The crustose lichen *Lecanora dispersa*. Lichen symbioses, associations between fungi and algae, have originated multiple times during fungal evolution. At least one successful establishment of symbiosis led to the more than 6000 species of the order Lecanorales, represented here by *L. dispersa*. The white-rimmed cups (between 0.3 and 0.7 millimeter in diameter) emerging from the rock substrate produce the meiotic spores of this fungal symbiont. See page 1492 and the News story on page 1437. [Photo: V. Wirth]

Multiple Origins of Lichen Symbioses in Fungi Suggested by SSU rDNA Phylogeny

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Phylogenetic hypotheses provide a context for examining the evolution of heterotrophic lifestyles. The lichen lifestyle, which is the symbiotic association of fungi with algae, is found in various representatives of Dicaryomycotina, both Ascomycetes and Basidiomycetes. A highly resolved parsimony analysis of small subunit ribosomal DNA (SSU rDNA) sequences suggests at least five independent origins of the lichen habit in disparate groups of Ascomycetes and Basidiomycetes. Because lichen associations arose from parasitic, mycorrhizal, or free-living saprobic fungi, neither mutualism nor parasitism should be construed as endpoints in symbiont evolution.

Lichens are a classic example of symbiosis ["Zusammenleben ungleichnamiger Organismen" (*1*)], with interactions ranging from mutualistic to parasitic (*2*). Hyphae of the fungal symbiont may lie within a matrix of algal cells, adhere to these cells as appressoria, invaginate these cells as haustoria, or occasionally penetrate cell walls and plasmalemmae (*3*). Long-term survival of the lichen association depends on balanced growth of the symbionts, yet this balance does not preclude killing or saprobic digestion of the algal symbiont (*4*). Lichen-forming fungi represent many diverse lineages of Dicaryomycotina (*5*) that traditionally have been studied under the rubric of lichenology. These lineages are not descended from a single lichen-forming ancestor, yet it is not known how many times, and in which groups, the lichen habit originated. By examining the phylogenetic position of lichen-forming fungi relative to saprobic or pathogenic fungi, we can address a fundamental question of symbiont evolution: whether mutualistic symbioses are derived from more parasitic forms (*6*).

To determine the origins of the lichen habit, we included lichen-forming fungi within a phylogenetic analysis of Amastigomycota, members of Eumycota that lack motile stages. The major lineages of Amastigomycota—Basidiomycetes, Ascomycetes, and the paraphyletic zygomycetous fungi (*7*)—have few comparable morphological characters to serve as the basis for phylogenetic hypotheses. For example, the

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group Dicaryomycotina is distinguished by the single morphological feature of dikaryotic hyphae (5). Even the sexual structures used to define these groups do not allow for comparisons. Additionally, many fungi lack sexual structures and cannot be unambiguously classified.

Unlike morphological characters, molecular characters allow direct comparisons among extant Eumycota. Specifically, sequences of small subunit ribosomal DNA (SSU rDNA) have been used to propose phylogenetic hypotheses for fungi (8). The inclusion of SSU rDNA sequences from four lichen-forming representatives has produced a highly resolved phylogeny for Ascomycetes and has demonstrated that lichen-forming fungi are a key to understanding ascomycete relations (9). Our study used SSU rDNA sequences from 10 lichen-forming fungi and 65 other fungi in a cladistic analysis, which produced two equally parsimonious cladograms that differed only in sister taxa relations within one clade of three fungi (10) (Fig. 1). These phylogenetic hypotheses support Dicaryomycotina, Basidiomycetes, and Ascomycetes as monophyletic and resolve their relations (11). In the topologies, we identified the phylogenetic positions of lichen-forming fungi and nodes where discordance in lifestyles among sister taxa indicates an independent gain or loss of the lichen habit. In each case, it is most parsimonious to interpret the lichen habit as gained (12).

Within Basidiomycetes, our phylogenetic hypothesis supports three independent origins of the lichen habit, each corresponding to groups supported by morphological characters (13). The basal origin of the basidiolichen habit is within the coral fungi; a few species of *Multiclavula* (Fig. 2A) form loose lichen associations with the green alga *Coccomyxa* or occasionally with cyanobacteria. An independent lichen association with *Coccomyxa* is represented by *Omphalina umbellifera* (Fig. 2B), which produces typical gilled mushrooms and is allied with the button mushroom *Agaricus bisporus* and the oyster mushroom *Pleurotus ostreatus*. Another basidiolichen origin is indicated by *Dictyonema pavonia* (Fig. 2C), whose unique haustoria penetrate the cyanobacterium *Scytonema*. This tropical inhabitant is most closely related to the wood-rotting *Schizophyllum commune*. Two of the three basidiolichens, *Multiclavula mucida* and *O. umbellifera*, produce fruiting structures that are algae-free and closely resemble those of their non–lichen-forming relatives (13). The loose associations these basidiolichens form with algal colonies require few changes in overall morphology. These basidiolichen associations may have arisen multiple times, perhaps quite recently, within each closely related group.

