

An Unusual New Rheophytic Species of the Liverwort Genus *Cephaloziella* from Panama

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Communicating Editor: James I. Cohen

Abstract—During bryophyte surveys in the Antón River watershed, Coclé Province, Panama, we discovered a minute rheophytic liverwort representing an undescribed species of Cephaloziellaceae. Phylogenetic analyses based on nuclear ITS1–2 and plastid *rbcL* DNA markers placed the new species within *Cephaloziella*, where it resolved in a clade with *C. microphylla*, *C. kiaeri*, and *C. recurvifolia* in the ITS tree, and with *C. microphylla* alone in the *rbcL* tree. We describe the species as *Cephaloziella minutiloba*. The new species is morphologically distinct by its minute size, flagelliform shoots, scattered violet rhizoids, succubous and highly unequally conduplicate leaves with a prominent ventral lobe and a strongly reduced dorsal lobe bent over the ventral lobe, and crenate-dentate leaf margins. The gynoecea on short ventral branches are made up of 2–3 series of highly connate, crenate bracts and a 5-plicate perianth with a fringed mouth. This new species expands our understanding of morphological diversity and rheophytic adaptation within *Cephaloziella*. The morphological affinities of the species are discussed, and the importance of integrative taxonomic approaches in liverwort systematics is emphasized.

Keywords—Central America, molecular systematics, rheophytes, tropical forests.

Bryophytes represent the second-most diverse lineage of the land plants and occupy a wide range of habitats, including aquatic environments, though being absent in marine ones (Gradstein et al. 2001; Glime 2024). Bryophytes in riparian habitats experience seasonal flooding and may complete their life cycle temporarily underwater, where they form diverse rheophytic (=rheophilous) communities (Shevock et al. 2017). The harsh conditions of seasonal flooding by river water currents experienced by rheophytic species are reflected in the evolution of distinct and specialized morphological traits. Rheophytic liverwort taxa often share robust stems, attached to the substrate by a creeping stoloniform shoot, and highly fertile, autoicous gametophytes (Gradstein et al. 2004, 2011). Owing to their specialized morphology, rheophytic liverworts are often placed in morphologically isolated genera, such as *Myriocolea* Spruce, *Schusterolejeunea* (Spruce) Grolle, and *Kymatocalyx* Herzog (Gradstein and Vána 1999; Gradstein et al. 2004; Sierra and Zartman 2023). However, some of these taxa have been shown to be nested within morphologically disparate genera, indicating convergent evolution in the rheophilous habit. Here, molecular phylogenetics has been instrumental in unraveling the taxonomic identities and evolutionary relationships of rheophytic bryophytes (e.g., Gradstein et al. 2011; Heinrichs et al. 2012; Sierra et al. 2018).

During recent bryophyte field sampling, an intriguing, tiny rheophytic liverwort, with leafy shoots less than half a millimeter wide, was discovered in the Antón river watershed, Coclé province, Panama. The new species seemed to be a member of Cephaloziellaceae s.s. (Crandall-Stotler et al. 2009; Gradstein 2021), because of its very small size, stems without hyalodermis, scattered rhizoids, succubous to subtransverse leaves not wider than the stem, lack of underleaves, and a sporophyte enclosed by a perianth. However, by its violet rhizoids and highly unequally conduplicate leaves consisting of a toothed ventral lobe (=leaf lamina) and a minute dorsal lobe bent over the ventral lobe, the plant did not match any of the members of this family described from tropical America (Gradstein et al. 2001; Flores et al. 2017; Gradstein 2021).

Because of its puzzling morphology, we decided to use DNA markers to explore its systematic affinity. Sequencing

of the nuclear and plastid genomic regions of Cephaloziellaceae species has given insights into the intricate phylogenetic relationships within the family (Gradstein et al. 2014; Mamontov and Vilnet 2017; Bakalin et al. 2024). By integrating molecular phylogeny and morphological analyses, we aimed to determine the taxonomic identity of this unusual rheophytic taxon from Panama. We applied a species concept based on monophyly and unique morphological traits (De Queiroz 2007). Our integrative approach provided evidence for the divergence of a novel taxonomic lineage, which is here formally described and illustrated. We also suggest future research directions for Cephaloziellaceae.

MATERIALS AND METHODS

Field Sampling—Field surveys resulting in the sampling of the new species were carried out in 1997, 2018, and 2023. The bryophyte surveys included inventorying of soil microhabitats along the Río Antón near the waterfall “El Chorro de Las Mozas”, a tourist locality near the town of El Valle de Antón, Coclé province, about 120 km away from Panama City (Figs. 1, 2). Geomorphologically, El Valle de Antón is situated within the caldera of an extinct volcano, at an elevation of 600 m.

Molecular Taxon Sampling—To address the phylogenetic position of the new species, we included 86 accessions, which included members of the family Cephaloziellaceae s.s. (Crandall-Stotler et al. 2009; Gradstein 2021), as well as species of the genera *Anastrophyllum* (Spruce) Steph., *Chaetophyllopsis* R.M.Schust., *Gottschelia* Grolle, *Nothogymnomitrium* R.M.Schust., *Obtusifolium* S.W.Arnell, *Scapania* (Dumort.) Dumort., and *Sphenolobus* (Lindb.) Berggr., as outgroups (Bakalin et al. 2024; Table 1). A total of 87 sequences were downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). All newly generated DNA sequences are deposited in GenBank. Species names and GenBank accession numbers for all sequences are listed in Table 1.

DNA Extraction and Sequence Assembly—Total plant genomic DNA was extracted from tissue of six pulverized leafy shoots of the new species using a cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle 1987). Polymerase chain reaction (PCR) was carried out to amplify two molecular markers: the plastid region *rbcL* with the primers *rbcL*-210-F and *rbcL*-1200-R (Gradstein et al. 2006) and the nuclear ribosomal internal transcribed spacer (ITS1–2) region nrITS1–5.8S–ITS2, with the primers Bryo18SF and Bryo26SR (Hartmann et al. 2006). Sequencing primers were the same as those used for the PCR reactions.

