

Dalip Kumar Upreti · Pradeep K. Divakar  
Vertika Shukla · Rajesh Bajpai *Editors*

# Recent Advances in Lichenology

Modern Methods and Approaches  
in Lichen Systematics and Culture Techniques,  
Volume 2

 Springer

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and Culture Techniques, Volume 2

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*Editors*

Dalip Kumar Upreti  
Lichenology Laboratory  
CSIR-National Botanical Research  
Institute  
Lucknow, Uttar Pradesh  
India

Vertika Shukla  
Lichenology Laboratory  
CSIR-National Botanical Research  
Institute  
Lucknow, Uttar Pradesh  
India

Pradeep K. Divakar  
Facultad de Farmacia, Departamento de  
Biología Vegetal II  
Universidad Complutense de Madrid  
Madrid, Madrid  
Spain

Rajesh Bajpai  
Lichenology Laboratory  
CSIR-National Botanical Research  
Institute  
Lucknow, Uttar Pradesh  
India

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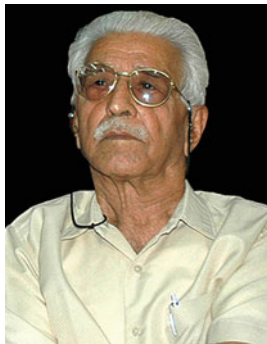
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*Dedicated to*



Dr. D.D. Awasthi  
Father of Indian Lichenology

## Foreword



### सीएसआईआर-राष्ट्रीय वनस्पति अनुसंधान संस्थान CSIR-National Botanical Research Institute

(वैज्ञानिक तथा औद्योगिक अनुसंधान परिषद, नई दिल्ली)  
राणा प्रताप मार्ग, लखनऊ - 226 001, उ.प्र., भारत  
(Council of Scientific & Industrial Research, New Delhi)  
Rana Pratap Marg, Lucknow - 226 001, U.P., India



डॉ. चन्द्र शेखर नौटियाल

टाटा इनोवेशन फेलो, एफएनए, एफएनएएससी, एफएनएएस  
निदेशक

**Dr. Chandra Shekhar Nautiyal**

TATA Innovation Fellow, FNA, FNASc, FNAAS

Director

### FOREWORD

Lichens are unique organisms which require multidimensional approach to explore its potential in various fields of environment, botany and chemistry. Modern techniques especially molecular, culture, remote sensing technique has considerably contributed in the field of lichens. Multivariate analyses together with GIS approaches have established lichens as an ideal and reliable indicator of air pollution. Advanced culture techniques have increased the pharmacological application which was earlier restricted due to meager biomass of lichens. Advent of sophisticated analytical instrumentations facilitated isolation and characterization of bioactive constituents of lichens even in lower concentration for their bioprospection.

I am delighted to see that the editors made an effort to compile the advances in the field of lichenology contributed by experts of various fields of lichenology from around the globe, which will not only help to introduce various multidisciplinary approaches and techniques in the field of lichenology but also create interest among researchers to take up research on these unique organisms.

I am glad that this work has been accomplished in the Lichenology Laboratory of CSIR-National Botanical Research Institute, Lucknow.

I congratulate the editors for their endeavour in composing a comprehensive and valuable contribution about advances in the study of lichens worldwide and hope this book will be immensely helpful for environmentalists, botanist and phytochemists.

Date: 15<sup>th</sup> July 2014

(C.S. Nautiyal)

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## Preface

Lichens present an excellent example of symbiotic association. The unique composition of alga and fungi in lichens not only results in conferring differential sensitivity towards a range of environmental factors but also makes them physiologically adapted and chemically diversified to combat various abiotic and biotic environmental stresses.

This book volume covers two very important aspects of lichens, i.e. biomonitoring and bioprospection. In order to understand the role of lichens as biomonitors, it is important to know various factors which influence the growth of lichens in natural conditions.

Lichens have been known for long as bioindicators of air pollution and various studies have explored biomonitoring potential of different lichen species. Recently more standardised protocols have been adopted in Europe and America, which ensures the authenticity and reproducibility of lichen biomonitoring data.

Standardised methods for lichen diversity assessment not only authenticate classical approaches of lichen diversity changes related to ecological studies and air quality, but also provide an opportunity to extend to the studies related to climate change and land use changes. Lichen diversity studies coupled with remote sensing data can be used for mapping lichen species, which provide vital information regarding present scenario and predict future model of climate change or land use change. Applicability of lichen diversity-remote sensing studies further extends to estimating and mapping influence of pollutant in unsampled location based on sampled locations, which help to track the long-range transport of semi-volatile pollutants to high altitude ecosystems.

Lichens also play a major role in functioning of the ecosystem by the process of soil formation (lichens have an ability to grow on barren rock), but this characteristic is disadvantageous for conservation of ancient monuments as lichen growth results in biodeterioration. Studies on restricting lichen growth and preserving the monument are a subject of research and need implementation of strategies for conservation of monuments.

