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Circumscription of the South American moonwort *Botrychium* (Ophioglossaceae)

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Abstract

The genus *Botrychium* (Ophioglossaceae) is comprised of about 30 widely distributed species. Because of their small size and few, yet variable, morphological characters, many questionable taxa have been described over the years. Since its description in 1906, *B. dusenii* has been treated as both a distinct species and variously divided into four taxa: *B. lunaria* var. *dusenii*, *B. lunaria* var. *typicum*, *B. lunaria* var. *antarctica*, and *B. ramosum* var. *patagonicum* (*B. matricariaefolium* subsp. *patagonicum*). The aim of this study is to evaluate the validity of these taxa using spore wall sculpture, spore size, allelic composition employing enzyme electrophoresis, and morphological characters. We conclude that only one species of *Botrychium* occurs in southern South America and recognize this as *B. dusenii*, a species distinct from *B. lunaria*. Spore size and results of enzyme electrophoresis suggest that *B. dusenii* is an allotetraploid derived through hybridization between a diploid member of the *B. lunaria* complex and a diploid member of the *B. campestre* complex. We provide information about habitat, distribution, and full exsiccate citations.

Keywords: *Allozymes*, *Botrychium*, *enzyme electrophoresis*, *fern spores*, *moonwort*, *taxonomy*

Introduction

Within the genus *Botrychium* (Ophioglossaceae), Clausen (1938) described three subgenera: *Eubotrychium* (= *Botrychium*), *Sceptridium* (Lyon) R.T. Clausen and *Osmundopteris* (Milde) R.T. Clausen. The first group, commonly called the moonworts, was strictly delimited by Hauk et al. (2003), and is comprised of about 30 species (Dauphin et al., 2014). Leaves of *Botrychium* have two distinct segments, which are variously pinnately compound. The trophophore (blade) bears sterile pinnae; and the sporophore bears stalked spherical sporangia. Branching patterns of the trophophore and sporophore somewhat mirror each other (Hauk, 1995; Stensvold 2008; Meza Torres & Ferrucci, 2013).

Botrychium s.s. occurs in cool temperate and boreal regions of Asia, Australia, Europe, New Zealand, North America and South America. *Botrychium lunaria* (L.) Sw., originally described in Europe, also occurs in Asia, Australia, New Zealand, North America and South America (Clausen, 1938).

In southern South America, moonworts were originally collected by P. Dusén and described by Christ (1906) as *Botrychium lunaria* (L.) Sw. var. *dusenii* Christ and *Botrychium ramosum* A. Gray var. *patagonicum* Christ. In his monograph of the Ophioglossaceae, Clausen (1938) retained Christ's varietal designation, *B. lunaria* var. *dusenii*, and synonymized *B. ramosum* var. *patagonicum* as *B. matricariaefolium* A. Braun subsp. *patagonicum* (Christ) R.T. Clausen. He also included an un-named specimen collected in Patagonia (Furlong 10, NY) as *B. lunaria* var. *typicum*, which was later known as *B. lunaria*. Additionally, *B. lunaria* var. *antarctica* Speg. has been described from Argentinean Patagonia. More recently, in a reconsideration of the taxonomic status of these plants, Alston (1960) studied morphology and elevated *B. lunaria* var. *dusenii* and *B. ramosum* var. *patagonicum* to species status as *Botrychium dusenii* (Christ) Alton. Only Christ's var. *dusenii* and Alston's *B. dusenii* have been widely recognized in the literature.

Because of morphological similarity between species, in the past, many distinct taxa have been lumped together as a single species. For example, Stensvold et al. (2002) described two new species formerly included within *B. lunaria*. Similarly, the South American taxon *B. dusenii* has also been lumped into the circumboreal species *B. lunaria*.

Spore ornamentation and size are important characters for delimiting species in the Ophioglossaceae. The first to use palynological studies was Prantl (1884). Others using palynology in *Ophioglossum* are Burrows (1997), Burrows and Edwards (1993), and Meza Torres et al. (2015); and for *Sceptridium*, Sahashi (1979, 1981a, 1981b, 1983). Spore descriptions of Botrychioid species were also made by Tryon & Lugardon (1991). Heusser (1971) and Morbelli (1980, 1983) provide palynological descriptions for southern South American Ophioglossaceae, and Moe (1974) provides descriptions and keys for northern European ferns and lycophytes.

In a revision of southern South American Ophioglossaceae (Meza Torres, 2012), the type collections of relevant *Botrychium* species were compared to South American plants labeled as *B. dusenii*. Because morphological characters were insufficient to clearly differentiate *B. dusenii* from all northern hemisphere taxa, sculpture and size of the spore wall were examined with scanning electron microscopy (SEM). We also compared genetic profiles of *B. dusenii* and related taxa through allozyme electrophoresis.

The aim of this study is to evaluate the southern South American moonworts to determine which taxa occur here. This will be accomplished using spore wall sculpture, spore size, allelic composition using enzyme electrophoresis and study of herbarium specimens. In addition, we re-evaluate the classification and nomenclature of southern South American *Botrychium*.

Materials and methods

Palynological methods

Spores of *B. lunaria* specimens from Europe and South America, including type specimens, were analyzed and compared. Qualitative characters of sporoderm morphology were studied using light microscopy and SEM. Spores were studied without chemical treatment, because the perispore of some species of Ophioglossaceae does not resist acetolysis treatment (Meza Torres et al., 2015). The terminology proposed by Tryon & Lugardon (1991) and Punt et al. (2007) were used for describing the spore surface and structure.

Spores of the following specimens were analyzed by SEM:

Botrychium lunaria: Sweden: Västerbotten Koddis, jul 1860, *Håkanson* (LIL 25771).

Botrychium lunaria var. *dusenii*: Argentina: Tierra del Fuego. Depto. Ushuaia. 54°52'10.8"S, 67°33'40.6"W, 47 msl, Puerto Almanza, Beagle Channel, 22 Jan 2009, *Meza Torres et al.* 1058 (CTES); Prov. Santa Cruz. Depto. Lago Argentino. Fósil River, near San Martín Lake, 800 msl, 22 Feb 1905, *Dusen without number* (S-08-9411).