Sequences were edited and aligned with Geneious Prime version 2024.0.4 (<https://www.geneious.com>). Alignments were performed automatically using the default settings (65% similarity, gap open penalty = 12, gap extension penalty = 3, refinement iterations = 2) and were later

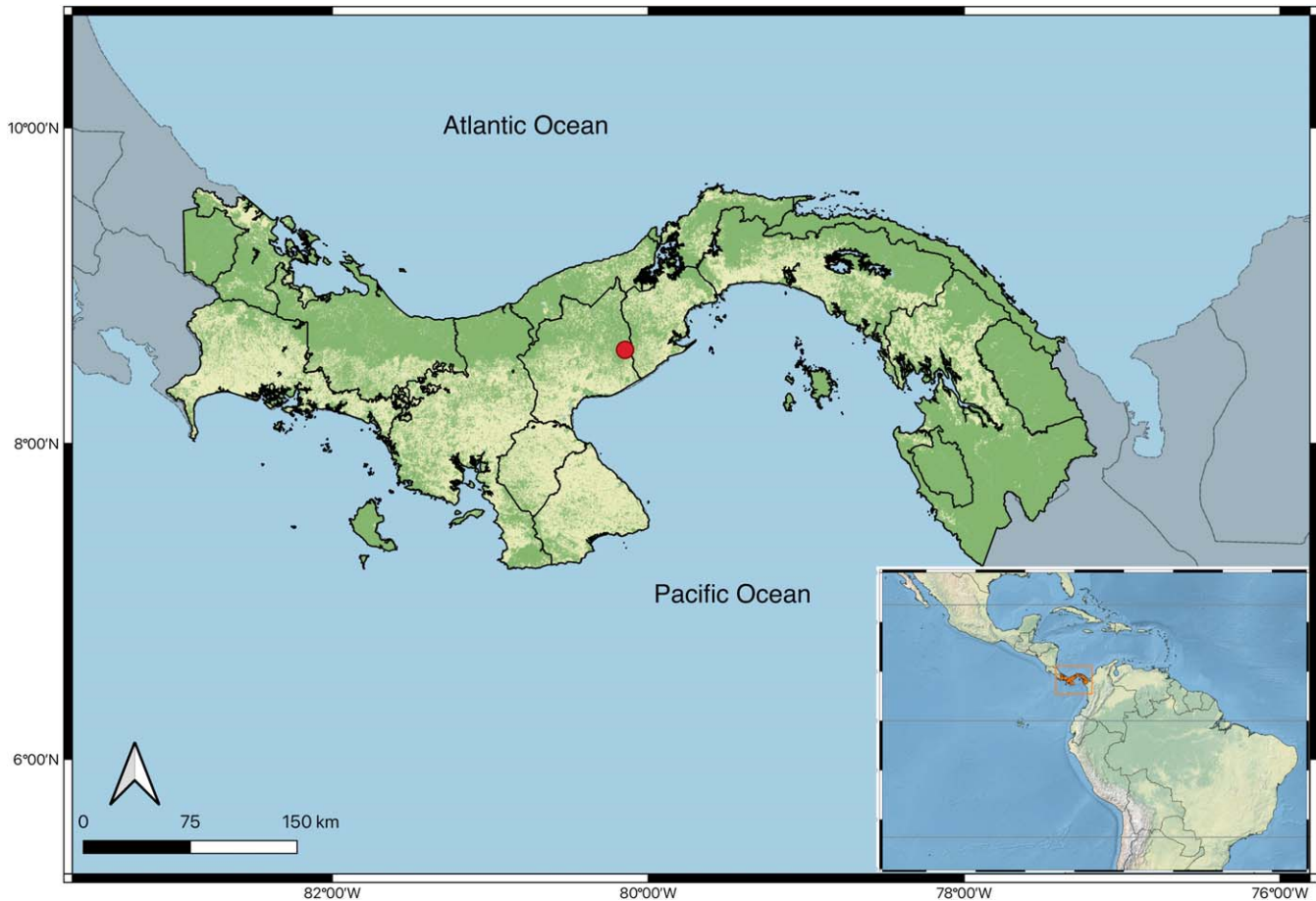


FIG. 1. The locality of *Cephaloziella minutiloba* in El Valle de Antón, Coclé, Panama (red dot).

checked manually. The alignment of 58 sequences of the nuclear ITS region comprises 1251 base pairs, of which 549 were parsimony informative. The chloroplast sequences for *rbcL* (48 sequences) comprised 1514 base pairs with 211 parsimony-informative sites. All sequence alignments and phylogenies have been deposited in the Dryad Digital Repository (Sierra et al. 2025).

Phylogenetic Analyses—Gene trees were inferred in IQ-tree using 1000 ultrafast bootstrap replicates, a maximum likelihood inference method with specific evolutionary models for each gene (ITS: GTR+F+G4; and *rbcL*: TIM+F+G4) using ModelFinder (Minh et al. 2013; Kalyaanamoorthy et al. 2017). The phylogenetic tree with ML result was manipulated and visualized using the packages *ape* and *phytools* (Zhang 2017; Paradis and Schliep 2019) in the R-software (R Core team 2024). Bootstrap values ≥ 70 were regarded as moderate and ≥ 85 as good support for the new species (Erixon et al. 2003). Bayesian inference was performed on the software MrBayes v. 3.2.6 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Two parallel Markov chain Monte Carlo (MCMC) runs adding up to ten million generations were conducted, with each run containing four chains with default priors on most parameters. Trees and estimated parameter values were sampled every 1000 generations, thus obtaining a total of 10,000 samples from which the first 1000 (10%) were discarded as burn-in. Convergence of runs was verified using Tracer 1.6 (Rambaut et al. 2014). A majority-rule consensus tree was computed to calculate the Bayesian posterior probability (BPP), in which values of BPP ≥ 0.95 were considered significant.

