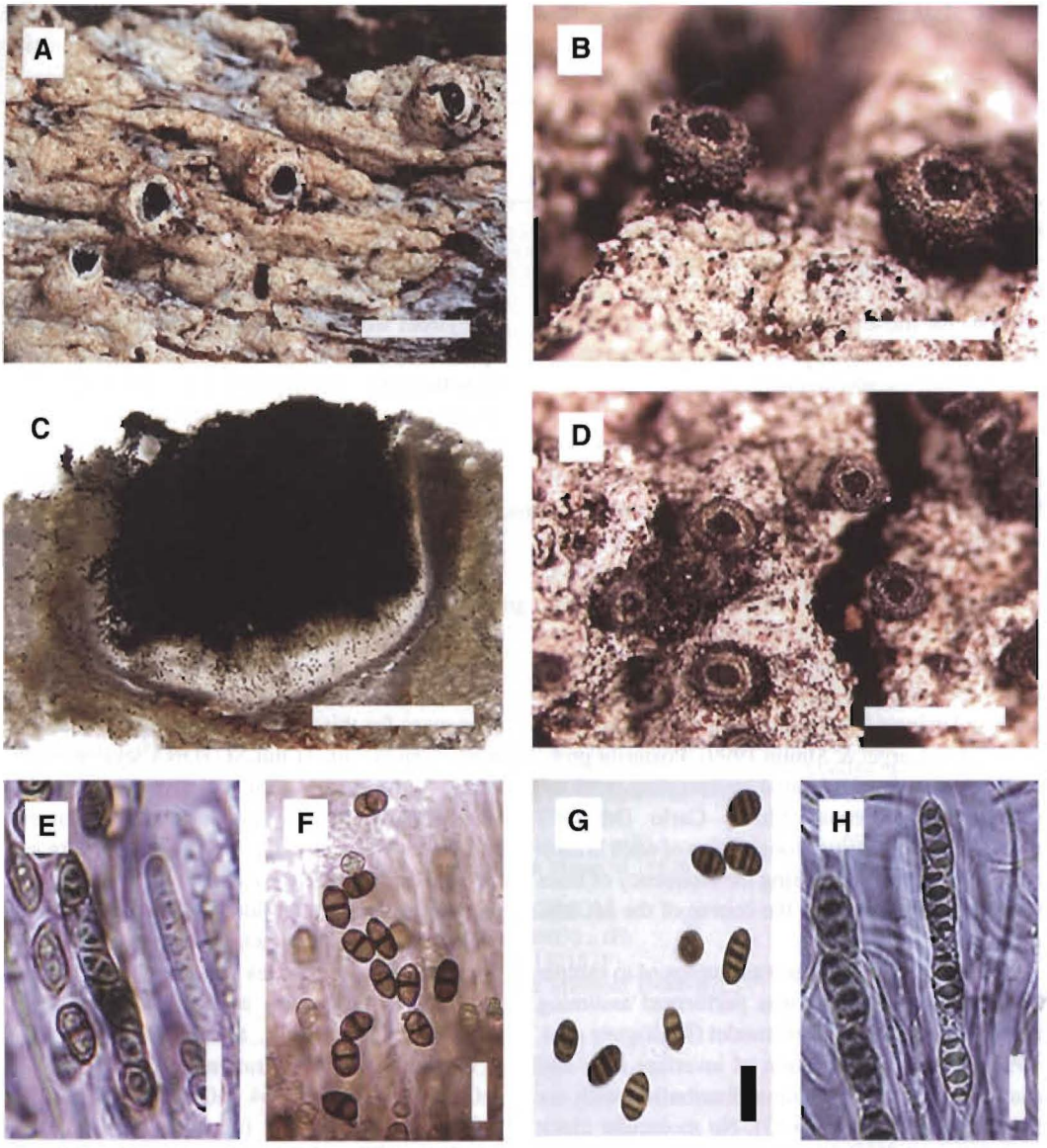

Figure 1. A–G. Morphology and anatomy of *Nadvornikia* and *Pyrgillus*. A. Morphology of *N. hawaiiensis*, Lumbsch & Mangold 19176q (F). B. & D. Morphology of *P. javanicus*, Lumbsch & Mangold 19115e (F). C. Section through an ascoma of *N. hawaiiensis*. E. Ascus and young spores of *N. hawaiiensis*. F. Mature spores of *N. hawaiiensis*. G. Spores of *P. javanicus*. H. Ascus and spores of *Pyrenula nitida*, 2002, Schmitt & Pauls (F). Bars 2 mm (A, D), 1 mm (B), 100 µm (C), 10 µm (E–H).

quencing and PCR amplifications were performed using the same sets of primers; the cloned products were sequenced with universal primers specific to the plasmid (T7 and SP6). Cycle sequencing was executed with the following program: 25 cycles of 95°C for 30 sec., 48°C for 15 sec., 60°C for 4 min. Sequenced products were precipitated with 10 µl of sterile dH_2O, 2 µl of 3 M NaOAc,

and 50 µl of 95% EtOH before they were loaded on an ABI 3100 (Applied Biosystems) automatic sequencer. Sequence fragments obtained were assembled with SeqMan 4.03 (DNASTAR) and manually adjusted.

Sequence alignments. Sequences were aligned using the software SAM (Karplus et al. 1998; http://www.cse.ucsc.edu/research/compbio/sam.html) that employs a linear Hidden Markov Model (HMM) for the alignment. Regions that could not be aligned with statistical confidence were excluded from the phylogenetic analysis.

Phylogenetic analysis. The alignment was analysed using the programs PAUP* 4.0b10 (Swofford 2003) and MrBAYES 3.0 (Huelsenbeck & Ronquist 2001). The polarity of characters was assessed selecting *Eurotium rubrum* as the outgroup, since the Eurotiales are regarded as the sister-group to Chaetothyriales in most recent phylogenetic studies (e.g. Lindemuth et al. 2001, Lutzoni et al. 2001, Lumbsch et al. 2004). The data were analysed using a Bayesian approach (Huelsenbeck et al. 2000; Larget & Simon 1999). Posterior probabilities were approximated by sampling trees using a Markov chain Monte Carlo (MCMC) method. The posterior probabilities of each branch were calculated by counting the frequency of trees that were visited during the course of the MCMC analysis.

