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## Research Article



# Phylogenetic and morphological infrageneric classification of the genus *Dendroceros* (Dendrocerotaceae; Anthocerotophyta), with the addition of two new subgenera

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*Dendroceros* is one of the most diverse genera of hornworts, with 41 species and a widespread distribution. The genus is highly distinctive by its epiphytic habitat, gametophytes with perforated wings, multicellular spores due to endosporic germination, highly modified pyrenoid, and desiccation tolerance. The genus is monophyletic and, until now, was divided into two subgenera (subg. *Apoceros* and subg. *Dendroceros*). This classification has not been tested within a phylogenetic framework. Using published phylogenetic data from four molecular markers (*rbcl*, *trnK* including *matK*, *rps4*, and *nad5*) as well as morphological observations of over 80 specimens, we chose 38 morphological characters, which were mapped in the best maximum likelihood tree and optimized for reconstruction of ancestral character. We circumscribe the genus *Dendroceros* and propose 4 subgenera: subg. *Apoceros* (eight species), characterized for having schizogenous cavities in the midrib; subg. *Dendroceros* (14 species) with undulate-crispate gametophyte wings, and spore distal surface covered by aculeate papilla; subg. *Nodulosus* (five species) with rectangular cells in the capsule with nodular wall thickenings; and the monotypic subg. *Cichoraceus* with a single species that has granular spore micro-ornamentations, and a macro-ornamentation formed by pustulate papilla.

**Key words:** biodiversity, bryophytes, classification, hornworts, neotropics, taxonomy

## Introduction

Molecular data have changed the way researchers view plant phylogenetic relationships, especially in character-poor lineages such as bryophytes (Duff, Villarreal, Cargill, & Renzaglia, 2007; Puttick et al., 2018). There are three main groups within the bryophytes, with highly disparate morphological features: liverworts (Marchantiophyta), mosses (Bryophyta), and hornworts (Anthocerotophyta). The evolutionary ties between the mosses and liverworts (setaphytes; Renzaglia, Villarreal, & Garbary, 2018) have been addressed multiple times and we are on the verge of a consensus about their relationship. The position of hornworts remains uncertain in recent molecular phylogenies. Hornworts are either part of the bryophyte monophylum or the sister group to all

land plants (Puttick et al., 2018; Wickett et al., 2014). Within hornworts, the generic relationships within the hornworts have been firmly established (Duff et al., 2007; Renzaglia, Villarreal, & Duff, 2009; Villarreal, Cusimano, & Renner, 2015), while their infrageneric relationships (subgenera and species) have only been addressed in two studies. One study, within the family Dendrocerotaceae, reconstructed the phylogeny of the mostly Neotropical genus *Nothoceros* (Villarreal & Renner, 2014); the second study focused on several species of *Megaceros* from Australasia (Cargill, Vella, Sharma, & Miller, 2013). Thus, the family Dendrocerotaceae seems to be the focus of the most recent phylogenetic studies in hornworts.

The other two genera within the Dendrocerotaceae, *Phaeomegaceros* and *Dendroceros*, remain largely under-studied from a systematic point of view. The genus *Dendroceros* is highly distinctive by its: (1)

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epiphytic habitat, (2) gametophytes with perforated wings; (3) multicellular spores due to endosporic germination; (4) highly modified pyrenoids (i.e., specialized compartments inside chloroplasts essential for carbon concentration mechanism); and (5) desiccation tolerance (Cargill, Renzaglia, Villarreal, & Duff, 2005; Duff, Cargill, Villarreal, & Renzaglia, 2004; Duff et al., 2007; Li, Villarreal, & Szövényi, 2017; Schuette & Renzaglia, 2010; Villarreal et al., 2015; Villarreal, Goffinet, Duff, & Cargill, 2010). *Dendroceros* is, also, one of the most diverse genera among the hornworts, with 41 species and a widespread distribution (Söderström et al., 2016; Villarreal & Renner, 2014). *Dendroceros* species grow mainly on tree bark, branches, and leaves in temperate and tropical forests, between 800–2000 m a.s.l. (Campbell, 1986; Garcia, Sérgio, Villarreal, Sim-Sim, & Lara, 2012; Gradstein, Churchill, & Allen, 2001; Hasegawa, 1980, 1983; Renzaglia et al., 2009; Villarreal, Cargill, Hagborg, Söderström, & Renzaglia, 2010).

*Dendroceros* is unambiguously monophyletic (Duff et al., 2007). However, more than 60% of the described species lack clear morphological descriptions, and ~34% have sequence data from 3–4 molecular markers, especially plastid markers (Söderström et al., 2016; Villarreal, et al., 2010). Schuster (1987) subdivided the genus into two subgenera (subg.): subg. *Apoceros* R. M. Schust., which is characterized by schizogenous cavities in the midrib; and the subg. *Dendroceros*, which possesses a solid midrib (Hasegawa, 1980; Piippo, 1993; Schuster & Engel, 1987; Stephani, 1917). Currently, only 17 species have sub-generic designations (Tab. S1; Söderström et al., 2016). It is evident that the lack of morphological and molecular studies of most of the *Dendroceros* species hamper any systematic evaluation of the subgenera and test character evolution of the subgenera and test character evolution within the genus (Renzaglia et al., 2009; Villarreal, et al., 2010; Villarreal & Renner, 2014). Based on the above, we tested the monophyly of both subgenera proposed by Schuster (1987) and we revised the current infrageneric classification of the genus using all available data and observations from over 80 specimens. The information presented here fills important gaps in our knowledge of hornworts and establishes a robust phylogenetic framework to test character evolution within *Dendroceros*.

## Materials and methods

### Taxon sampling

We studied ~80 specimens (28 species, including type material) assigned to *Dendroceros* held in public herbaria. We examined specimens from the CANB, CHR,

CONC, CONN, DUKE, G, NY, S herbaria (acronyms according to *Index Herbariorum*, Thiers, 2017). The specimens were examined under dissecting and compound microscopes, and by scanning electron microscopy, using standard protocols (Villarreal & Renzaglia, 2006a). Images were taken using a Zeiss Axio Lab A1 light microscope equipped with an Axion Erc5 digital camera, as well as a FEG – Quanta 200 FEI Scanning Electron Microscope, at the Microscopy Center of the Federal University of Minas Gerais, Brazil. Spore ornamentation nomenclature follows Lellinger (2002). To improve and complete the morphological information of many of the species, we used previously published taxonomic descriptions and their protologues, as well as the Plant Science photographic database from type specimens available at JSTOR (<http://plants.jstor.org>).

### Sampling for phylogenetic analyses

We downloaded GenBank sequences of three plastid regions (*rbcL*, *trnK* including *matK*, and *rps4*) and the mitochondrial *nad5* exon2 (Duff et al., 2004, 2007; Villarreal & Renner, 2012, 2013), from 14 species of *Dendroceros* (Table S1, see online supplemental material, which is available from the article's Taylor & Francis Online page at <http://dx.doi.org/10.1080/14772000.2019.1682080>), and six other members of the family Dendrocerotaceae: *Megaceros flagellaris* (Mitt.) Steph.; *Megaceros leptohymenius* (Hook., F. & Taylr.) Steph.; *Nothoceros giganteus* (Lehm. & Lindenb.) J. Haseg.; *Nothoceros renzagliensis* J.C. Villarreal et al.; *Phaeomegaceros fimbriatus* (Gottsche) Duff et al.; and *Phaeomegaceros hirticalyx* (Steph.) Duff et al. We chose *Phymatoceros phymatodes* (M. Howe) Duff et al. as the outgroup taxon, following published studies (Duff et al., 2007). All sequences used in this study are available on the GenBank public platform (<https://www.ncbi.nlm.nih.gov/nucleotide/?term=Dendroceros>). Voucher information and GenBank accession numbers are provided in Table S2 (see supplemental material online).

### Phylogenetic analyses

The GenBank sequences were edited using the software MEGA 6.0 (Tamura, Stecher, Peterson, Filipinski, & Kumar, 2013) and aligned using Muscle (Edgar, 2004), followed by manual adjustments. The alignment of the mitochondrial *nad5* gene and the plastid regions *trnK*, *rbcL*, *rps4* were concatenated using Mesquite 3.2 (Maddison & Maddison, 2017). The matrix consisted of 4,170 nucleotides. A maximum parsimony (MP) analysis was performed in PAUP\* (Swofford, 2002), using a heuristic search with 1000 repetitions employing Tree-

bisection and reconnection (TBR). The statistical support was obtained through non-parametric bootstrapping (Felsenstein, 1985) with 5,000 replicates.

