

**A NEW FAMILY OF LEAFY LIVERWORTS FROM THE MIDDLE EOCENE
 OF VANCOUVER ISLAND, BRITISH COLUMBIA, CANADA¹**

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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) *Anomoclada*-type endogenous branching.
- *Conclusions:* A new liverwort family, Appianaceae 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; jungermanniid; liverwort.

Fundamental aspects of the evolution of plant structure can be derived from the study of living plants. Comparative analyses 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 gleicheniaceus (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 coalified 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 sharonae* Hernick et al., a thalloid liverwort. The fossils are preserved as coalified 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 coalified 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 coalified 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 Sphaerocarpaceae (Harris, 1939; Krassilov and Schuster, 1984). Additional leafy liverworts from the Late Jurassic have been described, including *Cheirorhiza brittae* 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 Rouse, 1994). Within the nodules, the plant material is preserved by cellular permineralization in calcium carbonate. The nodules also contain marine fauna including corals, echinoderms, 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 framboids (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 framboids and is due to the reduction of marine sulphates by anaerobic bacterial decomposers 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 8800 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 *Anomoclada*-type lateral branching.

Etymology—*Appiana* is 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 anticlinal walls of a poorly preserved epidermal layer. This feature indicates the presence of a hyalodermis (outer layer of stem composed of large empty cells) characterized by thin external periclinal 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).

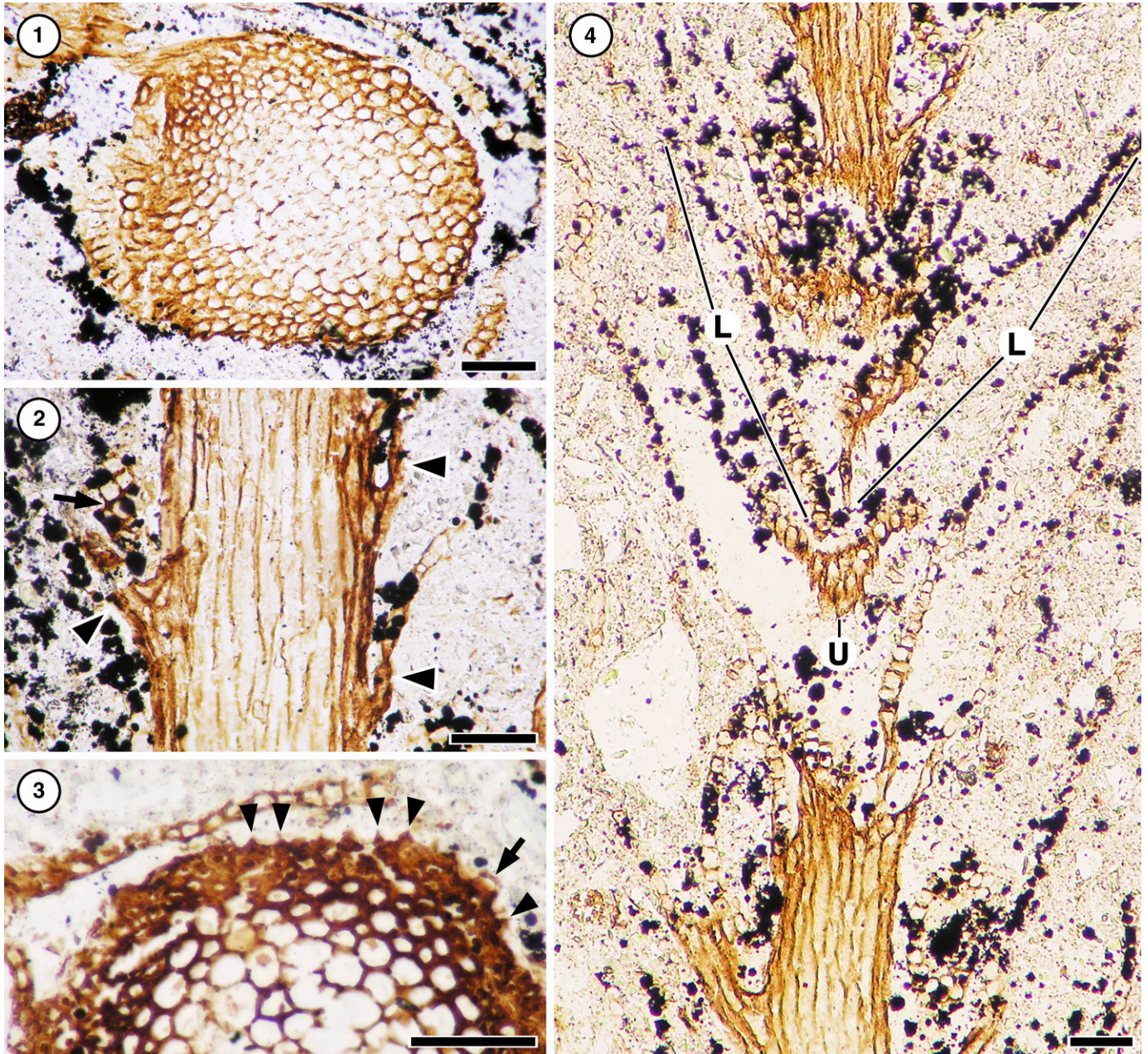
The leaves are unistratose and ecostate (Figs. 4, 7). Lateral leaves are inserted incubously (as shown in Fig. 4, the ventral margins of lateral leaves are attached closest to the immediately preceding underleaf, as opposed to farthest from it), whereas underleaves are inserted transversely (Fig. 4). 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 mm) 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 jungermanniopsisid 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) anisophylly, with three-ranked phyllotaxis and



