



OXFORD  
BIOLOGY



*the* **TIMETREE** *of* **LIFE**

*edited by* **S. BLAIR HEDGES** *and* **SUDHIR KUMAR**  
*foreword by* James D. Watson



# Liverworts (Marchantiophyta)

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## Abstract

Liverworts (Phylum Marchantiophyta) include 5000–8000 species. Phylogenetic analyses divide liverworts into Haplomitriopsida, Marchantiopsida, and Jungermanniopsida. Complex thalloids are grouped with Blasiales in Marchantiopsida, and leafy liverworts are grouped with Metzgeriidae and Pelliidae in Jungermanniopsida. The timetree shows an early Devonian (408 million years ago, Ma) origin for extant liverworts. The complex thalloid habit originated sometime in the Triassic (246–203 Ma). Both leafy and epiphytic habits are indicated as old features, but analyses also indicate possible extinctions during the Permian and Triassic (299–200 Ma) and rapid family and genus-level divergences during the Cretaceous and early Cenozoic (145–50 Ma).

The evolution of land plants marks one of the most important events in earth history. Because of their lengthy and well-documented fossil record, the major patterns in early land plant evolution have mainly been interpreted using macrofossil evidence from the vascular plant lineage (1). Liverworts (Fig. 1), in contrast, have a limited fossil record, they are easily neglected due to their small size, and their role in early land plant evolution is rarely emphasized. Nevertheless, growing evidence (1–7) indicates an early split in land plant evolution between the liverworts and all other land plants. This implies that liverworts occupy a critical position, and that they may help us understand the morphological and reproductive changes that favored the successful radiation of land plants and their adaptations to life in a terrestrial environment. Here we review recent progress in

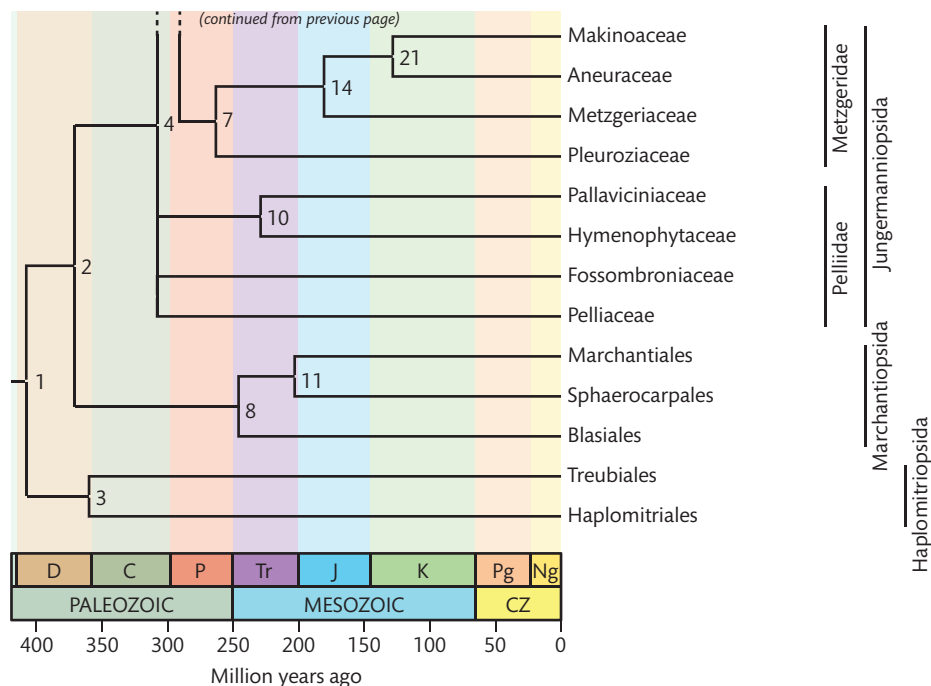
our understanding of phylogenetic relationships among major lineages and the origin and divergence times of those lineages.

Altogether, liverworts (Phylum Marchantiophyta) comprise an estimated 5000–8000 living species (8, 9). Early and alternative classifications for these taxa have been numerous [reviewed by Schuster (10)], but the arrangement of terminal taxa (species, genera) into larger groups (e.g., families and orders) based on morphological criteria alone began in the 1960s and 1970s with the work of Schuster (8, 10, 11) and Schljakov (12, 13), and culminated by the turn of the millenium with the work of Crandall-Stotler and Stotler (14). Three morphological types of plant bodies (gametophytes) have generally been recognized and used in liverwort classifications: “complex thalloids” including ~6% of extant species diversity and with a thalloid gametophyte that is organized into distinct layers; “leafy liverworts”, by far the most speciose group, including ~86% of extant species diversity and with a gametophyte that is differentiated into stem and leaves; and “simple thalloids” including ~8% of extant species diversity and with a more or less anatomically undifferentiated thalloid gametophyte.