We obtained the best partition for our data and the appropriate nucleotide substitution using Partitionfinder2 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016). The best partition scheme was by locus: *nad5* (1-1106, TRN +  $\Gamma$ ), *rbcl* (1107-2441, GTR + I +  $\Gamma$ ), *rps4* (2442-3024, TVM + I), and *trnK-matK* (3025-4170, K81UF +  $\Gamma$ ). Bayesian analyses (BI) were carried out with two simultaneous and independent runs with four chains (one cold and three heated chains) and 1,000,000 generations; posterior probabilities of tree topologies were estimated from both partitions and the trees were sampled every 100th generation using a Markov Chain Monte Carlo method implemented in MrBayes version 3.2 (Ronquist *et al.*, 2012). The first 25% of sampled trees were discarded for burn-in to make the analyses converge conservatively, and a majority rules consensus of the trees excluding the burn-in was produced with posterior probabilities above 0.95 as supported. The alignments and trees have been deposited on TreeBASE (<https://treebase.org/treebase-web/home.html>; accession no. 23631). We used the software FigTree to view and edit trees (Rambaut, 2017).

We performed maximum likelihood (ML) analyses on RAxML-HPC BlackBox v. 8.2 (Stamatakis, 2014) using Cipres Science Gateway (<https://www.phylo.org>). The substitution model GTR + CAT was used with four unlinked partitions and the statistical support was evaluated using 1000 ML start replicas using the same substitution model.

## Character optimization

We chose 38 morphological variables of categorical characters based on the analysed material and taxonomic descriptions (Garcia *et al.*, 2012; Hasegawa, 1980; Infante, 2010; Piippo, 1993; Proskauer, 1951, 1953). The characters related to the gametophytes were recorded from mature portions of each plant (e.g., mid region), the apical and basal regions of the plants were not taken into consideration, because these were undeveloped or deteriorated. On the sporophyte, the dehisced mature capsules were studied, especially in the middle region of the capsule.

A matrix of morphological characters was encoded with binary and multistate characters (Table S3, see supplemental material online) and mapping characters and reconstruction of ancestral character states were performed in WinClada 10.00.08 (Nixon, 2002) and Mesquite v.3.2 (Maddison & Maddison, 2017). Characters were mapped on the best maximum

likelihood tree and optimized to evaluate synapomorphies and homoplasies (Fig. S1, see supplemental material online). We explored delayed optimization (deltran) and rapid optimization (acctrans) to identify additional characters that would define certain clades (Agnarsson & Miller, 2008).

## Results

### Phylogenetic analyses

The phylogenetic analyses resulting from the combination of the coding regions *rbcl*; *nad5*; *trnK-matK*; *rps4*, Fig. 1.1) show that the genus *Phaeomegaceros* was not well-supported (bootstrap 59% MP). In contrast, the genera *Megaceros* and *Nothoceros* were identified as monophyletic, with high bootstrap (MPB, MLB) and high posterior probability (p.p., Fig. 1.1). The genus *Dendroceros* is monophyletic, with high support values (MPB 99%; MLB 100% and p.p. 1.0). The *Dendroceros* species analysed were aggregated into four clearly defined groups.

Clade A includes two species, *D. difficilis* and *D. cucullatus*, with good support (MPB 83%, MLB 97% and p.p. 1.0). Clade B is formed by the species *D. crispus*, *D. javanicus*, *D. validus*, *D. tubercularis*, *D. breutelii*, and *D. paivae*, and was highly supported, with values over 98% (MPB, MLB) and above 0.95 (p.p) in all the analyses. Clade C was highly supported (MPB 99%, MLB 100% and 1.0 p.p.) and comprises *D. borbonicus*, *D. africanus*, *D. crispatus*, and *D. granulatus*. Finally, *D. cichoraceus* is sister to the rest of the other taxa (Fig. 1.1).

### Character evolution

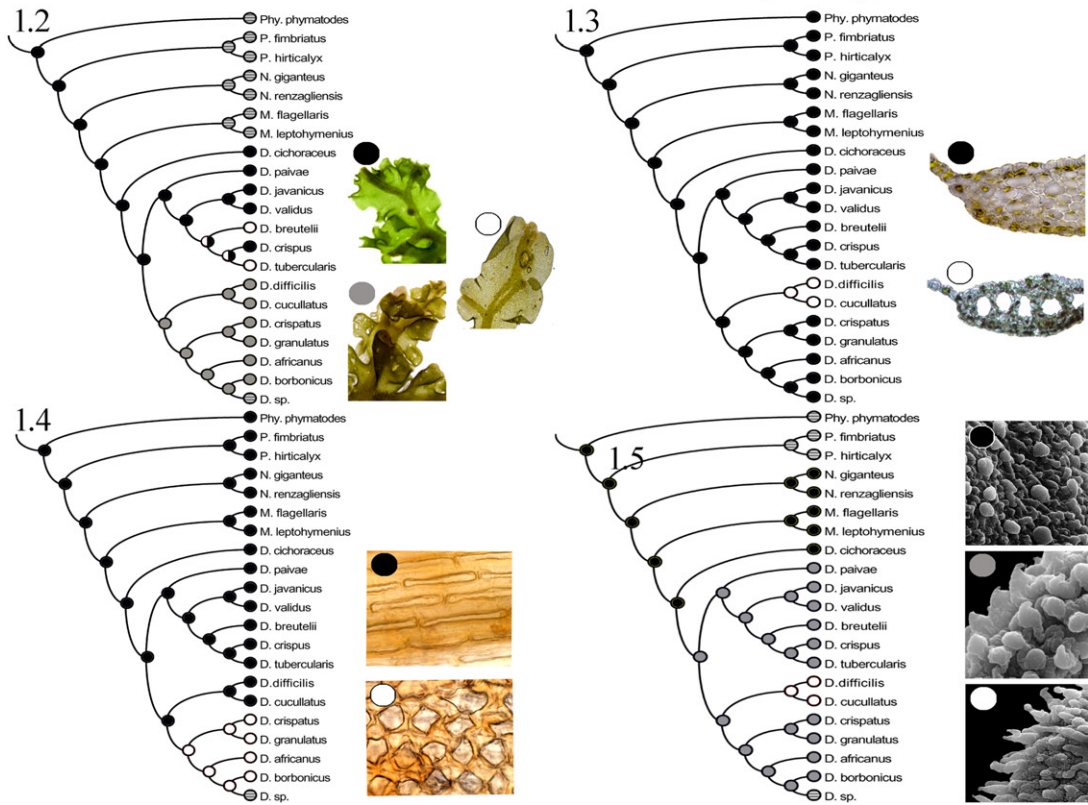
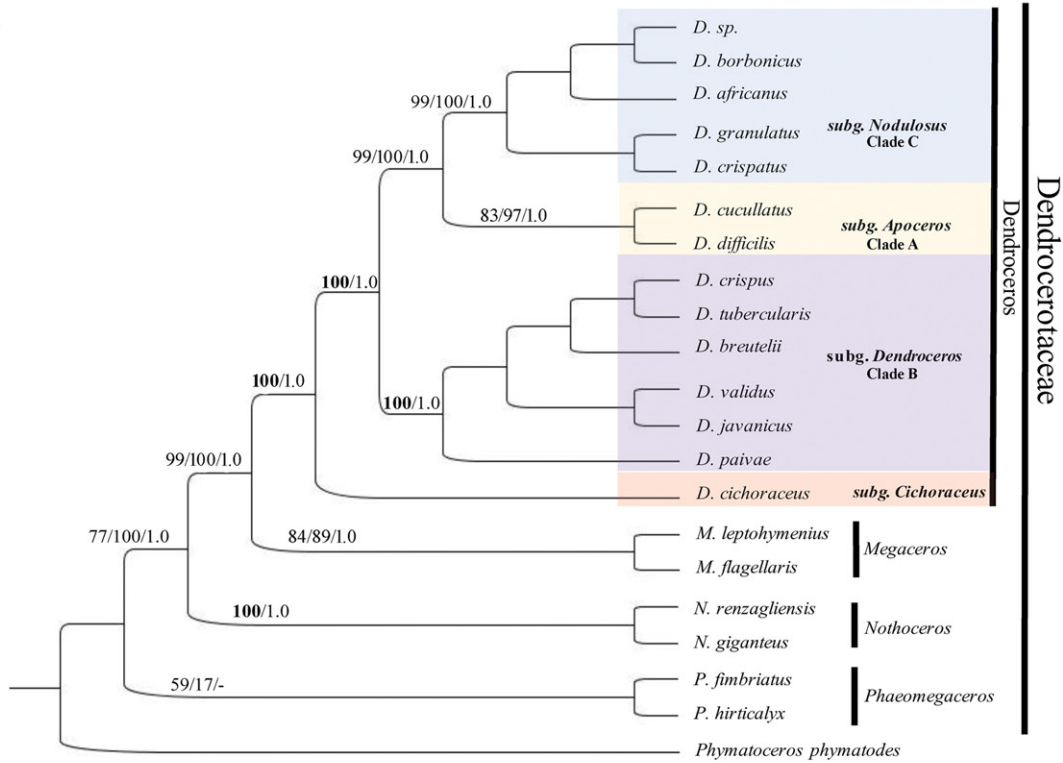
The morphological matrix of 38 characters included 24 gametophyte characters, 11 sporophyte characters, and two ecological characters (Table S3, Fig. S1, see supplemental material online). The most informative characters of the genus were:

