



## A synthesis of hornwort diversity: Patterns, causes and future work

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### Abstract

Hornworts are the least species-rich bryophyte group, with around 200–250 species worldwide. Despite their low species numbers, hornworts represent a key group for understanding the evolution of plant form because the best-sampled current phylogenies place them as sister to the tracheophytes. Despite their low taxonomic diversity, the group has not been monographed worldwide. There are few well-documented hornwort floras for temperate or tropical areas. Moreover, no species level phylogenies or population studies are available for hornworts. Here we aim at filling some important gaps in hornwort biology and biodiversity. We provide estimates of hornwort species richness worldwide, identifying centers of diversity. We also present two examples of the impact of recent work in elucidating the composition and circumscription of the genera *Megaceros* and *Nothoceros*. Important areas for further research are highlighted, particularly at taxonomic, ultrastructural, phylogenetic and genomic levels.

**Keywords:** Hornworts, biodiversity, diversification times, taxonomy, *Megaceros*, *Nothoceros*

### Introduction

The eukaryote Tree of Life is sprinkled with lineages of Paleozoic origin that have little extant diversity [Cycads (ca. 250 spp., Hill *et al.* 2003), Ginkgophyte (1 sp.), Gnetophytes (95 sp., (Carmichael & Friedman 1996), Sphenopsids (15 spp., Rothwell 1996)]. With distinct morphologies, these lineages are of paramount importance in understanding character transformations and the evolution of body form (Carmichael & Friedman 1996). Low extant diversity is often explained by an ancient radiation that was followed by multiple and massive extinctions through geological time (Kenrick & Crane 1997). For example, the sphenopsids are known from an extensive fossil record that first appeared in the Devonian. The group flourished in diversity with many genera and species in the Carboniferous, and through extinction events in the eons that followed are represented today by a single genus, *Equisetum* Linnaeus (1753: 1061–1062), with 15 species (Rothwell 1996; Smith *et al.* 2006). The fossil record, however, is extremely fragmentary for bryophytes, the first colonizing land plants, and does little in the way of resolving the earliest divergences and radiations among embryophytes.

Hornworts are the most species depauperate of all seedless plant phyla. Current hornwort diversity is estimated at 200–250 species, a small number in comparison to mosses (11000–13000 spp., Magill 2010), liverworts (7000–9000 spp., von Konrat *et al.* 2010), lycophytes (1285 spp., Frey & Stech 2009) and ferns (11000 spp., Smith *et al.* 2006). Despite low numbers of species, hornworts represent a key group in the

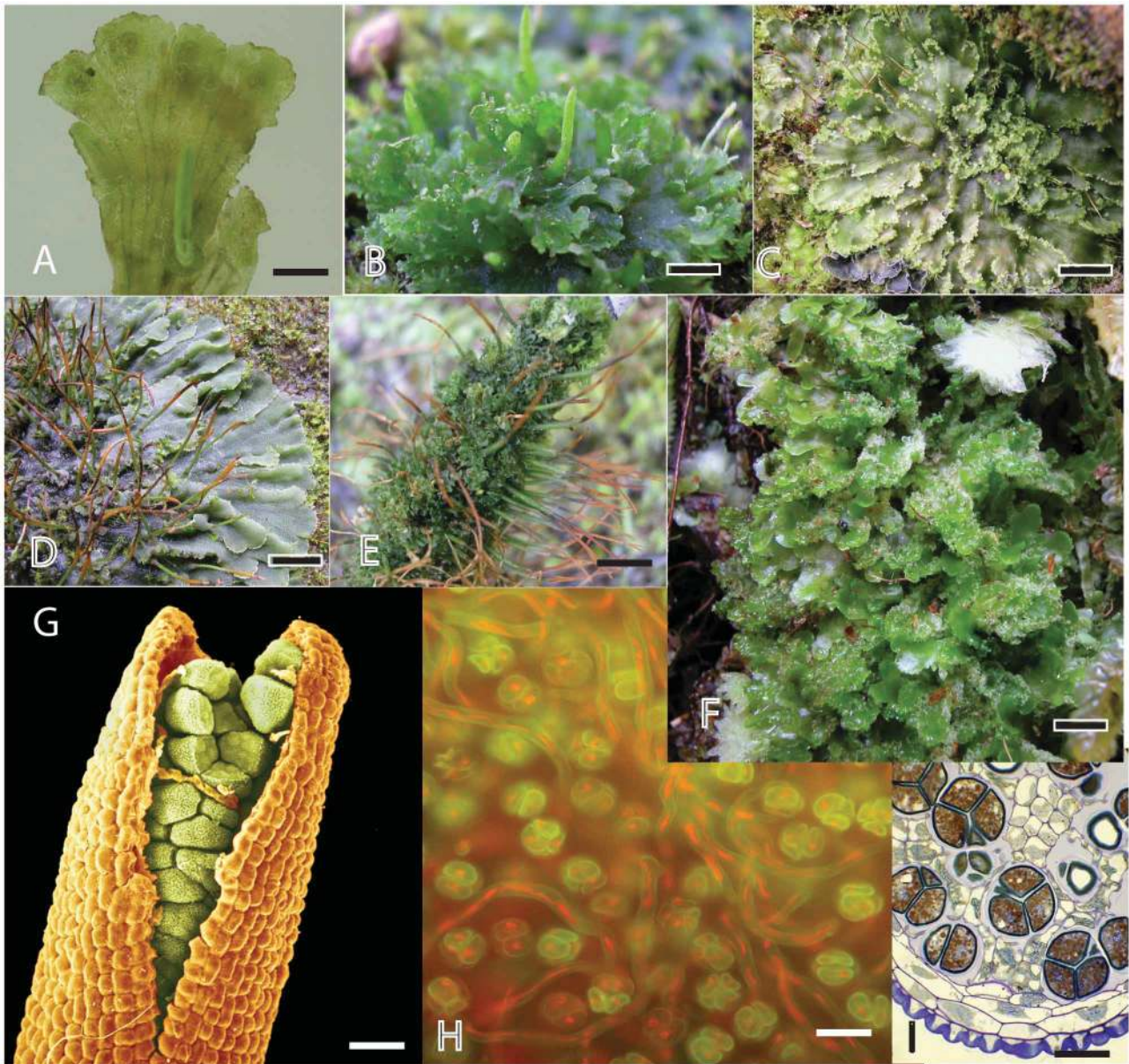
evolution of plant form because they are hypothesized to be sister to the tracheophytes (Qiu *et al.* 2006). Consequently, hornworts are the primary candidate in which to study the evolutionary conversion from a gametophyte to a sporophyte dominant life cycle. Although difficult to characterize, the mysteries of this radical transformation are hidden somewhere in the structural diversity that is diagnostic of this small, peculiar plant group.

Hornworts have long fascinated scientists because of their unique combination of morphological and developmental traits (Hofmeister 1862; Campbell 1895; Renzaglia 1978; Renzaglia *et al.* 2009). Most hornworts have an algal-like chloroplast with a central pyrenoid that contains the enzyme RuBisCO (Vaughn *et al.* 1990) and therefore exhibits a carbon concentration mechanism not seen in other land plants (Smith & Griffiths 1996; Hanson *et al.* 2002; Meyer *et al.* 2008). A cyanobacterial association is ubiquitous in hornwort gametophytes and is established via apically-derived, stoma-like clefts. Colonies of the cyanobacteria are internal and either discrete or develop with apical growth as central strands (Villarreal & Renzaglia 2006). The only other plant gametophyte that harbors a nitrogen-fixing bacterium is that of the liverworts in the Blasiales. In these plants, contrary to hornworts, the homoplastic development of *Nostoc* colonies is external to the thallus (Renzaglia *et al.* 2000).

Although the gametophyte alone is sufficient to distinguish hornworts from other embryophytes, it is the sporophyte that is truly exceptional (Fig. 1). The hornwort sporophyte is essentially a sporangium that grows from a basal meristem and continually produces spores from the tip downward. Hundreds of genetically different sporophytes may develop on a single gametophyte, progressively releasing meiotically-derived spores throughout the season. These morphological traits are unwavering within hornworts and unparalleled among living and extinct embryophyte lineages (Renzaglia *et al.* 2009), a fascinating but frustrating phenomenon as morphological synapomorphies with tracheophytes are virtually non-existent. Biochemical features of the cell wall (e.g. xylans, xyloglucans) have emerged as potential phylogenetic markers to support the hornwort-polysporangiophyte relationship (Carafa *et al.* 2006; Peña *et al.* 2008; Popper & Tuohy 2010). The morphological distance from other plants and the small size of the clade suggest that the group is an end-line that has suffered rampant decimation at sometime in the past. Whether existing diversity represents relicts of an early radiation or of more recent speciation events has not yet been evaluated.

Although there are few morphological traits that hornworts share with sister-groups, taxonomic boundaries within hornworts are blurred and species diversity is poorly known. This is true in spite of the paucity of documented cases of polyploidy and hybridization in hornworts. Indeed, the group is characterized by low and little variable chromosome numbers of 4 + 1 sex chromosomes in dioicous taxa and 5 to 6 chromosomes in monoicous taxa. Small genome sizes suggest low levels of paleo-polyploidization (Proskauer 1957; Newton 1983; Renzaglia *et al.* 1995). In spite of low species numbers, low chromosome counts, and limited biodiversity, hornworts remain a phylogenetically important group of plants that is inadequately characterized. The group has never been taxonomically revised on a global scale, and there are few well-documented floras, whether temperate (Proskauer 1958; Schuster 1992; Paton 1999) or tropical (Hasegawa 1980–1986; Asthana & Srivastava 1991; Singh 1994; Gradstein & Costa 2003). Moreover, no phylogenetic species-level or population studies on hornworts are available.

This paper is a first step in filling some of the critical gaps in knowledge about hornwort biology (Table 1, Supplemental information 1). We begin by estimating the time of divergence of hornworts from tracheophytes. We provide a synthesis of the scattered reports on hornwort fossils and we assign times of diversification within the group. We then turn our attention to current centers of diversity, to describe what is known and what can be learned. Finally, we use two case studies of the genera *Megaceros* and *Nothoceros* to explore hornwort species level diversity using morphology and sequence data.



