Progress in understanding the evolution and
classification of lichenized ascomycetes

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This article reviews the current state of knowledge about the evolutionary history of lichenized ascomycetes within an integrated system of all ascomycetes. Substantial progress has been made since molecular techniques were applied to unravel evolutionary relationships. Molecular approaches detect how often the lichen symbiosis arose, test current classification concepts, and find the proper placement for species with uncertain relationships. Uncertainties in some evolutionary hypotheses still need to be resolved by studying further genes. The currently available phylogenetic trees show a number of convergent phenotypic developments in lichenized ascomycetes. The current classification of ascomycetes is reviewed in the light of recent molecular data.

Keywords: Lichens, ascomycetes, systematics, phylogeny, evolutionary history.

The lichens are an immensely diverse group of organisms, which have adapted to an extremely wide range of ecological niches and conditions. They may thrive in all regions from the icy conditions beyond the arctic circles to tropical rain-forests, and they may occur on bare soil, underneath the surface of rocks, in shells of barnacles, on leaf surfaces, and many other substrates. More diversity is encountered in the fungal component (mycobiont) and, because this usually dominates the association, lichens are generally now regarded as a life form of fungi. The stable and self-supporting association of an exubitant mycobiont (where the fungus forms the outer tissue) with an alga (Hawksworth, 1988) represents - among other symbiotic forms of fungi, such as mycorrhiza-formers, pathogens, endophytes, etc. - a rather successful strategy, resulting in c. 13,500 species, or more than two fifths of all ascomycetes. The integration of this diversity into fungal systematics has been an ongoing task for mycologists and lichenologists that started with the delayed acceptance of Schwendener’s (1867) discovery of the symbiotic nature of lichens. The Finnish lichenologist Vainio (1890) stated that lichens with particular types of fruitbodies were heterogeneous and needed to be arranged according to their affinity with fungal genera to give an integrated system of lichens and ascomycetes. Despite this suggestion, lichens were usually classified separately until Santesson (1952) integrated lichens into a fungal system. Since then modern methods have enabled the relationships between lichens and other fungi to be seen in an evolutionary framework (Fig 1).

Lichens have diverse growth forms (crustose, foliose, beard-like, etc. (Figs 2 - 4)), sexual structures, and secondary chemistry. Although these phenotypic characters can be used to classify lichens, one of the previous obstacles to a fully integrated system was the lack of appropriate characters for higher level classifications. It was hardly possible to develop a clear hypothesis of the evolutionary history (phylogeny) of the major taxonomic groups because of the considerable number of non-homologous characters in fungi and the suspicion that convergent character developments had occurred at various levels. These problems have been overcome by the availability of DNA sequence data from ubiquitous genes. This amount of this data is steadily increasing, both in the number of organisms sequenced and in the number of genes studied (Lumbsch, 2000; and references therein), and resulting hypotheses are becoming more reliable.

Relationships between lichenized and non-lichenized fungi

An interesting and still controversial question is how often the lichen symbiosis has evolved in fungi. Lichen
forming fungi are currently classified in a dozen of the ascomycete orders, ten of which exclusively include lichenized ascomycetes (compare Apton, 1998; Table 1). The use of the polymerase chain reaction (PCR) in studies of lichens has been simplified by the use of fungal-specific primers (Gargas & Taylor, 1992; Gargas & DePriest, 1996; Döring et al., 2000; Zoller et al., 1999; Liu, Wheelan & Hall 1999), and the use of only DNA from the fungal component (Grube et al., 1995; Cubero et al., 1999; Wolinski, Grube & Blanz, 1999; Grube, 2001a). The relatively slowly evolving large and small subunit genes (LSU, SSU) of the nuclear ribosomal DNA (nrDNA) have been used to provide comparable measures to integrate the systematics of lichenized and non-lichenized fungi.