*Wing anatomy*: the undulate-crispate wings is the ancestral state in *Dendroceros*, with a shift to flat wings in the ancestor shared by *D. tubercularis*, *D. breutelii*, and *D. crispus*, and a reversal in the former species. There is one evolutionary transition to galeate wings in clade C (Fig. 1.2).

*Transverse section of the midrib*: the ancestral state is a solid midrib with a transition to thallus with schizogenous cavities in the clade A (Fig. 1.3).

*Thickenings of the cell walls in the capsule*: the rectangular cell shape is the ancestral state with a shift to nodular capsule cells in clade C (Fig. 1.4).

1.1



**Fig. 1.** Phylogenetic tree and main morphological characters of *Dendroceros* Ness genus. 1.1. Maximum likelihood Phylogenetic tree resulting from analyses based on the concatenated sequences from the *rbcL nad5*; *trnK-matK*; *rps4* regions. Values in the branches represent MP, ML bootstrap support (as a percentage), and posterior probabilities; when all of the values are equal they are presented in bold. We highlight the new subgenera classification. Key morphological characters used in the ancestral reconstruction analyses (see Tab. S3 and Fig. S1). 1.2. Format of the gametophyte wings undulate to crispate (black), flat (white) and galeate (gray); 1.3. Cross-section of the midrib solid (black) and with schizogenous cavities (white); 1.4. Capsule cell walls thickenings rectangular (black) and nodular (white); 1.5. Spore ornamentation at distal surface postulate papillae (black), aculeate papillae (gray) and spines (white). Striped circles, unknown characteristics.

**Table 1.** Comparison of the sizes of spores in the genus *Dendroceros* as determined by light and SEM microscopy. Values in  $\mu\text{m}$ . Measures were compared and complemented with Chantanaorrapint (2014), Chantanaorrapint *et al.* (2014), Garcia *et al.* (2012), Hasegawa (1980, 1982, 1986), Infante (2010), Piippo (1993), and Stephani (1917).

subg.	Species	Light		SEM	
		Length	Width	Length	Width
Apoceros	<i>Dendroceros difficilis</i>	40–65	50–85	30–45	42–68
Apoceros	<i>Dendroceros cavernosus</i>	45–55	45–60	19–35	35–55
Apoceros	<i>Dendroceros cucullatus</i>	—	—	30–60	45–100
Apoceros	<i>Dendroceros pedunculatus</i>	100–110	110–135	—	—
Apoceros	<i>Dendroceros muelleri</i>	54	—	34–55	59–94
Apoceros	<i>Dendroceros ogeramangus</i>	45–60	63–75	—	—
Apoceros	<i>Dendroceros subdifficilis</i>	50–65	60–85	—	—
Apoceros	<i>Dendroceros seramensis</i>	45–70	50–85	—	—
Dendroceros	<i>Dendroceros javanicus</i>	35–50	35–63	30–40	35–55
Dendroceros	<i>Dendroceros validus</i>	25–50	37–60	30–40	40–70
Dendroceros	<i>Dendroceros breutelii</i>	40–50	50–70	30–60	40–55
Dendroceros	<i>Dendroceros tubercularis</i>	30–55	45–70	24–40	40–60
Dendroceros	<i>Dendroceros herasii</i>	52–67	57–75	42–47	40–65
Dendroceros	<i>Dendroceros foliicola</i>	45–55	45–65	—	—
Dendroceros	<i>Dendroceros paivae</i>	—	—	45–62	50–63
Dendroceros	<i>Dendroceros rigidus</i>	50–55	55–77	30–50	50–65
Dendroceros	<i>Dendroceros crispus</i>	37–62	45–75	30–45	45–78
Dendroceros	<i>Dendroceros adglutinatus</i>	37.6	62.5	—	—
Dendroceros	<i>Dendroceros subplanus</i>	45–55	45–65	—	35–60
Dendroceros	<i>Dendroceros acutilobus</i>	40–65	40–80	—	—
Dendroceros	<i>Dendroceros crassinervis</i>	—	—	27–45	48–75
Dendroceros	<i>Dendroceros allionii</i>	—	—	40–45	65–75
Nodulosus	<i>Dendroceros granulatus</i>	30–70	40–100	15–33	40–60
Nodulosus	<i>Dendroceros africanus</i>	40–62	45–87	30–40	45–60
Nodulosus	<i>Dendroceros japonicus</i>	50–100	60–110	45–60	55–75
Nodulosus	<i>Dendroceros crispatus</i>	50–80	75–100	35–75	75–90
Nodulosus	<i>Dendroceros borbonicus</i>	57–85	50–112	30–50	40–80
Cichoraceus	<i>Dendroceros cichoraceus</i>	55–72	62–85	35–70	50–85

*Spore ornamentation on distal surface:* the ancestral state is the postulate papilla (macro-ornamentation in *D. cichoraceus*), with a shift to aculeate papilla observed in clades B and C; and spines are a synapomorphy for clade A (papilla shape, see Figs 1.5, 6).

### Taxonomic treatment

The analyses confirmed the existence of four distinct natural groups, or subgenera, within *Dendroceros* (Hasegawa, 1980; Piippo, 1993; Schuster, 1987; Söderström *et al.*, 2016; Stephani, 1917). To clarify some of the nomenclatural problems within the genus, we propose a new circumscription of the subgenera of *Dendroceros* Nees based on the phylogenetic inferences obtained here and the morphological characteristics analysed for the 28 species listed in Table 1.

### Key to *Dendroceros* Nees subgenera

1a Cross section of the thallus midrib showing schizogenous cavities; cells in the capsule with thickened non-nodular walls ... **subg. Apoceros**

1b Cross section of the thallus midrib solid; cells in the capsule with or without thickened non-nodular walls..... **2**

2a Capsule cells with nodular thickened walls; gametophyte with galeate (hood-like) wings; perforation on wings always present ... **subg. Nodulosus**

2b Capsule cells with non-nodular thickened walls; gametophyte flat, undulate, or crispate, wing perforations not present or rare ... **3**

3a Gametophyte wings with pores, margins entire; spores with less than a total of 20 cells, distal surface with macro-ornamentations of aculeate papilla 0.8–3.3  $\mu\text{m}$  long (under SEM) ... **subg. Dendroceros**

3b Gametophyte wings with almost no pores, margins crenate; spores with more than 21 cells, distal surface with macro-ornamentations of pustulate papilla 0.9–2.2  $\mu\text{m}$  long (under SEM) ..... **subg. Cichoraceus**

Family Dendrocerotaceae  
*Dendroceros* Nees, 1846

*Dendroceros* Nees subg. *Apoceros* Schust R. M. 1987  
(Figs 2, 6.1)

**Type species.** *Dendroceros cavernosus* by original designation in Schuster (1987).

**Diagnosis.** Gametophyte light-green when dried, varying from ochre-green and red to shades orange in the midrib region; wings of the gametophyte with macro-perforations; schizogenous cavities visible in cross sections of the midrib; involucre with ornamentation and a rough cuticle; cells of the capsule rectangular, with thickened non-nodular walls; distal spore surfaces with micro-ornamentations of warts and granules, and macro-ornamentation of spines 1–3 µm long.

