Arctoa (2015) **24**: *27-31*
doi: 10.15298/arctoa.24.05

LARRAINIA, A NEW GENUS OF AMBLYSTEGIACEAE FROM THE CAPE HORN REGION OF CHILE

LARRAINIA (AMBLYSTEGIACEAE), НОВЫЙ РОД ИЗ РАЙОНА МЫСА ГОРН В ЧИЛИ

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Abstract

Larrainia prorulosa W.R.Buck is described as a new genus and species of Amblystegiaceae from southernmost Chile. The new genus is characterized by a very small stature, differentiated branch and stem leaves, and short prorulose laminal cells. The two known collections are both sterile. Based on DNA sequence data, *Larrainia* is placed in the Amblystegiaceae, most closely related to *Vittia*. However, it is most similar morphologically to *Conardia*. The new species would probably have previously been placed in *Rhynchostegiella* in the Brachytheciaceae (as has been *Conardia*). The diversity within *Rhynchostegiella* is discussed.

Резюме

Описан новый род и вид из семейства Amblystegiaceae *Larrainia prorulosa* W.R.Buck с крайнего юга Чили. Для нового рода характерны очень мелкие размеры растений, дифференцированные стеблевые и веточные листья и короткие клетки пластинки листа с выступающими верхними углами. Оба известных образца вида стерильны. *Larrainia* отнесена к семейству Amblystegiaceae на основании данных последовательностей ДНК. По данным молекулярных маркеров наиболее близким к ней родом является *Vittia*, однако морфологически *Larrainia* больше похожа на *Conardia*. Новый вид сначала был предположительно отнесен к роду *Rhynchostegiella* из Brachytheciaceae (так же как и *Conardia*). Обсуждается морфологическое разнообразие рода *Rhynchostegiella*.

KEYWORDS: Amblystegiaceae, Brachytheciaceae, Chile, *Conardia*, *Eurhynchiella*, *Larrainia*, *Rhynchostegiella*, *Vittia*.

INTRODUCTION

Although short on land, southernmost South America is very rich in bryophytes. Occupying less than 0.01% of the Earth's land mass, the area harbors over 5% of the world's bryophytes (Rozzi *et al.*, 2008). Even though the region is a hot spot for bryophyte diversity, it is somewhat surprising that a significant number of taxa are being found in it new to science. Certainly this is partly due to historical factors. In the early days of sailing ship expeditions, most ships either went north of the region, through the Straits of Magellan, or south of the region, nearer Antarctica, to avoid the treacherous seas with numerous rocky shoals. Of course there are exceptions, the most famous of which was the Erebus and Terror Expedition (Hooker, 1844–1860). One might predict that the bryoflora of the region would be a synthesis of that of further north in Tierra del Fuego, and further south in Antarctica. However, fieldwork was needed to prove such a hypothesis. In conjunction with John Engel and Matt von Konrat at the Field Museum, a grant was awarded to the senior author from the U.S. National Science Foundation to intensively collect in the region and to prepare a bryoflora.

The investigated flora region is defined as the political boundaries of the Provincia Antártica Chilena (excluding continental Antarctica). The project is funded for four years, with a trip each year to a quadrant of the region. One might assume that the region is fairly uniform geologically and climatically, but such is not the case. The westernmost part of the region receives about 4 m of precipitation per year but at the eastern end of the region only about 700 mm of rain falls per year (Rozzi *et al*., 2004). During the 2013 field season, the primary focus was on the area around Isla Navarino, *i.e.*, the driest part of the flora region. During this time the only outcrops of calcareous substrates known locally were encountered. Previous fieldwork in the Cape Horn Archipelago has resulted in the discovery and description of several new species of mosses (Garilleti *et al.*, 2012; Andreas, 2013). These previous publications as well as the current one are ample evidence of the bryological diversity of the Cape Horn Archipelago.

