A NEW FAMILY OF LEAFY LIVERWORTS FROM THE MIDDLE EOCENE OF VANCOUVER ISLAND, BRITISH COLUMBIA, CANADA

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- Premise of the study: Morphology is a reflection of evolution, and as the majority of biodiversity that has lived on Earth is now extinct, the study of the fossil record provides a more complete picture of evolution. This study investigates anatomically preserved bryophyte fossils from the Eocene Oyster Bay Formation of Vancouver Island. While the bryophyte fossil record is limited in general, anatomically preserved bryophytes are even more infrequent; thus, the Oyster Bay bryophytes are a particularly significant addition to the bryophyte fossil record.

- Methods: Fossils occur in two marine carbonate nodules collected from the Appian Way locality on the eastern shore of Vancouver Island, British Columbia, and were prepared using the cellulose acetate peel technique.

- Key results: The fossils exhibit a novel combination of characters unknown among extinct and extant liverworts: (1) three-ranked helical phyllotaxis with underleaves larger than the lateral leaves; (2) fascicled rhizoids associated with the leaves of all three ranks; (3) Anomoocladella-type endogenous branching.

- Conclusions: A new liverwort family, Appianacae fam. nov., is established based upon the novel combination of characters. Appiana gen. nov. broadens the known diversity of bryophytes and adds a hepatic component to one of the richest and best characterized Eocene floras.

Key words: bryophyte; Canada; Eocene; fossil; jungermannioid; liverwort.

Fundamental aspects of the evolution of plant structure can be derived from the study of living plants. Comparative analysis of plant structure and the genetic mechanisms that control it can indicate which lineages are more basal and which more derived and suggest possible evolutionary trajectories. However, because this approach is based on living plants, it excludes many structures and combinations of characters that lack counterparts in the living flora, as well as many of the most basal forms. Extant biodiversity represents a small fraction of the diversity of life that spans Earth’s geologic history (Niklas, 1997). The fossil record, while not preserving genetic information, provides access to a comparatively extensive diversity of plant structure, lending to a more complete picture of evolution. Therefore, study of the fossil record allows for higher resolution in the understanding of evolutionary events in deep time.

The middle Eocene Appian Way fossil assemblages have yielded a taxonomically diverse flora that includes fruits from various taxa, conifer cones, fern, and fungal fossils, preserved along with numerous nondescript plant fragments. Fruits of Juglandaceae (Elliott et al., 2006) and Fagaceae (Mindell et al., 2007a) have been described, as well as inflorescences of Platanaceae (Mindell et al., 2006a). Taxodiaceous pollen cones (Hernandez-Castillo et al., 2005), as well as schizaeaceous (Trivett et al., 2006) and gleicheniaceous (Mindell et al., 2006b) fern remains and various fungi (Smith et al., 2004; Mindell et al., 2007b) have also been documented. We add an anatomically preserved leafy liverwort to the taxonomic diversity of the flora. This is a significant addition because leafy liverworts are not well represented in the fossil record. The Appian Way liverwort is the only bryophyte described to date from the locality and represents a new family.

The fossil record of liverworts and other bryophytes is sparse when compared to more derived embryophyte lineages. Comprehensive surveys of the bryophyte fossil record have been compiled by Steere (1946), Jovet-Ast (1967), Lacey (1969), Krassilov and Schuster (1984), Miller (1982), Oostendorp (1987), and Taylor et al. (2009). The scarcity of bryophyte fossils has been historically linked to a larger amount of attention given to the investigation of vascular plants, particularly in the early land flora. This may have led to bryophyte fossils being overlooked or misinterpreted (as suggested by Krassilov and Schuster, 1984; Smoot and Taylor, 1986). The scarcity of bryophyte fossils has also been attributed traditionally to a preservation bias determined by their diminutive size, delicate tissues, and simple structure (Steere, 1946). In that context, Edwards et al. (1995) and Edwards (2000) have emphasized difficulties posed by the small size and degree of fragmentation of putative fossils of early bryophytes. However, several experimental studies addressing the issue (Kroken et al., 1996; Hemsley, 2001; Graham et al., 2004) have challenged these ideas, showing that bryophytes have good preservation potential.

