Bucklandiella araucana (Grimmiaceae), a new species from Chile

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Abstract. The new species *Bucklandiella araucana* Larraín is described from southern Chile. It is characterized by its small size, epilose, almost cucullate leaves, lack of a differentiated basal marginal row of pellucid and straight-walled cells, bistratose and dorsally flat costa at midleaf, smooth to slightly bulging lamina cells, unistratose leaf lamina with scattered bistratose spots at distal margins, undivided, prong-like peristome teeth, and deeply lobed calyptra. The species seems to be an endemic of the volcanic range of the western slopes of the southern Andes (39–42°S), where it grows in *Nothofagus* forests and open lava fields on the hillsides of the many active volcanoes of the area. Molecular data support the distinctiveness of this new taxon and identify *Bucklandiella araucana* as sister to *B. curiosissima, B. didyma*, and *B. emersa*. A distribution map and illustrations of the new species are presented. The phylogenetic perspectives of our novel molecular results are discussed.

Keywords. Bryophyta, taxonomy, molecular phylogeny, *Racomitrium*.

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The genus *Racomitrium* Brid. (Grimmiaceae) has recently been divided by Ochyra et al. (2003) into four genera (*Racomitrium, Niphotrichum* (Bednarek-Ochyra) Bednarek-Ochyra & Ochyra, *Codriophorus* P. Beauv., and *Bucklandiella* Roiv.), a concept based on the infrageneric division originally proposed by Kindberg (1897). These four genera still form a monophyletic group, recognized by Ochyra et al. (2003) as a subfamily of the Grimmiaceae, the Racomitrioideae, and characterized by the plagiotropic growth, cladocarpy, the strongly nodulose basal laminal cell walls, the absence of a stem central strand, and the dioicous sexuality. Of these segregates, *Bucklandiella* is by far the most diverse, comprising the majority of the species formerly included in *Racomitrium*. The genus is characterized by the smooth laminal cells, (although in some cases the cells have thickened anticlinal cell walls, which may seem papillose in cross section), relatively short peristome teeth (always less than 500 μm), and smooth setae that are twisted to the left (clockwise if the observer looks from above, assuming torsion goes up).

Following the pioneering works by Hooker & Wilson (1844), Montagne (1845), and Müller (1849, 1885), in which the first species of *Bucklandiella* were described from southern South America and neighboring areas, several new species have been described from this region early in the last century (e.g.: Bartram 1946; Cardot 1900, 1905, 1908; Cardot & Brotherus 1923; Dusén 1907; Herzog 1954, 1957; Herzog et al. 1939; Roivainen 1955a, 1955b; Thériot

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1935), although some of them have been subsequently synonymized with previously described taxa. In the last half of the 20th century the taxonomic circumscription of the genus has been repeatedly revised (Bednarek-Ochyra 1993; Bednarek-Ochyra & Ochyra 1994; Bell 1974; Clifford 1955; Deguchi 1984; Frisvoll 1986; Lawton 1973; Ochyra et al. 1988; Roivainen 1955a). These studies differ in the number of accepted species, and hence in their diagnosis and in the diversity of the genus in South America. While Clifford (1955) merged almost all southern South American species of *Bucklandiella* in his wide concept of *Racomitrium crispulum* (Hook. f. & Wilson) Hook. f. & Wilson, Roivainen (1955a) presented a key to 16 *Bucklandiella* species in the same year just for Tierra del Fuego, and additionally described one more species in a separate paper in the same volume of the same journal (Roivainen 1955b). Ochyra et al. (2008) argued that Clifford overlooked many important characters for most austral species, and that *Bucklandiella crispula* (= *R. crispulum*) should be considered a narrow endemic, restricted to the Auckland islands and Campbell Island, south of New Zealand (Ochyra et al. 2008).

Our ongoing studies on South American Racomitrioideae suggest that 16 species of *Bucklandiella* occur in Latin America, with 12 of these restricted to the southern tip of the continent, south of 30° S. Some specimens from central-south Chile remained, however, undetermined as they combined traits diagnostic of *Bucklandiella didyma* (Mont.) Bednarek-Ochyra & Ochyra, *B. sudetica* (Funck) Bednarek-Ochyra & Ochyra, and *B. rupestris* (Hook. f. & Wilson) Bednarek-Ochyra & Ochyra. During a field trip in 2009 to Villarrica Vulcano, Cautín Province, central-south Chile, fertile populations of this challenging taxon were discovered allowing for a detailed description of all morpho-anatomical characters and providing fresh material for DNA extractions. The careful examination of these vouchers, together with several older collections from localities nearby, confirmed the hypothesis that these populations belong to a taxon clearly distinct from all sympatric species. Additional examination of collections and original descriptions of similar species from outside the study area indicated that the specimens correspond to an undescribed taxon.

Materials & Methods

Morphological study: Microscopic examination was made by traditional methods, dissecting the plants under a stereo microscope and preparing slides for examination under the light microscope. Cross sections were made throughout the leaves for observing the variation in the shape of the costa along the leaves. Permanent slides were mounted on Hoyer's solution (Anderson 1954). Many Chilean (and adjacent Argentinean) collections from several herbaria (BA, BM, BONN, CONC, F, FH, H, HIP, HIRO, JE, MA, MO, NY, PC, PUCV, S, SGO, US) were examined looking for more samples matching these vouchers in morphology, yielding additional records from the expected area.

