# The adaptive radiation of lichen-forming Teloschistaceae is associated with sunscreening pigments and a bark-to-rock substrate shift

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**Adaptive radiations play key roles in the generation of biodiversity and biological novelty, and therefore understanding the factors that drive them remains one of the most important challenges of evolutionary biology. Although both intrinsic innovations and extrinsic ecological opportunities contribute to diversification bursts, few studies have looked at the synergistic effect of such factors. Here we investigate the Teloschistales (Ascomycota), a group of >1,000 lichenized species with variation in species richness and phenotypic traits that hinted at a potential adaptive radiation. We found evidence for a dramatic increase in diversification rate for one of four families within this order—Teloschistaceae—which occurred ∼100 Mya (Late Cretaceous) and was associated with a switch from bark to rock and from shady to sun-exposed habitats. This adaptation to sunny habitats is likely to have been enabled by a contemporaneous key novel phenotypic innovation: the production in both vegetative structure (thallus) and fruiting body (apothecia) of anthraquinones, secondary metabolites known to protect against UV light. We found that the two ecological factors (sun exposure and rock substrate) and the phenotypic innovation (anthraquinones in the thallus) were all significant when testing for state-dependent shifts in diversification rates, and together they seem likely to be responsible for the success of the Teloschistaceae, one of the largest lichen-forming fungal lineages. Our results support the idea that adaptive radiations are driven not by a single factor or key innovation, but require a serendipitous combination of both intrinsic biotic and extrinsic abiotic and ecological factors.**

adaptive radiation | lichens | sunlight protection | substrate switch | Teloschistaceae

The rate at which species proliferate through evolutionary time varies greatly from one evolutionary lineage to another, forming one of the most notable characteristics of macroevolutionary change. Adaptive radiations, in which a period of increased speciation is caused by adaptation to novel environments, are particularly important in generating biological diversity (1–3). Understanding the drivers behind these events is thus an important goal. Two basic categories of drivers have been described: extrinsic ecological opportunities that are presented to the ancestors of radiations (e.g., refs. 4 and 5) and intrinsic evolutionary adaptations within the ancestral lineage that result in breakthroughs that spur diversification (6–10). At an extreme, the latter may involve only one trait—a "key innovation" (11–13). Although both processes are likely to be involved in any adaptive radiation, there have been few attempts to investigate how they may be entwined (14, 15): innovations may immediately generate an ecological opportunity by providing access to a new ecological niche; conversely, the exploitation of a new opportunity might generate strong directional selection, leading to rapid evolutionary change at the origin of the adaptation, the results of which permeate throughout the entire lineage.

A potential example of an evolutionary radiation can be found in a group of lichens [obligate mutualistic ectosymbioses between fungi and either or both green algae and cyanobacteria (photobionts) (16)]: the family Teloschistaceae. Although cosmopolitan, this taxon is especially associated with exposed habitats, being able to colonize some of the most arid regions of the world. Because many lichens can use fog and dew as their principal source of water and can be highly tolerant to desiccation, they might be regarded as well adapted to arid exposed habitats. However, many groups of lichens are limited in their ability to exploit such habitats, due, in part, to the high levels of UV-light exposure. Protection against excessive irradiation is required (17), especially for the photobiont partner (18, 19).

In the Teloschistaceae, the solution to this problem lies in their characteristic orange and yellow pigmentation, which results from the accumulation of cortical anthraquinones. These secondary metabolites are produced by the fungal partner, and their crystals alter the spectral composition of the light that goes through the cortex (20) by absorbing visible blue light, UV-B and -A radiations (21, 22). Synthesis of anthraquinones and other lichen pigments is stimulated by UV-B radiation (e.g., refs. 23–26), and they are thought to reduce UV-B–induced damage to

## Significance

**The tempo of diversification of life can be accelerated by fortuitous ecological opportunity or by phenotypic innovation. In this study, we document how both factors are likely to have played a role in the origin and success of a major fungal lineage, the Teloschistaceae (comprising ∼1% of all fungi). Anthraquinone pigments are found in a widespread, but scattered, range of fungi and plants, but are particularly abundant in the lichen-forming Teloschistaceae, where they provide sunlight protection, especially needed when growing in arid deserts of the world. We found that anthraquinones evolved in these lichens, in conjunction with an ecological switch to exposed, rocky environments, allowing them to colonize swathes of unexploited habitats worldwide and sparking an acceleration in diversification.**

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. KT291442–KT291669). Sequence alignment and tree file data are available from TreeBASE, treebase.org (Study id no. 18015).