In 1987 Schuster described this subgenus and included three species (*D. cavernosus*, *D. difficilis*, and *D. pedunculatus*). Piippo (1993) later published the species *D. ogeramnangus* and classified it within this group, together with *D. subdifficilis*. The species *D. muelleri* was studied by Stephani (1917), who placed it in the group of species with schizogenous cavities in the midrib. The Asiatic species *D. seramensis* also presents schizogenous cavities in the midrib (Hasegawa, 1986). The last two species, together with *D. cucullatus*, are currently classified among the incertae sedis of the genus, and we included them in subg. *Apoceros*, which is composed of eight species; two of which were included in our phylogenetic analysis.

*Dendroceros cavernosus* J. Haseg. 1980

**Type.** Malaysia, N. Borneo: between Sosopodon and S. Kelinggen, foot of Mt. Kinabalu, 1350–1400 m, on fallen branches, Mizutani M. 3861 (holotype NICH!).

**Description and/or illustration:** p. 308, Fig. 11 in Hasegawa (1980); 106 p. in Hasegawa (2002).

**Geographic distribution.** Malaysia, Papua New Guinea, Solomon Islands.

**Additional material examined.** CANB 578715, CHR 502079, CHR 501509, NY 03103379, NY 03103380.

*Dendroceros cucullatus* Steph. 1923

**Type.** Philippines, Mindanao, Butuan Subprovince, 15 m, on tree trunk, March–July 1911, Weber C. M. 1341 (holotype G 00061264!, isotype G 00061265!, isotype NY 253465!).

**Description and/or illustration.** p. 429 in Stephani (1923); Figs 9 b, c, f, h in Hasegawa (1980); p. 34, Figs 2, 5a–b in Chantanaorrapint (2014); p. 147, Figs 3–4 in Chantanaorrapint et al. (2014).

**Geographic distribution.** Philippines and Thailand.

*Dendroceros difficilis* Steph. 1917

**Type.** Indonesia. Java. Provo Preanger, infra lacum vulcanicum Telaga bodas, 1.550 m, November 1894, Schiffner V. #2 (holotype G 19700, not seen; isotype NY 231498!).

**Description and/or illustration.** p. 1009 in Stephani (1917); p. 304, Fig. 9 in Hasegawa (1980); p. 41, Fig. 9 in Piippo (1993); p.106 in Hasegawa (2002); p. 147, Figs 3–4 in Chantanaorrapint et al. (2014).

**Geographic distribution.** Indonesia, Malaysia, Philippines.

**Additional material examined.** G 00048022, NY 03103374.

*Dendroceros muelleri* Steph. 1889

**Type.** Australia, Bellender Ker Range, Coll. Herb. Melbourne 1886, Froggatt, W. & Sayer W. # s.n. (holotype G 00060899!).

**Description.** p. 133 in Stephani (1889); p. 1009 in Stephani (1917).

**Geographic distribution.** Australia.

*Dendroceros ogeramnangus* Piippo 1993

**Type.** Papua New Guinea. ‘Morobe Prov.: Rawlinson Range, near Ogeramnang airstrip. In extensively cultivated garden area with scattered trees along trail from Selimbeng (Serembeng) down toward Rulum River’, 1200–1450 m, 29°S, 142°E, collection site 10k. May 1981, Norris 59226 (holotype H; isotype JE, not seen).

**Description and/or illustration.** p. 40, Figs 7, 10 in Piippo (1993).

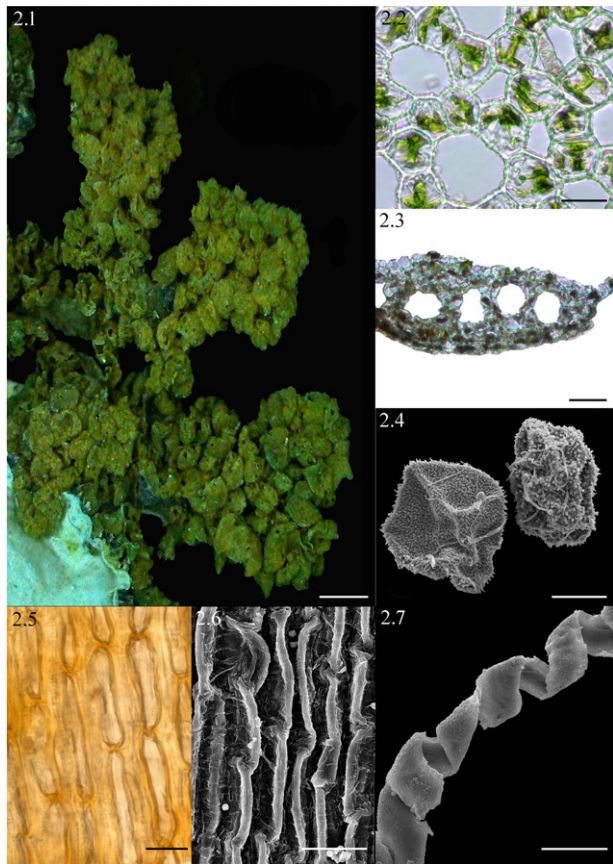
**Geographic distribution.** Papua New Guinea.

*Dendroceros pedunculatus* Steph. 1909

**Type.** Indonesia, Amboina, Karsten G. (holotype G 00061209, not seen).

**Description and/or illustration.** p. 1009 in Stephani (1917); p. 306, Fig. 10 in Hasegawa (1980); p. 43, Figs 7, 11 in Piippo (1993).

**Geographic distribution.** Indonesia.



**Fig. 2.** Morphological characteristics of subg. *Apoceros*: 2.1. *D. cucullatus*, gametophyte; 2.2. *D. cucullatus*, gametophyte wing cells; 2.3. *D. cavernosus*, cross-section of the midrib; 2.4. *D. muelleri*, spores proximal and distal (left to right) views; 2.5. *D. cavernosus*, rectangular cells of the capsule with cell wall thickenings (light microscope); 2.6. *D. cucullatus*, rectangular cells of the capsule, with cell wall thickenings (SEM); 2.7. *D. muelleri*, pseudoelaters. Scales: 2.1 = 2.1 mm; 2.2, 2.3 = 50  $\mu$ m; 2.4, 2.6 = 25  $\mu$ m; 2.5 = 20  $\mu$ m; 2.7 = 10  $\mu$ m.

***Dendroceros seramensis*** J. Haseg. 1986

**Type.** Indonesia. Seram. Kecamatan, Tehoru, between Wolu and Wae Waya, in a secondary lowland forest, on trunk of tree, 0–360 m, Akiyama, H. 10269 (holotype KYO, not seen).

**Description and/or illustration.** p. 10, Fig. 1 in Hasegawa (1986).

**Geographic distribution.** Indonesia.

***Dendroceros subdifficilis*** S. Hatt. 1951

**Type.** Indonesia, West Irian, Manokwari: Prafi, Bivouae, February 1943, Tsuyama 1178 (holotype TNS; isotype NICH, not seen).

**Description and/or illustration.** Fig. 2 in Hasegawa (1986); p. 44, Fig. 15 in Piippo (1993).

**Geographic distribution.** New Guinea.

***Dendroceros*** Nees subg. ***Dendroceros*** (Figs 3, 6.2)

**Type species.** *Dendroceros crispus* by original designation in Schuster (1987).

**Diagnosis:** Gametophyte light-green, dark-green when dry; flat undulate, or crispate; wings of the gametophyte with macro-perforations; pores mainly irregular; midrib solid; rectangular cells in the capsule with wall thickenings; pseudoelaters papillose or smooth; multicellular spores, proximal surface coarsely vermiculate, distal surface with granular and vermiculate micro-ornamentations, with macro-ornamentations of the aculeate papilla 0.8–3.3  $\mu$ m long.