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Nevertheless, lack of durable tissue and a weakly developed cuticle may lessen the chance of finely preserved bryophyte fossils, particularly when subjected to transport over long distances prior to burial.

Bryophyte fossils are preserved as either cellular permineralizations or coalfied compressions (sensu Schopf, 1975). The first mode of preservation retains a high level of anatomical detail, whereas the second mode retains a more limited range, if any (e.g., cuticular or tracheary element anatomy). However, due to the diminutive size of bryophytes, the limited amount of cellular detail preserved in compressions can sometimes represent a significant proportion of the organism’s anatomy. The oldest unequivocal macrofossil bryophyte with cellular detail is the Middle Devonian Metzgeriothallus sharonaec Hernick et al., a thalloid liverwort. The fossils are preserved as coalfied compressions exhibiting good epidermal cellular detail (Hernick et al., 2008). Older microfossils of potential liverwort affinities consist of spore masses reported from the Late Ordovician of Oman (Wellman et al., 2003).

The Mesozoic liverwort fossil record consists exclusively of coalfied compressions. The earliest leafy liverwort fossil is the early Permian Jungermannites selandicus Poulsen, represented by compressions with poor anatomical preservation (Oostendorp, 1987). The Mesozoic liverwort fossil record consists exclusively of coalfied compressions. Jungermannites includes four other species that span the Late Triassic through the Late Cretaceous. The Late Triassic Naiadita lanceolata Brodie is represented by compressions with good anatomical detail, allowing for its placement within the Calobryales or Sphaerocarpales (Harris, 1939; Krassilov and Schuster, 1984). Additional leafy liverworts from the Late Jurassic have been described, including Cheirorhiza britteae Krassilov (1973) and Laticaulina papillosa Krassilov (1973). The former provides anatomical details that allow for placement within the Jungermanniales, whereas the latter appears highly fragmented, with only a suggestion of jungermannialean affiliations (Krassilov and Schuster, 1984). Another leafy liverwort, the Early Cretaceous Diettertia montanensis Brown et Robinson (Brown and Robinson, 1974; Schuster and Janssens, 1989), also represented by compressions, exhibits anatomy allowing for unequivocal assignment to the Jungermanniales.

The Cenozoic hepatic fossil record is dominated by amber fossil floras that provide numerous examples of finely preserved leafy liverworts, which can be assigned to extant taxa (summarized by Taylor et al., 2009). These fossil floras are limited to the Baltic and Bitterfield ambers, both European amber deposits. The sediment bearing the Bitterfield amber has a well-constrained absolute age of 25.3–23.8 million years (Schmidt and Dörfelt, 2007). The age of Baltic amber is less well constrained and ranges from early to late Eocene, with material that is as old as 55 million years. All amber fossil liverworts have all been assigned to extant taxa. The Appian Way hepatic and the leafy liverworts preserved in amber are the only examples of complete anatomical preservation in the leafy liverwort fossil record. Whereas amber liverworts are typically characterized through examination of external morphology, the Appian Way fossils allowed for documentation of internal anatomy due to permineralization in calcium carbonate, a mode of preservation new to the study of leafy liverwort fossils.

MATERIALS AND METHODS

This study is based on 14 gametophyte stem segments, some exhibiting frequent branching, preserved in two carbonate nodules collected from the Appian Way locality (49°54′42″N, 125°10′40″W) on the eastern shore of Vancouver Island, British Columbia, Canada (Mindell et al., 2009). The nodules occur in the silty sandstone matrix of the middle Eocene Oyster Bay Formation, which is exposed at Appian Way on the northern margin of the Georgia Basin (Mustard and Rousse, 1994). Within the nodules, the plant material is preserved by cellular permineralization in calcium carbonate. The nodules also contain marine fauna including corals, echinodermes, gastropods, fish bones, and shark teeth (Mindell et al., 2009). The faunal assemblage and lithology of the silty sandstone matrix have been interpreted as reflecting a shallow marine environment (Haggart et al., 1997). Characteristic mollusks, decapods, and gastropods indicate an early to middle Eocene age (Haggart et al., 1997; Schweitzer et al., 2003; Cockburn and Haggart, 2007). Pollen has provided a less-constrained age for the locality, as both late Paleocene and early Eocene palynomorphs are present (Sweet, 2005).