**FIGURE 1.** A. *Leiosporoceros dussii* (Stephani 1893: 142) Hässel (1986: 255), Panama. Female plant with sporophyte. The gametophyte shows the blue-green looking *Nostoc* strands. B. *Anthoceros* sp., Australia. A monoicous plant showing some immature sporophytes. C. *Phaeomegaceros coriaceus* (Stephani 1916: 991) Duff *et al.* (2007: 241), New Zealand. Fan-shaped overlapping gametophytes with marginal gemmae in each lobe. D. *Phaeomegaceros hirticalyx* (Stephani 1916: 966) Duff *et al.* (2007: 241), New Zealand. The orange-brownish sporophytes contrast with the velvety appearance of the gametophytes. The gametophytes are covered with dorsal outgrowths. E. *Dendroceros validus* Stephani (1917: 1016), New Zealand. The species grows on shrubs and leaves. F. *Nothoceros giganteus* (Lehm. et Lindenb. in Lehmann 1832: 25) Villarreal *et al.* (2007: 283), New Zealand. The only species of the genus *Nothoceros* outside of the American continent. The luxurious appearance of the species is due to the extensive development of “wings” over the wide midrib, giving a “lettuce-like” look characteristic of the species. G. *Dendroceros crispatus* (Hooker 1813: 117) Gottsche *et al.* (1846: 579), Australia. Scanning electronic micrograph (SEM) of a dehiscing sporophyte with green multicellular spores and golden pseudoelaters. Notice the short epidermal cells. SEM colored by Andy Long. H. *Leiosporoceros dussii* (Steph.) Hässel, Panama. Autofluorescence of tetrads and elaters. The smooth bean-shaped spores are in bilateral-alterno opposite tetrads (yellow-green) and are interspersed by elongated pseudoelaters; both spores and pseudoelaters contain plastids (red). I. *Notothylas temperata* Hasegawa (1979: 20). Japan. Transverse section of the sporophyte showing tetrads (brown) with pseudoelaters in “shelves”. The central columella is physically connected to a pseudoelater chain. Scale bars: A–F= ca. 10 mm; G= 50  $\mu$ m; H= 30  $\mu$ m; I=40  $\mu$ m.

**TABLE 1.** GENERIC SYNOPSIS OF HORNWORTS (Modified from Renzaglia *et al.* 2009).

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<p><b><i>Leiosporoceros</i></b> Hässel (1986: 255), <b>1</b> species, <i>L. dussii</i> (Steph.) Hässel. Neotropical. Thallus typically solid; mucilage clefts absent in <i>Nostoc</i>-infected tissues, present in young uninfected plants. <i>Nostoc</i> colonies in longitudinally oriented strands in mucilage-filled schizogenous canals. Chloroplast 1 per cell. Pyrenoid lacking. Antheridia numerous (up to 70 per chamber) with a tiered jacket cell arrangement. Capsule with stomata. Massive sporogenous tissue (6–9 layers). Spore tetrads bilateral alterno-opposite. Spores yellow, minute, ovoid, nearly smooth; Y-shaped to monolete mark present. Pseudoelaters long, usually unicellular, thick-walled.</p> <p><b><i>Anthoceros</i></b> Linnaeus (1753: 1139), Ca. <b>83</b> species. Worldwide distribution, mostly tropical. Thallus and involucre with mucilage-containing schizogenous cavities. Chloroplast 1 (-4) per cell. Pyrenoid present or with a starch-free area. Antheridia numerous (4 to 45) per chamber with a tiered jacket cell arrangement. Capsules with stomata. Spores smoky gray, dark brown to blackish with a defined trilete mark; ornamentation spinose, punctate, baculate, jagged, or lamellate. Pseudoelaters short, thin-walled.</p> <p><b><i>Folioceros</i></b> Bhardwaj (1971: 9), <b>17</b> species. Mostly Pantropical to subtropical. Thallus and involucre with mucilage-containing schizogenous cavities. Chloroplast 1 (-2) per cell. Pyrenoid present or absent. Antheridia numerous (up to 60) per chamber with a tiered jacket cell arrangement. Capsules with stomata, except <i>Folioceros incurvus</i> (Steph.) D. C. Bhardwaj. Spores smoky gray, dark brown to blackish without a defined trilete mark; ornamentation spinose, baculate, jagged, mammillose or lamellate. Pseudoelaters long, strongly thick walled.</p> <p><b><i>Sphaerosporoceros</i></b> Hässel (1988: 78), <b>2</b> species, <i>S. adscendens</i> (Lehm. et Lindenb. in Lehmann 1832: 24) Hässel (1988: 79; United States) and <i>S. granulatus</i> (Gottsche 1863: 371) Hässel (1988: 79); Tropical America. Thallus and involucre with mucilage-containing schizogenous cavities. Chloroplast 1 (-2) per cell. Pyrenoid present. Capsules with stomata. Spores dark brown to blackish with a reduced defined trilete mark; ornamentation connate-cristate with ridges to short blunt-spines. Pseudoelaters with short ovoid to cylindrical cells, thin-walled.</p> <p><b><i>Notothylias</i></b> Sull. ex Gray (1846: 74), <b>21</b> species. Mostly tropical to temperate. Most species in the Indian sub-continent. Thallus solid. Chloroplast 1 (-3) per cell. Pyrenoid present or absent. Antheridia 2–4(–6) per chamber usually with a non-tiered jacket cell arrangement. Sporophytes short, lying horizontally in the thallus, mostly or totally enclosed within the involucre. Stomata absent. Massive sporogenous tissue (2-5 layers). Sutures elaborate, rudimentary or absent. Columella present or absent. Spores yellow to blackish with an equatorial girdle. Pseudoelaters absent to short to sub-quadrate with thickenings.</p> <p><b><i>Phaeoceros</i></b> Proskauer (1951: 346), ca. <b>41</b> species. Worldwide distribution, mostly tropical. Thallus solid. Marginal or short ventral tubers present or absent. Chloroplast 1 (-2) per cell. Pyrenoid present or absent. Antheridia (1-) 2–6 (–8) per chamber with a non-tiered jacket cell arrangement. Stomata present. Spores yellow to brownish when completely mature, with equatorial girdle. Ornamentation spinose to bumpy. Pseudoelaters short to elongated, thin-walled.</p> <p><b><i>Paraphymatoceros</i></b> Hässel (2006: 208), <b>1</b> species, <i>P. diadematus</i> Hässel (2006: 209). Chile. Thallus solid, usually narrow. Abundant marginal tubers. Chloroplast 1 (-2) per cell. Pyrenoid absent. Antheridia 2-5 per chamber with a non-tiered jacket cell arrangement. Stomata present. Spores yellow to blackish-brownish when completely mature, with equatorial girdle. Ornamentation of rounded protuberances in distal face with a proximal depression. Pseudoelaters short.</p> <p><b><i>Hattorioceros</i></b> (Hasegawa 1994a: 272) Hasegawa (1994b: 32), <b>1</b> species, <i>H. striatisporus</i> (Hasegawa 1994a: 268) Hasegawa (2000: 273). Fiji and Himalayas. Thallus solid. Chloroplast morphology and antheridium features unknown. Stomata present. Spores yellow to brownish. Spores small (usually less than 20 µm) without a triradiate mark, variable in shape, mostly ovoidal. Ornamentation surface deeply canaliculate-striate. Pseudoelaters short, unevenly thick-walled.</p> <p><b><i>Mesoceros</i></b> Piippo (1993: 30). <b>2</b> species. <i>M. mesophoros</i> Piippo (1993: 30); New Guinea and <i>M. porcatus</i> Piippo (1999: 279); China. Thallus solid. Chloroplast morphology unknown. Antheridia 2-3 per chamber with a non-tiered jacket cell arrangement. Spores dark brown papillate to connate with reticulate ridges. Pseudoelaters short, thin-walled.</p> <p><b><i>Phymatoceros</i></b> Stotler <i>et al.</i> (2005: 113), <b>2</b> species. <i>P. bulbiculosus</i> (Brotero 1804: 430) Stotler <i>et al.</i> (2005: 113); Europe–Israel and <i>P. phymatodes</i> (Howe 1898: 12) Duff <i>et al.</i> (2007: 240); Western United States.</p>
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**TABLE 1** (CONTINUED)

Thallus solid. Long-stalked ventral tubers. Chloroplast 1 (-2) per cell. Pyrenoid present or absent. Antheridia 1–3 (-4) per chamber with a non-tiered jacket cell arrangement. Stomata present. Spores yellow to brownish when completely mature, with equatorial girdle. Ornamentation finely vermiculate with distal bump. Pseudoelaters short, thin-walled.

***Dendroceros*** Nees in Gottsche *et al.* (1846: 579), **43** species. Mostly tropical to subtemperate.

Epiphytic and epiphyllic. Thallus solid (subg. *Dendroceros*) or with mucilage-containing schizogenous cavities (subg. *Apoceros*). Thallus with a conspicuous midrib and perforated wings. *Nostoc* present as globose colonies in the ventral and dorsal side of the thallus. Pit-field-like thickenings present in the thallus. Chloroplasts 1 per cell. Pyrenoid conspicuous with spherical incrustations. Antheridia 1 (-2) per chamber with a non-tiered jacket cell arrangement. Stomata absent. Spores multicellular due to endosporic germination, colourless to pale yellow, appearing green due to the chloroplasts. Ornamentation papillose to shortly tuberculate. Pseudoelaters long with helicoidal thickenings.

***Megaceros*** Campbell (1907: 484), **8** species. Paleotropical to subtemperate.

Thallus solid in rosettes. Pit-field-like thickenings present in the thallus. Chloroplast 1–8 (-12) per cell. Pyrenoid absent. Antheridia 1 (-2) per chamber with a non-tiered jacket cell arrangement. Stomata absent. Spores colourless to pale yellow, appearing green due to a chloroplast. Ornamentation mamilllose to tuberculate. Pseudoelaters long with helicoidal thickenings.