Gargas et al. (1995) were among the first to use molecular methods to investigate the evolutionary ‘origins’ of lichens. They suggested two separate origins of lichen symbioses within the ascomycetes, in the orders Arthoniales and Lecanorales, both supported as monophyletic (arisen from a single evolutionary line) sister groups of plant pathogenic or saprophytic fungi. Recently, Lutzoni et al. (2001) suggested that the loss of lichen symbiosis is more common than its gain. This could indicate that lichenization was a basal event in ascomyccete evolution, and that a number of important fungal groups may be derived from a lichenized ancestor. This is consistent with the presence of non-lichenized lichenicolous fungi in larger primarily lichenized groups, that are thought to have lost their lichenized life style. As an example, approximately twenty percent of the species in the lichen genera Arthonia and Opegrapha (Arthoniales) are lichenicolous fungi. Mycotrella and Merismatium are lichenicolous genera in the lichenized Verrucariales, and Carbonos, Coccidina and Epidich are classified in families of the lichenized Lecanorales (Coccidinae and Rhizocarpaceae). This placement is true at the genus level for obligated lichenicolous lichens, which are parasitic on other lichens but retain their lichenized habit. Apart from that, many facultative lichenicolous associations are common between lichens in the Lecanorales (Fig. 4). All this indicates that life style may be rather flexible in relation to evolutionary pathways.

Lutzoni et al. (2001) also suggested that lichen symbiosis arose much earlier than previously assumed, and that several major lineages of strictly non-lichenized fungi may stem from lichen-forming ancestors. This differentiation between primary and secondarily derived non-lichenized groups sheds new light on the evolution of ascomycetes. The occurrence of secondary metabolites has previously been used in lichen classification, especially at lower taxonomic levels. More than 1000 unique secondary metabolites are known from lichens, and the capacity to produce these may be maintained in secondarily de-lichenized lineages. Interestingly, Penicillium and Aspergillus, regarded as secondarily non-lichenized fungi by Lutzoni et al. (2001), produce numerous compounds, including antibiotics and mycotoxins, whereas Candida of non-lichenized origin, lacks such chemical diversity. This theory implies that the evolution of biochemical pathways leading to secondary metabolites, particularly the polyketides, is linked with lichenization, or at least the potential to form a lichen symbiosis.

Sterile lichens

A further challenge to lichenology that has been addressed by molecular studies, is the placement of taxa of uncertain taxonomic affinity (e.g. Arup & Grube, 1999; Sterroos & DePriest, 1998; Platt & Spatafora, 2000; Fig 1). Sterile lichens only reproduce asexually, and since they lack many of the features traditionally used for classification they are often unclassified. Others were classified at various hierarchic levels, e.g. at the family level (e.g. Simplicaceae, Thamnoliaceae; both now supposed to belong to Lecanophlebiaceae), the genus level (e.g. Cystocoleas, Racodium, Blaineyia) or the species level based on thallus morphology. A particular aspect of the last is the so-called species pair concept in lichenology (Du Rietz, 1924; Poelt, 1970). Such a pair consists of closely related species, one of which produces fruitbodies, whereas the other, often with a wider geographic distribution, only reproduces asexually. It has been suggested that the sexual species is primary and that the apomorphic relative stems from it secondarily. Recent molecular investigations show that the situation is more complicated: a species pair may consist of several previously unrecognized species (Kroken & Taylor, 2001), or, in other cases, no indication of a species border is detected (Mykls, Lohntander & Trehar, 2001). Both scenarios can include cases where the presence or absence of ascomata on the thalli could be modifications induced by environmental conditions.