DNA extraction, amplification and sequencing: DNA was extracted from herbarium specimens of exemplars of all sections (except *Laevifoliae*) of *Bucklandiella*, including all species morphologically similar to the new taxon. Representatives of *Racomitrium, Niphotrichum,* and *Codriophorus* were also sampled to serve as outgroups (**Appendix 1**). Shoots were cleaned manually under a stereo microscope, removing all eventual fungus infections or epiphytic algae and cleaning the selected shoots several times with distilled water. Extraction was made following the protocol described in Doyle & Doyle (1987) with some minor modifications for improving DNA precipitation due to the usually small amount of DNA gathered (i.e. keeping samples containing DNA in isopropanol for at least 10 hours at −20°C, and using refrigerated microcentrifuges and increasing the spinning time and speed during precipitation and washing stages). Amplification of the selected chloroplast regions (*rps*4-trnT-trnL intergenic spacers and trnK/*mat*K) and the nuclear region ITS 1 & 2 were done using EcoTaq® DNA Polymerase (Ecogen, Madrid) and prepared FastStart® Taq DNA Polymerase mix (Roche, Basel), respectively. In both cases we followed the manufacturer's instructions with slight modifications (described below) but for the FastStart® protocol we used 25 µL reactions instead of the recommended 50 µL reactions.

***rps*4–trnT–trnL intergenic spacers (*rps*4–trnL IGSs):** reactions for these spacers were performed in a total volume of 50 µL, adding 0.3 µL (1.5 U) of polymerase, 5 µL of polymerase buffer [10X], 2.5 µL $MgCl_2$ [50 mM], 5 µL of dNTP mix [0.2 mM], 2 µL

of each primer [20 μM], and 2 μL of DNA template, completing the volume with 31.2 μL of ultra-pure water. Primers used were rps4-166F (Hernández-Maqueda et al. 2008) and P6/7 (Quandt et al. 2004). Amplification cycles consisted in an initial period of 2 min at 94°C, followed by 29 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, ending with a final extension period of 5 min at 72°C. Reactions were performed in a Mastercycle® Gradient thermocycler (Eppendorf, Hamburg).

trnK/*mat*K: reactions for this region were performed in a total volume of 25 μL , adding 0.2 μL (1 U) of polymerase, 2.5 μL of polymerase buffer [10X], 2.5 μL $MgCl_2$ [50 mM], 2.5 μL of dNTP mix [0.2 mM], 1 μL of each primer [20 μM], and 1 μL of DNA template, completing the volume with 16.8 μL of ultra-pure water. In exceptional cases 0.5 μL of betaine and/or 1 μL KCl were added, or the primer quantity was increased to 2 μL each. Primers used were trnK-F (Wicke & Quandt 2009), and psbARbryo (Hernández-Maqueda 2007). In some cases, where this primer pair did not work, we used trnK-F together with the reverse primer trnK-R4 (Wicke & Quandt 2009). Amplification cycles consisted in an initial period of 3 min at 96°C, followed by 39 cycles of 30 s at 94°C, 90 s at 48°C, and 3 min at 72°C, ending with a final extension period of 20 min at 72°C. When the amplification failed we modified the parameters as follows: an initial cycle of 1 min at 96°C, 45 s at 50°C, 90 s at 68°C, followed by two cycles of 45 s at 95°C, 45 s at 48°C and 1 min at 68°C, and then 37 cycles of 30 s at 94°C, 30 s at 45°C and 1 min at 68°C, ending with a final extension period of 15 min at 68°C. Reactions were performed in the Eppendorf's thermocycler or in a T3 Thermocycler (Biometra, Göttingen).

ITS: reactions for this region were performed in a total volume of 25 μL, adding 12.5 μL of FastStart® polymerase mix, 5 μL of each primer [10 μM], and 1 μL of DNA template, completing the volume with 6.5 μL of ultra-pure water. Primers used were ITS4 (White et al. 1990), and 18S (Spagnuolo et al. 1999). Amplification cycles consisted in an initial period of 2 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 48°C, and 1 min at 68°C (with a time increment of 4°C/cycle), ending with a final extension period of 4 min at 68°C. Reactions were performed in the Biometra's thermocycler.

Sequencing was performed by Macrogen (Seoul). Sequences were edited and manually aligned using PhyDe ver. 0.995 (www.phyde.de) following the alignment rules and hotspot definitions presented in Kelchner (2000), Olsson et al. (2009), and Borsch & Quandt (2009). Phylogenetic inference was performed with MrBayes ver. 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), applying the GTR model of nucleotide substitution, assuming site-specific rate categories following a gamma distribution and a proportion of invariable sites. All characters were given equal weight and gaps were treated as missing data. The default settings for the a priori probabilities were used. Four runs with four chains (5,000,000 generations each) were run simultaneously, with the temperature of the heated chain set to 0.2 (default setting). Chains were sampled every 1000th generations. Calculation of the consensus tree and of the posterior probability of clades was done based upon the trees sampled after the chains converged (25%). Additionally, maximum parsimony (MP) ratchet analyses were conducted with the command line version of PAUP 4.0b10 (Swofford 2003) via the command files generated by PRAP2 (Müller 2004a), including 10,000 bootstrap replicates. Ratchet settings were as follows: 10 random addition cycles of 200 iterations each with a 25% of upweighting of the characters in the iterations. For each of the tree constructing methods mentioned above, we analyzed the concatenated data matrix of the three sequenced regions, as well as the plastid versus the nuclear data partition in order to detect incongruences in tree topology. Hot spot regions were excluded from analyses (see **Table 1**). In addition, the data matrix was analyzed separately with an indel matrix appended, both for the Bayesian and MP analyses, using the simple indel coding strategy (Simmons & Ochoterena 2000) via Seqstate (Müller 2004b). Trees were edited and support values added using TreeGraph2 (Stöver & Müller 2010). Alignment and trees are deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11880).

