CHARACTER EVOLUTION

# Morphological disparity in Cladoniaceae: The foliose genus *Heterodea* evolved from fruticose *Cladia* species (Lecanorales, lichenized Ascomycota)

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**Abstract** Phylogenetic relationships of the genera *Cladia* and *Heterodea* were reconstructed using a combined dataset of nuclear ITS, nuclear LSU and mitochondrial SSU rDNA sequences. Based on different analyses (Bayesian approach, maximum parsimony, maximum likelihood), the ingroup (*Cladia* + *Heterodea*) is strongly supported as monophyletic. *Pilophorus strumaticus* has a well supported sister-group relationship to the ingroup and together they form a sister group with a well-supported clade, which includes *Metus conglomeratus* and *Pycnothelia papillaria*. The Shimodaira-Hasegawa test and the ELW test significantly rejected monophyly of *Cladia* excluding *Heterodea*. Within *Cladia* three main clades can be distinguished which share morphological and chemical characters. The position of the foliose genus *Heterodea* within the fruticose *Cladia* is supported by anatomical and chemical characters. The species of clade II that includes two *Cladia* species and *Heterodea* share a similar type of upper cortex and two-layered medulla with an inner or lower medulla consisting of dark pigmented, thick-walled hyphae. Our phylogenetic estimate and the anatomical studies indicate that the foliose thallus of *Heterodea* originated from pseudopodetia of *Cladia*. It is discussed that the species currently classified in *Cladia* and *Heterodea* need to be placed in the same genus.

**Keywords** Bayesian approach; *Metus*; morphological evolution; *Pilophorus*; pseudopodetia; *Pycnothelia*; thallus

## ■ INTRODUCTION

Traditionally the growth form of lichens has played an important role in their classification. Many genera are primarily distinguished on the basis of different growth forms, such as the crustose genus *Caloplaca* and the foliose *Xanthoria*. Not surprisingly, such classifications based on a single vegetative character have been shown to create polyphyletic assemblages that do not reflect our knowledge of the evolution of these taxa (Gaya & al., 2003, 2008; Søchting & Lutzoni, 2003). However, most of the currently accepted genera in lichenized fungi contain only one type of growth form and variability within this character is restricted to a few groups. There are only few exceptions in which species with different growth forms were placed within a single genus. These include *Roccellina* (Tehler & Irestedt, 2007), *Stereocaulon* (Högnabba, 2006), and *Xanthoparmelia* (Blanco & al., 2004). This is surprising, since the growth forms—and the fact that lichens form persisting thalli—primarily reflects the necessity to expose a sufficient area containing a photosynthetic partner to light for photosynthesis. Further, extreme changes in thallus morphologies are well documented in some peltigeralean lichens. In the genus *Sticta*, for example, the same mycobiont species was shown to produce foliose thalli in the presence of a green algal partner but a fruticose thallus when associated with a cyanobacterium (James & Henssen, 1976; Armaleo & Clerc, 1991). Thus plasticity of thallus morphology is known to be much higher than in cormophytes (Jahns & Ott, 1994; Honegger, 1996; Honegger & al., 1996) and further examples of morphological plasticity can be expected among lichen-forming fungi. In this study we have focused on a group of species in Cladoniaceae.

The family Cladoniaceae (Lecanorales, Ascomycota) currently includes 16 genera (Lumbsch & Huhndorf, 2007) with over 400 accepted species. Most genera in this family have a dimorphic thallus with a crustose or foliose primary thallus and a vertical thallus that bears apothecia and conidia. This stalked apothecium is lichenized and often forms the main part of the thallus that is called a podetium, when derived from generative tissue or a pseudopodetium when derived entirely from vegetative tissue (Jahns, 1970; Jahns & al., 1995). The definition of the generative tissue and hence the distinction between podetia and pseudopodetia has been disputed (Hammer, 1993, 1995, 1998, 2001, 2003). In this paper we follow the interpretation and terminology as defined by Jahns for practical reasons (Jahns, 1970; Jahns & al., 1995). The term cladoniiform was proposed for such dimorphous lichens where the thallus is differentiated into horizontal and vertical structures (Ahti, 1982). Cladoniiform lichens occur in several unrelated groups of lichenized fungi and hence this morphological term does not describe homologous structures (Stenroos & DePriest, 1998). In

addition to the dimorphic growth form with a primary thallus and podetia, foliose thalli with unstalked apothecia occur in *Heterodea* (Jahns & Van der Knapp, 1973; Filson, 1978); foliose or squamulose thalli with shortly stalked apothecia where the stalks do not contain algae in *Calathaspis* (Lamb & al., 1972), *Gymnoderma* (Zhou & al., 2006) and *Myelorrhiza* (Verdon & Elix, 1986), and thalli with pseudopodetia in *Cladia* (Filson, 1981) and *Thysanothecium* (Galloway & Bartlett, 1982; Hammer, 2001). In an effort to enhance our understanding of the evolution of these different morphologies in Cladoniaceae, we focused on two genera with a center of distribution in Australia, namely *Cladia* and *Heterodea*. These two genera deviate somewhat morphologically from typical, dimorphic Cladoniaceae and have previously been placed in separate families. Based on the absence of a primary thallus (interpretations of a primary thallus in *Cladia* by previous authors were rejected by Filson, 1981) and the presence of pseudopodetia, *Cladia* was placed in the monotypic family Cladiaceae (Filson, 1981, 1992a). This family was originally proposed as Clathrinaceae (nomen nudum), based on a synonym of the name *Cladia* (Duvigneaud, 1944). Duvigneaud regarded the presence of pseudopodetia of thalline origin as sufficiently different to allow separation of the genus from Cladoniaceae. Galloway (1966) and Filson (1981) regarded Cladiaceae as close to other fruticose genera, such as *Alectoria*, *Oropogon* (both Parmeliaceae) or *Ramalina* (Ramalinaceae). However, molecular studies showed that Cladiaceae is not related to these genera, but nested within Cladoniaceae and was subsequently synonymized with this family (Wedin & al., 2000).