The program MrBayes was employed to sample the trees. The analysis was performed assuming the general time reversible model (Rodriguez et al. 1990) including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+ G). No molecular clock was assumed. A run with 2,000,000 generations starting with a random tree and employing 12 simultaneous chains was executed. Every 100th tree was saved into a file.

We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?i=tracer) and determined that stationarity was achieved when the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck & Ronquist 2001). The initial 1000 trees were discarded as burn-in before stationarity was reached. Using PAUP*, majority-rule consensus trees were calculated from 19,000 trees sampled after reaching likelihood convergence to calculate the posterior probabilities of the tree nodes. These are estimated probabilities of the clades under the assumed model and hence posterior probabilities equal to and above 95% are considered significant supports. In addition maximum parsimony (MP) trees were inferred using the heuristic search option with 200 random sequence additions. Gaps were treated as missing data. Branch lengths equal to zero were collapsed to polytomies. Nonparametric bootstrap support (Felsenstein 1985) for each clade was tested based on 2000 replications, using the heuristic bootstrap option of PAUP*4.0. Phylogenetic trees were drawn using TREEVIEW (Page 1996).

Results

We generated a total of 19 new nuclear LSU rDNA sequences for this study (Table 1). The sequences were aligned with 31 nuLSU rDNA sequences obtained from Genbank (Table 1) to produce a matrix of 889 unambiguously aligned nucleotide position characters in the nu LSU. 423 characters were variable. The alignment is available in TreeBASE (http://treebase. bio.buffalo.edu/treebase/).