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DNA in both the fungus and the alga of lichens (22). Anthraquinones are polyketide aromatic compounds, derived from the acetyl polymalonyl pathway and synthesized by multidomain polyketide synthases (PKSs), which are encoded by PKS gene clusters (19). Only one gene involved in anthraquinone synthesis in lichens has been characterized so far: the transcript of a nonreducing fungal type I PKS gene from the cultured mycobiont of *Xanthoria elegans* (27). There are various closely related anthraquinones in the Teloschistales, and each species may possess several in different proportions. The pattern of variation among taxa is still poorly understood, appears to have little phylogenetic signal, and may depend on environmental as well as genetic factors. Because all appear to serve the same function, here we simply consider the presence or absence of anthraquinones. The ubiquity of anthraquinones within the Teloschistaceae is, however, unique and hints at some evolutionary success favoring the production of these metabolites.

The Teloschistaceae, with ~650 described species (28) and an estimate of >1,000 extant species (29), shares common ancestry with three much less speciose families within the order Teloschistales: Brigantiaeaceae, Letrouitiaceae, and Megalosporaceae [<100 described species combined (30)]. Whereas the Teloschistaceae is a mainly rock-dwelling cosmopolitan clade with a diversity of growth habits ranging from crustose to fruticose, the Brigantiaeaceae and Letrouitiaceae (with a circumpacific center of diversity), as well as the Megalosporaceae (with an Australasian center of diversity), are generally epiphytic and crustose and are found mostly in the tropics (30).

In this study, we test the hypothesis that the high species richness of the Teloschistaceae is the result of an adaptive radiation, by