The nodules include numerous minute, opaque spheroids, which tend to aggregate around plant material. The spheroids, likely pyrite frambooids (spherical aggregates of minute crystals), are often associated with individual plant cells and can mark the position of already deteriorated cells. The good preservation of the plant fossils indicates that the depositional environment around the accumulating plant material was characterized by reducing conditions. Reducing bottom environments, especially in marine settings like that of the Oyster Bay Formation, promote pyrite precipitation in the presence of organic matter (Berner, 1984; Canfield and Raiswell, 1991; Wilkin and Barnes, 1997). This usually occurs in the form of frambooids and is due to the reduction of marine sulphates by anaerobic bacterial decomers living on the organic material.

Nodules were cut into serial slabs and peeled using the cellulose acetate peel technique (Joy et al., 1956). Microscope slides were prepared with Eukitt (O. Kindler, Freiburg, Germany) mounting medium. Images were captured using a Canon 8000 VR digital camera and a Nikon Eclipse E400 microscope, and processed with Adobe (San Jose, California, USA) Photoshop 5.0. All specimens and microscope slides are housed in the University of Alberta Paleobotanical Collections, Edmonton, Alberta, Canada (UAPC-ALTA).

SYSTEMATICS

Class—Jungermanniopsida Stotler and Crandall-Stotler.

Subclass—Jungermanniidae Engl.

Order—Incertae sedis.

Family—Appianaceae Steenbock et al. fam. nov.

Familial diagnosis—Gametophyte with stem and leaf organization; three-ranked helical phyllotaxis, anisophyllous; underleaf larger than lateral leaves; rhizoids fascicled and associated with leaf bases of all ranks.

Type genus—Appiana Steenbock et al. gen. nov.

Generic diagnosis—Characters of the genus as those of the family; leaves unistratose and ecostate, incubous lateral leaves, transverse underleaves; endogenous Anomocladia-type lateral branching.

Etymology—Appiana in named for the Appian Way locality that has contributed to knowledge of the middle Eocene flora and to the known diversity of leafy liverworts.

Type species—Appiana sillettiana Steenbock et al. sp. nov.

Specific diagnosis—Characters of the species as those of the genus. Gametophyte diminutive, branched at close but irregular intervals (0.5–1.5 mm); stem diameter 0.2–0.25 mm, cells longitudinally elongated, 4.6–18.6 µm in diameter, and at least 70 µm in length; leaves imbricate 60 µm apart on stem, lateral leaves are at least 630 µm long and 320 µm wide, underleaves at least
1.1 mm long and 330–390 µm wide, carinate, with base wrapped around ventral bases of lateral leaves; leaf cells isodiametric, 5.8–15.1 µm, with concave trigones; inflated cells at leaf margin are 11.5 mm in diameter; inflated cells at leaf base forming 2–3 cell wide band are 11.5–23.1 µm in diameter; rhizoids unicellular, 12 µm in diameter.

**Etymology**—In honor of Dr. Stephen C. Sillett of Humboldt State University, for providing guidance and inspiration in the resolution of the liverwort affinities of the fossils, as well as in recognition of his contributions to the knowledge of epiphytic bryophyte and lichen communities.

**Holotype hic designatus**—AW660 Gtop #15.

**Paratypes**—AW101 Ebot #91c (Fig. 19), #93 (Fig. 1); AW660 Gtop #1 (Figs. 9, 17), #3 (Fig. 16), #4 (Fig. 15), #6, #8, #12 (Fig. 5), #15 (Figs. 6, 11), #18 (Fig. 13), #20 (Figs. 3, 7), #57 (Fig. 10), #61 (Fig. 2), #63 (Fig. 4), #145 (Figs. 8, 12, 18, 20), #146 (Fig. 14).

