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1 **Lichenized fungi and the evolution of symbiotic organisation**

2

3

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9

10 **Summary.** Lichen symbioses comprise a fascinating relationship between algae and
11 fungi. The lichen symbiotic life style evolved early in the evolution of ascomycetes
12 and is also known from a few basidiomycetes. These lineages of ascomycetes have
13 diversified in the lichenized stage to give rise to a tremendous variety of
14 morphologies. Also, their thalli are often internally complex and stratified for
15 optimized integration of algal and fungal metabolisms. Thalli are frequently
16 colonized by specific non-lichenized fungi and occasionally also by other lichens.
17 Microscopy revealed various ways of these fungi to interact with their hosts. Beside
18 the morphologically recognizable diversity of the lichen mycobionts and
19 lichenicolous (lichen-inhabiting) fungi, many other microorganisms including other
20 fungi and bacterial communities are now detected in lichens by culture-dependent
21 and culture-independent approaches. The application of multi-omics approaches,
22 refined microscopic techniques, as well as physiological studies, have added to our
23 knowledge of lichens, not only about the taxa involved in the lichen interactions, but
24 also about their functions.

25

26 **1. Introduction And Historical Background**

27 Lichens were generally accepted as an independent group of organisms, when
28 Schwendener (1) discovered that lichens are the result of the association of fungi and
29 algae. This insight was not widely accepted by contemporaries, but in fact initiated a
30 scientific revolution as lichens later became the prime example of a mutualistic
31 symbiosis, and indeed the phenomenon for which the term symbiosis was originally
32 introduced in biology [(2) as “Symbiotismus”; (3)]. The lichen symbiosis has proved
33 to be one of the most important life styles in the Ascomycota, and is also known
34 from a few Basidiomycota. Approximately 20 000 currently known fungal species
35 live as lichens, mostly in species-rich lineages of Ascomycota (4). The traditional
36 view of lichens as a mutualistic symbiosis of a fungus and one or several green algae
37 or cyanobacteria, has always been under debate (5), but it has recently been more
38 challenged than ever by the discovery of numerous additional microorganisms that
39 potentially occur as obligatory participants in the symbiosis.

40 The self-supporting associations of fungi and their partners usually form a
41 compact and macroscopically recognizable structure, known as the lichen thallus.
42 Different from many other fungi, which reside beneath the surface of their substrates,
43 the lichen thallus is typically developed at the surface of the substrate or completely
44 exposed to the atmospheric conditions, apparently without exploiting the substrates’
45 nutrients (Fig. 1A-C).

46

47 **2. The Lichen Thallus**

48 The lichen thallus arose early in evolutionary history with the conquest of land, and
49 the earliest records of internally stratified cyanobacterial and green-algal lichens
50 were from the Lower Devonian (6). These lichens are of unclear relationship to

51 current lichen-forming lineages in the Ascomycota, which originated at least as early
52 as the Carboniferous, and radiated in the Jurassic and Cretaceous to generate the
53 diversity of the main modern groups (7). The ability to revitalize from dry stages
54 helps the lichen thallus in many species to survive environmental fluctuations with
55 extremes of desiccation and temperatures (8, 9). Lichens are thus prominent at high
56 altitudes and latitudes where conditions become adverse for most other multicellular
57 organisms. Especially in cool habitats, lichen biomass can be substantial and lichens
58 can then form a substantial part of the vegetation (Fig. 2A-C). By the balance of
59 respiration and photosynthetic energy production in the symbiotic system, many
60 lichen thalli grow slowly but are long-lived. Usually, the fungi dominate biomass and
61 dictate the shape of lichen thalli. The scientific name of lichens also by definition
62 refers to the fungal partner only.

63 Lichen fungi shelter the algal/cyanobacterial partners beneath a protective
64 peripheral cortex layer (Fig. 3). These layers are developed by the conglutination of
65 fungal hyphae, which seems to be correlated with increased branching and
66 anastomoses formation. The outer walls of the fungi are thickened to form sticky
67 contacts with neighboring hyphae. This appears to be a similar process as found
68 generally with the formation of fungal fruitbodies, but in the case of lichens
69 conglutination and compaction occurs in the vegetative mycelium, and this could be
70 one of the key evolutionary innovations to make lichen thallus evolution possible.
71 The conglutination of vegetative hyphae forms a more or less coherent layer by
72 which algal cells can be covered and under which algal populations can form more or
73 less coherent layers within fungal plectenchyma (i.e., tissue-like structure made of
74 fungal hyphae). This developmental process seems to be correlated with massive
75 branching and anastomosing of the hyphae. While genes regulating branching,

76 anastomosing and conglutination are known from model fungal systems, their
77 presence and regulation in lichenized fungi have so far not been studied. The
78 morphogenetic processes seem to be initiated as soon as the fungi contact appropriate
79 algal cells. Once the thallus morphogenesis is initiated, the algal symbionts can
80 proliferate massively under the fungal protectorate. The mycobionts are triggered by
81 the interaction with the algae to produce diverse, more or less complex thallus
82 morphologies, a wide diversity of lichen-derived secondary metabolites, as well as
83 fruitbodies for sexual fungal reproduction and/or asexual structures for combined
84 propagation of symbionts. Notably, strikingly convergent thallus morphologies have
85 evolved in several unrelated lineages of lichenized fungi (10). The convergent shapes
86 include complicated morphologies, for example umbilicate thalli (leafy structures
87 with a central, exclusively fungal holdfast), as well as reductions of complexity, and
88 reversal to simple crustose lichens or even parasitic life-styles, or the formation of
89 thalli composed of grain-like fungal-algal propagules.

90 In certain lineages, there is a strong selectional trend towards vegetative
91 reproduction solutions that disperse the fungal and algal partners. These include
92 soredia, which are granules of varying size that contain algal cells and fungal hyphae,
93 produced in structures of varying size and morphology termed soralia (Fig. 4A).
94 Other types of vegetative propagules are corticate, forming easily detached
95 outgrowths from the lichen thallus, called isidia (Fig. 4B). In several lineages of
96 lichens that reproduce by vegetative means, the production of sexual spore-producing
97 structures is rare or unknown.

98 While most species of lichenized fungi produce highly organized thalli with
99 internal stratification, several “borderline” lichens produce poorly differentiated
100 thalli (11, 12, 13). A small number of fungal species also seem to be facultatively

101 lichenized, in the sense that they sometimes are found loosely associated with algae,
102 and sometimes not (14, 15, 16). In the Stictidaceae, this facultative lichenization is
103 apparently determined by the substrate they grow on, as several species can grow as
104 saprotrophs on dead wood (Fig. 5A) and as loosely lichenized on bark (Fig. 5B). This
105 has been termed “optional lichenization” by Wedin et al. (17). These borderline and
106 optional lichens may be seen as present-day analogs of early thallus evolution, before
107 more advanced forms of thallus organization arose.

108

109 **3. The Lichen Fungi And Their Phylogenetic Relationships**

110 The integration of lichenized fungi into the classification of Fungi was slow at first
111 as very few lichenologists accepted the consequences of Schwendener’s discovery.
112 Vainio (18) was the first to classify at least some higher ranks of the lichen fungi
113 together with other fungi, but the very influential Zahlbruckner (19, 20) treated
114 lichens as a separate group, Lichenes, on a level equal to the Ascomycetes and the
115 Basidiomycetes, which prevented further progress for many years. Nannfeldt (21)
116 argued strongly for integrating lichenized fungi into a natural fungal system,
117 something that was acted upon by Santesson (22, 23, 24). Only in the second half of
118 the 20th century was this integrated classification widely accepted by most
119 mycologists, even if the study of lichen fungi nevertheless often still is considered a
120 matter for lichenologists, and quite distinct from the study of non-lichenized fungi.

121 Lichenized lineages are interesting from a phylogenetic-evolutionary
122 perspective, as they primarily have evolved and diverged in the lichenized condition
123 with similar partners. The self-supporting, self-sufficient lichen thallus is likely to be
124 the target of selective pressures very different from the selective forces working on

125 non-lichenized fungi, which creates a substantial difference from what saprobic or
126 parasitic fungi may undergo.

127 Within the lichens belonging to the Ascomycota, microscopic characters of the
128 sexual reproductive structures, in particular fruit-body development and organization
129 (25, 26, 27), and ascus structures (28, 29, 30), helped to refine the taxonomic
130 relationships and were largely the basis of classifications in the late pre-molecular
131 era. Already at that time, there was a vigorous debate about the number of times that
132 the lichen life style arose in the evolution of fungi. Since it was not feasible to grow
133 axenic fungal cultures, it was only possible to amplify mycobiont genes directly from
134 lichen samples after the development of fungal-specific PCR-primers, which
135 excluded the algal partners. With this technical advancement, Gargas et al. (31) were
136 able to provide a first phylogenetic analysis of 18S rRNA genes in lichenized fungi.
137 Their results suggested that the lichen life style arose at least twice in the evolution
138 of ascomycetes. Data from more sequences of lichenized lineages were analysed by
139 Lutzoni et al. (32) to revisit the fundamental question of the lichen origins. The idea
140 of two origins was not rejected, but neither was the possibility of a single origin of
141 lichens in Ascomycota. Both studies convincingly showed that the lichen life-style
142 was a rare innovation in the evolution of Ascomycota, while the latter study pointed
143 out that the symbiotic life style was more frequently lost in major ascomycete
144 groups. Later studies (33) again suggest that the lichenization originated multiple
145 times, which seems currently to be the generally agreed view.

146 During the past years our understanding of the phylogeny within the major
147 lineages of lichenized Ascomycota increased significantly through a number of
148 investigations with large numbers of species. An updated classification of
149 Ascomycota including the lichenized lineages can be found in Jaklitsch et al. (34),

150 reflecting these discoveries. Extant lichenized fungi occur in at least six of the major
151 lineages within the Ascomycetes; the Arthoniomycetes, Coniocybomycetes,
152 Dothideomycetes, Eurotiomycetes, Lecanoromycetes, and Lichinomycetes (35, 36,
153 37, 38). One additional lichenized lineage may belong to the Leotiomycetes.
154 Different ‘relaxed clock’ scenarios suggested that the origin and diversification of the
155 Pezizomycotina occurred in the Cambrian (38). Prieto & Wedin (38) provided
156 information about the timing of the main diversification events in Ascomycota,
157 including estimates for classes, orders and several families of both lichenized and
158 non-lichenized Ascomycota, many of which had not been previously dated. The
159 main lineages of lichen-forming Ascomycota were shown to all have originated at
160 least as early as the Carboniferous, with successive radiations in the Jurassic and
161 Cretaceous generating the diversity of the main modern groups.