The likelihood parameters in the sample had the following average values (± one standard deviation): base frequencies $\pi(A) = 0.237$ (±0.003), $\pi(C) = 0.228$ (±0.003), $\pi(G) = 0.307$ (±0.004), $\pi(T) = 0.228$ (±0.002), rate matrix $r(AC) = 1.245$ (±0.097), $r(AG) = 3.164$ (±0.229), $r(AT) = 1.53$ (±0.111), $r(CG) = 1.02$ (±0.089), $r(CT) = 8.936$ (±0.701), $r(GT) = 1.0$ (±0.0), gamma shape parameter alpha = 0.64 (±0.131), and the proportion of invariable site p(invar) = 0.335 (±0.065). The additionally performed MP analysis revealed basically the same topology as the Bayesian analysis with the exception that Agyriales were monophyletic. However, this received only 61% support. Our description below will concentrate on the Bayesian analysis. The bootstrap support values above 74% are indicated in Fig. 2 in addition to the posterior

Table 1. Species and specimens of lichenized and non-lichenized Ascomycota used in the current study. Taxa for which sequences have been newly obtained are in bold face. Herbarium acronyms follow Holmgren et al. (1990)

Species	Specimen	GenBank acc. no. nuLSU
Adelolecia pilatii	–	AY300826
Agonimia tristicula	–	AY300828
Berlesiella nigerrima	CBS 513.69	**AY605075**
Bryophagus gloeocapsa	–	AF465440
Ceramothyrium carniolicum	–	AY004339
Chroodiscus coccineus	–	AF465441
Coenogonium disjunctum	–	AF465443
Coenogonium flavicans	–	AF465444
Coenogonium luteum	–	AF279387
Diploschistes cinereocaesius	–	AY300835
Diploschistes diploschistoides	Australia, Lumbsch 19073b (F)	**AY605076**
Diploschistes muscorum	–	AY300836
Diploschistes ocellatus	Spain, 2.6.2003, Lumbsch (F)	**AY605077**
Diploschistes rampoddensis	–	AF274094
Diploschistes thunbergianus	–	AF274095
Eurotium rubrum	–	AY004346
Fissurina sp.	Costa Rica, Lücking 15070 a (F)	**AY605071**
Glyphium elatum	–	AF346420
Graphina poitiaei	–	AF465447
Gyalecta jenensis	–	AF279391
Gyalecta ulmi	–	AF465463
Lobaria pulmonaria	–	AF183934
Myriotrema bahianum	Costa Rica, Nelsen 2032 A (F)	**AY605067**
Myriotrema cinereum	Japan, Lumbsch 19056 b (F)	**AY605074**
Myriotrema desquamans	Australia, Lumbsch & Mangold 19166 o (F)	**AY605085**
Myriotrema laeviusculum	Costa Rica, Sipman 47896 (F)	**AY605070**
Myriotrema wightii	Costa Rica, Will-Wolf 10043 a (F)	**AY605079**
Nadvornikia hawaiiensis	Australia, Lumbsch & Mangold 19176 q (F)	**AY605080**
Norrlinia peltigericola	–	AY300845
Ocellularia perforata	Costa Rica, Sipman 44335 (B)	**AY605081**
Ocellularia rhodostroma	Costa Rica, Sipman 48032 a (F)	**AY605068**
Ocellularia sp. 1	Costa Rica, Lücking 15215 (F)	**AY605083**
Ocellularia sp. 2	Australia, Lumbsch & Mangold 19100 p (F)	**AY605082**
Ochrolechia balcanica	–	AF329171
Ochrolechia tartarea	–	AY300848
Pertusaria albescens	–	AF329176
Pertusaria erythrella	–	AF274100
Placopsis gelida	–	AY212836
Pyrrhospora quernea	–	AY300858
Pyrenula laevigata	–	AY607736
Pyrenula nitida	–	AY607737
Pyrgillus javanicus	Australia, Lumbsch & Mangold 19115e (F)	**AY605078**
Tephromela atra	–	AY300865
Thelotrema glaucopallens	Costa Rica, Lücking 15620 (F)	**AY605069**
Thelotrema lepadinum	–	AY300866
Thelotrema myriocarpum	Costa Rica, Lücking 15069 (F)	**AY605072**
Thelotrema suecicum	–	AY300867
Thelotrema trypethelioides	Australia, Lumbsch & Mangold 19092 t (F)	**AY605073**
Thelotrema weberi	Australia, Lumbsch & Mangold 19108 d (F)	**AY605084**
Trapelia placodioides	–	AF274103
Trapeliopsis granulosa	–	AF274119
Xylographa vitiligo	–	AY212878