***Nothoceros*** (Schuster 1987: 200) Hasegawa (1994: 32), **7** species. Austral America, New Zealand, Neotropical and Eastern United States.

Thallus solid, in a rosette or with a conspicuous midrib and imperforated wings. Pit-field-like thickenings present in the thallus. Chloroplasts 1–2 (-8) per cell. Pyrenoid absent, present or with a starch-free area. Antheridia 1 (-2) per chamber with a non-tiered jacket cell arrangement. Stomata absent. Spores colourless to pale yellow, appearing green due to a chloroplast. Ornamentation mamilllose to tuberculate similar to *Megaceros* in most species. Pseudoelaters long with helicoidal thickenings.

***Phaeomegaceros*** Duff *et al.* (207: 241), **7** species. Pantropical to subtemperate.

Thallus solid and large. Tubers typically absent, if present short ventral tubers. Chloroplasts 1–2 per cell. Pyrenoid absent. Antheridia 1 (-8) per chamber with a non-tiered jacket cell arrangement. Stomata present. Spores yellow to brownish when completely mature, with equatorial girdle. Ornamentation finely vermiculate with distal dimples. Pseudoelaters short to elongated, thin-walled to unevenly thick-walled.

## Extinct diversity: time estimates and the fossil record

A recent book, the Time Tree of Life, portrays diversification times across the entire spectrum of organisms from bacteria to mammals (Hedges & Kumar 2009). Among plants, the most conspicuous absence was hornworts. It is true that there are no studies that estimate divergence times within hornworts, thus no chapter dedicated to this clade is included. The main handicap to such studies is the lack of reliable fossil data for calibration of branching nodes.

A reasonable divergence time for hornworts from tracheophytes is late Ordovician/ early Silurian, some 430–450 MYA (Kenrick & Crane 1997; Wikstrom *et al.* 2009). Dated phylogenies constrained by fossil data, geological events and molecular calibrations suggest that the most recent common ancestor of liverworts originated around 450–475 MYA (Sanderson 2003; Wellman *et al.* 2003; Heinrichs *et al.* 2007). The evolution of the paraphyletic bryophytes with their green, branching gametophyte and monosporangiate sporophyte is widely accepted to precede that of tracheophytes, all of which produce polysporangiate sporophytes (Strother *et al.* 1996; Kenrick & Crane 1997; Langdale & Harrison 2008). The earliest whole plant fossils were polysporangiates that date back to the Silurian (ca. 425 MYA) (Kenrick & Crane 1997). Using 475 MYA as an age constraint for the origin of embryophytes, mosses are estimated to have evolved 454 MYA (Kenrick & Crane 1997 but see Newton *et al.* 2009); suggesting hornworts diversified within a window of about 30 million years. Indeed, the differentiation of all embryophyte lineages, except seed plants, is estimated to have happened over a total of 70 million years (Sanderson 2003; Magallón & Sanderson 2005; Magallón & Hilu 2009). It is precisely this rapid cladogenesis, coupled with a meager fossil record, that obscures calculations of divergence times of early land colonizers such as hornworts.

Using calibration points from published dated phylogenies and fossils of monilophytes, liverworts, and the limited hornwort fossil data available, the first divergence times within hornworts have been estimated (Villarreal unpublished data). This analysis suggests an early Devonian (ca. 365 MYA) origin for the most recent common ancestor of hornworts, with the crown group Dendrocerotaceae originating ca. 130 MYA. These preliminary results are in accordance with the divergence times estimated from other plant groups, and a little older than the estimates of the two hornworts presented by Newton *et al.* (2007) in their study of pleurocarpus diversification. Our estimate is based on 2 loci (*rbcL* and *nad5*) and approximately 60 hornwort species, providing a preliminary assessment of hornwort diversification. Further analyses including more fossil calibrations and more loci are underway to increase confidence in the results.

With a robust dated phylogeny and nuclear genomic resources, significant questions related to hornwort evolution may be addressed. Examples include the following: Did any hornwort lineages diversify in the Mesozoic?; Is the lack of extant hornwort diversity in any way correlated with extinctions in other plant groups?; Because genome doubling was noted as a possible advantage in the survival and propagation of vascular plants during the K-T extinction (Fawcett *et al.* 2009), did the lack of polyploidy in hornworts lead to extinctions in the group?; and, did an inability to undergo polyploidy events contribute to the limited extant diversity in hornworts?

The oldest fossil assigned to a hornwort lineage is the spore fossil *Stoverisporites lunaris* (Cookson & Dettmann 1958: 103) Burger *in* Norvick & Burger (1976: 118) from Argentina, dated to the Early Cretaceous (Archangelsky & Villar de Seoane 1996). This fossil resembles spores of *Phaeomegaceros*, one of the most nested hornwort clades. Fossil remains can potentially be assigned to *Notothylas* and *Phaeoceros laevis* (Linnaeus 1753: 1139) Proskauer (1951: 347)/*carolinianus* (Michaux 1803: 280) Proskauer (1951: 347)/*pearsonii* (Howe 1898: 8) Proskauer (1951: 347) (Chitale & Yawale 1980; Jarzen 1979; Archangelsky & Villar de Seoane 1996). Further examination of these collections for fossil bryophytes has the promise to provide calibration points for the crown hornwort group.

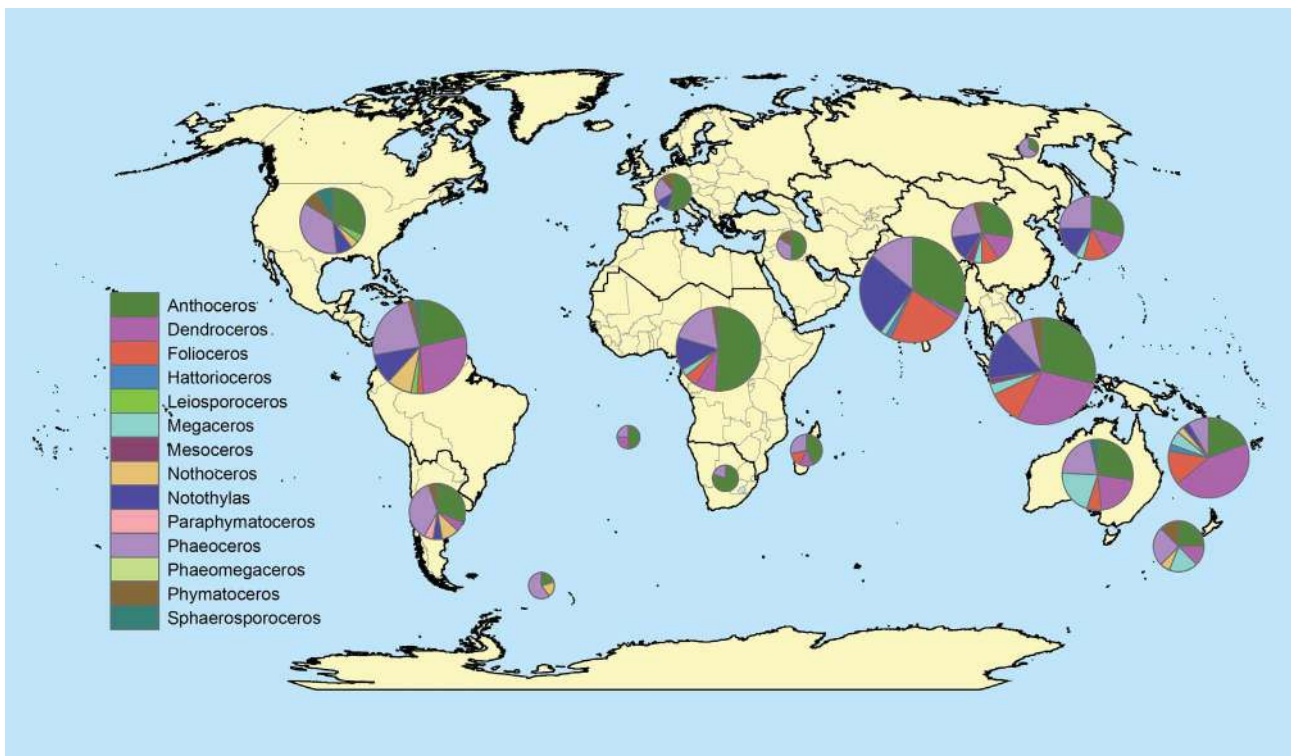
## Centers of extant hornwort diversity

Given the low number of documented species, it is surprising that global hornwort diversity is so poorly known. Fairly extensive treatments in temperate areas have been conducted, primarily in the northern hemisphere from more developed and accessible regions such as North America (Schuster 1992) and Europe (Proskauer 1958; Paton 1999). Within temperate areas of the southern hemisphere only New Zealand is currently represented (Campbell 1981–1995), and a recent exploration of the South Island of New Zealand (Cargill, Duckett and Slack unpublished data) has uncovered what appears to be a much more diverse group within the New Zealand *Megaceros*.

Thorough biogeographic analyses are limited in recent molecular phylogenies due to sparse taxon sampling, especially from tropical areas (Duff *et al.* 2007; Figure 2; supplemental information 1). It is difficult to obtain enough samples from remote areas around the world to represent known biodiversity, especially in *Anthoceros*, *Folioceros* and *Notothylas*. Nevertheless, some patterns related to hornwort evolution and centers of diversity have emerged from combined molecular and morphological studies (Fig 2). There were a number of unexpected results based on molecular phylogenies that revolutionized interpretations of interrelationships and informed biogeographic patterns. The monospecific Neotropical *Leiosporoceros* was identified as the earliest diverging hornwort (Duff *et al.* 2007; Villarreal *et al.* 2010). *Megaceros* s. lat. was found to be paraphyletic and has been redefined as a taxon that is restricted to the Old World tropics and is most closely related to *Dendroceros*. *Nothoceros* (see **Species delimitation**) was segregated from *Megaceros* s. lat., and is restricted to the American Continent, with the exception of *N. giganteus* from New Zealand, likely dispersed from the Americas.