*Dendroceros crispus*, *D. javanicus*, *D. tubercularis*, and *D. validus* were placed by Schuster (1987) and Piippo (1993) within this subgenus. Here we include *D. adglutinatus*, *D. allionii*, *D. breutelii*, *D. crassinervis*, *D. herasii*, *D. rigidus*, and *D. paivae*, which were classified as incertae sedis by Söderström *et al.* (2016; Fig. 1). The subg. *Dendroceros* therefore now consists of 14 species.

***Dendroceros acutilobus*** Steph. 1909

**Type.** Indonesia, Amboina, Ambon X, 1889, Karsten G. s.n. (holotype G, not seen).

**Description and/or illustration.** p. 298, Fig. 6 in Hasegawa (1980); p. 44, Figs 12, 13, 14 in Piippo (1993); p. 106 in Hasegawa (2002).

**Geographic distribution.** Indonesia, Papua New Guinea.

**Additional material examined.** G 00067713, NY 3103376.

***Dendroceros adglutinatus*** (Hook.f. & Taylor) Gottsche, Lindenb. et Nees 1846

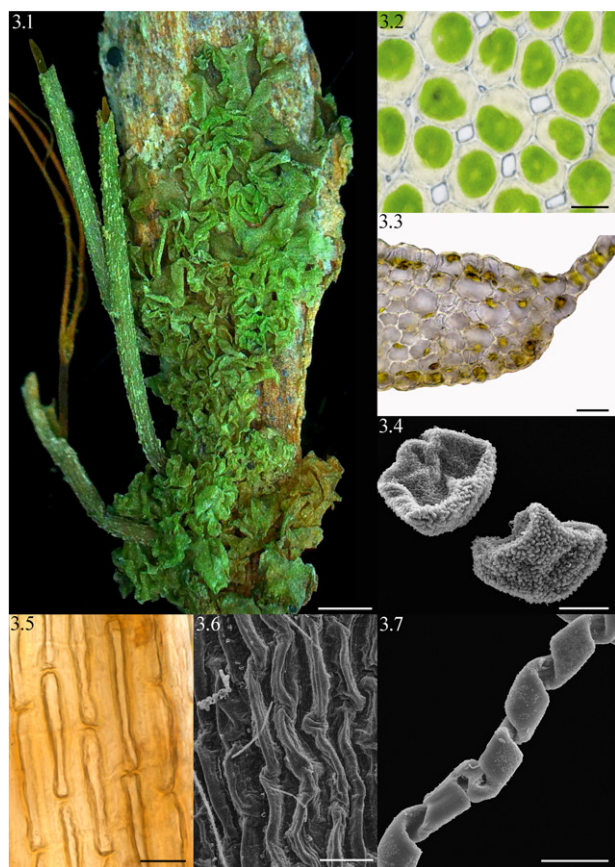
**Bas.** *Monoclea adglutinata* Hook.f. & Taylor 1846.

**Type.** Africa, Saint Helena. ‘On trees on Diana’s Peak’, at an elevation of 2000 feet. 1844 Hooker D.J (holotype not seen, isotype NY 920051).

**Description and/or illustration.** p. 580 in Gottsche *et al.* (1846); p. 1, Fig. 1G in Infante (2010).

**Geographic distribution.** Saint Helena.





**Fig. 3.** Morphological characteristics of subg. *Dendroceros*: 3.1. *D. crispus*, gametophyte and sporophyte; 3.2. *D. crispus*, gametophyte wing cells with pores; 3.3. *D. crispus*, cross-section of the midrib; 3.4. *D. crispus*, spores, proximal and distal views (left to right); 3.5. *D. crispus*, rectangular cells of the capsule, with cell wall thickenings (light microscope); 3.6. *D. allonii*, rectangular cells of the capsule, with cell wall thickenings (SEM); 3.7. *D. javanicus*, Pseudoelaters. Scales: 3.1 = 1 mm; 3.2, 3.4, 3.5 = 20  $\mu$ m; 3.3 = 30  $\mu$ m; 3.6 = 25  $\mu$ m; 3.7 = 10  $\mu$ m.

**Additional material examined.** S 165088.

*Dendroceros allionii* Steph. 1917

**Type.** Ecuador, Morona-Santiago – Bomboiza, Gualaquiza/Provincia de Morona Santiago, 900 m. Coll. Bryotheca Ecuadorensis 1901. Allioni M. 319. (holotype G 00060907!).

**Description.** p. 1014 in Stephani (1917).

**Geographic distribution.** Ecuador.

*Dendroceros breutelii* Nees 1846

**Type.** St. Kitts. Breutel s.n. (G- 00115617, not seen).

**Description.** p. 581 in Gottsche et al. (1846); p. 1015 in Stephani (1917).

**Geographic distribution.** Brazil, Cuba, Ecuador, Montserrat, Puerto Rico, Saint Kitts and Nevis.

**Additional material examined.** G 00060906, G 00115614, NY 00544177, NY 00544178, NY 00544179, NY 00544180, DUKE 0263664.

*Dendroceros crassinervis* (Nees) Gottsche, 1858.

**Bas.** *Anthoceros crassinervis* Nees, Syn. Hepat. 4: 589, 1846.

**Type.** Indonesia, Java. “*Inter Lichenes Indiae occidentalis legit Hampe, Vidi in hb. Hp.*” (J. Gottsche et al., 1846).

**Description.** p. 589 in Gottsche et al. (1846).

**Geographic distribution.** Indonesia.

**Additional material examined.** NY 03103378, NY 3103377.

*Dendroceros crispus* (Sw.) Nees 1844

**Bas.** *Anthoceros crispus* Sw., Prodr. (Swartz): 146, 1788.

**Type.** Jamaica, ‘In montibus altis ‘Liguaneis’ (‘in the mountains of New Liguane’ – P. Browne; meaning apparently the mountains closely NW of Kingston, rather than the whole of the Blue Mountain range). Swartz, O. s.n. (lectotype S-B20564 selected by Proskauer 1960, not seen).

**Description.** p. 146 in Swartz (1788); p. 581 in Gottsche et al. (1846); p. 1015 in Stephani (1917); p. 207 in Howe (1934).

**Geographic distribution.** Brazil, Colombia, Costa Rica, Cuba, Dominica, Ecuador

Guadeloupe, Jamaica, Martinique, Panama, Peru, Puerto Rico, Saint Helena, Suriname, Trinidad and Tobago, Uruguay, Venezuela.

**Additional material examined.** G 00115645, G 00115607, NY 231499, NY 01319034, NY 01319035, NY 01319036, NY 01319037, NY 01319038, NY 00544185, NY00544186, NY 00544187, NY 00544188, NY 00544189, NY 00544190, NY 00544191, NY

00544193, NY 00544194, NY 00544195, NY 00544196, NY 00544197, NY 00544199.

*Dendroceros foliicola* J.Haseg. 1980

**Type.** East Borneo. Munukan, N. of Tarakan, inner forest in N. part of the island near brooklet, on leaves of Salacca-palm, leg. W. Meijer B 5018 (NICH, not seen).

**Description and/or illustration.** p. 295, Fig. 5 in Hasegawa (1980).

**Geographic distribution.** East Borneo.

*Dendroceros herasii* M. Infante 2010

**Type.** Equatorial Guinea, Annobón, Mte. Quioveo, Bosque neblinoso de Schefflera mannii (Hook.f.) Harms en la cumbre, muy musgoso. Colgante en liana fina. 590m. October 2000. Infante M & Heras P, 27 (holotype VIT 25366, not seen).

**Description and/or illustration.** p. 285, Fig. 1 in Infante (2010).

**Geographic distribution.** Equatorial Guinea (Annobon) and Island of São Tomé.

*Dendroceros javanicus* (Nees) Nees 1844

**Bas.** *Anthoceros javanicus* Nees, Enum. Hep. Jav. (1830).

**Type.** Indonesia, Java, Nees (isotype G 19704, not seen).

**Description and/or illustration.** p. 582 in Gottsche et al. (1846); p. 1010 in Stephani (1917); p. 301, Fig. 8 in Hasegawa (1980); p. 46, Figs 12, 14, 15 in Piippo (1993); p. 119, Fig. 4 in Hasegawa (1995).