**Fig. 1** Leafy liverwort *Schistochila aligera* (Nees & Blume) J.B. Jack & Stephani, Schistochilaceae, Jungermanniales. Credit: X. He-Nygrén.





**Fig. 2** A timetree of liverworts. Divergence times are shown in Table 1. *Abbreviations:* C (Carboniferous), CZ (Cenozoic), D (Devonian), J (Jurassic), K (Cretaceous), Ng (Neogene), P (Permian), Pg (Paleogene), and Tr (Triassic). Jungermanniaceae-1 includes *Nardia scalaris* and *Jungermannia obovata*; Jungermanniaceae-2 includes *Jungermannia ovato-trigona* and *Leiocolea collaris*; and Jungermanniaceae-3 includes *Saccogyna viticulosa*, *Harpanthus flotovianus*, and *Geocalyx graveolens* in the original analyses by Heinrichs *et al.* (23).

may be an ancestral feature for liverworts as a whole, although formal reconstructions of ancestral morphological states have not been undertaken. The ancestral body plan is ambiguous because the Haplomitriopsida, which is well resolved as closest to Marchantiopsida and Jungermanniopsida, includes both leafy and more or less thallose types. Haplomitriopsida, the least diverse group in terms of living taxa, includes only the three genera *Haplomitrium*, *Treubia*, and *Apotreubia*. The Marchantiopsida include a monophyletic complex thalloid clade that is closest to the Blasiales, which has a simple thalloid morphology. The Jungermanniopsida, by far the most diverse group and possibly comprising 86% of extant species diversity, includes three distinct groups (subclasses): Pelliidae (including simple thalloid taxa and the more or less leafy Fossombroniales), Metzgeriidae (including simple thalloid taxa but also the leafy Pleuroziales), and Jungermanniidae (including all the leafy liverworts except Pleuroziales). Clearly, transitions between thallose and leafy body types have happened multiple times.

With the exception of the analyses by Wheeler (24) and Boisselier-Dubayle *et al.* (17), there are no recent studies targeting the resolution of relationships among complex thalloid taxa in Marchantiopsida. Standard molecular

markers, commonly used in the more inclusive analyses, display too little variation to establish well-supported relationships in this group (9). Some analyses place Sphaerocarpaceae as the closest relative of all remaining taxa (15, 16, 22, 23), and this is also reflected in the classification by He-Nygrén *et al.* (16), who recognized two orders in the complex thalloid clade, Sphaerocarpaceae and Marchantiales.

Relationships within each of the three Jungermanniopsida groups are better explored. A series of analyses have included a comprehensive sample of the simple thalloid groups Pelliidae and Metzgeriidae (9, 20, 22). Family-level relationships are comparatively well understood in both of these groups, although some of this knowledge has yet to be incorporated in a classificatory framework. Also the leafy group (Jungermanniidae) has been the focus of several analyses (15, 16, 18, 19, 23). These consistently identify two major groups (orders), Porellales and Jungermanniales, plus a smaller order, the Ptilidiales (containing Ptilidiaceae and Neotrichocoleaceae), whose relationship to the two larger clades is currently unresolved. Some analyses place the Ptilidiales in Jungermanniales (9, 19), but others indicate a closer relationship with the Porellales (16, 18, 23). The Ptilidiales may also be closest to a clade that

**Table 1.** Divergence times (Ma) and their confidence/credibility intervals (CI) among liverworts.

Timetree		Estimates							
Node	Time	Ref. (23)(a)		Ref. (23)(b)		Ref. (23)(c)		Ref. (27)	
		Time	CI	Time	CI	Time	CI	Time	CI
1	407.7	407.7	411–405	-	-	-	-	-	-
2	370.9	372.6	383–362	-	-	-	-	369.2	403–338
3	360.0	-	-	-	-	-	-	360.0	396–316
4	307.9	328.5	335–322	-	-	-	-	287.3	331–262
5	290.6	308.7	317–301	-	-	-	-	272.5	310–243
6	265.1	-	-	288.3	290–286	274.8	277–273	232.1	275–207
7	263.3	-	-	269.9	272–268	256.6	259–255	-	-
8	245.7	-	-	-	-	-	-	245.7	268–231
9	235.5	-	-	240.5	251–230	230.5	240–221	-	-
10	229.0	-	-	235.3	269–202	222.7	252–194	-	-
11	203.0	-	-	-	-	-	-	203.0	203–203
12	200.5	-	-	204.3	213–196	196.7	205–188	-	-
13	187.7	-	-	189.4	203–176	185.9	198–174	-	-
14	180.5	-	-	174.2	175–173	166.2	167–165	201.0	245–186
15	171.8	-	-	174.8	185–165	168.8	178–160	-	-
16	168.1	-	-	171.1	179–163	165.1	172–158	-	-
17	156.4	-	-	155.9	166–146	150.6	160–141	162.7	197–147
18	144.6	-	-	147.0	155–139	142.2	149–135	-	-
19	139.7	-	-	141.7	150–134	137.6	146–130	-	-
20	135.2	-	-	137.4	144–131	132.9	138–128	-	-
21	128.4	-	-	131.3	132–130	125.5	126–125	-	-
22	122.0	-	-	131.8	140–124	127.7	135–120	106.4	134–94
23	107.4	-	-	109.2	118–101	105.5	111–100	-	-
24	102.9	-	-	108.5	119–98	97.3	110–84	-	-
25	102.1	-	-	103.5	108–99	100.7	104–97	-	-
26	82.9	-	-	84.6	94–75	81.1	90–73	-	-
27	50.4	-	-	50.8	56–45	49.9	55–45	-	-
28	50.2	-	-	50.3	52–49	50.1	51–50	-	-