MATERIALS AND METHODS

DNA was extracted from a few branches of the holotype collection (*Buck 60369*, NY) using the NucleoSpin® plant kit from Macherey Nagel (Düren, Germany) fol-

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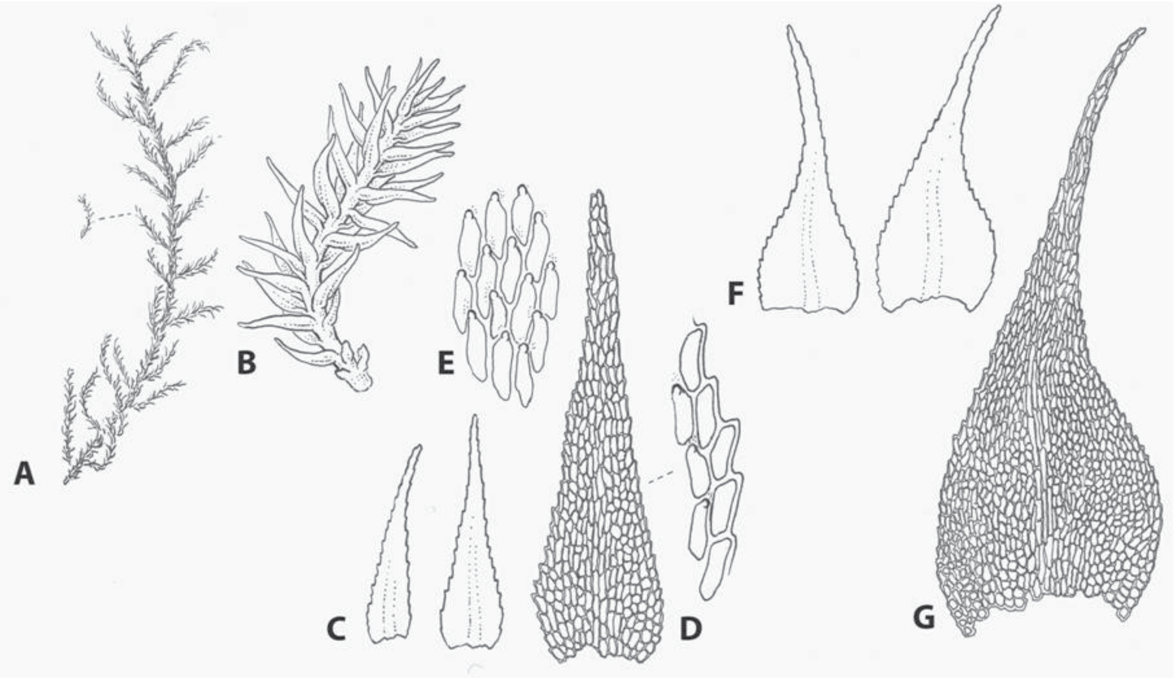