Rich tropical hornwort floras (Fig. 2) are known from limited regions, such as the Paleotropics, with studies coming from India (Asthana & Srivastava 1991; Singh 2002), West Africa (Wiggington 2004) and

Japan (Hasegawa 1980–1994). Biodiversity of certain genera in specified regions is also known, e.g., *Notothylas* in India/Nepal (Singh 2002), *Dendroceros* in Asia (Hasegawa 1980) and sections of *Phaeoceros*/*Anthoceros* in the Americas (Hässel de Menéndez 1989, 1990) and Australia (Cargill & Furher 2008). No comprehensive modern treatments for all genera exist for the Neotropics, tropical Africa, or China.



**FIGURE 2.** Proportion of hornwort species in the different genera across regions of the world. Size of the pie diagrams reflects the total number of species in that area (maximum 21 species). See also Supplemental Information 1.

The tropics harbor the highest diversity of known hornwort species per area, particularly the Indian sub-continent, tropical Asia and the Neotropics (Fig. 2). Additionally, India is a center of endemism (and perhaps diversification) for *Notothylas* and *Folioceros* (Fig 2). The extant diversity figures undoubtedly reflect areas accessible or explored by bryologists and not the true distribution and diversity in the group. Through the years there have been very few researchers with expertise in hornwort biology. India has been a center of interest and studies in the group, hence the recorded richness of diversity (Asthana & Srivastava 1991; Singh 2002). Of course, species numbers in a genus may also reflect the taxonomic philosophy and characteristics used by taxonomists to circumscribe species. For example, *Anthoceros* was the first hornwort described and species in this genus are well-represented all over the world; however, based on cursory examination, many of these taxa would likely be transferred to other genera if a modern revision were conducted (see Hässel de Menéndez 1989, 1990).

A contrasting case of inflated species numbers due to minor morphological variations is found in the genus *Dendroceros*. Thirteen species of *Dendroceros* have been described from tropical America (Stephani 1917, 1923), and based on examination of type material and field-collections, there are only 3 or 4 species that are widely distributed in the Neotropics (Villarreal unpublished data; Hässel de Menéndez pers. com. 2003). With inclusion of more taxa in molecular phylogenies, the numbers of species across the world will change from the current estimate of 200-250 species. Expeditions to uncharted regions and countries across the world are necessary to fully comprehend the genetic and structural potential in hornworts. This was proven true during the 2010 foray to New Zealand, even though Ella Campbell had collected and produced excellent publications on the hornworts throughout the country.

## Species delimitation

A. THE CASE OF *MEGACEROS*: Until recently, species concepts within the hornworts were based entirely on morphological features (Hasegawa 1980; Hässel de Menéndez 1989; Schuster 1992; Singh 2002). With the advent of molecular techniques coupled with ultrastructural data, particularly on the chloroplast and associated pyrenoid, species hypotheses are being reexamined. A prime example is the genus *Megaceros* s. lat. that was introduced above.

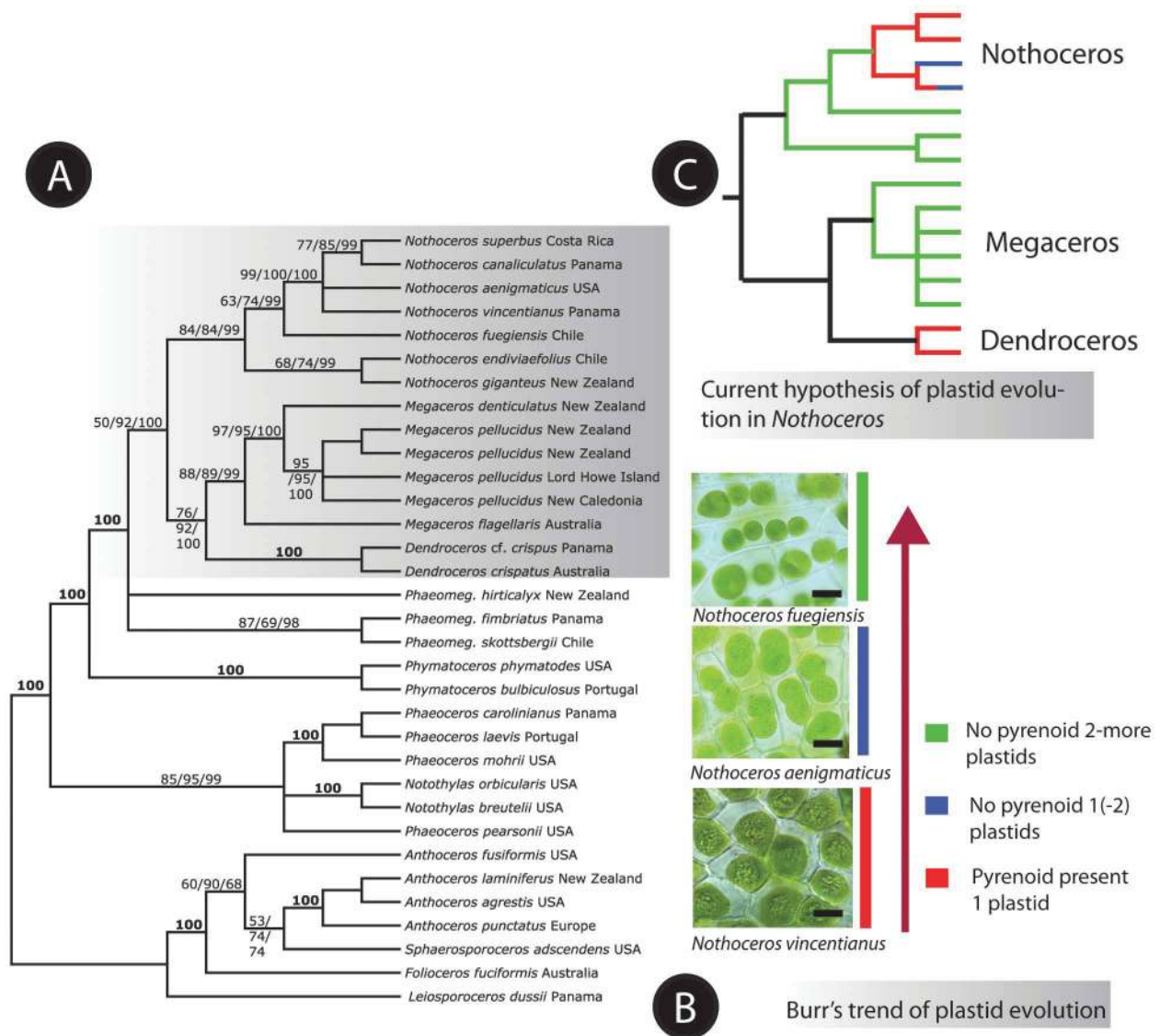
Described at the beginning of the 20<sup>th</sup> century, *Megaceros* was a morphological anomaly among hornworts with multiple chloroplasts lacking pyrenoids and spirally thickened pseudoelaters (Campbell 1907). New species were described and new combinations made as collections were made from the Paleotropics to Neotropics, namely Australasia, southern South America, Africa and Asia. In 1987, Schuster formally recognized two elements or subgenera – subg. *Megaceros* and subg. *Nothoceros* – that had previously caused confusion (Proskauer 1953; Hässel de Menéndez 1962). Subg. *Megaceros* was defined by a thallus with broad flat lobes and subg. *Nothoceros* Schuster (1987: 200) by a thallus with thickened midrib and lateral wings. Hasegawa later elevated *Nothoceros* [sic] to the status of genus (Hasegawa 1994). As noted above, both groups were separated geographically, with *Megaceros* restricted to the Paleotropics and *Nothoceros* to Austral America and New Zealand (Villarreal *et al.* 2010). Early recognition of these two entities was based solely on morphology but within a phylogenetic context (Hasegawa 1994). The generic split has gained further support from molecular data, which resolves the two genera as paraphyletic and not sister taxa (Fig 3.)

The taxonomic history of Australian elements of *Megaceros* s. str. is one that emphasizes the importance of combining morphological and molecular data. Fifteen species of *Megaceros* were described at the time that Hasegawa (1983) conducted a morphological assessment of the genus from Japan, South East Asia and the islands of the Pacific. His work led to the conclusion that many characters displayed a continuum across a broad geographical range (Hasegawa 1983). There is, according to Hasegawa, a single quite variable species in the region. Following these studies, Vella (2003) examined ornamentation patterns of spores in Australian populations (seven species have been described for Australia) and found four distinct distal spore patterns, which she ascribed to four distinct species. *Megaceros* taxonomy within the Australian context seemed cut and dried.