Phenotypic evolution

Molecular techniques can help to elucidate the evolution of phenotypic characters. Some phenotypic characters that are traditionally used for circumscriptions of taxa are not appropriate for this purpose for the majority of lichenized fungi. Evanscent, prototunicate asci and passive ascospore dispersal -i.e. the formation of a mazedium (a loose powdery spore mass formed by an ascoma) - used to be used to characterise an order of lichen-forming fungi, the Calicariales. Molecular data
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Families of uncertain position in
- Ascomycotina:
  - Aphanostomataceae
  - Aspergillaceae
  - Basidiomycetaceae
  - Cembrodaceae
  - Cephalothecaeeace
  - Coccocormataceae
  - Conidiothyriaceae
  - Dipodocurrentaceae
  - Epicoccaceae
  - Cladotheciaceae
  - Inomatosaceae
  - Korosholiaceae
  - Lantosporaceae
  - Mastodaceae
  - Microbiaceae
  - Myxococaceae
  - Orbiinae
  - Physalosiphaceae
  - Pleurotremaceae
  - Protosteliales
  - Pseudangiosporidaceae
  - Saccaceae
  - Seuriaceae
  - Sterigellaceae
  - Tetradiaceae
  - Thelotrichaceae
  - Thelostomataceae
  - Thrombidiaceae
  - Thrombidenaceae
  - Xyliomycetaceae
now show that much of the Caliciaceae forms a monophyletic group with Physciaceae (Wedin et al., 2000a), and Sphaerophoraceae, and that this group forms a distinct clade in Lecanorales, which also includes non-maculate genera (Wedin & Döring, 1999; Wedin et al., 2000b). Apparently, the evanescent asci evolved in several lineages of ascomycetes (and also in Arthoniales, see Grube, 1998, 2001b), and this could also be true for other type of asci found in the Lecanorales, all of which seem to be derived from asci with tube-like apical structures (Wedin et al., 2000b; Ekman & Wedin, 2000).

One of the characters that was previously widely used to circumscribe genera or even families was the growth form of the thallus. Recent studies show that various types of growth forms may occur in genera of different families in the Lecanorales (Arup & Grube, 1998, 1999, 2000; Grube & Arup, 2001), and these studies conflict with traditional generic classification. Genera defined by a crustose growth form that have been accepted for a long time, seem to be artificial in the light of molecular data, e.g. in the Bacidiaeae (Ekman, 2001). Different growth forms also appear to have evolved in the Arthoniales, where the family Roccellaceae includes mainly species with fruticose growth forms, and exceptionally some genera with crustose growth forms. Mill's et al. (1998) showed that the fruticose growth form may have arisen more than once independently in the family.

Photobiont selection

In a compilation of available data, Rambold et al. (1998) studied whether photobionts could be evolutionary indicators in Lecanorales. Some selective patterns of associations were detected. Cladonineae, for example, are mostly associated with the trebouxiod genus Asterocladus, while other algae, especially Trebouxia spp., are common photobionts in the rest of the Lecanorales. Apart from further patterns involving coccolid green algae reported by Rambold et al. (1998), it is interesting that phycosymbiophytes, which are symbioses of one fungal species with either coccolid green algae or cyanobacteria, are more common in not as Lecanoromycetaceae, particularly in the Peligerineae (Miadlikowska & Lutzoni, 2000). On the other hand, advanced lineages of ascomycetes might be restricted to particular types of photobionts. In Physciaceae, more restricted ranges of photobionts belonging to the genus Trebouxia are observed in morphologically advanced foliose groups, while crustose groups have a wider range of possible photobionts (Helm's et al., 2001). Similar distributions can also be recognized in the Arthoniomyctaceae. Basal groups, such as Arthonia may have either Treptophila, Phytophilia, or coccolid green algae as photobionts, or live as non-lichenized fungi on bark. In contrast, the more advanced Roccellaceae are confined to treptophilaic photobionts. Future work may show whether there is a general trend towards more restricted photobiont selection in lichens.

Photobiont selection could also be triggered in lichenicolous lichens and fungi by host association. The lichenicolous lichen Asparagopsis insiditas shares the same photobiont with its host Lecanora rugulosa (Grube, unpublished). Both genera are placed in Lecanorales in phylogenetic studies (e.g. Stenroos & DePriest, 1998). On the other hand, the lichenicolous lichen Diploschistes muscorum (Ostropales) on Cladonia (Lecanorales) replaces the photobiont after infection (Ostriches: Friedl, 1987). Further studies will also show whether non-lichenized lichenicolous fungi evolving from lichenized groups maintain an association with the host algae, and if lichenicolous fungi of predominantly non-lichenized groups prefer the fungal component of the host.