Results

Phylogeny. The concatenated data matrix of plastid (*rps*4–trnL and trnK/*mat*K) and nuclear (ITS1 & ITS2) sequence data comprised 5012 characters

Table 1. Location of mutational hotspots and corresponding region (location given as absolute position in the complete combined dataset of 5121 bp, before exclusion of hotspots).

Position	Region
202–207	rps4-trnT IGS
340–342	rps4-trnT IGS
615–617	trnT-trnL IGS
644–646	trnT-trnL IGS
752–755	trnT-trnL IGS
768–773	trnT-trnL IGS
1128–1130	trnK intron
1365–1367	trnK intron
1400–1401	trnK intron
3428–3447	matK-psbA IGS
3673–3677	ITS1
3937–3951	ITS1
4121–4128	ITS1
4409–4412	ITS1
4590–4592	ITS2
4909–4911	ITS2
5087–5104	ITS2

(excluding hotspots, compare **Table 1**). The data set included 288 parsimony informative characters. Indel coding added another 186 potentially phylogenetically informative sites. Phylogenetic analyses conducted on the individual data sets or the concatenated matrix using different tree constructing methods (results not shown, since no hard incongruences were observed), converged to the tree topology shown in **Fig. 1**. Three main clades were observed displaying a strong geographic pattern. The first lineage is represented by a Japan/Western North America clade consisting of *Bucklandiella lawtonae* and *B. latea* followed by a geographically diverse clade sister to a generally South Hemisphere clade (clades 1, 2 and 3, respectively, in **Fig. 1**). *Bucklandiella araucana* is resolved within a clade containing all of the Southern Hemisphere *Bucklandiella* species studied (with the exception of *B. valdon-smithii*, closely related to the bipolar *B. sudetica*).

The phylogenetic tree clearly separates *Bucklandiella araucana* from morphological similar species, such as *B. sudetica*, *B. rupestris*, and *B. didyma* (**Fig. 1**). Trees generated by Bayesian inference (BI) resolve *B. araucana* sister to a clade containing *B. didyma* and *B. emersa*, together with the New Zealand endemic *B. curiosissima*. Although this clade is not strongly supported by the MP analyses, when including indel coding to the Bayesian analysis the posterior probabilities increase considerably, but their value is still only 0.90, which may not be significant.

Discussion

Phylogenetic perspectives. Frisvoll (1988) provided the first attempt of an internal classification of the *Bucklandiella* clade, dividing it into six informal "subgroups", based mostly on Northern Hemisphere taxa. This classification largely relied on the morphology of the perichaetial leaves, the hyaline hairpoint, the basal marginal border, and basal cell walls. This classification was later adopted by Bednarek-Ochyra (1995) who included the Southern Hemisphere species, recognizing eight sections for *Racomitrium* subgen. *Ellipticodryptodon* (=*Bucklandiella*). Later, Ochyra et al. (2003) synonymyzed this subgenus with *Bucklandiella*. Recently, a ninth section was described by Köckinger et al. (2007) to accommodate the gemmiferous species of the group. The current sectional position of the studied species of the ingroup, based on Bednarek-Ochyra (1995), Bednarek-Ochyra & Ochyra (1996), and Köckinger et al. (2007), is shown in **Table 2**.

The present, and still preliminary phylogenetic inference for the Racomitrioideae sheds new insights into the relationships within the *Bucklandiella* clade. These results suggest that some of the morphological characters that have been used for circumscribing the infrageneric taxa of the genus do not reflect the evolutionary history of the group, with homoplasy due either to reversals to the plesiomorphic state, or independent evolution of derived states (i.e. shape of perichaetial leaves, presence of a basal marginal border of hyaline, straight-walled cells, plication of leaves, presence of gemmae, etc.). By contrast transformations of other characters as seta torsion, presence of hyaline hairpoints, and shape of costa, and the geographic distribution seem to better reflect the phylogenetic relationships.

Although no taxa from section *Laevifoliae* were sampled, our phylogenetic results support the monophyly of *Bucklandiella*, but suggest that the type

