

**On *Anthoceros phymatodes* M. Howe
and the hornwort genus *Phymatoceros* Stotler,
W. T. Doyle & Crand.-Stotl. (Anthocerotophyta)¹**

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Abstract – Based upon our study of type specimens, we confirm that *Anthoceros phymatodes* M. Howe from California is synonymous with *Phaeoceros bulbiculosus* (Brot.) Prosk. from Portugal. Furthermore, this taxon forms the basis for the recently named, monotypic genus *Phymatoceros* Stotler, W. T. Doyle & Crand.-Stotl. Our observations of the morphology, anatomy, and phenology of living populations from both California and Portugal reveal a suite of characters that discriminate this taxon, not only from *Phaeoceros* Prosk., but also from all other hornwort genera. These include rounded to spindle-shaped chloroplasts with abundant, bulging starch grains that may obscure a pyrenoid; highly dimorphic, dioicous thalli; a single antheridium per antheridial chamber, rather than 2 to 4 as in *Phaeoceros*; and spores that are fuscous at maturity.

Anthocerotophyta / California / Hornworts / Mediterranean / *Phaeoceros* / *Phymatoceros* / tubers

INTRODUCTION

Marshall Howe collected a hornwort on March 19, 1892 near “The Old Mill” in Marin County, California that although lacking reproductive structures, was yet of interest because it bore stalked ventral tubers. These tubers are initiated at the growing point apex and become ventral in the central midrib region with subsequent thallus growth (vide Howe, 1898). On February 22, 1896, he returned to the same location and collected it again, this time with very young sporophytes. These collections were then described as *Anthoceros phymatodes* M. Howe, sp. nov. (Howe, 1898). Although his account of this new species was

1. This paper is dedicated to the memory of H el ene Bischler-Causse (n ee Heiniger), who will be long remembered through her outstanding contributions to bryology.

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based upon both of these collections, Howe designated the 1896 collection with immature sporophytes as the type. The taxon was described as allied to *A. dichotomus* Raddi, but differing from it in the production of a somewhat larger costa, relative to thallus width, and in the development of a slightly thicker lamina. Distinctions made between the involucre width and mouth of these two species by Howe (1898) seem irrelevant since the *A. phymatodes* sporophytes were so immature. Howe (1898: 12) even stated "... it is quite possible that the description of the involucre given above will need some modification on the discovery of fully ripened sporogonia".

Müller (1916), in his discussion on the occurrence and distribution of *Anthoceros dichotomus*, stated that *A. phymatodes* was probably identical because of the shared occurrence of stalked ventral tubers. In his supplementary volume (Müller, 1939) and "Die Lebermoose Europas" (Müller, 1954), in fact, he included the provisional listing "?*Anthoceros phymatodes* Howe" in the synonymy of *A. dichotomus*. In the addendum to the anthocerotales for "Die Lebermoose Europas", Proskauer (1957) placed *A. dichotomus* under the earlier name *A. bulbiculosus* Brot. (= *Phaeoceros bulbiculosus* (Brot.) Prosk.), a reduction actually made some years earlier (Proskauer, 1954). It is of note that no mention of the name *A. phymatodes* was made in Proskauer's "Nachtrag" (1957) even though Müller, as discussed above, had associated that species with *A. dichotomus* in three of his earlier writings (Müller, 1916, 1939, 1954). Proskauer (1957: 1319), however, did write that there was a subspecies of *P. bulbiculosus* in California "In Kalifornien gibt es eine Unterart". This last statement is puzzling since in 1951 Proskauer had cited *A. phymatodes* as a synonym of *P. hallii* (Austin) Prosk. In this regard, it is of interest that the California "*phymatodes*" that Proskauer brought into his classes at Berkeley were, however, always labeled "*bulbiculosus*" by him (personal knowledge). We suspect that Proskauer changed his mind about the 1951 synonymy with *P. hallii*, but unfortunately, never published it.

The reduction of *A. phymatodes* to *P. hallii* by Proskauer (1951), nonetheless, impacted other nomenclatural treatments of *A. phymatodes*. Under the packet attached to the herbarium sheet containing a paratype specimen of *A. phymatodes* (UC Herbarium Sheet 45769) "Type?" "*A. phymatodes* = *Phaeoceros hallii* (Aust.) Prosk." is written in the hand of Proskauer. Based on this annotation, Stotler and Crandall-Stotler (1977) incorporated that synonymy into their North American checklist. Later, Hässel (1989: 738) published "*A. phymatodes* M. A. Howe = *P. hallii* (Aust.) Prosk." in her list of American synonyms although she did not include it in the synonym list under her actual treatment of *P. hallii*. Rather, she (Hässel, 1989: 731) cited the 1951 reduction by Proskauer.

The treatment of *A. phymatodes* by Frye and Clark (1947: 935-937) closely followed that of Howe (1898) but was supplemented with illustrations and descriptions of the sporophyte characters based upon a 1941 Mexican collection cited by them as "Clark and Frye Exsic. No. 68 (Frye & Frye 2993)". Their spore drawings clearly do not depict spores of either *P. hallii* or *P. bulbiculosus*. When Proskauer (1951) reduced *A. phymatodes* to *P. hallii*, he did so with the caveat that Frye and Frye 2993 did not belong there; i. e., it was not synonymous with *P. hallii*. *Anthoceros phymatodes* has never been formally transferred to the genus *Phaeoceros* Prosk. although Hasagawa (1984: 26) used that binomial (= nom. invalid.) in a tabular listing of the species of *Anthoceros* L. and *Phaeoceros* in Japan, North America, and Europe. It is somewhat ironic that he later (Hasegawa, 1991) listed it as a synonym of *P. laevis* (L.) Prosk. s. str. That error materialized

because he studied what he thought to be a specimen of genuine *A. phymatodes*, namely *Frye & Frye 2993* [NICH], and determined it to be *P. laevis*. Our study of a duplicate specimen of *Frye & Frye 2993* in WTU confirmed that this collection is unquestionably *P. laevis* (Stotler *et al.*, 2004).

Recently, Stotler and Crandall-Stotler (2005) recognized *Phaeoceros bulbiculosus* as an element of the hornwort flora of North America, citing *Anthoceros phymatodes* as a synonym of this taxon. In addition, *P. bulbiculosus* has been segregated from *Phaeoceros* into a new genus, *Phymatoceros* Stotler, W. T. Doyle & Crand.-Stotl. (Stotler *et al.*, 2005). The treatment herein provides the details of the studies that support these taxonomic decisions.