RESULTS

Molecular Phylogenetics—Phylogenetic analyses based on individual loci ITS1–2 and *rbcL* are presented in Figs. 3A, B. In both the ITS and *rbcL* maximum likelihood trees, the new species appears closely related to *Cephaloziella microphylla* and two further *Cephaloziella* species, *C. kiaeri* and *C. recurvifolia*,

which are frequently associated with *Cylindrocolea*. In the ITS tree (Fig. 3A), the new species is resolved in the *Cephaloziella* lineage (UFBoot = 93; BPP = 0.95), in a group with *Cephaloziella kiaeri*, *C. microphylla*, and *C. recurvifolia*, sister to a clade with the species *C. konstantinovae*, *C. spinicaulis*, *C. rubella*, and *C. elachista*. Within the subclade, the new species has weak bootstrap support (UFBoot < 50), yet Bayesian posterior probability is supported (BPP = 0.99). In the *rbcL* tree (Fig. 3B), it forms a weakly supported lineage with just *C. microphylla* (UFBoot = 69; BPP = 0.84), between a *Cephaloziella kiaeri*–*C. recurvifolia*–*Cephaloziopsis intertexta* clade (UFBoot = 61; BPP = 0.87).

TAXONOMIC TREATMENT

Cephaloziella minutiloba A.M. Sierra, Gradst. & Gudiño, sp. nov. TYPE: PANAMA, Prov. Coclé, El Valle de Antón, Parque Acuático Chorro Las Mozas, Río Antón, 8°35'33.4"N, 80°08'49.5"W, 607 m, sobre ladera rocosa a orillas del río, 4 December 2018, J. Gudiño-L. 3504, c.andr. & gyn. (Holotype: PMA!; isotypes: QFA!, SCZ!).

Diagnosis—Plants rheophytic, minute, leafy shoots ca. 0.5 cm long, 0.2–0.45 mm wide, often flagelliform, basal portion of main shoot stoloniform; stems of 50–60 rows of cells, epidermis cells smaller than medullary cells, all stem cells thin-walled; branching ventral-intercalary; rhizoids violet, scattered; leaves distant, succubous, not wider than the stem,



FIG. 2. The collecting site of *Cephaloziella minutiloba* along the Antón River near Las Mozas waterfall, El Valle de Antón, Panama, showing the river and its banks, a small waterfall, and surrounding forest vegetation.

leaf insertion not reaching the dorsal midline of stem, nearly transversely inserted and highly unequally conduplicate with a well-developed ventral lobe and a strongly reduced dorsal lobe, ventral lobe ovate-orbicular to ovate-triangular, margins obtusely crenate-dentate; leaf cells $15\text{--}20\text{--}(25) \times 12\text{--}16 \mu\text{m}$ in midleaf, thin-walled, without trigones; underleaves absent; androecia terminal or intercalary on main shoots; gynoecia on short-specialized ventral branches, with 2–3 series of bracts with crenate margins, inner bracts highly connate, inner bracteole reduced, perianth campanulate, 5-plicate, about as long as the inner bracts, mouth crenate to short-ciliate (Figs. 4, 5).

Plants autoicous, green, minute, leafy shoots ca. 0.5 cm long, and $(0.2\text{--})0.3\text{--}0.45$ mm wide, often flagelliform, basal portion of the main shoot stoloniform; branching sparse, ventral-intercalary (Fig. 4). **Stems** green, slightly zig-zag, $0.1\text{--}0.14$ mm in diameter, as wide as or wider than leaves, without hyalodermis, dorsal and ventral epidermal cells $10\text{--}20\text{--}(25) \times 8\text{--}12 \mu\text{m}$ in surface view, dorsal and ventral surface ca. 10–12 cells across; stem in cross section oval, slightly dorso-ventrally flattened, $1.5\times$ wider than high, made up of 50–60 rows of cells including 25–30 subquadrate epidermal cells and a similar number of larger medullary cells, epidermal cells $8\text{--}12 \mu\text{m}$ high, medullary cells $20\text{--}25 \mu\text{m}$ in diameter, all stem cells thin-walled. **Rhizoids** violet, sparse and scattered, frequently absent, where present sometimes forming small, dense mats, rhizoid tips often branched (Fig. 4). **Leaves** succubous, highly distant on main stems, more closely set and \pm contiguous on short branches and the apices of main shoots, nearly transversely inserted and highly unequally conduplicate with a well-developed ventral lobe and a strongly reduced dorsal lobe bent over the dorsal lobe, leaf insertion not reaching the dorsal midline of stem, leaving a (1)–5–8-cell wide leaf-free strip; ventral lobe (=leaf lamina) broadly ovate-orbicular to ovate-triangular, $0.07\text{--}0.15$ mm long, $0.1\text{--}0.12$ mm wide, sometimes smaller, flat, usually gradually narrowed to apex in the upper half, apex rounded to subacute, margins crenate to toothed by conically projecting cells, teeth up to ten per leaf, 1–2 cells long, obtuse, becoming smaller towards the apex (Figs. 5C, D); dorsal lobe very small, limited to the base of the ventral lobe, exceptionally larger and up to $1/2$ the length of the ventral lobe