**Locality**—Appian Way, south of Campbell River, British Columbia, Canada (49°54′42″N, 125°10′40″W; UTM 10U CA 5531083N, 343646E).

**Stratigraphic position and age**—Oyster Bay Formation, middle Eocene.

**DESCRIPTION**

The gametophyte stems are diminutive, ranging from 0.2 to 0.25 mm in diameter (Fig. 1), with the longest stem segment documented measuring at least 25 mm. Stem transverse sections (Fig. 1) show that cell diameters increase progressively from 4.6–9.3 µm at the periphery to 10.4–18.6 µm at center. The thickness of cell walls exhibits an opposite pattern, decreasing from the periphery (ca. 4 µm) toward the center (ca. 2 µm) of the stems. There is no evidence of specialized conducting tissue. Cells of the stem are 70–130 µm long, with no indication of radial variation in cell length (Fig. 2). Stem transverse sections display frequently occurring uneven protrusions from the outermost cell layer (Fig. 3), that are interpreted as anticalinal walls of a poorly preserved epidermal layer. This feature indicates the presence of a hyalodermin (outer layer of stem composed of large empty cells) characterized by thin external pericalinal cell walls, which did not fully preserve.

Leaves are anisophyllous and densely cover the stem. The bases of successive leaves are typically spaced at about 60 µm (Fig. 4), lending an imbricate appearance. Instances of more widely spaced leaves (ca. 100 µm) are associated with stem branching. In longitudinal sections, leaf bases can be seen running close to the stem, while their distal tips diverge outward at approximately 45°. The leaves are arranged in three ranks (vertical rows), including a rank of larger underleaves (amphigastria) and two ranks of smaller lateral leaves (Figs. 5–7). Underleaves appear carinate (shaped like the keel of a boat) in transverse section with a bulbous trough occupying the angle (Fig. 5). The bases of underleaves are wrapped around the stem for more than half of its circumference, covering the ventral margins of the lateral leaves, which can be seen tucked into the trough of the underleaf (Fig. 6).

The leaves are unistratose and ecostate (Figs. 4, 7). Lateral leaves are inserted incubously (as shown in Fig. 4, the ventral margins of lateral leaves are attached closest to the immediately preceding underleaf, as opposed to farthest from it), whereas underleaves are inserted transversely (Fig. 4). Underleaves are 330–390 µm wide at the base and at least 1.1 mm long. Lateral leaves are 320 µm wide and at least 630 µm long. Total leaf length was not measurable due to poor preservation of the distal areas of the leaves. Leaf lamina cells are roughly isodiametric (Figs. 4, 7, 8). They measure 5.8–8.1 µm in transverse section, 8.7–15.1 mm in longitudinal section and 11.5–14.4 mm in paradermal section. Concave trigones are visible in paradermal section (Fig. 18). Exceptions to leaf cell size are found at the leaf base and margin. Leaf margin cells appear slightly larger (ca. 11.5 µm) than laminar cells when leaves are viewed in transverse section (Fig. 11). Slightly inflated cells at the bases of leaves measure ca. 11.5–23.1 mm in diameter in longitudinal section (Fig. 9) and form a 2–3 cell wide band across the leaf base. The basalmost leaf cells, associated with the point of attachment to the stem, are elongated and have dimensions similar to those of adjacent stem cells (Fig. 10). A complete reconstruction of leaf shape or margin was not possible due to consistent poor preservation of distal leaf tissue. The incomplete preservation of leaves is likely related to taphonomy (fluvial transport) and to their delicate, unistratose anatomy. These same factors could have contributed to the absence of a sporophyte within the fossil assemblage.

The stems branch frequently at irregular intervals of 0.5–1.5 mm (Figs. 15, 19). One branch (Fig. 16) exhibits a finely preserved apical region covered in leaf primordia. Branches develop in the dorsal axil of lateral leaves and diverge at ca. 90° (Figs. 13, 17, 19). Branch primordia feature a dome-like covering which produces a collar typical of the branching of certain jungermanniopsid liverworts. In *Appiana*, the dome is one or more cell layers thick (Figs. 14, 20). The position of branches and the anatomy of the collar dome are characteristic of the Anomoclada-type branching (Crandall-Stotler, 1969).