MATERIALS AND METHODS

Detailed SEM and optical microscope studies were conducted of all type collections of *P. bulbiculosus*, *A. phymatodes* and *P. hallii* and representative fresh collections of plants referable to *P. bulbiculosus* from California and Portugal. These include, but are not restricted to, the following:

Herbarium Specimens: PORTUGAL. Coimbra. Cabrises, nos taludes de uma vala, *Fernandes, Neves & Santos s.n.* (UC-985943, neotype of *A. bulbiculosus*). UNITED STATES. California. [Alameda Co.], On slides near the Bay, Oakland, ex hb. Austin, *Bolander s.n.* (NY- paratype of *A. phymatodes* = *A. pearsonii* M. Howe); [Marin County], Mill Valley, Feb. 22, 1896, *Howe s.n.* (NY-00226661, 00226662, 00226663 & 00226664 = holotype of *A. phymatodes*), Mill Valley, Mar. 19, 1892, *Howe s.n.* (NY (2), UC, paratypes of *A. phymatodes*), Shaver Creek, April 10, 1927, *Sutcliffe s.n.* (CAS); Monterey County, Fort Ord Public Lands, along Crescent Bluff Road, on soil in open areas in chaparral, 24 April, 1998, *Doyle 9466* (ABSH). OREGON. [Marion County], 26, Springy Places, Silverton [Hall 26] (MANCH-EM74235=21217 lectotype of *A. hallii* Austin); Dripping rocks, Salem, Hall 35 (MANCH-EM74232=21214 & EM74237=21219 paratypes of *A. hallii* = *P. pearsonii* (M. Howe) Prosk.).

Fresh Collections: PORTUGAL. Baixo Alentejo, Parque Natural do Guadiana, Ribeira de Limas, 11.02.2004, *Sérgio s.n.* (LISU); Estremadura: Serra da Arrábida, Vila Nogueira de Azeitão, Casais de Porela, Piedade, Sito, 140m, 22.01.2005, *Sérgio s.n.* (ABSH, LISU). UNITED STATES. California. Santa Cruz County, Santa Cruz Mts., campus of U.C. Santa Cruz, soil along Fuel Break Road, 10 May, 1996, *Doyle 8384* (ABSH, UC); 30 March 2004, *Doyle 10919* (ABSH); 9 December 2004, *Doyle 11248* (ABSH); Santa Cruz Mts., meadow nr Waugh Ave, R1W, T11S, 12 Feb. 2004, *Doyle 10883* (ABSH).

Small samples of thalli and tubers from the type collections were prepared for SEM study using the Aerosol OT restoration and dimethoxymethane (FDA) dehydration methods of Hoffman *et al.* (1996), but with graded replacement of FDA with ethanol prior to critical-point drying. Freshly collected samples were fixed in a solution of 2% glutaraldehyde/2% paraformaldehyde in 0.1M sodium cacodylate buffer at pH 7.2, @ 4°C, overnight. After thorough rinsing in dH₂O, samples were post-fixed in 2% aqueous OsO₄ for 3 hours and then dehydrated in a graded ethanol series. All thallus samples were critical-point dried in a Tousimis Samdri-750 CPD, using CO₂ as the transition fluid. Spore samples were prepared by opening air-dried capsules onto a stub covered with sticky tape. All mounted specimens were coated with 400Å of gold/palladium in a Denton Desk II sputter coater. Specimens were viewed and digital images captured with a Hitachi H500 SEM.