**Geographic distribution.** Federated States of Micronesia, French Polynesia, Indonesia, Papua New Guinea.

**Additional material examined.** NY 3103366, NY 3103367, NY 3103368, NY3103370.

*Dendroceros paivae* see Garcia, C., Sérgio, C., Villarreal, J. C., Sim-Sim, M., & Lara, F. (2012).

**Type.** São Tomé e Príncipe. Ilha de São Tomé. Caminho para a Roça Trás-os-Montes. NKF3129. 1038m. 19-07-2007. Epiphyllous on leaves of *Syzygium jambos*. Alston. C. Garcia S. 125 (holotype LISU 237201!).

**Description and/or illustration.** p. 5, Figs 1–9; 26; 51–53 in Garcia et al. (2012).

**Geographic distribution.** Island of São Tomé.

*Dendroceros rigidus* Steph. 1917

**Type.** Brazil, São Paulo. May 1901. Schiffner V. 992 (holotype not seen; Isotype S B22423!).

**Description.** p. 1017 in Stephani (1917).

**Geographic distribution.** Brazil.

*Dendroceros subplanus* Steph. 1909

**Type.** Java, s.d. Solms 26c (holotype G, not seen).

**Description and/or illustration.** p. 294, Fig. 4 in Hasegawa (1980); p. 48 in Piippo (1993).

**Geographic distribution.** Java, Sumatra.

*Dendroceros tubercularis* S. Hatt. 1944

**Type.** Japan, Bonin Islands, Hahajima, April 1938, leg. Hattori, S. 3161 (holotype TNS, paratype NY 231509!).

**Description and/or illustration.** p. 6, Fig. 13 in Hattori (1944); p. 291, Fig. 3 in Hasegawa (1980).

**Geographic distribution.** Japan.

*Dendroceros validus* Steph. 1917

**Type.** Indonesia, Sumatra occid., in monte Singalang, in silvis primaevae as decliv. orient., 1760m, VIII 1894, Schiffner #12 (holotype G 00060897!, isotype NY 00231512!).

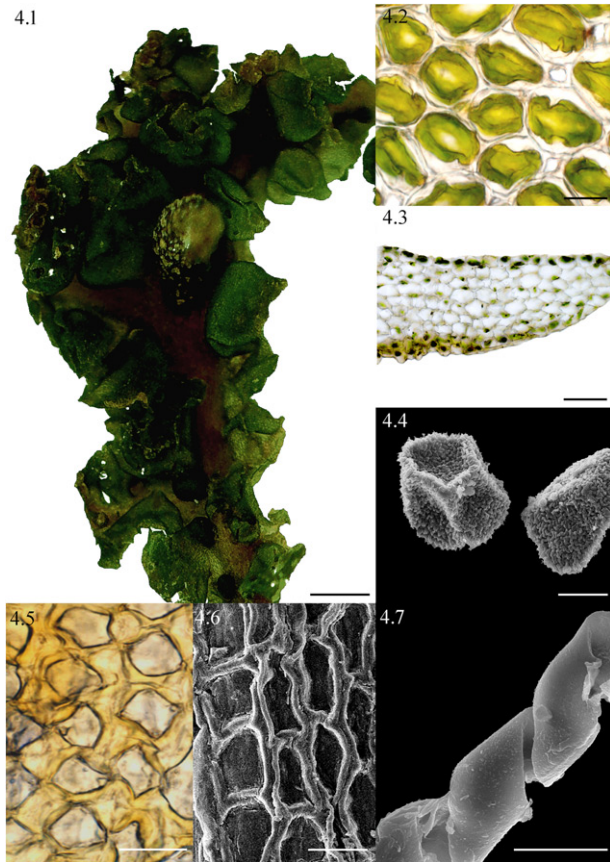
**Description and/or illustration.** p. 1016 in Stephani (1917); p. 300, Fig. 7 in Hasegawa (1980); p. 108 in Hasegawa (2002).

**Geographic distribution.** Indonesia and New Zealand.

**Additional material examined.** CHR 530624, CHR 530665, CANB 788972.

*Dendroceros* Nees subg. *Nodulosus* Peñaloza-Bojacá G. & Maciel-Silva A. subg. nov. (Figs 4, 6.3)

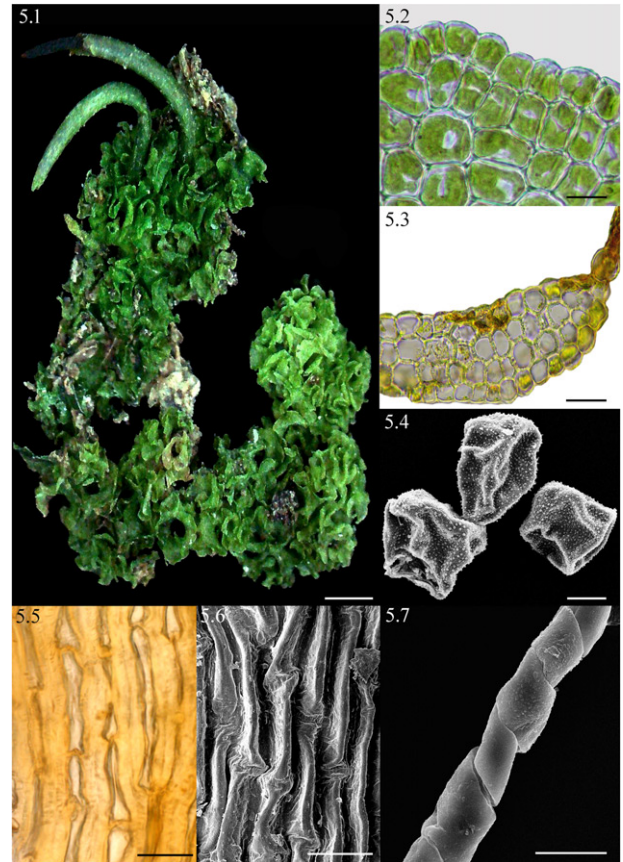
**Type species.** *Dendroceros crispatus* (Hook.) Nees



**Fig. 4.** Morphological characteristics of subg. *Nodulosus*: 4.1. *D. crispatus*, gametophyte; 4.2. *D. crispatus*, gametophyte wing cells with irregular pores; 4.3. *D. crispatus*, cross-section of the midrib; 4.4. *D. borbonicus*, spores, distal and proximal view (left to right); 4.5. *D. borbonicus*, rectangular cells of the capsule with thickening of the nodular cell walls (light microscope); 4.6. *D. africanus*, rectangular cells of the capsule, showing thickening of the cell walls (SEM); 4.7. *D. borbonicus*, pseudoelaters. Scales: 4.1 = 1 mm; 4.2, 4.5, 4.6 = 20  $\mu$ m; 4.3 = 75  $\mu$ m; 4.4 = 30  $\mu$ m; 4.7 = 10  $\mu$ m.

**Diagnosis.** Gametophyte galeate or with galeate wings, pale to dark green, but dull brown when dry; gametophyte wings reaching the midrib, with macro-perforations and pores; midrib solid; rectangular cells in the capsule with nodular wall thickenings; pseudoelaters papillose or smooth; multicellular spores, distal surface with granular and vermiculate micro-ornamentations, with macro-ornamentations of aculeate papilla being smaller (0.9–2.2  $\mu$ m long), with short, fine, and frequently straight apices.

*Dendroceros borbonicus* and *D. japonicus*, species previously included in subg. *Dendroceros* by Schuster (1987), were grouped in a new phylogenetic clade with *D. africanus*, *D. crispatus*, and *D. granulatus* (previously in uncertain positions based on Söderström et al. 2016). This subgenus therefore comprises a total of five species.



**Fig. 5.** Morphological characteristics of subg. *Cichoraceus*: 5.1–5.7 *D. cichoraceus*: 5.1. Gametophyte and immature sporophyte; 5.2. Margins of the gametophyte wings without pores; 5.3. Cross-section of the midrib; 5.4. Spores; 5.5. Rectangular cells of the capsule, showing thickening of the cell walls (light microscope); 5.6. Rectangular cells of the capsule, showing thickening of the cell walls (SEM); 5.7. Pseudoelaters; Scales: 5.1 = 1 mm; 5.2, 5.5 = 20  $\mu$ m; 5.3 = 30  $\mu$ m; 5.4, 5.6 = 25  $\mu$ m; 5.7 = 10  $\mu$ m.