(Fig. 5A), absent or rudimentary on flagelliform shoots (Figs. 5B, C, D), broadly triangular, to $50 \mu\text{m}$ long and $30 \mu\text{m}$ wide, bent over the ventral lobe, sometimes standing upwards, transversely inserted on the stem across 3–5 cells and attached to the leaf lobe by a 2–4-cells long keel, apex obtuse, margins irregularly crenulate to obtusely toothed, dorsal lobe on leaves of flagelliform shoots absent or rudimentary (Fig. 5A, B), on leaves near the apex of branches; cells of the ventral lobe thin-walled, smooth, irregularly quadrate-hexagonal to subrectangular, $15\text{--}20\text{--}(25) \times 12\text{--}16 \mu\text{m}$ in midleaf and near the base, at the margins smaller, ca. $7\text{--}12 \mu\text{m}$; cells of the dorsal lobe as in the ventral lobe but smaller; oil bodies degenerated, when present 1–3 per cell, orbicular to oblong, faintly granular. **Underleaves** absent. **Androecia** terminal to intercalary on long shoots, bracts in ca. 6–20 pairs, as large as vegetative leaves but much more densely positioned, imbricate, convex, unequally bilobed, dorsal leaf lobe about $1/2\text{--}2/3$ the size of ventral lobe, antheridia 1 per bract. **Gynoecia** on short-specialized branches (Fig. 5D), with 2–3 series of quadrate to ligulate bracts and bracteoles, inner bracts highly connate (Fig. 5F), ca. $0.25\text{--}0.3$ mm long, subequally bilobed, apex of the two lobes broadly rounded and irregularly crenate by conically projecting cells, inner bracteole inserted between the bracts, reduced; perianth 5-plicate, about as long as the inner bracts, campanulate, mouth very wide, crenate to shortly ciliate. **Sporophyte** immature. **Vegetative reproduction** not observed.

Etymology—The epithet refers to the dorsal lobe of the leaves, being usually reduced to a minute appendage at the dorsal base of the ventral lobe.

Distribution and Habitat—*Cephaloziella minutiloba* is hitherto only known from multiple specimens collected on the banks of the Antón River (Fig. 2) near the waterfall “El Chorro Las Mozas”, Province of Coclé, Panama. It was found on moist, shaded soil near the swiftly flowing waters of the river, growing deeply interwoven with the substrate, with branches over the substrate and associated with co-occurring bryophyte species, *Cyathodium cavernarum* Kunze, *Philonotis uncinata* (Schwägr.) Brid, *Fissidens flaccidus* Mitt., *Lejeunea laeta* (Lehm. & Lindenb.) Lehm. & Lindenb., and *Mittenothamnium scalpellifolium* (Müll. Hal.) H.A.Crum. The substrate was rich in filamentous algae (likely cyanobacteria). In addition,

TABLE 1. Accession sampling with species names and country of the specimens' sequences for the two genetic markers, ITS1-2 and *rbcL*, (Genbank accession number) used in this study. Bold font indicates DNA sequences generated for this study.

Species name	Country	ITS1-2	<i>rbcL</i>
<i>Cephaloziella minutiloba</i> A.M.Sierra, Gradst. & Gudiño	Panama	PX049169	PV999890
<i>Cephalomitron aterrimum</i> (Steph.) R.M.Schust.	New Zealand		KF852368
<i>Cephaloziella konstantinovae</i> Mamontov & Vilnet	Russia	KF471666	
<i>Cephaloziella crispata</i> N.Kitag.	Northwest Vietnam		OP205308
<i>Cephaloziella divaricata</i> (Sm.) Schiffn.	Russia	JX629921	
<i>Cephaloziella divaricata</i> (Sm.) Schiffn.	Czech Republic		KF852399
<i>Cephaloziella divaricata</i> (Sm.) Schiffn.	Russia	KF805881	
<i>Cephaloziella divaricata</i> (Sm.) Schiffn.	Germany		DQ312481
<i>Cephaloziella divaricata</i> (Sm.) Schiffn.	Russia	OP082396	OP205309
<i>Cephaloziella elachista</i> (J.B.Jack ex Gottsche & Rabenh.) Schiffn.	Russia	JX629918	
<i>Cephaloziella granatensis</i> (J.B.Jack ex Steph.) Fulford	Panama		KC184715
<i>Cephaloziella hirta</i> (Steph.) R.M.Schust.	Australia		DQ439682
<i>Cephaloziella kiaeri</i> (Austin) Douin	Korea	KF805887	
<i>Cephaloziella kiaeri</i> (Austin) Douin	Japan	KF805886	
<i>Cephaloziella kiaeri</i> (Austin) Douin	Borneo	KF805888	
<i>Cephaloziella kiaeri</i> (Austin) Douin	South Korea	OP082397	
<i>Cephaloziella microphylla</i> (Steph.) Douin	Korea	KF805889	
<i>Cephaloziella microphylla</i> (Steph.) Douin	Korea	KF805890	
<i>Cephaloziella microphylla</i> (Steph.) Douin	Thailand		KC184716
<i>Cephaloziella polystratosa</i> (R.M.Schust. & Damsh.) Konstant.	Russia	KF471665	
<i>Cephaloziella rubella</i> (Nees) Warnst.	Russia	KF805885	
<i>Cephaloziella rubella</i> (Nees) Warnst.	Russia	KF853559	
<i>Cephaloziella elachista</i> (J.B.Jack ex Gottsche & Rabenh.) Schiffn.	Russia	JX629919	
<i>Cephaloziella</i> sp.	Russia	JX629920	
<i>Cephaloziella konstantinovae</i> Mamontov & Vilnet	Russia	KF805884	
<i>Cephaloziella</i> sp.	Russia	KF805895	
<i>Cephaloziella</i> sp.	China	OP082398	
<i>Cephaloziella spinicaulis</i> Douin	Japan	JX629922	
<i>Cephaloziella spinicaulis</i> Douin	Russia	KF805896	
<i>Cephaloziella spinicaulis</i> Douin	Japan		AB476561
<i>Cephaloziella spinicaulis</i> Douin	Japan		KC184717
<i>Cephaloziella stellulifera</i> (Taylor ex Carrington & Pearson) Croz.	Netherlands	JX629923	
<i>Cephaloziella stellulifera</i> (Taylor ex Carrington & Pearson) Croz.	USA		KC184718
<i>Cephaloziella spinigera</i> (Lindb.) Warnst.	Russia	KF805865	
<i>Cephaloziella spinigera</i> (Lindb.) Warnst.	Russia	KF805864	
<i>Cephaloziella spinigera</i> (Lindb.) Warnst.	Russia	KF805866	
<i>Cephaloziella tahora</i> Bever. & Glenn	New Zealand		KT705315
<i>Cephaloziella turneri</i> (Hook.) Müll.Frib.	Russia	KF805892	
<i>Cephaloziella turneri</i> (Hook.) Müll.Frib.	Russia	KF805893	
<i>Cephaloziella turneri</i> (Hook.) Müll.Frib.	Russia	KF805894	
<i>Cephaloziella turneri</i> (Hook.) Müll.Frib.	USA		KC184719
<i>Cephaloziella varians</i> (Gottsche) Steph.	Russia	KF805876, KF805862	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Russia	KF805867	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Russia	KF805868	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Russia	KF805869	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Russia	KF805870	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Russia	KF805871, KF805861	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Russia	KF805872	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Russia	KF805875	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Norway	KF805877, KF805863	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Norway	KF805878	
<i>Cephaloziella varians</i> (Gottsche) Steph.	Norway	KF805880	
<i>Cephaloziella varians</i> (Gottsche) Steph.	New Zealand		DQ439689
<i>Cephaloziella kiaeri</i> (Austin) Douin	Northeast Vietnam		OP205311
<i>Cephaloziella kiaeri</i> (Austin) Douin	North Vietnam		OP205310
<i>Cephaloziella recurvifolia</i> (Steph.) S.Hatt.	Japan	KF805891	
<i>Cephaloziella recurvifolia</i> (Steph.) S.Hatt.	Japan		AM392306
<i>Cephaloziella recurvifolia</i> (Steph.) S.Hatt.	Japan		KF852297
<i>Cephaloziella recurvifolia</i> (Steph.) S.Hatt.	Japan		AB476564
<i>Cephaloziella recurvifolia</i> (Steph.) S.Hatt.	Japan		KC184722
<i>Cephaloziella recurvifolia</i> (Steph.) S.Hatt.	South Korea		OP205312
<i>Cephaloziopsis intertexta</i> (Gottsche) R.M.Schust.	Bolivia		KC184720
<i>Kymatocalyx dominicensis</i> (Spruce) Vána	Guadeloupe		KC184726
<i>Metacephalozia crispata</i> Bakalin, Maltseva & Troitzk.	Northeast Vietnam	OP082399	OP205307
Outgroup			
<i>Anastrophyllum assimile</i> (Mitt.) Steph.	Korea	KF836652	
<i>Anastrophyllum ellipticum</i> Inoue	Russia	KF836654, KF836632	
<i>Anastrophyllum michauxii</i> (F.Weber) H.Buch	USA		AY507390
<i>Chaetophyllopsis whiteleggei</i> (Carrington & Pearson) R.M.Schust. ex Hamlin	Australia		AY462292, KF852333
<i>Gottschelia schizopleura</i> (Spruce) Grolle	Malaysia		KF852362