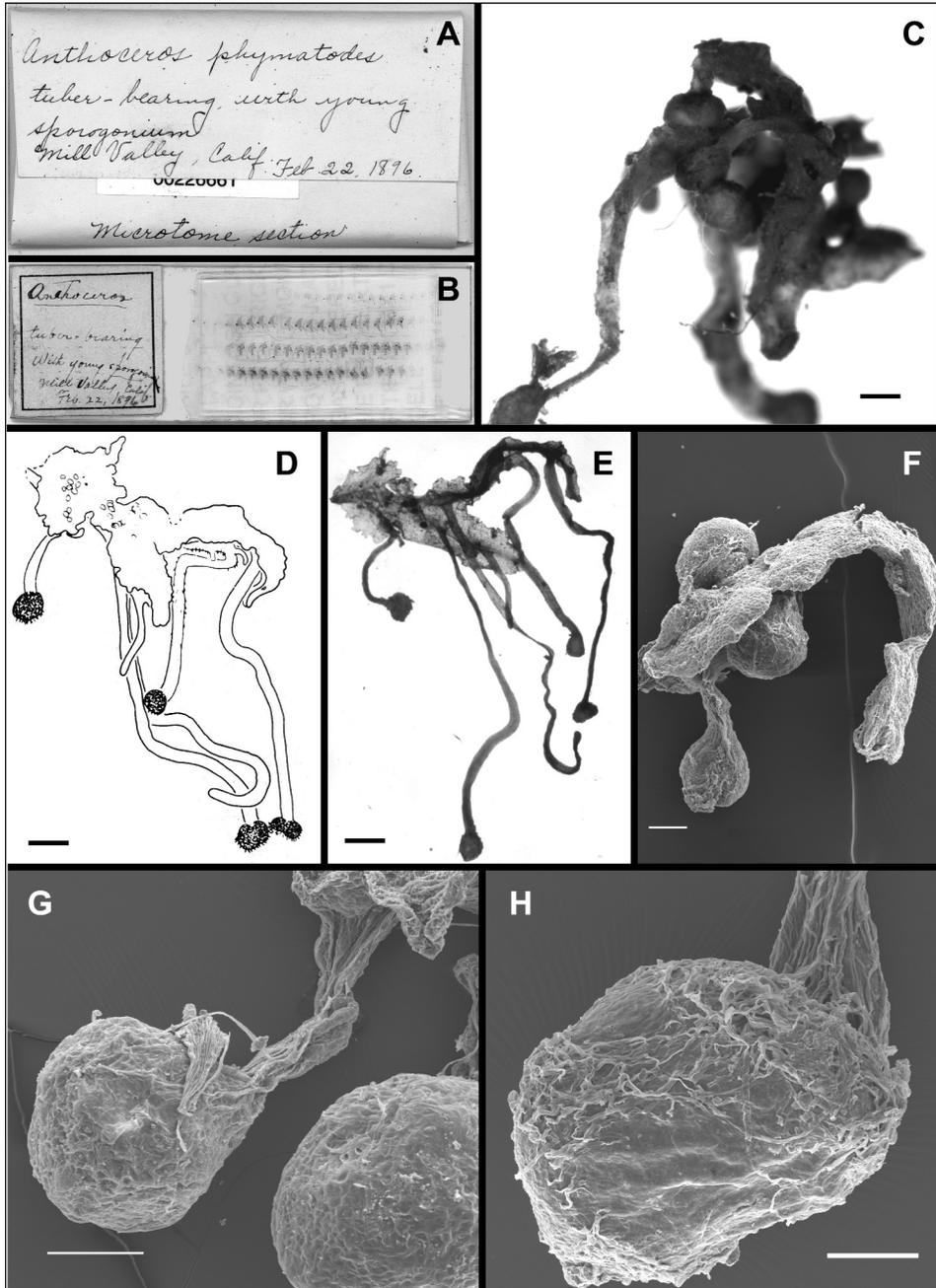
RESULTS AND DISCUSSION

Taxonomic considerations

The holotype of *Anthoceros phymatodes* is unique in that it consists only of serial microtome sections of plants mounted on glass microscope slides (Figs 1: A, B; 2: A-C). Four packets, each with labels that include the Howe holotype data for February 22, 1896 are extant in NY that contain serial sections of six different plants from the same gathering. Together, these comprise the holotype following Article 8.2 that allows for a type to consist of multiple small plants and Article 8.3 of the ICBN (Greuter *et al.*, 2000), which allows for a type specimen to be mounted as more than one preparation. Packet NY-00226661 contains two slides [one thallus bearing one young sporophyte]; NY-00226662 [two slides with thalli and tubers – series a = one thallus in serial transverse section and series b = one thallus in serial transverse section with good *Nostoc* colonies]; NY-00226663, one slide with thallus in longitudinal section [hits three tubers]; and NY-00226664, one slide of thallus in longitudinal section. Whole plants exist only in the paratype collections dated 1892 [NY (2), UC]. These specimens are consistent with the protologue and are more than sufficient to identify live plants collected in northern California throughout several growing seasons as this taxon. Comparisons of the types of *A. phymatodes* and numerous recent collections from California with the neotype of *Phaeoceros bulbiculosus*, supplemented with live material from Portugal, confirm that these taxa are, indeed, conspecific. The mature thalli of the 1892 paratypes of *A. phymatodes* are narrow, 1.0-1.8 mm wide, and produce numerous stalked tubers from the central region of the ventral thallus surface (Fig. 1: C, F). In both size and general facies, they closely resemble the tuber-bearing thalli of the neotype of *P. bulbiculosus* (Fig. 1: D, E). Furthermore, the stalked tubers of the two are morphologically identical, with mature tubers being more or less spheroidal, and often bearing randomly scattered, 2 or 3-celled slime hairs over their surfaces (Fig. 1: G, H).

The serial sections comprising the holotype of *A. phymatodes* show that the thallus is solid, with occasional enlarged mucilage cells, but without schizogenous mucilage canals (Fig. 2: A). The thallus is 9 to 12 cells thick near the middle, and tapers gradually to 3 cells thick at the margin. In transverse section, epidermal cells are 22-24 μm deep X 28-32 μm wide, internal cells are isodiametric, 48-64 μm in diameter, and mucilage cells are 90-100 μm in diameter. The scattered *Nostoc* Vaucher colonies are 100-150 μm in diameter and are encased by two rows of epidermal cells. The foot of the single immature sporophyte is 360 μm at its widest dimension and has small, haustorial cells at the foot/gametophyte juncture like *Phaeoceros* (see Renzaglia, 1978: Fig. 143). The sporangial wall is 5 cell layers

Fig. 1. Type specimens of *Anthoceros phymatodes* M. Howe and *Phaeoceros bulbiculosus* (Brot.) Prosk. **A, B.** Holotype of *A. phymatodes*, with A showing the packet label and B, one of the two slides of serial sections included therein. **C.** Light micrograph of a thallus with tubers from one of the paratypes of *A. phymatodes*, scale bar = 1 mm. **D.** Drawing of a tuber-bearing specimen of *P. bulbiculosus*, from Proskauer, 1957: 1318; scale bar = 2 mm. **E.** Light micrograph of a tuber-bearing thallus from the neotype of *P. bulbiculosus*; note the similarity to the Proskauer illustration in figure D; scale bar = 2 mm. **F, G.** SEM micrographs of thalli and tubers from one of the paratypes of *A. phymatodes*; scale bars = 200 μm . **H.** SEM micrograph of tuber from neotype of *P. bulbiculosus*; scale bar = 200 μm . A, B from Howe *s.n.* [holotype (NY-00226661)]; C, F, G from Howe *s.n.* [paratype (NY-00226658)]; D, from Proskauer, 1957: 1318; E, H from Fernandes, Neves & Santos *s.n.* [neotype (UC-985943)].



thick, and the involucre extends up to 1.1 mm in height. The several tubers that are included in the sections are fairly young, ranging only to 600 μm in diameter (Fig. 2: C). The tuber stalks are comprised of elongate cells, 40 μm wide X 80–120 μm long, and are 10 to 12 cell rows in thickness. The tuber proper consists of 2 or 3 layers of slightly elongate wall cells and an interior of deeply stained, isodiametric cells, 20–23 μm in diameter.

