RESEARCH ARTICLE

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Exploring the fossil history of pleurocarpous mosses: Tricostaceae fam. nov. from the Cretaceous of Vancouver Island, Canada[1]

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PREMISE OF THE STUDY: Mosses, very diverse in modern ecosystems, are currently underrepresented in the fossil record. For the pre-Cenozoic, fossil mosses are known almost exclusively from compression fossils, while anatomical preservation, which is much more taxonomically informative, is rare. The Lower Cretaceous of Vancouver Island (British Columbia, Canada) hosts a diverse anatomically preserved flora at Apple Bay. While the vascular plant component of the Apple Bay flora has received much attention, the numerous bryophytes identified at the locality have yet to be characterized.

METHODS: Fossil moss gametophytes in more than 20 carbonate concretions collected from the Apple Bay locality on Vancouver Island were studied in serial sections prepared using the cellulose acetate peel technique.

KEY RESULTS: We describe *Tricosta plicata* gen. et sp. nov., a pleurocarpous moss with much-branched gametophytes, tricostate plicate leaves, rhizoid-bearing bases, and delicate gametangia (antheridia and archegonia) borne on specialized branches. A new family of hypnanaean mosses, Tricostaceae fam. nov., is recognized based on the novel combination of characters of *T. plicata*.

CONCLUSIONS: *Tricosta plicata* reveals pleurocarpous moss diversity unaccounted for in extant floras. This new moss adds the first bryophyte component to an already diverse assemblage of vascular plants described from the Early Cretaceous at Apple Bay and, as the oldest representative of the Hypnanae, provides a hard minimum age for the group (136 Ma).

KEY WORDS Bryophyta; Cretaceous; fossil; gametangia; Hypnanae; moss; pleurocarpous; tricostate

Bryophytes predate the vascular plants and the fossil record of mosses can be traced back in time for at least 330 million years, into the Early Carboniferous (Hübers and Kerp, 2012). However, the long history of mosses is not matched by a corresponding richness of the fossil record of the group, especially for pre-Cenozoic times. Compared to an estimated 13 000 extant moss species (Goffinet et al., 2009) and to relatively numerous Cenozoic fossil mosses (many of which represent modern families, genera, and species; e.g., Miller, 1984; Taylor et al., 2009), the pre-Cenozoic moss fossil record, with only ca. 70 described species (e.g., Oostendorp, 1987; Ignatov, 1990; Taylor et al., 2009), represents a small fraction of known moss diversity. Considered in light of the long evolutionary history of the group, the marked scarcity of pre-Cenozoic mosses indicates that we are still missing most of the diversity representing the first 270 million years (to use a conservative estimate) of evolution in the group. Yet, only by discovering and characterizing this hidden diversity will we be able to understand patterns of moss diversity and evolution in deep time, with all their implications for understanding extant moss diversity. Paleobotanical studies of fossil mosses are our only way to access this hidden world of biological diversity that would remain unattainable otherwise.

Pre-Cenozoic fossil mosses are rarely placed into modern groups or such taxonomic assignments are tentative. Nevertheless, some of these fossils resemble modern groups or well-defined extinct lineages, demonstrating a potential to contribute to moss systematics. For example, the oldest unequivocal moss fossils represent leaf fragments from the Lower Carboniferous (Middle Mississippian,

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late Visean) of eastern Germany (Hübers and Kerp, 2012), some of which resemble the extinct Protosphagnales Neuburg, perhaps representing forms ancestral to both sphagnalean and nonsphagnalean mosses. In the Upper Jurassic of Russia, *Baigulia* Ignatov, Karasev et Sinitsa and *Bryokhutuliinia ingodensis* Ignatov show highly branched gametophytes and lateral bud-like structures interpreted as gametangial shoots (Ignatov et al., 2011). These fossil mosses, along with *Vetiplanaxis* N.E. Bell, are the only pre-Cenozoic that have putative affinities with the pleurocarpous mosses—a large group of mosses in which sporophytes are borne on reduced lateral shoots of gametophyte stems.

To date, Cretaceous moss diversity consists of less than ten genera (e.g., Debey and von Ettingshausen, 1859; Berry, 1928; Krassilov, 1973, 1982; Ignatov et al., 2011; Ignatov and Shcherbakov, 2011a), few of which preserve enough detail to support ordinal- or family-level placement. Species of *Vetiplanaxis*, a late Albian genus known from Burmese amber, are most comparable to the pleurocarpous Hypnodendrales (Hedenäs et al., 2014). Charcoalified gametophytes and sporophytes of *Campylopodium allonense* Konopka, Herendeen et Crane (1998) and *Eopolytrichum antiquum* Konopka, Herendeen, Merrill et Crane (1997) from the late Santonian of Georgia (USA) are assigned unequivocally to the families Dicranaceae and Polytrichaceae, respectively. Overall, we currently have a very incomplete image of what Cretaceous mosses looked like or where they fit among bryophytes and, therefore, of what they could teach us about moss diversity and evolution over time.

In terms of modes of preservation, most of the moss fossil record is represented by carbonaceous compressions. Anatomically preserved pre-Cenozoic moss fossils are rare and, prior to this study, have been limited to cuticular preservation of Mississippian moss leaves (Hübers and Kerp, 2012); charcoalified Late Cretaceous gametophytes and sporophytes (Konopka et al., 1997, 1998); permineralized Permian gametophytes of *Merceria augustica* Smoot et Taylor (1986); and amber preservation of mid-Cretaceous gametophytes (Hedenäs et al., 2014).

There is a growing realization that exquisitely preserved plant remains are present in marine carbonate concretions from Jurassic, Cretaceous, Paleogene, and Neogene sediments worldwide (e.g., Stockey and Rothwell, 2006), many of which contain remains of anatomically preserved bryophytes (e.g., Steenbock et al., 2011; Tomescu et al., 2012). Here we describe an anatomically preserved Early Cretaceous moss based on abundant permineralized specimens from the Apple Bay locality (Vancouver Island, British Columbia, Canada). This moss is described as a new genus and species characterized by highly branched gametophytes with perigonia and perichaetia on short lateral, bud-like branches, and tricostate leaves, a trait not recognized in extant mosses and documented only in a few Mesozoic fossils. It is one of the most complete pre-Cenozoic fossil mosses to date and represents the earliest record for pleurocarpy, as well as a new family within superorder Hypnanae. Along with other tricostate mosses (fossil genus *Tricostium* Krassilov), this moss brings to light a once widespread aspect of moss morphological diversity unknown in the extant bryoflora.

MATERIALS AND METHODS

Numerous moss gametophyte shoots are preserved by cellular permineralization in >23 carbonate concretions, as part of an allochthonous fossil assemblage deposited in nearshore marine sediments (e.g., Stockey and Rothwell, 2009). The concretions were collected from sandstone (greywacke) beds exposed on the northern shore of Apple Bay, Quatsino Sound, on the west side of Vancouver Island, British Columbia, Canada (50°36’21” N, 127°39’25” W; UTM 9U WG 951068) (e.g., Stockey and Rothwell, 2009). The layers containing the concretions are regarded as Longarm Formation equivalents and have been dated by oxygen isotope analyses to the Valanginian (Early Cretaceous, ca. 136 Ma) (Stockey et al., 2006; D. Gröcke, personal communication, 2013).

This Early Cretaceous flora includes lycophytes, equisetophytes, at least 10 families of ferns (Smith et al., 2003; Hernandez-Castillo et al., 2006; Little et al., 2006a, 2006b; Rothwell and Stockey, 2006; Stockey et al., 2006; Vavrek et al., 2006; Rothwell et al., 2014) and numerous gymnosperms (Stockey and Wiebe, 2008; Stockey and Rothwell, 2009; Klymiuk and Stockey, 2012; Rothwell and Stockey, 2013; Rothwell et al., 2014; Atkinson et al., 2014a, 2014b; Ray et al.,

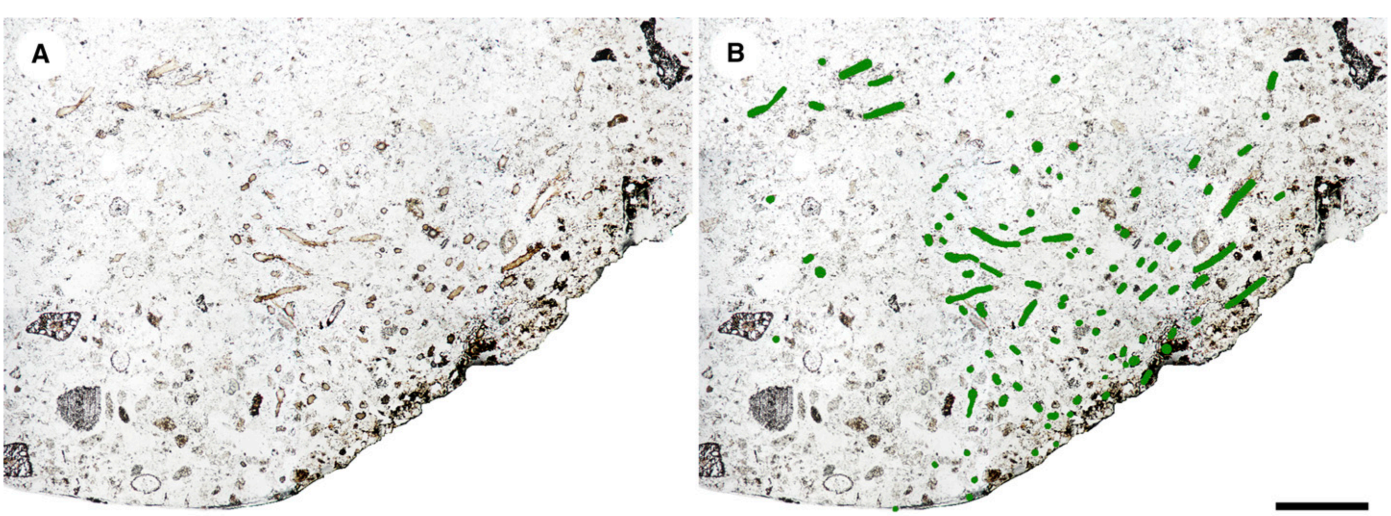

FIGURE 1 *Tricosta plicata* gen. et sp. nov. (A) Tuft of gametophytes seen in various planes of section. (B) Same image as A, with stems traced for clarity; scale bar = 2 mm; P13957 Btop #16.

