Phylogenetic position of the foliicolous genus *Chroodiscus* (Ostropales, Ascomycota) inferred from nuclear and mitochondrial ribosomal DNA sequences

Papong, K.[1], Corush[2], J., Mangold, A.[3], Lücking[2], R. and Lumbsch, H.T.[2*]

[1]Department of Biology, Faculty of Science, Mahsarakham University, Kantarawichai, Maha Sarakham Province 44150, Thailand
[2]Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, IL 60605, U.S.A.
[3]Ossastrasse 6, 12045 Berlin, Germany

Papong, K., Mangold, A., Corush, J. Lücking, R. and Lumbsch, H.T. (2009). Phylogenetic position of the foliicolous genus *Chroodiscus* (Ostropales, Ascomycota) inferred from nuclear and mitochondrial ribosomal DNA sequences. Fungal Diversity 38: 147-153.

The phylogenetic position of the lichenized, foliicolous fungal genus *Chroodiscus* was studied using a combined data set of nuLSU/mtSSU DNA sequence data. Species of *Chroodiscus* form a well-supported monophyletic clade that is sister to a clade including *Chapsa*, *Leucodecton* and *Thelotrema*. The analysis confirms the nested position of *Leucodecton* within *Chapsa* and suggests a repeated gain and/or loss of lateral paraphyses in this lineage: whereas *Acanthotrema*, *Chapsa*, and *Thelotrema* have lateral paraphyses, they are absent in *Chroodiscus* and *Leucodecton*.

Key words: *Graphidaceae*, lichens, phylogeny, taxonomy, *Thelotremataceae*.

Article Information
Received 14 December 2008
Accepted 14 July 2009
Published online 1 October 2009
*Corresponding author: Thorsten Lumbsch; e-mail: tlumbsch@fieldmuseum.org

Introduction

Leaves in tropical and oceanic regions are frequently colonized by bryophytes and fungi. Lichenized ascomycetes form an important component of the diversity of foliicolous organisms, with approximately 900 known species (Lücking, 2003, 2008). Species of different clades of lichen-forming ascomycetes grow on leaves. Some families that have their center of distribution in the tropics, such as *Gomphillaceae*, *Pilocarpaceae*, *Porinaceae*, or *Strigulaceae*, include numerous foliicolous species. In contrast, the largest family of crustose lichens, *Graphidaceae*, which now includes *Thelotremataceae* (Staiger *et al*., 2006; Mangold *et al*., 2008), includes only one genus that occurs typically on leaves: *Chroodiscus*. In its current circumscription (Frisch *et al*., 2006) the genus is restricted to strictly foliicolous species. Fourteen species are currently accepted in the genus (Lücking, 1999; Lücking and Grube, 2002; Papong *et al*., 2009). Previously, all thelotremoid Graphidaceae with chroodiscoid apothecia, i.e. ascomata exposed through radial fissures of the covering thallus layer, the latter eventually forming erect to recurved, triangular lobules bordering the disc, had been placed in *Chroodiscus* (Lumbsch and Vezda, 1990; Kalb and Vezda, 1992; Kantvilas and Vezda, 2000). However, morphological and molecular analyses suggested that chroodiscoid apothecia do not necessarily represent a synapomorphy (Frisch *et al*., 2006). Consequently, species with chroodiscoid ascomata were assigned to several genera, including: *Acanthotrema*, *Chapsa*, *Chroodiscus*, *Reimnitzia*, and *Topeliopsis* (Kalb, 2001; Frisch *et al*., 2006; Frisch and Kalb, 2006; Mangold *et al*., 2009). Among these, *Chroodiscus* is characterized by the lack of lateral paraphyses, rather lax paraphyses, thinly euseptate, I- negative ascospores, and the strictly foliicolous growth habit. Although the restriction to foliicolous species, which was originally proposed by Santesson (Santesson, 1952), resulted in a very