probabilities. Five most parsimonious trees 1821 steps long were found with consistency index 0.38 and retention index 0.64.

In the majority-rule consensus tree of 19,000 sampled trees (Fig. 2), Chaetothyriomycetes and Lecanoromycetes each form strongly supported monophyletic groups. *Pyrgillus javanicus* is nested within the genus *Pyrenula* (pp 1.0), which clusters within the Chaetothyriomycetes. *Pyrenula* plus *Pyrgillus* (Pyrenulales) form a sister group to a clade including three non-lichenized taxa (two Chaetothyriales and *Glyphium elatum*, which is currently unassigned to any order [Eriksson et al. 2004]), but this relationship lacks support. The lichen forming *Agonimia tristicula* and the lichenicolous *Norrlinia peltigericola* (Verrucariales) are sister to Pyrenulales plus Chaetothyriales.

In our analysis, *Nadvornikia* falls within Lecanoromycetes, which includes Lecanorales, Pertusariales, Agyriales, and Ostropales s. lat. However, there is no support for the supraordinal relationships in Lecanoromycetes. The Pertusariales are strongly supported (pp 0.99) and are sister to a clade formed by Agyriales plus Ostropales s. lat. Again, this relationship lacks support. Agyriales are represented by four species and appear paraphyletic in our analysis, but monophyletic in the MP analysis. Within Ostropales s. lat., which are not supported, three strongly supported clades can be distinguished: (1) *Gyalecta*, (2) Coenogoniaceae (including *Bryophagus*), and (3) Thelotremataceae, with Graphidaceae nested within (Thelotremataceae s. lat.).

The phylogeny within Thelotremataceae s. lat. is only partially resolved. In fact, only the relationships of closely related species show support. *Nadvornikia hawaiiensis* forms a well-supported sister group relationship with *Thelotrema myriocarpum* (pp 1.0). Three closely related species of the *Thelotrema lepadinum* group are strongly supported (pp 1.0) as monophyletic. Four *Myriotrema* (including *Thelotrema trypethelioides* that morphologically fits into *Myriotrema*) and four *Ocellularia* spp. form a well-supported clade (pp 0.99), but the representatives of the two genera are not separated within this clade. The genus *Diploschistes* is supported as monophyletic (pp 1.0), but without *D. ocellatus*, which remains unresolved within Thelotremataceae. Also, the relationships of *Myriotrema wightii* could not be established with this data set.

Two species currently classified in Graphidaceae were included in this study (*Fissurina* sp. and *Graphina poitiaei*). These two species show a strongly supported sister group relationship and are nested within Thelotremataceae. They appear on a clade with *Chroodiscus coccineus*, the *Thelotrema lepadinum* group, *Myriotrema desquamans* plus *Thelotrema glaucopallens*, and *Nadvornikia hawaiiensis* plus *Thelotrema myriocarpum*. The relationships of these groups, however, lack support.