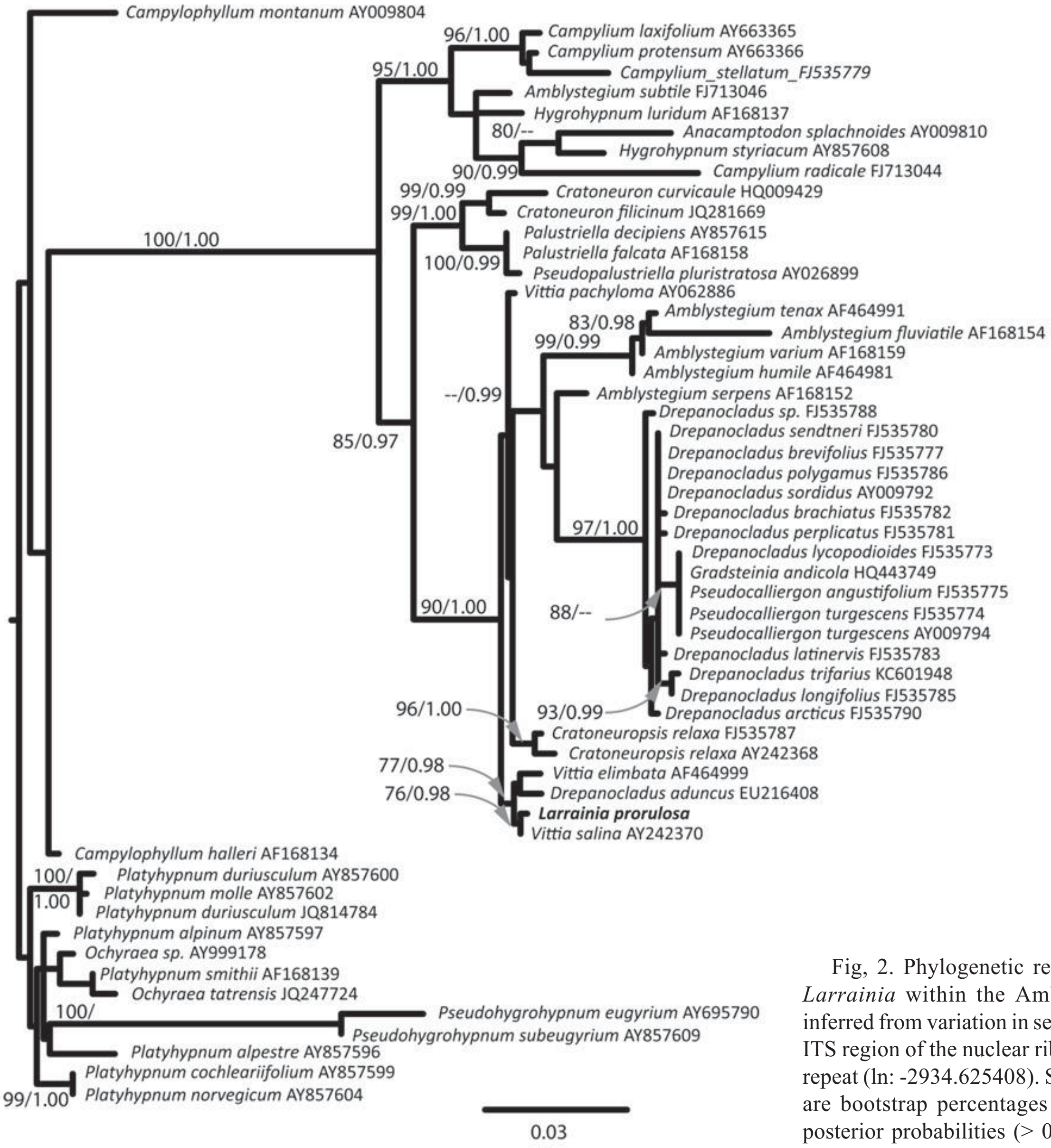
Fig. 1. *Larrainia prorulosa*. A. Habit, dry. B. Habit, moist. C. Branch leaves. D. Branch leaf areolation and marginal cells. E. Branch leaf cells showing prorulae. F. Stem leaves. G. Stem leaf areolation. Drawn from the holotype (*Buck 60369*, NY). See description for measurements.