The genus *Cladia* includes 14 species, most of which occur in Australia. The highest diversity of the genus is observed in Tasmania, where 11 species are known. The genus is also represented by numerous species in New Zealand and South America, from where one endemic species (*C. globosa*) has been described (Ahti, 2000). One species, *C. aggregata*, has a broad distribution, also occurring in Southeast Asia northwards to southern Japan and Korea, as well as India, South Africa, Central and South America (Filson, 1981; Kantvilas & Elix, 1999; Ahti, 2000; Hur & al., 2004). The genus is notoriously variable in morphology and secondary chemistry, making species determination difficult. Further, terminology and species circumscriptions have varied among authors. In his monograph of the genus, Filson (1981) accepted seven species, then nine (Filson, 1992a) and subsequently additional species were segregated based on morphological and chemical characters (Kantvilas & Elix, 1987, 1999; Ahti, 2000). *Cladia* is characterized by the presence of numerous perforations along the pseudopodetia (Fig. 1 on p. 844). The pseudopodetia are erect, branched, with a white or brownish black medulla. The genus contains a wide array of secondary compounds, especially β-orcinol depsides, depsidones, usnic acid, fatty acids and triterpenoids.

The genus *Heterodea* comprising two species, *H. beaugleholei* Filson and *H. muelleri* (Hampe) Nyl., is endemic to Australasia (Blackman & al., 1973; Filson, 1978). The thallus of *Heterodea* species is foliose and morphologically dissimilar from *Cladia* spp. (Fig. 3 on p. 846). The thallus has a white or brown medulla and lacks a lower cortex, the apothecia are sessile and secondary metabolites include usnic acid and the depsides, diffractaic and divaricatic acids.

The phylogenetic relationships of the genus *Heterodea* have remained uncertain for many years. While some authors assumed a relationship with Parmeliaceae, based on the foliose thallus and the secondary chemistry (Blackman & al., 1973), others classified it in Cladoniaceae (Jahns & Van der Knapp, 1973; Poelt, 1974). Subsequently, Filson (1978, 1992b) proposed the monogeneric family Heterodeaceae to accommodate this genus. Molecular studies, however, did not confirm the independence of Heterodeaceae, which appeared nested within Cladoniaceae (Wedin & al., 2000) and hence the genus is now classified in this family.

For this study we assembled a three-gene dataset to investigate the evolutionary history of *Cladia* and *Heterodea*.

## ■ MATERIALS AND METHODS

**Taxon sampling. —** Data of 26 representative samples of 18 taxa were assembled using sequences of nuITS, nuLSU and mtSSU. Specimens and sequences used for the molecular analyses are listed in the Appendix. NuITS, nuLSU and mtSSU sequences of three species (*Cladonia rangiferina* (L.) F.H. Wigg., *Pilophorus strumaticus* Nyl. ex Cromb., *Pycnothelia papillaria* Dufour) were downloaded from GenBank. These species and *Cladonia sulcata* A.W. Archer and *Metus conglomeratus* (F. Wilson) D.J. Galloway & P. James were used as outgroups based on previous phylogenetic studies in Cladoniaceae (Stenroos & DePriest, 1998; Wedin & al., 2000; Stenroos & al., 2002).

**DNA extraction, PCR amplification and sequence alignment. —** Fresh or herbarium material was used for extracting total genomic DNA. Thallus fragments of 2–15 mg were ground in liquid nitrogen. DNA was extracted using either the CTAB method (Doyle & Doyle, 1987) or the DNeasy™ Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

Primers for PCR amplification of three regions used for the study were: (1) nuITS regions: ITS1F-5′ (Gardes & Bruns, 1993) and ITS4-3′ (White & al., 1990); (2) nuLSU gene: nuLSU-0155-5′ (Döring & al., 2000), AL2R (Mangold & al., 2008), LR3, LR5, LR6 and LR7 (Vilgalys & Hester, 1990); and (3) mtSSU gene: mrSSU1-5′ (Zoller & al., 1999) and M2R, M3R & MSU 7-3′ (Zhou & Stanosz, 2001).

PCR reactions were performed in 25 µl volumes, including 8.75 µl of nuclease-free water, 2.5 µl of 10× buffer with 15 mM $MgCl_2$, 2.5 µl of 25 mM $MgCl_2$, 5 µl buffer, 0.5 µl of 10 mM dNTPs mix, 5 µl each of the primers at 10 mM concentration, 1.0 µl of DNA sample and 0.25 µl of *Taq* DNA polymerase. PCR was carried out for 30 cycles using the following program: 1 min at 95°C (denaturation), 1 min at 52°C–55°C (annealing) and 1 min at 72°C (extension) with final extension of 72°C for 10 min. Amplification products were cleaned using QIAquick PCR Purification Kit. The following cycle sequencing profile was used: denaturation for 3 min at 94°C and 25 cycles at: 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Sequenced

products were precipitated with 25 µL of 100% EtOH mixed with 1 µL of 3 M NaOAC, and 1 µL of EDTA, before they were loaded on an ABI PRISMTM 3100 or 3730 DNA Analyzer (Applied Biosystems, Carlsbad, California, U.S.A.). We assembled partial sequences using SeqMan v.4.03 (Lasergene, Madison, Wisconsin, U.S.A.) and edited conflicts manually.