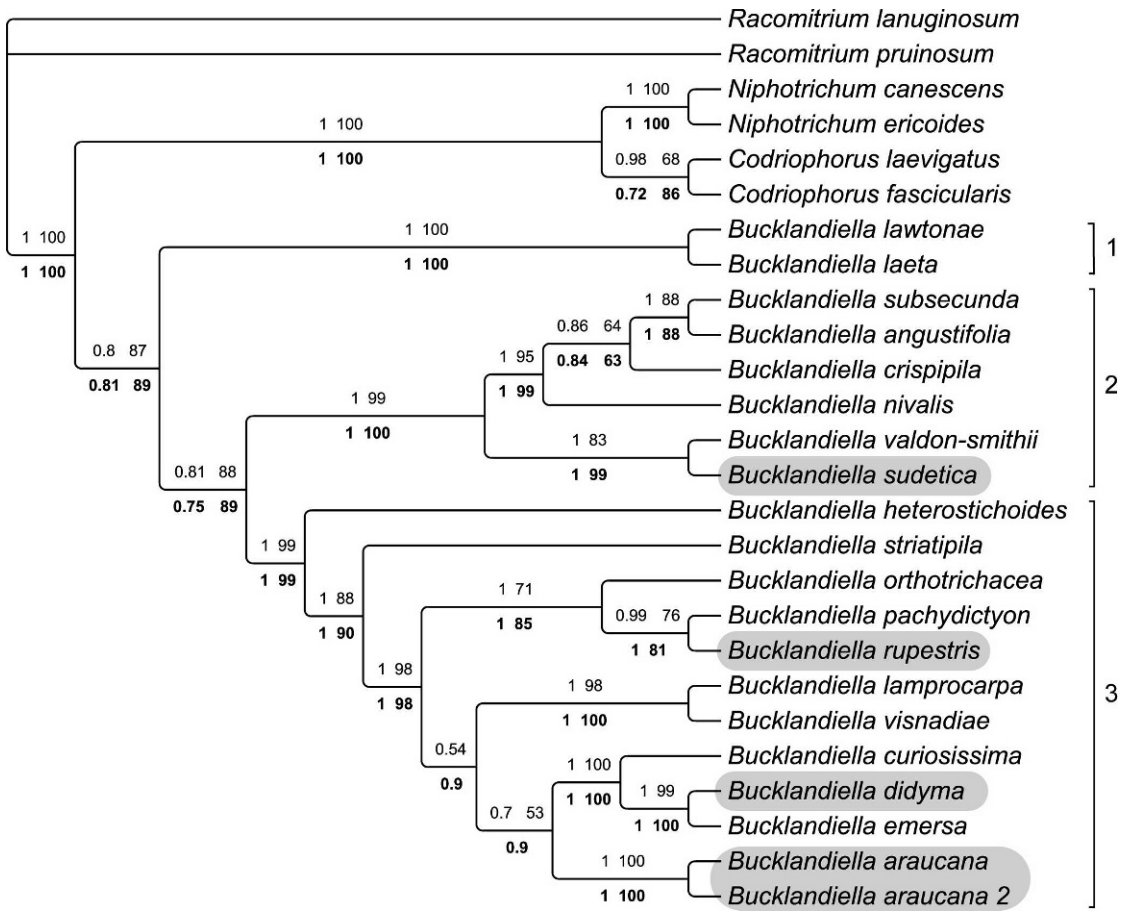

Figure 1. Phylogenetic relationships of *Bucklandiella araucana*. Bayesian 50% majority rule consensus tree inferred from the combined nuclear and chloroplast dataset. The new species is highlighted, as well as the morphologically most similar taxa. Numbers on branches indicate Bayesian posterior probabilities followed by MP bootstrap support (above branches without indel coding; below branches, in bold-face, including simple indel coding. Bootstrap support values below 50% are not shown).

section, together with sections *Marginatae*, *Subsecundae*, and *Emersae* should be redefined to satisfy a criterion of monophyly. Our trees accommodate the sampled species of *Bucklandiella* in three big clades: one including the two known species of section *Lawtoniae*, which is strongly supported as sister to the remainder of the genus (clade 1 in **Fig. 1**), a second one containing species from sections *Subsecundae*, *Marginatae*, *Gemmiferae*, and *Sudeticae* (clade 2 in **Fig. 1**), and a third one containing mostly strictly austral species belonging to sections *Ptychophyllae*, *Marginatae*, *Emersae*, and to the type section (clade 3 in **Fig. 1**). *Bucklandiella crispipila*, belonging to section *Marginatae* (*fide* Bednarek-Ochyra 1995) shows little genetic relation with the southern temperate species of the section, but it is molecularly related to species of section *Subsecundae*. This result is interesting because *B. crispipila* was for many years considered conspecific with the austral *B. striatipila*, yet is here resolved as distantly related to the latter, as recently suggested by Bednarek-Ochyra & Ochyra (2010).

The phylogenetic inference from molecular data also reveals the possible affinities of taxa currently unassigned to any section of *Bucklandiella*. The Chinese endemic *B. angustifolia* is closely related to *B. subsecunda* (**Fig. 1**), although it lacks the squarrose perichaetial leaves when wet, a trait that defines section *Subsecundae* (Frisvoll 1988, Bednarek-Ochyra 1995). *Bucklandiella rupestris* is nested within the clade containing *B. pachydictyon* (**Fig. 1**), with which it shares the costa shape in cross section, and *B.*

orthotrichacea, with which it shares the undivided peristome teeth. Finally, *B. valdon-smithii*, is infered as a close relative of *B. sudetica*, a relationship not considered by Ochyra & Bednarek-Ochyra (1999), although they share the costa cross section of three cell layers and dorsally convex throughout, the denticulate hyaline point, and the general aspect of plants when dry.

Bucklandiella araucana. The new species is easily overlooked in the field due to its small size and its overall similarity to dwarf expressions of the locally common *B. didyma*. When sporophytes are present, *B. araucana* is readily distinguished by the entire peristome teeth, smaller, ovoid to subglobose capsules (better seen when wet) and the shorter seta. Gametophytically, *B. didyma* usually has bistratose leaves for some marginal rows in the distal part, a conspicuous row of hyaline and straight walled cells in the leaf basal margins, and commonly a hyaline mucro or short hairpoint.

