



## ***Paspalum chilense* (Poaceae, Paspaleae): A new species from southern South America**

MARÍA PAZ CATANZARO<sup>1</sup>, MARISA G. BONASORA<sup>1</sup>, PABLO R. SPERANZA<sup>2</sup>, MARCELO MEDINA-NICOLAS<sup>3</sup>, JOSÉ F.M. VALLS<sup>4</sup> & GABRIEL H. RUA<sup>1</sup>

<sup>1</sup> Cátedra de Botánica Sistemática, Facultad de Agronomía, UBA. Av. San Martín 4453, C1417DSE Buenos Aires, Argentina.

E-mail: [catanzar@agro.uba.ar](mailto:catanzar@agro.uba.ar)

<sup>2</sup> Departamento de Biología Vegetal, Facultad de Agronomía-UDELAR, Uruguay.

<sup>3</sup> Laboratorio Taxonomía de Malezas, Servicio Agrícola y Ganadero (SAG), Región de la Araucanía, Temuco, Chile.

<sup>4</sup> Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Final W-5 Norte, Caixa Postal 02372, CEP 70770-917, Brasília, DF, Brasil.

### **Abstract**

*Paspalum chilense*, a new species from Chile belonging to the informal Notata group, is described and illustrated on the basis of morphological, cytological and molecular evidence. Our evidence supports its affinity with *P. barretoii* and *P. nummularium*, from Brazil, from which it probably diverged by vicariant speciation. The limited available material of the new species was previously identified as *P. pumilum* or *P. minus*. A new key to the species of the core Notata group is provided.

**Key words:** Notata group, *Paspalum chilense*, South America

### **Resumen**

*Paspalum chilense*, una nueva especie de Chile perteneciente al grupo informal Notata, es descrita e ilustrada en base a evidencia morfológica, citológica y molecular. Esta evidencia apoya su afinidad con *P. barretoii* y *P. nummularium* de Brasil, de las cuales probablemente ha divergido por especiación vicariante. El escaso material disponible de la nueva especie había sido previamente identificado como *P. pumilum* o *P. minus*. Se incluye una nueva clave para las especies del grupo core Notata.

**Palabras clave:** grupo Notata, *Paspalum chilense*, Sudamérica.

### **Introduction**

With about 350 species, *Paspalum* Linnaeus (1759: 855) is the largest genus in the Panicoideae. Its species are mostly distributed in natural grasslands in warm and temperate regions of the Americas. The genus is highly diverse in tropical regions but only a few species reach or exceed 40 degrees north and south in latitude.

The informal Notata group was first proposed by Chase (1929) to accommodate five perennial species with compressed culms, basal leaves, inflorescence of two conjugate racemes, and solitary spikelets. It included: *P. notatum* Flügge (1810: 106), after which the group was named, *P. serpentinum* Hochst. ex Steudel (1853: 22), *P. minus* Fournier (1886: 6), *P. pumilum* Nees (1829: 52) and *P. subciliatum* Chase (1929: 69). Barreto (1974) and Canto Dorow *et al.* (1996) expanded the original group and accepted nine species. Zuloaga *et al.* (2004) adopted a broadened concept by merging the group Linearia into Notata, and recognized 21 species. Recent phylogenetic analyses showed the Notata group *sensu lato* to be polyphyletic, but a core Notata clade is recovered in some analyses (Souza-Chies *et al.* 2006, Rua *et al.* 2010). This core Notata group should also include, on the basis of morphology, some other species not sampled in those phylogenies. Under such a concept, the core Notata group comprises the following species: *P. notatum*, *P. minus*, *P. pumilum*, *P. subciliatum*, *P. conduplicatum* Canto-Dorow, Valls & Longhi-Wagner (1995: 333),

*P. nummularium* Chase ex Send. & A.G. Burm. (1980: 487), *P. strigosum* Döll ex Chase (1929: 68), *P. bifidifolium* Soderstrom (1965: 3), which is probably a synonym of *P. pumilum*, and *P. barretoii* Canto-Dorow, Valls & Longhi-Wagner (1995: 332). This latter species has been considered a synonym of *P. minus* (Zuloaga & Morrone 2003, 2005, Zuloaga *et al.* 2004), but we tentatively prefer to maintain it as a separate species on the basis of morphological and cytological considerations as explained below. The taxonomic status of all species in the core Notata group will be further addressed in a forthcoming paper.