Sequence alignments were done separately for each dataset using ClustalW (Thompson & al., 1994). No introns were found. Ambiguous regions in the ITS alignment were removed manually before analysis.

**Phylogenetic analyses. —** To test for potential conflict, parsimony bootstrap analyses were performed on each individual dataset, and 75% bootstrap consensus trees were examined for conflict (Lutzoni & al., 2004). Since no conflicts (i.e., well supported differences in the topology) were found, multi-gene datasets were analyzed under maximum parsimony (MP), maximum likelihood (ML) and a Bayesian approach (B/MCMC).

MP analyses were performed using the program PAUP* v.4.0b (Swofford, 1993) with random additions and characters unordered and equally weighted. MulTrees option and branch swapping using TBR was in effect. Bootstrap analyses (Felsenstein, 1985) were performed with 2000 pseudoreplicates of random addition sequences. To assess homoplasy levels, consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated from each parsimony search.

ML analyses were performed using the program GARLI v.0.96 (Zwickl, 2006). The analyses were carried out assuming the general time reversible model of nucleotide substitution (Rodriguez & al., 1990), including estimation of invariant sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G). Bootstrap analysis was performed with 2000 pseudoreplicates.

A Bayesian analysis was performed using MrBayes v.3.1.1 (Huelsenbeck & Ronquist, 2001) using the GTR+I+G model. The dataset was partitioned into three parts including nuITS, nuLSU and mtSSU. Each partition was allowed to have its own parameter values (Nylander & al., 2004). No molecular clock was assumed. Heating of chains was set to 0.2. Posterior probabilities were approximated by sampling trees using a variant of Markov chain Monte Carlo (MCMC) method. Number of generations was 10 million. To avoid autocorrelation, only every 1000th tree was sampled. The first 4000 generations were discarded as burn in. We plotted the log-likelihood scores of sample points against generation time using TRACER v.1.4.1 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure that stationarity was achieved after the first 4000 generations by checking whether the log-likelihood values of the sample points reached a stable equilibrium value (Huelsenbeck & Ronquist, 2001). Additionally, we used AWTY (Nylander & al., 2007) to compare split frequencies in the different runs and to plot cumulative split frequencies to ensure that stationarity was reached. Of the remaining 1992 trees (996 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 with bootstrap support ≥70% under MP and ML and posterior probabilities ≥0.95 in the Bayesian analysis were considered as strongly supported. Phylogenetic trees were visualized using the program Treeview (Page, 1996).

**Hypothesis testing. —** Our phylogenetic analyses revealed a monophyletic genus with *Heterodea* nested within *Cladia*, thus contradicting their current classification. We also tested whether our data are sufficient to reject monophyly of the genera *Cladia* and *Heterodea* as distinct clades. For the hypothesis testing, we used two different methods: (1) Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa, 2001) and (2) expected likelihood weight (ELW) test (Strimmer & Rambaut, 2002). The SH and ELW test were performed using Tree-PUZZLE v.5.2 (Schmidt & al., 2002) with the combined dataset, comparing the best tree agreeing with the null hypotheses, and the unconstrained ML tree. These trees were inferred in Tree-PUZZLE using the GTR+I+G nucleotide substitution model.

**Anatomy. —** We have re-examined the morphology and chemistry of the three major clades of *Cladia* (incl. *Heterodea)* and also studied the thallus anatomy of the species in clade II in an effort to better understand the morphological disparity among species of that clade. Sections through thalli (20 µm thick) were prepared using a Leica CM1100 freezing microtome and stained in lactophenol-cotton blue. Microscopic examination was performed using a Zeiss Axioscope 2 plus compound microscope.

**Chemistry. —** The chemical constituents were identified using thin layer chromatography (TLC) (Culberson, 1972; Culberson & al., 1981; Culberson & Johnson, 1982; Lumbsch, 2002) and gradient-elution high performance liquid chromatography (HPLC) (Feige & al., 1993).

## ■ RESULTS

**Phylogenetic analyses. —** Fifty-six new sequences were generated for this study, including 18 nuITS, 19 nuLSU and 19 mtSSU sequences (Appendix). A matrix of 1718 unambiguously aligned nucleotide position characters with 450 positions in the nuITS, 420 positions in the mtSSU and 848 positions in the nuLSU dataset was used for the analyses. The number of constant characters was 1377.

MP analysis of the combined dataset yielded in nine most parsimonious trees, 675 steps long (CI = 0.627, RI = 0.711, RC = 0.445), 205 characters were parsimony-informative. The ML analyses of the combined dataset yielded a ML tree with a likelihood value of Ln = –6035.964.