Another aspect of lichens which is gaining importance in recent climate change studies is lichenometric studies, to observe glacier retreat phenomenon. As any change in shape and size of glacier provides vital information regarding climate change, therefore the age of lichens growing on exposed moraines is a possible clue to retreat of glacier.

Apart from being excellent biomonitors, another not much explored aspect of lichens is its chemistry. For lichens their unique chemistry supplements physiological adaptation for sustaining in extreme climates, but for mankind lichens are a treasure house of pharmacologically important bioactive constituents capable of curing simple cough and cold to HIV and cancer.

The therapeutic potential of lichen secondary metabolites is known since medieval times mainly known through folklore and ethnobotanical uses. The main constraint which restricts its commercial utilisation is the slow growth rate and low biomass of lichens. Recent advances in analytical instrumentation techniques have resulted in high throughput techniques for isolation, while highly sensitive detection techniques allow characterisation of bioactive compounds even in very low quantity. Structural characterisation of bioactive compound paves the way for its synthesis in the laboratory and further structural modifications.

Therefore, the book volume intends to introduce researchers to advancements in the field of lichenology with an aim to involve more active participation of multidisciplinary research in the study of lichens.



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## Editors and Contributors

### About the Editors

**Dalip Kumar Upreti** FNASc, Head of the Lichenology Laboratory, CSIR-National Botanical Research Institute (CSIR-NBRI), Lucknow, has a Ph.D. (1983) in Botany on *Studies on the lichen family Cladoniaceae from India and Nepal* from Lucknow University under the guidance of Dr. D.D. Awasthi. Dr. Upreti has over 35 years of research experience and has published more than 300 research papers in peer-reviewed journals and co-authored eight books.

Apart from taxonomy, Dr. Upreti has also carried out extensive research on ecology, lichen chemistry, pollution monitoring, in vitro culture and biodeterioration studies and bioprospection of Indian lichens. Dr. Upreti was in Antarctica in 1991–1992. He is the Indian corresponding member to the British Lichen Society and International Association for Lichenology.

**Pradeep K. Divakar** holds an M.Sc. in Botany and earned his Ph.D. (2002) on *Revisionary studies on the lichen genus Parmelia sensu lato in India* from Lucknow University. Dr. Divakar is Professor at the Department of Plant Sciences, Faculty of Pharmacy, Complutense University of Madrid, Spain. Dr. Divakar has published two books and more than 70 papers in national and international journals. Currently he is editor of *Biodiversity and Conservation*, *Myckeys*, *Organisms Diversity and Evolution*, and *Lazaroo* (Iberian Journal of Botany).

His research activities centre on molecular systematics, genetic diversity, evolution, speciation events, gene mapping, gene flow, TCS estimation of gene genealogies and phenotypic character mapping, ancestral character state, and genomics of fungi that form mutualistic symbioses with algae or cyanobacteria and mycobiont cultures.

**Vertika Shukla** holds an M.Sc. in Organic Chemistry and a Ph.D. (2003) on the *Chemical Study of Macrolichens of Garhwal Himalayas* from H.N.B. Garhwal University, Srinagar (Garhwal). She is currently working in the Lichenology Laboratory of CSIR-NBRI as a DST scientist and to date she has published more than 50 scientific articles.

Dr. Shukla has contributed to secondary metabolite chemistry, spatio-temporal behavior of pollutants in high altitude ecosystems, lichen responses to climate change and the role of lichens in bioremediation of atmospheric fallouts.

**Rajesh Bajpai** holds an M.Sc. in Environmental Science and received his Ph.D. in 2009 from Babasaheb Bhimrao Ambedkar (Central) University, Lucknow on *Studies on lichens of some monuments of Madhya Pradesh with reference to Biodeterioration and Biomonitoring*, is working as a DST scientist in Lichenology Laboratory of CSIR-NBRI, Lucknow. Dr. Bajpai has published 30 research papers in various national and international journals.

Dr. Bajpai has carried out extensive research work on the interaction of lichen with the environment, climate-change modeling, arsenic pollution and biodeterioration.

## Contributors

**Ólafur S. Andrésson** Faculty of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland

**Bhaskar C. Behera** Plant Science Division, Mycology and Plant Pathology Group, Agharkar Research Institute, Pune, India

**Ana Crespo** Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid, Spain

**Pradeep K. Divakar** Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid, Spain

**Christina Hametner** Department of Organismic Biology, University of Salzburg, Salzburg, Austria

**Kojiro Hara** Faculty of Bioresource Sciences, Akita Prefectural University, Akita, Japan

**David L. Hawksworth** Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid, Spain; Department of Life Sciences, The Natural History Museum, London, UK; Mycology Section, Kew, Surrey, UK