Within Ascomycetes the lichen habit arose at least twice, according to our phylogenetic hypothesis; other independent origins may be detected with the inclusion of other taxa (14). The most basal origin is represented by *Lecanora dispersa* (Fig. 2D), *Porpidia crustulata*, and *Sphaerophorus globosus* (Fig. 2E), members of Lecanorales (9) that commonly form intimate haustorial connections to coccoid green algae such as *Trebouxia*. The nearly 6000 species of Lecanorales include familiar crustose, foliose, and fruticose lichens with cup-shaped fruiting structures. Other lichen-forming groups such as Caliciales, as represented here by the saprobe *Mycocalicium albonigrum*, are sister taxa to Lecanorales. The calicialean lineage may have given rise to nonlichenized cleistothecial fungi, such as the laboratory model *Aspergillus*. A second origin of the ascolichen habit is in the lineage containing *Arthonia radiata* (Fig. 2F) and allied species of the order Arthoniales (15), whose hyphae may even penetrate

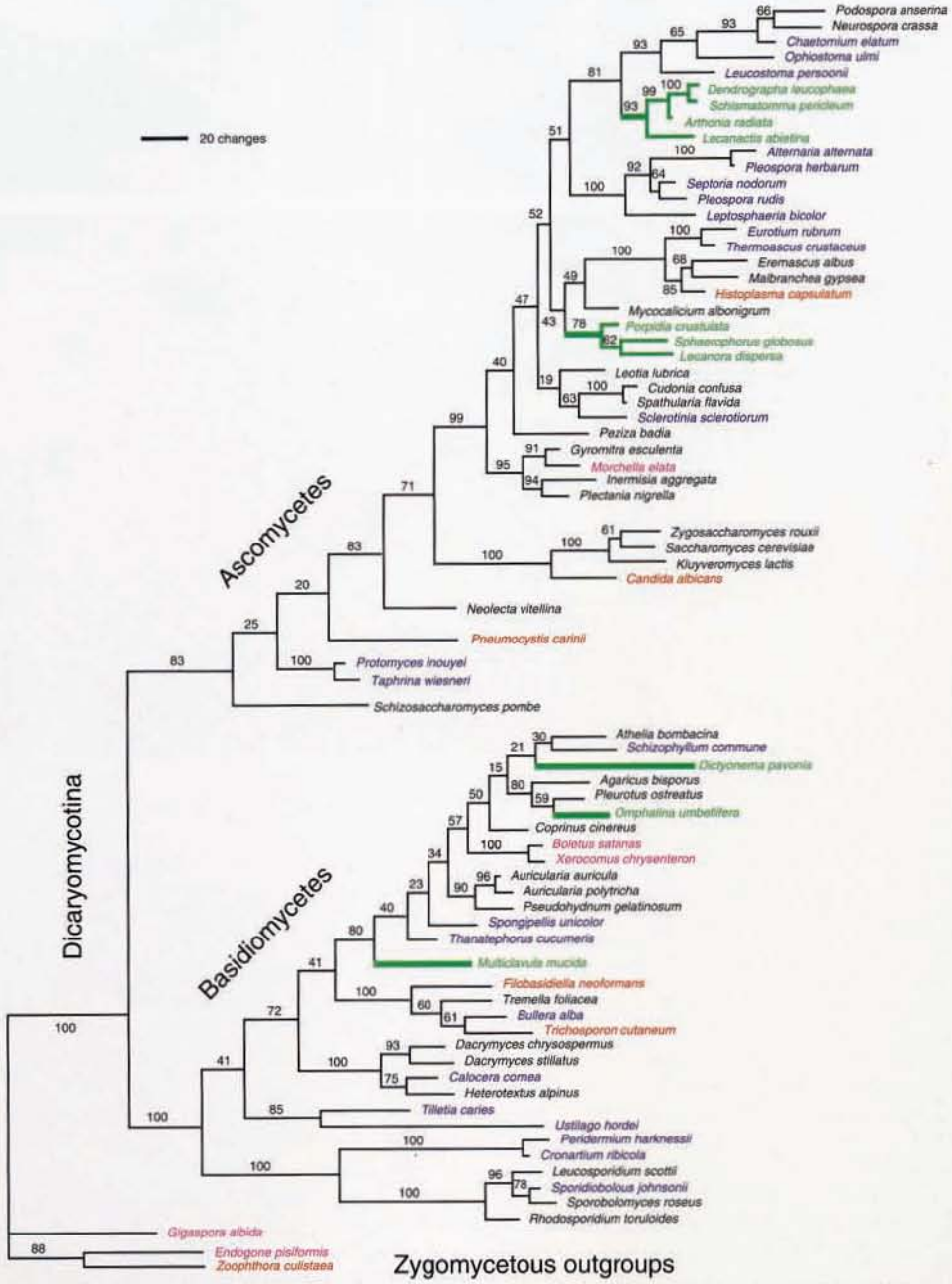

Fig. 1. Phylogenetic relations within Amastigomycota, as derived from parsimony analysis of 1927 nucleotides of SSU rDNA sequences from 75 representative fungi. One of two equally parsimonious cladograms of 3491 steps is shown; the cladograms differ only in sister taxa relations within the clade that includes *Agaricus bisporus*, *Omphalina umbellifera*, and *Pleurotus ostreatus*. Bootstrap percentages (21) from 200 replications are shown on each supported branch. The major fungal groups supported in this analysis are labeled adjacent to their nodes. Lichen-forming fungi are shown in green, mycorrhizal fungi in pink, plant pathogenic fungi in blue, animal pathogenic fungi in orange, and saprobic fungi in black; the green branches represent independent origins of the lichen habit.