In the B/MCMC analysis of the combined dataset, the likelihood parameters in the sample had the following values averaged for the three partitions (±standard deviation): base frequencies $\pi(A) = 0.271$ (±0.001), $\pi(C) = 0.223$ (±0.002), $\pi(G) = 0.264$ (±0.001), $\pi(T) = 0.242$ (±0.001), rate matrix r(AC) = 5.388 (±0.055), r(AG) = 0.1851 (±0.046), r(AT) = 0.139 (±0.0006), r(CG) = 4.416 (±0.054), r(CT) = 0.508 (±0.0002), r(GT) = 7.158 (±0.055), LnL = –6037.20 (±0.10), and the gamma shape parameter $\alpha = 0.5789$ (±0.002). The topology of the trees from the MP (strict consensus tree), ML and Bayesian analyses did

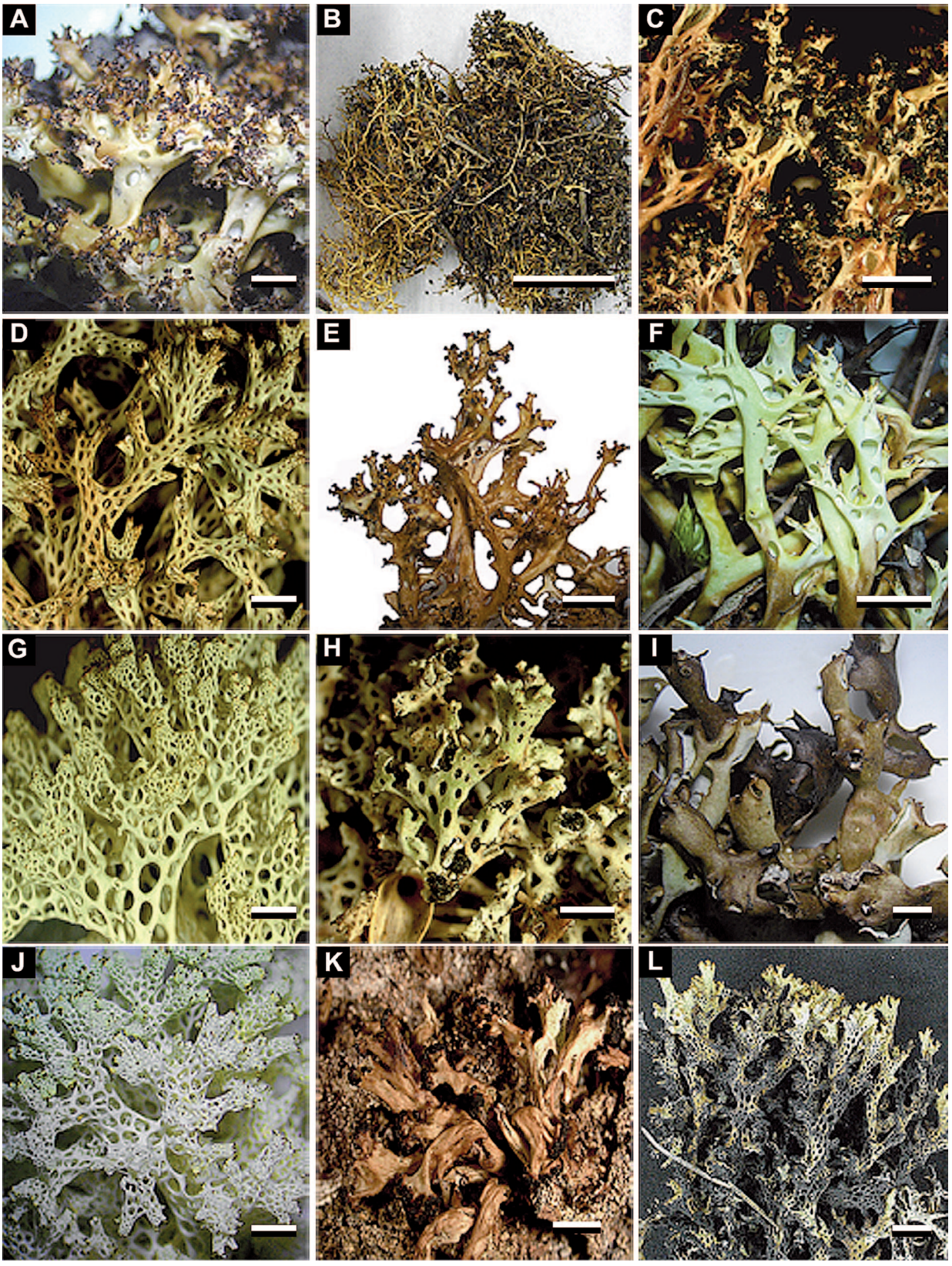


**Fig. 1.** Morphological characteristics of *Cladia* spp. **A–C,** *Cladia aggregata*, A, *Lumbsch 19994c* (F); B, (HO-528810); C, *Lumbsch 19975a* (F); **D,** *C. corallaizon*, *Elix 36942* (CANB); **E,** *C. deformis*, *Lumbsch 19994d* (F); **F,** *C. dumicola*, *Lumbsch 19993g* (F); **G,** *C. ferdinandii*, *Cranfield 22218* (CANB), **H,** *C. fuliginosa*, *Lumbsch 19989h* (F); **I,** *C. moniliformis*, *Lumbsch 19991f* (F); **J,** *C. retipora*, *Lumbsch 19976a* (F); **K,** *C. schizopora*, *Lumbsch 19974c* (F); **L,** *C. sullivanii*, *Lumbsch 19976b* (F). Scale bars = 5 mm.

not show any conflict and hence only the 50% majority-rule consensus tree of the Bayesian tree sampling is shown here (Fig. 2), with MP and ML bootstrap values above 75% as well as posterior probabilities equal or above 0.95 indicated by bold branches.