homogeneous group, the relationships to other genera in Graphidaceae remained uncertain. Previous molecular analyses that only included the type species (*C. coccineus*) failed to resolve the phylogenetic position of *Chroodiscus* with confidence (Frisch *et al*., 2006; Staiger *et al*., 2006; Mangold *et al*., 2008). Particularly the relationship to the other chroodiscoid genera *Acanthotrema* and *Chapsa* remained unclear. Although, the two genera did not cluster together in any of these studies, the lack of support for relationships between clades made it impossible to draw further conclusions. Within a clade of *Chapsa* and *Thelotrema* species that have ascomata with lateral paraphyses growing from the exciple into the hymenium, species of *Leucodecton* were nested, a genus lacking lateral paraphyses. However, again this relationship lacked significant support and hence needs confirmation by additional data.

In order to address the phylogenetic position of *Chroodiscus* and its relationship to other chroodiscoid genera and to evaluate the placement of taxa lacking lateral paraphyses nested with the *Chapsa/Thelotrema* clade, we generated new nuLSU and mtSSU sequences for five further species of *Chroodiscus* and analyzed them with a set of selected taxa in the thelotremoid Graphidaceae.

Materials and methods

Taxon sampling

A data matrix of 36 OTUs was assembled using sequences of nuclear large subunit and mitochondrial SSU rDNA sequences (Table. 1). We included four species of the *Myriotrema/Ocellularia* clade as outgroup in the analysis, since these genera were sister to clades including *Chroodiscus* and other genera with chroodiscoid ascomata in previous phylogenetic studies (Frisch *et al*., 2006; Mangold *et al*., 2008).

DNA extraction, amplification and sequencing

Total DNA was extracted from herbarium specimens using the DNeasy Plant Mini Kit (Qiagen) following the instructions of the manufacturer. Dilutions (10^{-1} up to 10^{-2}) of DNA were used for PCR amplifications. Primers for amplification were: (a) for the nuclear LSU rDNA: AL2R (Mangold *et al*., 2008) and nu-LSU-1125-3' (= LR6) (Vilgalys and Hester, 1990), and (b) for the mitochondrial SSU rDNA: mr SSU1 (Zoller *et al*., 1999) and MSU 7 (Zhou and Stanosz, 2001). 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 distilled 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 52 °C, 1 min at 73 °C, and a final elongation for 7 min at 73 °C. Amplification products were viewed on 1% agarose gels stained with ethidium bromide and subsequently purified using the QIAquick PCR Purification Kit (Qiagen) or Nucleo Spin DNA purification kit (Macherey-Nagel). Fragments were sequenced using the Big Dye Terminator reaction kit (ABI PRISM, Applied Biosystems). Sequencing and PCR amplifications were performed using the same sets of primers. 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 1 µL EDTA, 1 µL NaAc and 25 µL 100% EtOH, followed by 24.5 µL EtOH and 10.5 µL of sterile dH_2O. 10 µL Hi-Dye Formamide was added before loading on an ABI 3100 (Applied Biosystems) automatic sequencer. Sequence fragments obtained were assembled with SeqMan 4.03 (DNASTAR) and manually adjusted.

Sequence alignments and Phylogenetic analyses

Alignments for the single data sets were done using Clustal W (Thompson *et al*., 1994) and ambiguously aligned regions were manually excluded. The alignments were analysed by maximum parsimony (MP) and a Bayesian approach (B/MCMC). To test for potential conflict, MP bootstrap analyses were performed on each individual data set, and 75% bootstrap consensus trees were examine for conflict (Lutzoni *et al*., 2004).

Maximum parsimony analyses were performed using the program PAUP* (Swofford, 1993). Heuristic searches with 2000 random taxon addition replicates were

Table 1. Species and specimens used in the current study with Genbank accession numbers (newly obtained sequences in bold).