162 Comparatively few representatives of Basidiomycota are lichenized, but
163 recently, the *Cora-Dictyonema* complex was shown to contain a massive number of
164 earlier undistinguished species (39). An investigation focussing on the morphological
165 character evolution in this group showed that the lichenized thallus had evolved
166 progressively from loosely organized filamentous crusts to stereoid and corticioid
167 basidiomata more or less incorporated in the lichenized thallus (40). This indicates
168 that lichenized thalli may have evolved from reproductive structures in their non-
169 lichenized ancestors.

170 In the overview of lichenized Ascomycota below, we select some examples of
171 phylogenetic studies that contribute major advances in our understanding of
172 morphological and chemical character evolution, lichen biology and interactions, or
173 general understanding of major lineages, of each group.

174

175 **Arthoniomycetes.** The Arthoniomycetes contain a single order, Arthoniales, the
176 second-largest group of lichen-forming fungi, which is a morphologically diverse
177 group of predominantly lichenized representatives, where most form lichen
178 symbioses together with green algae of the Trentepohliaceae. Grube (41) presented a
179 pre-molecular era overview of taxonomic and phylogenetic concepts for the
180 Arthoniales. This account provided discussions of selected morphological
181 characters, and a key to the genera now placed in the order. This work served as a
182 baseline for subsequent molecular work. Ertz & Tehler (42) presented a first
183 comprehensive phylogeny of Arthoniales focusing on lineages previously assigned to
184 Opegraphaceae and Roccellaceae. Their two-locus phylogenetic study showed that
185 traditional morphological characters such as growth form, fruit body type, exciple (a
186 fungal layer of hyphae directly surrounding and derived from the ascoma),
187 hypothecium and ascospores colour, ascospores septation pattern, and secondary
188 metabolism are of limited use in delimiting families and genera. They concluded that
189 the high level of phenotypic plasticity might indicate that the Arthoniales is an old
190 group and that phenotypically characterized genera are paraphyletic. The recent
191 analysis of Frisch et al. (43) focused on the heteromorphic family Arthoniaceae and
192 demonstrated that previous classifications of this family did not reflect evolutionary
193 patterns. According to the phylogenetic hypothesis, lichen secondary metabolites,
194 such as pulvinic acid derivatives or red pigments, do not characterize monophyletic
195 groups above the genus level. The study also revealed that lichen-parasitic life style
196 in Arthoniaceae has evolved more than once and is found in four independent
197 lineages of the Arthoniaceae clade and in the *Bryostigma* clade. Arthoniomycetes
198 with chlorococcoid photobionts are restricted to the *Bryostigma* clade and
199 Chrysotrichaceae, while the only saprophytic *Arthonia* species in the phylogeny are

200 related to *Arthonia radiata* and group with lichenized taxa. The phylogenetic data
201 provide a coherent framework for delineating further monophyletic groups in
202 Arthoniaceae in the future.

203

204 **Coniocybomyces.** Coniocybomyces is a small group (one order and family,
205 currently two genera with ca. 30 species) of crustose lichens with prototunicate (thin-
206 walled and evanescent) asci and mazaedia-producing (mazaedium – an accumulation
207 of loose, maturing spores covering the ascoma surface), stalked ascomata. This is the
208 most recently described class of Ascomycota and is apparently related to the
209 Lichinomycetes (38), but this relationship is not supported by any obvious
210 morphological characteristics. The Coniocybomyces is one of the few lichen
211 groups from which a fossil is known, which Prieto & Wedin (7) also utilized in their
212 dating of the major groups of Ascomycota.

213

214 **Dothideomycetes.** The Dothideomycetes is a very large fungal group characterized
215 by ascolocular ascoma development, and only some representatives are lichenized. A
216 comprehensive phylogenetic study of representatives of 41 families using sequences
217 from five genes was presented by Schoch et al. (44). Notably, the ancestral
218 reconstruction of basic nutritional modes suggests numerous transitions from
219 saprobic life histories to either plant-associated or lichenised modes. Nelsen et al.
220 (37) integrated the primarily lichen-forming families Trypetheliaceae,
221 Monoblastiaceae and Arthopyreniaceae in a phylogeny of Dothideomycetes. Perez-
222 Ortega et al. (13) introduced the new order Collemopsidiales, which contains
223 Xanthopyreniaceae, an interesting family including borderline lichens, many of
224 which occur in rocky intertidal habitats, as well as lichenicolous fungi.

225

226 **Eurotiomycetes.** Eurotiomycetes is likewise a very large and morphologically
227 heterogenous group where only some representatives are lichenized, all within the
228 subclass Chaetothyriomycetidae, which is an assemblage of ecologically diverse
229 species, ranging from mutualistic lichenised fungi to human opportunistic pathogens.
230 A multigene phylogenetic study of rock-dwelling fungi concentrating on the
231 Verrucariales suggested that this lichenized order as one of the independent
232 ascomycete groups where lichenisation has evolved on a hostile rock surface (45).
233 The delimitation of orders and families in Chaetothyriomycetidae was re-assessed by
234 Gueidan et al. (35) in a broad phylogenetic study which classified four orders and ten
235 families in Chaetothyriomycetidae.

236

237 **Lecanoromycetes.** This class comprises the largest group of lichenized fungi and
238 one of the most species-rich classes in the kingdom of Fungi. Traditional concepts of
239 classification included reproductive structures, in particular of ascomata and ascus
240 apex structure (which often contain characteristic amyloid features). These
241 traditional concepts were challenged when comprehensive sequence sampling of taxa
242 was available (46, 47, 48). Miadlikowska et al. (49) demonstrated that the
243 Acarosporomycetidae and Ostropomycetidae are monophyletic subclasses, whereas
244 the delimitation of the largest subclass, the Lecanoromycetidae, remained uncertain.
245 This and the previous phylogenies confirmed that ascus apex morphology cannot be
246 consistently used as a cardinal character for family level classification of lichen-
247 forming fungi (50).

248 One important approach in understanding the evolution of phenetic features of
249 lichens is by studying large genera, which are pragmatically circumscribed by key

250 characters. In many studies these key characters proved to be misleading, and rather
251 were symptoms of massively parallel evolution and thus produced by unrelated
252 fungi. Examples of such misleading key characters are prototunicate asci and
253 mazaedia, which used to characterize the “old” order Caliciales (including pin- and
254 stubble-lichens), a group that in its “old” circumscription now has been shown to be
255 extremely polyphyletic (51, 38) and where the families Caliciaceae and
256 Sphaerophoraceae are now classified in the Lecanoromycetes (31, 38). Another
257 example of this phenomenon was provided by Schmutz’s et al. (52) analysis of the
258 heterogenous *Lecidea* (containing 1200 species), which are usually characterized by
259 black fruitbodies on a crust-like thalli and single-celled, hyaline ascospores. As
260 expected, the analysed species of *Lecidea* sensu lato and putatively related taxa were
261 scattered within Lecanoromycetidae, and some were even placed outside currently
262 recognized orders in Lecanoromycetidae.

263 Most of the lichen fungi that form symbioses with filamentous *Nostoc*
264 cyanobacteria belong to the Lecanoromycetes. *Nostoc* has a sheath around the
265 filamentous thread-like colonies that swells and becomes extremely gelatinous when
266 wet and this gives many cyanolichens a gelatinous habit when moist. Within the
267 Lecanoromycetes, most gelatinous lichens are classified in the Collemataceae. This
268 is yet another example of how classifications have been based on misleading key
269 characters as a number of gelatinous, *Nostoc*-containing groups formerly placed in
270 Collemataceae are nested within another Peltigerales family, the Pannariaceae (53,
271 54). Symbioses with *Nostoc* have clearly arisen several times. Depending on the
272 reconstruction method, most or all transformations in thallus structure within
273 Peltigerales took place from a non-gelatinous to a gelatinous, *Nostoc*-containing
274 thallus (53).

275 Within Lecanorales, the phylogeny of the largest lichen family Parmeliaceae
276 was studied in a number of papers, where Crespo et al. (55) showed that
277 morphological characters discriminated the main clades well, but that the
278 interpretation of the morphological diversity had been quite superficial. The
279 morphological diversity was indeed found to be substantial in this family when
280 Divakar et al. (56) showed that the lichenicolous lifestyle originated independently
281 three times within Parmeliaceae, ca. 24 million years ago.

282 Gaya et al (57) studied the Teloschistales using a supermatrix approach and
283 showed that a progressive, cumulative, addition of taxa to the matrix analysed with a
284 resulting increasing amount of missing data, did not affect the support and resolution
285 much, but that the monophyly of the order was inconsistent depending on the
286 combination of loci and taxa. In the Teloschistaceae, Arup et al. (58) proposed a
287 completely re-organized generic classification, and investigated how the apparently
288 large morphological plasticity affected the characterization of genera and species.
289 Secondary metabolites were frequently found to better serve as characterizing traits
290 than morphology, at least in parts of the family.

291 Within Ostropomycetidae Baloch et al. (59) suggested that the evolution of
292 life-styles and ascomatal morphologies in this group was very plastic, as shown by
293 the multiple evolution of perithecioid ascomata in the Gyalectaceae. Early
294 evolutionary splits in the Ostropomycetidae, in fact the second-most species-rich
295 subclass of lichenized Ascomycota, were considered by Resl et al. (60) in their
296 analysis of trapelioid fungi. The uncertain phylogenetic resolution of the c. 170
297 species prevented a clear backbone concept for the subclass. A monophyletic group
298 of nine core trapelioid genera was found, which do not form the sister group of
299 Ostropales, but the hypothesis of a sister group relationship of trapelioid genera and

300 Baeomycetaceae or Hymeneliaceae could not be rejected. Recently, Miadlikowska et
301 al. (61) provided the most comprehensive phylogenetic survey of the entire
302 Lecanoromycetes using a multigene maximum likelihood analysis with a cumulative
303 supermatrix approach. However the analysis of this massive data set (1139 taxa)
304 revealed that the cumulative addition of taxa with an increasing amount of missing
305 data leads to relatively stable representation of relationships for many families and
306 orders, but also in substantial loss of phylogenetic resolving power and support for
307 deep phylogenetic relationships.