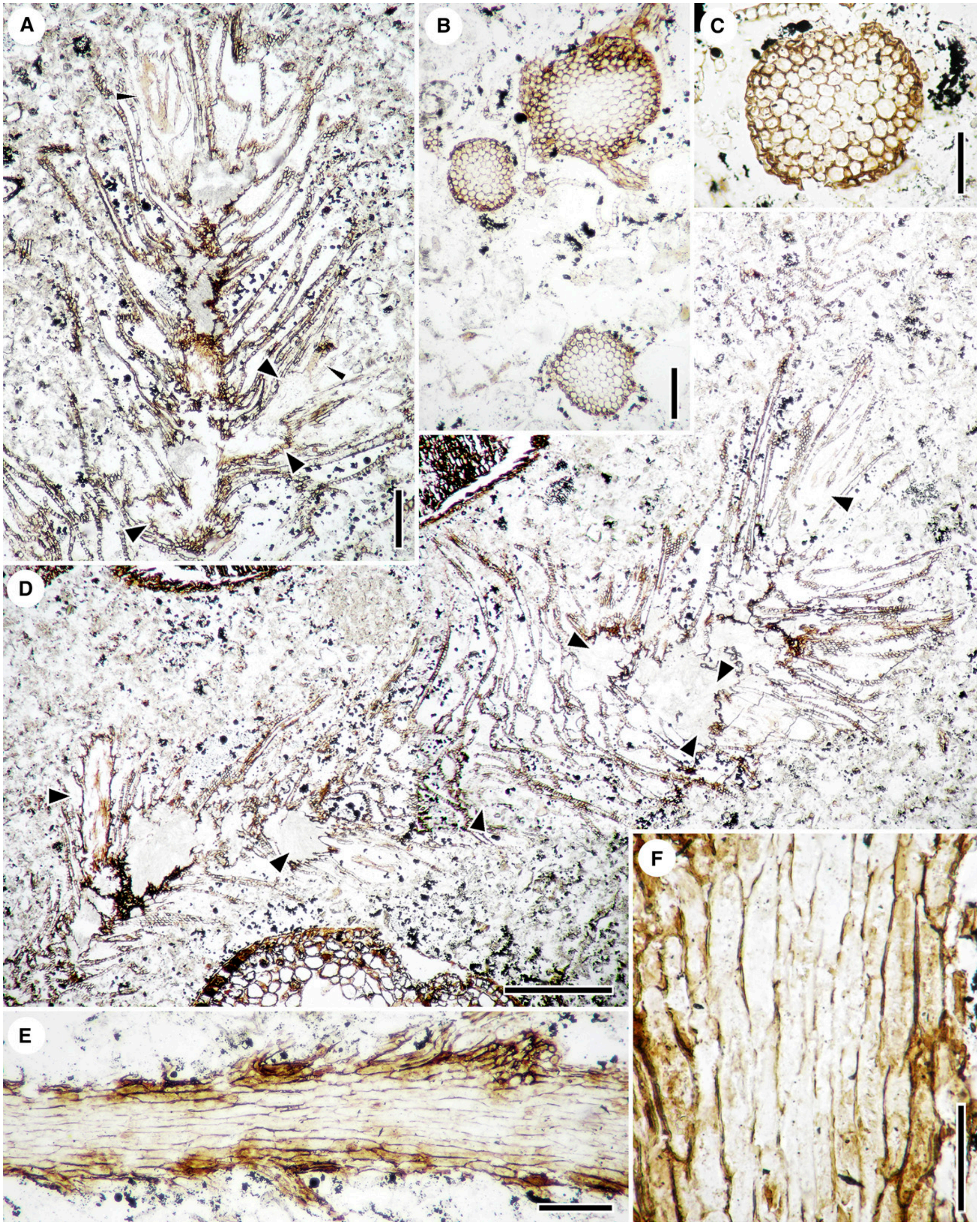

FIGURE 2 Habit, branching, shoot architecture, and stem anatomy of *Tricosta plicata* gen. et sp. nov. (A) Shoot in longitudinal section; narrow arrowheads show antheridia (upper arrowhead = sac; lower = stalk); all other arrowheads indicate perigonial branches; scale bar = 200 µm; P15425 C bot #38a. (B) Stems in transverse sections showing radially arranged cortical cells; scale bar = 100 µm; P13957 A #2. (C) Detail of B; note few, scattered, narrow cells near stem center; scale bar = 50 µm; P13957 A #2. (D) Composite image of much-branched shoot in longitudinal section; arrowhead at far left shows vegetative branch; all other arrowheads represent positions of perigonial branches; scale bar = 500 µm; P15425 Cbot #56a. (E) Stem in longitudinal section; scale bar = 100 µm; P13957 A #2. (F) Detail of E showing fusiform cortical cells; scale bar = 50 µm; P13957 A #2.

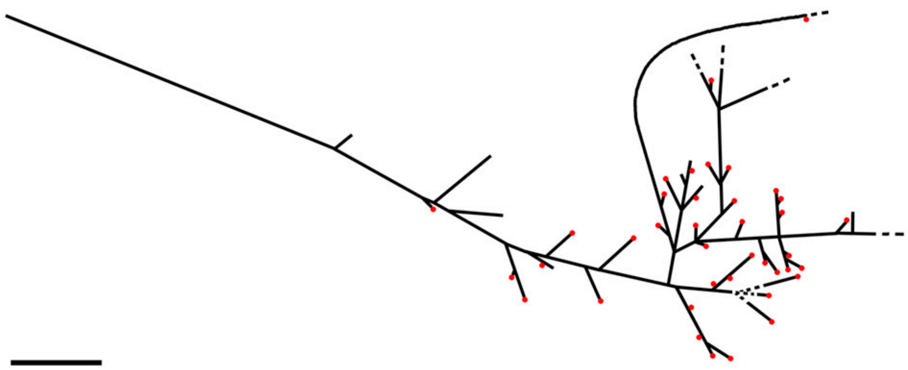

FIGURE 3 Branching architecture of *Tricosta plicata* gen. et sp. nov. reconstructed from serial sections (P15425 Cbot #1a-#92a); red dots represent perigonia; broken lines at apex of main stem indicate uncertain branch arrangement; other broken lines indicate saw cuts; scale bar = 1 mm.

2014; Klymiuk et al., 2015), as well as fungi (Smith et al., 2004; Bronson et al., 2013) and a lichen whose thallus shows modern heteromerous organization (Matsunaga et al., 2013). The Apple Bay flora is also emerging as the most diverse assemblage of fossil bryophytes known in the pre-Cenozoic worldwide (Tomescu et al., 2012), with leafy and thalloid liverworts, and more than twenty distinct moss morphotypes currently recognized. The mosses represent pleurocarpous, polytrichaceous, and leucobryaceous types, as well as several morphotypes of unresolved affinities including at least three distinct tricostate types.

Fossil-containing concretions were sliced into slabs and sectioned using the cellulose acetate peel technique (Joy et al., 1956). Slides were prepared using Eukitt, xylene-soluble mounting medium (O. Kindler GmbH, Freiburg, Germany). Micrographs were taken using a Nikon Coolpix E8800 digital camera on a Nikon Eclipse E400 compound microscope. Images were processed using Photoshop (Adobe, San Jose, California, USA). All specimens and preparations are housed in the University of Alberta Paleobotanical Collections (UAPC-ALTA), Edmonton, Alberta, Canada.

SYSTEMATICS

Class—Bryopsida Rothm.

Subclass—Bryidae Engl.

Superorder—Hypnanae W.R. Buck, Goffinet et A.J. Shaw.

Order—incertae sedis.

Family—Tricostaceae Shelton, Stockey, Rothwell et Tomescu, fam. nov.

Familial diagnosis—Gametophyte plants pleurocarpous. Stems regularly to irregularly pinnately branched, central conducting strand absent. Cortical cells thin-walled, hyalodermis or thick-walled outer cortex lacking. Paraphyllia absent. Leaves helically arranged, with three costae (tricostate) and conspicuous alar regions; laminal cells isodiametric to elongate. One to few gametangia borne on lateral specialized (perigonial, perichaetial) shoots.

Type genus—*Tricosta* Shelton, Stockey, Rothwell et Tomescu, gen. nov.

Generic diagnosis—Gametophytes much-branched; leaves isophyllous, partially overlapping and densely covering the stems. Branch primordia arising one or very few cells above subtending leaf. Multicellular rhizoids smooth. Leaves tricostate with costae symmetrically arranged, arising separately in leaf base and homogeneous in transverse section. Alar regions small; laminal cells smooth, thin-walled, elongate to oval, rhombic or repand, becoming isodiametric distally along lamina. Perigonia sessile on lateral branches, with one to a few antheridia; perigonial leaves like vegetative leaves but smaller. Perichaetia sessile, lateral along main stems, with few archegonia; perichaetial leaves different from vegetative leaves.

Etymology—*Tricosta* for the tricostate leaves.

Type species—*Tricosta plicata* Shelton, Stockey, Rothwell et Tomescu, sp. nov.

Specific diagnosis—Gametophytes in tufts at least 20 mm high, main stems once-pinnate. Branches inserted at 40-70° angles and 0.1-1.1 mm intervals. Stem diameter up to 0.2 mm, 10-14 cells across, epidermal cells narrower than cortical cells. Rhizoids at stem base ca. 24 μm in diameter. Leaves dense, 10-20 leaves per millimeter along stem; 3/8 phyllotaxis. Leaves straight, with 40-55° divergence angles, ca. 2.0 mm long, 0.5 mm wide at base, up to 0.9 mm wide midleaf. Leaves ovate, margins entire, apex acute. Leaves strongly plicate throughout; plications form adaxially concave longitudinal folds associated with costae. Leaf lamina ca. 18 cells wide between median and lateral costae, ca. 15 cells between lateral costae and leaf margin. Costae strong (ca. 0.9 of leaf length), median costa percurrent, up to 8 cells wide (cells 6-9 μm diameter), composed of three layers (1-2 layers distally). Abaxial cells of costa short, larger in diameter toward leaf apex. Median costa up to 55 μm wide, 30-40 μm thick; lateral costae 35 μm wide, 25-40 μm thick. Alar regions up to 9 cells wide; cells prominently inflated in transverse sections (diameter up to 34 μm), globose to elongate (up to 54 μm) in

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scale-like structure; subtending leaf base indicated by thin arrowhead; scale bar = 50 μm; P16435 Ctop #14. (F) Shoot transverse section showing leaf median costa (thick arrowhead) and one lateral costa (thin arrowhead) attached below point of leaf divergence; scale bar = 50 μm; P13131 Dtop #12c. (G) Serial section of F just above point of leaf divergence (with the median and lateral costa, arrowheads); scale bar = 50 μm; P13131 Dtop #13c. (H) Transverse section of shoot just above branching point showing unistratose and strongly plicate leaves with three costae per leaf (abaxial surface of one leaf underlined; c = costa); note paradermal section of part of leaf base (alar cells, arrowhead); scale bar = 100 μm; P13131 Dtop #3c.

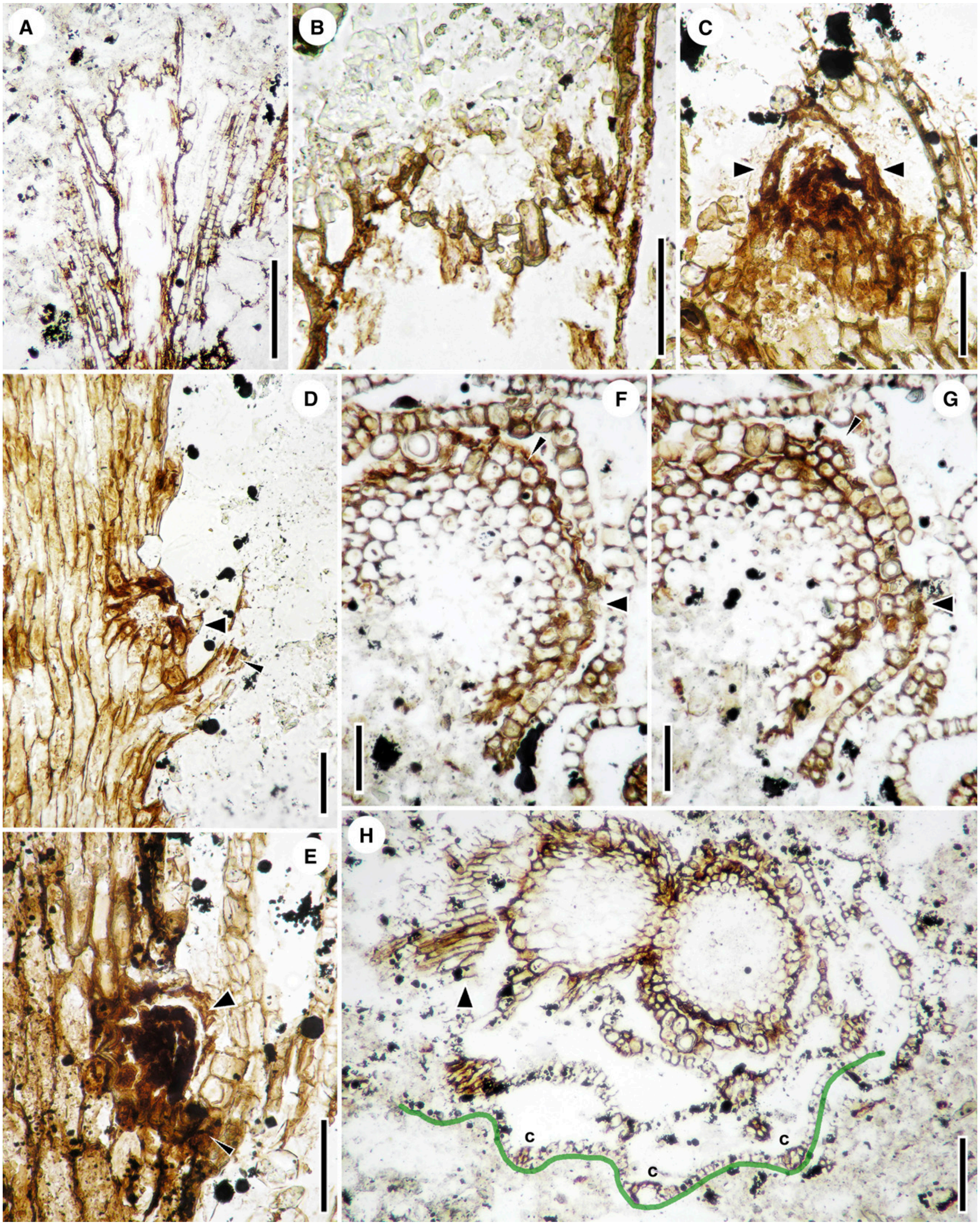

FIGURE 4 Shoot architecture and stem anatomy of *Tricosta plicata* gen. et sp. nov. (A) Vegetative shoot tip in longitudinal section; scale bar = 200 µm; P15425 Cbot #55a. (B) Detail of A showing a group of faintly colored apical cells; scale bar = 50 µm; P15425 Cbot #55a. (C) Darkened shoot tip showing scale-like leaf primordial (arrowheads); scale bar = 50 µm. P16435 Ctop #15. (D) Branch primordium in longitudinal section; subtending leaf distanced one or two cells from primordium; thick arrowhead indicates base of scale-like structure surrounding primordium; note dark hypha within costa (thin arrowhead); scale bar = 50 µm; P13957 Btop #25. (E) Branch primordium in longitudinal section directly subtended by leaf; thick arrowhead indicates