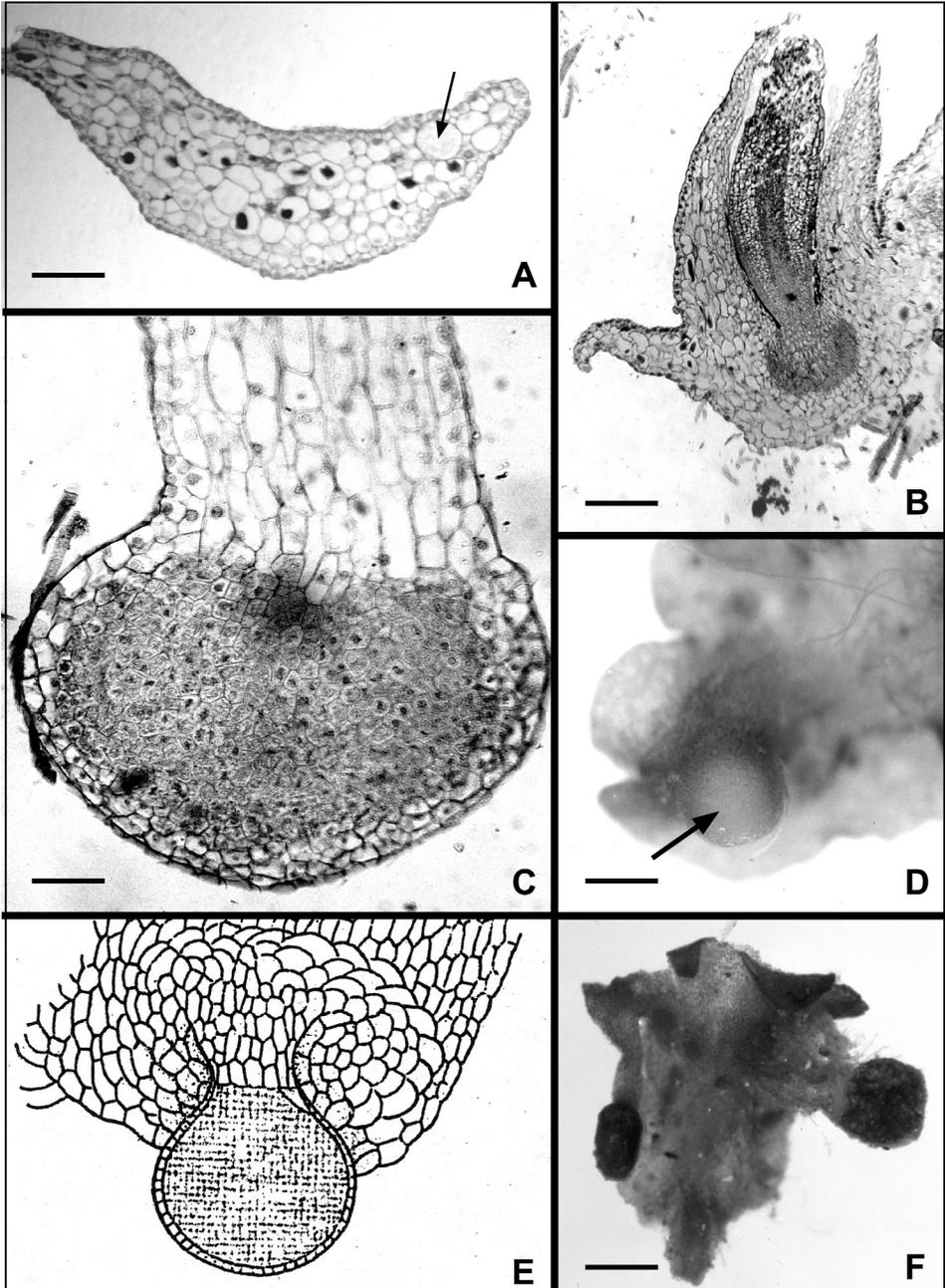
It is interesting to note the similarity of these sectioned tubers (Fig. 2: C) with a young tuber from live *P. bulbiculosus* from Portugal (Fig. 2: D) and the drawing in Fig. 2: E, which is an inverted copy of figure 5 from Lampa (1903: 437). In 1903, Emma Lampa described and illustrated what she considered to be exogenous antheridial development in *Anthoceros dichotomus* (= *A. bulbiculosus*), a claim accepted, unchallenged, by Davis (1904). As aptly discussed by Howe (1904), these so-called antheridia, which arose close to the thallus apex and had stalks 8 to 10 cells in thickness, are most likely developing ventral tubers. Howe's view is further supported here.

In his protologue, Howe (1898) also referred a Bolander specimen to his new taxon. We located a mica slide in NY with the following label data “*Anthoceros phymatodes* M. A. Howe ‘On slides near the Bay’, Oakland, Calif. Bolander, ex herb. Austin” (none was located in hb Austin at MANCH). That paratype specimen consists of thalli mounted on a mica slide, with average thallus width of 1.2 mm. The condition of these plants is quite poor making a positive identification questionable but it definitely is not *A. phymatodes* because the tubers are flat, marginal and only sometimes stalked (Fig. 2: F) rather than bulbate, stalked and ventral. A small piece of thallus that could be removed from the mica slide, in fact, closely resembles one of Howe's illustrations of *Anthoceros pearsonii* (Howe, 1898: Pl. 322, Fig. 6). Based upon plant size, habitat and tuber position, we refer this Bolander paratype to *Phaeoceros pearsonii*.

That *A. phymatodes* is not conspecific with *P. hallii* can also be confirmed, even without the characters provided by mature sporophytes. The thalli of *P. hallii* are broader, to 5.0 mm across, and thinner, 6–8 cells in depth, and possess only flattened, apical or marginal tubers. They in no way resemble the tuber-bearing thalli found in the types of *A. phymatodes*. In addition, our study of recent collections of both taxa from the Pacific Northwest confirm differences in spore wall architecture (compare Fig. 5 with Hässel de Menéndez, 1989: Fig. 13) and sexual condition, with monoicy in *P. hallii*, and dimorphic dioicy in *A. phymatodes*.

As a consequence of these comparative studies of living populations of *P. bulbiculosus* from California and Portugal, we have concluded that not only is *A. phymatodes* synonymous with *P. bulbiculosus*, but also that this taxon represents a genus distinct from *Phaeoceros*. We described it as the genus *Phymatoceros* (Stotler *et al.*, 2005), as will be discussed in detail in the section that follows.