Allozyme methods

Enzyme electrophoresis followed procedures described in Zika & Farrar, (2009). Individual plants were analyzed for 22 gene loci from 10 enzyme systems using three buffer systems from Soltis et al. (1983) buffer system 7 (0.038 M LiOH, 0.188 M boric acid) for resolving enzyme systems, aspartate aminotransferase (AAT), and triose-phosphate isomerase (TPI); buffer system 9 (0.065 M L-Histidine, 0.015–0.016 M citric acid, anhydrous) for resolving enzyme systems, malate dehydrogenase (MDH), phosphoglucosmutase (PGM), 6-phosphoglucuronate dehydrogenase (6-PGD), and phospho-glucosomerase (PGI); and buffer system 11 (0.4 M citric acid, trisodium salt) for resolving enzyme systems, aconitase (ACN), diaphorase (DIA), isocitrate dehydrogenase (IDH), shikimate dehydrogenase (SKDH). Alleles are expressed as numbers relative to distance of migration with 1 being the most distal from the origin.

Botrychium dusenii was collected from the Falkland Islands in 2003 and 2008 for allozyme analysis at Iowa State University (Stensvold & Farrar, 2010). Sites of these collections were Cape Pembroke (8 plants), Yorke Bay Pond (8 plants) and Bertha's Beach (11 plants). Upon arrival at the lab in Ames, Iowa, some plants were too damaged to allow for herbarium vouchering, and others were lost in return shipment to the Falkland Islands. Scanned images of all plants and a subset of herbarium vouchers are retained at Iowa State University (ISC).

Review of herbarium specimens

Specimens were examined from the following herbaria: BA, BAA, BAB, BM, CTES, K, LIL, LP, MERL, MO, NY, P, S, SGO, SI, and US. Of particular, relevance to this study are type collections from the British (BM), French (P), Swedish (S) Museums of Natural History, and from the Museum of La

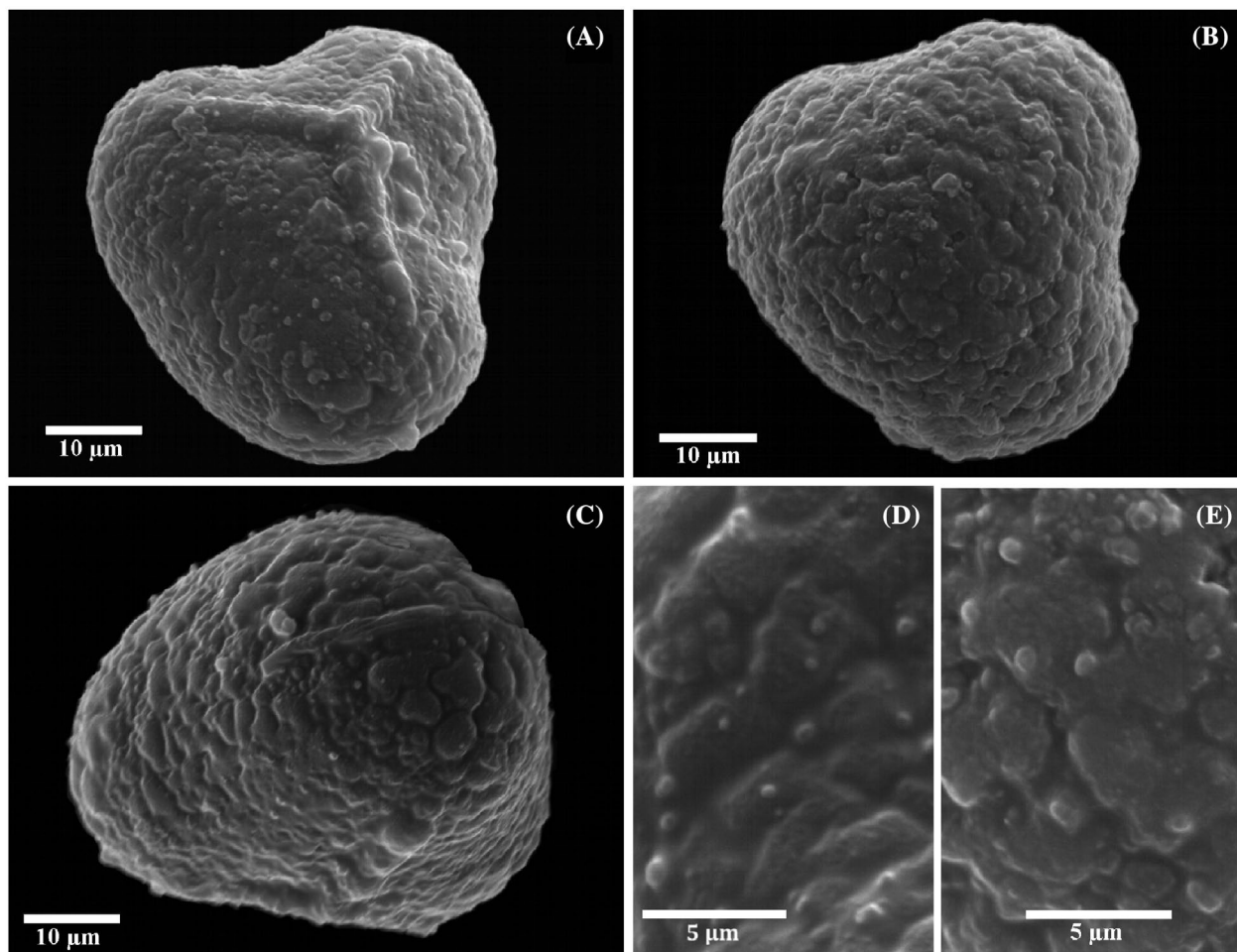


Figure 1. *Botrychium dusenii*. Scanning electron photomicrographs of spores of a type specimen, *Dusen s/n* (S). (A) Proximal view. (B) Distal view. (C) Equatorial view. (D) Detail of proximal view showing sculpture of the interlaesural region. (E) Detail of the ornamentation of the distal view.

Plata (LP), Argentina. Study of the specimens included label data (especially names), and morphological features.

Results

Palynological analysis

Analysis of spores from *B. dusenii* and *B. lunaria* showed differences in spore wall sculpture patterns and spore size. The principal characters differentiating these two taxa are described below and shown in Figures 1 and 2.