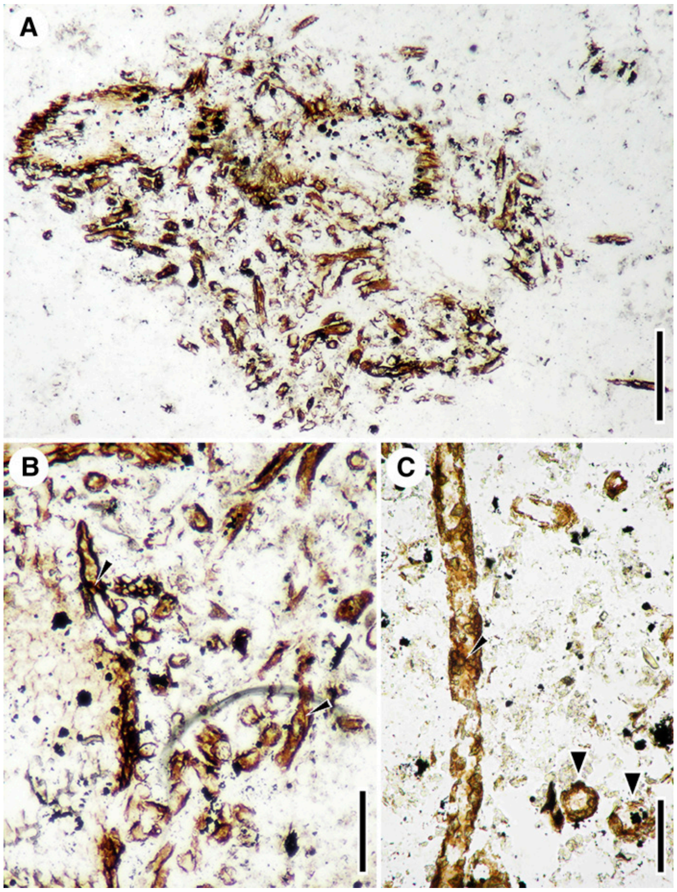

FIGURE 5 Rhizoids of *Tricosta plicata* gen. et sp. nov. (A) Base of gametophyte tuft showing several stems in transverse section bearing rhizoids; scale bar = 200 µm; P13256 Cbot #19. (B) Stem in transverse section (at left) surrounded by smooth-walled rhizoids; arrowheads indicate oblique end-walls within rhizoids; scale bar = 100 µm; P13256 Cbot #36. (C) Smooth-walled rhizoids in transverse (thick arrowheads) and longitudinal sections (at left); thin arrowhead indicates oblique end-wall; scale bar = 50 µm; P13256 Cbot #34.

longitudinal sections. Lamina ca. 13-19 µm thick; laminal cells forming mostly oblique files in base and midleaf; laminal cells form longitudinal files distally. Lamina cells at leaf base up to 5:1 (length/width ratio) and rectangular to rhombic; midleaf cells 2-3:1, up to 35 µm long and rhombic, repand or oval; distally, cells isodiametric and up to 23 µm diameter. Perigonial branches, ca. 1 mm long overall, bear ca. 4 erect leaves ca. 0.9 mm long, similar to vegetative leaves but with plications weak or absent on innermost leaves. Antheridia oblong, up to 350 µm long, borne on triseriate stalks. Perichaetia with few erect leaves; perigonial leaf cells narrow (ca. 4.5:1 and 40 µm long). Archegonia at least 200 µm long.

Etymology—Specific epithet *plicata* for the marked, characteristic plication of the leaves.

Holotype hic designatus—Gametophyte shoot in rock slab UAPC-ALTA P15425 C (slides Cbot series a) (Figs. 2A, D; 3; 4A, B; 6A, B; 7B, G; 8D-J; 9; 10).

Paratypes—UAPC-ALTA P13029 Dtop (Fig. 6B), P13131 Dtop (Figs. 4F-H; 6A, B; 8A), P13256 Cbot (Fig. 5), P13957 A (Figs. 2B, C, E, F), P13957 Btop (Figs. 1; 4D; 6A; 7H, I; 8C, K, L; 11), P15422 A (Fig. 7A, C-E), P16435 Ctop (Figs. 4C, E; 7F; 8B).

Locality—Apple Bay, Quatsino Sound, northern Vancouver Island, British Columbia (50°36’21” N, 127°39’25” W; UTM 9U WG 951068).

Stratigraphic position and age—Longarm Formation equivalent; Valanginian, ca. 136 Ma (Early Cretaceous).

Comments—*Tricosta plicata* also occurs in: UAPC-ALTA: P13032 F; P13171 E; P13172 G; P13174 C; P13175 E; P13218 F; P13311 I; P13308 J; P13483 C; P13616 E; P13957 C; P14560 B; P15393 B; P15422 B; P15800 C; P17515 B.

DESCRIPTION

Habit, branching, shoot architecture, and stem anatomy—*Tricosta plicata* is represented by more than 100 distinct gametophyte shoots. Gametophytes are diminutive, solitary or tufted (one tuft measures ca. 22 mm in height; Fig. 1). The most completely preserved individual shoot, whose branching architecture was reconstructed based on serial sections, is a fertile fragment 9 mm long (Fig. 2D). The base of this shoot is characterized by more widely spaced leaves and a thicker stem, while the apical region bears more densely spaced leaves on a narrower stem (Fig. 3). The incompletely preserved tip is flanked by perigonia (Fig. 3). Branching is frequent, irregularly to regularly pinnate, and roughly complanate. Branches are inserted at intervals of 0.1-1.1 mm and at 40-70° angles (Figs. 2A, D; 3). The basal 3.8 mm of the shoot bears no branches. Most branches along the main stem are relatively short, up to 0.85 mm long and unbranched. However, one lateral from the main stem generates a complex branching system perpendicular to the main stem and which bears four orders of branching, surpassing the main stem in length (Fig. 3).

Stem diameters range from 0.2 mm basally to 0.12 mm apically (with lateral branch diameters consistently smaller than main stem diameters) and the stems are ca. 10-14 cells across (Figs. 2B, C). Transverse sections show an epidermis of cells 16-23 µm in diameter and a cortex of slightly larger cells, 16-35 µm in diameter, with evenly thickened walls, circular to polygonal in shape (Figs. 2B, C). Stems occasionally bear one to a few narrow cells (5-12 µm in diameter) near the center but show no clear organization into a central conducting strand (Fig. 2C). Longitudinal sections show fusiform cortical cells 57-75 µm long and up to 18-23 µm wide (Figs. 2E, F). Epidermal cells are 35-60 µm long.

Vegetative shoot tips are incompletely preserved and show variation in preservation. The tips exhibit either large cells, faint in color (Figs. 4A, B), or small cells, darker in color (Fig. 4C)—the different colors may indicate different states of decomposition. Some of the shoots show leaf primordia (Fig. 4C) and branch primordia (Figs. 4D, E). Branch primordia occur in leaf axils, separated by at least one cell from their subtending leaf, and slightly sunken in the stem tissue. They are dome-shaped, up to 60 µm wide and 40 µm tall. Each branch primordium is covered by at least one over-arching scale-like structure (Figs. 4D, E). Preservation precludes resolving the origin of these structures, i.e., whether the

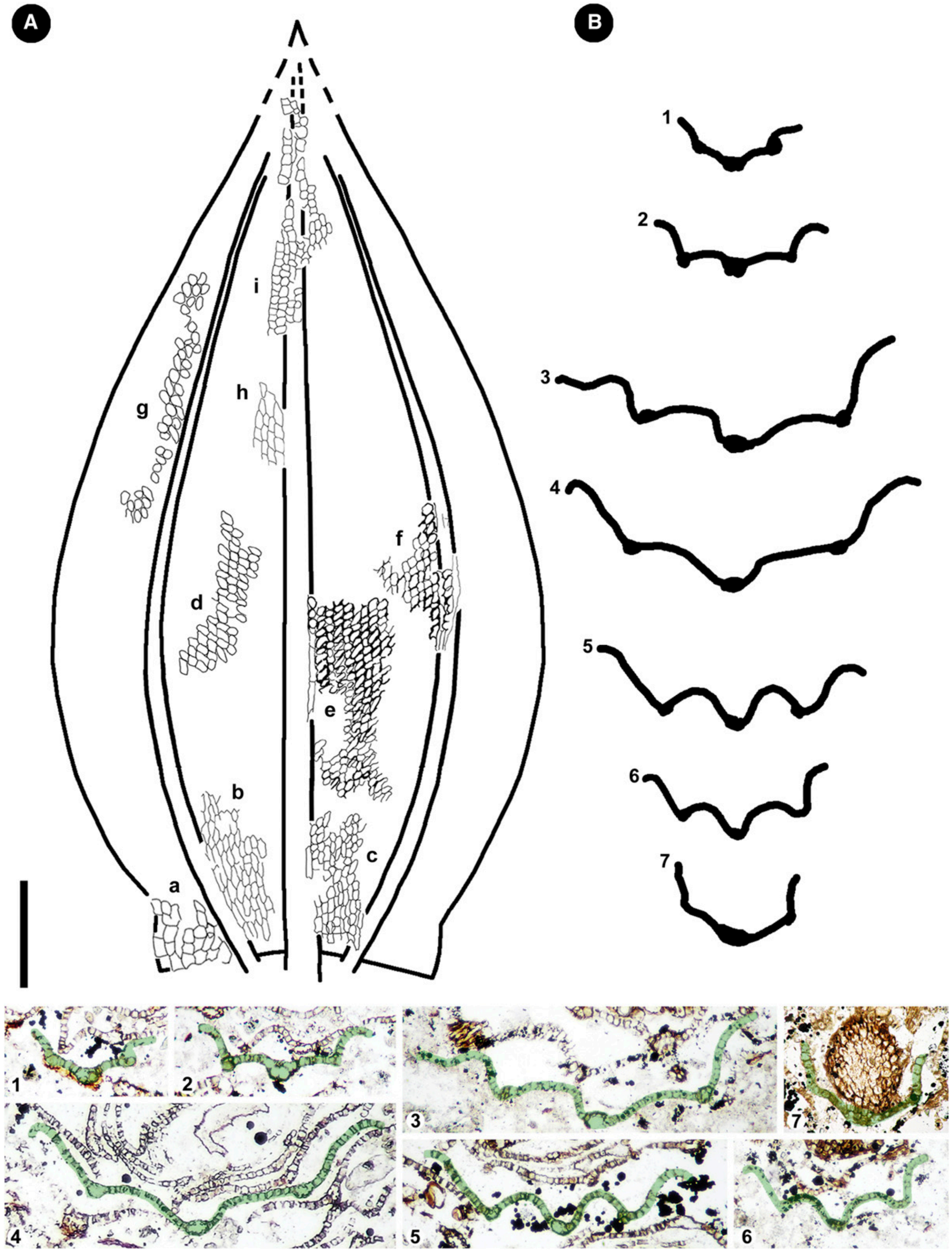

FIGURE 6 *Tricosta plicata* gen. et sp. nov. leaf model. (A) Cell morphology; note the thickened cell walls at midleaf (right side), representing cells whose wall outlines are followed by fungal hyphae; tracings are from nine different leaves: a—P13131 Dtop #4c; b—P13957 Btop #52; c—P15425 Cbot #47a; d—P15425 Cbot #49a; e—P15425 Cbot #49a; f—P15425 Cbot #47a; g—P15425 Cbot #14a; h—P15425 Cbot #54a; i—P13957 Btop #157. (B) Series of leaf tracings (right) in transverse section from apex (1) to base (7) demonstrate strong plication throughout; photos (below, with leaves highlighted) correspond to serial tracings with same number; leaf sections from seven different leaves; specimen numbers correspond to serial tracings from base to apex: P13029 D #21; P13029 D #21; P15425 Cbot #26a; P15425 Cbot 22a; P13131 Dtop #3c; P15425 Cbot #16a; P15425 Cbot #11a; scale bar = 200 µm for A and B.