Discussion

Our molecular phylogenetic analysis confirms recent classification proposals for the genera *Nadvornikia* and *Pyrgillus* based on morphological evidence. They support Tibell's hypothesis of the polyphyly of calicioid fungi. Calicioid lichens and fungi are dispersed over the phylogenetic tree of lichen forming fungi and belong to several unrelated orders, such as Lecanorales, Mycocaliciales, Ostropales, and Pyrenulales.

The placement of *Nadvornikia* in Thelotremataceae is somewhat surprising, given that characters such as a mazaedium, ascus or spore type, differ from typical Thelotremataceae (Figs 1 C, E & F). However, it shows that overall similarity in thallus and ascoma morphology can be of systematic importance (Fig. 1 A). Similar variation was also found in other groups that include calicioid lichens, such as Physciaceae (Wedin et al. 2002). Currently, the morphogenetic processes involving the change of dispersal strategies that lead to calicioid lichens and fungi are not understood.

Chemistry is another character set that was employed to support close relationship between *Nadvornikia* and Thelotremataceae (Harris 1990). *Nadvornikia* species contain lichexanthone or the stictic acid chemosyndrome (Harris 1990; Pant & Awasthi 1989; Tibell 1984, 1990). Although this chemistry is common in Thelotremataceae, these substances also abound in other groups of lichen