Fig. 2. Comparisons of type specimens of *Anthoceros phymatodes* M. Howe and Portuguese specimens of *Phaeoceros bulbiculosus* (Brot.) Prosk. **A.** Thallus section from holotype slide of *A. phymatodes*, with large mucilage cell at arrow; scale bar = 150 μm . **B.** Light micrograph of young sporophyte from holotype of *A. phymatodes*; scale bar = 200 μm . **C.** Young tuber in section from holotype slide of *A. phymatodes*; scale bar = 100 μm . **D.** Developing ventral tuber (at arrow) from living collection of *P. bulbiculosus*; scale bar = 500 μm . **E.** Inverted drawing of so-called ‘superficial antheridia’ described and illustrated by Lampa, 1903. **F.** Portion of a thallus from the Oakland paratype of *A. phymatodes*, with marginal rather than ventral tubers [= *P. pearsonii*]; scale bar = 600 μm . A, C from Howe *s.n.* [holotype (NY-00226662a)]; B from Howe *s.n.* [holotype (NY-00226661)]; D from 22.01.2005, Sérgio *s.n.* (ABSH, LISU); E modified from Lampa, 1903: 437; F from Bolander *s.n.* [paratype (NY-00226665)].



The Biology of *Phymatoceros bulbiculosus* (Brot.) Stotler, W. T. Doyle & Crand.-Stotl.

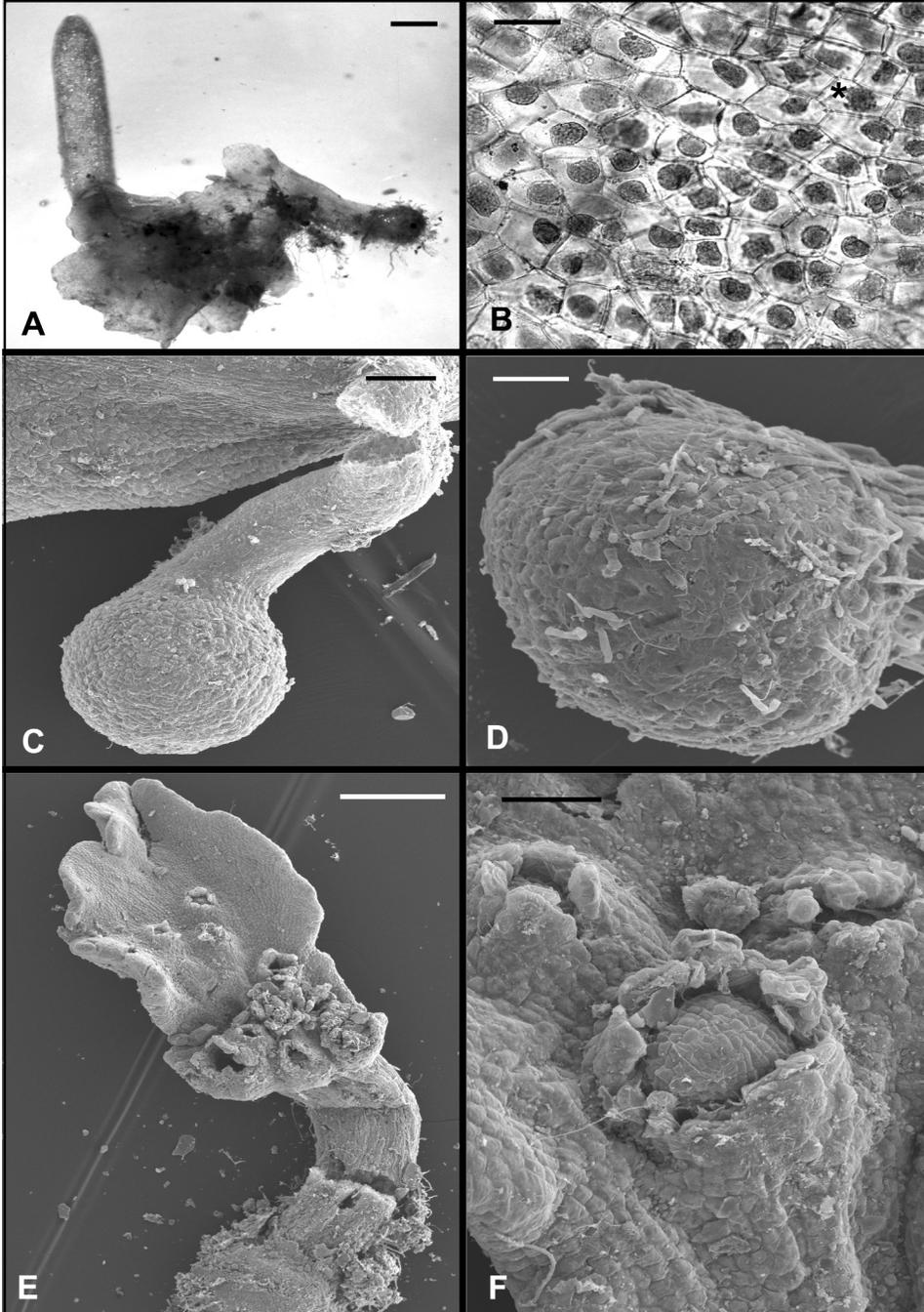
Phymatoceros bulbiculosus is an ephemeral of seasonally dry habitats, with a reported distribution in the Mediterranean region of Europe and Africa, Brazil, Argentina, and central and northern California (Schuster, 1992; Stotler & Crandall-Stotler, 2005; Stotler *et al.*, 2005). In the California sites, where our field studies have been focused, *P. bulbiculosus* forms small, compact mats on gravelly soil of open or partially shaded sites in meadows, hillsides, old logging roads and along trails at elevations of 250-650 m in the western foothills of the Sierra Nevada, slightly lower, 20-575 m, in the coastal mountains. The small, infrequently branched, strap-shaped thalli germinate from dormant tubers during the rainy season, as early as December or January; typically by late April spore maturation is complete and the plants die back, leaving their tubers and spores in the diaspore bank.

A single, erect cylindrical axis, approximately 0.5 mm in diameter and 1.0 mm (male tuberlings) to 2.5 mm (female tuberlings) long, emerges from the germinating tuber although occasionally two such axes may be formed. Gametangia develop as soon as the plant assumes horizontal growth and the flattened, somewhat canaliculate thallus forms at the apex of the tuberling (Fig. 3: A, E). Sporelings grown in axenic culture display this same developmental sequence.