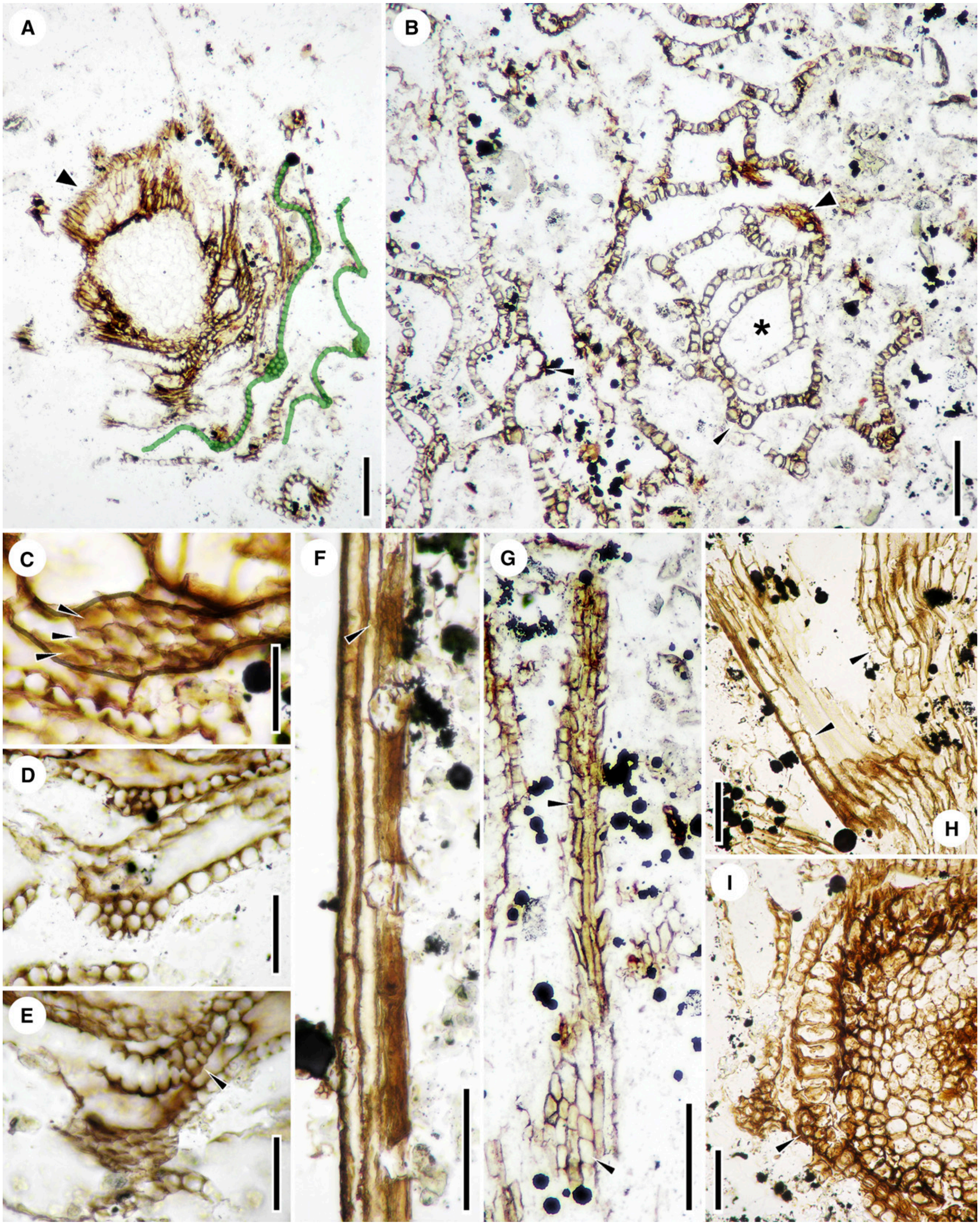

FIGURE 7 Leaf anatomy of *Tricosta plicata* gen. et sp. nov. (A) Shoot transverse section showing tricostate leaves with strong plications (two leaves highlighted); arrowhead indicates alar region in paradermal section; scale bar = 50 µm; P15422 A #1. (B) Perigonial shoot in transverse section (center indicated by asterisk); innermost leaves (ca. 4) perigonial, other leaves vegetative; note protruding bistratose abaxial costa-layer (thin arrowheads); thick arrowhead indicates bundle of epiphyllous fungal hyphae; scale bar = 100 µm; P15425 Cbot #16a. (C) Leaf transverse section showing three-layered median costa (ab-, adaxial surfaces traced; arrowheads = costal layers); scale bar = 50 µm; P15422 A #1. (D) Leaf transverse section showing

scale-like structures are derived from the delicate primordium tissue or the epidermis of the surrounding stem, i.e., either a "scale leaf" or "pseudoparaphyllium" origin, respectively—*sensu* Newton and De Luna (1999). The branch primordia are bordered by a palisade of radially arranged cells with circular to wedge shapes (up to 10 × 24 μm) in longitudinal sections (Figs. 4D, E).

One specimen represents the base of a small tuft (i.e., several shoots originating from a small number of branching stems) covered in rhizoids (Fig. 5A). The rhizoids, densely arranged, are multicellular, with characteristic oblique end-walls (Figs. 5B, C), diameters of 17-30 μm, and extend up to 700 μm from the stems. Branched rhizoids were not observed.

Tricosta plicata is isophyllous and leaves are partially overlapping, densely covering the stems, with ca. 9 leaves mm^{-1} in proximal regions of the shoots, and up to 23 leaves mm^{-1} distally (e.g., Fig. 2A). Phyllotaxis is helical, following a 3/8 phyllotactic ratio. Leaves are erect with divergence angles of 40-55° or wider where they subtend branches (Fig. 2A). Paraphyllia were not observed.

Leaf morphology and anatomy—In terms of overall shape, the leaves are symmetrical, ovate, have entire margins, and are broadly attached to the stems (Figs. 6; 4F, G). The leaves are ca. 0.5 mm wide at the base, reaching a maximum width of 0.9 mm and length of 2.1 mm. Incomplete preservation of leaf tips permits only close approximation of total leaf length. Leaf apices, when preserved, are acute (Figs. 8K, L).

Leaves are unistratose, strongly plicate, and tricostate (Figs. 4F-H; 6; 7A, B). Plication forms three adaxially concave longitudinal folds, each associated with a costa. The median fold and costa extend from the leaf base into the apex (i.e., percurrent), whereas the two lateral folds and costae are shorter, extending from the leaf base to somewhere below the apex (i.e., attenuated) (Figs. 8K, L). At the widest point, the lamina is ca. 18 cells wide between the median and lateral costae and ca. 15 cells between the lateral costae and leaf margin (Fig. 6). Median costae end apically within 4-5 cells from the leaf margin while lateral costae end 3-4 cells from the margin (Fig. 6). The three costae of a leaf originate separately and slightly below the level of leaf divergence (Figs. 4F, G). Leaf margins are unistratose and gently recurved (curved abaxially) throughout (Fig. 6).

Median costae are ca. 55 μm wide and 27-42 μm thick in the basal half of the leaf, while lateral costae are ca. 35 μm wide and 25-42 μm thick (e.g., Figs. 7C-E). Costae are tristratose at the base, becoming bistratose in the upper half of the leaf (e.g., Figs. 4H; 6; 7A, B). Costae consist of cylindrical elongate cells which form three layers: adaxial, median and abaxial (Figs. 7C-E). In paradermal and longitudinal sections, costal cells are 40-138 μm long, with one or both ends tapered (Figs. 7F-G). Adaxial and median costa layers are up to six cells wide basally, becoming one to two cells wide apically, with cells 8-16 μm in diameter. The abaxial layer is up to eight cells wide basally (cells 6-9 μm in diameter; Fig. 7C), and just one or two cells wide distally (cells up to 23 μm in diameter; Fig. 7B).

Prominent alar regions are present at the leaf base corners (Figs. 4H; 6; 7A, H, I; 8A-D). They are up to nine cells wide and five cells tall. Alar cells are inflated in transverse sections (e.g., Figs. 7I; 8A), ca. 15-34 μm wide, up to 54 μm long, and globose to elongate in paradermal and longitudinal sections (e.g., Figs. 8B, D). Laminal cells (Figs. 6; 8E-L) are 13-19 μm thick throughout. Toward leaf bases they have a length/width ratio of up to 5:1 and are ca. 40 μm long (up to 62 μm) with elongate and rectangular to rhombic shapes. In the midleaf, cells are ca. 2-3:1 and ca. 25 μm long (up to 35 μm) with mostly rhombic or oval shapes. In the distal half of the leaf, cells become isodiametric, with diameters of 10-23 μm. Laminal cells adjacent to the costae are comparable in size to neighboring laminal cells and have various, typically elongate shapes: rhombic, repand, rectangular, and isodiametric (Figs. 6; 7G). Throughout the basal half of the leaf, laminal cells typically form oblique files, whereas longitudinal files (of isodiametric cells) are typical in the distal leaf half (Fig. 6). Walls of laminal cells are smooth and thin (ca. 1.0 μm thick; Fig. 8E).

Specialized branches—At least two specimens exhibit perigonial branches. One of these is an extensively branched gametophyte with diminutive perigonial shoots borne apically or laterally on nearly all branches (Figs. 3; 2A; 9). Perigonial axes are 115-200 μm long, 95-115 μm thick, and bear ca. 4 leaves (Figs. 10A-C). The perigonial leaves are erect or spreading and anatomically similar to cauline leaves, except for a smaller size (e.g., lengths ca. 0.9 mm), weaker plications, and weaker costae in innermost perigonial leaves (Figs. 10A, F). All perigonial axes bear one antheridium at their tip (Figs. 10B, C). The antheridia are oblong (up to 350 μm long and 150 μm wide; Fig. 10C) and borne on triseriate stalks (145-150 μm long and 44-50 μm thick; Figs. 10B-E). The stalks are ca. 10-14 cells tall (Fig. 10D). Antheridial jackets are composed of narrow (7-8 μm) cells showing irregular shapes in paradermal sections (Fig. 10G). Paraphyses and sperm cells were not observed.

At least three shoots bear perichaetial branches (Fig. 11). These specialized branches are extremely short and borne laterally along main stems (Figs. 11A, B) which occur near the periphery of an extensively branched gametophyte tuft (Fig. 1). The numerous other shoot tips of the tuft are vegetative, incompletely preserved, or occupied by perithecioid fungal fruiting bodies. The perichaetia terminate short, bud-like branches that are constricted at the base where they attach to the main stem (Fig. 11A); the stem itself shows no change in diameter where the perichaetial branch is attached. Perichaetia consist of few densely arranged, straight and erect leaves which are crowded from their bases to near the apices (Figs. 11A-C). The leaves are composed of narrow cells (up to 4.5:1 and ca. 40 μm long midleaf) with rectangular or rhombic shapes throughout the lower half of the leaf (Figs. 11C, D). Perichaetial leaf apices were not observed. The branch tips are conic (Fig. 11D) or narrowly dome-shaped (Fig. 11G)

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three-layered median costa (below) and bilayered lateral costa (above); scale bar = 50 μm; P15422 A #1. (E) Leaf transverse section showing three-layered median costa (below); note alar region (above) and adjacent lateral costa (arrowhead); scale bar = 50 μm; P15422 A #1. (F) Costa in longitudinal section showing linear cells with tapered (arrowhead) or transverse end-walls; scale bar = 50 μm; P16435 Ctop #13. (G) Costa in paradermal section showing linear cells with tapered or transverse end-walls, and juxtacostal cells (arrowheads); scale bar = 100 μm; P15425 Cbot #54a. (H) Two leaf bases in longitudinal section showing few cells (arrowheads) of two different alar regions; scale bar = 50 μm; P13957 Btop #52. (I) Alar region in transverse section; arrowhead indicates adjacent lateral costa; note stem center occupied by hyphae; scale bar = 50 μm; P13957 Btop #25.

FIGURE 8 Leaf anatomy of *Tricosta plicata* gen. et sp. nov. (A) Shoot in transverse section showing inflated alar cells of (shaded) clasping leaf base (arrowhead = median costa); scale bar = 100 µm; P13131 Dtop #6c. (B) Densely foliated shoot in longitudinal section (leaf bases at left, stem at right); arrowhead indicates inflated alar cell; scale bar = 50 µm; P16435 Ctop #10. (C) Cells of leaf base in paradermal section (center) and few alar cells in section (thick arrowhead); thin arrowhead = lateral costa; scale bar = 50 µm; P13957 Btop #52. (D) Alar region in paradermal section; scale bar = 50 µm; P15425 C bot #44a. (E) Leaf cells in paradermal section showing thin walls indicative of the absence of fungal hyphae or taphonomic alterations; scale bar = 30 µm;