In the present paper we describe and illustrate *Paspalum chilense*, a new species endemic to southern Chile, at the southern extreme of the distribution of the genus. The new species belongs to the core Notata group and has remained unnoticed because the scarce material available had been previously misidentified as *P. pumilum* or *P. minus* (Acevedo de Vargas 1947, Zuloaga *et al.* 2004). Morphological, cytological, and molecular evidence from new collections confirms the affinity of this new species with the other species of this group, and especially with the southern Brazilian species *P. barretoii* and *P. nummularium*, from which it probably diverged when the western populations became isolated. A new key to the species of the core Notata group is provided.

## Materials and methods

Descriptions and illustrations were based on field collections and herbarium material (BAA, M, SI). Morphological characters were studied under a Wild Heerbrugg M5 stereoscopic microscope. Measures of spikelet length and width were scored from herbarium material (Appendix 1) of the new species (four specimens) and the most closely related species: *P. barretoii* (five specimens), *P. pumilum* (26 specimens), *P. nummularium* (five specimens), *P. conduplicatum* (three specimens), and *P. minus* (14 specimens). Each specimen was represented by measurements from one spikelet taken from the middle, most homogeneous portion of a raceme. The spikelet length/width ratio was plotted against spikelet length to graphically represent allometric differences. The specimens, which are listed on Appendix 1, were plotted on a map to compare their geographical distributions.

When the type collection (Rua *et al.* 918) was made, some living tillers were separated and kept under cultivation at the 'Lucien Hauman' Botanical Garden of the University of Buenos Aires (Buenos Aires, Argentina) for cytogenetic and anatomical studies. For cytogenetic analyses, young inflorescences were collected from these plants fixed in 3:1 (absolute ethanol: glacial acetic acid) for 24 h and stored in ethanol 70% at 4°C. Microsporocytes were prepared by squashing and staining the inflorescences with acetic carmine (Burson 1992). Photographs were taken with a Zeiss AxioPlan optical microscope and a Zeiss AxioCam ERc 5S camera, and edited using standard image-edition software.

Leaf blades obtained from living material were fixed in 70% ethanol. Freehand transverse sections were obtained from the middle portion of the blades and maintained in fresh water. The histological samples were washed in an aqueous solution 1:1 of sodium hypochlorite, rinsed, stained with Safranin, and mounted in Glycerol jelly (D'Ambrogio de Argüeso 1986, Zarlavsky 2014). Observations were made using a Zeiss AxioPlan optical microscope and a Zeiss AxioCam ERc 5S camera. For anatomical descriptions we followed the terminology proposed by Ellis (1976).

Phylogenetic relationships of *P. chilense* and related species were assessed using cpDNA sequences from the following regions: the *trnL*(UAA) intron, the *trnL*(UAA)–*trnF*(GAA) spacer, the *atpB*–*rbcl* spacer, and the *trnG*(UCC) intron. DNA from *P. chilense*, *P. barretoii*, *P. pumilum*, *P. nummularium*, *P. conduplicatum*, and *P. minus* was extracted, and the four target regions were amplified and sequenced following the procedures described in Rua *et al.* (2010), so that 24 new sequences were generated (Table 1). Additionally, sequences of *P. notatum*, *P. plicatulum* Michaux (1803: 45), and *P. dilatatum* Poiret (1804: 35) from a previous study (Rua *et al.* 2010) were included in the analysis. The sequences of *P. dilatatum* were used for tree rooting. Voucher specimens are listed in Table 1, indicating species, origin, chromosome number, and GenBank accession numbers for new and previously published sequences.

The sequences were aligned with the Clustal-W (Thompson *et al.* 1994) algorithm implemented in BioEdit (ver. 7.0.9.0, Hall 1999) and the resulting alignments were manually adjusted. The final matrix contained 2554 positions, of which only 12 were informative. Indels were coded as present/absent when the alignment of the flanking sequences was unambiguous. Following that criterion only two indels were considered informative. Parsimony analysis was performed using TNT ver. 1.1 (Goloboff *et al.* 2003b, 2008) after disabling uninformative characters. Since the data set was small, an exhaustive search was performed using implicit enumeration ("branch-and-bound" algorithm). Group support was quantified through Bremer support (Bremer 1994) and symmetric jackknife frequencies (Goloboff *et al.* 2003a). The data matrix and trees were submitted to TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2: S16968>).