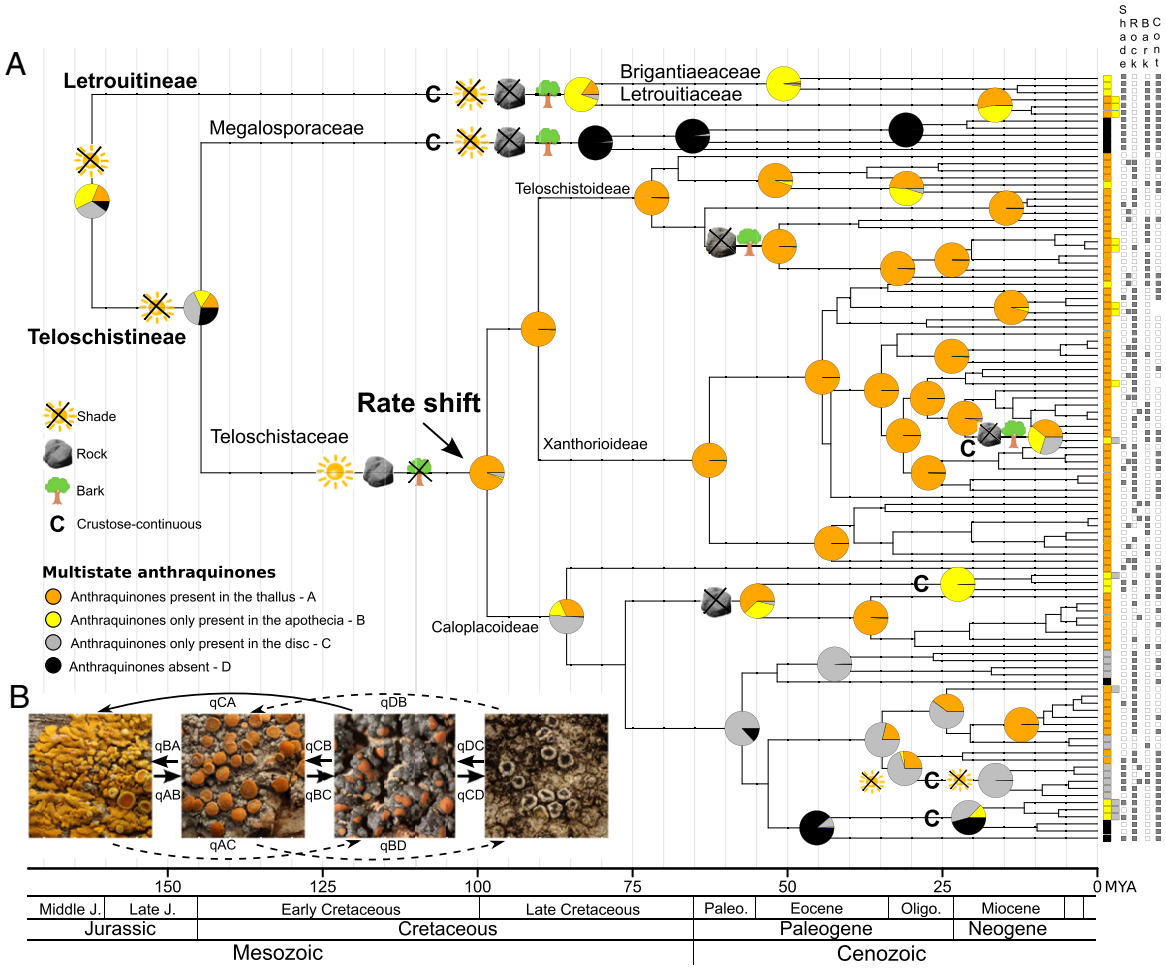


**Fig. 1.** Time-calibrated phylogeny of the order Teloschistales depicting multistate ancestral character state reconstructions of anthraquinone evolution. (*A*) Pies represent probabilities of each ancestor being in each of the four potential states for the presence/absence of anthraquinones. Colored squares at the tips of the tree indicate anthraquinone character states for extant taxa using the same color scheme as for the pie charts. Polymorphic taxa have more than one colored square. In addition, we mapped the presence/absence of other characters with symbols for ancestral states (on branches) and with black and white boxes for extant taxa (at the tips of the tree): a sun for light exposure (shade column), a rock for rock substrate (rock column), a tree for epiphytism (bark column), and a letter C for growth form crustose-continuous (cont column). These same symbols when crossed represent absence of the trait. We only show these symbols on the topology itself if we established high confidence in the state of the trait at a node and if we inferred a gain or loss of the trait at that particular node. These characters were coded and analyzed as binary character states (*SI Appendix*, Fig. S4). Geological times are indicated at the axis of the tree. This reconstruction illustrates a full anthraquinone ancestor living in sunny and rocky habitats as the most probable state at the crown node of the large family Teloschistaceae. These features are maintained throughout much of the history of the family. The same internode was significantly characterized by a major increase in diversification rates (Fig. 2). (*B*) Evolutionary model selected for the anthraquinone multistate reconstruction. Photographs illustrate the four different states of anthraquinone presence/absence in the lichen. Arrows indicate transitions between the possible character states. In this model, transitions and reversals are treated as equal, but single-step transitions and qCA (solid arrows) have higher rates than the remaining two-step transitions (dashed arrows), and three-step transitions are not allowed.

reconstructing the phylogeny of the order Teloschistales, dating the origin and diversification of this family, performing ancestral character state reconstructions of key phenotypic and ecological traits, and estimating diversification rates and how they relate to traits, in order to detect what might be responsible for the evolutionary success of the Teloschistaceae.

## Results and Discussion

**Divergence Times of Teloschistalean Lichens.** Our results (Fig. 1 and *SI Appendix*, Fig. S1) suggest that the first split between the tropical suborder Letrouitineae and the rest of the Teloschistales dates back to 162 Mya (193.19–132.84 Mya), in the Middle Jurassic when the climate was warm and arid (31). This divergence time overlaps with the split of another major lichen taxon, the Lecanorales (Lecanoromycetidae; 160.65 Mya) (32, 33). The next divergence at ∼145 Mya (167.72–122.56 Mya), in the Early Cretaceous, gave rise to the families Megalosporaceae and Teloschistaceae, concurrent with a cooling trend, with seasonal snow and small ice caps in the poles (34, 35). The Teloschistaceae started diversifying ∼98 Mya (113.47–82.73 Mya). The divergence of extant Teloschistaceae matches that of other lichen families, such as the Parmeliaceae, ∼109 Mya [136.55–85.52 Mya (32)], whose genera diversified mainly during the cooling periods of the Cenozoic.