Species	Sample	mtSSU	nuLSU
Acanthotrema brasilianum		DQ384916	DQ431928
Chapsa astroidea		EU075566	EU075614
Chapsa leprocarpa		EU075568	EU075615
Chapsa niveocarpa	Australia, Queensland, *Lumbsch 19151p & Mangold* (F)	FJ708494	FJ708487
Chapsa phlyctioides		EU075569	EU075617
Chapsa pulchra		EU075571	EU075619
Chroodiscus argillaceus	Thailand, Khao Yai National Park, *Papong 6069* (RAMK)	FJ708495	FJ708488
Chroodiscus australiensis	Australia, Queensland, *Lumbsch 5437 & Mangold* (F)	FJ708496	FJ708489
Chroodiscus coccineus		DQ384915	AF465441
Chroodiscus defectus	Thailand, Khao Sok National Park, *Papong 5118* (KKU)	FJ708497	FJ708490
Chroodiscus homchantarae	Thailand, Khao Sok National Park, *Papong 5289* (KKU)	FJ708498	-
Chroodiscus khaolungensis	Thailand, Khao Sok National Park, *Papong 4853* (KKU)	FJ708499	-
Leptotrema wightii		EU075574	EU075622
Leucodecton compuctellum	Australia, Queensland, *Lumbsch 19161vA & Mangold* (F)	FJ708500	FJ708491
Leucodecton subcompunctum		EU075575	EU075623
Leucodecton subcompunctum		EU075576	EU075624
Myriotrema microporum		EU075578	EU075626
Myriotrema olivaceum		EU075579	EU075627
Ocellularia diacida		EU075583	EU075630
Ocellularia profunda		EU075588	EU075635
Schizotrema schizolomum	Australia, New South Wales, *Mangold 24c* (F)	FJ708501	FJ708492
Schizotrema zebrinum		EU075608	EU075652
Thelotrema crespoae	Australia, New South Wales, *Mangold 27v* (F)	EU075606	FJ708493
Thelotrema diplotrema		EU075599	EU075643
Thelotrema nureliyum		EU075597	EU075647
Thelotrema nureliyum		EU075604	EU075649
Thelotrema cf. *nureliyum*		EU075603	EU075648
Thelotrema porinaceum		EU675291	EU126651
Thelotrema porinoides		EU675292	EU126652
Thelotrema saxatile		EU075602	EU075645
Thelotrema subtile		DQ871020	DQ871013
Thelotrema monosporum		EU075596	EU075644
Thelotrema suecicum		AY300917	AY300867
Topeliopsis decorticans		EU075609	EU075654
Topeliopsis meridensis		EU075610	EU075655
Topeliopsis muscigena		EU075611	EU075656

conducted with TBR branch swapping and MulTrees option in effect, equally weighted characters and gaps treated as missing data. Bootstrapping (Felsenstein, 1985) was performed based on 2000 replicates with random sequence additions. The B/MCMC analysis was conducted using the MrBayes 3.1.1 program (Huelsenbeck and Ronquist, 2001). It was performed assuming the general time reversible model of nucleotide substitution (Rodriguez *et al*., 1990) including estimation of invariant sites, assuming a discrete gamma distribution with six rate categories and no molecular clock was assumed. A run with 4,000,000 generations starting with a random tree and employing 6 simultaneous chains was executed. Every 100th tree was saved into a file. The first 200,000 generations (i.e. the first 2000 trees) were deleted as the "burn in" of the chain. We plotted the log-likelihood scores of sample points against generation time using TRACER 1.0 (http://evolve.zoo.ox.ac.uk/software.html?id=tracer) to check whether a plateau was achieved after the first 200,000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value. Additionally, we used AWTY (Nylander *et al*., 2007) to

compare splits frequencies in the different runs and to plot cumulative split frequencies to ensure that stationarity was reached. Of the remaining 76,000 trees (38,000 from each of the parallel runs), a majority rule consensus tree with average branch lengths was calculated using the sumt option of MrBayes. Posterior probabilities were obtained for each clade. Only clades that received bootstrap support equal or above 75% under MP and ME, and posterior probabilities ≥ 0.95 were considered as strongly supported. Phylogenetic trees were drawn using the program Treeview (Page, 1996).