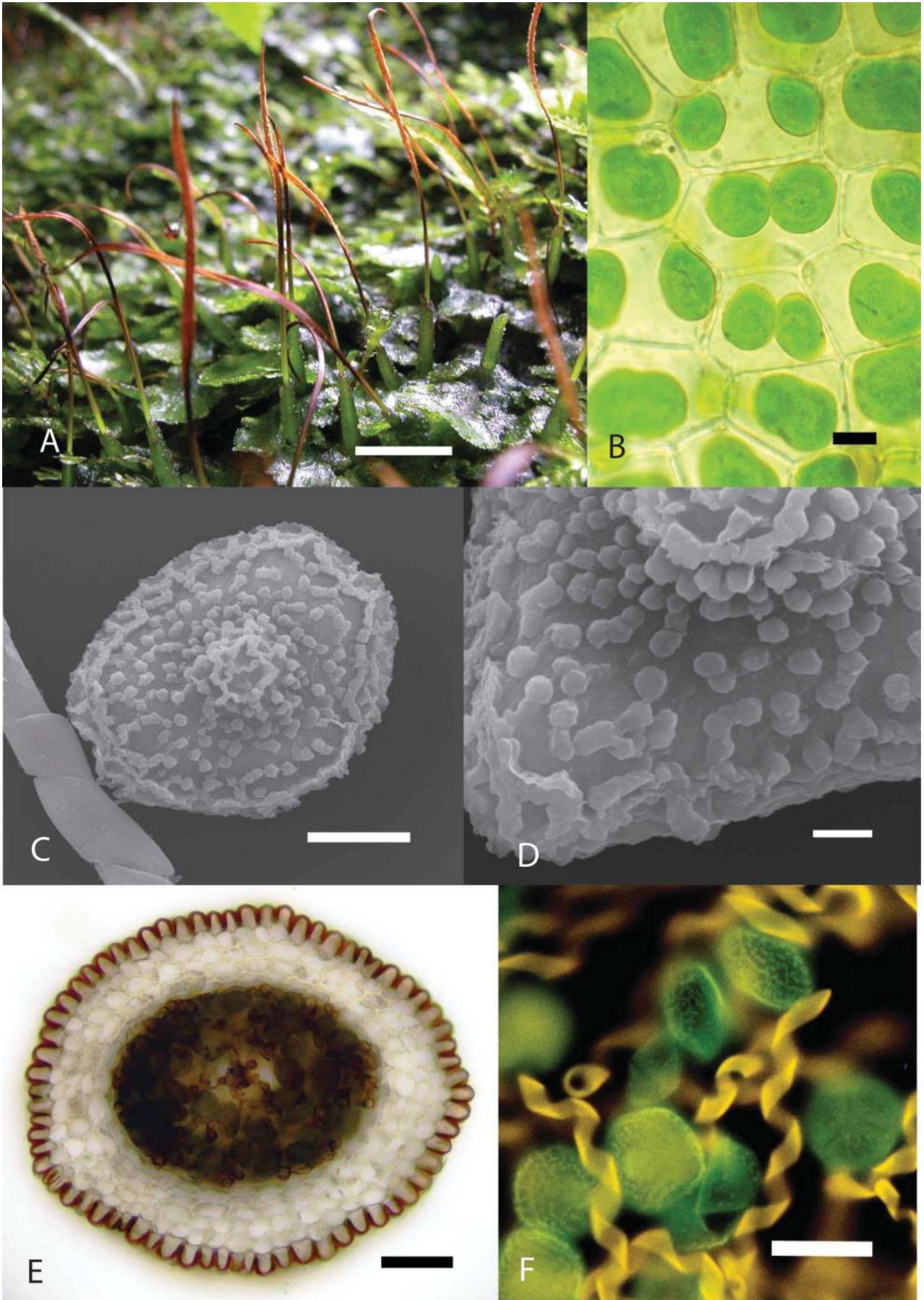
However, further study of the group expanded to include taxa from regions geographically close to Australia (Cargill unpublished data). Molecular data were also employed to test existing species concepts. The findings revealed three distinct clades: a tropical low altitude element (*M. flagellaris* (Mitten 1873: 419) Stephani 1916: 951), a geographically widespread, temperate or tropical high altitude element (*M. pellucidus* (Colenso 1885: 263) Hodgson (1972: 115)/*leptohymenius* (Hooker & Taylor 1844: 575) Stephani (1916: 955) complex) and a third element unique to New Zealand (*M. denticulatus* (Lehmann 1857: 25) Stephani 1916: 956). Spore patterns correlate with each of the three clades: (1) the tropical *M. flagellaris* clade has spores with a tessellated pattern around the circumference; (2) the second clade is defined by a spore pattern that is the most variable morphologically, with large tubercles or ribs on the central protuberance and around the rim of the distal face; and (3) the spores of *M. denticulatus* are characterized by large tubercles scattered over the distal face (Cargill *et al.* unpublished) (Fig. 3, 4).

A species concept based on the morphology of the spores for Australasian taxa is congruent with the molecular hypothesis. However, an extensive sampling of New Zealand taxa has not yet been included in the molecular dataset. As noted above, recent collections from the South Island have revealed remarkably diverse morphological variation in the thallus including collections that produce gemmae not previously recorded in this genus and not seen in Australian populations (Cargill and Duckett unpublished data). However, a comparable diversification of thallus morphology and spore patterning in Old World taxa is not echoed in Neotropical taxa of *Nothoceros*.





**FIGURE 3.** A. Majority rule consensus tree of phylogenetic relationship of hornwort genera with their respective localities inferred from *rbcL* and *nad5* genes (redrawn from Villarreal *et al.* 2010). The three integers above branches represent MP, ML bootstrap support and posterior probabilities (as a percentage), except when all values are the same. The subfamily Dendroceroideae is highlighted (grey box). B. Schematic representation of Burr's hypothesis (1970) of chloroplast evolution in *Nothoceros* (formerly *Megaceros*). Color coded boxes: Green for taxa with no pyrenoid and multiple plastids (e.g. *N. fuegiensis*); Blue for taxa with no pyrenoid and one (rarely two) plastid (*N. aenigmaticus*) and Red for monoplastidic taxa with a pyrenoid (*N. vincentianus* s. str.). A plesiomorphic condition of a pyrenoid present in *N. vincentianus*, an intermediate step of monoplastidic cells without pyrenoid up to more specialized cells with multiple plastids without a pyrenoid in *N. fuegiensis*. C. A diagram of a subtree with species of the genera *Nothoceros*, *Megaceros*, *Dendroceros* and *Dendroceros* (subfamily Dendroceroideae, gray box). Using *Megaceros* as an outgroup, the trend of plastid evolution in *Nothoceros* is more complex than previously hypothesized (Burr 1970). The multiple plastids without pyrenoid seem to be the plesiomorphic condition in *Nothoceros* (Austral *Nothoceros*) with most nested taxa (Neotropical and Eastern US taxa) having a single plastid with or without pyrenoid (see text for more explanation). Scale bars: 10  $\mu$ m in Figure 3B.



**FIGURE 4.** *Megaceros flagellaris*. A. Habit. B. Pyrenoidless chloroplasts of gametophore dorsal epidermal cells. C. SEM of distal face of spore. D. High magnification of patterning of distal face of spore. E. Cross section through sporophyte illustrating single outer epidermal layer, 4–5 layers of assimilative layer and inner sporogenous layer. F. Fluorescent microscopy of spores and pseudoelaters. Scale bars: A= 7 mm; B= 15 µm; C= 8 µm; D= 2 µm; E= 70 µm; F=25 µm.

B. THE CASE OF *NOTHOCEROS*: Using a total evidence approach, Villarreal *et al.* (2010) recognized seven species of *Nothoceros* (*N. aenigmaticus* (Schuster 1992: 830) Villarreal & McFarland (2010:109), *N. canaliculatus* (Pagán 1942: 111) Villarreal *et al.* (2007: 283), *N. endiviifolius* (Montagne 1845: 211) Villarreal *et al.* (2007: 283), *N. fuegiensis* (Stephani 1911: 91) Villarreal (2010:111), *N. giganteus* (Lehm. et Lindenb. in Lehmann 1832: 25) Villarreal *et al.* (2007: 283), *N. superbus* Villarreal *et al.* (2007: 280) and *N. vincentianus* (Lehm. et Lindenb. in Lehmann 1834: 16) Villarreal (2010: 109) s. lat.; Fig. 3) that exhibit broad morphological amplitude. Out of the 16 species previously described under *Megaceros* from Tropical America, most are likely synonyms of the widespread *Nothoceros vincentianus*. However, fresh collections from several type localities are not available for a critical re-examination. Spores of all Neotropical *Nothoceros* examined to date are characterized by the presence of tubercles aggregated in the center of the distal face as well as around the periphery (Villarreal *et al.* 2007; 2010). This spore type is reminiscent of the Australasian *Megaceros pellucidus/leptohymenius* complex (Campbell 1982b, 1984; Duff *et al.* 2007). Spore architecture, often a key feature to separate hornwort species, is conserved across *Nothoceros*; small differences in spore sizes have the potential to be informative as taxonomic characters. In contrast, the Austral *Nothoceros giganteus*, *N. endiviifolius* and *N. fuegiensis* have tubercles uniformly distributed on the distal face without any central clustering (Hässel de Menéndez 1962; Campbell 1986; Duff *et al.* 2007).

Unlike Old World *Megaceros* and most hornworts, a combination of vegetative, not spore-related, features of *Nothoceros* delineate species. Species such as *N. superbus*, *N. canaliculatus*, *N. giganteus* and *N. endiviifolius* develop thalli differentiated into midrib and imperforate wings (Fig. 1). The typical “*Megaceros*-like” thallus is found in *N. vincentianus*, *N. fuegiensis* and one phenotype of *N. aenigmaticus*. Within species, phenotypes include both a broad thallus and narrow, highly branched habit that resembles *Riccardia* Gray (1821: 679) (e.g., *N. aenigmaticus* and *N. cf. canaliculatus*). In addition, dorsal epidermal chloroplast structure within *Nothoceros* may be highly informative at species level. Either single (e.g. *N. aenigmaticus*, *N. vincentianus*) or multiple chloroplasts occur in each cell (*N. fuegiensis*, *N. endiviifolius/giganteus*) of a single plant and these typically lack a central pyrenoid. However, other taxa (e.g. one phenotype of *N. vincentianus*, *N. superbus* and *N. canaliculatus*) have monoplastidic cells and a central pyrenoid that is moderately electron dense at the ultrastructural level when compared with pyrenoids of other genera (Renzaglia *et al.* 2007; Villarreal, unpublished).

In spite of recent advances in taxonomy, data from morphological and taxonomic studies are badly needed to evaluate character transformation within *Nothoceros*. Nevertheless, based on our preliminary observations on chloroplast structure it is possible to revisit Burr’s hypothesis (1970) on the evolution of the chloroplast in the genus *Megaceros* s. lat. (mostly using species now recognized as *Nothoceros*) (Fig. 3). Burr suggested that in hornworts there is a trend from a single plastid with a compact pyrenoid (in *Phaeoceros/Notothylas* species), with intermediate steps of modified pyrenoids in *N. vincentianus*, to a more derived condition of multiple plastids and no pyrenoids (*N. endiviifolius*). Renzaglia *et al.* (2007) discussed multiple losses and gains of pyrenoids and modifications of pyrenoid substructure across hornworts. Concentrating on *Nothoceros*, Villarreal *et al.* (2010; Figure 3) presented a phylogenetic hypothesis that suggests an interesting variation on Burr’s hypothesis. Using *Megaceros* as an outgroup, the plesiomorphic condition in *Nothoceros* is pyrenoidless plastids (*N. endiviifolius/giganteus/fuegiensis*). More nested species usually have a single plastid with a modified pyrenoid (*N. vincentianus*, *N. canaliculatus*, *N. superbus*) or have a single chloroplast (or rarely 2–3) that lack a pyrenoid (*N. aenigmaticus* and one phenotype of *N. vincentianus*). This evolutionary transformation in chloroplast structure may have been in response to climate change (e.g. carbon dioxide concentration), and as such is a fertile ground for further research.