**Jae-Seoun Hur** Korean Lichen Research Institute, Sunchon National University, Sunchon, Republic of Korea

**Zophonías O. Jónsson** Faculty of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland

**Yogesh Joshi** Lichenology Division, Department of Botany, Kumaun University, Almora, Uttarakhand, India

**Hiroko Kawakami** Faculty of Bioresource Sciences, Akita Prefectural University, Akita, Japan

**Hangun Kim** College of Pharmacy and Research Institute of Life and Pharmaceutical Sciences, Sunchon National University, Sunchon, Republic of Korea

**Kyung Keun Kim** Medical Research Center for Gene Regulation, Chonnam National University Medical School, Gwangju, Republic of Korea

**Masashi Komine** Faculty of Bioresource Sciences, Akita Prefectural University, Akita, Japan

**Steven D. Leavitt** Committee on Evolutionary Biology, University of Chicago, Chicago, IL, USA; Department of Science and Education, Field Museum of Natural History, Chicago, IL, USA

**Vivian P.W. Miao** Department of Microbiology and Immunology, University of British Columbia, Vancouver, Canada

**Corrie S. Moreau** Department of Science and Education, Field Museum of Natural History, Chicago, IL, USA

**Gerhard Rambold** Mycology Department, University of Bayreuth, Bayreuth, Germany

**Preeti Shukla** Lichenology Laboratory, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India

**Elfie Stocker-Wörgötter** Department of Organismic Biology, University of Salzburg, Salzburg, Austria

**H. Thorsten Lumbsch** Department of Science and Education, Field Museum of Natural History, Chicago, IL, USA

**Manish Tripathi** Lichenology Division, Department of Botany, Kumaun University, Almora, Uttarakhand, India

**D.K. Upreti** Lichenology Laboratory, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India

**Neeraj Verma** Plant Science Division, Mycology and Plant Pathology Group, Agharkar Research Institute, Pune, India

**Silke Werth** Faculty of Life and Environmental Sciences, University of Iceland, Reykjavik, Iceland; Swiss Federal Research Institute WSL, Birmensdorf, Switzerland; Institute of Plant Sciences, University of Graz, Graz, Austria

**Yoshikazu Yamamoto** Faculty of Bioresource Sciences, Akita Prefectural University, Akita, Japan

**Luciana Zedda** BIO-Diverse, Bonn, Germany

# Lichenization: The Origins of a Fungal Life-Style

David L. Hawksworth

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## Abstract

Following a discussion on the naming of lichens and a definition of “lichen”, hypotheses on the origins of lichenization and lichen-forming fungi are reviewed. It is emphasized that lichen associations strictly have no scientific name, while the partners in the symbiosis do. As fungi have a wide range of associations with algae and cyanobacteria, the definition of “lichen” must include the fungal partner enclosing the photosynthetic, and the photosynthetic partner not being incorporated into fungal cells. Hypotheses put forward to explain lichenization are examined in the context of the evidence from the fossil record and molecular biology. There are uncertainties over the interpretation of many of the pre-Devonian fossils, but stratified undisputed lichen-like associations were present in the Lower Devonian, and material referable to modern genera is preserved in Eiocene and Miocene amber. Some early molecular studies suggested that the earliest ascomycetes may have been lichenized, but as more fungi have been sequenced, it has emerged as more likely that there have been repeated lichenization and de-lichenization events in different lineages over time. Some caution is necessary as the molecular trees do not include data from extinct lineages. The possibility that there were early lichen-like fungal associations as far back as the late Pre-Cambrian or early Cambrian cannot be discounted on the basis that they are not recognizable in the fossil record.

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D.L. Hawksworth (✉)  
Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal, 28040 Madrid, Spain  
e-mail: d.hawksworth@nhm.ac.uk

D.L. Hawksworth  
Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

D.L. Hawksworth  
Mycology Section, Royal Botanic Gardens, Kew, Kew, Surrey TW9 3DS, UK

### Keywords

Algae · *Ascomycota* · Cyanobacteria · Evolution · Lichens · Nomenclature · Palaeomycology · Symbiosis

## 1.1 Introduction

The origin and nature of lichens was a conundrum for the earliest naturalists, although many species were described and illustrated as plants from the mid-seventeenth century. Even Luyken (1809), in whose dissertation numerous generic names of Acharius were introduced, considered that they were compounded of air and moisture. Acharius (1810) asserted they were distinct from algae, hepatics and fungi. As regards development, Hornschurch (1819) thought that they arose from a vegetable “infusorium” which became green and developed by the action of light and air while, from the observations on the lichenization of *Nostoc* colonies to form a *Collema*, the renowned algologist Agardh (1820) viewed them as transformed algae. By the mid-nineteenth century, it was becoming increasingly clear from microscopical studies that the “gonidia” in lichens were not produced from hyphae inside the lichen thallus but were indeed algal cells, although this interpretation was not universally accepted immediately and was hotly debated (Lorsch 1988; Mitchell 2002, 2005, 2007). The term “symbiosis” was introduced for the lichen association by Frank (1876, as “symbiotismus”)<sup>1</sup> as a result of his studies on the anatomy of five<sup>2</sup> crustose lichens (Sapp 1994; Hawksworth 1995). Interestingly, it was Frank who later also coined the word “mycorrhiza” in 1885 for the fungus-root “Pilzensymbiosis” (Sapp 1994).