The thalli are dioicous, with the male plants being no more than half the size of the females (compare Fig. 3: E and Fig. 4: A). The male plants are very short-lived, and begin to form numerous ventral tubers as soon as the first-formed antheridia mature. The antheridial chambers are crowded near the base of the thallus, with each chamber forming but a single antheridium (Fig. 3: E, F). Juxtaposed chambers may be separated from each other by only a single layer of thallus cells; sometimes when these juxtaposed chambers open, the cells separating them tear, merging the chambers into a single cavity. Such merged cavities appear, then, to contain 2 or more antheridia. Mature antheridia are globose, 280-296 μm in diameter, with a single pale yellow chromoplast in each of the randomly arranged jacket cells; they are subtended by a very short 4-seriate stalk, 2 cell tiers in length.

Female thalli are more persistent than the males, forming tubers only on branches that lack sporophytes (Fig. 4: A, C, D). On branches with developing sporophytes a thickened area may develop ventrally below the sporophyte, but this does not mature into a tuber until spore maturation is complete. A few scattered *Nostoc* colonies are usually present in the female thalli, but are often lacking in the short-lived males.

Fig. 3. *Phymatoceros bulbiculosus* (Brot.) Stotler, W. T. Doyle & Crand.-Stotl. from California. **A.** Female thallus recently emerged from a tuber, and already bearing a young sporophyte; note that this thallus is comparable in size and development to the holotype; scale bar = 1 mm. **B.** Portion of a thallus wing showing a single spheroidal to ellipsoidal plastid, bearing numerous starch grains, in each epidermal cell; in the cell marked with an asterisk, one can see a somewhat rounded, central dark area that may be a pyrenoid; scale bar = 38 μm . **C.** SEM micrograph of a stalked, ventral tuber; scale bar = 200 μm . **D.** SEM micrograph of a tuber showing scattered slime cells and rhizoids; scale bar = 100 μm . **E.** SEM micrograph of a male thallus, recently emerged from a tuber, with several antheridial chambers, scale bar = 600 μm . **F.** SEM micrograph of a single antheridial chamber, showing a single globose antheridium per chamber; scale bar = 100 μm . A, B from Doyle 10883 (ABSH); C from Doyle 8384 (ABSH); D from Doyle 10919 (ABSH); E, F from Doyle 11248 (ABSH).