308 In the Graphidaceae, a very large family of >2000 species, Lumbsch et al. (62)
309 studied character evolution and found that certain characters (secondary metabolites,
310 in particular) had a high frequency of reversible phenotypic state changes , whereas
311 others, such as photobiont, hymenial persistence or ascoma aggregation, exhibited
312 low frequency of transformations. But even in the character with the highest number
313 of state changes (changes in secondary metabolite composition), the shifts were
314 highly structured phylogenetically, suggesting that the evolution of the character,
315 rather than the character state itself, can be used to predict phylogenetic relationships
316 with certain accuracy.

317

318 **Leotiomyces.** *Trizodia acrobata* is a borderline lichen and the sister group of
319 Leotiomyces. The lineage was discovered by Stenroos et al. (63) in a phylogenetic
320 analysis of bryophilous ascomycetes. *Trizodia* is ecologically unique by its
321 association with cyanobacterial colonies (mostly *Nostoc*) growing on the tips of peat
322 mosses (*Sphagnum* spp.). *Trizodia* was consistently present in all *Sphagnum*–*Nostoc*
323 associations studied. It envelops the cyanobacterial colonies both on the moss surface
324 as well as inside the leaf but does not form organized thallus structures.

325

326 **4. The Upcoming Genomics Perspective For Understanding Lichen Symbioses**

327 Increasing the number of loci used to determine phylogenetic relationships is now
328 possible through characterization of entire genomes of lichens. Genome-scale
329 datasets may lead to the development of consistent, well-supported hypotheses about
330 the evolution of lichenized fungi. A first step towards a phylogenomic analysis of
331 lichen-forming fungi was undertaken in an exemplary phylogenomic study of the
332 genus *Rhizoplaca* (Lecanoromycetes) by Leavitt et al. (64).

333 Current high-throughput sequencing technology has opened new opportunities
334 for studying organisms that grow slowly and are difficult to establish and grow in
335 axenic cultures. These difficulties explain why comparative genomics of lichen fungi
336 lags behind the advances made so far in other fungal groups. Meanwhile, genetic
337 manipulation, such as transformation, of lichens has been attempted (65). Numerous
338 lichen genome projects have been started in the past years, and some preliminary data
339 are already available. Basically, two strategies are followed in lichen genomics. The
340 more traditional approach is the acquisition of genetic information from the
341 individually cultured symbiotic partners. With the alternative metagenomic approach
342 the total symbiotic association is first sequenced and the genetic information is
343 afterwards assigned to symbiotic partners by bioinformatic analysis. Such progress is
344 possible by exploiting technologies that have significantly improved the quality of
345 sequence assemblies (e.g., by “mate-pair” sequencing) and by powerful
346 bioinformatics pipelines. Apart from gaining information on basic genomic features
347 such as genome size and predicted numbers of genes (Tab. 1), several studies
348 provided more detailed analyses of interesting functions.

349 Some of these analyses focused on the biosynthetic pathways of secondary
350 metabolites of lichenized fungi (including depsides and depsidones, as classes of
351 coupled phenol carboxylic acids). Sequencing the 34 Mb genome of the *Cladonia*
352 *grayi* mycobiont revealed new insights in the production of the lichen depsidone
353 grayanic acid (66), and suggests that a single polyketide synthase (PKS) synthesizes
354 two aromatic rings on tandem acyl carrier proteins and links them into a depside, and
355 that the transition from depside to depsidone requires only a cytochrome P-450 mono-
356 oxygenase. Sequencing of the *Cladonia uncialis* mycobiont revealed a putative
357 biosynthetic gene cluster leading to usnic acid, a dibenzofuran derivative (67).
358 Although no typically crystallized lichen substances have been recorded for *P.*
359 *membranacea*, a large number of mycobiont and photobiont genes and gene clusters
360 associated with secondary metabolite biosynthetic pathways have been identified in
361 its metagenome, and an unusual trans-AT polyketide biosynthetic pathway of a type
362 known only from other bacterial-eukaryote symbiosis has been identified in the
363 *Nostoc* photobiont (68).

364 In addition, the genome sequencing provided evidence that the tight
365 association of fungi and prokaryotes might have favored horizontal gene transfer
366 (HGT) events. One such event was detected in the methylammonium permease family
367 between prokaryotes and the *Cladonia grayi* mycobiont (69). Subsequently
368 McDonald et al. (70) suggested that lichen-forming fungi are losing this gene family
369 at slower rate than other fungal lineages.

370 The integration of further available (meta)omics approaches, such as
371 (meta)transcriptomics or (meta)proteomics, provides new and complementary insights
372 into the lichen symbiosis. In this context, transcriptomics reveals which of the total set
373 of genes are activated under certain conditions, whereas proteomics suggests which

374 functions are actually translated into protein functions. An initial full-length cDNA
375 library as a reflection of the transcriptome was provided by Wang et al. (71), using the
376 cultured mycobiont of the desert lichen *Endocarpon pusillum*. However, because a
377 symbiotic context is missing, the significance of the detected gene expression for
378 symbiosis is unclear. In a subsequent study of the same lichen, Wang et al. (72) also
379 analysed dehydrated thalli and confirmed expression of 23 candidate stress responsive
380 genes, selected from a larger set found with mycobiont cultures exposed to PEG-
381 induced drought stress. There is also ongoing RNA-Seq work aiming to compare gene
382 expression in the mycobiont alone in pure culture versus the mycobiont in the
383 symbiotic state to identify genes that are differentially expressed and might therefore
384 be correlated with symbiotic interactions. Gene expression studies elucidate relative
385 levels of active genes (73). Also, epigenetic modifications such as the presence of 5-
386 methycytosine in lichen genomes can be determined in conjunction with next-
387 generation sequencing platforms.

388 Moving a step further, Juntilla and Rudd (74) used high-throughput next
389 generation sequencing and expressed sequence tag (EST) sequence data to present a
390 first transcriptome of the eukaryotic partners in the thalli of the reindeer lichen
391 *Cladonia rangiferina* (with 62.8% reads of fungal and 37.2% of algal origin). Even
392 though a higher percentage of algal reads was found in the wetted thalli used, gene
393 ontology (GO) terms (<http://geneontology.org/>) and identified KEGG pathways
394 (www.genome.jp/kegg/) largely agreed with eukaryotic metaproteome patterns found
395 by Schneider et al. (75). Juntilla et al. (76) tracked the expression profiles during
396 desiccation and rehydration using microarray analyses, but the data do not provide
397 detailed insights into the regulatory processes. Most of the differentially expressed

398 genes do not show sequence similarity to known genes. It is, however, remarkable
399 that largest changes of gene expression are observed only minutes after rehydration.

400 Given the complexity of lichens it is not surprising that the functional
401 contributions of genes are organ-specific and modified by pertinent ecological and
402 developmental conditions. The partially annotated *Peltigera membranacea*
403 metagenome revealed the presence of mycobiont genes encoding galectin-like
404 proteins, which are family of proteins defined by their binding specificity for β -
405 galactoside sugars (77). RNA-Seq data further showed that one of these genes, *lec-1*,
406 was differentially expressed in rhizines, a purely fungal tissue, when compared to the
407 remainder thallus, composed of both mycobiont and photobiont (73).

408 While forthcoming comparative genomics studies will inform us about the
409 evolutionary dynamics of lineages diverging in lichen-symbiotic stages,
410 transcriptomics, proteomics, and metabolomics will improve our understanding of the
411 symbiotic regulation and processing. *Lobaria pulmonaria*, a tripartite lichen
412 (including both a green algae and a cyanobacterium) widely distributed in the
413 Northern hemisphere, has been featured in publications that have explored
414 metaproteomic issues (75, 78). Most algal proteins were assigned to energy
415 production and conversion. Carbohydrate transport and metabolism were significant
416 in both eukaryotic partners, but fungal functions were more diverse, with substantial
417 read numbers suggesting biogenesis and posttranslational modification. The bacterial
418 fraction (see also further below) in the metaproteome was dominated by proteins from
419 Alphaproteobacteria. The identified proteins are primarily involved in energy
420 conversion and carbohydrate metabolism, as well as responses to stress. Also, some of
421 the bacterial proteome spectra suggested a role of bacteria in secondary metabolite
422 synthesis, but this could not be resolved in full detail so far.

423

424 **5. Fungal-Algal Connections And Interactions In The Thallus.**

425 The main role of the algal partners is the provision of photosynthetically fixed
426 carbohydrates as the energetic basis for the self-sustained life style. The lichen-
427 forming fungi take up osmotically active monomeric sugars from the algae, which are
428 then further metabolized. Typically, green algae supply polyols (ribitol in
429 chlorococcal algae and erythritol in filamentous Trentepohliales), whereas glucose is
430 provided by cyanobacterial algae. Apparently the process of lichenization also
431 stimulates upregulation of photo-protective mechanisms in the photobiont (79). In
432 turn, the photobiont stimulates the antioxidant system of the mycobiont (8, 9).
433 Currently it is not well known, which chemical signals are transferred during the
434 initiation and onset of lichenized stages, and what effects they have in the symbiosis.
435 By comparison of algal strains, Meeßen and Ott (80) detected characteristic
436 metabolites in lichen-forming algae, such as the cyclic dipeptides cyclo-L-leucyl-L-
437 tyrosine and cyclo-(L-tryptophyl-L-tryptophyl), rhamnose and indole-3-carbaldehyde,
438 a precursor or a degradation product of the phytohormone indole-3-acetic-acid.
439 Because hyphal branching is not stimulated with these substances alone, but when
440 unicellular algae grow in proximity (81), further metabolites seem to be involved in
441 the signalling of lichenization. Such molecules probably include phytohormones
442 abscisic acid and ethylene, as well as others (82, 83, 84).

443 The fungal partner frequently produces attachment or penetration structures
444 (appressoria or haustoria, respectively) of different kinds at the mycobiont-photobiont
445 interface. Water and dissolved nutrients are can move readily between the bionts at
446 the contact zone, within a hydrophobic coat that the fungus produces over the algal
447 cell surface (85, 86).