The ingroup (*Cladia* + *Heterodea*) is strongly supported as monophyletic (Fig. 2). *Pilophorus strumaticus* has a well supported sister-group relationship to the ingroup and they form a sister group with a well-supported clade, which includes *Metus conglomeratus* and *Pycnothelia papillaria*. Within the ingroup three major clades can be distinguished, one of them (clade III), however, lacks support. Clade I includes *Cladia aggregata* (Sw.) Nyl., *C. deformis* Kantvilas & Elix, *C. dumicola* Kantvilas & Elix, *C. inflata* (F. Wilson) D.J. Galloway, *C. moniliformis* Kantvilas & Elix, and *C. schizopora* (Nyl.) Nyl. (Fig. 1A–C, E, F, I, K). The two samples of *C. dumicola* cluster together, while the samples of *C. aggregata* do not form a monophyletic clade, indicating that species circumscription in this clade requires further study. The samples of *C. aggregata* included in this study represent different chemotypes of that species. The following chemosyndromes were detected in these samples: atranorin and stictic acid (sample 4), barbatic acid (1, 2), fumarprotocetraric acid (5), and stictic acid (3). The well supported clade II includes two distinct morphological groups belonging to genus *Cladia* (*C. fuliginosa* Filson and *C. sullivanii* (Müll. Arg.) W. Martin) and *Heterodea* (*H. beaugleholei* Filson and *H. muelleri* (Hampe) Nyl.) (Fig. 1H, L, Fig. 3). The two species of *Cladia* and *Heterodea* in this clade each form strongly supported, monophyletic groups. Clade III is monophyletic, but lacks support. It includes two specimens of *C. ferdinandii* (Müll. Arg.) Filson, which form a strongly supported monophyletic taxon and a strongly supported clade including *C. corallaizon* F. Wilson ex Filson and *C. retipora* (Labill.) Nyl. (Fig. 1D, G, J). The relationships between the three ingroup clades remain unclear, since the sister-group relationship between clades I and II is not supported.

Hypothesis testing by both the SH and ELW tests for significant results ($P = 0.002$ and $P = 0.008$, respectively), rejected the monophyly of *Cladia* as currently circumscribed.

**Anatomical and chemical studies. —** The chemosyndromes present in the specimens examined are listed in Table 1.

Clade I includes species with hollow pseudopodetia that lack an inner-medulla and have few fenestrations (Fig. 1A–C, E, F, I, K). Further, apothecia proliferate and form tiers. Chemically this clade is very diverse with a number of depsides, depsidones, fatty acids, and triterpenoids present. In contrast, clade III is chemically uniform, with all species containing atranorin, usnic acid and several fatty acids and triterpenoids. Morphologically, this group is characterized by numerous perforations (Fig. 1D, G, J). *Cladia corallaizon* and *C. retipora* have a stranded inner medulla throughout the central portion of the pseudopodetia, which, however, is lacking in *C. ferdinandii*.

We focused our anatomical re-examination on clade II. Although the morphology of *Cladia fuliginosa* and *C. sullivanii* is fruticose and very different from the foliose *Heterodea* spp. (Fig. 3), there are striking anatomical similarities. The cortex