Rhizoids are unicellular and form fascicles associated with the bases of underleaves and the axils of lateral leaves. Rhizoid fascicles arising in the basiscopic flank of branches are interpreted as occurring in the axil of lateral leaves, as branching has been shown to occur in the dorsal axil of lateral leaves (Figs. 12, 14, 20). Underleaves also bear fascicles of rhizoids below their bases (Fig. 13). Individual rhizoids are ca. 12 µm in diameter and extend at least 180 µm from the point of attachment.

**DISCUSSION**

The preservation of the Appian Way specimens by cellular permineralization provides anatomical detail that allows for comparisons with extant ecostate mosses and leafy liverworts. The fossil gametophytes exhibit three-ranked helical phyllotaxis (two ranks of lateral leaves and one rank of underleaves), isodiametric leaf cells, an apparent lack of conducting tissues (thin-walled water-conducting cells as seen in *Haplomitrium* are possible), unicellular rhizoids, and a branching mode specific to leafy liverworts. These features, considered collectively, indicate that the Appian Way fossils are leafy hepatics. Furthermore, they combine a series of characters (discussed below) that set them apart from all known extant and extinct leafy hepatics: (1) fasciculate rhizoids associated with leaves in all three ranks; (2) anisophyllly, with three-ranked phyllotaxis and
underleaves larger than the lateral leaves; and (3) endogenous, *Anomocladia*-type lateral branching. This unique combination of characters warrants the creation of a new genus, *Appiana*, to accommodate the Appian Way specimens.

The fossil record of leafy liverworts consists of only five other extinct genera, none of which would have provided an adequate taxonomic placement for the Appian Way fossils. The amount of detail and types of characters preserved vary widely between these fossil genera (Table 1). *Jungermannites* (Goppert) Steere is defined by a diagnosis that is too inclusive to be taxonomically insightful given the amount of character detail known for *Appiana*. *Jungermannites* is defined only by the presence of lateral leaves and of a terminal sporophyte with a long seta. The genus is used exclusively for leafy liverwort fossils that preserve very little morphological detail (Steere, 1946). The remaining four fossil taxa all exhibit characters that exclude...
to leaf bases and their grouping into fascicles may reflect a phylogenetic signal. The association of fasciculate rhizoids with basal lineages is one of the few proposed morphological evolutionary trends that have been supported by molecular data (Davis, 2004). Despite this substantiation, exceptions to this rule found throughout numerous lineages of the Jungermanniidae (e.g., Balantiopsidaceae, Antheliaceae, Grolleaceae; Crandall-Stotler et al., 2009) hamper the narrowing of the taxonomic circumscription of Appiana based on rhizoid characters. Rhizoids associated with both lateral and underleaves are an exceedingly rare occurrence, being found in only a small number of extant basal genera (e.g., Herberta, Lophochaete, Zoopsis). Schuster (1984) proposed that early leafy liverworts would have had rhizoids associated with all leaf ranks (although not necessarily fasciculate), which could suggest that Appiana has affinities with such basal lineages as the Porellales and Ptilidiales. Further, to the best of our knowledge, no extant or fossil liverwort is characterized by fasciculate rhizoids attached in the axil of lateral leaves.