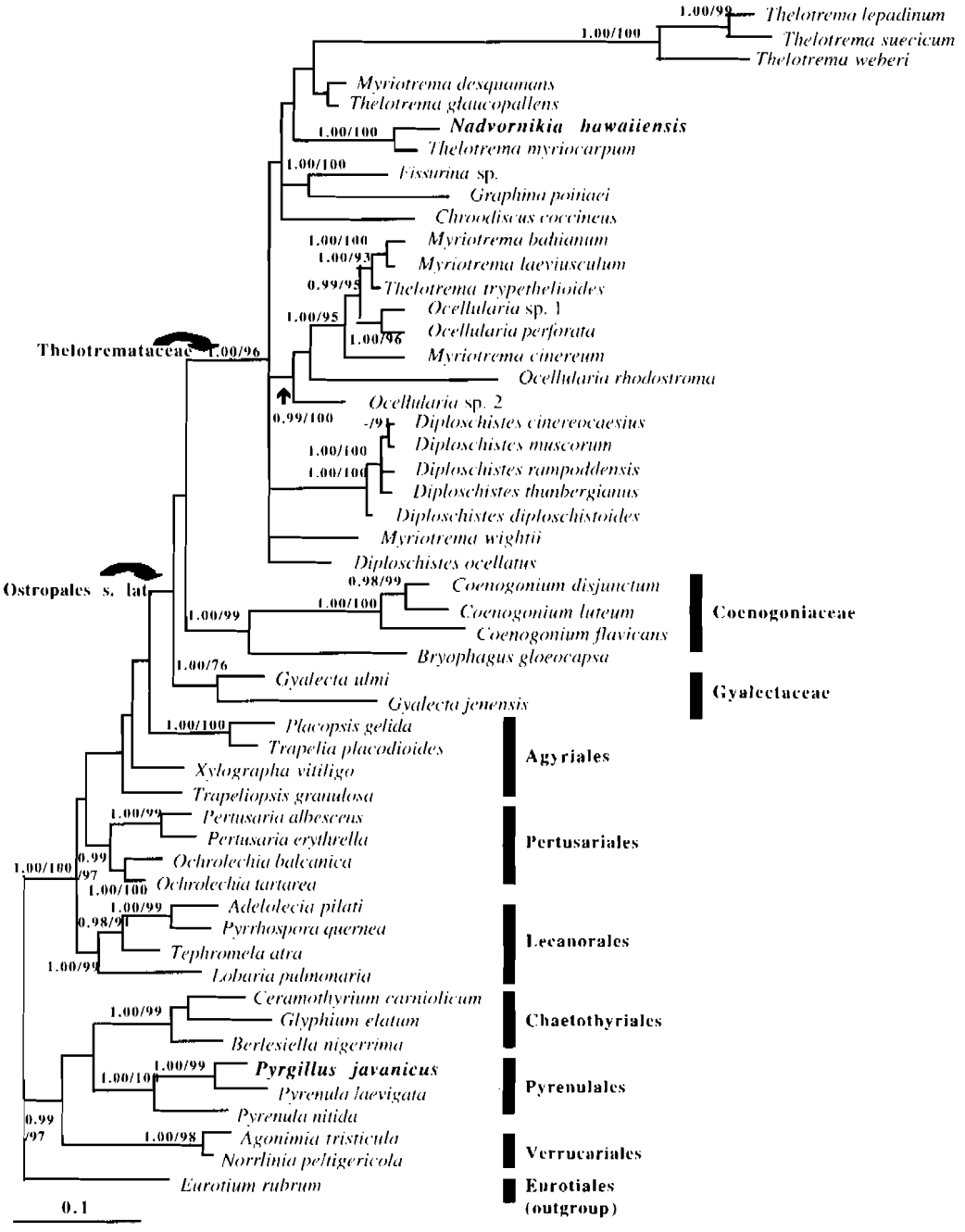

Figure 2. Phylogenetic placement of the genera *Nadvornikia* and *Pyrgillus* (both marked in bold) within the euascomycetes, based on partial nuLSU rDNA sequences. This is a majority-rule consensus tree based on 19,000 trees from a B/MCMC tree sampling procedure. Posterior probabilities equal or above 0.95 indicated at branches first, followed by the bootstrap value equal or above 75% obtained in the MP analysis. Ordinal and/or class placement of taxa indicated at margin.

forming fungi, and their presence may not be phylogenetically informative.

The genus *Pyrgillus*, on the other hand, has a number of features in common with other species of Pyrenulaceae. The ascomata clearly resemble perithecia (Figs 1 B & D), and the spores contain lens-shaped lumina, which is a common feature in that family (Figs 1 G–H). Additionally, lichexanthone, which occurs in *Pyrgillus javanicus*, is also a common substance in Pyrenulaceae (Harris 1990). The generic status of *Pyrgillus* and its relationships to *Pyrenula* require further studies.

Although the single gene data set is sufficient to evaluate the phylogenetic position of the two genera, we were unable to make any further inferences on the phylogeny within Thelotremataceae and Ostropales. The topology in this part of the tree was only partially resolved, and several clades did not receive significant support. The currently accepted core genera in the Thelotremataceae (*Thelotrema*, *Myriotrema*, *Ocellularia*) are not supported in our analysis. However, insufficient taxon sampling and lack of phylogenetic signal in the nuclear LSU rDNA may be responsible for this, and therefore no further conclusions can be drawn.

Our taxon sampling was also insufficient to resolve any detailed questions regarding relationships within Chaetothyriomycetes. However, the currently accepted orders Verrucariales, Pyrenulales, and Chaetothyriales were significantly supported. Supraordinal relationships were not supported, with the exception of the two classes Chaetothyriomycetes (including Chaetothyriales, Pyrenulales, and Verrucariales) and Lecanoromycetes (including Lecanorales, Pertusariales, Agyriales, and Ostropales s.lat.). Despite these shortcomings, our molecular data were sufficient to re-evaluate and satisfactorily confirm the phylogenetic position of *Nadvornikia* and *Pyrgillus*.

Acknowledgements

We dedicate this paper to Leif Tibell on the occasion of his 60th birthday. Leif has had the courage to dismantle his favourite group of lichens, the Caliciales. With molecular data we have only been able to confirm what he has already known.

We wish to thank Mathew Nelsen, Susan Will-Wolf (both Madison) and Harrie Sipman (Berlin) for supplying us with fresh material for molecular analysis. DNA extraction and sequencing has been performed in the Real Jardín Botánico de Madrid funded by the EU programme BIOD-Iberia, and in the Field Museum's Pritzker Laboratory for Molecular Systematics and Evolution, supported by a start-up fund of the Field Museum to HTL. Iyabo Osifeso (Chicago) is thanked for performing some of the molecular work. Imke Schmitt and Bryan Stuart (both Chicago) are thanked for their helpful comments on an earlier draft of this manuscript.