*Dendroceros africanus* Steph. 1909

**Type.** Africa, Cameroon, Bibundi. Jungner J. R. #9 1891 (holotype BM, not seen; isotype G 00045214!)

**Description and/or illustration.** p. 1013 in Stephani (1917); p. 421, Fig. 286 in Jones et al. (2004); p. 284 in Infante (2010).

**Geographic distribution.** Cameroon and Tanzania.

*Dendroceros borbonicus* Steph. 1893

**Type.** Africa, Reunion Island, 1875, De l'Isle, (holotype G-00045215!).

**Description.** p. 31 in Stephani (1893); p. 1011 in Stephani (1917).

**Geographic distribution.** Reunion Island and Mauritius.

**Additional material examined.** S 7510, BR Theo Arts 153-51.

*Dendroceros crispatus* (Hook.) Nees 1846

**Bas.** *Monoclea crispata* Hook., Bot. Misc. 1: 117, 1829.

**Type.** Lesser Antilles, Saint Vincent, in ramis arborum. Guilding, L. (lectotype S B1983!).

**Description and/or illustration.** p. 117, Fig. 27 in Hooker (1830); p. 579 in Gottsche *et al.* (1846); p. 573 in Spruce (1885); p. 8, Figs 27–36, 51 in Garcia *et al.* (2012).

**Geographic distribution.** Australia, Brazil, Cook Islands, Costa Rica, Cuba, Ecuador,

Guadeloupe Island, Hawaii, Jamaica, Martinique, Norfolk Island, New Caledonia,

São Tomé, Saint Vincent, Panamá, Peru, Puerto Rico, Tahiti.

**Additional material examined.** NY 1319033, NY 1319031, NY 1319048, NY 1319049, NY 1319050, NY 1319054.

*Dendroceros granulatus* Mitt. 1871

**Type.** Samoa, *s.l.*, component A, Powell, T. 144, (lectotype NY.00231502!).

**Description.** p. 419 in Mitten (1871); p. 1018 in Stephani (1917); p. 98 in Hasegawa (1982);

**Geographic distribution.** Samoa.

**Additional material examined.** NY 231538 A (Col: T. Powell 419), NY 00231541, NY 00231542.

*Dendroceros japonicus* Steph. 1909

**Type.** Japan, Shikoku – Mount Yokogura, May 1901. Yoshinaga T 26 (holotype G 00067715!).

**Description and/or illustration.** p. 288, Fig. 1 in Hasegawa (1980).

**Geographic distribution.** Japan.

**Additional material examined.** S B205906, NY 03103373.

*Dendroceros* Nees subg. *Cichoraceus* Peñaloza-Bojacá G. & Maciel-Silva A. subg. nov. (Figs 5, 6.4).

**Type species.** *Dendroceros cichoraceus* (Mont.) Gottsche.

**Diagnosis.** Gametophyte crispate, green to light-yellow when dried; wing cells oval to square-rectangular, without perforations (or rarely a few macro-perforations); wing margins crenulate; cells of the midrib square to rectangular; midrib solid; involucre cells with rough surfaces; capsule cells with rectangular wall thickenings; papillate pseudoelaters; multicellular spores, with more than 21 cells in total, distal surface with micro-ornamentations of granules and macro-ornamentations of pustulate papilla.

*Dendroceros cichoraceus* is newly identified as the sister to all other *Dendroceros* species (Duff *et al.*, 2007; Villarreal *et al.*, 2015; Villarreal & Renner, 2012; Fig. 1), supporting the inclusion of this new subgenus. The phylogenetic results and the morphological analyses dispel the confusion between this species and *D. crispus*. *Dendroceros cichoraceus* is differentiated from *D. crispus* by the lack of pores on the wings almost; having crenulate, margins and spores with macro-ornamentations of pustulate papilla (Howe, 1934; Proskauer, 1953; Stephani, 1917).

*Dendroceros cichoraceus* (Mont.) Gottsche 1858

**Bas.** *Anthoceros cichoraceus* Mont. 1845

**Type.** Chile australioria ad terram muscosam legit cl. Gay (PC 502; it is possible holotype, not seen).

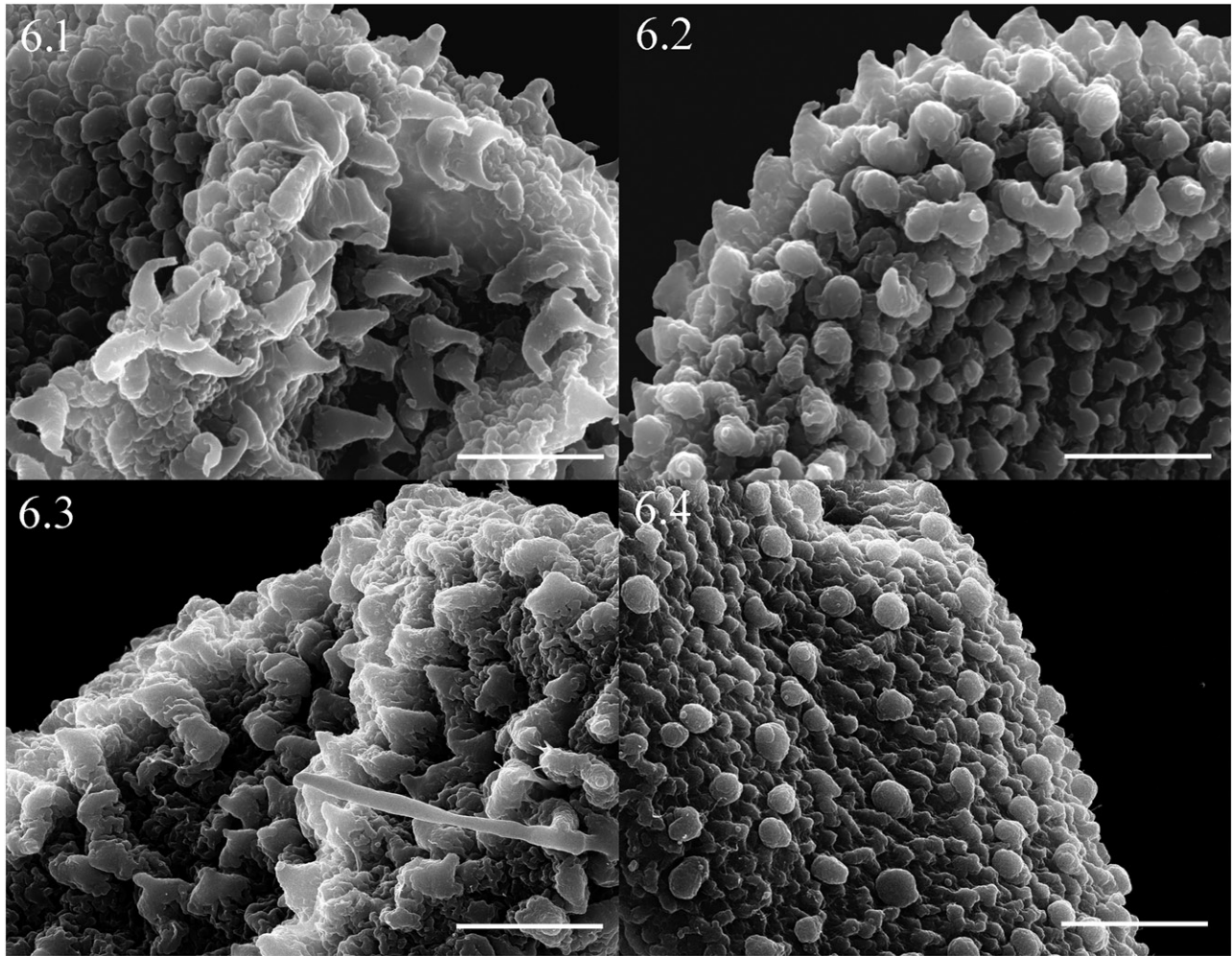
**Description.** p. 355 in Montagne (1845); p.1013 in Stephani (1917); p. 69 in Proskauer (1953).