Figs. 1–4. *Appiana sillettiana* gen. et sp. nov. Gametophyte stem with lateral leaves and underleaves. **1.** Transverse section of stem showing progressive increase in cell diameter and decrease in cell wall thickness from periphery to center. AW101 Ebot #93(c). **2.** Longitudinal section of stem with three leaf bases (arrowheads); cell length shows no radial variation within the stem. Note trigones of cells in the small scrap of leaf paradermal section visible above the axil of the leaf base at left (arrow). AW660 Gtop #61. **3.** Transverse section of stem and leaf (top left); protrusions from the outermost cell layer (arrowheads) represent remnants of anticlinal walls of a poorly preserved hyalodermis; arrow indicates a preserved hyalodermal cell. AW660 Gtop #20. **4.** Longitudinal section of stem with closely spaced leaves; U: carinate trough of underleaf in transverse section; L: longitudinal sections of lateral leaves. The ventral margins of lateral leaves (L) are attached closest to the immediately preceding underleaf (U), as opposed to farthest from it, consistent with incubous insertion. Note strings of black pyrite framboids indicating the positions of leaf cells. AW660 Gtop #63. Scale bars = 50 μ m.

underleaves larger than the lateral leaves; and (3) endogenous, *Anomoclada*-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

TABLE 1. Diagnostic characters used in descriptions of extinct genera of leafy liverworts (Harris, 1939; Steere, 1946; Krassilov, 1973; Schuster and Janssens, 1989) and comparison with *Appiana*.

Genera	Leaf arrangement	Leaf morphology	Leaf cells	Rhizoids	Branching	Sporophyte	Growth habit and organization
<i>Naiadita</i>	Helical	Narrow base, rounded apex; costate	Rectangular; thin walled	Unicellular	Infrequent	Spherical capsule; wall one cell thick	Stem not more than 2 cm long
<i>Diettertia</i>	Two ranked	Oval; ecostate	Elongate; hexagonal; thick walled	—	Frequent; intercalary and terminal	—	Dorsiventral; stem 1.0–1.5 mm wide
<i>Jungermannites</i>	Two or three ranked; underleaves absent or present	—	—	—	—	Terminal; capsule 4-valved on long seta	Bilateral organization, leafy stem
<i>Cheirorhiza</i>	—	Lateral: rounded and asymmetrical; bisected; entire margin. Underleaves: decurrent; lobate to trichoid	Polygonal	Unicellular or septate	Intercalary and terminal	—	Leafy stem; 1 mm wide
<i>Laticaulina</i>	—	Lateral: bisected. Underleaves: massive	Rectangular or polygonal	—	—	—	Broad, flattened; 1 mm wide
<i>Appiana</i>	Helical; three ranked	Underleaves larger than lateral leaves	Isodiametric	Unicellular; fasicled	Frequent; <i>Anomoclada</i> type	—	Stem likely upright; 0.25 mm wide

the Appian Way fossils: helical, but not three-ranked, leaf arrangement in *Naiadita*, absent or sparse rhizoids in *Diettertia* and *Cheirorhiza*, and a broad and flattened stem in *Laticaulina* (Krassilov, 1973; Krassilov and Schuster, 1984). Thus, distinct differences prevent inclusion of the Appian Way fossils in the above fossil taxa and further warrants the creation of the new genus.