**Increase in Diversification Rate Within the Teloschistales.** We found evidence for heterogeneous diversification dynamics within the Teloschistales, with the most likely scenario being one shift in diversification rate (Bayes factor: 1 shift vs. 0 shifts = 3.88). This shift seems to have taken place at the root of the Teloschistaceae (Bayes factor = 156.6 for evidence of a shift in diversification rate along this edge; Fig. 2 *A*, *C*, and *D*) and involved a jump in diversification rates along the stem lineage of this family. This higher level of diversification then continued to increase more gradually until the present day (*SI Appendix*, Figs. S2 and S3). The difference between the history of the Teloschistaceae and the other families is emphasized by macroevolutionary cohort analysis (Fig. 2*B*), which summarizes the extent to which any two species share a common macroevolutionary rate dynamic (36). The three tropical families and the family Teloschistaceae behave in general as two separate macroevolutionary cohorts with high probability (a clade within the Xanthorioideae in the Teloschistaceae providing a mild exception to this rule; depicted by red branches in Fig. 2 *A* and *B*).

These results are consistent with one signature of an adaptive radiation: a rapid jump in diversification rates. Conversely, a second hallmark of adaptive radiations is absent: a subsequent decrease in diversification rates, which might be expected to occur as a result of the newly available ecological niches being filled. One reason for this absence is that we simply lack the statistical power to detect this drop in diversification in this ancient event. An alternative hypothesis, however, is that the ecological opportunity that was exploited by the Teloschistaceae resulted in a shift to an environment that inherently provoked sustained diversification—one that was less stable, more transient, harsher, or patchier, for example.

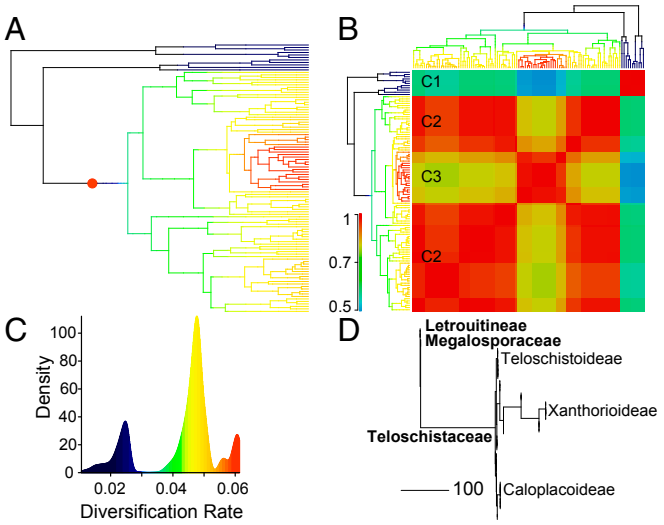


**Fig. 2.** Dynamics of species diversification rates in Teloschistales estimated with BAMM using the maximum clade credibility tree (MCCT) (59). (*A*) Phylorate plot for net diversification rates; colors at each point in time along branches indicate the mean evolutionary rate across all shift configurations sampled during simulation of the posterior. Core shift identified with Bayes Factors is represented by a single rate shift in the ancestor of the Teloschistaceae (red dot). (*B*) Macroevolutionary cohort matrix for speciation. Each cell in the matrix is coded by a color denoting the pairwise probability that two species share a common macroevolutionary rate regime. The MCC phylogeny is shown for reference on the left and upper margins of each cohort matrix with branches colored according to the phylorate plot shown in *A*; blue and red colors denote decoupled macroevolutionary dynamics, and the legend on the left gives the cohort probability of shared dynamics. Three major cohorts can be identified: one for the tropical families (C1), one for most of the Teloschistaceae (C2), and a third one for a small clade within the subfamily Xanthorioideae in the Teloschistaceae (red branches; C3). (*C*) Histogram showing posterior density of diversification rates. (*D*) Graphical representation of the Bayes factors for rate shifts along any branch of the Teloschistales MCCT for diversification. There is high evidence that a shift in the rate of diversification occurred along the branch leading to the Teloschistaceae (BF = 156.6).

**Macroevolution of Phenotypic Traits.** To examine which phenotypic changes might have shaped the evolution of the Teloschistaceae, and might have led to its evolutionary radiation, we first carried out extensive ancestral character state estimation for a set of binary coded traits (anthraquinones, light exposure, substrate, and growth form; *SI Appendix*, Fig. S4), using in addition to likelihood reconstruction, three different methods that account for several biases [BayesTraits-Bayes (37), Binary State Speciation and Extinction (BiSSE)-ML (38), and *corHMM*-ML (39, 40)]. In general, reconstructions were concordant among methods; we dealt with discrepancies by selecting the methods best indicated by statistical evidence for the different biases (*Materials and Methods*).