## Future work

In the “genomic era” extensive taxonomic and ultrastructural studies of hornworts are urgently needed. Ultrastructural features such as the placental region have provided a wealth of information for cellular processes and stand out as phylogenetic markers at generic level (Ligrone *et al.* 2003; Vaughn & Hasegawa 1993; Vaughn pers. com.). Chloroplast microstructure appears to be informative in taxon delimitation (see Asthana & Srivastava; Singh 2002) but has not been explored because of poor preservation of plastids in dried material. The potential homology of hornwort mucilage clefts with sporophytic stomata and the underlying genetic control of pore formation in hornworts compared with tracheophytes is awaiting investigation using phylogenomics, proteomic and developmental approaches (Ziegler 1987; Duckett *et al.* 2010). These new research venues provide a rich field for young scientists who are intrigued by the evolution of terrestrial plant life (Langdale & Harrison 2008).

A holistic approach to species circumscription or delimitation is becoming more commonplace. Molecular data is by far the fastest growing dataset in systematic and taxonomic studies. Molecular markers have been refined and utilized for phylogenetic studies at the deeper levels of plant evolution, but at the species level, ideal markers are still being sought. Chloroplast spacers (e.g. trnL region, *rps4-trnS* spacer) are promising markers to obtain resolution at species level in hornworts. The lack of a reference nuclear genome is hampering the development of single copy nuclear markers for phylogenetic reconstructions. A nuclear genome is also essential to pursue deep genomic and developmental genetic studies that will contribute to the elucidation of the bigger picture of early land plant evolution. A combination of ultrastructural, anatomical, phylogenetic and genomic research will unveil the secrets of poor diversification in hornworts and provide clues to the evolutionary conversion from gametophyte to sporophyte dominant life cycles in land plants.

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## References

- Archangelsky, S. & Villar de Seone, L. (1996) Estudios palinológicos de la formación Baqueró (Cretácico), provincia de Santa Cruz, Argentina. *Ameghiniana* 35: 7–19.
- Asthana, A.K. & Srivastava, S.C. (1991) Indian Hornworts (A Taxonomic Study). *Bryophytorum Bibliotheca* 42: 1–158.
- Bhardwaj, D.C. (1971) On *Folioceros*, a new genus of Anthocerotales. *Geophytology* 1: 6–15.
- Brotero, F.A. (1804) *Flora Lusitanica, Vol 1*. Olissipone [Lisboa], 607 pp.
- Burr, F.A. (1970) Phylogenetic transitions in the chloroplasts number of the Anthocerotales I. The number and ultrastructure of the

mature plastids. *American Journal of Botany* 57: 97–110

- Campbell, D.H. (1895) *The Structure and Development of Mosses and Ferns* (Archegoniatae). Macmillan, New York, 658 pp.
- Campbell, D.H. (1907) Studies on some Javanese Anthocerotae. I. *Annals of Botany* 21: 467–486.
- Campbell, E.O. (1981a) Notes on some Anthocerotae of New Zealand. *Tuatara* 25: 7–13.
- Campbell, E.O. (1981b) Notes on some Anthocerotae of New Zealand (2). *Tuatara* 25: 65–70.
- Campbell, E.O. (1982a) Notes on some Anthocerotae of New Zealand (3). *Tuatara* 26: 20–26.
- Campbell, E.O. (1982b) Some Anthocerotae of New Zealand with particular reference to their geographical distribution. *Journal of the Hattori Botanical Laboratory* 52: 37–44.
- Campbell, E.O. (1984) Notes on some Anthocerotae of New Zealand (4). *Tuatara* 27: 105–120.
- Campbell, E.O. (1986) Notes on some Anthocerotae of New Zealand (5). *Tuatara* 26: 83–94.
- Campbell, E.O. & Hasegawa, J. (1993) *Phaeoceros hirticalyx* (Steph.) Haseg. (Anthocerotae) new to New Zealand. *New Zealand Journal of Botany* 31: 127–131.
- Campbell, E.O. (1995) *Phaeoceros delicatus* a new species of Anthocerotae from New Zealand. *New Zealand Journal of Botany* 33: 285–290.
- Carafa, A., Duckett, J.G., Knox, J.P. & Ligrone, R. (2005) Distribution of xylans in bryophytes and tracheophytes: new insights into basal interrelationships of land plants. *New Phytologist* 168: 231–240.
- Cargill, D.C. & Fuhrer, B.A. (2008) Taxonomic studies of the Australian Anthocerotophyta II: The genus *Phaeoceros*. *Fieldiana. Botany N.S.*, No. 47: 239–253.
- Carmichael, J.S. & Friedman, W.E. (1996) Double fertilization in *Gnetum gnemon* (Gnetaceae): Its bearing on the evolution of sexual reproduction within the Gnetales and the Anthophyte clade. *American Journal of Botany* 83: 767–780.
- Chitale, S.D. & Yawale, N.R. (1980) On *Notothylites nirulai* gen. et sp. nov. a petrified sporogonium from the Deccan-Intertrappean beds of Mohgaonkalan M. P. (India). *The Botanique* 9: 111–118.
- Colenso, W. (1885) A description of some newly-discovered cryptogamic plants; being a further contribution toward making known the botany of New Zealand. *Transactions and proceedings of the New Zealand Institute* 17: 237–265.
- Cookson, I.C. & Dettmann, M.E. (1958) Some trilete spores from Upper Mesozoic deposits in the Eastern Australian Region. *Proceedings of the Royal Society of Victoria* 70: 95–128.
- Duckett, J.G., Pressel, S., P'ng, K.M.Y. & Renzaglia, K.S. (2010) The function and evolution of stomata in bryophytes. *Field Bryology* 101: 38–40.
- Duff, R.J., Villarreal, J.C., Cargill, D.C. & Renzaglia, K.S. (2007) Progress and challenges toward developing a phylogeny and classification of the hornworts. *The Bryologist*. 110: 214–243.
- Fawcett, J.A., Maere, S. & Van de Peer, Y. (2009) Plants with double genomes might have had a better chance to survive the Cretaceous–Tertiary extinction even. *Proceedings of the National Academy of Sciences* 106: 5737–5742.
- Frey, W. & Stech, M. (2009) Marchantiophyta, Bryophyta, Anthocerotophyta. In Frey, W. (ed.), *Syllabus of Plant Families - A. Engler's Syllabus der Pflanzenfamilien, 13<sup>ed</sup>. Part 3: Bryophytes and seedless Vascular Plants*: Borntraeger, Berlin, 13–115 pp.
- Gottsche, C.M. (1863) De Mexikanske Levermooser, efter Prof. Fr. Liebmanns Samling. *Det kongelige danske videnskabs-selskabs skrifter* 6: 97–380.
- Gottsche, C.M., Lindenberg, J.B.G. & Nees von Esenbeck, S.G. (1846) *Synopsis Hepaticarum, fasc. 4*. Hamburg, pp. 465–624.
- Gradstein, S.R. & Pinheiro da Costa, D. (2003) *The Hepaticae and Anthocerotae of Brazil*. Memoirs of the New York Botanical Gardens. Vol. 87: 1–318.
- Gray, A. (1846) Musci Alleghaniensis, sive spicilegia muscorum atque hepaticarum quos in itinere a Marylandia usque ad Georgiam per tractum montium, Fasc. I, II. *American Journal of Science and Arts, serie 2* 1: 70–81.
- Gray, S.F. (1821) *Natural Arrangement of British Plants. Vol. I*. London, 824 pp.
- Hanson, D., Andrews, T.J. & Badger, M.R. (2002) Variability of the pyrenoid-based CO<sub>2</sub> concentrating mechanisms in hornworts (Anthocerotophyta). *Functional Plant Biology* 29: 407–416.
- Hasegawa, J. (1979) Taxonomical studies on Asian Anthocerotae (1). *Acta Phytotaxonomica et Geobotanica* 30: 15–30.
- Hasegawa, J. (1980) Taxonomical studies on Asian Anthocerotae. II. Some Asian species of *Dendroceros*. *Journal of the Hattori Botanical Laboratory* 47: 287–309.
- Hasegawa, J. (1983) Taxonomical studies on Asian Anthocerotae. III. Asian species of *Megaceros*. *Journal of the Hattori Botanical Laboratory* 54: 227–240.
- Hasegawa, J. (1994a) A remarkable new species of *Phaeoceros* (Anthocerotae) with canalliculate-striate spore surface. *Journal of the Hattori Botanical Laboratory* 75: 267–273.
- Hasegawa, J. (1994b) New classification of Anthocerotae. *Journal of the Hattori Botanical Laboratory* 76: 21–34.
- Hasegawa, J. (2000) *Hattorioceros striatisporus* (Hasegawa) Hasegawa newly found in Fiji. *Bryological Research* 7: 273–275.
- Hässel de Menéndez, G.G. (1962) Estudio de las Anthocerotales y Marchantiales de la Argentina. *Opera Lilloana* 7: 1–207.
- Hässel de Menéndez, G.G. (1986) *Leiosporoceros* Hässel n. gen. and Leiosporocerotaceae Hässel n. fam. of Anthocerotopsida. *Journal of Bryology* 14: 255–259.
- Hässel de Menéndez, G.G. (1988) A proposal for a new classification of the genera within the Anthocerotophyta. *Journal of the Hattori Botanical Laboratory* 64: 71–86.
- Hässel de Menéndez, G.G. (1989) Las especies de *Phaeoceros* (Anthocerotophyta) de América del Norte, Sud y Central; la ornamentación de sus esporas y taxonomía. *Candollea* 44: 717–739.
- Hässel de Menéndez, G.G. (1990) Las especies de *Anthoceros* y *Folioceros* (Anthocerotophyta) de América del Norte, Sud y Central; la ornamentación de sus esporas y taxonomía. *Candollea* 45: 201–220.
- Hässel de Menéndez, G.G. (2006) *Paraphymatoceros* Hässel, gen. nov. (Anthocerotophyta). *Phytologia* 88: 208–211.
- Hedges, S.B. & Kumar, S. (2009) *The Time Tree of Life*. Oxford University Press, New York, 551 pp.