The new species shares with *Bucklandiella rupestris* the lack of a basal marginal band of pellucid cells with straight walls and the undivided peristome teeth but does not have the characteristic concatenated leaf arrangement of the later nor the conspicuous transverse thickenings of the leaf cells in superficial view. Plants of *B. rupestris* are often coarser; the costa in cross section below midleaf is tristratose, dorsally prominent and convex, and asymmetric.

It could also be confused with *Bucklandiella sudetica*, a morphologically highly variable taxon with occasionally entire peristome teeth, short setae, absence of basal marginal band of differentiated cells, no hairpoint, and epilose perichaetial leaves. Again, the most constant and reliable differentiating character is the shape of the costa in cross section, which at midleaf is convex to rounded-reniform, three-stratose and consisting of more or less homogeneous cells in the Chilean populations of *B. sudetica*, while in *B. araucana* it is flatter and mostly bistratose and it has a clearly differentiated row of enlarged ventral cells and a dorsal row of smaller cells.

Bucklandiella araucana Larraín *sp. nov.* **Figs. 2–3**

Plantae parvae, superne pallidae vel obscuro viride, inferne viride vel fuscae. Caules reptos vel erectos, 0.5–1.5 cm longi, breviter vel dicotomico ramulosis. Folia sine pilum hyalinum producta, apicis cucullatis. Cellulis laminae ubique unistratosis, raro in splenium bistratosis, laevibus vel pergrandibus. Costa superne magis lata qui subter, canaliculata, in basi bi(-tri)stratosi, superne bistratosa, cellulis ventralibus (2–)3–5(–6) praedita. Cellulis alaribus nullis vel parce differens, et fascia supraalaribus hyalinis esinuosis absens. Peristomii dentes 16, indivissus. Calyptrae basis profunde laciniata.

TYPE: CHILE. PROVINCIA DE CAUTÍN: Parque Nacional Villarrica, base del volcán Villarrica, poco más arriba de la entrada a las cuevas volcánicas, 39°22′38″ S, 71°56′31″ W, 1150 m, en afloramientos rocosos sobre el límite de la vegetación arbórea, sobre rocas; 18-Sep-2009. Leg. *Larraín 31884* (holotype: CONC; isotypes: KRAM, MA, NY).

Description. Plants small, dark green to yellowish in older herbarium specimens, forming compact tufts of intermingled stems. Stems 5–15 mm long, sympodially branched, without central strand, in transverse section composed of 1–3 rows of thick-walled cells surrounding a medulla of 6–9 rows of enlarged, thin-walled cells, 160–180 μm in diameter, bearing brownish rhizoids in the lower parts; axillary hairs uniseriate, 6–10 cells long, made up of 2–3 enlarged barrel-shaped basal cells gradually becoming smaller towards the tip. Leaves lanceolate, straight to slightly secund, epilose, often with cucullate apex, (1.1–)1.3–2.1(–2.5) mm long, 0.3–0.5 mm wide at widest part. Margins recurved in the proximal part of leaves, sometimes recurved towards the leaf apex. Costa symmetric, mostly bistratose throughout, with some tristratose spots in the proximal part, reaching the leaf apex or vanishing some cells below the leaf tip, mostly flat in cross section, 50–60 μm wide at base, made up of (2–)3–5(–6) ventral cells, and a dorsal band of thick-walled cells in the proximal part, becoming both rows undifferentiated at midleaf and above, where the ventral cells are reduced to 2(–3). Lamina unistratose throughout, seldom with scattered bistratose spots in the upper margins. Laminal cells with nodulose walls, sometimes with straight walls in the distal part of leaves, long-rectangular below, becoming shorter above,

Table 2. Systematic position of the sequenced species before this study, indicating their distribution (SH=Southern Hemisphere, meaning more than one subantarctic island or continental land below 30°S).

Section	Species	Distribution
Bucklandiella	*Bucklandiella lamprocarpa*	SH, with small populations in Europe
	B. visnadiae	Brazil endemic
Emersae	*B. emersa*	SH, SE Asia, Réunion Is.
Gemmiferae	*B. nivalis*	Austria endemic
Lawtoniae	*B. laeta*	Japan, China, Korea
	B. lawtonae	Western North America
Marginatae	*B. crispipila*	Pantropical
	B. didyma	SH
	B. heterostichoides	SH
	B. orthotrichacea	SH
	B. pachydictyon	SH
	B. striatipila	SH
Ptychophyllae	*B. curiosissima*	New Zealand endemic
Subsecundae	*B. subsecunda*	Pantropical
Sudeticae	*B. sudetica*	Bipolar
incertae sedis	*B. angustifolia*	China endemic
	B. araucana	Chile endemic
	B. rupestris	SH
	B. valdon-smithii	Marion Is. endemic

occasionally very short to almost quadrate above, or with rows of oblate cells near the leaf apex, basal cells (25–)30–60(–70) × 7–9 μm, medial cells (12–)15–50(–60) × 7–10 μm, upper cells (4–)5–10(–20) × 8–10 μm. Basal marginal cells undifferentiated, only very rarely forming a short band of 3–4 slightly differentiated hyaline cells with straight walls. Alar cells undifferentiated.