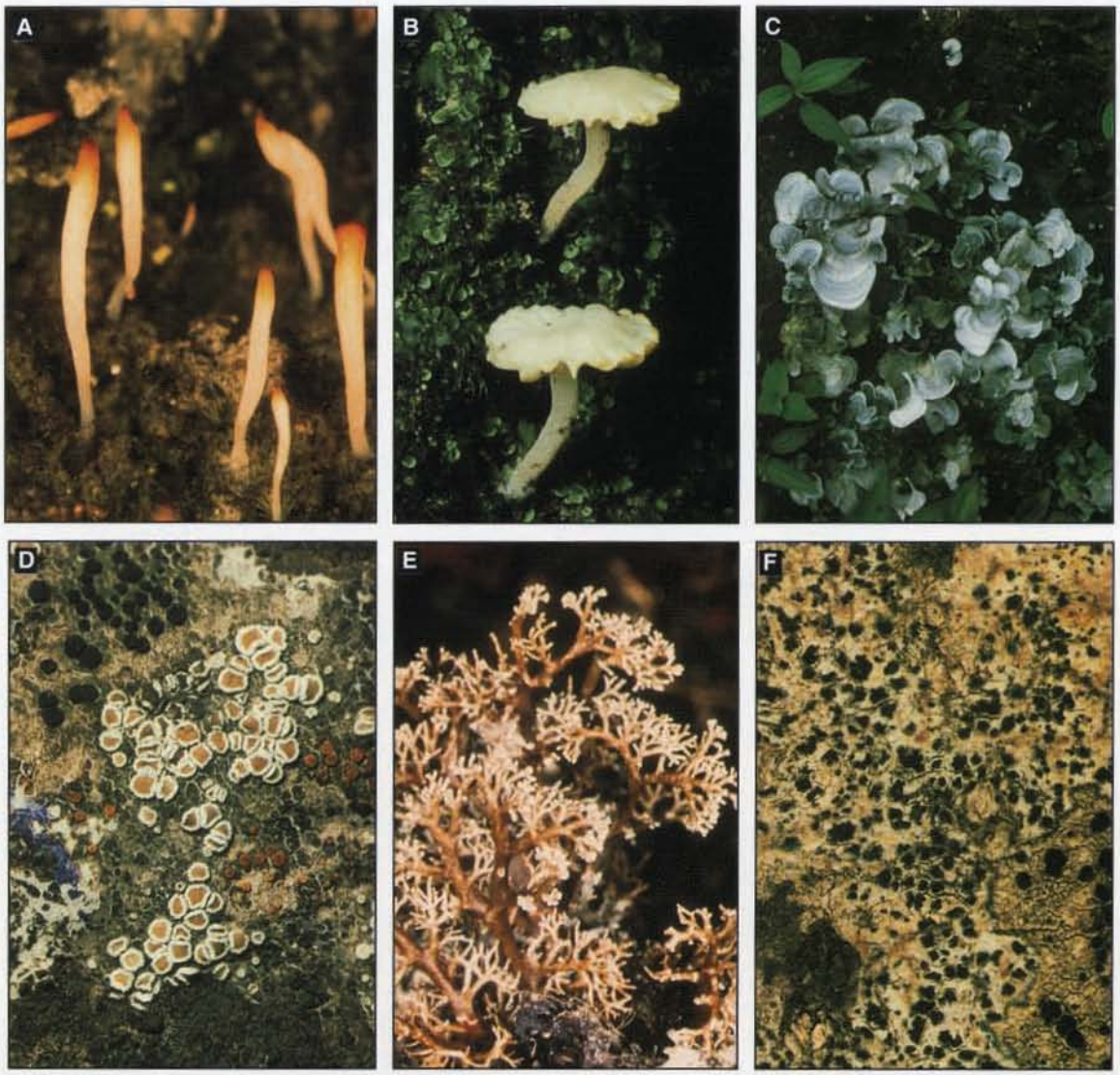

Fig. 2. Representatives of the five independent origins of lichen symbioses. (**A**) The coral fungus *Multiclavula vernalis* (Schw.) R. H. Petersen, a close relative of *M. mucida*, on decaying wood. (**B**) The mushroom *Omphalina hudsoniana* (Jenn.) Bigelow, a close relative of *O. umbellifera*, on organic materials. (**C**) The foliose *Dictyonema pavonia* (Sw.) Parmasto on tropical soil. (**D**) The crustose *Lecanora dispersa* (Pers.) Sommerf. on rock. (**E**) The fruticose *Sphaerophorus globosus* (Hudson) Vainio on organic materials. (**F**) The crustose *Arthonia radiata* (Pers.) Ach. on bark. (A) through (C) are Basidiomycetes, (D) through (F) are Ascomycetes, and (D) and (E) are members of the order Lecanorales. Photographs (*22*) are by C. Scheidegger (A), V. Wirth (B, C, D, and F), and K. Rasbach (E).

their algal partner, typically the filamentous green alga *Trentepohlia*. This order of nearly 2000 species is closely related to perithecial fungi, including the model fungi *Neurospora crassa* and *Podospora anserina*. Separate origins of the lichen habit in Lecanorales and Arthoniales explain the notable differences in their morphology, haustoria, and algal symbionts. In contrast to the basidiolichens, ascolichens are intricate associations in which the lichen morphology differs from that produced by either of the symbionts cultured axenically. In Ascomycetes, lichen formation is a dominant lifestyle, with nearly half of the species associated with algae, and lichen origins are relatively ancient and evolutionarily successful.

Mycologists would never propose that all fungi pathogenic to plants, or all fungi that form mycorrhizae, are separate cohesive units. However, fungi that form lichens have been studied and classified in isolation from other fungi, even very recently (*16*). Here, we show that lichen symbioses arose at least five times in phylogenetically distant groups; the concept of lichen is thus ecologically meaningful (*17*) but not phylogenetically meaningful. Within this phylogenetic context, the most parasitic lichen-forming fungi, Arthoniales (*18*), are closely related