448 Lichen fungi associate with only about 120 known species of algae, most of
449 them green algae, and a few cyanobacterial algae (86), although the species taxonomy
450 is so poorly understood in lichenized cyanobacteria that these currently cannot be
451 identified to species level (87). The photosynthetic partners are green algae in about
452 90% of lichen species. Hence 10 % of the lichens take advantage of bacterial nitrogen
453 fixation and associate with cyanobacterial algae, most commonly with filamentous
454 *Nostoc* strains. Another strategy is the additional association with *Nostoc* strains in
455 specialized organs (cephalodia; Fig. 6A) in or on a green-algal thallus (88), resulting
456 in tripartite lichens, which apparently evolved as a segregation of functions. The
457 higher proportion of heterocysts suggests a main role of cephalodia in nitrogen
458 fixation (89). On the other hand, several cyanobacterial lichens may also have green
459 algae in addition to the cyanobacteria in their photosynthetic layer (90), which might
460 widen their ecological amplitude in cool habitats. Few fungal species are even able to
461 use either eukaryotic green or prokaryotic blue-green algae to develop independent
462 symbiotic thalli (91). These phycosymbiodemes, primarily found in the order
463 Peltigerales of Lecanoromycetes either have similar morphologies, or are shaped
464 profoundly differently (Fig. 6B). When growing separately they were described in
465 independent genera, which obviously has blurred the understanding of their
466 phylogenetic relationships (92).

467 With very few exceptions, the green algae propagate clonally and do not form
468 sexual structures in the lichenized stage. The apparent suppression of the sexual
469 capacity of the algal partner by the lichen-forming fungi could be interpreted as a
470 selfish strategy to avoid genetic diversification of the partner and to maintain
471 efficient control over the algal physiology.

472 Refined molecular analyses meanwhile have helped to improve the knowledge
473 about lichen photobiont diversity as well, both their phylogenetic relationship as well
474 as their patterns of association with their fungal hosts. Major mycobiont lineages
475 seem to vary by their overall spectra of preferences for algal groups. While the
476 majority of Arthoniomycetes have a preference for Trentepohliaceae (except for
477 some species assigned to *Arthonia*), Lecanoromycetes and particular members of
478 Chaetothyriomycetes (above all Verrucariales) associate with a broader range of
479 green algae (43, 61, 93).

480 Recent work suggests that species of lichenized fungi frequently vary in their
481 algal specificity and selectivity. This correlates with the range of the species and
482 climatic differences, i.e. the same lichens occurring in different habitats often
483 associate with related algal species with different ecological preferences (94, 95).
484 This flexibility supports the hypothesis of habitat-adapted symbioses proposed by
485 Rodriguez et al. (96) which suggests that the environment determines the optimal
486 partnership of symbionts. Wedin et al. (97) showed that *Diploschistes muscorum* has
487 an even more flexible photobiont strategy than earlier believed. *Diploschistes* starts
488 as a parasitic fungus infecting the unrelated lichen *Cladonia* (Fig. 7), eventually
489 taking over and forming an independent thallus. Although the *Cladonia* used the
490 same photobiont at all investigated sites, *Diploschistes* associated with different
491 photobionts at all three sites, keeping the original *Cladonia* alga at one site but
492 replacing it with others in the two other sites (97). This suggests a very generalistic
493 photobiont strategy in this lichenicolous (=lichen-inhabiting) lichen.

494 Rikkinen et al. (98) suggested that lichen fungi in the Peltigerales form
495 ecological photobiont guilds, within which the fungi share related photobiont
496 cyanobacteria. Such associations can be seen both among an ecological assemblage

497 of epiphytic macrolichens, the *Nephroma*-guild, and among a group of
498 predominantly terricolous cyanolichens, the *Peltigera*-guild, where the photobionts
499 with each guild are closely related *Nostoc*-strains. Several authors have likewise
500 observed similar photobiont-mediated guilds in green-algal lichens (99, 5).

501

502 **6. Additional Fungi In The Lichen Symbiosis**

503 As lichen thalli are very persistent and variable morphologically, they provide a rich
504 diversity of small-scale niches for various microorganisms, in particular fungi and
505 bacteria (treated below). Lichenicolous (lichen-inhabiting) fungi comprise all fungal
506 species living in or on lichens, apart from the thallus-forming fungus itself (the
507 mycobiont). This biological group has been studied extensively, and was even
508 recognized before the symbiotic nature of their lichen hosts was established. More
509 than 1,800 species of lichenicolous fungi have been described (100), but as numerous
510 new species are still being discovered, their precise number is unknown but is clearly
511 currently much underestimated. The evolutionary origin of the lichenicolous life
512 style is diverse, but a substantial number of lichenicolous fungi apparently evolved
513 after delichenization of originally lichenized lineages. As most lichenicolous fungi
514 seem to exploit their hosts without rapid destruction, they are highly specific for their
515 hosts, and for particular symbionts of the hosts.

516 Typically lichenicolous fungi are recognizable by conspicuous reproductive
517 structures or by their symptoms such as discolorations or gall-like
518 hypertrophifications. Some other species associate with lichens but remain cryptic.
519 Isolation techniques and axenic cultivation revealed that these species either reside
520 on the surfaces of the thalli (101, 102, 103), or occur internally in lichen host thalli
521 (also known as “endolichenic fungi”, 104). Given the abundance and the potential of

522 culturable endolichenic fungi to produce secondary metabolites, it might be argued
523 whether these fungi might have a biological effect on their host, or influence the
524 phenotype in various ways. However, even though microscopic evidence already
525 demonstrates the endolichenic growth of fungi beside the mycobiont in lichens (86),
526 it is not clear which of these fungi grow in lichens or reside as spores or otherwise in
527 dormant stages.

528 Culture techniques have retrieved a surprisingly large number of fungal species
529 from lichens (105), but these numbers only represents a subset of the total fungal
530 diversity present in environmental samples. Therefore, culture-independent methods
531 are now also employed to characterize the mycobiome of lichen thalli. Using DNA-
532 fingerprinting techniques, Fleischhacker et al. (106) found a high diversity of lichen-
533 associated fungi without correlation with the presence of externally visible
534 lichenicolous fungi. Zhang et al. (107) provided an overview of diversity and
535 distribution of fungi in lichens from an Arctic habitat using next generation
536 sequencing. Their study of the lichen mycobiome indicated that lichens harbor fungi
537 related to those with diverse ecological context. Lichens thus represent a reservoir or
538 an evolutionary hotbed for fungi that may have a role in other habitats, but the
539 specific conditions in fungi with recurrent and prolonged cryptobiotic stages do not
540 favor the rise of, for example, a biotrophic plant pathogen. Studies in *Lobaria*
541 revealed the high specificity of *Tremella lobariacearum* (108), but whether the same
542 is true for non-symptomatic occurrences of Tremellomycetes is unknown. Refined
543 microscopic techniques can reveal to what extent the scattered occurrence of
544 tremellalean fungi might influence the morphology of the host. Millanes et al. (109)
545 described Cystobasidiomycetes as a new lineage of Pucciniomycotina. The

546 lichenicolous members of this lineage, which primarily comprises yeasts, cause
547 conspicuous fertile hypertrophifications of their hosts.

548

549 **7. Bacterial Participation In Lichen Thalli**

550 Fungi associated with lichens have been studied extensively for a long time, but the
551 ubiquitous presence of bacteria in lichen thalli received more attention only
552 comparatively recently. Sequencing of lichen-associated bacterial communities
553 revealed information about their diversity on lichen thalli (110, 111, 112, 113, 114,
554 115, 116, 117, 118). Alphaproteobacteria usually dominate the bacterial communities
555 in lichens, while other bacterial groups (frequently Acidobacteria, Actinobacteria,
556 and Betaproteobacteria) are also found in significant numbers. One group of
557 Alphaproteobacteria, the lichen-associated Rhizobiales (LAR1) is a clade of bacteria
558 so far known only from lichens (113; 115). Sequence-based data are complemented
559 with microscopic information, in particular employing fluorescence *in situ*
560 hybridization (FISH)(119). The distribution of bacteria belonging to certain groups
561 can be visualized under the microscope using specific probes (confocal laser
562 scanning microscopy, CLSM). Bacteria often form biofilm-like communities on the
563 lichen thalli and are usually tightly connected with the fungal structures, in particular
564 with hydrophilic surfaces of the lichens. The bacteria can also intrude to various
565 depths in the intercellular matrix of the upper cortex, and are occasionally also found
566 inside the hyphae of the fungal hosts (120). The lichen-associated bacterial
567 communities so far investigated are host-specific in their composition (112; 115). A
568 clear shift in microbiome profile is observed when the parasitic lichen *Diploschistes*
569 *muscorum* infects, and eventually overcomes *Cladonia symphycarpa*, to form a
570 thallus of its own (97; Fig. 7). Here, the Alphaproteobacteria population dominating

571 the *Cladonia* microbial community decreases during the transition, and numbers of
572 Betaproteobacteria or Chloroflexi increase substantially when *Diploschistes* takes
573 over, supporting the view that the microbial community is highly species-specific in
574 lichen thalli.

575

576 **Conclusions**

577 Phylogenetic studies significantly improved the understanding of evolution and
578 phenotypic diversification of lichenized fungi and their partners. Lichen thalli are
579 dominated by the primary fungal symbiont, which controls the characteristic
580 photosynthetic partners. Beside these commonly known symbionts, an unknown
581 numbers of additional organisms can participate in the symbiont system (121). The
582 analysis of massive amounts of molecular data from lichens, which has just started,
583 will help to further understand the role of these complex and fascinating partnerships.

584

585 **References**

- 586 **1. Schwendener S.** 1869. Die Algentypen der Flechtengonidien. Basel.
- 587 **2. Frank AB.** 1877. Über die biologischen Verhältnisse des Thallus einiger
588 Krustenflechten. *Cohn, Beiträge zur Biologie der Pflanzen* **2**:123-200.
- 589 **3. de Bary A.** 1879. *Die Erscheinung der Symbiose*. Strasburg.
- 590 **4. Sipman HJM, Aptroot A.** 2001. Where are the missing lichens? *Mycol Res*
591 **105**:1433-1439.
- 592 **5. Piercey-Normore MD, Deduke C.** 2011. Fungal farmers or algal escorts: lichen
593 adaptation from the algal perspective. *Molecular Ecology* **20**:3708-3710.