(Continued)

TABLE 1. (CONTINUED).

Species name	Country	ITS1-2	rbcL
<i>Gottschelia schizopleura</i> (Spruce) Grolle	Reunion		FJ984938
<i>Gottschelia schizopleura</i> (Spruce) Grolle	Reunion		FJ984939
<i>Herzogobryum vermiculare</i> (Schiffn.) Grolle	French Southern and Antarctic Lands		KF943587
<i>Nothogymnomitrium erosum</i> (Carrington & Pearson) R.M.Schust.	Australia	GQ900006	GQ900318
<i>Obtusifolium obtusum</i> (Lindb.) S.W.Arnell	Russia	DQ875118	
<i>Obtusifolium obtusum</i> (Lindb.) S.W.Arnell	Russia		KF852303
<i>Obtusifolium obtusum</i> (Lindb.) S.W.Arnell	Russia	OP082400	OP205313
<i>Oleolophozia perssonii</i> (H.Buch & S.W.Arnell) L.Söderstr.	Russia	MT504417	
<i>Oleolophozia perssonii</i> (H.Buch & S.W.Arnell) L.Söderstr.	Russia	MT504418	
<i>Scapania nimbosea</i> Taylor	United Kingdom		KF852408
<i>Scapania undulata</i> (L.) Dumort.	Finland		AY149840
<i>Scapania undulata</i> (L.) Dumort.	USA	JN631489	
<i>Scapania undulata</i> (L.) Dumort.	Portugal	JN631487	
<i>Sphenolobus minutus</i> (Schreb. ex D. Crantz) Berggr.	Russia	MT422255	
<i>Sphenolobus minutus</i> (Schreb. ex D. Crantz) Berggr.	Svalbard	EU791789	
<i>Sphenolobus minutus</i> (Schreb. ex D. Crantz) Berggr.	Norway		DQ312475

tubers of *Cyathodium spruceanum* Prosk. were observed, although no thalli of this species were seen. The frequent association of the new species with *Cyathodium cavernarum*, a characteristic bryophyte species of eroded riverbanks and creeks, and also found in caves in Panama and neighboring countries in Central America (Salazar Allen 2005), suggests that the new species is more widespread and has been overlooked.

Additional Specimens Examined—Panama, Prov. Coclé, El Valle de Antón, Parque Acuático Chorro Las Mozas, Río Antón, 8°35'34.9"N, 80°08'48.5"W, 631 m, 4 Dec 2018, N. Salazar-A. 21695, ster. (Paratype: PMA!); *ibid.*, sobre ladera, 30 Jul 1997, N. Salazar-A. 16263, ster. (PMA!); *ibid.*, along rocky trail on the gently sloping margin of the river near the picnic area, creeping in a mat of *Lejeunea laeta* (Lehm. & Lindenb.) Lehm. & Lindenb. and *Cyathodium cavernarum* Kunze on moist soil over rock, 14 Nov 2023, S. R. Gradstein s.n., c.andr. (PMA!).