Classification and evolution

Several studies of SSU rDNA have shown low genetic divergence at the ordinal/familial level, especially in the Lecanorales, and so higher taxonomic groupings in the order are poorly supported in phylogenetic analyses (Wedin & Döring, 1999). The situation is improved by inclusion of data from LSU rDNA (Lutzoni et al., 2001), but nevertheless tree topologies remain uncertain. These results might be due to rapid and early radiation in these groups (Berbee & Taylor, 1995; Berbee, Carman & Winka, 2000), events that have also been suggested for the origin of green plants. Possibly, the evolution of land plant diversity and the availability of new substrates over geological time may have been a catalyst for the radiation of lichenized fungi. Another factor for low divergence could be structural constraints on the secondary structure of SSU rDNA, requiring compensatory mutations in stem regions, which in sequence analysis could increase the confidence in the recovery of lineages, but may obscure relationships between them. Analysis of further genes will certainly improve these hypotheses.

*Fig 1 Cladogram illustrating the classification outlined in Table 1. The tree is based on SSU sequences retrieved from GenBank, some sequences are unpublished and supplied by the authors. This tree does not report any statistical support for the groups present, and has not been subject to rigorous analytical tests. However, the relationships agree with the majority of published phylogenetic investigations.*
Several orders that were recognized from phenotypic characters in early classifications have now been confirmed as monophyletic groups from sequence data. The ranks of these groups in a traditional hierarchic classification have already been revised, either according to their phylogenetic position or by classification of signature sequences. I.e. short pieces of sequence characteristic of a taxonomic group (see Eriksson, 1995; Eriksson & Winka, 1998).

The increasing amount of molecular data and the better resolution of evolutionary relationships forced some authors to suggest the application of a rank-free classification. The application of a rank-free classification is a matter of controversy, not least because of the poor support for basal linkages in the Ascomycota (Berbee et al. 2000). However a rule system has been proposed by the PhyloCode (Cantino & DeQueiroz, 2000). Adoption of an evolutionary based classification in ascomycetes would be unwise at the present time (see also Nixon & Carpenter, 2000).

**The main groups of lichenized fungi**

The classification used here is based on the most recent Outline of Ascomycota (Eriksson et al., 2001) and Eriksson & Winka (1997, 1998) (Table 1).

**Arthoniomycetes**

Arthoniales: the order includes >1200 species (Hawksworth et al., 1995) and is characterised by an ascostomal development which shares both ascomolar and ascocellular characters (‘Zwischengruppe’ according to Henssen & Jahn, 1973). The order includes the previously separated Opegraphales (Tehrler, 1990). Several SSU sequences are available from the Arthoniaceae and Roccellaceae (Myllys et al., 1998), and according to these, unilirate pyrenomycetes appear as the sister group of Arthoniales (Fig. 1). Sporestigma (Crube 2001b) (Fig 5), Tylophorellae, and Wigea, three genera with calicoid characters placed in Arthoniales, could represent basal linkages in the class.

**Lecanoromycetes**

In earlier versions of the Outline of Ascomycota (Eriksson & Winka 1998), the orders in this class were submerged into several tribes. As a result of molecular studies, most orders have now been re-established.

Agryriales: this order was re-established by Lumbsch et al. (2001) based on spectral analysis and maximum parsimony analysis, as well as phenotypic characters.

Lecanorales: this is the largest order of lichenized fungi with 7108 species. SSU sequences are readily available, and have been used to find the position of the order in the ascomycetes (Gargas et al., 1995), and to focus on relationships between families (e.g. Wedin et al., 2000a, b). The resolution of basal linkages in the suborder Lecanorinae from SSU rDNA data sets is generally low. However, the suborder Peltigerinae appears consistently at the base of the Lecanorinae clade (Wedin et al., 2000a, b), and represents an isolated, possibly old lineage in the order (Fig 1). This isolated position is also corroborated by characters of the ascus.