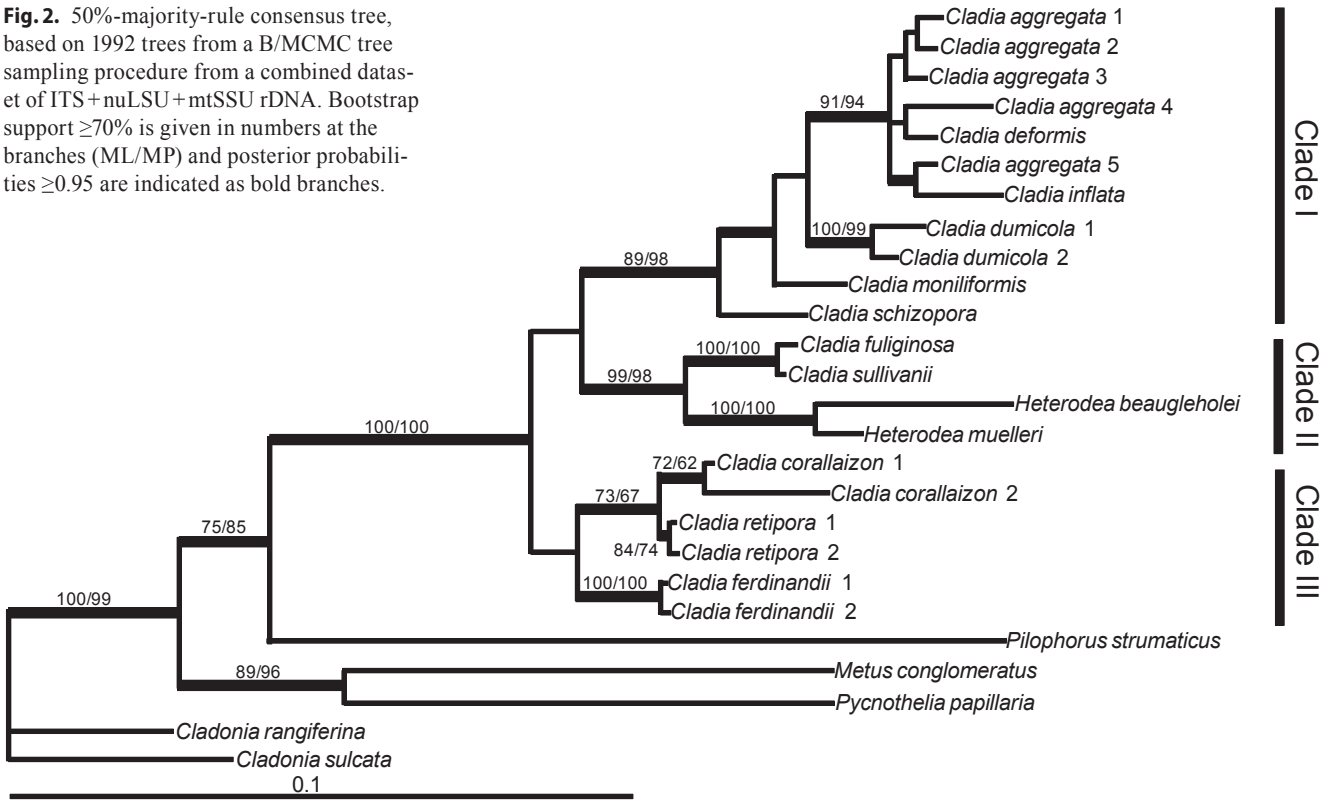


**Fig. 2.** 50%-majority-rule consensus tree, based on 1992 trees from a B/MCMC tree sampling procedure from a combined dataset of ITS + nuLSU + mtSSU rDNA. Bootstrap support ≥70% is given in numbers at the branches (ML/MP) and posterior probabilities ≥0.95 are indicated as bold branches.

in all four species of clade II is composed of conglutinated, longitudinal hyphae that form a dense, paraplectenchymatous cortex. The hyphae are more or less longitudinally to slightly irregular oriented (Fig. 3C, F, I). The upper parts of the medulla in *Heterodea*, *C. fuliginosa* and *C. sullivanii* are white. In the two *Cladia* species and in *Heterodea muelleri*, a pigmented lower or inner medulla is also developed. This pigmented part of the medulla is brown to black and consists of thick hyphae with thick cell walls that form an irregular, loosely woven network (Fig. 3 B, E, H). This inner medulla forms the network of black veins at the lower surface of *H. muelleri* and the inner medulla of the pseudopodetia of *C. fuliginosa* and *C. sullivanii*. There is also a chemical similarity among species in clade II. The divaricatic acid chemosyndrome occurs in *Heterodea beaugleholei*, *Cladia fuliginosa* and *C. sullivanii*. Further, all four species lack atranorin but contain usnic acid. Diffractaic acid, which is present in *H. muelleri*, occurs in a rare chemotype of *C. aggregata* which we have not investigated.

## ■ DISCUSSION

This study has revealed a remarkable example of the morphological disparity of vegetative characters in lichenized fungi, namely the evolution of a foliose growth form from within a group of fruticose lichens. Disparity of growth forms has been reported from several, unrelated clades of lichen-forming fungi (Arup & Grube, 2000; Grube & Arup, 2001; Gaya & al., 2003; Søchting & Lutzoni, 2003; Blanco & al., 2004; Högnabba, 2006; Tehler & Irestedt, 2007). These results