DISCUSSION

The molecular-phylogenetic results confirmed that the new species is a distinctive new member of the genus *Cephaloziella*, with a distinct combination of morphological characters supporting its recognition as a new species. According to Dauphin et al. (2025), four species of *Cephaloziella* occur in Central America: *C. divaricata* (Sm.) Schiffn., *C. fragillima* (Spruce) Fulford, *C. hampeana* (Nees) Schiffn. and *C. subtilis* (Lindenb. & Gottsche) Steph. Of these species, only *C. fragillima* has been reported for Panama (Bocas del Toro, Chiriquí, and the Naso Tjër Di comarca), commonly under the name *C. granatensis* (Steph.) Fulford, which is a synonym of *C. fragillima* (Gradstein 2021). The species described here is very different from the known *Cephaloziella* taxa from Central America, and it does not match any other member of the genus recorded from tropical and southern South America (Fulford 1976).

The most outstanding and quite unusual morphological features that characterize the new species are the strongly unequally conduplicate leaves with a well-developed ventral lobe and a very poorly developed dorsal lobe, being reduced to a minute appendage at the dorsal base of the ventral lobe. Indeed, our first impression of the plant was that it had undivided leaves. However, near the tips of the main shoots or distally swollen lateral branches, the dorsal lobe may be more distinct and exceptionally even reach up to half the length of the ventral lobe (Fig. 5A); on flagelliform portions of the shoots, in contrast, the dorsal leaf lobe is completely absent

(Fig. 5B, C). The leaves are almost transversely inserted on the stem, and the insertion line ends well before the dorsal midline of the shoots, leaving a dorsal leaf-free strip. The width of this strip depends on the size of the dorsal leaf lobe and is very wide (5–8 cells) on stem portions where the dorsal leaf lobe is absent or minute. Where the dorsal leaf lobe is rather well developed, however, the strip is much narrower (Fig. 5A).

Another unusual feature of the new species is the violet rhizoids. Violet rhizoids are common in the thalloid Fossombroinales but are very rare among leafy liverworts, where they are known from a few Asiatic species of *Solenostoma* Mitt. (Solenostomataceae) and the rare *Lethocolea naruto-toganensis* Furuki from Japan (Acrobolbaceae). We are not aware of any other member of Cephaloziellaceae with violet rhizoids.

The stems of *Cephaloziella minutiloba* are relatively thick, consisting of 50–60 rows of cells, and the shoots are often flagelliform, and these might be interpreted as adaptations to the rheophytic habitat (e.g., Vitt and Glime 1984, Gradstein et al. 2011). Indeed, stems of numerous rows of cells are also seen in the genus *Kymatocalyx*, another rheophytic member of Cephaloziellaceae. In *Kymatocalyx*, however, the stems are differentiated in a thick-walled cortex, 1–3 cell rows across, surrounding a thin-walled medulla (Gradstein and Vána 1999). In the new species, in contrast, all stem cells are thin-walled, and a thick-walled cortex is not developed.

The molecular-phylogenetic results indicate that the new species is most closely related to *Cephaloziella microphylla* (Steph.) Douin, yet with low support (Fig. 3). The latter species is widespread in Eastern Asia and shows several important similarities to *C. minutiloba*, such as the ventral-intercalary branches, the thin-walled cells of stems and leaves, the frequently unequally bifid leaves, the crenate-dentate leaf margins, plicate perianth, and the highly connate female bracts and bracteole (Kitagawa 1969). The size of the leaf cells is also rather similar in the two species. The gynoecea in *C. microphylla* can be positioned at the tip of the main shoots or on short ventral branches.

The degree of asymmetry of the two leaf lobes in *C. microphylla*, with the ventral lobe being larger than the dorsal lobe, seems to be rather variable in *C. microphylla*, being particularly distinct in plants described as *C. inaequiloba* Douin. The latter species was reduced to a synonym of *C. microphylla* by Kitagawa (1969). The reduction of the dorsal leaf lobe is much less pronounced in *C. microphylla* than in *C. minutiloba*;