***Botrychium dusenii*.** Spores yellow, trilete, trilobate, 39 (46.47) 54 µm in equatorial diameter; 32 (39.32) 47 µm in polar diameter. Proximal face flat or somewhat convex, irregularly papillate-verrucate, with verrucae smaller than those on the distal face; laesural arms prominent, formed by fusing ridges, conspicuous nearly to the equator, 12 (19.60) 25 µm long. Distal face mildly hemispherical, verrucate,

fossulate-foveolate, with polygonal verrucae of different sizes, sometimes fused.

***Botrychium lunaria*.** Spores yellow, trilete, trilobate, 34 (37.64) 42 µm in equatorial diameter; 28 (37.64) 38 µm in polar diameter. Proximal face plane or mildly convex; irregularly rugulate-verrucate; laesural arms straight and prominent, with irregular margins, 10 (14) 19 µm long. Distal face hemispherical, irregularly fossulate, with broad muri, about 3.5 wide.

Allozyme analysis

We obtained scorable allele patterns at 19 gene loci for *B. dusenii* and compared these (Table I) to results previously obtained for these loci in related North American and European species (Stensvold, 2008; Farrar, 2009; Zika & Farrar, 2009). Alleles listed in Table I are as visualized in individual plants. Allelic composition in *B. dusenii* is highly constant across individuals and populations. Variability among plants

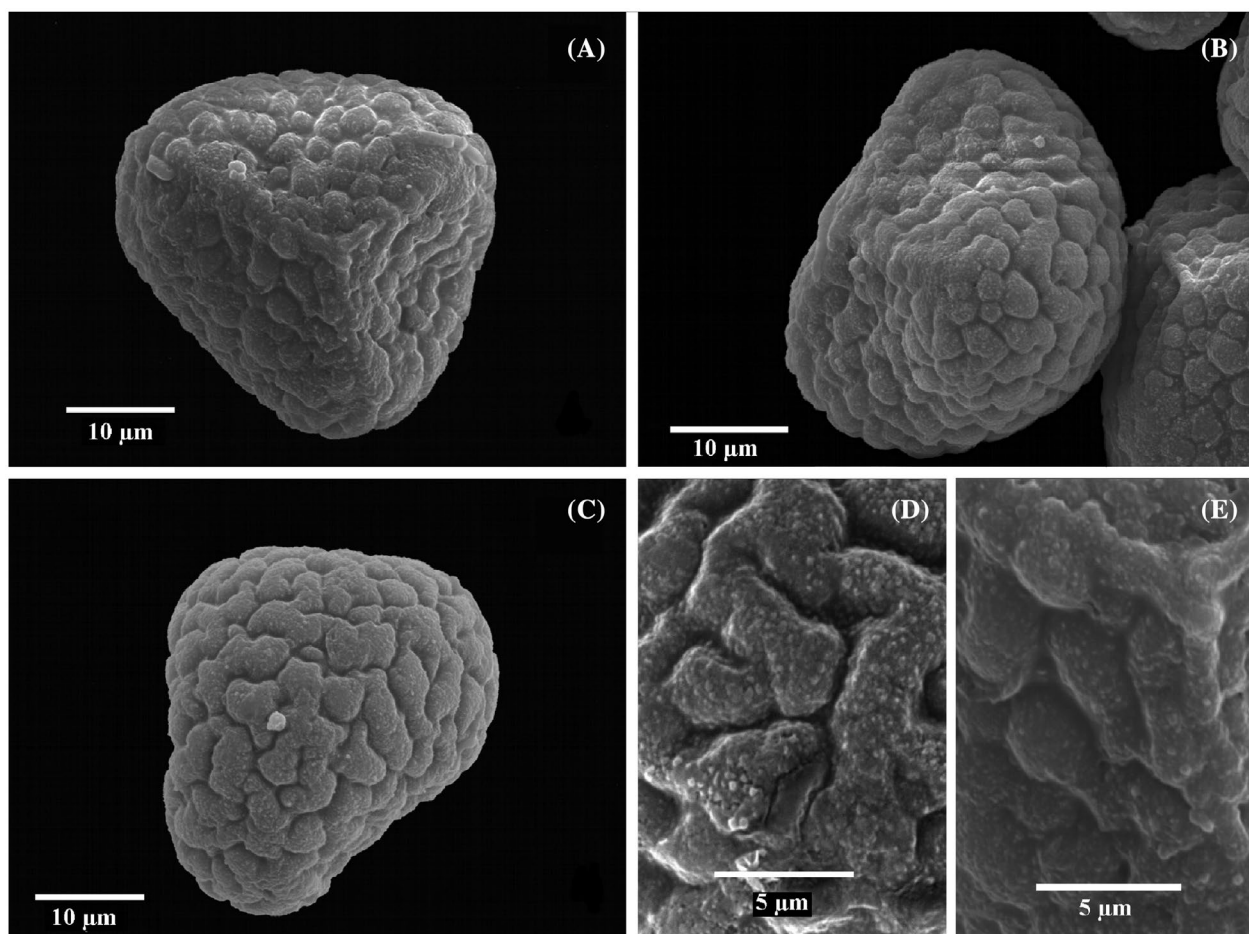


Figure 2. *Botrychium lunaria*. Scanning electron photomicrographs of spores from a European specimen, *Håkanson* (LIL 25771). (A) Proximal view. (B) Equatorial view (C) Distal face. (D) Detail of the ornamentation of the distal view. (E) Detail of proximal view showing laesura and sculpture of the interlaesural region.

was observed at only two loci, this expressed as absence of one of the two redundant alleles at loci displaying fixed heterozygosity. Fixed heterozygosity (present in all plants at DIA-4, MDH-1 and PGI-2) or partially fixed heterozygosity (present in most plants at IDH and MDH-2) was displayed at five loci.

Botrychium dusenii expressed alleles consistent with parentage by a member of the *B. lunaria* complex of taxa at 17 of 19 loci, and required this parentage (alleles not present elsewhere in *Botrychium*) at 6 loci. At 5 loci, *B. dusenii* expressed alleles not present in the *B. lunaria* complex, but present in the once-pinnate *B. campestre* complex in North America.