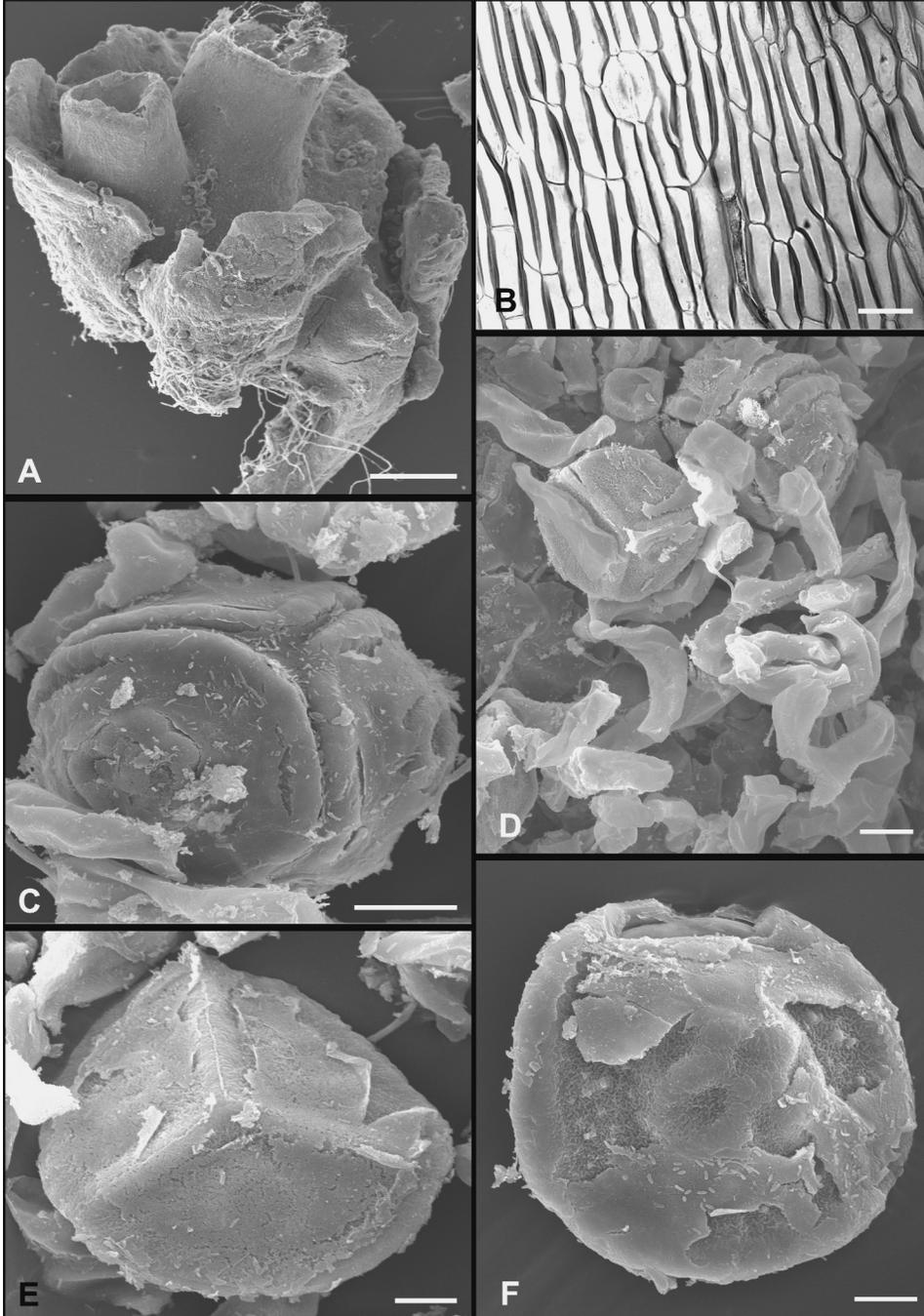


Proskauer (1958: 1319) described the plastids of *P. bulbiculosus* as being of a distinctive form. In surface view (Fig. 3: B), the cells of the dorsal epidermis are isodiametric to slightly rectangular, 30-38 μm in width. The single chloroplast in each dorsal epidermal cell is ellipsoidal to spindle-shaped, from 15-19 μm in length and distended by numerous large, discoid starch grains that are scattered throughout. These many starch grains obscure the internal structures of the chloroplasts. In fact, details of chloroplast organization are equivocal. Our original description of *Phymatoceros* indicated that it is set apart from elements of the *Phaeoceros laevis* group by the absence of a pyrenoid in the chloroplast (Stotler *et al.*, 2005). This conclusion was based on surface view observations of epidermal cells in freshly collected specimens from both Portugal and California, in which the plastids appear as described above (Fig. 3: B). K. Renzaglia (personal communication) has subsequently informed us that there definitely are pyrenoids in the plastids in *P. bulbiculosus* from Portugal. Our review of the serial sections of thalli in the holotype of *A. phymatodes* show that there is, in fact, a small, central darkened region that might well be a pyrenoid in the spindle-shaped plastid. The plastids in these sections, in both shape and presumed pyrenoid position, resemble those illustrated by Burr (1970: Fig. 8) in cross-sections of *Notothylas orbicularis* (Schwein.) Sull. ex A. Gray. While these observations would appear to confirm the presence of *Notothylas*-like pyrenoids in *Phymatoceros*, further studies at the ultrastructural level are needed to verify this conclusion.

The tubers are morphologically similar to those described earlier for the type specimens. Those formed on the female plants are generally larger than those formed on the males. Tests of fresh tubers with IKI were negative, suggesting that starch, while likely present, is not the major storage product of the tuber. It is probable that oils and proteins serve as the major storage products as reported for other tuberous hornworts by Ashworth (1896) and Ligrone and Lopes (1989).

The young sporophytes emerge from their involucre within a month of tuber germination. The involucre are erect, 1.0 to 1.2 mm long by 0.5-0.7 mm wide, with the unlobed mouth somewhat clasping the sporophyte (Fig. 4: A). Stomata are scattered throughout the mature areas of the capsule (Fig. 4: B), with the guard cells averaging 64 μm in length by 18 μm in width. The longitudinal radial walls of the epidermal cells are uniformly thickened while the transverse radial walls remain thin. Under field conditions the color of the capsule changes during development from green (very young) to yellowish-orange (immature spores) to dark brown or smoky black (mature, field dehiscence stage). Capsules that are collected prior to field dehiscence will darken and split open as they dry, but the spores contained therein are still somewhat immature. Such, in fact, is the case in the neotype of *Phaeoceros bulbiculosus* (Fig. 5). Fully mature capsules are erect or slightly bent, from 1.5-2.0 cm long, and dehisce along a single longitudinal slit.

Fig. 4. *Phymatoceros bulbiculosus* (Brot.) Stotler, W. T. Doyle & Crand.-Stotl. from California. **A.** SEM micrograph of a female thallus bearing involucre and sporophytes; scale bar = 600 μm . **B.** Sporangial wall in surface view, showing a stoma and the longitudinal thickenings of the radial walls; scale bar = 44 μm . **C.** Spore tetrad, showing the enclosing spore mother cell wall covering the distal surfaces of the spores; scale bar = 20 μm . **D.** Intermixed spores and pseudoelaters from the medial part of the sporangium; scale bar = 20 μm . **E, F.** Recently separated spores showing proximal (E) and distal (F) surfaces; note that in both cases the enclosing special walls of the spore tetrad are beginning to deteriorate, revealing the fibrillar spore wall ornamentation beneath; scale bars = 10 μm . A from *Doyle 10919* (ABSH); B-F from *Doyle 8384* (ABSH).