Concepts of the Calicioid 202 species have changed significantly since SSU rDNA became available. While the lichenicous Mycocalicellaceae are now regarded as a separate order (Tibell & Wedin, 2000), Calicioida s.str. are members of the Lecanorales, and form a monophyletic group with the Physciaceae (Wedin et al., 2000a). This is surprising and more data are now being obtained to further investigate this relationship. Nevertheless, the evolution of ascus characters, which are important in traditional classification of Lecanorales, seems to be more complex than previously thought (Wedin et al., 2000b). Relationships between many families of the Lecanorales are still only poorly studied, and although the SSU rDNA seems to be too conservative to solve these questions, the ITS regions are often too variable and difficult to align. In such cases, mitochondrial SSU rDNA could provide better resolution (e.g. in Parmeliaceae; Crespo, Blanco & Hawksworth 2001).

Lichinales: members of this order are almost exclusively associated with cyanobacteria and therefore often kept separate from Lecanorales. The origin of the order was studied by Schultz, Arendholz & Bädel (2001). The order is clearly supported as a monophyletic group, however there is no support for a close relationship to Lecanorales (see Fig 1). Ascus characters are varied in the Lichinales, as in the Lecanorales, and prototunicate asc and passive ascospore dispersal are suggested to be a derived feature (Schultiz et al., 2001). In Lutzoni et al. (2001), the order is placed as a sister group to Arthoniomycetes and Sordariomycetes.

Ostropales: Winka et al. (1998) showed that Diplochistes and Graphis, as members of the Graphidales, can be included in Ostropales (Fig 6). The order Ostropales (1854 spp.) consists primarily of non-lichenized fungi and also includes the Thelotremataceae.

Pertusariales: recent molecular studies have suggested that Pertusaria and Laevosporopsis together form a sister group to Bacillariaceae (Platt & Spatafora, 2000). This, however, needs confirmation from other sequences. Lumbsch, Schmitt & Mesuelli (2001) found that Coccotremataceae belong to this order as well.

The Umbilicariaceae appear outside the Lecanorales in SSU rDNA phylogenies (Fig 1), and the group is treat-
Fig 2 A lichen community found at windswept alpine heaths. The fruticose species *Alectoria ochroleuca* and the foliose species *Cetraria nivalis* (Lecanorales), both coloured yellow green by usnic acid, grow together with the white *Thamnolia vermicularis* (Icmadophilaceae), containing thamnolic acid.

*Fig 1* Caloplaca eleganssima (Lecanoromycetes) from the Namib desert. Typical for most members of *Caloplaca* are red colored anthraquinones as secondary metabolites.

*Fig 4* Lecanora muralis (centre, green-yellow), *Candeariella* sp. (bright yellow), *Dinarchus* sp. (pale yellowish) growing on *Aspicilia* sp. (grey; all Lecanoromycetes). Many species of Lecanorales have biological relationships with other members of the order. Three species grow here as facultative lichenicolous lichens.

ied as a family of uncertain position in Lecanoromycetes by Eriksson et al. (2001). We suggest an isolated position for this order, which can not fully be resolved by rDNA phylogenies.

Icmadophilaceae and Baeomyctaceae are treated as families *incertae sedis* by Eriksson et al. (2001). Platt & Spatafora (2000) investigated the relationships of the sterile lichen genera *Siphula* and *Thamnolia* which
belong to a clade which also included *Icnadothelia* and *Dibritis*, representative of the Icnadophiliaceae. Stergoos & DePriest (1998) and Platt & Spatafora (2000) showed that neither Bacomyctaceae nor Icnadophiliaceae are related to Leotiales as previously suggested, and Platt & Spatafora (2000) claimed that both families represented independent lichenization events (Fig 1). In their study, *Baeomyces* was the sister group to Pertusariales (Fig 7). Lumbsch et al. (2001), found that Pertusaria, Diplomithecaceae and members of Agyriaceae form a sister group to members of Umbilicariaceae.