The issue of how the lichen symbiosis had arisen remained obscure, but the integration of

lichenized fungi into the overall system of fungal classification, the advent of molecular systematics, and critical work on newly recognized fossils have all led to new insights which are reviewed here. A prerequisite for a discussion on this topic is, however, an understanding of the status of names given to lichens and how the term “lichen” is defined.

## 1.2 Names Given to Lichens

While lichens were considered as plants and as single organisms, names were unwittingly applied to the associations rather than to their separate components. When the fungal components were isolated into culture, however, they looked so different from the lichenized thalli that Thomas (1939) opted to give them separate scientific names, such as *Cladoniomyces pyxidatae* for the fungal partner of *Cladonia pyxidata*. This view was not shared by the International Botanical Congress (IBC) in Stockholm in 1950 which ruled that “for nomenclatural purposes names given to lichens shall be considered as applying to their fungal components” (Lanjou et al. 1952). Notwithstanding that decision, in a series of papers from 1952 to 1957, Ciferri and Tomaselli (e.g. Tomaselli and Ciferri 1952) introduced numerous generic names with the suffix “-myces”, and also names at higher ranks, for fungal partners. They made proposals to modify the rules to permit this, but they were rejected by the Montreal IBC in 1959, and Tomaselli (1975) subsequently agreed that this was the right decision. The result of this ruling is that lichen associations do not have a separate scientific name from the partners in the symbiosis. Consequently, whole lichens have no names (Hawksworth 1999), and it is more correct to speak of “lichenized fungi”, “lichen-forming fungi” or “lichen fungi”, rather than “lichens”, when using scientific names. A corollary of this ruling is that where the same fungus forms morphologically distinct lichens with different photosynthetic partners, so-called photosymbiodemes or “phototypes”, the same fungal name has to be used for both morphs; for example, the

<sup>1</sup> Often cited as published in 1877, but the article is indicated as written in “Marz 1876”, and the first two parts of volume 2 of the journal are listed as received in the 11 October 1876 issue of *Flora* (59: 530).

<sup>2</sup> *Arthonia radiata* (as *A. vulgaris*), *Arthopyrenia cerasi*, *Graphis scripta*, *Lecanora albella* (as *L. pallida*), and *Pertusaria pertusa* (as *Variolaria communis*).

shrubby cyanobacteria morph and the green algal foliose morph of *Sticta felix* are both referred to by that name.

As it is the fungal and algal partners of lichens that have scientific names, not the associations, the nomenclature of the fungal partners comes under the auspices of a single Nomenclature Committee established by successive IBCs, at least since 1950. The committee was, however, named as “for Fungi and Lichens” from 1954 to 1993, when it reverted to “for Fungi”, a logical change as lichen associations do not have names apart from those of their fungal components. Since the IBC in 1981, lichenized and non-lichenized fungi have been treated together under “Fungi” in the lists of conserved and rejected names adopted by IBCs. Lichenologists are traditionally included amongst the members of the Nomenclature Committee for Fungi (NCF). This provision does not preclude the establishment of a separate international committee to advise on names of lichenized fungi, as proposed by Lendemer et al. (2012), as there is already a precedent with a separate body that considers fungi with a yeast morph, the International Commission on Yeasts (ICY), established in 1964. It is, however, the NCF which reports to the IBC and makes formal recommendations relating to all organisms treated as fungi under the *International Code of Nomenclature for algae, fungi and plants* (McNeill et al. 2012).

### 1.3 What Is a Lichen?

While the answer to the question “What is a lichen?” may seem obvious when considering cases in which distinctive thalli are formed, the issue becomes more complex when the full range of associations between fungi, algae and cyanobacteria is considered. Early definitions and debates over them have been summarized elsewhere (Hawksworth 1988), and the generally

accepted definition currently in use is: “An ecologically obligate, stable mutualism between an exhabitant fungal partner (the mycobiont<sup>3</sup>) and an inhabitant population of extracellularly located unicellular or filamentous algal or cyanobacterial cells (the photobiont<sup>3</sup>)” (Hawksworth and Honegger 1994). The emphasis on the fungal partner forming the outer tissues (as “exhabitant”) excludes some fungal–algal associations in which the algae forms the outer tissue, as is the case with, for example, *Blodgettia confervoides*, *Mycophycias ascophylli* (syn. *Stigmidium ascophylli*) and *Phaeospora lemaneae*. The mention of “extracellularly located” is to exclude the glomeromycete *Geosiphon pyriforme* which has cyanobacteria inside bladder-like cells of the fungal partner (Schüßler 2012).