Like many jungermanniid liverworts, Appiana has anisophyllous stems with a three-ranked phyllotaxis. The underleaves of Appiana can be loosely compared with the large underleaves seen in some members of extant Porellales. However, Appiana is unusual in having underleaves that are larger than the lateral leaves, a characteristic that has not been documented in any leafy hepatic. The phylogenetic significance of the size relationship between lateral leaves and underleaves has been discussed by Schuster (1984), who suggested that size reduction in underleaves could have been driven by evolution of a prostrate growth habit, whereby the third rank was positioned on the lower side of horizontal stems and, thus, had reduced photosynthetic importance. Considering Schuster’s reasoning, it seems unlikely that Appiana, with comparatively larger underleaves, had a prostrate habit with underleaves in a ventral position. Alternatively, if the underleaves had evolved to occupy the dorsal position on the stem, they would have limited the photosynthetic capacity of lateral leaves (Fig. 5). As such a growth habit does not appear advantageous, we suspect that Appiana was
Figs. 5–7. *Appiana sillettiana* gen. et sp. nov. Serial transverse sections of gametophyte stem with lateral leaves and underleaves demonstrating phylloaxis: Fig. 5 is proximal and Fig. 7 distal; tracings on right panel are blue for underleaves and yellow for lateral leaves. 5. Leaves are arranged in three ranks, including a rank of larger underleaves and two ranks of smaller lateral leaves; underleaves are carinate with a rounded trough (arrowhead). AW660 Gtop #12. 6. Underleaves wrap around the stem for nearly half of its circumference, covering a margin of each lateral leaf; note successive underleaf (U2). AW660 Gtop #15. 7. Successive lateral leaves are apparent (L2) as the underleaf (U2) displays characteristic trough (arrowhead). Previous lateral leaves (L1) and underleaf (U1) have diverged away from axis. AW660 Gtop #20. Scale bar for all images = 50 µm.
Figs. 8–17. *Appiana sillettiana* gen et sp. nov. gametophyte. 8. Leaf paradermal section; in conjunction with Figs. 4 and 7, this demonstrates isodiametric leaf cells; note trigones. AW660 Gtop #145. 9. Stem and leaf longitudinal section; note inflated basal leaf cells. AW660 Gtop #1. 10. Leaf base with elongated cells associated with point of attachment. AW660 Gtop #57. 11. Leaf transverse section with inflated marginal cell (arrow). AW660 Gtop #15. Scale bar = 10 mm. 12. Fascicled, unicellular rhizoids. AW660 Gtop #145. 13. Rhizoid fascicle arising between successive underleaves (arrowheads); distal portions of the rhizoids are preserved in the left half of the image. Arrow indicates developing branch (with leaf primordia) on dorsal side of stem (i.e., opposite the underleaves). AW660 Gtop #18. 14. Stem longitudinal section with rhizoid fascicle basiscopic to branch collar dome (arrow). AW660 Gtop #146. 15. Stem longitudinal section showing irregularly spaced branches (arrowheads); the arrow indicates the location of a branch observed in an adjacent plane of section. AW660 Gtop #4. Scale bar = 100 µm. 16. Longitudinal section of stem apex with leaf primordia. AW660 Gtop #3. 17. Branch development in axil of lateral leaf (arrowhead). AW660 Gtop #1. Scale bars = 50 µm, except where otherwise noted.
accord with the common practice in paleobotany whereby taxa exhibiting novel combinations of characters are used to define new families or even higher taxa (e.g., orders Calamopityales, Buteoxygenales; Taylor et al., 2009; family Kaplanopteridaceae; Tomescu et al., 2006). Specifically, in the case of leafy liverworts, Schuster and Janssens (1989) created a new family to accommodate Dieteritina, a fossil taxon that also lacked the sporophyte.

**Conclusions**—Leafy liverwort gametophytes are anatomically preserved by cellular permineralization in marine carbonate nodules of the middle Eocene Oyster Bay Formation, at Appian Way on Vancouver Island. These fossils are a significant addition to the limited fossil record of liverworts and one of the few examples of anatomical preservation in the group. Additionally, they exhibit a combination of characters unknown among extinct and extant hepatics: (1) three-ranked, helical phyllotaxis with underleaves larger than the lateral leaves; (2) fascicled rhizoids associated with the leaves of all three ranks; and (3) Anomoclada-type endogenous branching. This combination of characters warrants the creation of a new genus (*Appiana*) and family (Appianaceae) of jungermanniid liverworts. By adding hepatics to the already diverse Appian Way fossil assemblage, *Appiana* broadens the taxonomic range of the Oyster Bay Formation flora.

**LITERATURE CITED**