- 594 **6. Honegger R, Edwards D, Axe L.** 2013. The earliest records of internally
595 stratified cyanobacterial and algal lichens from the Lower Devonian of the Welsh
596 Borderland. *New Phytologist* **197**:264-275.
- 597 **7. Prieto M, Wedin M.** 2013. Dating the diversification of the major lineages of
598 Ascomycota (Fungi). *PLoS One* **8**(6): e65576..
- 599 **8. Kranner I, Cram JW, Zorn M et al.** 2005. Antioxidants and photoprotection in a
600 lichen as compared with its isolated symbiotic partners. *Proc Natl Acad Sci USA*
601 **102**:3141-3146.
- 602 **9. Kranner I, Beckett RP, Hochman A, Nash TH III.** 2008. Desiccation tolerance
603 in lichens: a review. *Bryologist* **111**:576-593.
- 604 **10. Grube M, Hawksworth DL.** 2007. Trouble with lichen: the re-evaluation and re-
605 interpretation of thallus form and fruit body types in the molecular era.
606 *Mycological Research* **111**:1116-1132.
- 607 **11. Kohlmeyer J, Hawksworth DL, Volkmann-Kohlmeyer B.** 2004. Observations
608 on two marine and maritime “borderline” lichens: *Mastodia tessellata* and
609 *Collemopsidium pelvetiae*. *Mycological Progress* **3**:51-56.
- 610 **12. Muggia L, Gueidan C, Knudsen K, Perlmutter G, Grube M.** 2013. The lichen
611 connections of black fungi. *Mycopathologia* **175**:523-535.
- 612 **13. Pérez-Ortega S, Garrido-Benavent I, Grube M, Olmo R, de los Rios, A.** 2016.
613 Hidden diversity of marine borderline lichens and a new order of fungi:
614 Collemopsidiales (Dothideomyceta). *Fungal Diversity* in press.
- 615 **14. Hawksworth DL.** 1988. The variety of fungal-algal symbioses, their
616 evolutionary significance, and the nature of lichens. *Botanical Journal of the*
617 *Linnean Society* **96**:3-20.

- 618 **15. Aptroot A.** 1991. A monograph of the Pyrenulaceae (excluding *Anthracothecium*
619 and *Pyrenula*) and the Requienellaceae, with notes on the Pleomassariaceae, the
620 Trypetheliaceae and *Mycomicrothelia* (lichenized and non-lichenized
621 Ascomycetes). *Bibliotheca Lichenologica* **44**:1-178.
- 622 **16. Aguirre-Hudson B.** 1991. A taxonomic study of the species referred to the
623 ascomycete genus *Leptorhaphis*. *Bulletin of the British Museum (Natural History),*
624 *Botany* **21**:85-192.
- 625 **17. Wedin M, Döring H, Gilenstam G.** 2004. Saprotrophy and lichenization as
626 options for the same fungal species on different substrata: environmental plasticity
627 and fungal lifestyles in the *Stictis-Conotrema* complex. *New Phytologist* **164**:459-
628 465.
- 629 **18. Vainio EA.** 1890. Étude sur classification naturelle et morphologie des lichens
630 du Brésil. *Acta Societatis pro Fauna et Flora Fennica, Helsinki* **7**:1-256.
- 631 **19. Zahlbruckner A.** 1907. *Lichenes* (Flechten) B. Spezieller Teil, p 49–249. *In*
632 Engler A, Prantl K (ed), *Die natürlichen Pflanzenfamilien. 1. Teil*, Leipzig.
- 633 **20. Zahlbruckner A.** 1926. *Lichenes* (Flechten) B. Spezieller Teil, p 61–270. *In*
634 Engler A, Prantl K (ed), *Die natürlichen Pflanzenfamilien. 2. Auflage, Vol. 8,*
635 *Leipzig.*
- 636 **21. Nannfeldt JA.** 1932. Studien über die Morphologie und Systematik der nicht-
637 lichenisierten inoperculaten Discomyceten. *Nov Act Reg Soc Upsal* **8**:1-368.
- 638 **22. Santesson R.** 1952. Foliicolous lichens I. A revision of the taxonomy of the
639 obligately foliicolous, lichenized fungi. *Symbolae Bot Upsal* **12**:1-590.
- 640 **23. Santesson R.** 1953. The new systematics of lichenized fungi, p 809-810. *In*
641 Osvold H, Aberg E (ed) *Proceedings of the 7th International Botanical Congress,*
642 *Stockholm 1950, Almquist and Wiksell, Waltham.*

- 643 **24. Santesson R.** 1954. Fungal symbionts of lichens. *Taxon* **3**:147-148.
- 644 **25. Henssen A, Jahns M.** 1974. *Lichenes. Eine Einführung in die Flechtenkunde.*
645 Stuttgart: G. Thieme.
- 646 **26. Henssen A, Keuck G, Renner B, Vobis G.** 1981. The lecanoralean centrum, p
647 138-234. In Reynolds DR (ed.), *Ascomycete Systematics. The Luttrellian Concept.*
648 Springer-Verlag, New York, Heidelberg, Berlin.
- 649 **27. Döring H, Lumbsch HT.** 1998. Ascoma ontogeny: is this character set of any
650 use in the systematics of lichenized ascomycetes. *Lichenologist* **30**:489-500.
- 651 **28. Hafellner J.** 1984. Studien in Richtung einer natürlicheren Gliederung der
652 Sammelfamilien Lecanoraceae und Lecideaceae. Beiträge zur Lichenologie.
653 Festschrift J. Poelt. *Beihefte zur Nova Hedwigia*, **79**:241-371.
- 654 **29. Rambold G, Triebel D.** 1992. The inter-lecanoralean associations. *Bibliotheca*
655 *lichenologica* **48**:1-201.
- 656 **30. Hafellner J, Hertel H, Rambold G, Timdal E.** 1993. *A new outline of the*
657 *Lecanorales.* Graz: Privately published by the authors.
- 658 **31. Gargas A, DePriest PT, Grube M, Tehler A.** 1995. Multiple origins of lichen
659 symbioses in fungi suggested by SSU rDNA phylogeny. *Science* **268**:1492-1495.
- 660 **32. Lutzoni, F., Pagel, M., Reeb, V.** 2001. Major fungal lineages are derived from
661 lichen symbiotic ancestors. *Nature* **411**:937-940.
- 662 **33. Schoch CL, Sung GH, López-Giráldez F, Townsend JP, Miadlikowska J,**
663 **Hofstetter V, Robbertse B, Matheny B, Kauff F, Wang Z, Gueidan C, Andrie**
664 **RM, Trippe K, Ciuffetti LM, Wynns A, Fraker E, Hodkinson BP, Bonito G,**
665 **Groenewald JZ, Arzanlou M, De Hoog GS, Crous PW, Hewitt D, Pfister D,**
666 **Peterson K, Gryzenhout M, Wingfield MJ, Aptroot A, Suh SO, Blackwell M,**
667 **Hillis DM, Griffith GW, Castlebury LA, Rossman A, Lumbsch HT, Lücking**

668 **R, Büdel B, Rauhut A, Diederich P, Ertz D, Geiser DM, Hosaka K,**
669 **Inderbitzin P, Kohlmeyer J, Volkmann-Kohlmeyer B, Mostert L, O'Donnell**
670 **K, Sipman H, Rogers JD, Shoemaker R, Sugiyama J, Summerbell RC,**
671 **Untereiner W, Johnston PR, Stenroos S, Zuccaro A, Dyer PS, Crittenden PD,**
672 **Cole MS, Hansen K, Trappe JM, Yahr R, Lutzoni F, Spatafora JW. 2009a.**
673 The Ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and
674 evolution of fundamental reproductive and ecological traits. *Systematic Biology*
675 **58:224-239.**

676 **34. Jaklitsch W, Baral HO, Lücking R, Lumbsch HT. 2016. *Syllabus of plant***
677 ***families. Part 1/2 Ascomycota.* Borntraeger Science Publishers, Stuttgart.**

678 **35. Gueidan C, Aptroot A, Da Silva ME, Cáceres Badali H, Stenroos S. 2014. A**
679 **reappraisal of orders and families within the subclass Chaetothyriomycetidae**
680 **(Eurotiomycetes, Ascomycota). *Mycological Progress* 13:1027-1039.**

681 **36. James TY, Kauff F, Schoch C, Matheny PB, Hofstetter V, Cox C J, Celio G,**
682 **Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V,**
683 **Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D,**
684 **O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson**
685 **AW, Schüßler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D,**
686 **Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DD,**
687 **Humber RA, Morton JB, Sugiyama J, Rossman A, Rogers JD, Pfister DH,**
688 **Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J,**
689 **Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW,**
690 **Matsura K, Langer E, Lanfer G, Untereiner WA, Lücking R, Büdel B, Geiser**
691 **DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS,**
692 **Lutzoni F, McLaughlin DJ, Spatafora JW and Vilgalys R. 2006.**

693 Reconstructing the early evolution of fungi using a six-gene phylogeny. *Nature*
694 **443**:818-822.

695 **37. Nelsen MP, Lücking R, Mbatchou JS, Andrew CJ, Spielmann AA, Lumbsch**
696 **HT.** 2011. New insights into relationships of lichen-forming Dothideomycetes.
697 *Fungal Diversity* **51**:155-162.

698 **38. Prieto M, Baloch E, Tehler A, Wedin M.** 2013. Mazaedium evolution in the
699 Ascomycota (Fungi) and the classification of mazaediate groups of unclear
700 relationship. *Cladistics* **29**:296-308.

701 **39. Lücking R, Dal-Forno M, Sikaroodi M, Gillevet PM, Bungartz F, Moncada**
702 **B, Yáñez-Ayabaca A, Chaves JL, Coca LF, Lawrey JD.** 2014. A single
703 macrolichen constitutes hundreds of unrecognized species. *Proceedings of the*
704 *National Academy of Sciences U.S.A.* **111**:11091–11096.

705 **40. Dal-Forno M, Lawrey JD, Sikaroodi M, Bhattarai S, Gillevet PM,**
706 **Sulzbacher M, Lücking R.** 2013. Starting from scratch: Evolution of the lichen
707 thallus in the basidiolichen *Dictyonema* (Agaricales: Hygrophoraceae). *Fungal*
708 *Biology* **117**:584-598.

709 **41. Grube M.** 1998. Classification and phylogeny in the Arthoniales (lichenized
710 Ascomycetes). *The Bryologist* **101**: 377-391.

711 **42. Ertz D, Tehler A.** 2011. The phylogeny of Arthoniales (Pezizomycotina)
712 inferred from nuLSU and RPB2 sequences. *Fungal Diversity* **49**:47-71.

713 **43. Frisch A, Thor G, Ertz D, Grube M.** 2014. The Arthonialean challenge:
714 restructuring Arthoniaceae. *Taxon* **63**:727-744.