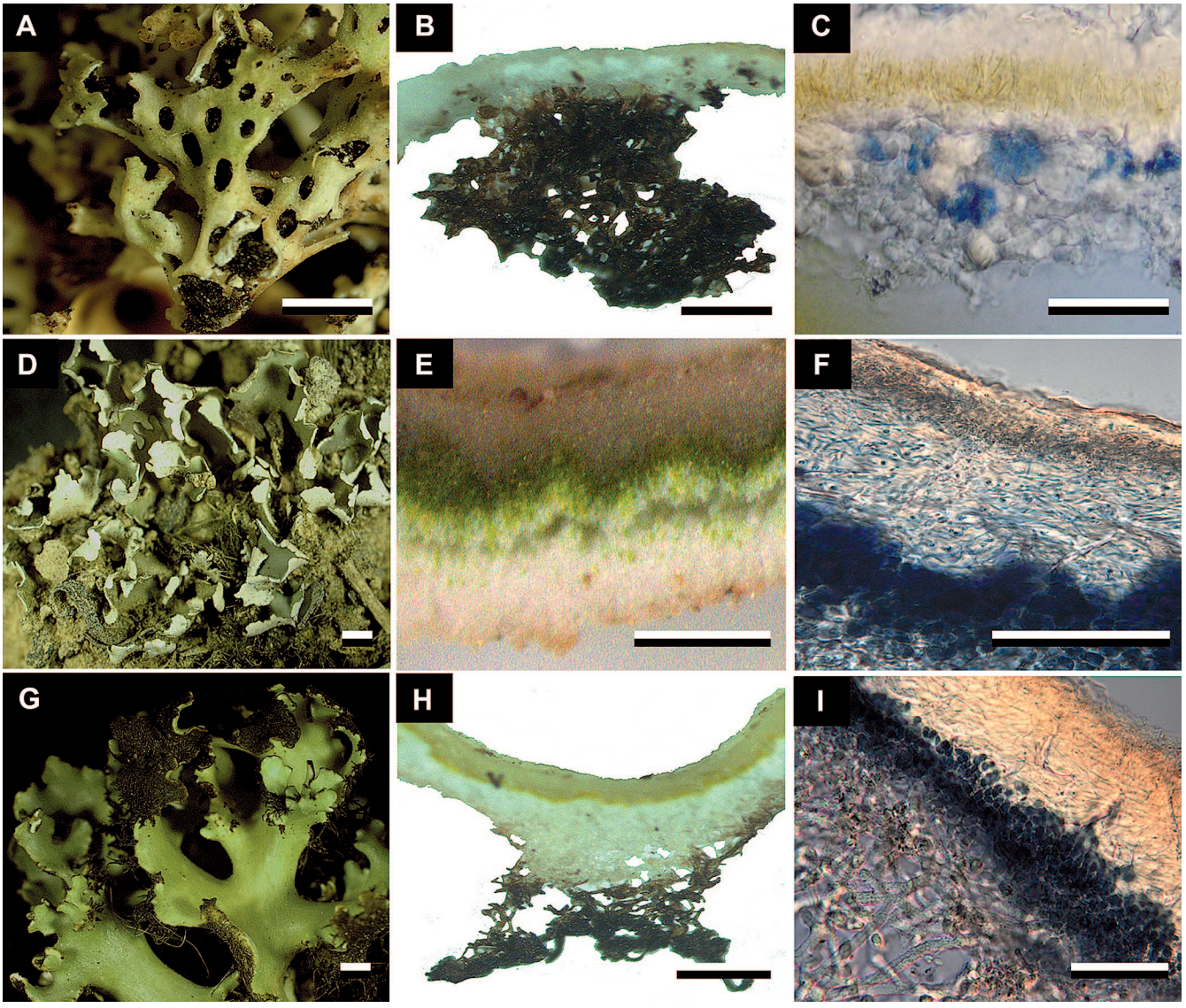


**Fig. 3.** Morphological and anatomical characteristics of the cortex of *Cladia fuliginosa* and *Heterodea* spp. **A–C,** *Cladia fuliginosa*, *Lumbsch 19989h* (F): **A,** habit; **B,** internal medulla; **C,** upper cortex; **D–F,** *H. beaugleholei*, *Elix 39644* (CANB): **D,** habit; **E,** internal medulla; **F,** upper cortex; **G–I,** *H. muelleri*, *Elix 39643* (CANB): **G,** habit; **H,** internal medulla; **I,** upper cortex. Scale bars for A, B, D, E, G, H = 5 mm; C, F, I = 8 µm

confirm that vegetative characters are not always reliable phylogenetic discriminators, probably due to the lack of specialization of cell-types and the lack of true tissue in fungi (Jahns & Ott, 1994). The morphological divergence in thalline morphology in these lichens may be caused by adaptations to particular ecological factors (Grube & Hawksworth, 2007), but it would require studies on the ecophysiology of *Cladia* and *Heterodea* species to prove this hypothesis (Grube & Kroken, 2000; Grube & Hawksworth, 2007). A more detailed discussion of thallus forms and their possible origins can be found elsewhere (Grube & Hawksworth, 2007). Nevertheless, the plasticity of morphological features in lichenized fungi demonstrates that caution is needed when using these characters for taxonomic purposes without the background of a phylogenetic overview derived from an independent dataset, such as DNA sequences.

**Table 1.** Chemosyndromes present in examined specimens of the genera *Cladia* and *Heterodea*.

| Species | Chemosyndromes present |
|---|---|
| *Cladia aggregata* 1 | Barbatic acid |
| *C. aggregata* 2 | Barbatic acid |
| *C. aggregata* 3 | Stictic acid |
| *C. aggregata* 4 | Stictic acid |
| *C. aggregata* 5 | Fumarprotocetraric acid |
| *C. corallaizon* 1 | Atranorin, protolichesterinic acid, ursolic acid, usnic acid |
| *C. corallaizon* 2 | Atranorin, protolichesterinic acid, ursolic acid, usnic acid |
| *C. deformis* | Fumarprotocetraric acid, stictic acid |
| C. *dumicola* 1 | Caperatic acid |
| *C. dumicola* 2 | Caperatic acid |
| *C. ferdinandii* 1 | Atranorin, rangiformic acid, ursolic acid, usnic acid |
| *C. ferdinandii* 2 | Atranorin, rangiformic acid, ursolic acid, usnic acid |
| *C. fuliginosa* | Divaricatic acid, ursolic acid |
| *C. inflata* | Fumarprotocetraric acid |
| *C. moniliformis* | Homosekikaic acid, unidentified fatty acids |
| *C. retipora* 1 | Atranorin, protolichesterinic acid, ursolic acid, usnic acid |
| *C. retipora* 2 | Atranorin, fumaprotocetraric acid, protolichesterinic acid, ursolic acid, usnic acid |
| *C. schizopora* | Fumarprotocetraric acid |
| *C. sullivanii* | Divaricatic acid, ursolic acid |
| *Heterodea beaugleholei* | Divaricatic acid, usnic acid |
| *H. muelleri* | Diffractaic acid, usnic acid |

Anatomical similarities between the pseudopodetia in *Cladia* and the foliose thallus of *Heterodea* indicate that these structures may be homologous and that the thallus of *Heterodea* may represent a highly modified, flattened pseudopodetium. Such an interpretation would also explain the absence of a stalk in the apothecium of *Heterodea* spp. (Jahns & Van der Knapp, 1973; Filson, 1978), since all other taxa in Cladoniaceae have at least shortly stalked ascomata.

Our analyses support previous studies (Wedin & al., 2000; Wiklund & Wedin, 2003; Myllys & al., 2005) that *Heterodea* belongs to Cladoniaceae and is closely related to *Cladia*. Interestingly, a close relationship between *Cladia* and *Heterodea* has not been raised in the literature, with the notable exception of Poelt (1974), who wrote "The foliose *Heterodea* ... may be related to *Cladia*." without giving any explanation for his statement. Using an extended taxon sampling (including 13 of 16 described species in the complex) we could demonstrate that *Heterodea* is not only closely related but nested within *Cladia*. The three major clades within *Cladia* s.l. (incl. *Heterodea*) largely parallel groups previously segregated using morphological and chemical characters (Galloway, 1977; Filson, 1981, 1992a; Kantvilas & Elix, 1987, 1999). Interestingly, although the chemistry in *Cladia* is known to be diverse and hypervariable in the *C. aggregata* group, it appears to be a good phylogenetic indicator for characterizing two of the three clades within *Cladia* s.l.