Note: Node times in the timetree represent the mean of time estimates from different studies. For Node 5, maximum (317 Ma) and minimum (301 Ma) ages from Heinrichs *et al.* (23) (a) were used as alternative calibration points in their second series of analyses yielding maximum (b) and minimum (c) age estimates for each node. Minimum age constraints forced were 112 Ma (Node 9), 203 Ma (Node 11), 90 Ma (Node 17, ref. 23), and 50 Ma (Node 28).

includes both the Jungermanniales and the Porellales. This uncertainty bears directly on our interpretation of ventral lobe (lobule) and water sac evolution in the leafy liverworts (9). A jungermannialian relationship for the Ptilidiaceae–Neotrichocoleaceae clade indicates that the elaboration of ventral lobes into water sacs may be a feature that is shared by a larger group than Porellales, where it is most commonly seen. Schuster (8) interpreted

these lobules as functioning in water retention and as an adaptation for an epiphytic habit (hence the term water sacs). An alternative interpretation is that they serve a nutritional purpose (25). The loss of a mycorrhiza-like association with Glomeromycota, shared by all Jungermanniopsida and possibly associated with a change in habit from terrestrial to epiphytic, support such an interpretation (26).

He-Nygrén *et al.* (16) recognized three suborders in the Porellales: Ptilidiineae (Ptilidiaceae and Neotrichocoleaceae), Porellineae (Porellaceae, Goebeliellaceae, Radulaceae, Frullaniaceae, Jubulaceae, and Lejeuneaceae), and Lepidolaenineae (Lepidolaenaceae). However, other analyses do not support monophyly of Porellineae and indicate alternative placements for both Porellaceae and Goebeliellaceae (9, 15, 19, 23) (Fig. 2).

Recent analyses, based on multigene data sets, support monophyly of four larger groups within the Jungermanniales (9, 16, 19, 23), and these groups largely correspond to the four suborders recognized by He-Nygrén *et al.* (16): Perssoniellineae, Cephaloziineae, Jungermanniineae, and Lophocoleineae. We should expect changes with respect to the circumscription and comprehensive inclusiveness of each of these groups in the future. They are all diverse groups and future analyses, with an even more comprehensive taxon sample, will likely improve on our current understanding. The Myliaceae (*Mylia taylorii*), for example, was only recently included in the analyses, but their relationships are still poorly supported (23).

Despite the rapid accumulation of molecular sequence data for liverworts, there are only two papers published that provide molecular estimates of liverwort divergence times at the hierarchical level covered in the present review. Both used a penalized likelihood approach and accounted for uncertainties in branch lengths and topology by analyzing 100 trees and parameter estimates drawn from the Bayesian posterior distribution of their phylogenetic analyses. The first study included 34 liverwort taxa but mainly focused on the diversification of mosses (Bryophyta) (27). Their analysis used a plastid two-gene data set (*rbcL* and *rps4* genes) and a fixed calibration point at 450 Ma for extant land plants (27). Spore tetrads are considered diagnostic of land plants, and the calibration point was based on a conservative date for their appearance in the fossil record (1). Seven minimum-age constraints were enforced in their analyses, six among vascular plants and one among liverworts. The liverwort constraint concerned the split between Marchantiales and Sphaerocarpaceae, forcing all estimates to be at least 203 Ma.