lowing the manufacturer's instructions. The ITS region (ITS1 + 5.8S + ITS2) was amplified using the universal primers ITS1 and ITS4 (White *et al.*, 1990). The amplicon was sequenced using the PCR primers and these reactions were performed using the ABI PRISM® BigDye™ Terminators v. 1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.) optimized for half- or quarter-size reactions. Sequencing products were purified using Sephadex G-50 (Amersham, Piscataway, NJ, U.S.A.) gel filters, and then separated by capillary electrophoresis using an ABI PRISM® 3100 Genetic Analyzer. Nucleotide sequences were edited using Sequencher v. 3.1.1 (Gene Codes Corp., Ann Arbor, MI, U.S.A.). The ITS sequences (GenBank KJ736771) were added to a matrix of 54 selected Amblystegiaceae sequences available on GenBank. Sequences were aligned with MUSCLE v. 3.8.31 (Edgar, 2004) and all 664 sites were retained for the analyses. Phylogenetic relationships were inferred under the criterion of maximum likelihood (ML) and using Bayesian inference (BI) methods. The data were partitioned into ITS1, 5.8S and ITS2. Maximum likelihood analyses were carried out using RAxML v. 7.2.3 (Stamatakis, 2006). The topology with the highest likelihood score was chosen as the best tree. Statistical supports for branches were obtained via non-parametric bootstrapping with 100 pseudoreplicates. Bayesian inference was conducted using MrBayes v. 3.0b4 (Ronquist & Huelsenbeck, 2003) assuming a GTR+G model of substitution for all partitions. Parameters for the different partitions were allowed to vary independently and branch lengths and topology were linked. Two runs each with four chains were performed with trees and parameters sampled every 1000th generation. Burn-in and convergence were assessed using the likelihood of the runs plotted against generations using Tracer v. 1.5 (Rambaut & Drummond, 2009). Posterior probabilities (PP) were estimated by sampling trees from the PP distribution. Trees were summarized after removing the burn-in samples. Finally, a 50% majority-rule consensus tree was built in MrBayes.

TAXONOMY

Larrainia prorulosa W. R. Buck, gen. et sp. nov. Fig. 1

Plants small, bright green; stems creeping, to ca. 1.5 cm long, irregularly pinnately branched; in cross-section with small, thick-walled cells in 1–2 row(s) surrounding abruptly larger, thin-walled cells, central strand small; rhizoids in clusters on the undersides of stems, abaxial to leaf insertions, smooth; pseudoparaphyllia foliose, ± triangular; stem and branch leaves somewhat differentiated. Stem leaves erect and ± flexuose when dry, erect-spreading to spreading when moist, ovate, 0.55–0.72 × 0.20–0.28 mm, ± abruptly long-acuminate, the acumen ca. 1/2 the leaf length; margins plane or narrowly and irregularly recurved at base, subentire toward apex, otherwise serrulate to serrate to supra-alar regions, with teeth spreading; costa single, ending in the acumen, subpercurrent to percurrent, ± flexuose, not becoming more slender above and apex not projecting as a spine, but costal cells sometimes apically prorulose; laminal cells ± hexagonal, 8.5–23 × 5–6 µm, ca. 1.5–3(–4):1, sparsely prorulose at upper ends, especially above, becoming more elongate in the acumen and shorter juxtacostally toward the insertion; alar cells well developed, oblate, quadrate



Fig, 2. Phylogenetic relationships of *Larrainia* within the Amblystegiaceae inferred from variation in sequences of the ITS region of the nuclear ribosomal DNA repeat (ln: -2934.625408). Support values are bootstrap percentages (> 70%) and posterior probabilities (> 0.95).

and short-rectangular, 0.8–3:1, extending up the margins by 4–8 cells, not reaching the costa. Branch leaves erect-spreading and ± flexuose when dry, spreading to wide-spreading when moist, lanceolate to narrowly ovate, 0.45–0.55 × 0.11–0.14 mm, ± gradually acuminate; margins plane, serrulate to serrate ± throughout but more weakly so in the acumen; costa single, subpercurrent to percurrent, with cells often prorulose apically but apex not projecting as a spine; laminal cells ± rounded short-rectangular, 11–17 × 5.5–8 µm, ca. 1.5–3:1, mostly prorulose at upper ends; alar cells quadrate to short-rectangular, ca. 1–2:1, extending up the margins by 3–6 cells, reaching the costa or separately from it by a single cells. Inflorescences and sporophytes unknown.