- Heinrichs, J., Hentschel, J., Wilson, R., Feldberg, K. & Schneider, H. (2007) Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. *Taxon* 56: 31–44.
- Hill, K.D., Chase, M., Stevenson, D.W., Hills, H.G. & Schutzman, B. (2003) The families and genera of cycads: A molecular phylogenetic analysis of Cycadophyta based on nuclear and plastid DNA sequences. *International Journal of Plant Sciences* 164: 933–948.
- Hodgson, E.A. (1972) New Zealand Hepaticae (Liverworts) - XX: A miscellany taxonomic notes, Part 3. *Journal of the Royal Society of New Zealand* 2: 109–118.
- Hofmeister, W. (1862) *On the germination, development and fructification of the higher Cryptogamia and on the fructification of the Coniferae*. Ray Society, London. [English translation (by F. Currey)]
- Hooker, J.D. & Taylor, T. (1844) Hepaticae Novae-Zelandiae et Tasmaniae; being characters and brief descriptions of the Hepaticae discovered in the Islands of New Zealand and Van Diemen's Land, during the voyage of H.M. Discovery ships Erebus and Terror, together with those collected by R.C. Gunn and W. Colenso Esqrs. III. Species of New Zealand. *London Journal of Botany* 3: 556–577.
- Hooker, W.J. (1830) *Monoclea crispata*. *Botanical miscellany* 1: 117–118.
- Howe, M.A. (1898) The Anthocerotaceae of North America. *Bulletin of the Torrey Botanical Club* 25: 1–24.
- Jarzen, D.M. (1979) Spore morphology of some Anthocerotaceae and the occurrence of *Phaeoceros* spores in the Cretaceous of North America. *Pollen et Spores* 21: 211–231.
- Kenrick, P. & Crane, P. (1997) *The origin and early diversification of land plants: A cladistic study*. Smithsonian Institution Press, Washington DC, 441 pp.
- Langdale, J. & Harrison, C.J. (2008) Developmental transitions during the evolution of plant form. In A. Minelli & G. Fusco, *Evolving Pathways: Key themes in Evolutionary Developmental Biology*. Cambridge University Press, UK, pp. 297–314.
- Lehmann, J.G.C. (1832) *Novarum et minus cognitarum stirpium pugillus IV*. Hamburg, 64 pp.
- Lehmann, J.G.C. (1834) *Novarum et minus cognitarum stirpium pugillus VI addita enumeratione plantarum omnium in his pugillis descriptarum*. Hamburg, pp. 1–66.
- Lehmann, J.G.C. (1857) *Novarum et minus cognitarum stirpium pugillus X addita enumeratione plantarum omnium in his pugillis descriptarum*. Hamburg, 40 pp.
- Ligrone, R., Duckett, J.G. & Renzaglia, K.S. (1993) The gametophyte–sporophyte junction in Land Plants. *Advances in Botanical Research* 19: 231–318.
- Linnaeus, C. (1753) *Species Plantarum, ed. 1*. Holmiae [Stockholm], 1200 pp.
- Magallón, S. & Hilu, K.W. (2009) Land Plants (Embryophyta). In Hedges, S.B. & Kumar, S. (eds.), *The Time Tree of Life*. Oxford University Press, New York, 133–137 pp.
- Magallón, S. & Sanderson, M.J. (2005) Angiosperm divergence times: The effect of genes, codon positions and time constraints. *Evolution* 59: 1653–1670.
- Meyer, M., Seibt, U. & Griffiths, H. (2008) To concentrate or ventilate? Carbon acquisition, isotope discrimination and physiological ecology of early land plant life forms. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* 363: 2767–2778.
- Magill, R.E. (2010) Moss Diversity: new look at old numbers. *Phytotaxa* 9: 167–174.
- Michaux, A. (1803) *Flora Boreali-Americana* 2. Paris, 340 pp.
- Mitten, W. (1873) Jungermanniae and Marchantiae. In: Seeman, B. (Ed), *Flora vitiensis*. Reeve: London, pp. 325–453.
- Montagne, J.F.C. (1845) Plantes cellulaires. In: Hombron, M.M. & Jaquinot, H. (Eds), *Voyage au Pôle Sud et dans l'Océanie sur les corvettes l'Astrolabe et la Zelée, exécuté par ordre du roi pendant les années 1837-1838-1839-1840, sous le commandement de M. J. Dumont d'Urville*. Botanique, vol. 1. Gide & Cie: Paris, pp. 1–349.
- Musser, A.M. (2003) Review of the monotreme fossil record and comparison of palaeontological and molecular data. *Comparative Biochemistry and Physiology Part A*. 136: 927–942.
- Newton, A.E., Wikström, N. & Shaw, A.J. (2009) Mosses (Bryophyta). In Hedges, S.B. & Kumar, S. (eds.), *The Time tree of life*. Oxford University Press, New York, 138–145 pp.
- Newton, A.E., Wikström, N., Bell, N., Forrest, L.L. & Ignatov, M. (2007) Dating the diversification of the pleurocarpous mosses. In: Newton, A.E. & Tangney, R.S., *Pleurocarp Mosses: Systematics and Evolution*, Systematic Association Special Volume Series No 71. CRC Press, Boca Raton, Florida, 337–366 pp.
- Newton, M. (1983) Cytology of the Hepaticae and Anthocerotae. In R.M. Schuster (ed.), *New Manual of Bryology*, vol. 1. Hattori Botanical Laboratory, Nichinan, Japan, 117–148 pp.
- Norvick, M.S. & Burger, D (1975) Palynology of the Cenomanian of Bathurst Island, Northern Territory, Australia. Australian Government Publishing service, Bulletin 151. Canberra, 169 pp.
- Pagán, F.M. (1942) A new species of *Dendroceros* from Puerto Rico. *The Bryologist* 45: 111–115.
- Peña, M.J., Darvill, A.G., Eberhard, S., York, W.S. & O'Neill, M. (2008) Moss and liverwort xyloglucans contain galacturonic acid and are structurally distinct from the xyloglucans synthesized by hornworts and vascular plants. *Glycobiology* 18: 891–904.
- Paton, J. (1999) *The Liverwort Flora of the British Isles*. Harley Books. England. 626 pp.
- Piippo, S. (1993) Bryophyte flora of the Huon Peninsula, Papua New Guinea. LIV. Anthocerophyta. *Acta Botanica Fennica* 148: 27–51.
- Piippo, S. (1999) *Mesoceros porcatus*, a new hornwort from Yunnan. *Haussknechtia, Beiheft* 9: 279–282.
- Pooper, Z.A. & Tuohy, M.G. (2010) Beyond the Green: Understanding the evolutionary puzzle of plant and algal cell walls. *Plant Physiology* 153: 373–383.
- Proskauer, J. (1951) Studies on Anthocerotales. III. *Bulletin of the Torrey Botanical Club* 78: 331–349.