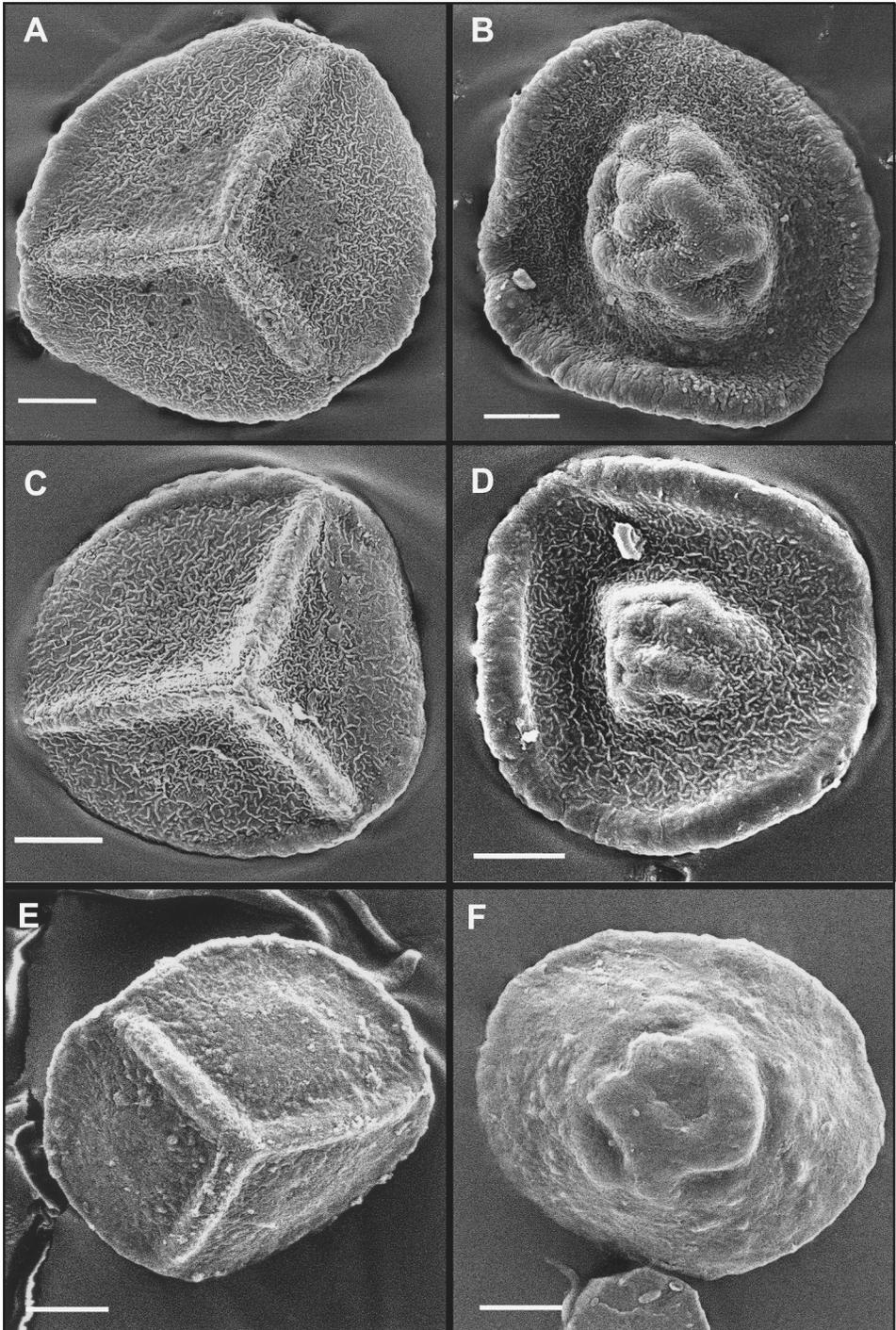


Spore wall architecture has long been used as a reliable character in hornwort systematics (e.g., Proskauer, 1964; Hässel de Menéndez, 1989, 1990). Within a single capsule, however, one can find a progression of architectural changes from meiotic tetrads to mature stages (Fig. 4: C-F; Fig. 5). To assure that spores are truly mature, it is necessary to allow populations with capsules to mature and dry naturally in the field before collection. In *P. bulbiculosus*, nearly mature spores from capsules collected prior to dehiscence, as for example, in the neotype, are yellow and exhibit fine fibrils on both the proximal and distal spore surfaces (Fig. 5: A-D). In spores that have just recently separated from the tetrad, remnants of the sporocyte wall and intrasporal septum obscure these fibrils (Fig. 4: C, E, F). These remnants are removed as the spores mature, exposing the fibrils in midstage spores, but another coating of unknown material is deposited over the spores just prior to capsule dehiscence. This latter material changes spore color from yellow to brownish black and again obscures the fibrils (Fig. 5: E, F). Thus, as one progresses from the base to the apex of a truly mature capsule, one will find yellow spores with a fine reticulum of fibrils in the middle part of the capsule, but fuscous spores with roughened surfaces at the apex (Fig. 5). The origin of this coating material is unknown, but its very late deposition over the exine of nearly mature spores is suggestive of a perine-like layer.

In herbarium specimens, most of the fully mature spores in a capsule are discharged before or during the drying process so what remains in these capsules are maturing spores that lack the fuscous layer. Such spores will exhibit the basic fibrils and yellow color of the immature spores. The same is essentially true for those herbarium capsules that are “nearly” mature, but indehiscent. No doubt, many of the published SEMs of hornwort spores from type specimens are of spores that still remained in the capsule and thus spores that were maturing when death prematurely came upon them. The spores of the neotype of *P. bulbiculosus* (Fig. 5: C, D) and those from *Sutcliffe s.n.* (Fig. 5: A, B) are examples of maturing, but not fully mature spores, while those from *Doyle 9466* (Fig. 5: E, F) are fully mature spores that have been removed from field-dehisced capsules. Proskauer (1964: 110), in fact, pointed out this phenomenon many years ago, writing: “In some cases the basic markings are obscured by secondary external wall deposition.”

It is important to note that we have not observed this late deposition of a fuscous coating material in *Phaeoceros laevis*, *P. carolinianus* (Michx.) Prosk., *P. hallii*, *P. mohrii* (Austin) Hässel and *P. oreganus* (Austin) Hässel. In these taxa the spores remain yellow all the way to the apex of field-ripened sporophytes, and distinctive patterns of exine architecture remain unchanged with maturation. In this character *Phymatoceros* is clearly distinct from many, but not all, species currently placed in *Phaeoceros*; e. g., *Phaeoceros pearsonii* possesses similar fuscous spores at maturity.

Fig. 5. Spores of *Phymatoceros bulbiculosus* (Brot.) Stotler, W. T. Doyle & Crand.-Stotl. **A, B.** Proximal (A) and distal (B) surfaces of spores before final “ripening” and deposition of the fuscous coating; at this stage the spores are yellow with both surfaces covered by a fine network of vermiculae or fibrils; scale bar = 10 μ m. **C, D.** Proximal (C) and distal (D) surfaces of spores from immature capsules of the neotype of *Phaeoceros bulbiculosus* (Brot.) Prosk.; note the similarity in architecture with the immature spores of the California plants in figures A & B; scale bars = 10 μ m. **E, F.** Proximal (E) and distal (F) surfaces of fuscous spores from field-ripened sporophytes, showing how the deposition of material just prior to spore release covers the fine vermiculae of the immature spore; scale bars = 10 μ m. A, B from *Sutcliffe s. n.* (CAS); C, D from *Fernandes, Neves & Santos s. n.* [neotype (UC-985943)]; E, F from *Doyle 9466* (ABSH).



Spores in *Phymatoceros* range from (49-) 52–64 (-69) μm in equatorial diameter. The distal spore face is ornamented with a central elevated protuberance that is formed of thick irregular ridges, and the proximal faces are more or less smooth, with a prominent triradiate ridge. A fine network of fibrils that is visible only in somewhat immature spores ornaments the exine of both surfaces. Pseudoelaters are comprised of two or three cells, are 15–18 μm in diameter, have unthickened walls, and are fuscous at maturity.

Affinities of *Phymatoceros* Stotler, W. T. Doyle & Crand.-Stotl.

The segregation of *Phymatoceros bulbiculosus* from *Phaeoceros* is supported by a combination of gametophytic and sporophytic characters. The resolution of *Phaeoceros* as a paraphyletic assemblage in recent molecular phylogenetic studies (Duff *et al.*, 2004) further suggests that with continued study, still other segregates will be identified. While discrete as a genus, *Phymatoceros*, nonetheless, shares the following characters with *Phaeoceros*: a solid thallus that lacks schizogenous cavities, antheridia with randomly arranged jacket cells, sporophyte foot with numerous, small haustorial cells, capsule with stomates, and thin-walled, two- or three-celled pseudoelaters. Consequently, *Phymatoceros* is classified in Notothyladaceae (Milde) Müll. Frib. *ex* Prosk. subf. Phaeocerotoidae Hässel (Stotler & Crandall-Stotler, 2005).

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