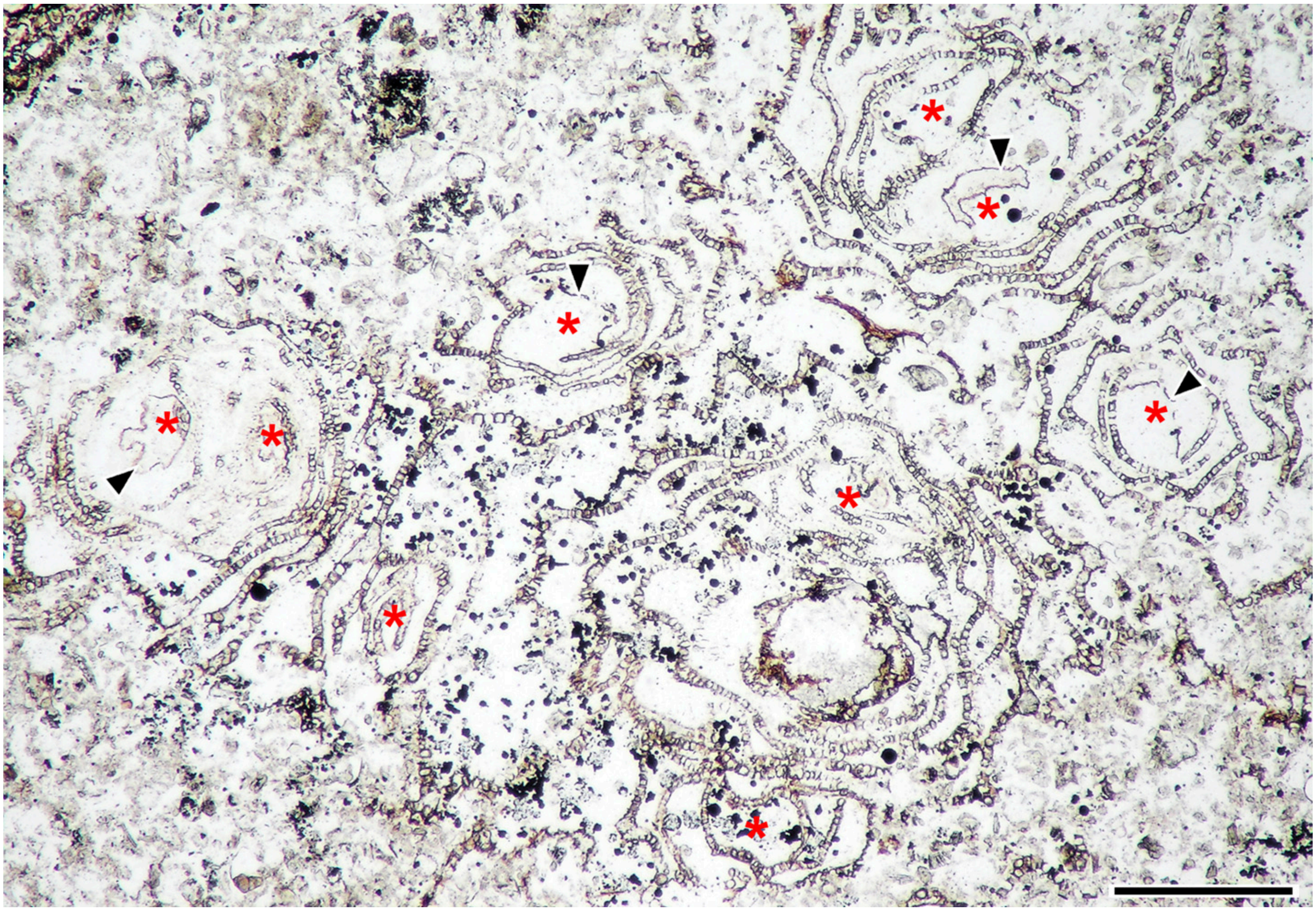

FIGURE 9 *Tricosta plicata* gen. et sp. nov. Several perigonia in transverse sections (asterisks), some showing antheridia (arrowheads); scale bar = 200 µm; P15425 Cbot #13a.

and bear a small number of pale-colored archegonia (Figs. 11C, D, G). The archegonia are at least 200 µm long, with a venter up to 50 µm across (Figs. 11F), and lack a distinct stalk (e.g., Figs. 11D, G). In one specimen seen in oblique-longitudinal section (Fig. 11E, F), the neck canal is seen at the center, with a single layer of neck cells and few layers of delicate venter tissue (Fig. 11F).

DISCUSSION

The tricostate condition—The costa (also termed midrib or nerve) is a multistratose region of the leaf forming a longitudinal band that is anatomically different from the rest of the lamina. Most moss leaves bear a single costa, which varies greatly in anatomy and morphology among taxa (Goffinet et al., 2009). The condition in which a costa is divided at the base or along its length (e.g., Goffinet et al., 2009) is treated as a single "forked" costa, which makes sense from a developmental standpoint. Whereas ecostate mosses (mosses that lack costae or have costae of insignificant length) are found among diverse lineages (e.g., *Sphagnum* L., *Buxbaumia* Hedw., *Erpodium* Brid., *Pleurophascum* Lindb., *Hedwigia* Beauv.), mosses bearing multiple costae per leaf (pluricostate or multicostate) are typically found among pleurocarpous taxa (e.g., *Thamniopsis* M. Fleisch., *Antitrichia* Brid., *Neckera* Hedw.; Goffinet et al., 2009). Extant pluricostate mosses typically bear two short costae per leaf and instances of two strong costae (e.g., some Hookerales) or more than three costae are rare (e.g., *Antitrichia,* which features a median costa and a variable number of shorter accessory costae; e.g., Lawton, 1971). None of these pluricostate conditions conforms to the tricostate condition of *Tricosta plicata*, in which three

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P15425 Cbot #49a. (F) Leaves in paradermal section showing laminal cell shapes near leaf base (lower left) and in lower half of leaf (right); scale bar = 100 µm; P15425 Cbot #47a. (G) Laminal cell shapes in distal half of leaf; scale bar = 50 µm; P15425 Cbot #49a. (H) Leaves in paradermal sections showing cell shapes of midleaf; scale bar = 100 µm; P15425 Cbot #49a. (I) Leaf paradermal section showing cell shapes at midleaf; note fungi (arrowheads); scale bar = 100 µm; P15425 Cbot #48a. (J) Paradermal section showing leaf cell shapes at midleaf; scale bar = 100 µm; P15425 Cbot #48a. (K) Leaf apex in paradermal section; note leaf margins not shown; scale bar = 50 µm; P13957 Btop #157. (L) Detail of K showing laminal cell shapes and median costa (upper arrowhead shows linear cells of adaxial costal layer; lower arrowhead shows short cells of abaxial costal layer); scale bar = 50 µm; P13957Btop #157.

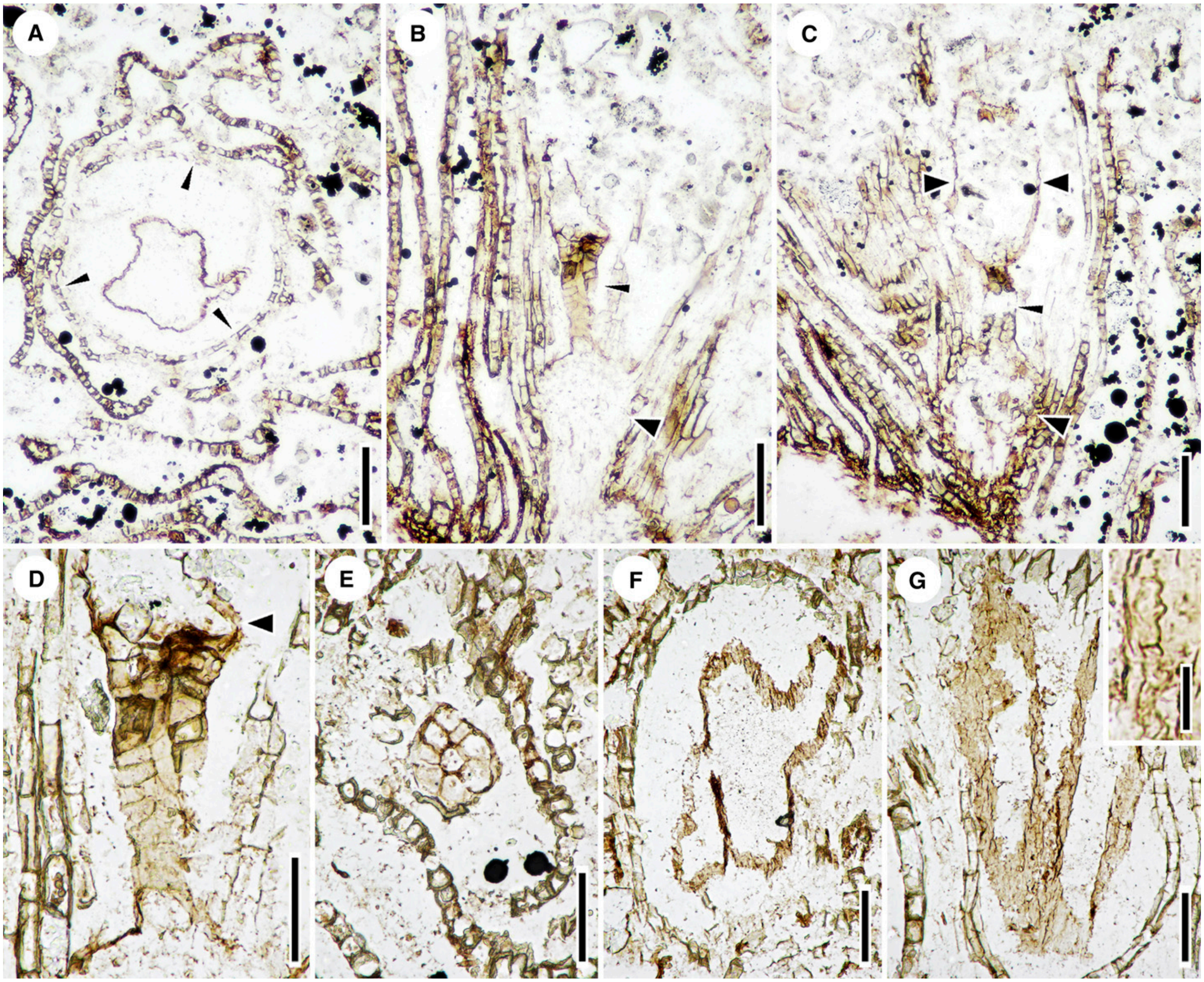

FIGURE 10 Perigonia of *Tricosta plicata* gen. et sp. nov. (A) Perigonial shoot in transverse section showing antheridium at center and innermost perigonial leaves (arrowheads) with weak costae and plication; scale bar = 100 µm; P15425 Cbot #25a. (B) Perigonium in longitudinal section showing incompletely preserved swollen axis (thick arrowhead) and well-preserved antheridial stalk (thin arrowhead; note base of sac attached to stalk); scale bar = 100 µm; P15425 Cbot #38a. (C) Perigonium in longitudinal section showing incompletely preserved axis (thick arrowhead, bottom), antheridial stalk (thin arrowhead), and antheridial sac (between thick arrowheads); scale bar = 100 µm; P15425 Cbot #36a. (D) Detail of B showing antheridial stalk in longitudinal section; arrowhead indicates base of antheridial sac; scale bar = 50 µm; P15425 Cbot #38a. (E) Antheridial stalk in transverse section; scale bar = 50 µm; P15425 Cbot #28a. (F) Perigonium transverse section showing convoluted antheridial jacket and innermost perigonial leaf; scale bar = 50 µm; P15425 Cbot #38a. (G) Antheridial jacket in longitudinal section showing irregular and narrow cell shapes (e.g., inset; scale bar = 10 µm); scale bar = 50 µm; P15425 Cbot #38a.

strong costae originate independently at the leaf base and extend well beyond the midleaf. In this context, the tricostate condition present in both *T. plicata* and the Mesozoic genus *Tricostium* clearly sets these species apart from all other living and extinct mosses.

Tricostate analogues in extant mosses—Although no mosses with three strong costae are recognized in modern floras, a few extant mosses exhibit multilayered bands of cells *additional* to the median costa that can be morphologically similar to lateral costae: (1) multistratose longitudinal thickenings (or multistratose "streaks") composed of cells more or less similar to those of the lamina; and (2) multistratose intramarginal limbidia (intramarginal borders or teniolae), which are bands of cells running parallel with and internal to the leaf margin by 1-3 cells. It is important to note that none of the rare studies of leaf development in mosses (e.g., Frey, 1970) has addressed the homology of multistratose structures of the lamina and we can only base comparisons on anatomy.