Dioicous. Perigonia not seen. Outer perichaetial leaves straight when wet, similar in shape to vegetative leaves, epilose; innermost perichaetial bracts strongly modified, widely ovate, with obtuse apex and entire to uneven border, epilose, hyaline throughout except at the extreme apex, where they are sometimes slightly chlorophyllose, laminal cells with thin and straight walls. Seta yellowish to dark brown, smooth, twisted to the left, straight, short, (2.2–)3.0–5.0(–6.0) mm long, with longitudinal thickenings clearly seen in cross section, composed of 2–3 rows of thick walled cortical cells, and 4–5 layers of thin walled enlarged hyaline cells in the medulla. Capsule ovoid to subglobose, becoming oblong when dry, 1.2–1.8 mm × 0.4–0.6 mm in diameter, yellow to brown, smooth, with scattered stomata in the lower part. Peristome teeth prong-like, densely papillose, 250–400 μm long, consistently undivided. Spores spherical, slightly rough, 12–14 μm in diameter. Calyptra mitrate, deeply lobed, with 4–5 spreading lobes, sometimes seemingly cucullate by the unequal splitting of the lobes.

Etymology. The species name honors the original inhabitants of the area where this plant grows, the Mapuche people, so called ''araucanos'' by the Spanish conquers.

Distribution. The species has been so far collected only on the western slopes of the Andean Range, between 39° S and 42° S (**Fig. 4**), which corresponds to the administrative Regions Araucanía (IX), Los Ríos (XIV) and Los Lagos (X) of Chile. Most of the collections come from protected land, i.e. National Parks Villarrica, Puyehue, and Vicente Pérez Rosales; thus the species would be currently protected, at least from massive human disturbance.

Ecology. *Bucklandiella araucana* grows in areas dominated by pyroclastic volcanic rocks, where the secondary rock corresponds to andesitic basalts, commonly present in the volcanic slopes of the southern Chilean Andes (Lara et al. 2001). All the volcanoes where the species has been found have experienced recent eruptions (Casertano & Lombardi