In addition to the phylogenetic analyses, we used alternative hypothesis testing to evaluate whether our data are sufficient to reject monophyly of *Chapsa*+*Chroodiscus* (=*Chroodiscus* sensu Kantvilas & Vezda, 2000). For the hypothesis testing two different methods were employed: 1. Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) and 2. expected likelihood weight (ELW) test following Strimmer & Rambaut (Strimmer and Rambaut, 2002). The SH and ELW tests were performed using Tree-PUZZLE 5.2 (Schmidt *et al*., 2002) with the combined data set on a sample of 200 unique trees, the best trees agreeing with the null hypotheses, and the unconstrained ML tree. These trees were inferred in Tree-PUZZLE employing the GTR+I+G nucleotide substitution model.

Results

Phylogenetic analyses

Fifteen new DNA sequences were obtained for this study. The combined alignment of the nuclear LSU and mitochondrial SSU rDNA included 1088 unambiguously aligned nucleotide position characters, 505 of which were variable and 360 parsimony-informative. The MP bootstrap support method for testing data sets for incongruence indicated no strongly supported conflict (data not shown) and hence combined analyses was performed.

MP analysis of the combined data set yielded 111 most parsimonious trees (1623 steps long, CI=0.45, RI=0.64). The strict consensus tree of the MP analysis did not contradict the Bayesian tree topology and hence only the majority-rule consensus tree of the Bayesian tree sampling is shown here (Fig. 1).

In the B/MCMC analysis of the combined data set, the likelihood parameters in the sample had the following mean (Variance): LnL = -9324.11 (0.002), base frequencies π(A) = 0.294 (0.00014), π(C) = 0. 168 (0.00011), π(G) = 0.262 (0.00013), π(T) = 0.276 (0.00013), the gamma shape parameter alpha = 0.449 (0.00069), and p(invar) = 0.238 (0.00053).

In our phylogenetic analysis species of *Chroodiscus* form a strongly supported monophyletic group. The phylogenetic tree indicates that *Chroodiscus* belongs to a well-supported clade including also *Chapsa*, *Leucodecton*, and *Thelotrema*. This clade is sister to the monotypic genus *Acanthotrema*, a relationship that is also supported. However, the relationships of *Chroodiscus* to *Chapsa*, *Leucodecton and Thelotrema* are not resolved with confidence. The genera *Leucodecton* and *Thelotrema* are supported as monophyletic, while *Chapsa* is paraphyletic with *Leucodecton* nested within. The two other genera characterized by having lateral paraphyses, *Schizotrema* and *Topeliopsis*, each form strongly supported monophyletic groups and are sister clades to each other, but the latter relationship lacks support. The two genera are sister to the *Acanthotrema/Chapsa/ Chroodiscus/Leucodecton/Thelotrema* clade, but again without support.

The alternative hypothesis testing significantly rejects a monophyly of *Chroodiscus* sensu Kantvilas & Vezda (2000), i.e. including *Chapsa* species ($p \leq 0.001$ in both tests). However, monophyly of *Chroodiscus* and *Chapsa* including *Leucodecton* is not rejected (p=0.43 in SH test, p=0.21 in ELW test).

Some relationships within the genus *Chroodiscus* were supported. The morphologically close species *C. khaolungensis* and *C. argillaceus* form a strongly supported group that together with *C. defectus* and *C. homchantarae* form a well-supported clade, which corresponds to group A in Lücking *et al*. (Lücking *et al*., 2008). *Chroodiscus australlienis* (group B in Lücking *et al*., 2008) has a strongly supported sister-group relationship to these taxa. Sister to the rest of included