715 **44. Schoch CL, Crous PW, Groenewald JZ, Boehm EWA, Burgess TI, de**
716 **Gruyter J, de Hoog GS, Dixon LJ, Grube M, Gueidan C, Harada Y,**
717 **Hatakeyama S, Hirayama K, Hosoya T, Huhndorf SM, Hyde KD, Jones**

718 **EBG, Kohlmeyer J, Krus Å, Li YM, Lücking R, Lumbsch HT, Maranová L,**
719 **Mbatchou JS, McVay AH, Miller AN, Mugambi GK, Muggia L, Nelsen MP,**
720 **Nelson P, Owensby CA, Phillips AJL, Phongpaichit S, Pointing SB, Pujade-**
721 **Renaud V, Raja HA, Rivas Plata E, Robbertse B, Ruibal C, Sakayaroj J,**
722 **Sano T, Selbmann L, Shearer CA, Shirouzu T, Slippers B, Suetrong S,**
723 **Tanaka K, Volkmann-Kohlmeyer B, Wingfield MJ, Wood AR, Woudenberg**
724 **JHC, Yonezawa H, Zhang Y, Spatafora JW. 2009b. A class-wide phylogenetic**
725 **assessment of Dothideomycetes. *Studies in Mycology* 64:1-15.**

726 **45. Gueidan C, Villaseñor CR, de Hoog GS, Gorbushina AA, Untereiner WA**
727 **and Lutzoni F. 2008. A rock-inhabiting ancestor for mutualistic and pathogen-**
728 **rich fungal lineages. *Studies in Mycology* 61:111-119.**

729 **46. Lumbsch HT, Schmitt I, Palice Z, Wiklund E, Ekman S, Wedin M. 2004.**
730 **Supraordinal phylogenetic relationships of Lecanoromycetes based on a Bayesian**
731 **analysis of combined nuclear and mitochondrial sequences. *Molecular***
732 ***Phylogenetics and Evolution* 31:822-832.**

733 **47. Wedin M, Wiklund E, Crewe A, Döring H, Ekman S, Nyberg Å, Schmitt I,**
734 **Lumbsch HT. 2005. Phylogenetic relationships of Lecanoromycetes**
735 **(Ascomycota) as revealed by analyses of mtSSU and nLSU rDNA sequence data.**
736 ***Mycological Research* 109:159-172.**

737 **48. Ekman S, Andersen H L, Wedin M. 2008. The limitations of ancestral state**
738 **reconstruction and the evolution of the ascus in the Lecanorales (lichenized**
739 **Ascomycota). *Systematic Biology* 57:141-156.**

740 **49. Miadlikowska J, Kauff F, Hofstetter V, Fraker E, Grube M, Hafellner J,**
741 **Reeb V, Hodkinson BP, Kukwa M, Lücking R, Hestmark G, Otalora MG,**
742 **Rauhut A, Büdel B, Scheidegger C, Timdal E, Stenroos S, Brodo IM,**

743 **Perlmutter GB, Ertz D, Diederich P, Lendemer JC, May PF, Schoch C,**
744 **Arnold AE, Gueidan C, Tripp E, Yahr R, Robertson C, Lutzoni F.** 2006. New
745 insights into classification and evolution of the Lecanoromycetes
746 (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal
747 RNA- and two protein-coding genes. *Mycologia* **98**:1088-1103.

748 **50. Tibell, L.** 1998. Practice and prejudice in lichen classification. *Lichenologist*
749 **30**:439-453.

750 **51. Tibell L.** 1984. A reappraisal of the taxonomy of Caliciales. *Beiheft zur Nova*
751 *Hedwigia* **79**:597-713.

752 **52. Schmull M, Miadlikowska J, Pelzer M, Stocker-Wörgötter E, Hofstetter V,**
753 **Fraker E, Hodkinson BP, Reeb V, Kukwa M, Lumbsch HT, Kauff F, Lutzoni**
754 **F.** 2011. Phylogenetic affiliations of members of the heterogeneous lichen-
755 forming fungi of the genus *Lecidea* sensu Zahlbruckner (Lecanoromycetes,
756 Ascomycota). *Mycologia* **103**:983-1003.

757 **53. Wedin M, Wiklund E, Jørgensen PM, Ekman S.** 2009. Slippery when wet:
758 phylogeny and character evolution in the gelatinous cyanobacterial lichens
759 (Peltigerales, Ascomycetes). *Molecular Phylogenetics and Evolution* **53**:862-871.

760 **54. Otálora MAG, Aragón G, Molina MC, Martínez I, Lutzoni F.** 2010.
761 Disentangling the *Collema-Leptogium* complex through a molecular phylogenetic
762 study of the Collemataceae (Peltigerales, lichen-forming Ascomycota). *Mycologia*
763 **102**:279-290.

764 **55. Crespo A, Mattsson JE, Blanco O, Divakar PK, Lumbsch HT, Articus K,**
765 **Wiklund E, Bawingan PA, Wedin M.** 2007. Testing morphology-based
766 hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using

767 three ribosomal markers and the nuclear RPB1 gene. *Molecular Phylogenetics*
768 *and Evolution* **44**:812-824.

769 **56. Divakar PK, Crespo A, Wedin M, Leavitt SD, Hawksworth DH, Myllys L,**
770 **McCune B, Randle T, Bjerke JW, Ohmura Y, Schmitt I, Boluda CG, Alors**
771 **D, Roca-Valiente B, Del-Prado R, Ruibal C, Buaruang K, Núñez-Zapata J,**
772 **Amo de Paz G, Rico VJ, Molina MC, Elix JA, Esslinger TL, Tronstad IKK,**
773 **Lindgren H, Ertz D, Gueidan C, Saag L, Mark K, Singh G, Dal Grande F,**
774 **Parnmen S, Beck A, Navarro Benatti M, Blanchon D, Candan M, Clerc P,**
775 **Goward T, Grube M, Hodkinson BP, Hur JS, Kantvilas G, Kirika PM,**
776 **Lendemer J, Mattsson JE, Messuti MI, Miadlikowska J, Nelsen M, Ohlson**
777 **JI, Pérez-Ortega S, Saag A, Sipman HJM, Sohrabi M, Thell A, Thor G,**
778 **Truong C, Yahr R, Upreti DK, Cubas P, Lumbsch HT.** 2015. Evolution of
779 complex symbiotic relationships in a morphologically derived family of lichen-
780 forming fungi. *New Phytologist* **208**:1217-1226.

781 **57. Gaya E, Högnabba F, Holguin, Á, Molnar K, Fernández-Brime S, Stenroos**
782 **S, Arup U, Søchting U, van den Boom P, Lücking R, Sipman HJM, Lutzoni**
783 **F.** 2012. Implementing a cumulative supermatrix approach for a comprehensive
784 phylogenetic study of the Teloschistales (Pezizomycotina, Ascomycota). -
785 *Molecular Phylogenetics and Evolution* **63**:374-387.

786 **58. Arup U, Søchting U, Frödén P.** 2013. A new taxonomy of the family
787 Teloschistaceae. *Nordic Journal of Botany* **31**:016-083.

788 **59. Baloch E, Lücking R, Lumbsch HT, Wedin M.** 2010. Major clades and
789 phylogenetic relationships between lichenized and non-lichenized lineages in
790 Ostropales (Ascomycota: Lecanoromycetes). *Taxon* **59**:1483-1494.

- 791 **60. Resl P, Schneider K, Westberg M, Printzen C, Palice Z, Thor G, Fryday A,**
792 **Mayrhofer H, Spribille T.** 2015. Diagnostics for a troubled backbone: testing
793 topological hypotheses of trapelioid lichenized fungi in a large-scale phylogeny of
794 Ostropomycetidae (Lecanoromycetes). *Fungal Diversity* **73**:239-258.
- 795 **61. Miadlikowska J, Kauff F, Högnabba F, Oliver JC, Molnár K, Fraker E,**
796 **Gaya E, Hafellner J, Hofstetter V, Gueidan C, Otálora MAG, Hodkinson B,**
797 **Kukwa M, Lücking R, Björk C, Sipman HJM, Burgaz AR, Thell A, Passo A,**
798 **Myllys L, Goward T, Fernández-Brimem S, Hestmark G, Lendemer J,**
799 **Lumbsch HT, Schnull M, Schoch CL, Sérusiaux E, Maddison DR, Arnold**
800 **AE, Lutzoni F, Stenroos S.** 2014. A multigene phylogenetic synthesis for the
801 class Lecanoromycetes (Ascomycota): 1307 fungi representing 1139 infrageneric
802 taxa, 317 genera and 66 families. *Molecular Phylogenetics and Evolution* **79**:132-
803 168.
- 804 **62. Lumbsch HT, Parnmen S, Kraichak E, Papong KB, Lücking R.** 2014. High
805 frequency of character transformations is phylogenetically structured within the
806 lichenized fungal family Graphidaceae (Ascomycota: Ostropales). *Systematics*
807 *and Biodiversity* **12**:271-291.
- 808 **63. Stenroos S, Laukka T, Huhtinen S, Döbbeler P, Myllys L, Syrjänen K,**
809 **Hyvönen J.** 2010. Multiple origins of symbioses between ascomycetes and
810 bryophytes suggested by a five-gene phylogeny. *Cladistics* **26**:281-300.
- 811 **64. Leavitt SD, Grewe F, Widhelm T, Muggia L, Wray B, Lumbsch HT.** 2016.
812 Resolving evolutionary relationships in lichen-forming fungi using diverse
813 phylogenomic datasets and analytical approaches. *Scientific reports* **6**: 22262.