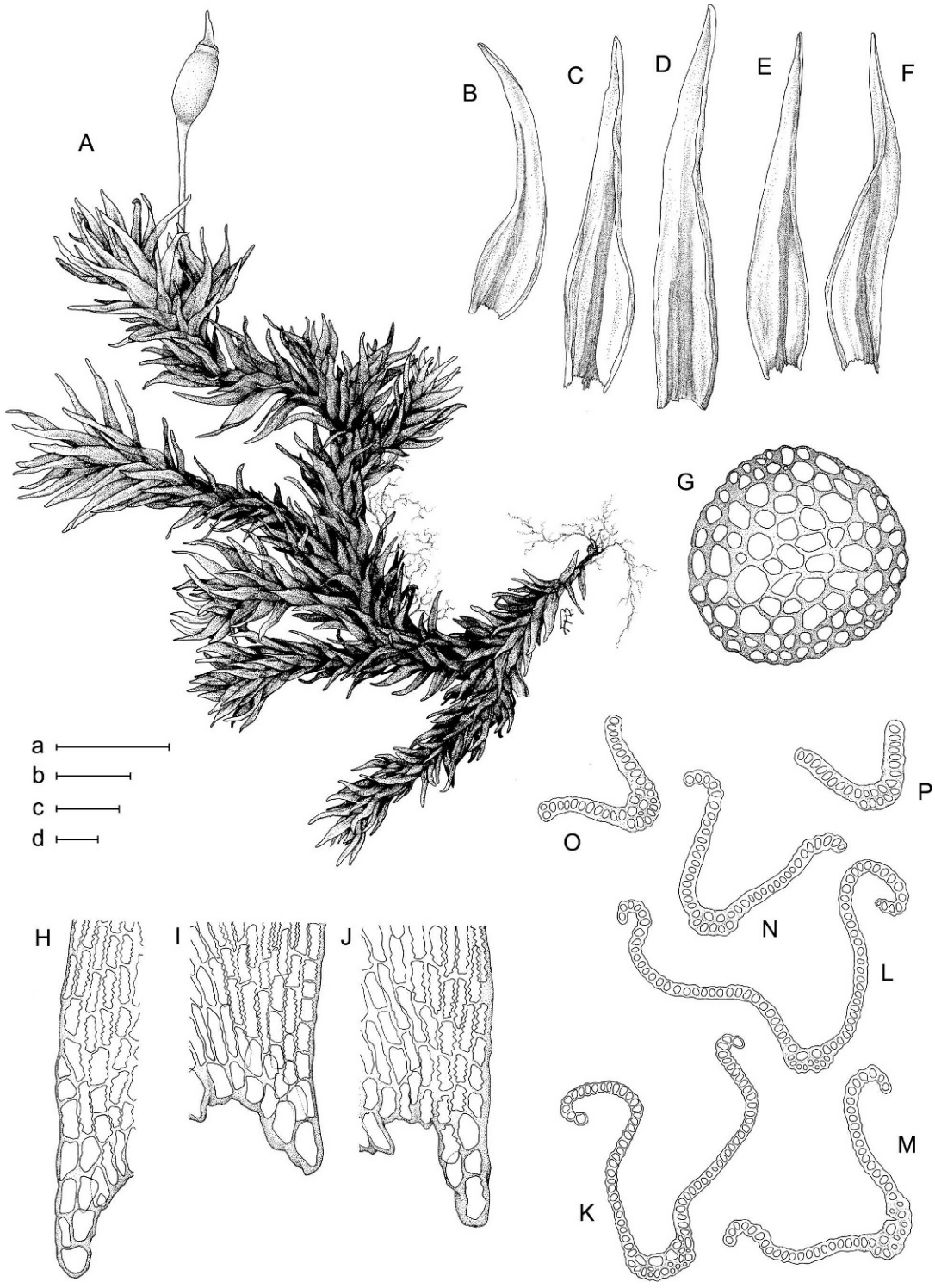

Figure 2. *Bucklandiella araucana* Larraín. **A**. Habit of female plant. **B–F**. Leaves. **G**. Stem cross section. **H–J**. Basal marginal cells. **K–P**. Leaf cross sections, from base to apex. (A drawn from *Deguchi 25237* [HIRO], B-P drawn from the holotype, *Larraín 31884* [CONC]). Scales: a. 2 mm (A); b. 400 µm (B-F); c. 50 µm (G-J); d. 50 µm (K-P).

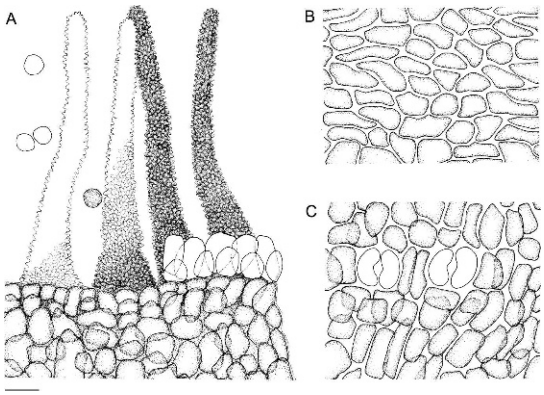

Figure 3. *Bucklandiella araucana* Larraín. **A**. Mouth of capsule showing peristome teeth, annulus, and spores. **B**. Exothecial cells just below capsule mouth. **C**. Exothecial cells at capsule base, showing stomata (all from *Deguchi 25237* [HIRO]). Scale bar: 25 μm (A–C).

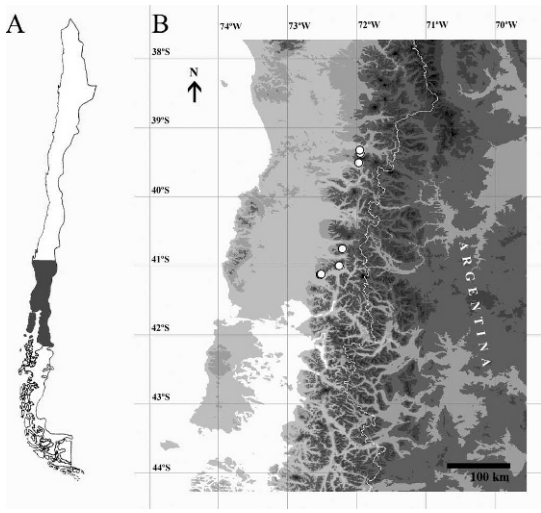

Figure 4. Distribution map of *Bucklandiella araucana*. **A**. Map of Chile showing in black the area detailed at right. **B**. Detail of Chile between 38° and 44°S, indicating studied specimens (white dots).

1963). *Bucklandiella araucana* has been collected growing mostly in volcanic boulders, basalt outcrops, and soil (as described on herbarium labels, presumably lying over rocks), on dry exposed places above the timberline, as well as on boulders in the interior of dense humid mixed forests, between 700–1250 m a.s.l. The tree species indicated in some of the herbarium labels include *Nothofagus dombeyi*, *N. pumilio*, *Drimys winteri*, *Fitzroya cupressoides*, and *Podocarpus* sp. Among the accompanying moss species found in the examined material are species of *Schistidium, Acroschisma wilsonii, Racomitrium geronticum*, *Bucklandiella didyma, B. striatipila*, and *B. heterostichoides.*