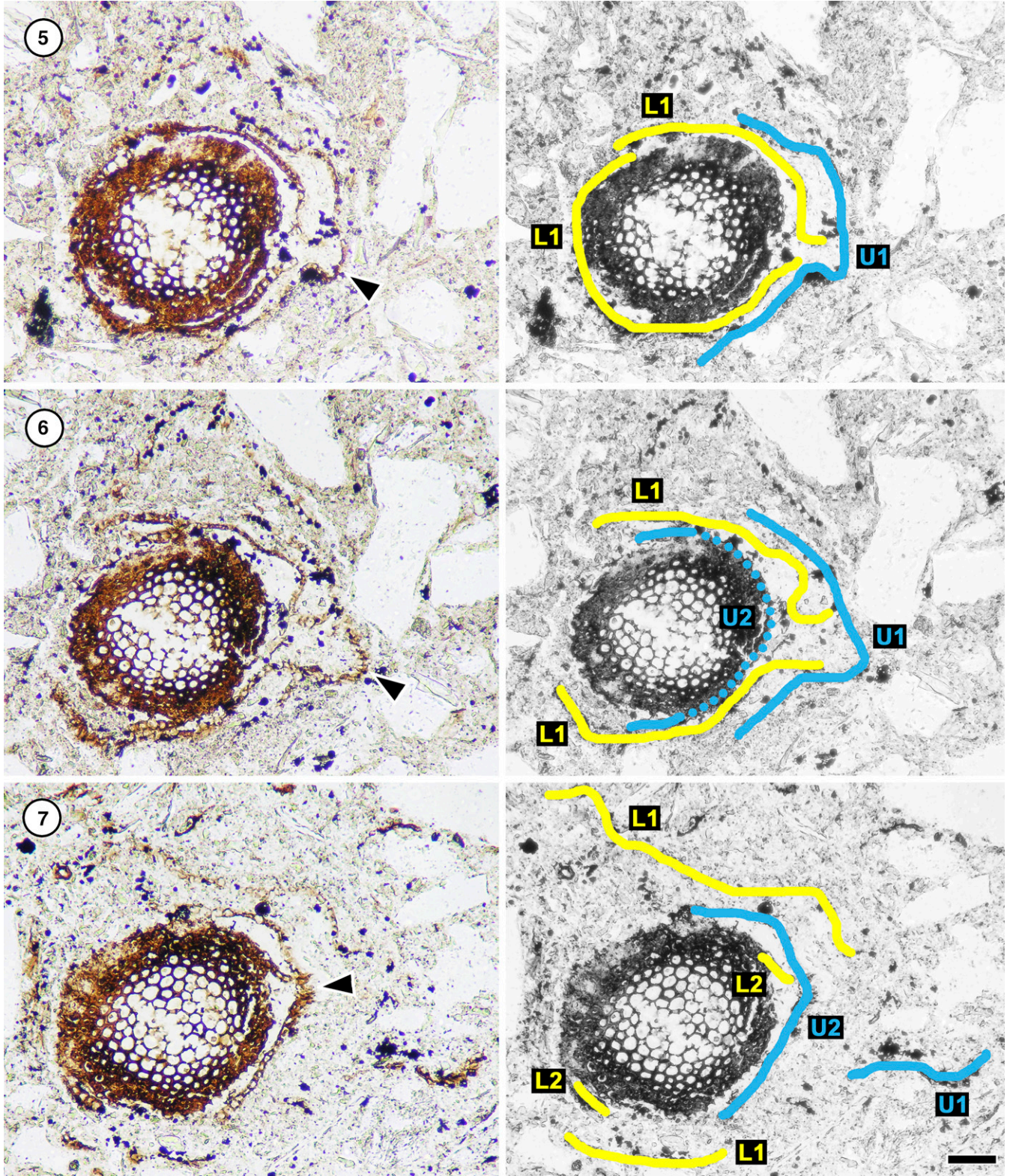
Aside from a small number of fossils assigned to *Jungermannites*, all Cenozoic leafy liverworts have been assigned to living genera (Chandra, 1995; Taylor et al., 2009). The great degree of homoplasy of many liverwort gametophyte characters and the lack of sporophyte characters in the Appian Way fossils make their taxonomic placement beyond the subclass rank (Jungermanniidae) difficult. Subclass Jungermanniidae includes leafy, anisophyllous plants with two ranks of ventral leaves and one rank of morphologically different underleaves and does not specifically exclude any type of branching or rhizoid distribution habit (Crandall-Stotler et al., 2009).