- 814 **65. Park SY, Jeong MH, Wang HY, Kim JA, Yu NH, Kim S, Cheong YH, Kang**
815 **S, Lee YH, Hur JS.** 2013c. *Agrobacterium tumefaciens*-mediated transformation
816 of the lichen fungus, *Umbilicaria muehlenbergii*. *PLoS One* **8**:e83896.
- 817 **66. Armaleo D, Sun X, Culberson C.** 2011. Insights from the first putative
818 biosynthetic gene cluster for a lichen depside and depsidone. *Mycologia* **103**:741-
819 754.
- 820 **67. Abdel-Hameed M, Bertrand RL, Piercey-Normore MD, Sorensen JL.** 2016.
821 Putative identification of the usnic acid biosynthetic gene cluster by de novo
822 whole-genome sequencing of a lichen-forming fungus. *Fungal Biol* **120**:306-316.
- 823 **68. Kampa A, Gagunashvili AN, Gulder TA, Morinaka BI, Daolio C,**
824 **Godejohann M, Miao VP, Piel J, Andrésson Ó.** 2013. Metagenomic natural
825 product discovery in lichen provides evidence for a family of biosynthetic
826 pathways in diverse symbioses. *Proc Natl Acad SCI USA* **110**:E3129-3137.
- 827 **69. McDonald TR, Dietrich FS, Lutzoni F.** 2012. Multiple horizontal gene
828 transfers of ammonium transporters/ammonia permeases from prokaryotes to
829 eukaryotes: Toward a new functional and evolutionary classification. *Mol Biol*
830 *Evol* **29**:51-60.
- 831 **70. McDonald TR, Mueller O, Dietrich FS, Lutzoni F.** 2013. High-throughput
832 genome sequencing of lichenizing fungi to assess gene loss in the ammonium
833 transporter/ammonia permease gene family. *BMC Genomics* **14**:1.
- 834 **71. Wang YY, Zhang T, Zhou QM, Wei JC.** 2011. Construction and
835 characterization of a full-length cDNA library from mycobiont of *Endocarpon*
836 *pusillum* (lichen-forming Ascomycota). *World J Microbiol Biotechnol* **27**:2873-
837 2884.

- 838 **72. Wang YY, Zhang XY, Zhou QM, Zhang XL, Wei JC.** 2015. Comparative
839 transcriptome analysis of the lichen-forming fungus *Endocarpon pusillum*
840 elucidates its drought adaptation mechanisms. *Sci China Life Sci* **57**:89-100.
- 841 **73. Miao VPW, Manoharan SS, Snæbjarnarson V, Andrésón ÓS.** 2012.
842 Expression of *lec-1*, a mycobiont gene encoding a galectin-like protein in the
843 lichen *Peltigera membranacea*. *Symbiosis* **57**:23-31.
- 844 **74. Juntilla S, Rudd S.** 2012. Characterization of a transcriptome from a non-model
845 organism, *Cladonia rangiferina*, the grey reindeer lichen, using high-throughput
846 next generation sequencing and EST sequence data. *BMC Genomics* **13**:575.
- 847 **75. Schneider T, Schmid E, de Castro JV, Cardinale M, Eberl L, Grube M, Berg**
848 **G, Riedel K.** 2011. Structure and function of the symbiosis partners of the lung
849 lichen (*Lobaria pulmonaria* L. Hoffm.) analyzed by metaproteomics. *Proteomics*
850 **11**:2752-2756.
- 851 **76. Juntilla S, Laiho A, Gyenesei A, Rudd S.** 2013. Whole transcriptome
852 characterization of the effects of dehydration and rehydration on *Cladonia*
853 *rangiferina*, the grey reindeer lichen. *BMC Genomics* **14**:1.
- 854 **77. Manoharan SS, Miao VPW, Andrésón ÓS.** 2012. LEC-2, a highly variable
855 lectin in the lichen *Peltigera membranacea*. *Symbiosis* **58**:91-98.
- 856 **78. Grube M, Cernava T, Soh J, Fuchs S, Aschenbrenner I, Lassek C, Wegner U,**
857 **Becher D, Riedel K, Sensen CW, Berg G.** 2015. Exploring functional contexts of
858 symbiotic sustain within lichen-associated bacteria by comparative omics.
859 *International Society for Microbial Ecology J* **9**:412-424.
- 860 **79. Kosugi M, Arita M, Shizuma R, Moriyama Y, Kashino Y, Koike H, Satoh K**
861 2009. Responses to desiccation stress in lichens are different from those in their
862 photobionts. *Plant Cell Physiol* **50**:879–888.

- 863 **80. Meeßen J, Ott S.** 2013. Recognition mechanisms during the precontact state of
864 lichens: I. Mycobiont-photobiont interactions of the mycobiont of *Fulgensia*
865 *bracteata*. *Symbiosis* **59**:121-130.
- 866 **81. Meeßen J, Epstein S, Ott S.** 2013. Recognition mechanisms during the
867 precontact state of lichens: II. Influence of algal exudates and ribitol on the
868 response of the mycobiont of *Fulgensia bracteata*. *Symbiosis* **59**:131-143.
- 869 **82. Dietz S, Hartung W.** 1999. The effect of abscisic acid on chlorophyll
870 fluorescence in lichens under extreme water regimes. *New Phytol* **143**:495-501.
- 871 **83. Ott S, Krieg T, Spanier U, Schieleit P.** 2000. Phytohormones in lichens with
872 emphasis on ethylene biosynthesis and functional aspects on lichen symbiosis.
873 *Phyton* **40**:83-94.
- 874 **84. Wang XY, Wei XL, Luo H, Kim JA, Jeon HS, Koh YJ, Hur JS.** 2010. Plant
875 hormones promote growth in lichen-forming fungi. *Mycobiology* **38**:176-179.
- 876 **85. Honegger R.** 1991. Functional aspects of the lichen symbiosis. *Annual Review of*
877 *Plant Physiology and Plant Molecular Biology* **42**:553-578.
- 878 **86. Honegger R.** 2012. The symbiotic phenotype of lichen-forming ascomycetes and
879 their endo- and epibionts, p 287-339. In Hock B (ed), *The Mycota 9. Fungal*
880 *Associations*. 2nd ed. Springer, Berlin.
- 881 **87. Rikkinen J.** 2015. Cyanolichens. *Biodiversity and Conservation* **24**:973-993.
- 882 **88. James PW, Henssen A.** 1976. The morphological and taxonomic significance of
883 cephalodia. In DH Brown, DL Hawksworth & RH Bailey (eds) *Lichenology:*
884 *Progress and Problems*. Academic Press, London, pp. 27-77.
- 885 **89. Hyvärinen M, Härdling R, Tuomi J.** 2002. Cyanobacterial lichen symbiosis:
886 the fungal partner as an optimal harvester. *Oikos* **98**:498-504.

- 887 **90. Henskens FL, Green TGA, Wilkins A.** 2012. Cyanolichens can have both
888 cyanobacteria and green algae in a common layer as major contributors to
889 photosynthesis. *Annals of Botany* **110**:555-563.
- 890 **91. Armaleo D, Clerc P, 1991.** Lichen chimeras: DNA analysis suggests that one
891 fungus forms two morphotypes. *Experimental Mycology* **15**:1-10.
- 892 **92. Magain N, Goffinet B, Sérusiaux E.** 2012. Further photomorphs in the lichen
893 family Lobariaceae from Reunion (Mascarene archipelago) with notes on the
894 phylogeny of *Dendriscoaulon* cyanomorphs. *Bryologist* **115**:243-254.
- 895 **93. Thüs H, Muggia L, Pérez-Ortega S, Favero-Longo SE, Joneson S, O'Brien**
896 **H, Nelsen MP, Duque-Thüs R, Grube M, Friedl T, Brodie J, Andrew CJ,**
897 **Lücking R, Lutzoni F, Gueidan C.** 2011. Revisiting photobiont diversity in the
898 lichen family Verrucariaceae (Ascomycota). *European Journal of Phycology*
899 **46**:399-415.
- 900 **94. Blaha J, Baloch E, Grube M.** 2006. High photobiont diversity associated with
901 the euryoecious lichen-forming ascomycete *Lecanora rupicola* (Lecanoraceae,
902 Ascomycota). *Biological Journal of the Linnean Society* **88**:283-293.
- 903 **95. Fernández-Mendoza F, Domaschke S, García MA, Jordan P, Martín MP,**
904 **Printzen C.** 2011. Population structure of mycobionts and photobionts of the
905 widespread lichen *Cetraria aculeata*. *Molecular Ecology* **20**:1208-1232.
- 906 **96. Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith**
907 **F, Kim Y-O, Redman RS.** 2008. Stress tolerance in plants via habitat-adapted
908 symbiosis. *The ISME Journal* **2**:404-416.
- 909 **97. Wedin M, Maier S, Fernandez-Brime S, Cronholm B, Westberg M, Grube**
910 **M.** 2016. Microbiome change by symbiotic invasion in lichens. *Environmental*
911 *Microbiology* **18**:1428-1439.

- 912 **98. Rikkinen J, Oksanen I, Lohtander K.** 2002. Lichen guilds share related
913 cyanobacterial symbionts. *Science* **297**:357-357.
- 914 **99. Rikkinen J.** 2003. Ecological and evolutionary role of photobiont-mediated
915 guilds in lichens. *Symbiosis* **34**:99-110.
- 916 **100. Lawrey JD, Diederich P.** 2003. Lichenicolous fungi: interactions, evolution,
917 and biodiversity. *The Bryologist* **106**:80-120.
- 918 **101. Petrini O, Hake U, Dreyfuss MM.** 1990. An analysis of fungal communities
919 isolated from fruticose lichens. *Mycologia* **82**:444-451.
- 920 **102. Girlanda M, Isocrono D, Bianco C, Luppi-Mosca AM.** 1997. Two foliose
921 lichens as microfungal ecological niches. *Mycologia* **89**:531-536.
- 922 **103. Prillinger H, Kraepelin G, Lopandic K, Schweigkofler W, Molnár O,**
923 **Weigang F, Dreyfuss MM.** 1997. New species of *Fellomyces* isolated from
924 epiphytic lichen species. *Systematic and Applied Microbiology* **20**:572-584.
- 925 **104. Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P, Way A,**
926 **Hofstetter V, Kauff F, Lutzoni F.** 2009. A phylogenetic estimation of trophic
927 transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic
928 fungal diversification? *Systematic Biology* **58**:283-297.
- 929 **105. Crittenden PD, David JC, Hawksworth DL, Campbell FS.** 1995. Attempted
930 isolation and success in the culturing of a broad spectrum of lichen-forming and
931 lichenicolous fungi. *New Phytologist* **130**:267-297
- 932 **106. Fleischhacker A, Grube M, Kopun T, Hafellner J, Muggia L.** 2015.
933 Community analyses uncover high diversity of lichenicolous fungi in alpine
934 habitats. *Microbial Ecology* **70**:348-360.