TYPE: CHILE. Prov. Antártica Chilena, Comuna Cabo de Hornos, Isla Grande de la Tierra del Fuego, Ventisquero Serka, NW of Bahía Yendegaia, 54°48’02”S, 68°58’23”W, ca. 80 m; *Nothofagus pumilio* forest SE of glacier; on shaded calcareous rock ledge; 14 January 2013, *W. R. Buck 60369* (holotype NY, holotype fragment CONN).

ADDITIONAL SPECIMEN SEEN: CHILE. Prov. Antártica Chilena, Comuna Cabo de Hornos, Parque Nacional Alberto de Agostini, Isla Hoste, Península Dumas, Bahía Yekadahby, 55°03’47”S, 68°25’19”W; moist *Nothofagus betuloides–N. pumilio-Maytenus* forest on E-facing slope with extensive rock outcrops; on humus; 15 January 2013, *W. R. Buck 60404* (MO, NY).

ETYMOLOGY: This new genus is named in honor of Chilean bryologist, Juan Bernardo Larraín Benoit (1979–), who is also a collaborator of the senior author on the bryoflora of the Cape Horn Archipelago. Juan has accompanied him on all the field work associated with this project and has been an ideal field companion since, with his youthful enthusiasm, he reaches altitudes I only look at. The specific epithet refers to the prorulose laminal cells, especially on the branch leaves.

DISCUSSION

Larrainia is characterized by very small plants with no leaves more than 0.8 mm long, slightly differentiated branch and stem leaves, a strong single costa, relatively short, prorulose laminal cells, and well-differentiated alar cells. The type collection was growing tightly appressed to calcareous rock on a shaded ledge but the other collection was on humus in a non-calcareous habitat.

Initially it was unclear as to the relationships of this small pleurocarp. Because of the small size, a number of moss families seemed possible. The two most probable families, for a temperate unicostate pleurocarp, are the Brachytheciaceae and Amblystegiaceae.

Larrainia differs from all other sympatric species of Brachytheciaceae by its small size, percurrent costa and prorulose laminal cells. It is probably most similar to *Eurhynchiella acanthophylla* (Mont.) M. Fleisch., newly found this far south (at the same locality as the paratype, on a shaded vertical rock wall, *W. R. Buck 60426*, NY) but that species has smooth, linear laminal cells, although it is of similar stature.

If this taxon had been described in the last century, it would probably have been placed in the genus *Rhynchostegiella* (Schimp.) Limpr. However, this genus has been restricted to a core of European-North African species (Aigoin *et al.*, 2009). The three European species traditionally placed in *Rhynchostegiella* (*e.g.*, Brotherus, 1925) but which molecularly were discordant with the others, were placed in three, newly described unispecific genera. Unfortunately, although morphology was discussed and a matrix made, the various genera, including *Rhynchostegiella* itself, were not morphologically characterized in a helpful manner. For example, during the study of this group in preparation of describing *Larrainia*, the senior author found that one of the most diagnostic characters for *Rhynchostegiella* s.str. is collenchymatous exothecial cells. None of the other segregate genera has this feature. However, it was not even mentioned by Aigoin *et al.* (2009).

Even more unfortunate, over half the species placed in *Rhynchostegiella* but which are extra-European were not even mentioned for the most part. Therefore, in trying to taxonomically position these mostly Asian species, all the material at NY was examined. The morphological diversity found among the Asian/Pacific species of *Rhynchostegiella* s.l. is staggering. Unquestionably more genera will need to be described. For example, *R. smithii* E.B. Bartram, from Fiji, is totally unlike the other species, with its ovate, obtuse leaves and setae only 1–2 mm long. There is also a group of species, including *R. mindorensis* (Broth.) Broth., *R. opacifolia* Dixon and *R. papuensis* E.B. Bartram, that are all similar with more or less acute, weakly decurrent leaves, margins serrate throughout, with a strong, single costa ending about 3/4 the leaf length and projecting apically as a spine, short laminal cells, and well-developed alar cells. Previously, Ignatov & Huttunen (2002) resurrected a *Rhynchostegiella* segregate for a group of Asian species, *Remyella* Müll. Hal. In this genus, the leaves are ovate-lanceolate and serrulate to base, often with the leaf apex somewhat twisted. The costa ends at or below midleaf and the alar region is scarcely differentiated. The exothecial cells have thick vertical walls and thin horizontal walls, and the annulus is well developed.