Branching in *Appiana* conforms to the *Anomoclada* type (as described by Crandall-Stotler, 1969, 1972)—it is endogenous (arising from cells beneath the stem epidermis), has an associated collar structure with branch primordia and arises from the dorsal area of lateral leaf axils. Seemingly rare, the *Anomoclada*-type branching is reported in a small number of genera (e.g., *Evianthus* and *Anomoclada*) in the most recent liverwort classification (Crandall-Stotler et al., 2009) and, thus, is not diagnostic for any subfamily or higher ranked taxon. A survey of the phylogenetic distribution of different branching types in the same recent classification indicates that lateral exogenous branching (whereby branches originate from cells of the stem epidermis) is generally associated with basal leafy hepatic lineages and shows a progression toward endogenous branching in more derived lineages. Based on this, we could conclude that *Appiana*, with its endogenous branching, should be placed among the more derived Jungermanniidae, therefore, potentially among the Jungermanniales.

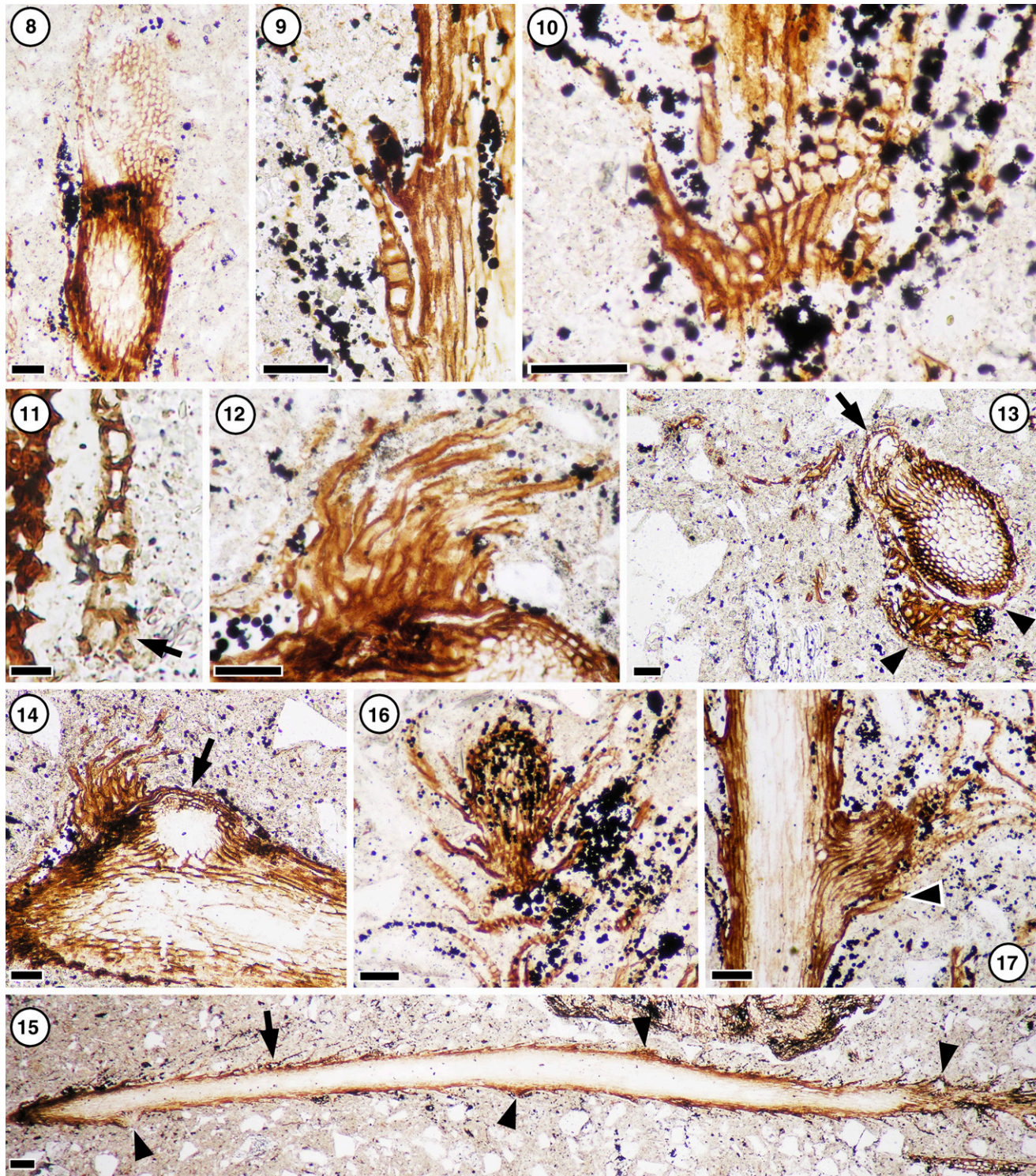
The rhizoids of *Appiana* are unicellular and form fascicles attached at the base of underleaves and in the axil of lateral leaves. According to Schuster (1984), the restriction of rhizoids

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 fascicled rhizoids attached in the axil of lateral leaves.