**Chaetothyriomycetes**

Verrucariales: Lutzoni et al. (2001) and Winka & Eriksson (unpublished) suggested a relationship of this order (720 species) to Chaetothyriomycetes, see also Fig 1. There are only a few published molecular studies within the order, and an infrageneric account on *Dermatocarpon* is presented by Heldmarssen (in press; Fig 8). The placement of Verrucariales with Chaetothyriomycetes is supported by ascromial characters, namely the presence of periphyses.

Pyrenulales: a representative of this unclassified order forms a sister group to Chaetothyriomycetes in the phylogenetic tree of Lutzoni et al. (2001). This is also seen in Fig 1, where Pyrenula forms a group with *Rhychnostoma* which is classified as Chaetothyriomycetes *incertae sedis* (Eriksson et al. 2001). The morphology of Pyrenulales is quite different from the Chaetothyriomycetes, and characterized by a hamatheceum (interascal tissue) similar to that of members of Plecosporales.

**Unclear groups**

Many families of lichens have not been included in molecular studies, as seen in Table 1. Among these are several groups of folliculous lichens, e.g. the discocarpous Comphillaceae, which are perhaps related to Ostropales, or the pyrenocarpous (producing perithecia) families Aspidothelaeaceae and Phyllobathelaeaceae. While these families are well known, some pyrenocarpous fungi that have loose photobiont associations are still poorly understood, e.g. the enigmatic *Mastodia tesselata*, that has been placed in the unassigned family Mastidiaceae. In this species the mycobiont exerts only a slight morphogenetic influence on the photobiont in young algal thalli, and this association cannot clearly be interpreted as a lichen symbiosis (Lad. Huisken & Ott 2001). Another almost unknown group is the Morioaceae, that have been described as forming goniocysts in the vegetative thallus. Goniocysts are thin-walled brownish hyphal cells enclosing roundish structures formed by algal cells. A relationship between this family and the Verrucariales, in particular the genus *Mertinia*, has been suggested (Triebel 1989). The latter comprises lichenicolous species with more or less loose host-associations. Some species may form goniocysts with free-living algae, a kind of facultative lichenization which might here represent a throwback to primarily lichenized ancestors.

**Conclusions**

The results from the molecular studies of fungi suggest that phenotypic evolution in lichenized fungi is more complex that previously thought. It is not clear whether lichenization may lead to complex secondary metabolites or vice versa. The picture of fungal evolution is still preliminary and many potential key-groups of lichenized and non-lichenized fungi have not yet been included in molecular studies. Ambiguous or short lignages seen in the SSU trees need to be confirmed by data from other genes in order to obtain a stable and natural classification of both lichenized and non-lichenized fungi.

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**References**


**Fig 5** *Synastygna reclusa* (Arthoniales, Arthoniomycetes). This species from Florida has evanescent ascospores. The ascospores of this unclassified member of the Arthoniales are deposited on the surface of the thallus and passively dispersed.

**Fig 6** *Graphis scripta* (Ostropales). Elongate ascocarps are found in many members of Graphiaceae. The family has its centre of diversity in the tropics. The illustrated species is one of the few occurring in temperate regions.

**Fig 7** *Baemopyrus rufus* (Baemopyraceae). This species has stalked fruitbodies and is found on acid ground. While photosynthesis is carried out by the algal symbiont in a layer of the externally visible thallus, a section through the substrate shows that rhizine of the mycobiont may reach several centimeters deep in the substrate.

**Fig 8** *Dermatocarpon minutum* (Verrucariales, Chaetothyriomycetes). The lophose growth form is developed in only few genera of Verrucariales, and could represent a monophyletic group within the order.