Discussion

Spore sculpture elements observed in *Botrychium lunaria* are consistent with the descriptions of Moe (1974) for Fennoscandian specimens. The spores described by Tryon & Lugardon (1991), under the

name *B. lunaria* from Patagonia, and the descriptions of Heusser (1971), and Morbelli (1980), for specimens from the Patagonian area, are consistent with the characteristics given here for *B. dusenii*. There are significant differences between the two taxa in spore ornamentation and laesura. *Botrychium dusenii* displays verrucate ornamentation, with polygonal verrucae, while *B. lunaria* shows fosulate ornamentation, with muri averaging 3.5 µm wide. The spores of Patagonian specimens have an average diameter nearly 10 µm greater than *B. lunaria*, a size commonly associated with tetraploid taxa of *Botrychium* (Wagner & Wagner, 1990; Stensvold et al., 2002). Tryon & Tryon (1982) note the large size of Patagonian *B. lunaria* var. *dusenii* spores and suggest this large size reflects polyploidy.

In a genetic study of putative taxa in the *B. lunaria* complex, Stensvold (2008) identified three varieties within *B. lunaria* and three entities separable from *B. lunaria* at the species level, all in Europe and North America. Members of the *B. lunaria* complex display a suite of alleles that both unite the complex and sep-

Table I. Alleles expressed at enzyme-coding loci in *B. dusenii* and its putative diploid parent species. Allele numbers joined by “+” are non-recombining homoologous alleles contributed by parent taxa expressing different alleles at a given locus. Expression of a single allele in *B. dusenii* where parent taxa express different alleles presumably results from failed expression (silencing) of one of its two inherited loci. Allele numbers reflect relative migrating positions within genus *Botrychium*, with “1” representing the allele migrating most distally from the origin.

Locus	<i>B. lunaria</i> complex ^a	<i>B. dusenii</i>	<i>B. campestre</i> complex ^b
	<i>n</i> = 1007	<i>n</i> = 27	<i>n</i> = 603
Aat-1	2	2	3
Aat-2	3	3	3
Aco-2	1 or 3	1	1 or 3
Dia-1	2 or 4 or 5	2	2 or 4
Dia-2	1	1	1
Dia-3	3	2	2 or 3
Dia-4	8	7 + 8	7
Idh-1	2 or 4	2 or 2 + 4	1 or 2
Mdh-1	1.5 or 3	1 + 1.5	1 or 2
Mdh-2	3 or 5	3 or 3 + 5	3
Mdh-3	1 or 2	2	1 or 2
Mdh-4	<i>n</i>	<i>n</i>	<i>n</i> or 2
6Pgd	5	1	1
Pgi-2	4 or 5	2 + 4	2
Pgm-1	1	1	1
Pgm-2	2 or 3	2	2
Skdh	1 or 2	1	1 or 2
Tpi-1	3 or 4	4	3
Tpi-2	3 or 4	3	3

^a*B. lunaria* and *B. neolunaria* Stensvold ined. Other taxa in the complex display additional alleles not present in *B. dusenii* or in the *B. campestre* complex (data from Stensvold, 2008).

^b*B. campestre* and *B. lineare* (data from Farrar, 2009).

arate it from other *Botrychium*. Genetic analysis of *B. dusenii* concluded that it was most likely an allotetraploid species having a member of the *B. lunaria* complex as one of its ancestral parents (Stensvold & Farrar, 2010).

Analysis of these data indicates presence of alleles in *B. dusenii* at 17 of 19 loci that could have been contributed by a member of the *B. lunaria* complex, and presence at 5 loci that have not been detected in the *B. lunaria* complex, but are present in members of the *B. campestre* complex in North America (Farrar, 2009; Zika & Farrar, 2009). Alleles present at all 19 loci in *B. dusenii* can be accounted for by combinations derived from the *B. lunaria* complex and the *B. campestre* complex. Taken together with its fixed heterozygosity and large spore size, these data strongly suggest that *B. dusenii* is an allotetraploid species derived through hybridization between a diploid member of the northern hemisphere *B. lunaria* complex and a diploid, once-pinnate member of the North American *B. campestre* complex. The genotype of *B. dusenii* most closely resembles North American *B. spathulatum* W. H. Wagner, which also matches morphologically. *B. spathulatum* has a putative origin of *B. campestre* x *B. lunaria* (Wagner and Wagner, 1990). No diploid species of *Botrychium* have been documented in South America, but evidence for dispersal of *Botrychium* from the Northern to the Southern Hemisphere is supported by a match of the single genotype of *B. lunaria* in New Zealand with a genotype of *B. lunaria* present in Alaska (Stensvold, 2008). Genetic relationships of *B. dusenii* to these

and other known allotetraploid *Botrychium* species are under study.

The synonymy of *B. dusenii* suggests the possible occurrence of more than one *Botrychium* taxon in southern South America, but careful analysis of the original Dusén collection supports only a single taxon with intrapopulational variability. Clausen (1938) synonymized *B. ramosum* under *B. lunaria* var. *typicum*, and applied this synonymy in recognizing *B. lunaria* var. *dusenii*. Although Clausen also accepted Christ's *B. ramosum* subsp. *patagonicum* (as a new combination, *B. matricariifolium* subsp. *patagonicum*), he states that he did not see a specimen. Christ's description of this taxon is similar to that of var. *dusenii*, and its co-occurrence with var. *dusenii* at the type locality suggests that Christ's two taxa represented only morphological variation within the population.

In the protologues of *B. lunaria* var. *dusenii* and *B. ramosum* var. *patagonicum*, Christ presented illustrations of four specimens of var. *dusenii*, and two of var. *patagonicum*. The description of *B. ramosum* var. *patagonicum* gives the same locality and collector as for *B. dusenii*, again suggesting that Christ's taxa might reflect only intrapopulational variation.

In elevating the status of *B. lunaria* var. *dusenii* to species level as *B. dusenii*, Alston (1960) also synonymised *B. ramosum* var. *patagonicum* into *B. dusenii*, citing a specimen of the type collection deposited at the British Museum (BM). This sheet (BM000936591) has a single plant, which corresponds exactly to the drawing (No. 4) of var. *dusenii* in