Lichens are essentially “the symbiotic phenotype of lichen-forming fungi” (Honegger 2012), and encompass not only a wide range of morphologies, of which an elegant overview is provided by Honegger (2012), but also differences in the extent to which the associations are obligate. Examples are known of single fungal species able to form either lichen thalli, or survive as saprobes, depending on whether they grow on bark or wood; these were formerly placed in different genera on that basis until proved conspecific by molecular methods (Wedin et al. 2004). Over 20 genera are now known to include both lichen-forming species and ones which are either lichenicolous (i.e. growing on lichens) or saprobic, for example, *Arthonia*, *Caloplaca*, *Diploschistes*, *Diplo-tomma*, *Mycomicrothelia*, *Opegrapha* and *Thelocarpon*. There are also examples of fungal genera, such as *Arthopyrenia*, *Chaenothecopsis*, *Cyrtidula*, *Leptorhaphis* and *Stenocybe*, which have traditionally been studied by lichenologists, but which appear not to be obligately associated with any algal or cyanobacterial partner.

In the last few years, a further complication over a definition of “lichen” has arisen from the discovery that lichen thalli can routinely include specialized non-photosynthetic bacteria which only occur within their tissues, some producing novel compounds (Cardinale et al. 2006; Grube et al. 2012). In addition, the presence of fungi

<sup>3</sup> I regard the terms “mycobiont” and “photobiont” as unnecessary jargon as “fungal partner” and “photosynthetic partner” are more immediately understood by non-specialists.



other than the fungal partner, and which do not represent lichenicolous species, but which occur in lichen thalli, so-called endolichenic fungi, have been revealed by molecular methods (U'Ren et al. 2010). It has been suggested from ancestral-state molecular reconstructions that some of these endolichenic fungi may have been the source of endophytic fungi in plants and ancestors of plant pathogens and saprobes (Arnold et al. 2009). Evidence that many of these endolichenic fungi actually live inside the lichen thalli, has, however, yet to be convincingly demonstrated, although hyphae other than of the fungal partner can sometimes be revealed by scanning electron microscopy (Honegger 2012).

A further dimension arises with respect to the photosynthetic partners, as different algal species, and sometimes genera, may form morphologically identical lichens with the same fungal species. Three different *Trebouxia* species, for example, have been isolated from *Parmelia saxatilis* thalli (Friedl 1989). Individual thalli may also contain more than a single algal species. There is also a tendency for lichens in the same habitat to have the same photosynthetic partner; for example, species of four genera in the maritime Antarctic shared the same *Nostoc* strain (Wirtz et al. 2003).

Other issues, not pertinent to expand on here, include the occurrence of different fungal genotypes of the same species, or in some cases different species, in the same lichen thallus, and the probability that some crustose lichens on bark also obtain some nutrients from their hosts and are not totally self-supporting. It is becoming increasingly evident, therefore, that the term "lichen" includes a spectrum of associations of varying degrees of morphological and biological specialization, and that their thalli are not always two-partner systems, but may rather be considered as more complex ecosystems.

Despite the recognition of the partners in lichen associations as different organisms meriting their own scientific names, Margulis (1993) nevertheless, somewhat bizarrely, introduced the phylum name *Mycophycophyta* for the association, ignoring all molecular and morphological evidence. While this has been ignored by

lichenologists and other mycologists, it is regrettable that the term has been taken up in numerous introductory texts (e.g. Allen 2012) and on websites (7,200 hits in Google on 18 Aug. 2014). All researchers who work with lichens are urged to discourage this practice.

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## 1.4 Hypotheses on the Origins of the Lichen Life-Style

Lindsay (1856) considered that lichens must have been the first colonizers of land early in Earth history, before any soil was formed, but did not speculate on their origin. Church (1921) hypothesized that seaweeds, trapped on the land as pools dried, lost their chloroplasts, became in effect primitive fungi and were invaded by or captured unicellular algae. Corner (1964) concurred with Church's view that lichens originated during the migration of life from sea to land, and referred to them as the "land seaweeds".

Cain (1972), however, hypothesized that the first ascomycetes arose from autotrophic "ascophytes", on soil in moist tropical sites, before the origin of vascular plants and perhaps even before green algae. He suggested that the ascophytes would have resembled modern lichens, but were not xerophytic and lacked algal partners, later evolving into lichens through acquiring cyanobacteria initially, and eventually green algae. Eriksson (1981) did not accept Cain's views, and argued that heterotrophic fungi evolved first from heterotrophic or parasitic algae in rock pools and lagoons, where they became lichenized through association with cyanobacteria. He suggested that these early lichens had lecanoralean-type asci from which were derived the non-lichenized ascohymenial and ascolocular ascomycetes, with non-fissitunicate and fissitunicate asci, respectively. Independently, a similar conclusion was reached from a study of the numbers of obligately lichenicolous fungi on different families of host lichens, highlighting the *Peltigeraceae* (Hawksworth 1982).