- Proskauer, J. (1953) Studies on Anthocerotales IV. *Bulletin of the Torrey Botanical Club* 80: 65–75.
- Proskauer, J. (1957) Studies on Anthocerotales V. *Phytomorphology* 7: 113–135.
- Proskauer, J. (1958) Nachtrag zur Familie Anthocerotaceae. In: K. Müller (Ed.) *Die Lebermoose Europas, Rabenhorst's Kryptogamen-Flora* 3rd edn., 1303–1319 pp.
- Qiu, Y-L, Li, L, Wang, B., Chen, Z., Knoop, V., Groth-Malonek, M., Dombrowska, O., Lee, J., Kent, L., Rest, J., Estabrook, G.F., Hendry, T.A., Taylor, D.W., Testa, C.M., Ambros, M., Crandall-Stotler, B., Duff, R. J., Stech, M., Frey, W., Quandt, D. & Davis, C.C. (2006) The deepest divergences in land plants inferred from phylogenetic evidence. *Proceedings of the National Academy of Sciences* 103: 15511–15516.
- Renzaglia, K.S. (1978) A comparative morphology and developmental anatomy of Anthocerotophyta. *Journal of the Hattori Botanical Laboratory* 44: 31–90.
- Renzaglia, K.S., Duff, R.J., Nickrent, D.L. & Garbary, D. (2000) Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. *Transactions of the Royal Society, London* 355: 769–793.
- Renzaglia, K.S., Rasch E.M. & Pike, L.M. (1995) Estimates of nuclear DNA content in bryophyte sperm cells: phylogenetic considerations. *American Journal of Botany* 82: 18–25.
- Renzaglia, K.S., Schuette, S., Duff, R.J., Ligrone, R., Shaw, A.J., Mishler, B.D. & Duckett, J.G. (2007) Bryophyte phylogeny: Advancing the molecular and morphological frontiers. *The Bryologist* 110: 179–213.
- Renzaglia, K.S., Villarreal, J.C. & Duff, R.J. (2009) New insights into morphology, anatomy, and systematics of hornworts. In: Goffinet, B. & Shaw, A. J. (Eds), *Bryophyte Biology*. 2<sup>nd</sup> ed Cambridge University Press. UK. 565 pp.
- Rothwell, G.W. (1996) Pteridophytic evolution: An often underappreciated phytological success story. *Review of Palaeobotany and Palynology* 90: 209–222.
- Sanderson, M.J. (2003) Molecular data from 25 proteins do not support a Precambrian origin of land plants. *American Journal of Botany* 90: 954–956.
- Schuster R.M. (1987) Preliminary studies on Anthocerotae. *Phytologia* 63: 193–201.
- Schuster R.M. (1992) *The Hepaticae and Anthocerotae of North America, East of the Hundredth Meridian*. Vol. 6. Field Museum of Natural History: Chicago. 937 pp.
- Singh, D.K. (1994) Distribution of Family Notothylaceae in India and its phytogeographical significance. *Advances in Plant Sciences Research* 11: 28–41.
- Singh, D.K. (2002) *Notothylaceae of India and Nepal (A morpho-taxonomic revision)*. Bishen Singh Mahendra Pal Singh, India. 271 pp.
- Smith, A.M., Pryer, K.M., Schuettepelz, E., Korall, P., Schneider, H. & Wolf, P.G. (2006) A classification of extant ferns. *Taxon* 55(3): 705–731.
- Smith, E.C. & Griffiths, H. (1996) A pyrenoid-based carbon concentrating mechanism is present in terrestrial bryophytes of the class Anthocerotae. *Planta* 200: 203–212.
- Stephani, F. (1893) Hepaticarum species novae. Pars II. *Hedwigia* 32: 137–147.
- Stephani, F. (1911) Botanische Ergebnisse der schwedischen Expedition nach Patagonien und dem Feuerlande 1907-1909. II. Die Lebermoose. *Kungliga Svenska Vetenskapsakademiens Handlingar* 46: 1–92.
- Stephani, F. (1916) *Species Hepaticarum* 5. Genève & Bale, pp. 849–1008.
- Stephani, F. (1917) *Species Hepaticarum*. Vol. 5. George and Cie, Geneva.
- Stephani, F. (1923) *Species Hepaticarum*. Vol. 6. George and Cie, Geneva.
- Stotler, R.E., Doyle, W.T. & Crandall-Stotler, B.J. (2005) *Phymatoceros* Stotler, W. T. Doyle & Crand. -Stotl., gen. nov. (Anthocerotophyta). *Phytologia* 87: 113–116.
- Strother, P.K., Al-Hajri, S. & Travers, A. (1996) New evidence for land plants from the lower middle Ordovician of Saudi Arabia. *Geology* 24: 55–58.
- Vaughn, K.C., Campbell, E. O., Hasegawa, J., Owen, H. A. & Renzaglia, K. S. (1990) The anthocerote chloroplast: a review. *New Phytologist* 120: 169–190.
- Vaughn, K.C., & Hasegawa, J. (1993) Ultrastructural characteristics of the placental region of *Folioceros* and their taxonomic significance. *The Bryologist* 96: 112–121.
- Vella, N. (2003) *Centre for Plant Biodiversity Research*. Available from: <http://www.anbg.gov.au/cpbr/summer-scholarship/2003-projects/vella-megaceros.html> (April, 2010)
- Villarreal, J.C. & Renzaglia, K.S. (2006) Structure and development of *Nostoc* strands in *Leiosporoceros dussii* (Anthocerotophyta): a novel symbiosis in land plants. *American Journal of Botany* 93: 693–705.
- Villarreal, J.C., Hässel de Menéndez, G.G. & Salazar Allen, N. (2007) *Nothoceros superbus* (Dendrocerotaceae), a new species of hornwort from Costa Rica. *The Bryologist* 110: 279–85.
- Villarreal, J.C., Goffinet, B., Duff, R.J. & Cargill, D.C. (2010) Phylogenetic delineation of the genera *Nothoceros* and *Megaceros*. *The Bryologist* 113: 106–113.
- von Konrat, M., Söderström, L., Renner, M.A.M., Hagborg, A. & Briscoe, L. (2010) Early Land Plants Today (ELPT): How many liverwort species are there? *Phytotaxa* 9: 22–40.
- Wellman, C.H., Osterloff, P.L. & Mohiuddin, U. (2003) Fragments of the earliest land plants. *Nature* 425: 282–285.
- Wigginton, M.J. (ed.) (2004) E.W. Jones's liverwort and hornwort flora of West Africa. *Scripta Botanica Belgica*. Meise: National Botanic Garden of Belgium. 30 (xii): 1–443.
- Wikström, N., He-Nygrén, X. & Shaw, A.J. (2009) Liverworts (Marchantiophyta). In: Hedges, S.B. & Kumar, S. (eds.), *The Time tree of life*. Oxford University Press, New York, pp. 146–152.
- Ziegler, H. (1987) The evolution of stomata. In: Ziegler, E., Farquhar, G.D. & Cowan, I.R. (eds.), *Stomatal function*, Stanford University Press, California, pp. 29–57.

**Supplemental information 1:** Hornwort species diversity partitioned by genera across distinct geographic areas (see Fig. 2 for a graphical representation).

Region	Genus	Species count
	<i>Anthoceros</i>	8
	<i>Dendroceros</i>	6
	<i>Folioceros</i>	2
<b>Australia</b>	<i>Megaceros</i>	6
	<i>Phaeoceros</i>	6
<b>Australia Total</b>		<b>28</b>
	<i>Anthoceros</i>	6
	<i>Dendroceros</i>	3
	<i>Folioceros</i>	2
	<i>Megaceros</i>	1
	<i>Mesoceros</i>	1
	<i>Notothylas</i>	3
<b>China</b>	<i>Phaeoceros</i>	5
	<i>Phymatoceros</i>	1
<b>China Total</b>		<b>22</b>
	<i>Anthoceros</i>	7
	<i>Dendroceros</i>	3
	<i>Folioceros</i>	3
	<i>Megaceros</i>	1
<b>East Asia</b>	<i>Notothylas</i>	4
	<i>Phaeoceros</i>	6
<b>East Asia Total</b>		<b>24</b>
	<i>Anthoceros</i>	5
	<i>Notothylas</i>	1
<b>Europe</b>	<i>Phaeoceros</i>	2
	<i>Phymatoceros</i>	1
<b>Europe Total</b>		<b>9</b>
	<i>Anthoceros</i>	19
	<i>Dendroceros</i>	1
	<i>Folioceros</i>	13
	<i>Hattorioceros</i>	1
<b>Indian Subcontinent</b>	<i>Megaceros</i>	1
	<i>Notothylas</i>	15
<b>(including Sri Lanka)</b>	<i>Phaeoceros</i>	8
<b>Indian Subcontinent Total</b>		<b>58</b>
<b>Middle Atlantic Ocean</b>	<i>Anthoceros</i>	2
	<i>Dendroceros</i>	1
	<i>Phaeoceros</i>	1
<b>Middle Atlantic Ocean Total</b>		<b>4</b>
	<i>Anthoceros</i>	10
	<i>Dendroceros</i>	13
	<i>Folioceros</i>	1
	<i>Leiosporoceros</i>	1
	<i>Nothoceros</i>	4
	<i>Notothylas</i>	5
	<i>Phaeoceros</i>	11
<b>Neotropics</b>	<i>Phaeomegaceros</i>	2
	<i>Phymatoceros</i>	1
	<i>Sphaerosporoceros</i>	1
<b>Neotropics Total</b>		<b>49</b>
	<i>Anthoceros</i>	4
	<i>Dendroceros</i>	2
	<i>Megaceros</i>	3
	<i>Nothoceros</i>	1
<b>New Zealand</b>	<i>Phaeoceros</i>	4
	<i>Phaeomegaceros</i>	2
<b>New Zealand Total</b>		<b>16</b>



	<i>Anthoceros</i>	8
	<i>Leiosporoceros</i>	1
	<i>Nothoceros</i>	1
	<i>Notothyllas</i>	2
	<i>Phaeoceros</i>	9
	<i>Phymatoceros</i>	2
<b>North America</b>	<i>Sphaerosporoceros</i>	2
<b>(including Mexico)</b>		
<b>North America Total</b>		<b>25</b>
	<i>Anthoceros</i>	7
	<i>Dendroceros</i>	16
	<i>Folioceros</i>	5
	<i>Hattorioceros</i>	1
	<i>Megaceros</i>	2
<b>Pacific Islands</b>	<i>Nothoceros</i>	1
	<i>Notothyllas</i>	1
	<i>Phaeoceros</i>	3
<b>Pacific Islands Total</b>		<b>36</b>
<b>Russian Far East</b>	<i>Anthoceros</i>	1
	<i>Phaeoceros</i>	2
<b>Russian Far East Total</b>		<b>3</b>
	<i>Anthoceros</i>	3
	<i>Phaeoceros</i>	2
<b>South West Asia</b>	<i>Phymatoceros</i>	1
<b>South West Asia Total</b>		<b>6</b>
<b>Southern Africa</b>	<i>Anthoceros</i>	4
	<i>Phaeoceros</i>	5
<b>Southern Africa Total</b>		<b>9</b>
	<i>Anthoceros</i>	6
	<i>Dendroceros</i>	1
	<i>Nothoceros</i>	2
	<i>Notothyllas</i>	1
	<i>Paraphymatoceros</i>	1
	<i>Phaeoceros</i>	7
<b>Southern South America</b>	<i>Phaeomegaceros</i>	3
	<i>Phymatoceros</i>	1
<b>Southern South America Total</b>		<b>22</b>
	<i>Anthoceros</i>	20
	<i>Dendroceros</i>	3
	<i>Folioceros</i>	2
	<i>Megaceros</i>	1
	<i>Notothyllas</i>	5
	<i>Phaeoceros</i>	7
<b>Tropical Africa</b>	<i>Phymatoceros</i>	1
<b>Tropical Africa Total</b>		<b>39</b>
	<i>Anthoceros</i>	17
	<i>Aspiromitus</i>	1
	<i>Dendroceros</i>	17
	<i>Folioceros</i>	6
	<i>Megaceros</i>	2
	<i>Mesoceros</i>	1
	<i>Notothyllas</i>	9
	<i>Phaeoceros</i>	5
<b>Tropical Asia</b>	<i>Phaeomegaceros</i>	2
<b>Tropical Asia Total</b>		<b>60</b>
	<i>Anthoceros</i>	3
	<i>Dendroceros</i>	1
<b>Western Indian Ocean</b>	<i>Folioceros</i>	1
	<i>Phaeoceros</i>	2
<b>Western Indian Ocean Total</b>		<b>7</b>