Our reconstructions infer that the presence of anthraquinones throughout the thallus originated during the initial split of the Teloschistaceae (BayesTraits, *corHMM*) or slightly later (BiSSE) and spread throughout the family. Within the Caloplacoideae (Fig. 1), a loss and secondary gain at the tips (BayesTraits, *corHMM*) or just a recent acquisition (BiSSE) may have occurred (*SI Appendix*, Fig. S4).

The incidence of anthraquinones in the Teloschistales, however, is more complex than just presence or absence of orange pigments in the thallus: although anthraquinones are sometimes found throughout the surface of the organism (which we coded as A in Fig. 1), in other instances, pigments are restricted to the apothecia (B), and sometimes they are present only in the disk of these apothecia (C). In several species, pigments are entirely absent (D). We therefore also performed a multistate analysis, and detected evidence for progressive loss and gain of anthraquinones in our analyses (Fig. 1*B*). The best-fit model using BayesTraits had transitions and reversals possible at the same rate, but higher rates for one-step changes (solid arrows in Fig. 1*B*), than for two-step changes (except for one two-step reversal, qCA), and with three-step changes not possible at all (qAD = qDA = 0). Under this model, presence of anthraquinones in thallus and apothecia was reconstructed as the ancestral state in the most recent common ancestor of the Teloschistaceae, and is the most common state within the family (Fig. 1*A*). The pigment was progressively lost in a few cases, notably within the subfamily Caloplacoideae with some clades retaining the pigment in the apothecium, and subsequently reducing it to the disk, the most

valuable portion of the apothecium (which contains the ascospores), until anthraquinones disappeared completely. Only one clade in the Caloplacoideae (*Caloplaca aurantia* group; *SI Appendix*, Fig. S4*A*) seems to have experienced a regain of anthraquinones in the thallus from a state of only disk presence.

Although our analyses show that the spread of anthraquinones throughout the thallus originated at the split of the family Teloschistaceae, the original acquisition of anthraquinones per se most likely took place at the root of the Teloschistales, as shown in *SI Appendix*, Fig. S4*B*, and was secondarily lost in the Megalosporaceae. This conclusion is reinforced by the fact that these pigments are mostly absent from the closest relatives to the Teloschistales (i.e., Caliciales, Lecanorales). The uncertainty in the multistate analysis (Fig. 1) might be an artifact of how we coded a continuously varying trait: whereas most Letrouitineae possess anthraquinones only in their apothecia, a few species of *Letrouitia* also have anthraquinones in their thalli, but compared with the Teloschistaceae, these pigments are far less abundant and often disappear completely in shady habitats.

Exposure to shade was estimated as the ancestral state at the root of the Teloschistales and has been maintained throughout the tropical Letrouitineae and also in the Megalosporaceae. This trait was lost—corresponding to a shift from shaded to sunlit habitats—along the stem lineage of the Teloschistaceae (Fig. 1 and *SI Appendix*, Fig. S4). Subsequently, there were several secondary gains, mostly within the Caloplacoideae, that were not recovered by all reconstruction methods.

Following the same pattern, the colonization of rocky substrates evolved during the early history of the Teloschistaceae and spread throughout the family, although it was secondarily lost on a few occasions (i.e., *Fulgensia* and *Teloschistes* clades; Fig. 1 and *SI Appendix*, Fig. S4*D*). The reconstruction of bark (epiphytism) as a substrate preference complements that of rock colonization. Although there is considerable uncertainty at the root, the predominance of bark as a substrate for tropical Letrouitineae and Megalosporaceae and its loss associated with the split of the Teloschistaceae is unequivocal. Most clades that showed secondary losses of rock as a substrate were replaced by secondary gains of bark, with the exception of the *Fulgensia* clade, where species mostly grow on soil and mosses (Fig. 1 and *SI Appendix*, Fig. S4*E*).

The evolution of growth forms (e.g., crustose, foliose, and fruticose thalli) showed the most discrepancies among methods and least well-supported ancestral estimation. We found that the trait crustose-continuous thallus seems to have been present at the origin of the tropical families (Fig. 1): although BayesTraits and *corHMM* approaches support the hypothesis that the trait was ancestral to the Teloschistales as a whole, lost in certain clades within the Teloschistaceae, and secondarily gained in a few instances, our BiSSE analysis supports several independent gains within the family (*SI Appendix*, Fig. S4). The reconstruction of attachment structures, which is associated with the evolution of the growth forms foliose and fruticose (i.e., a greater level of separation of the thallus from its substrate, compared with crustose lichens), was also quite uncertain. It seems plausible that the trait evolved repeatedly in several clades within the Teloschistaceae.