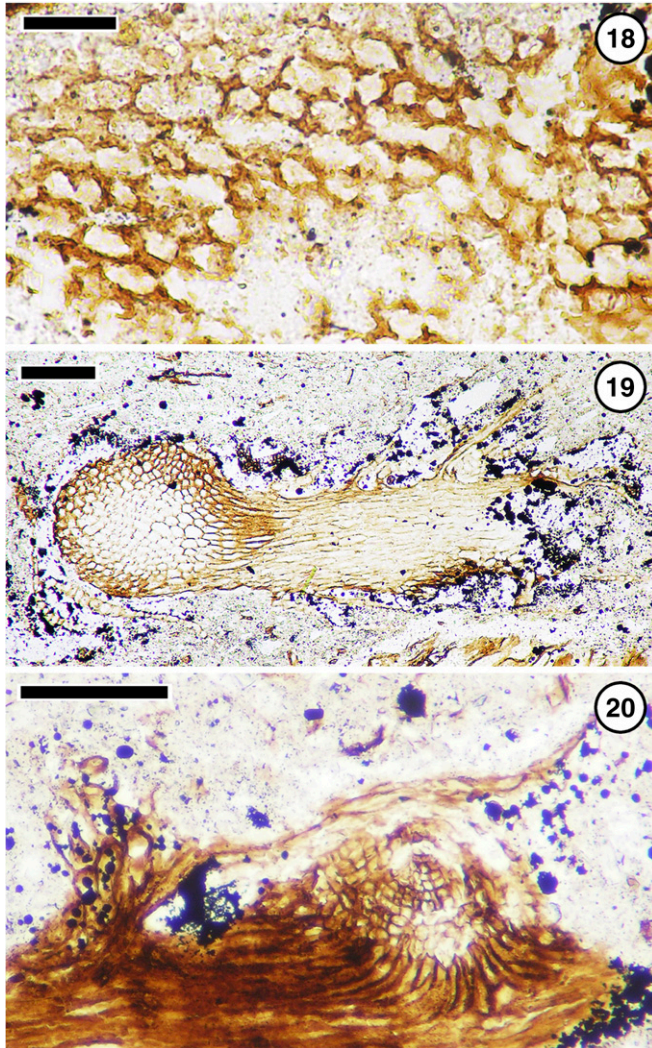
Like many jungermanniid liverworts, *Appiana* has anisophyllous stems with a three-ranked phyllotaxis. The underleaves of *Appiana* can be loosely compared with the large underleaves seen in some members of extant Porellales. However, *Appiana* is unusual in having underleaves that are larger than the 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 then 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 phyllotaxis; 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 μ m.



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 μ m. **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 μ m. **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 μ m, except where otherwise noted.



Figs. 18–20. *Appiana sillettiana* gen et sp. nov. gametophyte. **18.** Leaf paradermal section showing concave trigones (detail of Fig. 8). AW660 Gtop #145. Scale bar = 50 μ m. **19.** Stem (transverse section) with attached branch (longitudinal section). AW101 Etop #91(c). Scale bar = 100 μ m. **20.** Branch primordium enclosed by collar. Rhizoid fascicle (left) basicopic to the branch primordium. AW660 Gtop #145. Scale bar = 100 μ m.

characterized by an erect growth habit. Regardless of the evolutionary processes leading to the development of enlarged underleaves, the uniqueness of this condition in *Appiana* sets it apart from all other hepatic taxa.

Is a new family necessary to accommodate *Appiana*? The relative size of the underleaves of *Appiana*, paired with the distribution of its rhizoids, are not consistent with any known leafy liverwort family. The absence of data on the sporophyte, which prevents reconstruction of the whole plant, would typically weigh against creation of a new family. However, (1) the gametophytes of *Appiana* preserve enough anatomical and morphological details that exclude this genus from all known families, and (2) even if known, the sporophyte would not resolve familial placement, as concluded from a review of the variation of sporophyte characters among modern families as presented by Crandall-Stotler et al. (2009). These facts support the creation of a new family to accommodate *Appiana*'s unique features. This is in

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, Buteoxytonales; 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 *Diettertia*, 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) *Anomoclad*-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.

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