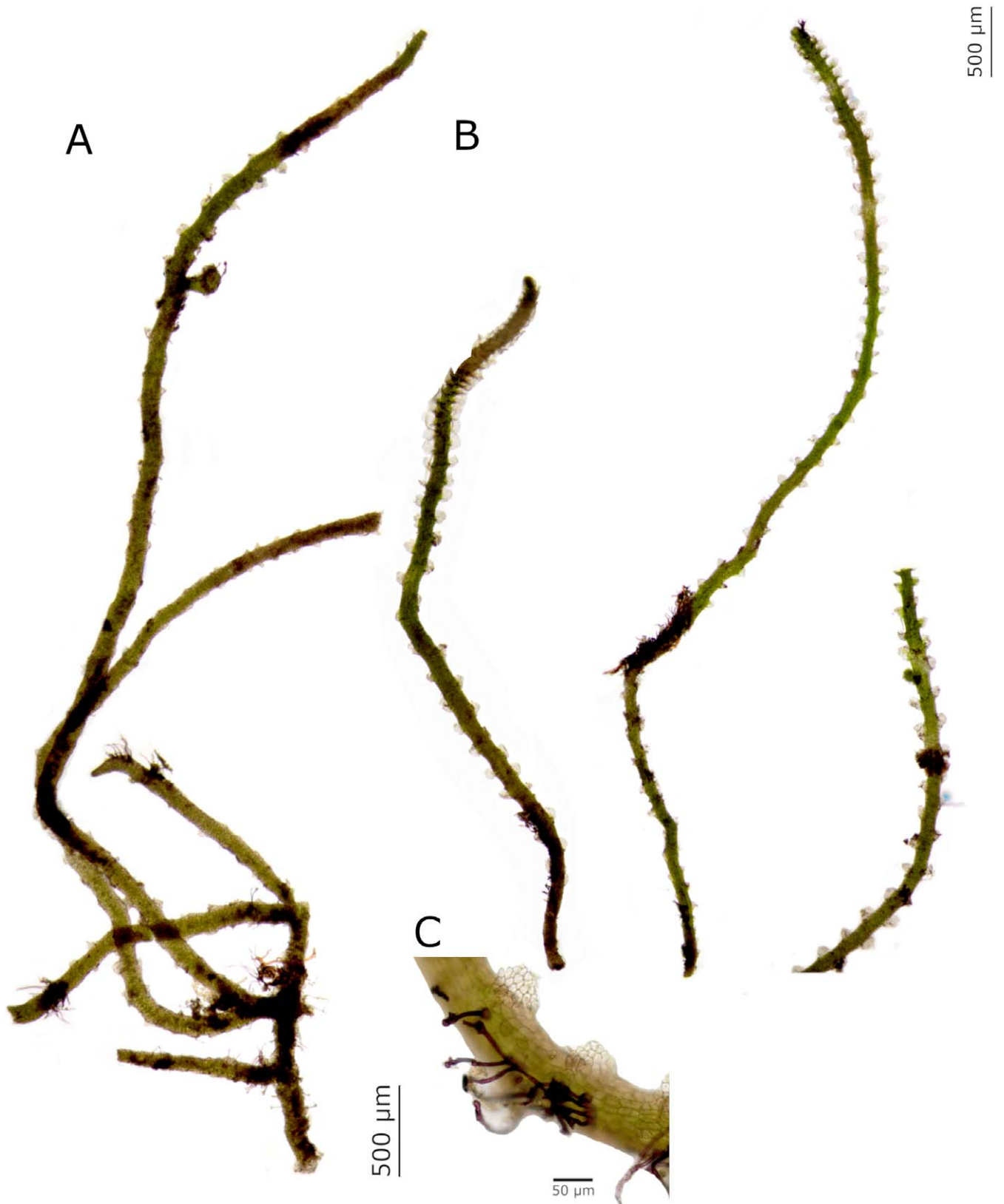


FIG. 4. Habit of *Cephaloziella minutiloba*. A. Ramifications departing from the stoloniform shoot base. B. Upper portion of branches showing variation in leaf lobe shape and size. C. Shoot with purple rhizoids. Photographs taken with a light microscope from the Holotype.