Multistratose thickenings similar to costae are seen in *Coscinodon arctolimnius* Steere and *C. cribrosus* Spruce (Grimmiaceae), in which leaves bear a median costa and two lateral multistratose thickenings that run along leaf plications (Hastings and Deguchi,

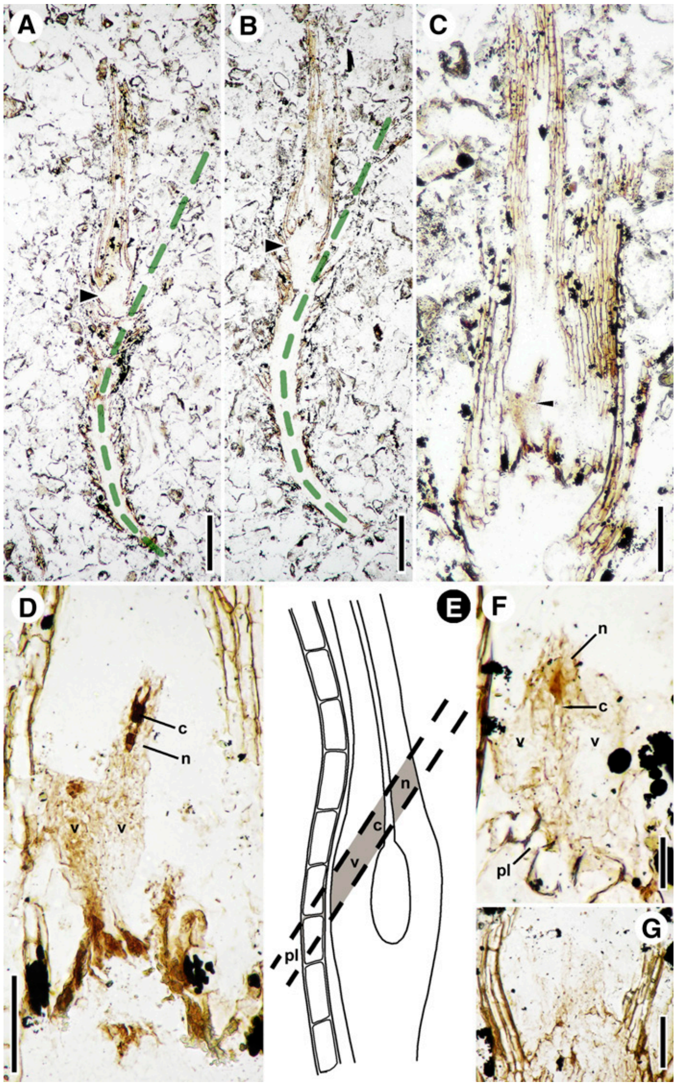

FIGURE 11 Perichaetia and archegonia of *Tricosta plicata* gen. et sp. nov. (A-B) Stem (dashed line) and lateral perichaetial branch (arrowhead) in longitudinal section; the stem that bears the perichaetial branch exits the plane of section in which the latter is seen; note constricted base of perichaetial branch where it is attached to main stem; scale bars = 300 µm; A, P13957 Btop #129; B, P13957 Btop #131. (C) Perichaetium in longitudinal section; scale bar = 100 µm; P13957 Btop #132. (D) Detail of C showing incompletely preserved perichaetium axis and bases of archegonia (bottom); at least two archegonia shown with delicate venters (v) overlapping; scale bar = 100 µm; P13957 Btop #132. (E) Archegonium diagram demonstrating plane of section (in between dashed lines) shown in F; approximately to scale of F. (F) Oblique section of archegonium showing narrow neck canal (c), single layer of neck cells (n) and few layers of delicate venter (v); scale bar = 20 µm; P13957 Btop #121. (G) Perichaetium longitudinal section showing archegonial base attached to apex of perichaetial branch; scale bar = 50 µm; P13957 Btop #89. c = neck canal; n = neck; pl = perichaetial leaf; v = venter.

1997). These thickenings consist of cells similar in anatomy to those of the costa. While the multistratose thickenings of *Coscinodon* Spreng. are comparable to costae in featuring elongated cells, costae and multistratose thickenings are probably developmentally different as suggested by: (1) the fact that cells in the streaks are shorter than those of the median costa; (2) irregular width, thickness, and position of the streaks on the leaf; and (3) an absence of cell differentiation in the streaks similar to that seen in the costa (i.e., stereids are present in the costae and not in the streaks).

Multistratose intramarginal limbidia are seen in a few genera—those of *Calymperes* Sw., *Teniolophora* W.D. Reese, and *Limbella* Müll. Hal. (e.g., Gradstein et al., 2001) show the closest apparent similarity to the tricostate condition of *Tricosta plicata*. In *Calymperes* and *Teniolophora*, the cross-sectional anatomy of limbidia is simpler than that of the costa, suggesting different developmental origins of the two types of structures. In *Limbella tricostata* (Sull.) Bartr. (=*Sciaromium tricostatum* (Sull.) Mitt.) the intramarginal limbidia have cross-sectional anatomy similar to that of the costa (e.g., Lawton, 1971). Although among extant mosses the intramarginal limbidia of *Limbella* are most similar to the lateral costae of *Tricosta*, these limbidia are much closer to the leaf margin (only one to two cells away; e.g., Lawton, 1971) than the costae of *Tricosta* (with leaf margins 10-15 cells wide).

Overall, multilayered structures of the lamina known in extant mosses that approach the tricostate condition are anatomically different from, and probably not homologous to costae, as discussed in the paragraphs above. This suggests that extant moss diversity does not include any structures equivalent to the lateral costae of *Tricosta*.

Taxonomic placement of* Tricosta plicata *gen. et sp. nov.—*Justification for a new genus*—Mosses with tricostate leaves have been previously reported only from Mesozoic (Triassic to Early Cretaceous) rocks in Russia and Mongolia (potentially extending into the Permian; Ignatov and Shcherbakov, 2011b), where they are preserved as compressions (Krassilov, 1973; Ignatov and Shcherbakov, 2011a, b). These mosses have been assigned to the genus *Tricostium*, with three species: *Tricostium triassicum* Ignatov et Shcherbakov, *T. papillosum* Krassilov, and *T. longifolium* Ignatov et Shcherbakov. The genus *Tricostium* is diagnosed as having partially overlapping, flat, unistratose leaves with three costae (Krassilov, 1973).

The unique nature of three strong costae per leaf suggests a close relationship among all tricostate mosses. However, several characters differentiate *Tricosta plicata* from the genus *Tricostium* (Table 1), indicating that it represents a new genus. Aside from the tricostate leaves, *Tricosta plicata* is similar to *Tricostium* only in terms of leaf divergence angles (ca. 40–45°), leaf width (ca. 1.0 mm), and in having strong costae, and short laminal cells (Table 1). Of the three species of *Tricostium*, *T. papillosum* is most similar to *Tricosta plicata*, comparing favorably in leaf shape and length, and the width of the median costa. However, *Tricosta plicata* differs from *Tricostium papillosum* in branching angle, leaf density, leaf profile, leaf apex, laminal cell arrangement, laminal cell shape, laminal cell dimensions, and leaf cell wall texture.

Furthermore, the difference in modes of preservation leads to a strong disparity between *Tricosta* and *Tricostium* in the type and number of taxonomically informative characters, as well as the degree of morphological and anatomical detail available. The compression fossils assigned to *Tricostium* provide information on few characters, including leaf shape, size, angle of divergence, and leaf density along the stems, as well as branching pattern (if present) and leaf areolation (Table 1). As a result, *Tricostium* is defined chiefly on leaf characters, as the fossils lack detail on other characters;

TABLE 1. Summary of *Tricosta plicata* gen. et sp. nov. defining characters and a comparison with species of *Tricostium*.

Character	*Tricosta plicata*	*Tricostium longifolium*	*Tricostium papillosum*	*Tricostium triassicum*
Stem length (min.)	22 mm	10 mm	3.9 mm	?
Branch length (min.)	500 µm	5.5-7.5 mm	1.3 mm	?
Distance between branches (min.)	480 µm	5.0 mm	?	?
Stem diameter	0.2 mm	ca. 0.3 mm	?	?
Branching angle	(41)-55-(75°)	ca. 25-35(60°)	ca. 43°	?
Density of foliation	dense, (11)-18-(23) leaves mm^{-1}	sparse; ca. 1.6 leaves mm^{-1}	dense, ca. 3-5 leaves mm^{-1}	?
Leaf divergence	erect-spreading (38)-45-(55°)	15-45° at base; distal half recurved	patent (ca. 25-40°)	?
Leaf orientation	straight	recurved	straight	?
Leaf shape	ovate	lanceolate	ovate (to narrowly ovate)	narrowly lanceolate (?or oblong)
Leaf concavity	plicate	some keeled	flat (?slightly undulate)	flat (?to concave)
Leaf margin	entire	?	serrate distally	entire
Leaf length	ca. 2.0 mm	4-6 mm	1.2-1.8 mm	4-5 mm
Leaf width	0.8-1.0 mm	up to 1.5 mm	ca. 0.5-1 mm	0.9 mm
Leaf apex	acute (?to acuminate)	acute	obtuse to acute	acute?
Leaf base	clasping	truncate?	clasping (?or auriculate)	truncate?
Median costa length (% of leaf length)	at least 95 (attenuated to percurrent)	at least 90	90-95 (attenuated to percurrent)	at least 80
Median costa width	ca. 54 µm	60-80 µm	ca. 50 µm	80 µm
Lateral costa length (% of leaf length)	at least 90	at least 90	70-90	at least 80
Lateral costa width	ca. 35 µm	ca. 25 µm	ca. 20-30 µm	30-40 µm
Alar region	conspicuous; cells inflated	?	?	?
Laminal cell arrangement	oblique files near midleaf; longitudinal files in distal half	?oblique files	longitudinal files	longitudinal files
Laminal cell shape	rhombic, repand, oval to isodiametric	isodiametric (?rounded or polygonal)	polygonal, isodiametric	quadrate to short rectangular
Laminal cell size	up to 5:1 (ca. 40 µm long) basally; 2-3:1 at midleaf (ca. 25 µm long); isodiametric up to 23 µm distally	13-17 µm	15-18 µm	13-16 µm wide
Leaf cell wall thickenings	absent	?	?thickened corners	?
Laminal cell surface texture	smooth	?	pluripapillate (8-10 papillae per cell)	?

consequently, none of the *Tricostium* species is reconstructed as a whole plant. Therefore, *Tricostium* is best regarded as a morphogenus (i.e., a taxon defined based only on a subset of characters of the whole plant; Bell and York, 2007) erected for moss leaves displaying a tricostate condition. In contrast, *Tricosta plicata* preserves information on several additional characters including branching architecture, phyllotaxis, stem diameters, stem anatomy, detailed leaf anatomy from various planes of section, costal anatomy, and fertile structures (perigonial and perichaetial shoots). Consequently, *Tricosta plicata* is characterized in much more detail than any of the species of *Tricostium* and represents a natural taxon based on a whole-plant concept for the gametophyte. Taken together, all these considerations warrant placement of the Apple Bay material in the new genus, *Tricosta*.

Tricosta plicata as a hypnanaean pleurocarp—In a strict sense, pleurocarpy refers to the production of sporophytes (thus, perichaetia with archegonia) on typically bud-like lateral shoots. Recognition of pleurocarpy is complicated by the fact that in some acrocarpous mosses (e.g., *Hedwigia ciliata* (Hedw.) P.Beauv.) new vegetative branches can be initiated immediately below perichaetia that terminate long branches; in such cases, the new vegetative branch displaces the perichaetium laterally, leading to a pseudo-pleurocarpous branching pattern (Mishler and De Luna, 1991). In *Tricosta plicata* the perichaetial branches are short, bud-like and, importantly, they are attached by a constricted base to the main stem; additionally, the main stem shows no constriction at the points of attachment of perichaetial branches. Together, these observations indicate that the perichaetial branches are true laterals and support interpretation of *T. plicata* as a true pleurocarp. Furthermore, the abundance of lateral bud-like perigonial branches, a feature that suggests a similar branching pattern for the perichaetia (N. E. Bell, personal communication, 2013; L. Hedenäs, personal communication, 2013) corroborates this interpretation.

Aside from the superorder Hypnanae, pleurocarpy is present in some members of the rhizogoniaceous grade of lineages basal to the Hypnanae (Bell and Newton, 2004), specifically of the Orthodontiales, Rhizogoniales, and Aulacomniales (Bell et al., 2007). Of these groups, which form a clade informally referred to as pleurocarpids (Bell et al., 2007), only the hypnanaean pleurocarps (or subsets of this group) combine the set of gametophyte features documented in *Tricosta plicata*: (1) monopodial and much-branched (±pinnate) primary stems; (2) pluricostate, (3) homocostate, (4) and strongly plicate leaves; (5) leaf cells elongate and rhombic at mid leaf, with (6) thin walls, and (7) arranged in oblique files; (8) the presence of well-differentiated alar regions; and (9) the absence of a central conducting strand in the stems (e.g., Lawton, 1971; Vitt, 1982, 1984; Hedenäs, 1994; La Farge-England, 1996; Newton and De Luna, 1999; Ignatov and Shcherbakov, 2007; Newton, 2007; Goffinet et al., 2009). While none of these characters considered individually is exclusively diagnostic of the Hypnanae, they each occur only sporadically outside of this group, and are not known to occur in combination in any extant nonhypnanaean.

Within the Hypnanae (the clade comprising the orders Hypnodenrales, Ptychomniales, Hookeriales, and Hypnales), homogenous costae characterize only the clade consisting of the Ptychomniales + Hookeriales + Hypnales [= the homocostate pleurocarp clade of Bell et al. (2007)]. Consequently, the combination of gametophyte traits of *Tricosta* supports placement in superorder Hypnanae and suggests that, within this superorder, *Tricosta* could be a member of the homocostate pleurocarp clade.

Justification for a new hypnanaean family—Among the homocostate pleurocarps, the Ptychomniales often have plicate leaves, while some Hookeriales are bicostate in a similar manner to the way in which *Tricosta* is tricostate. Based on the Early Cretaceous age and the combination of characters of *Tricosta*, one could speculate that the tricostate-plicate condition in this fossil was ancestral to both the plicate (but sometimes ecostate) state found in many Ptychomniales and the bicostate (but nonplicate) condition found in some Hookeriales.