Additional specimens examined. CHILE. REGIÓN DE LA ARAUCANÍA. PROVINCIA DE CAUTÍN: Parque Nacional Villarrica, base del volcán Villarrica, justo encima de la entrada a las cuevas volcánicas, 39°22′38″ S, 71°56′31″ W, 1150 m, 18-Sep-2009, *Larraín 31885* (CONC); Parque Nacional Villarrica, north slope of volcán Villarrica, 2.3 km by road south of park entrance, *Nothofagus dombeyi-Weinmannia-Gaultheria phillyreafolia* semiscrub forest, on rock, 39°20′ S, 71°57′ W, 800 m, 19-Jan-1976, *Crosby 11830* (MO). REGIÓN DE LOS RÍOS. PROVINCIA DE VALDIVIA: comuna de Panguipulli, camino de Coñaripe a Parque Nacional Villarrica sector Quetrupillán, 1 km antes del cruce hacia las termas Geométricas, sobre roca de origen volcánico junto al camino vehicular, 39°31′ S, 71°58′ W, 900 m, 1-Apr-2010, *Larraín 32683b* (CONC). REGIÓN DE LOS LAGOS. PROVINCIA DE OSORNO: SW-Hang vom Vulkan Osorno ca 8 km nördl. Ensenada (ca 45 km NO von Puerto Montt), an der Piste zum Gipfel, auf trockenen Vulkangestein in Geröllfeld, 41°08′ S, 72°31′ W, 1000 m, 31-Mar-1999, *Müller C315* (CONC); ibidem, auf Vulkangestein in Schuttfeld, +/− trocken, *Müller C332* (CONC); ladera del volcán Osorno, sobre rocas, 700 m, *Pizarro E4* (PUCV); volcán Puntiagudo, faldeo río Alerzal, sobre roca y suelo, 1100 m, *Pizarro E21* (PUCV); upper part of refugio Antillanca, Parque Nacional Puyehue, in *Nothofagus pumilio* forest and grassland, ca. 1000 m, 24-Nov-1987, *Deguchi 31322* p.p. (HIRO). PROVINCIA DE LLANQUIHUE: Parque Nacional Vicente Pérez Rosales, ladera sur del volcán Osorno, sobre tierra arenoso volcánica, 13-May-1975, *Pizarro E2* (PUCV); around refugio, southern slope of volcán Osorno, in highland shrubby zone, 1200–1250 m, 9-Nov-1981, *Deguchi 25237* (HIRO), *25239* (HIRO); around refugio, southern slope of volcán Osorno, in closed *Nothofagus* forest, ca. 900 m; *Deguchi 25885* (HIRO); around refugio, southern slope of volcán Osorno, in *Nothofagus* forest, 680 m, *Deguchi 25216* (HIRO).

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Appendix 1. List of specimens used for DNA analyses, indicating origin and voucher information, with GenBank accession numbers (*rps*4–trnL/*mat*K-trnK/ITS) for the regions sequenced. *Rps4-trnL* sequences for *Bucklandiella didyma and B. crispipila,* and ITS sequences for *B. didyma* were retrieved from Hernández-Maqueda (2007). Nomenclature follows Ochyra et al. (2003).

Bucklandiella angustifolia: China. Fugong, *Shevock 31052* (CONC), HE586600/HE585276/HE584699; ***B. araucana***: Chile. Villarrica, *Larraín 31884* (CONC, holotype), HE586612/HE585284/HE584700; ***B. araucana* 2**: Chile. Villarrica, *Larraín 31885* (CONC, paratype), HE586613/HE585283/HE584701; ***B. crispipila***: Colombia. Especial, *Churchill & Muñoz 19337* (MA), EU246864, EU246899,EU246924/HE600698/—; ***B. curiosissima***: New Zealand. Canterbury Land, *Wilson s/n* (CHR 510953), HE586611/—/HE584702; ***B. didyma***: Chile. Llanquihue, *Holz & Franzaring CH 00-4* (MA), EU246865,EU246900&EU246925/HE600699/EU343799; ***B. emersa***: Reunion Is. Cratere Commerson, *Frahm REU-342* (BONN), HE586614/HE585285/HE584703; ***B. heterostichoides***: Kerguelen. Grande Terre, *Ochyra 3822/06* (CONC), HE586604/HE585278/HE584704; ***B. laeta***: Japan. Shikoku, *Deguchi s/n* (MA), HE586598/HE585269/HE584705; ***B. lamprocarpa***: Chile. Aisén, *Larraín 27861 A* (CONC), HE586609/HE585282/HE584706; ***B. lawtonae***: U.S.A. Alaska, *Schofield 109246* (CONC), HE586597/HE585275/HE584707; ***B. nivalis***: Austria. Carinthia, *Köckinger 03-453* (CONC, isotype), HE586601/HE585286/—; ***B. orthotrichacea***: Chile. Villarrica, *Larraín 31999* (CONC), HE586606/HE585280/HE584708; ***B. pachydictyon***: Chile. Aisén, *Larraín 27072* (CONC), HE586607/HE585281/HE584709; ***B. rupestris***: Chile. Aisén, *Larraín 27070 A* (CONC), HE586608/ —/HE584710; ***B. striatipila***: Chile. Aisén, *Larraín 26643* (CONC), HE586605/HE585279/HE584711; ***B. subsecunda***: Bolivia. Tarija, *Churchill et al. 23559* (MA), HE586599/HE585287/HE584712; ***B. sudetica***: U.S.A. California, *Shevock 18497* (MA), HE586603/HE585277/HE584713; ***B. valdon-smithii***: Marion Is., *Ochyra & Smith 738/99* (CONC, isotype), HE586602/HE585271/HE584714; ***B. visnadiae***: Brazil. Minas Gerais, *Buck 27053* (CONC, isotype), HE586610/—/HE584715; ***Codriophorus fasicularis***, Poland. Tatra Mountains, *Cykowska 2559* (CONC), HE586596/HE585274/HE584716; ***C. laevigatus***: Chile. Aisén, *Larraín 27017* (CONC), HE586595/HE585273/HE584717; ***Niphotrichum canescens***: Poland. Tatra Mountains, *Cykowska 1558* (CONC), HE586591/HE585272/HE584718; ***N. ericoides***: Poland. Tatra Mountains, *Cykowska 320* (CONC), HE586594/HE585270/HE584698; ***Racomitrium lanuginosum***: Ecuador. Azuay, *Jorgensen et al. 1589* (MA), HE586592/HE588126/HE584719; ***R. pruinosum***: New Zealand. Nelson, *Streimann 61054* (MA), HE586593/HE588127/HE584720.