We refrain from discussing species circumscription in *Cladia* here, since this is beyond the scope of this paper. However, polyphyly of the chemically diverse *C. aggregata* group indicates that additional studies with extended sampling of this clade are necessary to address species delimitation in these fungi.

As a consequence of the above study the generic concept in the *Cladia* clade needs revision. Species currently placed in *Cladia* and *Heterodea* should be included in the same genus as *Cladia*, but a much less well-known name, *Heterodea* has priority. Consequently publication of the two new combinations that would be needed in *Cladia* is deferred and instead *Cladia* has been proposed for conservation against *Heterodea* (Lumbsch & al., 2010) to include species previously classified in *Heterodea*.

## ■ ACKNOWLEDGEMENTS

We are grateful to Gintaras Kantvilas (Hobart) for providing us with material for this study, for helping us organize a field trip to Tasmania and for his support. We also wish to thank Erin Sackett-Hermann and Fabian Ernemann (both Chicago) for their significant assistance with the molecular work. The majority of new sequences in this study were generated at the Pritzker Laboratory for Molecular Systematics and Evolution at The Field Museum (Chicago). We also wish to thank the RAMK herbarium staff for their kindness and support. This work was financially supported by a Scholarship of The Field Museum to the first author and the National Research Council of Thailand.

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**Appendix.** Species and specimens used in this study, with location, reference collection details, and GenBank accession numbers for nuITS, nuLSU, mtSSU (new accession numbers are in bold). Sequences downloaded from GenBank are indicated by an asterisk (*).

*Cladia aggregata* 1 (Sw.) Nyl., Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19970f* (F), GQ500914, **GQ500969**, **GQ500939**. *Cladia aggregata* 2, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19994c* (F), GQ500917, **GQ500966**, **GQ500940**. *Cladia aggregata* 3, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19975a* (F), **GQ500909**, **GQ500965**, **GQ500937**. *Cladia aggregata* 4, Australia, Tasmania, *Kantvilas, s.n.* (HO-58646), **GQ500922**, **GQ500960**, **GQ500936**. *Cladia aggregata* 5, Australia, Tasmania, *Kantvilas, s.n.* (HO-528810), **GQ500921**, **GQ500972**, **GQ500938**. *Cladia corallaizon* 1 F.Wilson ex Filson, Australia, Victoria, *Elix 36942* (CANB), **GQ500926**, **GQ500956**, **GQ500929**. *Cladia corallaizon* 2, Australia, Australian Capital Territory, *Elix 39642b* (CANB),**GQ500908**, **GQ500957**, **GQ500930**. *Cladia deformis* Kantvilas & Elix, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19994d* (F), **GQ500923**, **GQ500967**, **GQ500935**. *Cladia dumicola* 1 Kantvilas & Elix, Australia, Tasmania, *Kantvilas, s.n.* (HO-524809), **GQ500924**, **GQ500970**, **GQ500941**. *Cladia dumicola* 2, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19993g* (F), **GQ500915**, **GQ500968**, **GQ500933**. *Cladia ferdinandii* 1 (Müll.Arg.) Filson, Australia, Western Australia, *Cranfield, s.n.* (CANB-22218), GQ500927, **GQ500974**, **GQ500950**. *Cladia ferdinandii* 2, Australia, Western Australia, *McCrum, s.n.* (CANB), GQ500928, **GQ500973**, **GQ500951**. *Cladia fuliginosa* Filson, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19989h* (F), **GQ500916**, **GQ500954**, **GQ500944**. *Cladia inflata* (F.Wilson) D.J. Galloway, Australia, Tasmania, *Kantvilas, s.n.* (HO-548645), **GQ500925**, **GQ500955**, **GQ500943**. *Cladia moniliformis* Kantvilas & Elix, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19991f* (F), **GQ500910**, **GQ500971**, **GQ500934**. *Cladia retipora* 1 (Labill.) Nyl., Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19976a* (F), **GQ500918**, GQ500963, GQ500931. *Clalia retipora* 2, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19989g* (F), **GQ500911**, GQ500964, GQ500932. *Cladia schizopora* (Nyl.) Nyl., Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19974c* (F), **GQ500919**, **GQ500952**, **GQ500942**. *Cladia sullivanii* (Müll.Arg.) Martin, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19976b* (F), **GQ500920**, **GQ500953**, **GQ500945**. *Cladonia rangiferina* (L.) Weber ex F.H. Wigg., AF458306*, AY533000*, AY300881*. *Cladonia sulcata* A.W. Archer, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19975i* (F), GQ500913, **GQ500959**, **GQ500949**. *Heterodea beaugleholei* Filson, Australia, Australian Capital Territory, *Elix 39644* (CANB), **GQ500907**, **GQ500961**, **GQ500946**. *Heterodea muelleri* (Hampe) Nyl., Australia, Australian Capital Territory, *Elix 39643* (CANB), **GQ500906**, GQ500962, GQ500947. *Metus conglomeratus* (F. Wilson) D.J. Galloway & P. James, Australia, Tasmania, *Lumbsch, Parnmen & Widhelm, HTL 19982b* (F), **GQ500912**, GQ500958, GQ500948. *Pilophorus strumaticus* Nyl. ex Cromb., AF517931*, AY340560*, AY340517*. *Pycnothelia papillaria* (Ehrh.) L.M. Dufour, AF453271*, DQ986800*, DQ986783*.