When compared to individual hypnanaean families, *Tricosta* is most similar to the Pilotrichaceae (Hookeriales) and families of the Hypnales (Table 2). The vast majority of pleurocarp diversity belongs to the Hypnales, which comprises more than 40 families and 400 genera (Goffinet et al., 2009). There are numerous families within this group that have several conspicuous traits in common with *Tricosta plicata,* e.g., monopodial and pinnate branching, absence of paraphyllia, lack of a conducting strand, helically arranged leaves, conspicuous alar regions, and laminal cell morphology (Lawton, 1971; Vitt, 1982; Chiang, 1995; Gradstein et al., 2001; Goffinet et al., 2009; Eckel, 2011; Ramsay, 2012a, b). Families exhibiting some combination of these traits are included in Table 2. Of these families, Amblystegiaceae, Regmatodontaceae, Hypnaceae, and Rhytidiaceae are most similar to *Tricosta* (Table 2). However, each of these families exhibits significant differences from *Tricosta* (Table 2). Additional differences not listed in Table 2 include: (1) stem anatomy (in Pilotrichaceae: a few outer cortex layers with narrow, thick-walled cells and, typically, a hyalodermis); and (2) isodiametric distal leaf cells, present in *Tricosta* but not known in any of the families listed above. Together, the differences suggest that none of these families is a good placement for *Tricosta* and, along with the unique tricostate condition, warrant erection of a new family, Tricostaceae.

Pleurocarpous mosses in the pre-Cenozoic fossil record—Few pre-Cenozoic mosses have been discussed in terms of putative pleurocarpy. In such discussions, pleurocarpy has been suggested based on characters that are not exclusively diagnostic of this condition when considered independently (e.g., much-branched gametophytes, equivocal reproductive structures). *Uskatia* Neuburg, described from the Permian of Russia, has been compared to pleurocarps by Oostendorp (1987), based on abundantly branched pinnate stems with small leaves. However, Ignatov and Shcherbakov (2007) have suggested that the genus is part of a different group, due to the presence of leaves attached to the stem only by their costa, a character unknown in any living mosses. *Capimirinus riopretensis* Christiano De Souza, Ricardi Branco et Leon Vargas

TABLE 2. Comparison of *Tricosta plicata* gen. et sp. nov. to some monopodially branched pleurocarpous mosses[1,2].

Character	Pilotrichaceae	Amblystegiaceae	Regmatodontaceae	Hypnaceae	Rhytidiaceae	Pylaisiadelphaceae	Sematophyllaceae	*Tricosta plicata*
Branching	irregular to pinnate	irregular to subpinnate	irregular to subpinnate	pinnate	pinnate	pinnate	irregular to pinnate	irregular to pinnate
Stem conducting strand	absent	usually present	weak	present	narrow	usually absent	absent	absent
Paraphyllia	absent	occasional	absent	usually absent	absent	?absent	absent	absent
Leaf orientation	straight	straight to falcate-secund	straight	often falcate or falcate-secund	often ±secund	straight; few falcate	occasionally secund, rarely falcate-secund	straight
Leaf surface topography	some concave	rarely plicate; some concave	some concave	often concave (or plicate)	plicate, rugose	some concave	concave	strongly plicate
Costa(e)	strong, double	mostly single, often variable	single	short and double or absent	single, strong	short and double or none	short and double or none	three, strong
Laminal cell shape	various	short to linear	short to elongate	mostly linear	linear	mostly linear	mostly linear	short to elongate
Laminal cell surface and walls	smooth or papillose; porose or not	smooth or rarely prorulose, some mammillose or papillose	smooth	smooth or papillose	strongly porose, prorulose	smooth, sometimes papillose	smooth or papillose	smooth
Alar cells	undifferentiated	not to strongly differentiated	not or barely differentiated	usually well-differentiated, quadrate to inflated	well-differentiated	few, quadrate, usually not inflated	well-differentiated; basal 1-2 rows strongly inflated	few, well-differentiated, ±inflated (rarely quadrate)

[1] The classification follows Goffinet et al. (2009); Pilotrichaceae within Hookeriales; all other families within Hypnales.
[2] Based on Lawton (1971), Vitt (1982), Chiang (1995), Gradstein et al. (2001), Goffinet et al. (2009), Eckel (2011), Ramsay (2012a, b).

(2012), known from Permian rocks of Brazil, shows sparse dichotomous branching, leaves ca. 1.4 × 0.5 mm, and a putative sporophyte attached to a short lateral shoot. However the sporophytic nature of this structure is equivocal because of its unusually small dimensions. Because of the uncertain nature of this structure and the lack of other informative characters, the placement of *Capimirinus riopretensis* among pleurocarpous mosses is uncertain.

Palaeodichelyma sinitzae Ignatov et Shcherbakov (2007), described from the Jurassic (Lower Cretaceous?) of Russia, has characters that suggest pleurocarpy, such as lateral bud-like structures. This species exhibits traits seen in the pleurocarpous family Fontinalaceae, i.e., strong costae, keeled leaves, tristichous phyllotaxis, and elongate laminal cells (Ignatov and Shcherbakov, 2007). However, pleurocarpy of *Palaeodichelyma* is conjectural, because the exact nature of its lateral bud-like structures is not known, and the laminal cells have transverse end-walls, which are rare among the pleurocarpous mosses.

Bryokhutuliinia Ignatov, preserved as compressions in the Jurassic (Lower Cretaceous?) of Russia and Mongolia (Ignatov and Shcherbakov, 2007, 2011a; Ignatov et al., 2011), has pinnately branched shoots and bud- or rosette-like structures interpreted as gametangial branches. Although pinnate branching is indicative of pleurocarpy and some of the leaf traits suggest Hookerialean affinities (e.g., ecostate and complanate leaves; Ignatov and Shcherbakov, 2007), additional evidence is needed to unequivocally establish pleurocarpous affinities for this moss. This is also the case for *Vetiplanaxis*, described from Cretaceous Burmese amber. This fossil moss compares favorably to the pleurocarpous Hypnodendrales based on branching patterns and laminal cell morphology (Hedenäs et al., 2014), but additional evidence is needed to support assignment to the group.

Overall, among the pre-Cenozoic mosses, *Palaeodichelyma*, *Bryokhutuliinia*, and *Vetiplanaxis* compare most favorably to extant pleurocarps (e.g., Hedenäs et al., 2014). However, in these taxa, pleurocarpy is suggested based on only a few characters encountered in extant pleurocarpous mosses (e.g., general appearance, pinnate branching), rather than on a well-defined, extensive set of diagnostic criteria. In this context, the suite of traits listed above in support of the systematic affinities of *Tricosta plicata* provides the strongest evidence to date for pleurocarpy and, more specifically, for placement in the Hypnanae of any pre-Cenozoic moss.

Gametangia in the pre-Cenozoic fossil record—The only previously described fossil bryophyte with preserved archegonia is the leafy liverwort *Naiadita* Brodie from the Triassic of England (Harris, 1938). The fossil record of antheridia borne on free-living gametophytes is sparse. A few Early Devonian vascular plant gametophytes from the Rhynie chert (*Remyophyton delicatum* Kerp, Trewin et Hass, *Kidstonophyton discoides* Remy et Hass, *Lyonophyton rhyniensis* Remy et Remy) show well preserved antheridia (Taylor et al., 2009). *Eopolytrichum antiquum* (Konopka et al., 1997) is the only previously known instance of preservation of antheridia in the *moss* fossil record. Aside from that, a very small number of equivocal splash cups or perigonia are known (Townrow, 1959; Ignatov and Shcherbakov, 2007; Christiano De Souza et al., 2012). The antheridia and archegonia of *Tricosta plicata* are, thus, a welcome addition to this sparse fossil record.

Lastly, the presence of only one sex per gametophyte on fertile *Tricosta* specimens suggests dioicy, i.e., one extensively branched gametophyte (Fig. 3) bears numerous perigonial branches, whereas another gametophyte tuft with hundreds of branches (Fig. 1) bears only a few perichaetial branches. Although we cannot rule out the possibility that *Tricosta* gametophytes were monoicous and bore gametangia of both types, the fact that the most extensive specimens are unisexual is consistent with dioicy.

CONCLUSIONS

Throughout the Mesozoic and late Paleozoic, anatomical preservation among fossil mosses is rare (Smoot and Taylor, 1986; Konopka et al., 1997, 1998; Hübers and Kerp, 2012; Hedenäs et al., 2014). The anatomical and morphological detail preserved in *Tricosta plicata* allows for the most complete reconstruction of a fossil moss gametophyte to date, from rhizoid-bearing plant bases to shoot tips bearing gametangia. *Tricosta plicata* represents a new family, genus, and species, and is the first bryophyte component described from the Early Cretaceous Apple Bay flora of Vancouver Island. This fossil species adds another taxon to the still sparse picture of pre-Cenozoic mosses, allowing a better glimpse of what Cretaceous mosses looked like and where they fit among bryophytes. The antheridia and archegonia of *Tricosta plicata* add to a very sparse fossil record of bryophyte gametangia.

The combination of gametophytic traits exhibited by *Tricosta* indicates that it is a hypnanaean pleurocarpous moss. A few other pre-Cenozoic fossil mosses have been reported as putative pleurocarps (e.g., *Uskatia*, *Capimirinus*, *Palaeodichelyma*, *Bryokhutuliinia*, and *Vetiplanaxis*), but *Tricosta plicata* provides the strongest and oldest evidence to date for pleurocarpy and, more specifically, for placement in the Hypnanae. As such, *Tricosta* provides a hard minimum age for the hypnanaean clade—Valanginian, 136 Ma.

Exhibiting a previously unknown combination of characters, *Tricosta* represents a new moss family with no living representatives. Its similarity and possible affinities with the genus *Tricostium*, which is known from the Triassic through Cretaceous of Asia, suggest that the Tricostaceae may have been widely spread during the Mesozoic. Such fossil occurrences and the groups they represent (see also Steenbock et al., 2011) are constant reminders that the extant flora does not hold the complete answer to the overall patterns of bryophyte diversity over space and time. Aside from populating gaps in the knowledge of overall plant diversity that would remain open otherwise, fossil species are crucial to addressing patterns of deep phylogeny. Their study broadens the range of taxon sampling by adding well-characterized lineages with novel combinations of characters and whose existence could not have been foreseen from studies based exclusively on extant plants. Every time phylogenetic studies have sampled systematically the fossil record, their results have provided new perspectives (e.g., Rothwell, 1999; Rothwell and Nixon, 2006; Hilton and Bateman, 2006). Together, all of these are significant and irreplaceable contributions that the study of fossil plants brings to the study of evolution.

Studies of anatomically preserved fossil bryophytes and the types of data they provide for use in comparisons with extant bryophytes for taxonomic placement emphasize the need, also stressed elsewhere (Câmara and Kellogg, 2010), for thorough, taxonomically broad surveys of anatomy and development in extant bryophytes. Such studies would both enhance the precision of taxonomic placement of fossils and increase resolution of overall moss systematics and phylogeny.

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LITERATURE CITED

Atkinson, B. A., G. W. Rothwell, and R. A. Stockey. 2014a. *Hubbardiastrobus cunninghamioides* gen. et sp. nov., evidence for a Lower Cretaceous diversification of cunninghamioid Cupressaceae. *International Journal of Plant Sciences* 175: 256–269.

Atkinson, B. A., G. W. Rothwell, and R. A. Stockey. 2014b. *Hughmillerites vancouverensis* sp. nov. and the Cretaceous diversification of Cupressaceae. *American Journal of Botany* 101: 2136–2147.

Bell, N. E., and A. E. Newton. 2004. Systematic studies of non hypnanaean pleurocarps: Establishing a phylogenetic frame work for investigating the origins of pleurocarpy. *In* B. Goffinet, V. C. Hollowell, and R. E. Magill [eds.], Molecular systematics of bryophytes. *Missouri Botanical Garden Monographs in Systematic Botany* 98: 290-319.

Bell, N. E., D. Quandt, T. J. O'Brien, and A. E. Newton. 2007. Taxonomy and phylogeny in the earliest diverging pleurocarps: Square holes and bifurcating pegs. *Bryologist* 110: 533–560.

Bell, N. E., and P. V. York. 2007. *Vetiplanaxis pyrrhobryoides*, a new fossil moss genus and species from Middle Cretaceous Burmese amber. *Bryologist* 110: 514–520.

Berry, E. W. 1928. Contributions to the Mesozoic flora of the Atlantic coastal plain. *Bulletin of the Torrey Botanical Club* 55: 441–448.

Bronson, A. W., A. A. Klymiuk, R. A. Stockey, and A. M. F. Tomescu. 2013. A perithecial sordariomycete (Ascomycota, Diaporthales) from the Lower Cretaceous of Vancouver Island, British Columbia, Canada. *International Journal of Plant Sciences* 174: 278–292.

Câmara, P. E. A. S., and E. A. Kellogg. 2010. Morphology and development of leaf papillae in Sematophyllaceae. *Bryologist* 113: 22–33.

Chiang, T. Y. 1995. Phylogeny and morphological evolution of *Macrothamnium* M. Fleisch. and related taxa (Bryopsida: Hypnaceae). *Botanical Bulletin of Academia Sinica* 36: 143–153.