The second paper focused mainly on the Jungermanniopsida clade of liverworts (23). This analysis included a twofold strategy where an internal calibration point for liverworts was obtained in a first series of analyses using a broader sample of land plants. This first series of analyses used a two-gene data set (*rbcL* and *rps4* genes) and included 56 taxa (49 liverworts, two mosses,

one hornwort, three tracheophytes, and one algal outgroup). The first split among tracheophytes was used as calibration point and fixed at 430 Ma and eight minimum-age constraints were enforced during the analyses, all of which concerned liverworts. They also constrained the embryophyte crown group at a maximum age of 475 Ma based on the occurrence of spore monads and diads from the Ordovician (488–444 Ma) that have been considered to represent liverworts (28). The analyses established upper and lower bound age estimates for the split between the Metzgeriidae and Jungermanniidae and these estimates were used as calibration points in their second series of analyses.

The second set of analyses focused on the Jungermanniidae and used a three-gene data set (*rbcL*, *rps4*, and *psbA* genes) and 86 taxa (75 Jungermanniidae, five Metzgeriidae, five Pelliidae, and one Marchantiopsida outgroup). No less than 10 minimum-age constraints were enforced during the analyses, and the split between the Metzgeriidae and Jungermanniidae was fixed at 301 and 317 Ma in two consecutive analyses based on results from their first series of analyses. Age estimates are also affected in these analyses by the constraints and there are nodes that in all analyses are forced toward their constrained ages (Table 1). Furthermore, using ages obtained from one analysis as constraints and/or calibration points in subsequent analyses is risky. Nevertheless, these analyses provide working hypotheses for the time course of liverwort diversification.

The liverwort timetree (Fig. 2) shows an initial split between the Haplomitriopsida and remaining taxa in the early Devonian (408 Ma), and this estimate is comparable to that indicated by the macrofossil record. *Pallaviciniites devonicus* has been associated with liverworts, and this fossil taxon has been documented from the late Devonian (29, 30). Krassilov and Schuster (29) considered this fossil taxon as possibly related to the Pallaviciniaceae or Hymenophytaceae, but their derived positions and considerably younger ages indicate this to be incorrect.

The living lineages of Marchantiopsida diversified in the early Triassic (246 Ma), but neither analysis (23, 27) provides a molecular-based estimate for the origin of the complex thalloid clade as a whole. Newton *et al.* (27) constrained the node at a minimum age of 203 Ma based on macrofossil evidence, but this forces the node to be 203 Ma in all analyses. In Heinrichs *et al.* (23) the node was also constrained at a minimum age of 225 in their first series of analyses, but they only report results for a few nodes from this analysis (Table 1). Unconstrained



analyses would likely indicate a younger age for this node. Branch lengths are considerably shorter among the complex thalloid taxa, indicating a decrease in evolutionary rate (9), and although the analyses incorporate such a decrease, we may still underestimate the magnitude of this deceleration. This would lead to molecular age estimates that are too young and if we accept the fossil-based information (31), this is what we are seeing.

Leafy liverworts are the most successful group of liverworts in terms of species diversity and possibly account for as much as 86% of all extant liverworts. Although uncertainty remains about the origin of their leafy habit, even a conservative estimate places the origin in the Paleozoic. The earliest divergence among living Porellales and Jungermanniales appears to be of Permian age (265 Ma) and the leafy habit clearly had evolved by this time. The Pleuroziaceae, in the Metzgeriidae, are also leafy and leafyness may have originated already in the Early Permian (309 Ma). Although the origin of the leafy habit appears to have been a Paleozoic event, both the leafy groups Porellales and Jungermanniales show a Permian to Triassic pattern with extensive periods of time with little or no cladogenesis. This pattern corresponds with that indicated for mosses (27), and likely relates to an increased rate of extinctions during this time (32, 33).

Also epiphytism appears to have originated early during liverwort evolution. A constant presence of epiphytism in all families of Porellales indicates an origin already by the late Permian.

Much of the family- and genus-level divergences in the leafy Porellales and Jungermanniales appear in the Mesozoic (Fig. 2) and early Cenozoic [see (23) for genus-level divergences]. Heinrichs *et al.* (23) linked this pattern, at least in part, to the development of tropical angiosperm-dominated forests, and they speculated that it corresponds to the pattern reported for other lineages of land plants such as lycopods (34) and leptosporangiate ferns (35). Crown-group Lejeuneaceae, for example, is dated as late Cretaceous (23), and is a highly diverse group, with as many as 1000 extant species, and they are predominantly epiphytic or epiphyllous. That the development of tropical angiosperm-dominated rain forests in the late Cretaceous and early Cenozoic triggered and mediated this diversification seems entirely plausible.

Although the molecular estimates of liverwort divergence times that have been published so far (23, 27) cannot capture every aspect of the dynamics associated with the origin and diversification of liverworts into their present-day diversity, they have brought new insights into our understanding of liverwort evolution. No doubt,

this molecular approach has opened up a new avenue for tracing the origin and evolution, not only of liverworts, but also of any group of organisms with a limited fossil record.

## Acknowledgments

Support was provided by the Swedish Research Council to N. W. and by the U.S. National Science Foundation to A. J. S.

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