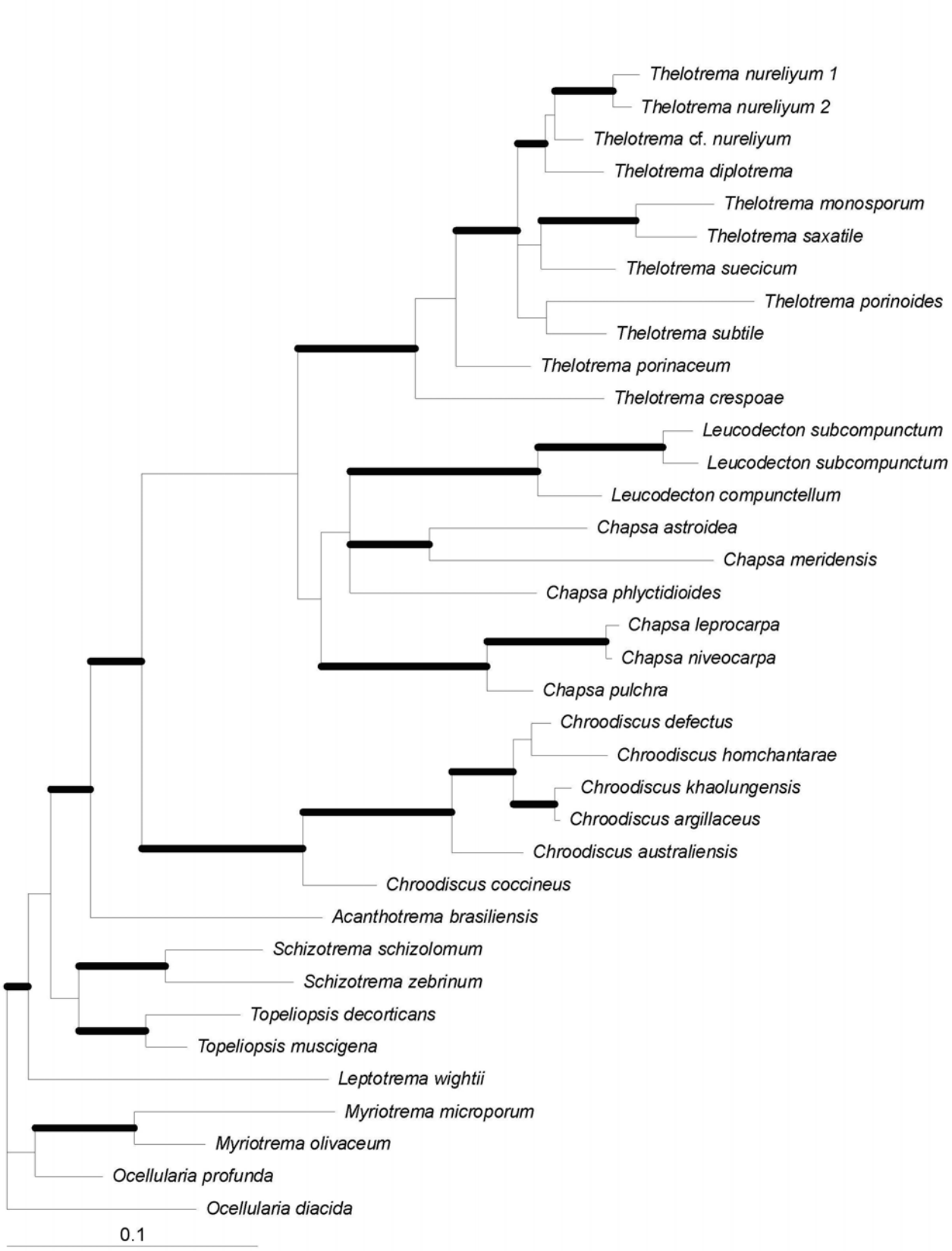

Fig. 1. Phylogenetic placement of *Chroodiscus* as inferred from a two gene-partition analysis. This is a 50% majority-rule consensus tree based on a B/MCMC tree sampling procedure. Branches with posterior probabilities equal or above 0.95 and MP bootstrap support values above 70% are indicated in bold.

Chroodiscus species is the type species, *C. coccineus* (belonging to group D in Lücking *et al*., 2008).

Discussion

Previous molecular analyses only included the type species of *Chroodiscus* and found the genus isolated within Graphidaceae (Staiger *et al*. 2006; Mangold *et al*. 2008). Our study with an extended taxon sampling, however, indicates a close relationship of *Chroodiscus* to the to other chroodiscoid genera: *Acanthotrema* and *Chapsa*, as well as the genus *Thelotrema*. This placement is in agreement with phenotype-based phylogenies (Lücking *et al*. 2008;). Although we could demonstrate that *Chroodiscus* belongs to the *Acanthotrema/Chapsa/Leucodecton/Thelotrema* clade, its phylogenetic placement within this clade remains uncertain. Our data are not sufficient to reject a scenario in which both *Chroodiscus* and *Leucodecton* would be nested within *Chapsa*. Additional studies employing additional markers and a larger taxon sampling will be necessary to further evaluate the evolution of characters within this clade.