In summary, the origin of the Teloschistaceae corresponds with interrelated transitions in the phenotypic evolution of anthraquinones spreading through the thallus and the ecological evolution from shaded epiphytic habitats to exposed rocky substrates.

**Testing for State-Dependent Diversification Rates.** The origin of the Teloschistaceae was associated with both an increase in the rate of diversification and the evolution of key phenotypic traits. We therefore investigated more directly whether there was a link between these phenotypic traits and the shift in diversification rates, using BiSSE and accounting for the incomplete sampling of taxa in our dataset (41).

We found evidence that presence of anthraquinones in the thallus increased the rate of diversification, whereas living in shady habitats or possessing a crustose-continuous growth form both decreased the rate of diversification. In the first case, the effect was significant only when we assumed that 70% or fewer of species had been sampled ($\Delta$AIC = 2.2; $P$ = 0.04 for 70% sampling), which is the most reasonable assumption: current estimates are of at least 1,000 species (29). In the case of shade and crustose-continuous thalli, the effect was significant even when we assumed all species were sampled ($\Delta$AIC = 7, $P$ = 0.003 for shade; $\Delta$AIC = 12, $P$ = 0.0003 in crustose-continuous for 100% sampling). In contrast, we found no evidence that substrate (rock or bark) or attachment structures influenced the rate of diversification (*SI Appendix*, Table S1).

Evolutionary shifts in these phenotypic traits may also be directly related to cladogenesis [sensu (42)], where character changes are associated with speciation events. We tested for cladogenetic effects using BiSSEness (42). Here we found evidence for significant cladogenetic effects with shade and with the rock and bark substrates (*SI Appendix*, Table S2), but not with anthraquinones nor with crustose-continuous growth forms or with attachment structures.

In summary, our results support the hypothesis that phenotypic innovations allow diversification through the exploitation of novel habitats. The presence of anthraquinones in the thallus and the colonization of rocks in unshaded habitats have both been central to the success of the Teloschistaceae. Beyond that, our results also indicate that phenotypic traits (anthraquinones and crustose-continuous) are especially correlated with diversification rates, whereas ecological substrate traits (rock and bark) are particularly linked to cladogenesis.

**Setting Events in a Time Frame.** Our results demonstrate an adaptive radiation, beginning at the onset of the diversification of the large Teloschistaceae family, and associated with a shift to sunny, rocky habitats and the innovation of using anthraquinones as a sun-protecting pigment. These events are likely to have occurred in the Early Cretaceous, ~100 Mya, during the Cretaceous Thermal Maximum (112–89 Mya). Several events seem to concentrate around that time. The onset of diversification of the Teloschistaceae could be linked to the increase in temperatures in the Cretaceous, leading to the colonization of newly formed arid and exposed regions, possibly augmented by greater provincialization (43) and changes in orographic landmarks, such as the separation of America and Africa (119–105 Mya) (44). Alternatively, the greater seasonality and the spread of deciduousness in both angiosperms and gymnosperms in the cooling period that followed the Cretaceous Thermal Maximum (43) may have changed the light environment for the epiphytic ancestors of the Teloschistaceae, leading to adaptation to higher light exposure. In this latter scenario, the intermediate light exposure offered by deciduous habitats might be seen as providing a "stepping stone" in the adaptive landscape, leading to the evolution of light-protective anthraquinones throughout the thallus and allowing the subsequent colonization of fully exposed habitats.

Several other examples of diversification events have been found in epiphytic groups, during the same period as the diversification of the Teloschistales, subsequent to the radiation of angiosperms [194 (210–162) Mya (45), 217 (257–182) Mya (46, 47), and 240–175 Mya (48)], suggesting that the terrestrial dominance of the latter in the Cretaceous, and the advent of the Pentapetalae radiation ~110 (115–105) Mya (45), triggered an ecological opportunistic response, including some fern groups (49, 50), epyphitic Lycophytes (51), and other groups of lichenized fungi [Lecanoromycetidae and Ostropomycetidae (52)]. However, in all of the above-mentioned studies, diversification is linked to the exploitation of novel epiphytic niches generated by angiosperms' more diverse and structured habitats and by the expansion of rain forests. The radiation of