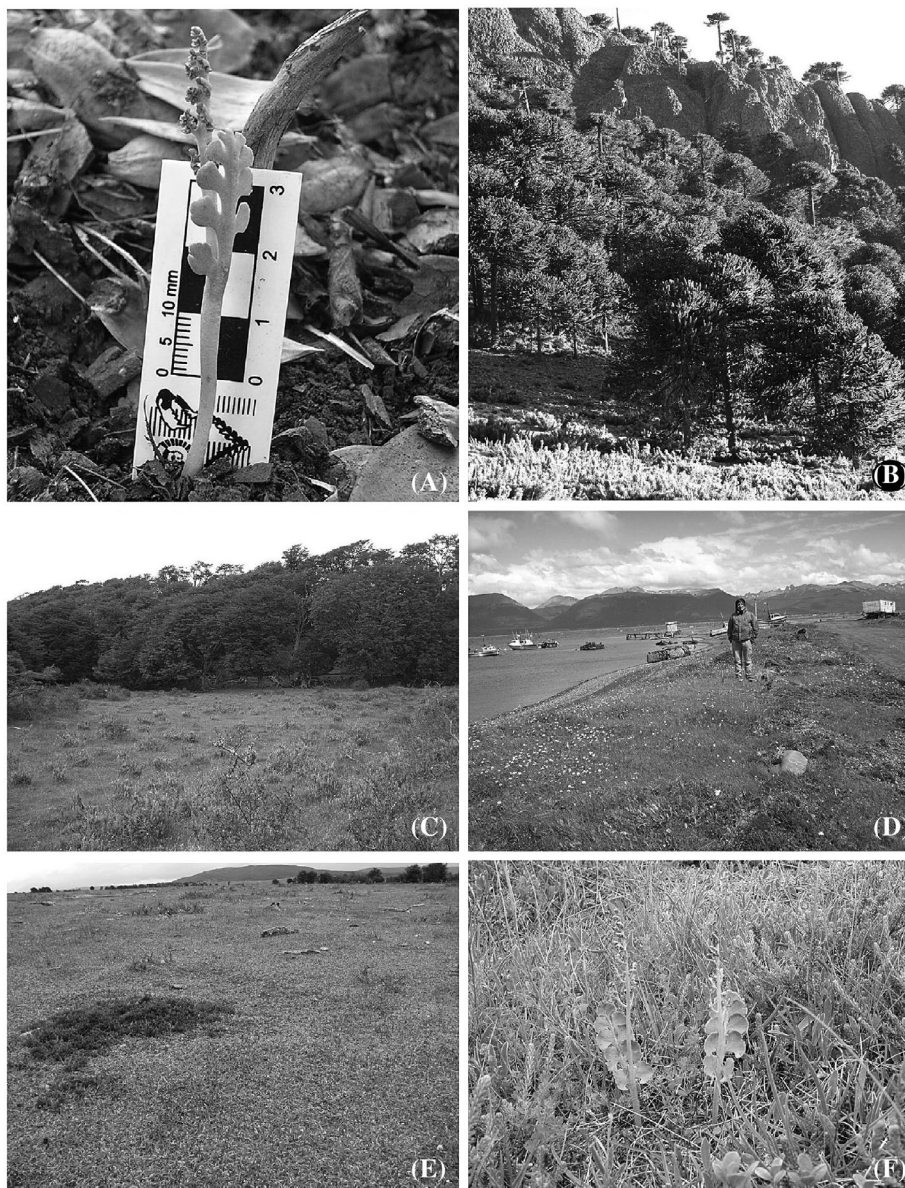


Figure 3. (A) Photograph of *Botrychium dusenii* in its natural environments B–D Environments where this species was collected. (A,B) *Araucaria araucana* Forest, Neuquén Prov., Depto. Picunches, Argentina. (C) Border *Nothofagus* forest, Tierra del Fuego Prov. Depto. Ushuaia, Argentina. (D) Low grassland, in the first line of littoral vegetation, Tierra del Fuego Prov., Depto. Ushuaia, Beagle Channel, Argentina. (E) Hills of Punta Arena (Region XII, Chile), in 2009 this area was completely colonized by the alien plant *Hieracium pilosella*. (F) *Botrychium lunaria*, growing in maritime beach dune vegetation, Orrestranden Norway. (A,B) Meza Torres *et al.* 684 (CTES).

the original publication by Christ. The Swedish Museum of Natural History (S) holds a sheet (S08-9411) with 6 plants collected by Dusén in Patagonia and determined by Christ as *B. lunaria* var. *dusenii*. Two of these specimens are identifiable with Christ's concept of var. *dusenii*, because they correspond with Figures 5 and 7 of the original publication (Christ 1906). Consequently, we consider this herbarium sheet to be the more appropriate holotype for *B. dusenii*. The herbarium sheet (P0125382) from the Natural History Museum, in Paris (P) contains two smaller plants collected by Dusén, and has an

initial determination with the name *Botrychium matricariaefolium*. These plants correspond to Figures 2 and 3 of the protologue of *B. ramosum* var. *patagonicum*, and for this reason is certainly the type of Christ's description of that variety. It can be inferred that Christ's illustrations were from a single collection of at least 9 specimens, and that these were separated and ultimately deposited in BM and S (as both *B. lunaria* var. *dusenii*), and in P (as *B. matricariaefolium* var. *patagonicum*).

Spegazzini (1924), in the protologue of his description of *B. lunaria* var. *antarctica* cited two