A morphological and ecological characterization of the *Acanthotrema/Chroodiscus/ Chapsa/Leucodecton/Thelotrema* clade is not easy. Although most species in the group have either chroodiscoid or lepadinoid apothecia, the clade shows quite some variation in morphological and ecological features. It includes corticolous, foliicolous, muscicolous, and saxicolous taxa, as well as representatives from tropical rainforest, exposed coastal vegetation, and subtropical-temperate regions. The ecological variability of this clade is exemplified by species such as *Chapsa meridensis* (subparamo, muscicolous), *Chroodiscus coccineus* (tropical rainforest, foliicolous), *Leucodecton compunctellum* (exposed coastal vegetation, corticolous), and *Thelotrema lepadinum* (tropical montane to temperate, corticolous and saxicolous).

Yet, tendencies can be observed in this clade: the predominance of thalli with rudimentary cortex or a lacking cortex, the reduction of the endospore in the frequently I-negative ascospores, and the predominance of species with secondary substances of the stictic acid chemosyndrome, or lacking substances. This is in contrast to the *Ocellularia-Myriotrema-Stegobolus* clade (Frisch *et al*., 2006; Mangold *et al*., 2009), which includes chiefly tropical species with usually well-developed cortex, often I+ violet-blue ascospores with distinct endospore, and a predominance of the psoromic acid chemosyndrome. Thus, while the phylogeny of the former Thelotremataceae is far from being clarified, a certain combination of morphological, ecological, and chemical characters seems to be predictive for these two major clades.

The inclusion of six of the 14 accepted species of *Chroodiscus* in this analysis permits a first glance at the phylogeny within the genus. In previous phenotype-based phylogenetic analyses (Lücking and Grube, 2002; Lücking *et al*., 2008), four major clades were distinguished. Species of three of these four clades were included in this study, but only the argillaceus clade (group A) had more than one species represented. While this clade is confirmed as monophyletic, more species of the other clades need to be sequenced to test monophyly and relationships of these clades. Relationships between clades within *Chroodiscus* differ between this study and previous phenotype-based studies in that the polarity of the clade is reversed. However, additional species need to be included in the molecular analyses before any further conclusions can be drawn.

Acknowledgements

This work was financed by a NSF grant (DEB-0516116) to The Field Museum (PI: HTL, Co-PI: RL). Newly obtained DNA sequences were generated in the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum. Fabian Ernemann (Chicago) is thanked for performing some of the work in the molecular laboratory.

References

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

Frisch, A. and Kalb, K. (2006). The lichen *Topeliopsis*, additions and corrections. Lichenologist 38: 37-45.

Frisch, A., Kalb, K. and Grube, M. (2006). Contributions towards a new systematics of the lichen family Thelotremataceae. Bibliotheca Lichenologica 92: 1-539.

Huelsenbeck, J. P. and Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755.

Kalb, K. (2001). The lichen genus *Topeliopsis* in Australia and remarks on Australian Thelotremataceae. Mycotaxon 79: 319-328.

Kalb, K. and Vezda, A. (1992). Neue foliicole Flechten I. Nova Hedwigia 55: 195-209.

Kantvilas, G. and Vezda, A. (2000). Studies on the lichen family Thelotremataceae in Tasmania. The genus *Chroodiscus* and its relatives. Lichenologist 32: 325-357.

Lücking, R. (1999). Addiciones y correcciones al conocimiento de la liquenoflora foliícola de Costa Rica. La familia Asterothyriaceae y el género *Chroodiscus* (Thelotremataceae), con un análisis filogenético Cryptogamie, Mycologie 20: 193-224.