Larrainia prorulosa is resolved, however, based on inferences from the nuclear ITS locus, within a core group of Amblystegiaceae composed primarily of *Amblystegium* and *Drepanocladus* (Fig. 2). Within this group *Larrainia* is most closely related to *Vittia salina* Hedenäs & J.Muñoz and, with *V. elimbata* Hedenäs, Vanderpoorten & Goffinet and a specimen identified as *D. aduncus* from Argentina (*Biasuso et al. 105*, S), and together they compose a weakly supported lineage. The identification of the latter was confirmed and its sequence, deviating from other *D. aduncus* samples, was already discussed by Hedenäs (2008). *Vittia* Ochyra is resolved as paraphyletic but considering the short branches, it is possible that *V. pachyloma* (Mont.) Ochyra, the type of *Vittia*, belongs to the same lineage with the other species of *Vittia*, the anomalous *D. aduncus* sample from Argentina, and *Larrainia*.

Phylogenetic resolution of aquatic or rheophilous species with bistratose lamina and terrestrial species with unistratose leaves in a single monophyletic group is not novel (see also *Gradsteinia* [Fig. 2] and *Donrichardsia* [Huttunen & Ignatov, 2010]), and simply suggests that shifts in habitat trigger morphological transformations and that the latter should perhaps not be assigned taxonomic weight above the species rank. The polarity of these shifts is not well established and recent inference within *Rhynchostegium* suggests a perhaps counter-intuitive trend from aquatic to terrestrial habitats (Huttunen & Ignatov, 2010). Although *Larrainia* is tentatively resolved as a sister-taxon to *Vittia salina*, it may be premature to accommodate the Cape Horn material within *Vittia*, given the lack of robustness of the relationships within *Vittia*, including the monophyly of the genus. Thus, we prefer to recognize these collections as a distinct genus on the basis of morphological differences until the phylogeny of the Amblystegiaceae and the affinities of all *Vittia* species are fully resolved.

Although the molecular data indicate a close relationship with *Vittia*, morphologically *Larrainia* seems most similar to *Conardia* H. Rob., as first suggested by Bruce Allen. *Conardia* was described to accommodate a single North Temperate species, *C. compacta* (Müll. Hal.) H. Rob., previously most often placed in *Amblystegium*, but also *Brachythecium* and *Rhynchostegiella*. *Larrainia prorulosa* differs from *Conardia* by its smaller size, smooth rhizoids, foliose pseudoparaphyllia, differentiated branch and stem leaves, considerably shorter and prorulose laminal cells, and lack of vegetative propagula on the abaxial surface of the costa, as well as its very different distribution. *Conardia compacta* is autoicous; inflorescences and sporophytes are unknown in *Larrainia* and thus it is probably dioicous. However, molecular data (Vanderpoorten *et al.*, 2002) indicate that *Conardia* is not at all close to the Amblystegiaceae or *Vittia*.

ACKNOWLEDGMENTS

WRB thanks the U.S. National Science Foundation for grant DEB 0948380 (Collaborative Research on the Marchantiophyta, Anthocerophyta and Bryophyta of the Cape Horn Archipelago: Floristics and Implications for Conservation), which funded the fieldwork of this project. Bruce Allen first suggested a relationship to *Conardia* and we thank him for his discussion of the new taxon. The molecular component was supported by DEB-1212505 (AToL: Assembling the Pleurocarp Tree of Life: Resolving the Rapid Radiation Using Genomics and Transcriptomics) to BG. We thank Nicholas Moskwa and Dr. Yang Liu (UCONN) for sequencing the type and analyzing the molecular data, respectively.

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