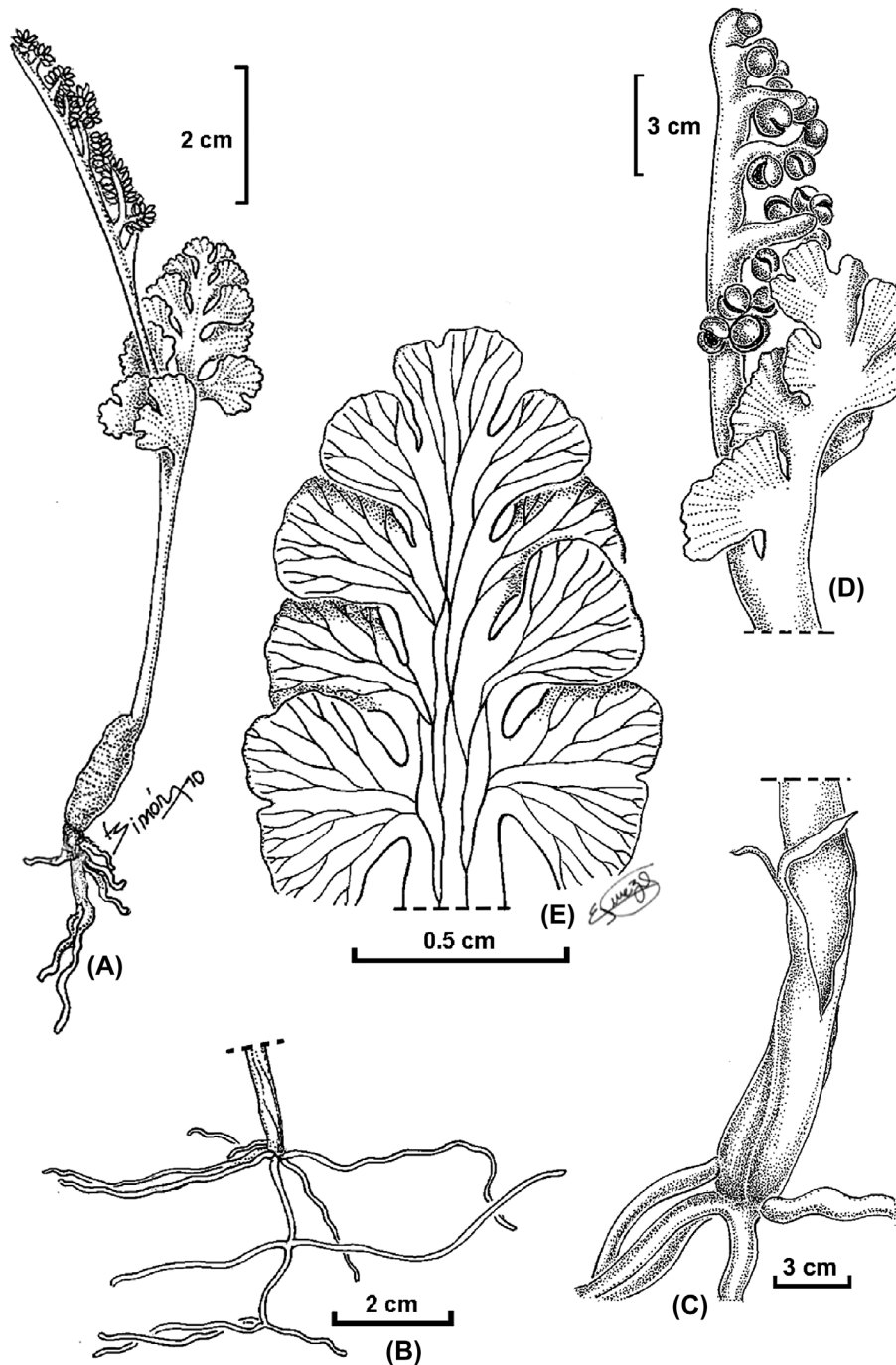


Figure 4. *Botrychium dusenii*. (A) General view. (B) Underground root system. (C) Rhizome and base of the petiole showing the sheath. (D) Detail of upper part of a fertile frond. (E) Venation detail. (A–E) Meza Torres et al. 1058 (CTES).

collections, the first collected on Staten Island in March 1882, the second from Lapataia beach on Tierra del Fuego Island. The sheet from Staten Island holds many plants, while the sheet from Tierra del Fuego Island with fewer specimens contains annotations by Spegazzini comparing *B. dusenii* and *B. lunaria*. Because the annotations were made by the author, we choose the collection from Tierra del Fuego

as lectotype for Spegazzini's var. *antarctica*. Our examination of plants from Tierra del Fuego and the Falkland Islands in southern South America indicates that these do not differ significantly from plants at the type locality of *B. dusenii*.

Data we obtained from scanning electronic microscope studies of spore structure, spore size, genetic analysis and study of herbarium material

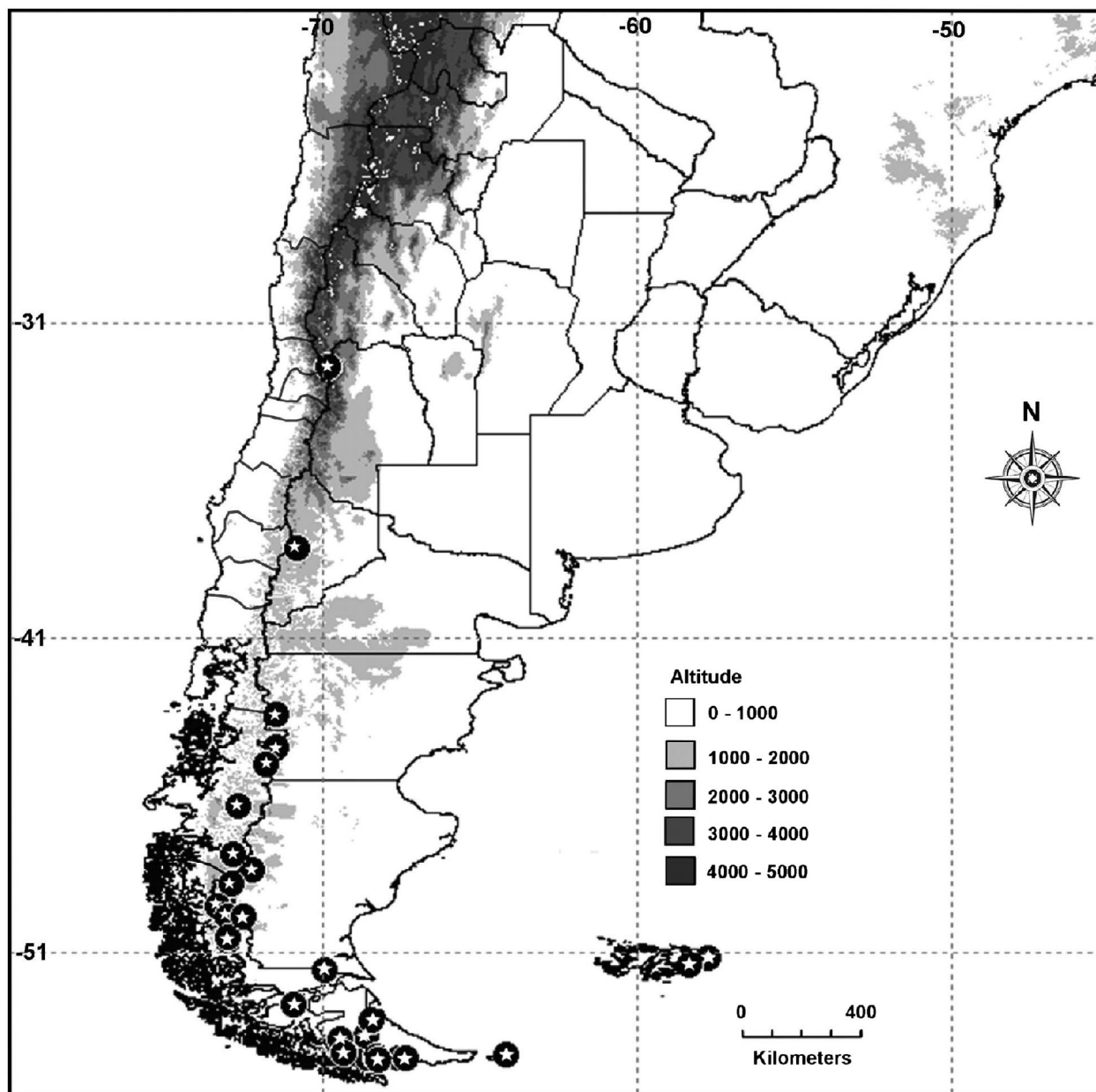


Figure 5. Distribution map of *Botrychium dusenii*.