References

Aptroot, A. 1991. A monograph of the Pyrenulaceae (excluding *Anthracothecium* and *Pyrenula*) and the Requienellaceae, with notes on the Pleomassariaceae, the Trypetheliaceae and *Myconicrothelia* (lichenized and non-lichenized ascomycetes). – Bibliotheca Lichenol. 44: 1–178.

Döring, H., Clerc, P., Grube, M. & Wedin, M. 2000. Mycobiont-specific PCR primers for the amplification of nuclear ITS and LSU rDNA from lichenized ascomycetes. – Lichenologist 32: 200–204.

Eriksson, O. E., Baral, H.-O., Currah, R. S., Hansen, K., Kurtzman, C. P., Rambold, G. & Laessoe, T. 2004. Outline of Ascomycota. – Myconet 9: 1–89.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. – Evolution 39: 783–791.

Gargas, A., DePriest, P. T., Grube, M. & Tehler, A. 1995. Multiple origins of lichen symbioses in fungi suggested by SSU rDNA phylogeny. – Science 268: 1492–1495.

Harris, R. C. 1989. A sketch of the family Pyrenulaceae (Melanommatales) in eastern North America. – Mem. New York Bot. Gard. 49: 74–107.

Harris, R. C. 1990. Some Florida Lichens. – Publ. by the Author. Bronx, N.Y.

Hawksworth, D. L. 1994. Ascomycete Systematics. Problems and Perspectives in the Nineties. – Plenum Press, New York.

Henssen, A. & Jahns, H. M. 1974. Lichenes. – Georg Thieme Verlag, Stuttgart.

Huelsenbeck, J. P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. – Bioinform. 17: 754–755.

Huelsenbeck, J. P., Rannala, B. & Masly, J. P. 2000. Accomodating phylogenetic uncertainty in evolutionary studies. – Science 288: 2349–2350.

Karplus, K., Barrett, C. & Hughey, R. 1998. Hidden Markov models for detecting remote protein homologies. – Bioinform. 14: 846–856.

Larget, B. & Simon, D. L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. – Molec. Biol. Evol. 16: 750–759.

Lumbsch, H. T., Schmitt, I., Palice, Z., Wiklund, E., Ekman, S. & Wedin, M. 2004. Supraordinal phylogenetic relationships of Lecanoromycetes based on a Bayesian analysis of combined nuclear and mitochondrial sequences. – Molec. Phylogen. Evol. 31: 822–832.

Lutzoni, F., Pagel, M. & Reeb, V. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. – Nature 411: 937–940.

Martín, M. P. & Winka, K. 2000. Alternative methods of extracting and amplifying DNA from lichens. – Lichenologist 32: 189–196.

Nylander, W. 1857. Enumeration Generale des Lichens. – Suppl. Mem. Soc. Sci. Cherbourg 5: 332–339.

Nylander, W. 1860. Synopsis methodica lichenum omnium hucusque cognitorum praemissa introductione lingua gallica tractata. – L. Martinet, Paris.

Page, R. D. M. 1996. Treeview: an application to display phylogenetic trees on personal computers. – Comp. Appl. Biosci. 12: 357–358.

Pant, G. & Awasthi, D. D. 1989. Caliciales from India and Nepal. – Biovigyanam 15: 3–27.

Poelt, J. 1973. Classification. – In: Ahmadjian, V. & Hale, M. E. (eds). The Lichens. Academic Press, New York & London. pp. 599–632.

Rodriguez, F., Oliver, J. F., Martín, A. & Medina, J. R. 1990. The general stochastic model of nucleotide substitution. – J. Theor. Biol. 142: 485–501.