- 935 **107. Zhang T, Wei XL, Zhang YQ, Liu HY, Yu LY.** 2015. Diversity and
936 distribution of lichen-associated fungi in the Ny-Ålesund Region (Svalbard, High
937 Arctic) as revealed by 454 pyrosequencing. *Scientific Reports* **5**:14850.
- 938 **108. Werth S, Millanes AM, Wedin M, Scheidegger C.** 2013. Lichenicolous fungi
939 show population subdivision by host species but do not share population history
940 with their hosts. *Fungal Biology* **117**:71-84.
- 941 **109. Millanes AM, Diederich P, Wedin M.** 2016. *Cyphobasidium* gen. nov., a new
942 lichen-inhabiting lineage in the Cystobasidiomycetes (Pucciniomycotina,
943 Basidiomycota, Fungi). *Fungal Biology*, doi: 10.1016/j.funbio.2015.12.003.
- 944 **110. Cardinale M, Puglia AM, Grube M.** 2006. Molecular analysis of lichen-
945 associated bacterial communities. *FEMS Microbiology Ecology* **57**:484-495.
- 946 **111. Grube M, Berg G.** 2009. Microbial consortia of bacteria and fungi with focus
947 on the lichen symbiosis. *Fungal Biology Reviews* **23**:72-85.
- 948 **112. Grube M, Cardinale M, de Castro JV, Müller H, Berg G.** 2009. Species-
949 specific structural and functional diversity of bacterial communities in lichen
950 symbioses. *The ISME Journal* **3**:1105-1115.
- 951 **113. Hodkinson BP, Lutzoni F.** 2009. A microbiotic survey of lichen-associated
952 bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* **49**:163-180.
- 953 **114. Selbmann L, Zucconi L, Ruisi S, Grube M, Cardinale M, Onofri S.** 2010.
954 Culturable bacteria associated with Antarctic lichens: affiliation and
955 psychrotolerance. *Polar Biology* **33**:71-83.
- 956 **115. Bates ST, Cropsey GW, Caporaso JG, Knight R, Fierer N.** 2011. Bacterial
957 communities associated with the lichen symbiosis. *Applied and Environmental*
958 *Microbiology* **77**:1309-1314.

- 959 **116. Bjelland T, Grube M, Hoem S, Jorgensen SL, Daae FL, Thorseth IH,**
960 **Øvreås L.** 2011. Microbial metacommunities in the lichen–rock habitat.
961 *Environmental Microbiology Reports* **3**:434-442.
- 962 **117. Mushegian AA, Peterson CN, Baker CC, Pringle A.** 2011. Bacterial diversity
963 across individual lichens. *Applied and Environmental Microbiology* **77**: 4249–
964 4252.
- 965 **118. Hodkinson BP, Gottel NR, Schadt CW, Lutzoni F.** 2012. Photoautotrophic
966 symbiont and geography are major factors affecting highly structured and diverse
967 bacterial communities in the lichen microbiome. *Environmental Microbiology*
968 **14**:147-161.
- 969 **119. Cardinale M, de Castro JV, Müller H, Berg G, Grube M.** 2008. *In situ*
970 analysis of the bacterial community associated with the reindeer lichen *Cladonia*
971 *arbuscula* reveals predominance of Alphaproteobacteria. *FEMS Microbiology*
972 *Ecology* **66**:63-71.
- 973 **120. Erlacher A, Cernava T, Cardinale M, Soh J, Sensen CW, Grube M, Berg G.**
974 2015. Rhizobiales as functional and endosymbiotic members in the lichen
975 symbiosis of *Lobaria pulmonaria* L. *Frontiers in microbiology* **6**.
- 976 **121. Honegger R.** 1992. Lichens: mycobiont-photobiont relationships, p. 225-275.
977 *In* Reisser, W (ed) *Algae and symbiosis*. Biopress, Bristol.
- 978 **122. Juntilla S.** 2015. Characterisation of *Cladonia rangiferina* transcriptome and
979 genome, and the effects of dehydration and rehydration on its gene expression.
980 Ann Univ Turkensis, Doctoral thesis.
- 981 **123. Park SY, Choi J, Kim JA, Jeong MH, Kim S, Lee YH, Hur JS.** 2013a. Draft
982 genome sequence of *Cladonia macilenta* KoLRI003786, a lichen-forming fungus
983 producing biruloquinone. *Genome Announc* **1**: e00695-13.

- 984 **124. Park SY, Choi J, Lee GW, Kim JA, Oh SO, Jeong MH, Yu NH, Kim S, Lee**
985 **YH, Hur JS.** 2014a. Draft genome sequence of lichen-forming fungus *Cladonia*
986 *metacorallifera* strain KoLRI002260. *Genome Announc* **2**: e01065-13.
- 987 **125. Wang YY, Liu B, Zhang XY, Zhou QM, Zhang T, Li H, Yu YF, Zhang XL,**
988 **Hao XY, Wang M, Wang L, Wei JC.** 2014. Genome characteristics reveal the
989 impact of lichenization on lichen-forming fungus *Endocarpon pusillum* Hedwig
990 (Verrucariales, Ascomycota). *BMC Genomics* **15**:34.
- 991 **126. Park SY, Choi J, Kim JA, Yu NH, Kim S, Kondratyuk SY, Lee YH, Hur JS.**
992 2013b. Draft genome sequence of lichen-forming fungus *Caloplaca*
993 *flavorubescens* strain KoLRI002931. *Genome Announc* **1**: e00678-13.
- 994 **127. Park SY, Choi J, Lee GW, Jeong MH, Kim JA, Oh SO, Lee YH, Hur JS.**
995 2014b. Draft genome sequence of *Umbilicaria muehlenbergii* KoLRILF000956, a
996 lichen-forming fungus amenable to genetic manipulation. *Genome Announc*
997 **2**:e00357-14.
- 998

999 **Figure legends**

1000 Fig. 1. Diverse shapes of lichen thalli: A. Coral-like fruticose (shrub-like) thallus of
1001 *Cladia retipora*. Photo: Birgitta Strömbäck. B. Foliose (leaf-like) thalli of *Lobaria*
1002 *pulmonaria* and *Lobarina scrobiculata* covering the trunk of a *Salix caprea* in an old-
1003 growth spruce-dominated forest. Photo: Mats Wedin. C. Crustose (crust-like) thallus
1004 of *Acarospora fuscata* growing on a siliceous rock. Photo: Martin Westberg.

1005

1006 Fig. 2. Substantial biomass of lichens: A. Scandinavian costal lichen heath dominated
1007 by reindeer lichens (*Cladonia* spp.). Photo: Birgitta Strömbäck. B. Lichen-dominated
1008 soil crust community on the Great Alvar of Öland (Sweden). Photo: Martin Westberg.
1009 C. Abundant lichen cover dominated by *Pseudocyphellaria homoeophylla*, in a New
1010 Zealand cool temperate rain forest. Photo: Birgitta Strömbäck.

1011

1012 Fig. 3. Vertical section through a thallus of *Parmelia saxatilis*, a foliose lichen. A
1013 distinct layer of green algal cells is clearly visible under the uppermost cortex; all
1014 other structures are made up by fungal hyphae. Photo: Einar Timdal.

1015

1016 Fig. 4. Vegetative dispersal in lichens: A. Soralia formed along the thallus margins in
1017 *Vulpicida pinastri*. These structures produce soredia, small granules of algal cells
1018 surrounded by fungal hyphae. Photo: Einar Timdal. B. Simple, cylindrical isidia
1019 formed on the thallus upper surface by *Parmelia saxatilis*. Photo: Einar Timdal.

1020

1021 Fig. 5. Optional lichenization: *Schizoxylon albescens*. A. Apothecium of a
1022 saprotrophic colony on dead aspen (*Populus tremula*) wood. Photo: Lucia Muggia. B.

1023 Lichenized morph on aspen bark. Note the green algal colonies around the young
1024 apothecium. Photo: Lucia Muggia.

1025

1026 Fig. 6. A. *Cephalodia* visible as dark structures that include cyanobacteria, on
1027 *Peltigera aptosa*. Photo: Einar Timdal. B. Phycosymbiodemes with different
1028 morphologies: *Sticta* with joined photomorphs. Note green-algal foliose parts growing
1029 out from the basal cyanobacterial fruticose parts. Photo: Mats Wedin.

1030

1031 Fig. 7. Symbiotic invasion: The transition of *Cladonia* thallus into *Diploschistes*
1032 thallus, by invasion of the latter. A. Uninfected *Cladonia symphycarpa* thallus. Photo:
1033 Einar Timdal. B. *Cladonia* thallus with clear *Diploschistes* infection (whitish areas)
1034 with typical *Diploschistes* apothecia (dark patches in the whitish areas). Photo: Einar
1035 Timdal. C. An almost complete take-over by *Diploschistes*, with only small remnants
1036 of *Cladonia* . Photo: Martin Westberg.

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1042 **Table 1.** Lichenized fungal species for which genome information is available (as of

1043 March 2016).

Species	Class	Genome size (Mb)	Gene Number	Reference
<i>Acarospora strigata</i>	Lecanoromycetes	27	?	70
<i>Arthonia rubrocincta</i>	Arthoniomycetes	26	?	70
<i>Cladonia rangiferina</i>	Lecanoromycetes	32	9 211	122
<i>Cladonia grayi</i>	Lecanoromycetes	34	?	Armaleo, http://genome.jgi.doe.gov/Clagr2/Clagr2.home.html
<i>Cladonia macilenta</i>	Lecanoromycetes	37,1	7 322	123
<i>Cladonia metacorallifera</i>	Lecanoromycetes	36,6	11 361	124
<i>Cladonia uncialis</i>	Lecanoromycetes	30	?	67
<i>Dibaeis baeomyces</i>	Lecanoromycetes	35	?	70
<i>Endocarpon pallidulum</i>	Lecanoromycetes	41	?	70
<i>Endocarpon pusillum</i>	Lecanoromycetes	37.5	9 285	125
<i>Graphis scripta</i>	Lecanoromycetes	36	?	70
<i>Gyalolechia flavorubescens</i>	Lecanoromycetes	34,4	9 695	126
<i>Leptogium austroramericanum</i>	Lecanoromycetes	46	?	70
<i>Peltula cylindrica</i>	Lecanoromycetes	32	?	70
<i>Physcia stellaris</i>	Lecanoromycetes	59	?	70
<i>Umbilicaria muehlenbergii</i>	Lecanoromycetes	34.8	8 294	127
<i>Xanthoria parietina</i>	Lecanoromycetes	32	10 800	Kuo et al.,

				http://genome.jgi.doe.gov/Xanpa1/Xanpa1.home.html
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