the Teloschistaceae stands out in suggesting a contemporaneous move away from epiphytic habitats to saxicolous (rock-dwelling) ones, which leaves us with several open questions. Were the nascent Teloschistaceae outcompeted in the newly arisen forest canopies and forced to occupy more extreme but available habitats? In this scenario, anthraquinones evolved to occur in the thallus as a relatively trivial consequence of the exposed conditions. Or did the innovation of anthraquinones being present in the thallus allow the opportunistic colonization of exposed rocky habitats and accelerate diversification? In line with the first of these hypotheses, the sister groups of the Teloschistaceae (Letrouitineae and Megalosporaceae) do not show signs of diversification outbursts and remain species-poor, despite being epiphytic, and predominantly tropical in distribution. Conversely, the facts that anthraquinones are found in only a very few other lichen species and that other successful lichen lineages were adopting epiphytism at the same time as the Teloschistaceae were colonizing open habitats point to the idea that anthraquinones were important factors in the taxon's evolutionary radiation.

These considerations—along with our direct findings that anthraquinones and ecological traits share a similar evolutionary history and are both related to diversification—lead us to conclude that rather than one or other playing a dominant role, ecological opportunities and phenotypic innovations are intricately entwined as factors causing adaptive radiations. On the one hand, anthraquinones represent a clear case of an innovation—a phenotypic trait that lies at the heart of the radiation. On the other hand, it is equally clear that this innovation occurred in concert with the exploitation of novel, UV-exposed habitats. Interestingly, these changes appear to have co-occurred with the transformation of the terrestrial landscape by gymnosperm- and angiosperm-dominated forests. Therefore, our results suggest the intriguing possibility that ecological opportunities related to global climate and vegetation also played a central role in the evolutionary origin of anthraquinones themselves.

## Materials and Methods

**Taxon and Locus Sampling.** We selected taxa using our previous sampling of the Teloschistales (30) as a reference. Six loci were targeted for this study: nuclear ribosomal ITS, large subunit (nrLSU), and mitochondrial small subunit (mtSSU), and nuclear protein-coding genes *RPB*1 (one locus) and *RPB*2 (two amplicons, considered here as two separate loci). See *SI Appendix*, Table S3 for voucher and GenBank accession number information, and see *SI Appendix, SI Materials and Methods* for details on alignments and datasets.

**Phylogenetic Analyses.** A synopsis of datasets used in our analyses is shown in *SI Appendix*, Table S4. A total of 1,000 replicates of maximum-likelihood (ML) bootstrapping with a GTRGAMMA model were performed in RAxML (Version 7.2.8) (53, 54) to test for topological incongruence among each locus separately and on all possible combinations (55). For the *RPB*2 locus, this criterion was applied on each amplicon separately. Phylogenetic relationships and confidence on the combined dataset were inferred primarily with Bayesian analyses by using MrBayes (Version 3.2.2) (56). In addition, support was also inferred using ML bootstrap proportions computed in RAxML (see *SI Appendix, SI Materials and Methods* for details on conflict and Bayesian and ML analyses settings).

Overall, a well-resolved and supported topology of the Teloschistales was recovered by both ML and Bayesian approaches on the six-locus combined dataset (*SI Appendix*, Fig. S5). The taxonomy of this group of lichens, specifically the family Teloschistaceae, has been highly controversial, and several classifications have recently been proposed with some newly delimited genera not significantly supported (e.g., refs. 29 and 57). On the grounds of practicality, here we use the traditional generic names for the group, recently included in a "without-prejudice" list of generic names of fungi for protection under the International Code of Nomenclature (58), being aware that their current circumscription is polyphyletic in some cases (see *SI Appendix, SI Materials and Methods* for more details).

**Dating Analyses.** The times of divergence were estimated by using a two-step approach. Because of the lack of fossils related to the group of interest, the results of a first dating analysis (eukaryotes dataset; *SI Appendix*, Fig. S6) was used to obtain calibrations of the root and a few major clades using a subset of the Teloschistales. This first analysis was based on eight fossils, two secondary molecular-based calibrations across the eukaryotes, and several nodes constrained as monophyletic (*SI Appendix*, Tables S5 and S6). The second analysis then focused on the order Teloschistales by using time estimates from the first analysis as secondary calibrations to date divergences within the more exhaustive sampling of Teloschistales (Teloschistales dataset; *SI Appendix*, Fig. S1 and Tables S7 and S8). Settings for these two analyses are described in *SI Appendix, SI Materials and Methods*. A relaxed-clock model of molecular evolution was allowed in both analyses.