In order to stimulate debate on evolutionary pathways in ascomycetes, Dick and Hawksworth (1985) endeavoured to construct a diagram

displaying the then-recognized orders, lichenized and non-lichenized, taking into account ascus type and ecology; in their construction, cyanobacterial *Peltigerales* emerged as the deepest rooted amongst the lichenized orders. In the light of molecular data and recently discovered fossils, Eriksson (2005, 2006) developed his earlier ideas on the origin of filamentous ascomycetes. He considered the different possibilities for the origin of all ascomata-forming ascomycetes (other than *Neolecta*), i.e. subphylum *Pezizomycotina*, and concluded that while origins on early vascular plants, bryophytes or macroalgae were very unlikely, an origin on microalgae and cyanobacteria was very probable. The subphylum was suggested to be derived from a group of hypothetical lichenized ancestors, *Protolichenes*, which were living symbiotically with algae and cyanobacteria long before land plants developed. Eriksson termed this the “Protolichenes Hypothesis”, and considered that the many types of asci found in modern lichen-forming fungi indicated that they had evolved on several evolutionary lines over extended periods of time, and that saprobic and parasitic ascomycetes had arisen later through the loss of symbiosis.

In an innovative analysis of fungi in the context of the origin of life and its emergence onto land, Moore (2013) independently concurred that from the beginning, fungi formed lichens with cyanobacteria and noted that these would have been able to colonize terrestrial habitats as they formed about 1.5 Gyr<sup>4</sup> ago.

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## 1.5 Fossil Evidence

The oldest report of a fossil lichen, named *Thuchomyces lichenoides*, is from 2.8 Gyr-old Pre-Cambrian of South Africa. It was interpreted as a palisade of podetium-like structures, measuring  $2\text{--}3 \times 0.5\text{--}0.6$  mm, was dismissed as an artefact of the extraction method (Cloud 1976), but later shown to be real from subsequent observations (MacRae 1999). Somewhat similar

but more urn-like structures, also from the Pre-Cambrian of South Africa but dated to 2.2 Gyr ago, have recently been described as *Diskagma buttonii* and compared with *Cladonia* and *Geosiphon* (Retallack et al. 2013). In neither case is there incontrovertible evidence that these Pre-Cambrian organisms were lichens, i.e. fungi with an included photosynthetic partner. If these, and some other early enigmatic structures from China and Namibia, commented on by Retallack (1994) and Retallack et al. (2013), were truly fungal, this would have major implications for views on the origins of eukaryotic life; the earliest definite fossil evidence for cyanobacteria and eukaryotes has been considered to date to  $\sim 2.5$  Gyr and 1.78–1.68 Gyr ago, respectively (Rasmussen et al. 2008).

The last era of the Pre-Cambrian, the Neoproterozoic Ediacaran (*ca* 635–542 Mya<sup>5</sup>), also has fossils interpreted as lichens (Retallack 1994), amongst which are three species from South Australia, described in *Dickinsonia*, one reaching 1.4 m in length (Retallack 2007). However, a dual nature could not be established and that interpretation is questionable (Waggoner 1995). Also in this period, there is evidence of cyanobacterial and closely associated filaments, considered to be fungal, from marine deposits of 635–551 Mya in southern China. These have been interpreted as lichen-like (Yuan et al. 2005), though the actual nature of the relationship is obscure, but the fungi produced structures recalling spores of *Glomeromycota*, rather than of *Ascomycota*.

There are few lichen candidates from the earliest periods of the Palaeozoic, the Cambrian and Ordovician, but spores recovered from the Silurian suggest that ascomycetes were already diverse by that time (Sherwood-Pike and Gray 1985). Whether any of these spores are from lichenized fungi cannot be ascertained.

Structured lichens were, however, definitely present in the mid-Palaeozoic Devonian, and several cases have been critically documented. The earliest are *Chlorolichenomycites salopensis*

<sup>4</sup> Gyr = Giga years (i.e. billions of years).

<sup>5</sup> Mya = Million years ago.

and *Cyanolichenomycites devonicus*, from the Lower Devonian (415 Mya) of Wales, and these had internal stratification and green algal and cyanobacterial partners, respectively (Honegger et al. 2012). Preservation of the structures may have been facilitated by charring. The hyphae were septate, ascomata were absent, but pycnidia occurred in *C. devonicus*; the two genera were tentatively referred to the *Pezizomycotina*. *Winfrentia reticulata*, described from the Lower Devonian Rhynie Chert from Scotland (400 Mya), consisted of a reticulum of fungal hyphae with included cyanobacterial cells (Taylor et al. 1997, 2009). It lacked stratified tissues and had a fungal partner that may have belonged to *Zygomycota* rather than *Ascomycota*; it consequently differed from extant lichens, but could represent an extinct lichenized lineage (Honegger 2012). A stronger candidate from this period, however, is the genus *Spongiophyton*, species of which are reported from the Lower to Middle Devonian in Bolivia, Brazil, Canada, Ghana and the USA. They have a structure reminiscent of the cortex of extant foliose lichens (Taylor et al. 2004) but no sporing structures are known. What does seem clear is that lichenized and non-lichenized fungi together with free-living algae and cyanobacteria, may have formed extensive rock and soil-crust communities before the evolution of land plants (Honegger 2012).