Swofford, D. L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. – Sinauer Associates. Sunderland, Massachusettes.

Tehler, A. 1996. Systematics, phylogeny and classification. – In: Nash III, T. H. (ed.). Lichen Biology. Cambridge University Press, Cambridge, pp. 217–239.

Tibell, L. 1984. A reappraisal of the taxonomy of Caliciales. – Beih. Nova Hedwigia 79: 597–713.

Tibell, L. 1996. Caliciales. – Flora Neotropica 69: 1–78.

Tibell, L. & Wedin, M. 2000. Mycocaliciales, a new order for nonlichenized calicioid fungi. – Mycologia 92: 577–581.

Tuckerman, E. 1867. Lichens of the Hawaiian Islands. – Proc. Amer. Acad. Arts Sci. 7: 223–235.

von Höhnel, F. 1919. Fragmente zur Mykologie. Nr. 1188. – Sitzungsber. Akad. Wiss. Wien, math. naturw. Kl. Abt. 1, 128: 616–621.

Wedin, M. & Döring, H. 1999. The phylogenetic relationship of the Sphaerophoraceae, *Austropeltum* and *Neophyllis* (lichenized Ascomycota) inferred by SSU rDNA sequences. – Mycol. Res. 103: 1131–1137.

Wedin, M. & Tibell, L. 1997. Phylogeny and evolution of Caliciaceae, Mycocaliciaceae, and Sphinctrinaceae (Ascomycota), with notes on the evolution of the prototunicate ascus. – Canad. J. Bot. 75: 1236–1242.

Wedin, M., Tehler, A. & Gargas, A. 1998. Phylogenetic relationships of Sphaerophoraceae (Ascomycetes) inferred from SSU rDNA sequences. – Pl. Syst. Evol. 209: 75–83.

Wedin, M., Döring, H., Nordin, A. & Tibell, L. 2000. Small subunit rDNA phylogeny shows the lichen families Caliciaceae and Physciaceae (Lecanorales, Ascomycotina) to form a monophyletic group. – Canad. J. Bot. 78: 246–254.

Wedin, M., Baloch, E. & Grube, M. 2002. Parsimony analyses of mtSSU an nITS rDNA sequences reveal the natural relationships of the lichen families Physciaceae and Caliciaceae. – Taxon 51: 655–660.

Winka, K., Alberg, C. & Eriksson, O. E. 1998. Are there lichenized Ostropales? – Lichenologist 30: 455–462.

Zahlbruckner, A. 1926. Lichenes. B. Spezieller Teil. – In: Engler, A. (ed.). Die natürlichen Pflanzenfamilien, 2 ed., vol. 8. Borntraeger, Leipzig, pp. 61–270.

SLU

Department of Conservation Biology

November 16, 2004

Dear M.P. Martin

Thanks a lot for your contribution to the Tibell festschrift and your patience with our questions and the tight time schedule! Your copy/copies (one copy per author) of the volume as well as your reprints are enclosed. Leif received his copy on Friday 12 November. It came as a total surprise for him. In the evening we were 18 lichenologists including some from Germany, Finland and Estonia who had dinner together with Leif in the herbarium of UPS. Leif entertained us with playing his fiddle.

If anyone wishes to buy more copies of the volume, they are available, probably for c. 300 SEK, from acta@ub.uu.se. Anders Nordin will send you a PDF file with your paper. As you will note, there are some spelling errors on the back. We have no idea when they appeared since the second proof was without these errors.

Yours sincerely

Göran Thor and Anders Nordin

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

Postal address	*Visiting address*	*Tel.*	*Fax*	*E-mail*
P.O. Box 7002	Bäcklösav 10	+46 (0)18-67 10 00	+46 (0)18-67 35 37	Goran.Thor@nvb.slu.se
S-750 07 UPPSALA	ULTUNA	+46 (0)18-67 24 19		
SWEDEN				