Christiano De Souza, I. C., F. S. Ricardi Branco, and Y. Leon Vargas. 2012. Permian bryophytes of western Gondwanaland from the Parana Basin in Brazil. *Palaeontology* 55: 229–241.

Debey, M. H., and C. R. von Ettingshausen. 1859. Die Urweltlichen Acrobryen des Kreidegebirges von Aachen und Maastricht. *Denkschriften der Kaiserlichen Akademie der Wissenschaften, Mathematisch-naturwissenschaftliche Klasse. Vienna* 17: 183–248.

Eckel, P. M. 2011. Pilotrichaceae. Bryophyte flora of North America, provisional publication. Missouri Botanical Garden. Website http://www.mobot.org/plantscience/bfna/V2/Pilotrichaceae.htm [accessed 01 May 2015].

Frey, W. 1970. Blattenwicklung bei Laubmoosen. *Nova Hedwigia* 20: 463–556.

Goffinet, B., W. R. Buck, and A. J. Shaw. 2009. Morphology, anatomy, and classification of the Bryophyta. *In* B. Goffinet and A. J. Shaw [eds.], Bryophyte biology, 2nd ed., 55-138. Cambridge University Press, Cambridge, UK.

Gradstein, S. R., S. P. Churchill, and N. Salazar-Allen. 2001. Guide to the bryophytes of tropical America. New York Botanical Garden Press, Bronx, New York, USA.

Harris, T. M. 1938. The British Rhaetic flora. British Museum (Natural History), London, UK.

Hastings, R. I., and H. Deguchi. 1997. Taxonomy of *Coscinodon arctolimnius* (Bryopsida, Grimmiaceae) including a new subspecies from Asia. *Bryologist* 100: 50–55.

Hedenäs, L. 1994. The basal pleurocarpous diplolepidous mosses—a cladistic approach. *Bryologist* 97: 225–243.

Hedenäs, L., J. Heinrichs, and A. R. Schmidt. 2014. Bryophytes of the Burmese amber forest: Amending and expanding the circumscription of the Cretaceous moss genus *Vetiplanaxis. Review of Palaeobotany and Palynology* 209: 1–10.

Hernandez-Castillo, G. R., R. A. Stockey, and G. W. Rothwell. 2006. *Anemia quatsinoensis* sp. nov. (Schizaeaceae), a permineralized fern from the Lower Cretaceous of Vancouver Island. *International Journal of Plant Sciences* 167: 665–674.

Hilton, J., and R. M. Bateman. 2006. Pteridosperms are the backbone of seed-plant phylogeny. *Journal of the Torrey Botanical Society* 133: 119–168.

Hübers, M., and H. Kerp. 2012. Oldest known mosses discovered in Mississippian (late Visean) strata of Germany. *Geology* 40: 755–758.

Ignatov, M. S. 1990. Upper Permian mosses from the Russian platform. *Palaeontographica. Abteilung B. Paläophytologie* 217: 147–189.

Ignatov, M. S., E. V. Karasev, and S. M. Sinitsa. 2011. Upper Jurassic mosses from Baigul (Transbaikalia, South Siberia). *Arctoa* 20: 43–64.

Ignatov, M. S., and D. E. Shcherbakov. 2007. Did pleurocarpous mosses originate before the Cretaceous? *In* A. E. Newton and R. S. Tangney [eds.], Pleurocarpous mosses: Systematics and evolution, 321-336. CRC Press, Boca Raton, Florida, USA.

Ignatov, M. S., and D. E. Shcherbakov. 2011a. Lower Cretaceous mosses from Khasurty (Transbaikalia). *Arctoa* 20: 19–42.

Ignatov, M. S., and D. E. Shcherbakov. 2011b. Lower Triassic mosses from Yaman Us (Mongolia). *Arctoa* 20: 65–80.

Joy, K. W., A. J. Willis, and W. S. Lacey. 1956. A rapid cellulose peel technique in palaeobotany. *Annals of Botany* 20: 635–637.

Klymiuk, A. A., and R. A. Stockey. 2012. A Lower Cretaceous (Valanginian) seed cone provides the earliest fossil record for *Picea* (Pinaceae). *American Journal of Botany* 99: 1069–1082.

Klymiuk, A. A., R. A. Stockey, and G. W. Rothwell. 2015. Plant-arthropod interactions in *Acanthostrobus edenensis* (Cupressaceae), a new conifer from the Upper Cretaceous of Vancouver Island, British Columbia. *International Journal of Plant Sciences* 176: 378–392.

Konopka, A. S., P. S. Herendeen, and P. R. Crane. 1998. Sporophytes and gametophytes of Dicranaceae from the Santonian (Late Cretaceous) of Georgia, U.S.A. *American Journal of Botany* 85: 714–723.

Konopka, A. S., P. S. Herendeen, G. L. Smith Merrill, and P. R. Crane. 1997. Sporophytes and gametophytes of Polytrichaceae from the Campanian (Late Cretaceous) of Georgia, U.S.A. *International Journal of Plant Sciences* 158: 489–499.

Krassilov, V. A. 1973. Mesozoic bryophytes from the Bureja Basin, Far East of the USSR. *Palaeontographica. Abteilung B. Paläophytologie* 143: 95–105.

Krassilov, V. A. 1982. Early Cretaceous flora of Mongolia. *Palaeontographica. Abteilung B. Paläophytologie* 181: 1–43.

La Farge-England, C. 1996. Growth form, branching pattern, and perichaetial position in mosses: Cladocarpy and pleurocarpy redefined. *Bryologist* 99: 170–186.

Lawton, E. 1971. Moss flora of the Pacific Northwest. Hattori Botanical Laboratory, Nichinan, Japan.

Little, S. A., R. A. Stockey, and G. W. Rothwell. 2006a. *Solenostelopteris skogiae* sp. nov. from the Lower Cretaceous of Vancouver Island. *Journal of Plant Research* 119: 525–532.

Little, S. A., R. A. Stockey, and G. W. Rothwell. 2006b. *Stramineopteris aureopilosus* gen. et sp. nov.: Reevaluating the role of vegetative anatomy in the resolution of leptosporangiate fern phylogeny. *International Journal of Plant Sciences* 167: 683–694.

Matsunaga, K. K. S., R. A. Stockey, and A. M. F. Tomescu. 2013. *Honeggeriella complexa* gen. et sp. nov., a heteromerous lichen from the Lower Cretaceous of Vancouver Island (British Columbia, Canada). *American Journal of Botany* 100: 450–459.

Miller, N. G. 1984. Tertiary and Quaternary fossils. *In* Schuster R. M. [ed.], New manual of Bryology, vol. 1, 1194-1232. Hattori Botanical Laboratory, Nichinan, Japan.

Mishler, B. D., and E. De Luna. 1991. The use of ontogenetic data in phylogenetic analyses of mosses. *Advances in Bryology* 4: 121–167.

Newton, A. E. 2007. Branching architecture in pleurocarpous mosses. *In* A. E. Newton and R. S. Tangney [eds.], Pleurocarpous mosses: Systematics and evolution, 287-307. CRC Press, Boca Raton, Florida, USA.

Newton, A. E., and E. De Luna. 1999. A survey of the morphological characters for phylogenetic study of the transition to pleurocarpy. *Bryologist* 102: 651–682.

Oostendorp, C. 1987. The bryophytes of the Palaeozoic and the Mesozoic. *Bryophytorum Bibliotheca* 34: 1–112.

Ramsay, H. P. 2012a. Australian mosses online. 1. Sematophyllaceae. Website http://www.anbg.gov.au/abrs/Mosses_online/01_Sematophyllaceae/Sematophyllaceae_family.pdf [accessed 09 May 2015].

Ramsay, H. P. 2012b. Australian mosses online. 14. Pylaisiadelphaceae. Website http://www.anbg.gov.au/abrs/Mosses_online/05_Pylaisiadelphaceae/Pylaisiadelphaceae_family.pdf [accessed 09 May 2015].

Ray, M. M., G. W. Rothwell, and R. A. Stockey. 2014. Anatomically preserved Early Cretaceous bennettitalean leaves: *Nilssoniopteris corrugata* n. sp. from Vancouver Island, Canada. *Journal of Paleontology* 88: 1085–1093.

Rothwell, G. W. 1999. Fossils and ferns in the resolution of land plant phylogeny. *Botanical Review* 65: 188–218.

Rothwell, G. W., and K. C. Nixon. 2006. How does the inclusion of fossil data change our conclusions about the phylogenetic history of euphyllophytes? *International Journal of Plant Sciences* 167: 737–749.

Rothwell, G. W., and R. A. Stockey. 2006. Combining characters of Pteridaceae and tree ferns: *Pterisorus radiata* gen. et sp. nov., a permineralized Lower Cretaceous filicalean with radial sori. *International Journal of Plant Sciences* 167: 695–701.

Rothwell, G. W., and R. A. Stockey. 2013. Evolution and phylogeny of gnetophytes: Evidence from the anatomically preserved seed cone *Protoephedrites eamesii* gen. et sp. nov. and the seeds of several bennettitalean species. *International Journal of Plant Sciences* 174: 511–529.

Rothwell, G. W., R. A. Stockey, and M. A. Millay. 2014. Evolution of modern Marattiales; superficially *Christensenia*-like synangia in the Early Cretaceous. Botany 2014: Annual meeting of the Botanical Society of America, Boise, Idaho, USA [online abstract]. Website http://2014.botanyconference.org/engine/search/index.php?func=detail&aid=283.

Smith, S. Y., R. S. Currah, and R. A. Stockey. 2004. Cretaceous and Eocene poroid hymenophores from Vancouver Island, British Columbia. *Mycologia* 96: 180–186.

Smith, S. Y., G. W. Rothwell, and R. A. Stockey. 2003. *Cyathea cranhamii* sp. nov. (Cyatheaceae), anatomically preserved tree fern sori from the Lower Cretaceous of Vancouver Island, British Columbia. *American Journal of Botany* 90: 755–760.

Smoot, E. L., and T. N. Taylor. 1986. Structurally preserved fossil plants from Antarctica: II. A Permian moss from the Transantarctic Mountains. *American Journal of Botany* 73: 1683–1691.

Steenbock, C. M., R. A. Stockey, G. Beard, and A. M. F. Tomescu. 2011. A new family of leafy liverworts from the Middle Eocene of Vancouver Island, British Columbia, Canada. *American Journal of Botany* 98: 998–1006.

Stockey, R. A., and G. W. Rothwell. 2006. The last of the pre-angiospermous vegetation: A Lower Cretaceous flora from Apple Bay, Vancouver Island. Presentation No. 45. *In* Advances in paleobotany—recognizing the contributions of David L. Dilcher and Jack A. Wolfe on the occasion of their 70th year, Florida Museum of Natural History, University of Florida, Gainesville, Florida, USA [online abstract]. Website http://www.flmnh.ufl.edu/paleobotany/meeting/abstract.htm#Stockey.

Stockey, R. A., and G. W. Rothwell. 2009. Distinguishing angiophytes from the earliest angiosperms: A Lower Cretaceous (Valanginian-Hauterivian) fruit-like reproductive structure. *American Journal of Botany* 96: 323–335.

Stockey, R. A., G. W. Rothwell, and S. A. Little. 2006. Relationships among fossil and living Dipteridaceae: Anatomically preserved *Hausmannia* from the Lower Cretaceous of Vancouver Island. *International Journal of Plant Sciences* 167: 649–663.

Stockey, R. A., and N. J. B. Wiebe. 2008. Lower Cretaceous conifers from Apple Bay, Vancouver Island: *Picea*-like leaves, *Midoriphyllum piceoides* gen. et sp. nov. (Pinaceae). *Botany* 86: 649–657.

Taylor, T. N., E. L. Taylor, and M. Krings. 2009. Paleobotany: The biology and evolution of fossil plants, 2nd ed. Academic Press, Burlington, Massachusetts, USA.

Tomescu, A. M. F., C. Steenbock, M. Friedman, and R. A. Stockey. 2012. The Early Cretaceous Apple Bay flora of Vancouver Island: A hotspot of fossil bryophyte diversity. Botany 2012: Annual meeting of the Botanical Society of America, Columbus, Ohio, USA [online abstract]. Website http://2012.botanyconference.org/engine/search/index.php?func=detail&aid=962.

Townrow, J. A. 1959. Two Triassic bryophytes from South Africa. *South African Journal of Botany* 25: 1–22.

Vavrek, M. J., R. A. Stockey, and G. W. Rothwell. 2006. *Osmunda vancouverensis* sp. nov. (Osmundaceae), permineralized fertile frond segments from the Lower Cretaceous of British Columbia, Canada. *International Journal of Plant Sciences* 167: 631–637.

Vitt, D. H. 1982. Bryopsida. *In* S. P. Parker [ed.], Synopsis and classification of living organisms, 307-336. McGraw-Hill, New York, New York, USA.

Vitt, D. H. 1984. Classification of the Bryopsida. *In* R. M. Schuster [ed.], New manual of bryology, vol. 2, 696-759. Hattori Botanical Laboratory, Nichinan, Japan.