**Geographic distribution.** Chile.

**Additional material examined.** CONC Larrain 42330, CONC Larrain 31162.

## Discussion

We revisited and partly corroborated Schuster's (1987) subgenera treatment and provided a novel assessment of character evolution in the genus *Dendroceros*. Species with schizogenous cavities in the midrib remain gathered into clade A, with a high phylogenetic support – subg. *Apoceros*; while species with a solid thallus were grouped into three distinct clades with an also good phylogenetic support: species with galeate gametophyte, wing perforations always present and capsule cells with



**Fig. 6.** Ornamentation of spores in species of *Dendroceros*. 6.1. *D. cucullatus* (subg. *Apoceros*); 6.2. *D. crassinervis* (subg. *Dendroceros*); 6.3. *D. crispatus* (subg. *Nodulosus*); 6.4. *D. cichoraceus* (subg. *Cichoraceus*). Scales: 6.1-6.4 = 5  $\mu\text{m}$ .

nodular thickened walls in clade B – subg. *Nodulosus*; gametophyte wings with pores and spore distal surface with macro-ornamentations as aculeate papilla in clade C – subg. *Dendroceros* and gametophyte wings with almost no pores and spore distal surface with macro-ornamentations as pustulate papilla in subg. *Cichoraceus*. We will highlight the main morphological characters of the genus in light of the phylogenetic results of our study.

### Gametophyte

*Dendroceros* gametophytes have different morphologies in the wings, as seen in subg. *Dendroceros* (undulate and crispate) and subg. *Cichoraceus* (only crispate), with a state shift to galeate wings in subg. *Apoceros* and subg. *Nodulosus*. Galeate wings are an apomorphy within the genus and a unique character for these two

subgenera. The presence of a multistratose midrib and wings have evolved independently in *Dendroceros* and few species of *Nothoceros* (e.g., *N. endiviifolius*, *N. superbus*; Villarreal, Menéndez, & Allen, 2007). The basic body plan of *Dendroceros* (and few species of *Nothoceros*), a midrib with monostromatic wings may be related to the adaptive response to the hydration and dehydration cycles present in an epiphytic environment (Schuette & Renzaglia, 2010; Wood, 2007).

Another character of the genus is the midrib anatomy, already recognized by Stephani (1909, 1917). The solid midrib, present in the subg. *Dendroceros*, subg. *Nodulosus*, and subg. *Cichoraceus*, is commonly found across the genus with a single transition to cavernous thallus (subg. *Apoceros*). Nevertheless, schizogenous cavities in the middle region of the thallus seem to be a homoplastic trait in hornworts, equally found in the family Anthocerotaceae and some gametophytes of

*Leiosporoceros dussii* (Steph.) Hässel (Renzaglia, 1978; Villarreal & Renzaglia, 2006b). Mucilage-filled cavities are ubiquitous in hornworts, especially in the sporophyte of species bearing stomata (Duckett & Pressel, 2018; Renzaglia, 1978). Mucilage-filled cavities harbour antheridia and nitrogen-fixing cyanobacteria (Renzaglia, Duff, Nickrent, & Garbary, 2000; Renzaglia *et al.*, 2009). At a functional level the mucilage-filled schizogenous cavities are thought to be related to desiccation tolerance in hornworts (Renzaglia *et al.*, 2000).

Pores (2–15 µm) are found at the junction of the cells in monostromatic wings of *Dendroceros*. These wing pores are commonly confused with trigones in the literature (Hasegawa, 1980; Infante, 2010; Piippo, 1993). Trigones are collenchymatous thickenings of the cell walls present at the junctions of three or more cells (Glime, 2017). In older *Dendroceros* specimens, the spaces between wing cells resemble cell wall thickenings. However, recently collected specimens clearly showed these spaces between cells (Fig. 3.2). Those pores still differ from the macro-perforations (40 to over 200 µm) seen on gametophyte wings in several tropical species (e.g., *D. crispatus* and *D. javanicus*; Chantanaorrapint *et al.*, 2014; Hasegawa, 1980, 1982; Infante, 2010). The development and function of pores and macro-perforations remain unknown.

### Capsules and spores

Epidermal cells in the *Dendroceros* capsule have a rectangular shape with different types of wall thickenings (nodular and rectangular), which have been used by different authors to separate species (Chantanaorrapint, 2014; Chantanaorrapint *et al.*, 2014; Garcia *et al.*, 2012; Hasegawa, 1980, 1982; Infante, 2010; Pagán, 1942; Stephani, 1909, 1917). In our study, this character was useful to distinguish the species of subg. *Nodulosus*, which have nodular thickenings in their cell walls; different from other subgenera with rectangular thickenings.

The thickening of the epidermal cells starts in the basal region of the capsule still covered by the involucre (Ligrone & Renzaglia, 1990; Renzaglia, 1978). During these early stages of development, epidermal cells do not show clear thickenings. The thickenings are easily observed as the sporophyte grows and emerges from the involucre (Renzaglia & Garbary, 2001; Renzaglia *et al.*, 2009). In *D. crispatus*, for example, the cells at the base of the capsule do not show nodular thickenings. Functions of these thickenings are not well defined, although we believe they can play a role in the capsule dehiscence, such as pseudoelateres with helical

thickenings and the spiral columella that facilitate spore separation and dispersal (Renzaglia *et al.*, 2009).

*Dendroceros* spores germinate while still inside the capsule, which results in a range of size and morphologies (Chantanaorrapint, 2014; Garcia *et al.*, 2012; Hasegawa, 1980; Schuette & Renzaglia, 2010). Measurements of spore length in our study agree with previous observations in literature, with a range of 45–135 µm for species in the subg. *Apoceros*, 40–112 µm in subg. *Nodulosus*, and 85 µm in the subg. *Dendroceros* and subg. *Cichoraceus* (Hasegawa, 1980, 1986; Infante, 2010; Piippo, 1993; Stephani, 1917). However, there is a considerable amount of variability in published records. For instance, in *D. pedunculatus*, there are records of large spores (e.g., 110–135 µm; Hasegawa, 1980; Stephani, 1917) or rather small spores (e.g., 70–83 µm; Piippo, 1993). We suspect the technique used to measure spores may generate this variation (Table 1).

Due to endospory there is considerable variation in the number of cells (5 to <20), which depends directly on the state of development of the capsule (Schuette & Renzaglia, 2010). Endospory is only found in all *Dendroceros* species and *Nothoceros renzagliensis* (Villarreal, Campos, Uribe, & Goffinet, 2012). It is plausible that endospory is an adaptation to environments under periodic desiccation (Schuette & Renzaglia, 2010; Wood, 2007). Endosporic germination is highly homoplastic in bryophytes, being reported from disparate orders such as *Pelliales*, *Metzgeriales*, *Marchantiales* (liverworts) and *Andreaeales*, *Bryales*, *Orthotrichales* (mosses, Frey & Stech, 2009; Schuette & Renzaglia, 2010; Villarreal *et al.*, 2012).

An important taxonomic feature across hornworts is the spore ornamentation. *Dendroceros* species have been typically described as having papillate spores and few studies have stressed the importance of this character in the genus. However, we found that micro-ornamentation is useful to define infrageneric taxa in *Dendroceros* (Fig. 6). For example, the spores of subg. *Apoceros* have wart-like micro-ornamentations (< 0.2 µm long), as well as macro-ornamentation of spines (1–3 µm long). In the subg. *Dendroceros*, the micro-ornamentation consists of granules and vermiculates, with the macro-ornamentation consisting of aculeate papilla 0.8–3.3 µm long, with a small bend at the apex. In the subg. *Nodulosus* the micro-ornamentation is similar to the species of the subg. *Dendroceros*, but the macro-ornamentation of aculeate papilla is smaller (0.9–2.2 µm long) with short, fine, and frequently straight apices. In these two subgenera the format of macro-ornamentations evolved from the ancestral state, pustulate papilla, present in subg. *Cichoraceus* and some species of

*Nothoceros* and *Megaceros* (Garcia et al., 2012; Villarreal, 2010; Villarreal et al., 2007).

The present study brings light to the subgenera relationships within the hornwort genus *Dendroceros*. Additionally, we emphasize the unique biological features of *Dendroceros* such as its epiphytic habitat, pyrenoid with protein incrustations and desiccation tolerance. Based on the results shown above, we present a new taxonomic treatment for the genus.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).


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## Supplemental data

Supplemental data for this article can be accessed here: <https://doi.org/10.1080/14772000.2019.1682080>.

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