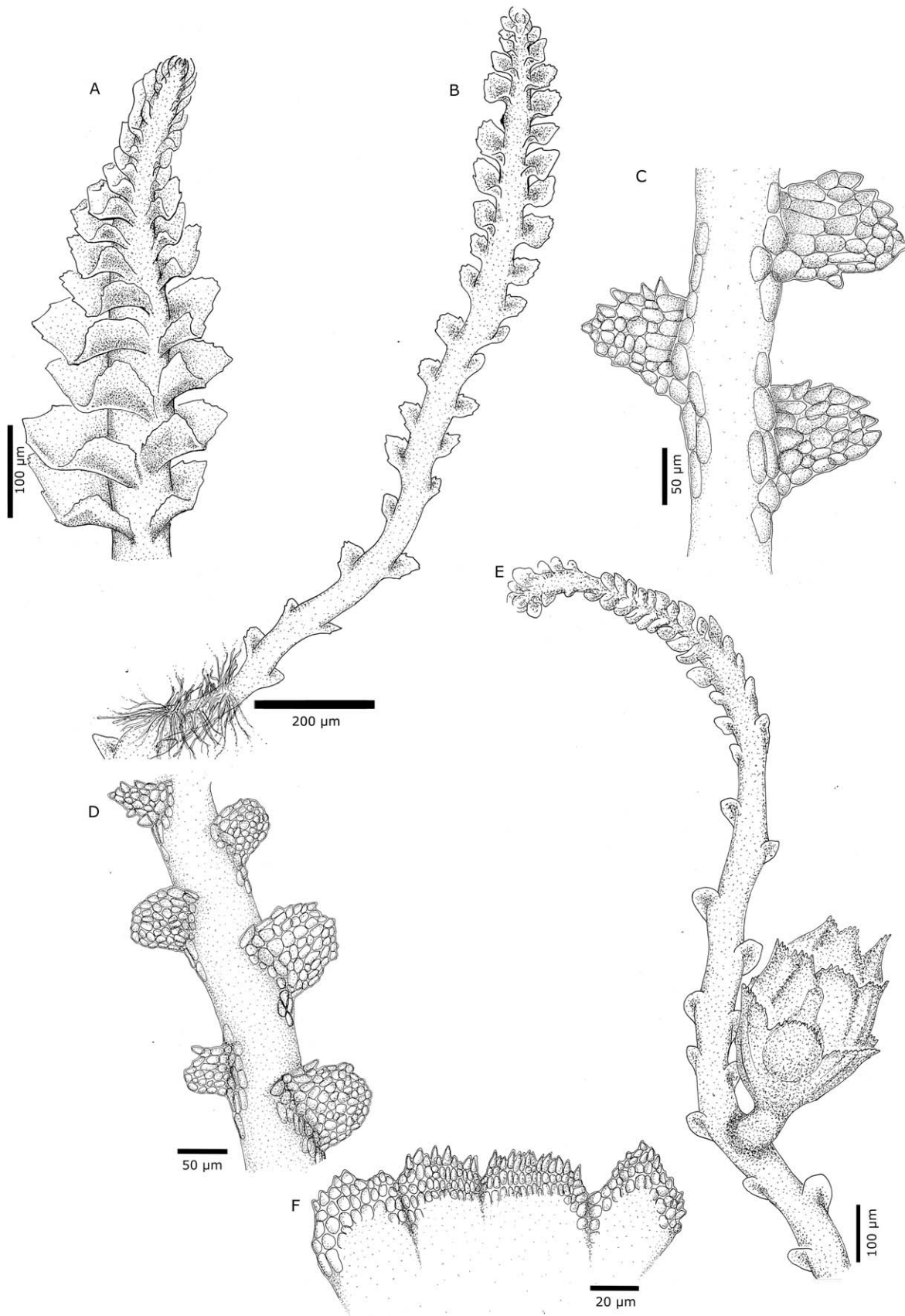


FIG. 5. Morphological features of *Cephaloziella minutiloba*. A–C. Shoots in dorsal view. A. Branch apex with unusually large dorsal leaf lobes, to 1/2 of ventral lobe length. B. Flagelliform shoot in dorsal view, showing absence of dorsal leaf lobes. C. Shoot with three leaves in dorsal view. D–E. Shoots in ventral view. D. Flagelliform shoot in ventral view. E. Fertile shoot in ventral view showing androecia and a gynoecium. F. Highly connate gynoecial bracts. Illustration from the holotype.

moreover, the leaves of *C. microphylla* are not conduplicate, with the dorsal lobe being bent over the ventral one. In addition, *C. microphylla* clearly differs from *C. minutiloba* in the colorless rhizoids, mammillose leaf cells projecting on the dorsal leaf surface, and the frequent presence of underleaves (Inoue 1976; Mamontov and Vilnet 2017).

Unequally conduplicate leaves also occur in the recently described *C. konstantinovae* Mamontov and Vilnet from Eastern Asia (Mamontov and Vilnet 2017). However, the dorsal leaf lobes in *C. konstantinovae* are less reduced than in *C. minutiloba*, to maximally half the size of the ventral leaf lobes, like in *C. microphylla*.

A dorsal leaf-free strip on the stem, seen in the new species, has not been reported for *Cephaloziella microphylla*. We examined herbarium material of the species from Japan (Honsu, Shizuoka Pref., Tagata, 8 Oct 1980, leg. et det. *M. Mizutani s.n.*, GOET [ex hb. NICH179197]; Kiushu, Miyazaki Pref., Nichinan, Inotani valley, 17 May 1977, *Iwatsuki & Mizutani s.n.*, *Hepaticae Japonicae Exsiccatae 1006*, GOET) and found that the leaves were inserted up to the dorsal midline of the stem; a leaf-free strip was absent. The presence of a leaf-free strip in *C. minutiloba* and its absence in *C. microphylla* is intriguing since its presence is usually considered a diagnostic feature of the genus *Cylindrocolea* R.M.Schust., separating the latter genus from *Cephaloziella*. In the molecular-phylogenetic analyses, *C. minutiloba* and *C. microphylla* were resolved near *Cephalozella kiaeri* and *Cephaloziella recurvifolia*, both of which possess a dorsal leaf-free strip and are therefore sometimes placed in *Cylindrocolea* (e.g., Vána et al. 2013; Bakalin et al. 2024). This taxonomic placement is controversial, however; other characters of the two species, such as very small leaf cells and the presence of gemmae, are characteristic features of *Cephaloziella*. The species are therefore placed here in *Cephaloziella* (Fig. 3; Table 1), following Mamontov and Vilnet (2017), Gradstein and Lee (2021), and earlier authors.

Cylindrocolea is a tropical genus of 18 species (Söderström et al. 2016). Only three species (including the two mentioned above, here placed in *Cephaloziella*) have thus far been sequenced; more than 80% of the species of *Cylindrocolea* (and of Cephaloziellaceae as a whole), including the type of *Cylindrocolea*, have not been studied by means of molecular phylogenetic methods (Gradstein et al. 2014; Bakalin et al. 2024). The generic status of *Cylindrocolea* thus remains unresolved despite claims of Bakalin et al. (2024) to the contrary, and the dorsal leaf-free strip seems to be an unstable generic feature in this group. Furthermore, the molecular markers used to date are poorly informative to draw solid phylogenetic inferences and generic classification of the family. A detailed molecular-phylogenetic study of *Cylindrocolea*, including a wide, representative sampling of the species of the genus and using informative regions of the plastid or nuclear genomes, is recommended.

Overall, liverwort systematics remains a challenging field. From extant species to fossil taxa, homoplastic characters and highly variable morphologies continue to obscure generic boundaries. These issues are particularly pronounced in small, morphologically reduced liverworts such as those in the family Cephaloziellaceae, where few diagnostic characters are available (Feldberg et al. 2013). Although molecular-phylogenetics has greatly improved our understanding of the backbone phylogeny of major bryophyte orders and families, resolving relationships at lower taxonomic levels remains difficult (Bechteler et al. 2023; Li et al. 2024). To address these

limitations, integrative approaches, wide-genome datasets (including complete plastome and nuclear genome), detailed morphological studies, and advanced phylogenomic tools will be highly essential for reconstructing liverwort systematics at all taxonomic ranks.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Dr. Noris Salazar Allen and Prof. Dr. Juan Carlos Villarreal A. for their invaluable support throughout the development of this work. This research was supported through the Canada Research Chair (950-232698) funds awarded to Prof. Villarreal (Université Laval). We also thank the curators and staff of the herbaria SCZ, PMA, QFA, and GOET for their assistance. This study was conducted with the authorization and support of the local and governmental authorities of Panama. We thank the comments provided by two anonymous reviewers.

AUTHOR CONTRIBUTIONS

AMS contributed with species description, molecular laboratory work, phylogenetic analysis, and illustrations. SRG participated in fieldwork, first recognized the new species, and contributed to the discussion and species description. JGL contributed with fieldwork, species description, and illustrations. All authors contributed equally to the writing of the manuscript.

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