The Early to Mid-Devonian (400–350 Mya) enigmatic *Prototaxites* has also been posited as a possible lichen. It is known from many parts of the world and formed pillar-like structures to 9 m tall and 1.5 m diam. It has been considered a basidiomycete, possessing septal pores and unfused clamp connections (Hueber 2001), but no basidia were found. In order to sustain itself, these massive structures must have had a carbon source, and it has been suggested that came from green algae embedded in the thallus, and this would also explain some of the polymers found (Selosse 2002). An alternative subsequent interpretation was that it represented rolled-up mats of liverworts intermixed with fungal and cyanobacterial associates, but that hypothesis does not seem sustainable (Boyce and Hotton 2010). *Nematothallus* species also have a complex stratified anatomy

again recalling lichenized fungi (Edwards et al. 2013), and that genus and *Prototaxites* are placed in the extinct order *Nematophytales* which is considered to have affinities with *Mucoromycotina* or *Glomeromycota* (Retallack & Landing 2014).

*Honeggeriella complexa* is an undoubted stratified foliose or squamulose lichen from the Mesozoic Early Cretaceous of British Columbia dated at ca 133 Mya. It lacked rhizines and had a green algal partner with intracellular haustoria (Matsunaga et al. 2013); no ascomata were found, but the sections show a clear upper and lower cortex, a delimited algal layer and a less compacted medulla, similar to modern foliose *Lecanoromycetes*.

More fossils recalling extant lichens survive from the Cenozoic. *Pelichothallus villosus*, described on leaves of *Chrysobalanus* from Eocene (56–34 Mya) deposits in the USA, appears to represent a species of *Strigula*, with a *Cephaleuros* algal partner and pycnidia recalling those of *Strigula* (Sherwood-Pike 1985). A wide and increasing range of lichens trapped in amber dating from the Eocene into the Miocene are being described, especially from the Baltic and Dominican Republic. These finds are listed in Matsunaga et al. (2013), and correspond to modern genera, including *Alectoria*, *Anzia*, *Calicium*, *Chaenotheca*, *Hypotrachyna*, *Parmelia* and *Phyllopsora*. There is also an impression recalling *Lobaria pulmonaria* from the Miocene of California (Peterson 2000).

In view of the abundance of lichens today and the antiquity of the symbiosis, there are remarkably few fossils which have been recognized as lichens. This may be partly because they tend to occur in dry habitats, and so fail to preserve, but it is, perhaps, more likely that the crux of the issue “is simply the inability of paleobiologists to recognize them in the fossil record” (Taylor and Osborn 1996). In that connection, it may be significant that the recognition of the earliest undoubted lichens involved an experienced lichenologist (Honegger et al. 2012). There is an extensive early literature on fossil fungi (Pirozynski 1976), and there are 950 named spore types in the fossil record which are considered to

be fungal (Kalgutkar and Jansonius 2000). In the light of modern technologies, it may well be that there are reports to be re-discovered and re-interpreted, as well as fresh discoveries to be made from the examination of fossil remains. Further, perhaps indications of the abundance of lichens might be deduced not from recognizable fossils, but rather from their mineralogical effects, such as laminar calcretes which arise from the action of lichens on exposed calcareous rock surfaces (Klappa 1979).

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## 1.6 Molecular Evidence

The first major work assessing the importance of lichenization in the evolution of fungi was that of Lutzoni et al. (2001), who concluded that the major lineages in *Ascomycota* were derived from lichenized ancestors. The rates of loss of lichenization exceeded gains, and where there was loss, the fungi became lichenicolous, plant pathogens or saprobes. As more examples of orders and families were sequenced, a fuller picture emerged through a collaborative effort by numerous researchers (Lutzoni et al. 2004). An earlier origin of the lichen symbiosis was indicated by strong support for a close relationship of the lichen-forming *Lichinomycetes*, *Thelocarpaceae* and *Biatoridium* to the *Eurotiomycetes-Lecanoromycetes* group, indicating a deep transition to lichenization, and supporting the hypothesis of low numbers of lichenization events. In contrast, in *Basidiomycota*, lichenization had been a relatively recent and not an ancestral event.

As more genes and more taxa were sampled, hypotheses were tested and modified. The Lutzoni et al. (2001) hypothesis of lichenized ancestors was not supported (Lücking et al. 2009), but, due to early radiations, assessing the number of lichenization events could not be resolved. The situation is complicated by previously unsampled lineages being found to be independent and deeply rooted, such as the cyanobacterial-partnered *Lichinomycetes* (Reeb et al. 2004) which also have

a specialized polysaccharide chemistry distinct from *Lecanoromycetes* (Prieto et al. 2008). The current molecular evidence, based on data from six genes, does, however, suggest that there have been multiple independent origins of the lichen symbiosis, with losses mainly limited to terminal closely related species (Schoch et al. 2009).