to groups that include virulent plant pathogens (*Leucostoma* and *Alternaria*), which suggests that lichen symbionts arose from parasitic fungi. Lichen formation is not a primitive lifestyle; other lichen symbionts arose from predominantly mycorrhizal fungi (basidiolichens) or from saprobic fungi (Lecanorales). Indeed, lecanoralean lichen symbionts may have given rise to fungi parasitic on lichens (*19*). Fungi are opportunists: Saprobes become symbionts; symbionts switch between mutualism and parasitism. On the basis of this phylogenetic analysis of fungi in symbiotic lichen associations, we find no support for the tenet that there is a general evolutionary progression from parasitism to mutualism.

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11. This phylogenetic hypothesis supports the monophyly of most groups recognized in traditional classifications, and the addition of taxa may resolve finer relations. For example, in Basidiomycetes, Auriculariales (represented by *Auricularia auricula, A. polytricha*, and *Pseudohydnum gelatinosum*) is recognized as monophyletic, yet its placement within the Homobasidiomycetes clade (defined by *Multiclavula mucida, Thanatephorus cucumeris, Spongipellis unicolor*, and *Athelia bombacina*) is problematic. In Ascomycetes, only the order Pezizales (represented by *Peziza badia, Gyromitra esculenta, Morchella elata, Inermisia aggregata*, and *Plectania nigrella*), here recognized as paraphyletic, differs from the first author's previous molecular phylogenies (*9*).
12. In Ascomycetes, the hypothesis of two independent gains of the lichen symbiosis is more parsimonious than that of a single origin with three subsequent losses. In Basidiomycetes, the hypothesis of three independent gains of the lichen symbiosis is more parsimonious, because a single origin would require one gain and eight losses. Although gain and loss can be variably weighted so that the loss of the lichen symbiosis is easier than its gain, this strategy ignores the necessary replacement of the lost lichen symbiosis with a gained saprobic, symbiotic, or parasitic habit.
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