support a distinction between *B. dusenii* and *B. lunaria*. We propose that all South American plants discussed herein be treated as *B. dusenii*.

Taxonomic treatment

Botrychium dusenii (Christ) Alston, Lilloa 30: 107. 1960. TYPE: ARGENTINA. Prov. Santa Cruz, Depto. Lago Argentino, Río Fósil, near to San Martín lake, 800 m, 22 Mar 1905. *Dusén s/n. pro parte* (Holotype: S!; isotype BM!). Basionym: *Botrychium lunaria* (L.) Sw. var. *dusenii* Christ, *Ark for Bot.* 6: 5. Figures 4–8. 1906.

Heterotypic synonyms:

Botrychium ramosum Asch. var. *patagonicum* Christ, *Ark. for Bot.* 6(3): Figures 2–3. 1906. \equiv *B. matricariifolium* A. Braun subsp. *patagonicum* (Christ) R.T. Clausen, *Mem Torrey Bot Club* 19 (2): 89. 1938. – TYPE: Holotype: ARGENTINA. *Ad Rio Fosiles haud procul a lacu San Martin in declibus denudatis c 800 msl*, 2 Mar 1905, *Dusén* 5939 (Holotype: P0125382!).

B. lunaria (L.) Sw. var. *antarctica* Speg., *Bol Acad Nac Ci* 27: 338. 1924 – LECTOTYPE here designated by Meza Torres: ARGENTINA. Prov. Tierra del Fuego. Depto. Ushuaia, Lapataia beach, 15 Jan 1924, *Spegazzini* 14243 (LP!). – PARATYPE: Isla

de los Estados, Puerto Roca (Penguin Rookery), 14 Mar 1882, *Spegazzini 14242* (LP!).

Description: Terrestrial plants, 3–11 cm high. Rhizomes erect, slender, roots fleshy generally perpendicular to the rhizome. Petioles succulent, base of petiole widened into a marcescent sheath, whitish or pale green. Trophophores sessile to short-stalked. Blades succulent, glabrous, up to 3.5 (4.5) cm long, 2.5 (3.5) cm wide, once pinnate. Pinnae lobed, spatulate or flabellate, arranged in 3–7 (9) pairs. Sporophores pinnate to bipinnate. Sporangia eusporangiate, brown, with transverse dehiscence (Figures 3(A) and 4).

Etymology: This species was dedicated to the Swedish naturalist, Dr Per Karl Hjalmar Dusén (1855–1926), who collected the type specimens of *Botrychium lunaria* var. *dusenii* and *B. ramosum* var. *patagonicum*.

Habitat and distribution: *Botrychium dusenii* is a species of a sub-antarctic climate, and occurs in Staten Islands, Malvinas Islands (Falkland Islands) and Argentinean and Chilean Patagonia, ascending the Andes Cordillera to Mendoza Province, near 32° south. In the classification of Cabrera and Willink (1980), these occurrences are within the Insular, Subantarctic and lower limit of High-Andean phytogeographical provinces (Figure 5). The elevational range is 0–3100 m above sea level, and its upper limit is recorded on The Inca's Bridge, Mendoza, Argentina (2740 m above sea level), it is possible that *B. dusenii* may occur at higher elevations. In Patagonia, this species grows in colonies of a few square meters (8–9 m² at most) in several habitats, such as: *Araucaria araucana* forests (Figure 3(B)), *Nothofagus* forest edges (Figure 3(C)), and on sea beach meadows (Figure 3(D)). The plants from Tierra del Fuego Island beaches appear to be salt tolerant, because they grow adjacent to salt water, on sandy, stony, or soil derived from the accumulation of marine mollusk shells. These areas may be submerged during extreme high tides or are exposed to the saline breezes from the sea. This species also occurs near small watercourses and mountain lakes. *Botrychium* grows in similar maritime habitats in southern Alaska, eastern Canada and Scandinavia.

Conservation status: For *B. dusenii*, the extent of occurrence is 1,023,683.552 km², and the area of occupancy (based on a user-defined cell width of 0.5 km, because the colonies of the subpopulations are very small) is 7.750 km². Field surveys conducted by the first author since 2008 in Argentine and Chilean Patagonia, found that this species is rare and difficult to find. In addition, populations appear to be decreasing in number. Some populations recorded in 2008 and 2009 could not be relocated in subsequent years. A possible cause for this is the emergence of invasive plants. For example, in the high hills of Punta Arena

(Region XII, Chile) where Hicken collected specimens in 1912, were in 2009 completely colonized by the invasive *Hieracium pilosella* L. with nearly 100% cover. Invasive plants are increasingly invading and occupying native plant habitat (Figure 3(E)).

The climate where *B. dusenii* grows appears to be warmer and drier than in the past, possibly a response to climate change. *Botrychium* in the northern hemisphere have successfully migrated northward with post-Pleistocene warming, now occupying previously glaciated areas in northern Europe, Iceland, and across northern North America (Stensvold, 2008). Many species have also migrated to previously glaciated high elevations in North America and Europe. While migration to higher altitudes may be possible for some populations of *B. dusenii*, southward migration is not an option for the southernmost populations. In addition to migration, another option, phenological adaptation to growth during a cooler, wetter season may be possible. This option appears to have been realized for *Botrychium campestre*, a species occupying the southernmost range of the genus in the Great Plains grasslands of North America. There *B. campestre* grows among native grassland vegetation, but only in early spring when weather conditions mimic those of mountain meadows and northern grasslands in mid-summer (Farrar, 2009).