There have been various attempts to apply molecular clocks to the dating of divergences, but calibration is a problem as it relies on the interpretation of fossils and this is often unclear. For example, if the fossil non-lichenized *Paleopyrenis devonicus* is considered to belong to (1) *Sordariomycetes*, the lineages of the five fungal phyla would originate at 1.489 Gya. (2) If recognized as at the base of *Peizizomycotina*, it would give an age of 792 Mya; while (3) if treated as a providing a minimum age for *Ascomycota*, a figure of 452 Mya is obtained (Taylor and Berbee 2006). Lücking et al. (2009) revisited this case using uniform calibration points; this placed the origin of fungi between 1.06 Gya and 760 Mya, and the origin of the *Ascomycota* at 650–500 Mya. These results correlate with the fossil records of fungi and plants, and do not require the postulation of hypothetical “protolichens” (see above).

Calibrated molecular phylogenetic trees are increasingly revealing that many extant lichen genera and species can be of considerable antiquity. In the case of *Parmeliaceae*, using two dated fossils referred to *Alectoria* and *Parmelia* (see above), Amo de Pas et al. (2011) demonstrated that the parmelioid lichens diversified around the K/T boundary at the end of the Cretaceous (*ca* 66 Mya) as the dinosaurs became extinct, and the major clades diverged during the Eocene and Oligocene (Tertiary period). The radiation of the genera in this family was dated to the early Oligocene, Miocene and early Pliocene (i.e. from *ca* 33 Mya) and considered to be linked to changing climatic conditions. *Flavoparmelia*, for example, was estimated to have split from *Parmotrema* in South America at the Eocene-Oligocene transition about 33 Mya, with the Australian groups arising much later, at around 5.4–6.5 Mya (Del Prado et al. 2013).

## 1.7 Origins of the Lichen Life-Style

Early hypotheses regarding the origin of the lichen life-style have now been challenged by new discoveries of fossils and molecular dating techniques. The view now emerging is that the earliest filamentous fungi were probably saprotrophs, and parasites of the earliest land plants, and mutualistic relationships with algae need not be invoked to explain these data (Lücking et al. 2009). Different fungal lineages appear to have established and lost symbiotic associations with algae independently, and there also may be cases of re-lichenization. It, therefore, seems that Aptroot (1998) was prescient when he estimated that there could have been “at least over a 100” lichenization events in the evolution of fungi.

The changing biology of fungi in and out of lichenization events in different phyla, classes, orders and families, mean that it is essential to study the evolution and classification of lichen-forming fungi in the context of the fungi as a whole. Lichenology must therefore be considered a sub-discipline of mycology, exploring a particular fungal life-style, on a par with mycologists and mycological organizations that are devoted to fungi exhibiting other biologies (e.g. medical fungi, mycorrhizas, plant pathogens, yeasts).

A final note of caution may be appropriate, however, as molecular data are derived from the organisms known to be living on Earth today. While regressive extrapolations can be made, these cannot account for fungi of ancient lineages that may have become extinct (see above), or for major lineages of fungi that have only recently been recognized (e.g. *Cryptomycota*). Consequently, while it may not be necessary to postulate “ascophyte” or “protolichen” ancestors of modern fungi to explain their evolution, it cannot be ignored that there may also have been early lichen-like fungal-cyanobacterial or algal associations of which no unequivocal fossils or ancestors remain—or perhaps still await reinterpretation or discovery. With the current revival of interest in palaeomycology, who knows what exciting discoveries lie ahead....

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# The Dynamic Discipline of Species Delimitation: Progress Toward Effectively Recognizing Species Boundaries in Natural Populations

Steven D. Leavitt, Corrie S. Moreau and H. Thorsten Lumbsch

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## Abstract

Species represent a fundamental unit in evolutionary biology and provide a valuable context for organizing, evaluating, and communicating important biological concepts and principles. Empirical species delimitation is a dynamic discipline, with ongoing methodological and bioinformatical developments. Novel analytical methods, increasing availability of genetic/genomic data, increasing computational power, reassessments of morphological and chemical characters, and improved availability of distributional and ecological records offer exciting avenues for empirically exploring species delimitation and evolutionary relationships among species-level lineages. In this chapter, we aim to contribute a contemporary perspective on delimiting species, including a brief discussion on species concepts and practical direction for empirical species delimitation studies. Using lichen-forming fungi as an example, we illustrate the importance and difficulties in documenting and describing species-level biodiversity.

## Keywords

Barcoding · Coalescence · DNA taxonomy · Fungi · Gene tree · Genomics · Lichens · Species circumscription · Species concept · Species tree

S.D. Leavitt (✉)  
Committee on Evolutionary Biology,  
University of Chicago, Chicago, IL, USA  
e-mail: sleavitt@fieldmuseum.org

S.D. Leavitt · C.S. Moreau · H. Thorsten Lumbsch  
Department of Science and Education Field Museum  
of Natural History, Chicago, IL, USA