**Testing for Shifts in Diversification Rates.** We used a Bayesian framework to model diversification using the BAMM program (59) and analyzed the results using the R package BAMMtools (36) to account for temporal and branch-specific shifts in diversification rates and to investigate whether regimes of net diversification (speciation rate − extinction rate) changed within the tree. Our dated phylogeny of the Teloschistales incorporates 108 species (Fig. 1*A*), ∼10% of the currently ascribed total (29), but the polyphyletic nature of the very large *Caloplaca* genus means that it is impossible to reliably assign unsampled species to clades within the Teloschistaceae. In our analyses using BAMM, we therefore made estimates of our sampling frequency and accounted for missing species using clade-specific sampling fractions for our four major lineages as follows: 0.089 for Teloschistaceae, 0.116 for Megalosporaceae, 0.11 for Brigantiaeaceae, and 0.176 for Letrouitiaceae. These fractions were based on current species estimates from the Index Fungorum database (accessed August 21, 2014) (see *SI Appendix, SI Materials and Methods* for details of the BAMM analyses).

**Ancestral Character State Estimations.** The traits anthraquinones in thallus, presence of anthraquinones, exposure to light, rock substrate, bark substrate, and growth form crustose-continuous were coded from our own sampled collections (*SI Appendix*, Fig. S4 and Table S3) and complemented with the literature. All traits were treated as binary and reconstructed on a selection of 40 nodes that represented interesting and well-supported clades by using, in addition to likelihood reconstruction, three separate methods that take into account possible biases caused by phylogenetic uncertainty, state-dependent differences in diversification rates and incomplete taxon sampling, and branch-dependent transition rates (using BayesTraits-Bayes, BiSSE-ML, and *corHMM*-ML, respectively), with the settings described in *SI Appendix, SI Materials and Methods*. The plotting of different methods and traits can be found in *SI Appendix*, Fig. S4, and model performance results are provided in *SI Appendix*, Tables S1, S9, and S10. We established a statistical threshold to deal with discrepancies among methods and to determine for which nodes we could confidently estimate character states (Fig. 1*A*). For each trait and for each node, we calculated the mean estimated probability of trait presence across methods. When this value fell between 0.25 and 0.75, the state for that node was considered ambiguous and was excluded. We selected which methods to include in this calculation based on statistical tests of state- and branch-dependent diversification rates. Thus, for the crustose-continuous character, which showed a high significance for state-dependent diversification rates, we used only BiSSE results. Similarly, for shade, we used the two-rate model *corHMM* and BiSSE. For all other traits, we used all four methods.

Additionally, we performed a multistate reconstruction in BayesTraits treating the anthraquinones trait as polymorphic depending on the pigments' location. We wanted to test for directionality in the loss of anthraquinones, as initially hypothesized. The same settings as in the binary analyses were applied; several runs were performed, starting with RJ and trying different constraints and hyper priors. Here 15 models with different constraints describing trait evolution were compared that can be grouped in models allowing transitions and reversals with different rates; transitions and reversals with the same rate; and stepwise models where one-step changes have different rates than two-step changes, independently of the transitions and reversals. Also, the root was "fossilized" (i.e., fixed to each possible state) by using the 'fossil' command of BayesTraits. Log likelihoods and BFs were compared.

**Testing for State-Dependent Diversification Rates.** We also examined whether any of the reconstructed traits was correlated with increased rates of diversification using BiSSE (38). BiSSE uses ML to estimate absolute rates of asymmetric character change ($q$), speciation ($\lambda$), and/or extinction ($\mu$) by maximizing the likelihood of these parameters for a given topology with branch lengths. For each character, we compared two models: (*i*) an unconstrained model in which q and $\lambda$ were allowed to vary; and (*ii*) a constrained model in which $\lambda$ and $\mu$ was set equal for each character. If diversification rates are

correlated with character states, then the unconstrained model should be favored over the constrained model. We carried out likelihood ratio tests ($\chi 2$ approximation) and compared $\Delta$AIC scores to assess the fit of models. Because the BiSSE model accounts for only the character change that occurs along lineages, we also implemented "BiSSE–node enhanced state shift" [BiSSE-ness (42)] model to allow for cladogenetic change in addition to anagenetic change, which thus enables us to address the mode of character change (along-lineage vs. speciational change) while at the same time accounting for the effect the character has on rates of speciation and extinction, i.e., trait-dependent diversification. We compared the fit of five different models, ranging from character change occurring only along lineages to when it occurred only at speciation, under different taxon sampling scenarios as described in *SI Appendix, SI Materials and Methods*.

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