Because of its rarity, habitat loss due to invasive plants and the potential effects of climate change, and following the IUCN red list categories (IUCN 2010), we consider to *Botrychium dusenii* to be Critically Endangered CR A2a,c,e.

Additional specimens examined: **Argentina. Prov. Chubut.** Depto. Río Singer, Lago Fontana, 11 Feb 1932, *Castellanos s/n* (BA 5901). Depto. Tehuelches, Lago Vintter, suelo arenoso, inmediaciones del lago, 16 Jan 1948, *Krapovickas 4154* (BAB). **Prov. Mendoza.** Depto. Las Heras, Puente del Inca, camino a Polvareda, 9 km E del Puente del Inca, 13 Jan 1963, *Boelcke et al. 9847* (BAA, SI). **Prov. Neuquén.** Depto. Picunches. Paso del Pino Achado, 38°39'57.24"S 70°50'21.84"W, 1562 msl, ladera sur de cerro, sotobosque de *Araucaria araucana*, junto a otras hierbas bajas, mantillo grueso, pocos individuos, aproximados en grupos de a 3 generalmente, 15 Jan 2008, *Meza Torres et al. 684* (CTES). **Prov. Santa Cruz.** Depto. Lago Argentino, Parque Nacional Los Glaciares, brazo norte, brazo del Ventisquero Upsala, bahía Chaura, 50°00'S 73°20'W, 19 Jan 1967, *Boelcke 12555* (BAA, BAB); in the valleys and mountains of the Cordillera of the Andes, in the vicinity of the south shores of Lake Argentine, from 30 to 50 km west of the Santa Cruz River, Nov 1907–Mar 1908, *Furlong 10* (NY); Glacier Upsala, Refugio Upsala, en costa pedregosa, lecho de antiguo río, 19°54'23"S 73°10'42"W, 13 Dec 2002,

Guerrido & Fernández 631 (SI); Montañas al Oeste del Lago Argentino Feb 1914, *Hauman s/n* (BA 1); Parque Nacional Los Glaciares, Jan–Feb 1953, Pérez Moreau 69745 (CTES); Ea. Fitzroy, Río de las Vueltas, ca. 850 msl, raro 4 Jan 1951, *Sleumer 1450* (LIL). **Prov. Tierra del Fuego.** Depto. Río Grande, Castillo 30 Jan 1942, *Castellanos s/n* (BA 45525); Ea. San Julio, 20 Dec 1972, *Goodall 4428* (BAB). Depto. Ushuaia. Hito 19, 6 Feb 1942, *Castellanos s/n* (BA 45524); Ea. Harberton, small bay at west end of airfield, end of flat in packed sand of upper beach, growing in *Azorella trifurcata* and *A. filamentosa*, 12 Nov 1967, *Goodall 982* (US); costado de la ruta “J”, 54°52'50,4"S 67°15'11,6"W, 15 msl, sotobosque de *Nothofagus*, único individuo, raro, 16 Jan 2009, *Meza Torres et al. 986* (CTES); Puerto Almanza, Canal de Beagle, 23 Dec 1932, *Castellanos s/n* (BA 7524); *ibidem*, Ea. Harberton, Campo Tropilla, Co. Fox Trap, 54°51'S 67°20'W, ca 30 msl, among Gunnera, in open ground, 5 Jan 1968, *Moore 1328* (BAA, K); Ushuaia, a 6–8 km de la población (54°50'47.70"S 68°18'39.83"W), entre el césped de la península, escasa, 9 Jan 1950, *Ruiz Leal & Carretero 12910* (LP, MERL); *ibidem*, road side, 30 Jan 1940, *Santesson s/n* (S 08–9408). Isla de los Estados, Puerto Roca, 26 Feb 1934, *Castellanos* (BA 12790), *ibidem* (Penguin Rookery), 14 Mar 1882, *Spegazzini 14242* (LP). Isla Soledad (East Falkland): Cape Penbroke at head of Whalebone Cove, very local in sandy soil in crevices of eroding sandstone on W side of Mary Hill, 10 Jun 164, *More 530* (K, LP, S, US). **Chile.** XI Región Aysen, Prov. Capitán Prat, Com. O'Higgins, Ventisqueros, ca. de 400 msl, 15 Feb 1932, *Donat 503* (SGO, SI). Prov. Coyhaique, Com. Coyhaique, en la orilla del camino de Coyhaique a Coyhaique Alto, lugar expuesto, con suelo pedregoso, 25 Nov 1981, *Barrera s/n* (SGO 100235 – SGO 100249). Prov. General Carrera, Com. Chile Chico, Guadal, *Nishida s/n* (SGO 106201). XII Región Magallanes y de la Antártica Chilena, Prov. Antártica Chilena, Com. Cabo de Hornos, Isla Navarino, Caleta Segura, ca. 11 km E of Puerto Navarino, ca 45 km W of Puerto Williams, 54°55'20"S, 68°12'52"W, ca. 0–6 msl, small rocky península of Beagle Channel, 24 Nov 2001, *Buck 41274* (NY). Prov. Magallanes, Com. Punta Arena, Punta Arena, 1 Oct 1899, *Hatcher s/n* (NY); *ibidem*, Feb 1922, *Gusinde s/n* (S08-9407); *ibidem*, frecuente en las lomas algo altas, entre el césped, 12 Jan 1904, *Hicken 21–22* (SI). Prov. Tierra del Fuego, Com. Timaukel, Estancia Vicuña, Pirámide 19 del límite internacional, 26 Feb 1931, *Donat 313* (BA, K, LIL, NY, SGO). Com. Timaukel, Fiordo de Parry, Seno Almirantazgo, en asociación herbácea costera, suelo arenoso, 13 Dec 1970, *Pisano 2866* (LP, MO); Fiordo de Parry, Bahía Cuevas, costa y bosque del litoral, en asociación herbácea costera, suelo ripio-

arenoso-orgánico, 21 Feb 1971, *Pisano 3072* (LP). Prov. Última Esperanza, Com. Torres del Paine, Lago Pehué, alt. ca. 200 msl, 26 Feb 1931, *Donat 410* (BA, K, LIL, SI); Parque Nacional Torres del Paine, clearing with low and varied plants, apparently natural, above first phase of *Nothofagus* forest..., growing on slopes, sandy soil, herb rare, 1 Jan 1981, *Sobel & Strudwick 2650* (NY).

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