Lücking, R (2003). Takhtajan's floristic regions and foliicolous lichen biogeography: a compatibility analysis. Lichenologist 35: 33-54.

Lücking, R. (2008). Foliicolous lichenized fungi. Flora Neotropica 103: 1-867.

Lücking, R. and Grube, M. (2002). Facultative parasitism and reproductive strategies in *Chroodiscus* (Ascomycota, Ostropales). Stapfia 80: 267-292.

Lücking, R., Papong, K., Thammathaworn, A. and Boonpragob, K. (2008). Historical biogeography and phenotype-phylogeny of *Chroodiscus* (lichenized Ascomycota: Ostropales: Graphidaceae). Journal of Biogeography 35: 2311-2327.

Lumbsch, H.T. and Vezda, A. (1990). Foliicolous lichens of the Australian rainforests. 1. Nova Hedwigia 50: 245-254.

Lutzoni, F., Kauff, F., Cox, C., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T.Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V., Schoch, C., Arnold, A. E., Miadlikowska, J., Spatafora, J., Johnson, D., Hambleton, S., Crockett, M., Shoemaker, R., Sung, G.-H., Lücking, R., Lumbsch, T., O'Donnell, K., Binder, M., Diederich, P., Ertz, D., Gueidan, C., Hansen, K., Harris, R.C., Hosaka, K., Lim, Y.-W., Matheny, B., Nishida, H., Pfister, D., Rogers, J., Rossman, A., Schmitt, I., Sipman, H., Stone, J., Sugiyama, J., Yahr, R. and Vilgalys, R. (2004). Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. American Journal of Botany 91: 1446-1480.

Mangold, A., Elix, J.A. and Lumbsch, H.T. (2009). Thelotremataceae. Flora of Australia 57: 195-420.

Mangold, A., Martin, M.P., Lucking, R. and Lumbsch, H. T. (2008). Molecular phylogeny suggests synonymy of Thelotremataceae within Graphidaceae (Ascomycota: Ostropales). Taxon 57: 476-486.

Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. and Swofford, D.L. (2007). AWTY (Are We There Yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24: 581-583.

Page, R.D.M. (1996). Treeview: an application to display phylogenetic trees on personal computers. Computer Applied Biosciences 12: 357-358.

Papong, K., Lücking, R., Thammathaworn, A. and Boonpragob, K. (2009). Four new taxa of *Chroodiscus* (Ascomycota: Ostropales: Graphidaceae) from Thailand. Bryologist 111: 152-163.

Rodriguez, F., Oliver, J.L., Marin, A. and Medina, J.R. (1990). The general stochastic-model of nucleotide substitution. Journal of Theoretical Biology 142: 485-501.

Santesson, R. (1952). Foliicolous Lichens I. A revision of the taxonomy of the obligately foliicolous, lichenized fungi. Symbolae Botanicae Upsalienses 12: 1-590.

Schmidt, H.A., Strimmer, K., Vingron, M. and von Haeseler, A. (2002). TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics 18: 502-504.

Shimodaira, H. and Hasegawa, M. (1999). Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16: 1114-1116.

Staiger, B., Kalb, K. and Grube, M. (2006). Phylogeny and phenotypic variation in the lichen family Graphidaceae (Ostropomycetidae, Ascomycota). Mycological Research 110: 765-772.

Strimmer, K. and Rambaut, A. (2002). Inferring confidence sets of possibly misspecified gene trees. Proceedings of the Royal Society of London Series B-Biological Sciences 269: 137-142.

Swofford, D.L. (1993). PAUP - a computer program for phylogenetic inference using maximum parsimony. Journal of General Physiology 102: A9-A9.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL-W - improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680.

Vilgalys, R. and Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238-4246.

Zhou, S. and Stanosz, G.R. (2001). Primers for amplification of mt SSU rDNA, and a phylogenetic study of *Botryosphaeria* and associated anamorphic fungi. Mycological Research 105: 1033-1044.

Zoller, S., Scheidegger, C. and Sperisen, C. (1999). PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. Lichenologist 31: 511-516.