**TABLE 1:** List of *Paspalum* species studied for phylogenetic analysis, voucher specimens and GenBank accession numbers (sequences in bold are new in this study).

Species	Voucher	<i>atpB</i> – <i>rbcL</i>	<i>trnG</i> intron	<i>trnL</i> intron	<i>trnL</i> – <i>trnF</i>
<i>P. barretoii</i>	Rua & Córdova 736 (BAA)	<b>KM396455</b>	<b>KM437864</b>	<b>KM437858</b>	<b>KM437875</b>
<i>P. chilense</i>	Rua <i>et al.</i> 918 (BAA)	<b>KM437870</b>	<b>KM437865</b>	<b>KM437859</b>	<b>KM437876</b>
<i>P. conduplicatum</i>	Valls <i>et al.</i> 14854 (CEN)	<b>KM437871</b>	<b>KM437866</b>	<b>KM437860</b>	<b>KM437877</b>
<i>P. dilatatum</i>	MVFA26505 (MVFA)	EU627221	EU627299	EU627377	U627455
<i>P. minus</i>	Valls <i>et al.</i> 14573 (CEN)	<b>KM437872</b>	<b>KM437867</b>	<b>KM437861</b>	<b>KM437878</b>
<i>P. notatum</i>	Rua <i>et al.</i> 296 (BAA)	EU627250	EU627328	EU627406	EU627484
<i>P. nummularium</i>	Rua & Córdova 737 (BAA)	<b>KM437873</b>	<b>KM437868</b>	<b>KM437862</b>	<b>KM437879</b>
<i>P. plicatulum</i>	Honfi 14 (CTES)	EU627257	EU627335	EU627413	EU627491
<i>P. pumilum</i>	Rua 716 (BAA)	<b>KM437874</b>	<b>KM437869</b>	<b>KM437863</b>	<b>KM437880</b>

## Taxonomic treatment

*Paspalum chilense* Catanzaro & G.H. Rua, *sp. nov.* (Figs. 1, 2A–C).

Type:—CHILE. Región de los Ríos: provincia del Ranco, Lago Ranco, río Calcurrupe, 40°13'32.8"S, 72°14'22.4"W, 81 m, 18 December 2007, G.H. Rua, M. Medina-Nicolas & E. Piel 918 (Holotype (here designated) BAA, isotype CONC).

*Paspalum nummularium* simile, sed spiculis elongatoribus, breviteribus apiculatis et ligulis conspicue longioribus differt.

Cespitose perennials forming dense tufts. Tillers radiate spreading to ascending, their bases forming short slender rhizomes; culms 3–12 cm tall, ca. 0.9 mm diameter, 2–3-noded, unbranched, internodes compressed, glabrous. Leaves mostly crowded at the base. Leaf sheaths striate, keeled, glabrous, the lower short, overlapping. Ligule ca. 1.4 mm long, with a row of hairs ca. 0.3 mm long behind it. Blades flat, 2–4(–12) cm long, 2–4 mm wide, coarse pubescent on both surfaces; the blade of the uppermost leaf sharply reduced. Peduncles 2–6.5 cm long, glabrous. Inflorescence shortly exserted, composed of 2 subconjugate racemes, ascending to reflexed, 1.4–2.5 cm long; rachis ca. 0.8 mm wide. Spikelets 1.8–2 mm long, 1.4–1.5 mm wide, widely elliptical, apiculate at the apex; glume and sterile lemma subequal, firm, smooth and shiny, glume 5-nerved with the lateral nerves approximating the margins, lemma 3-nerved; upper floret ca. 1.7–1.9 mm long, widely elliptical, pale, minutely papillose, the palea slightly depressed, lodicules 2, ca. 0.3 mm long, truncate; stamens 3, anthers 0.6–0.8 mm long, purple; stigmas 2, deep purple. Caryopsis not seen.

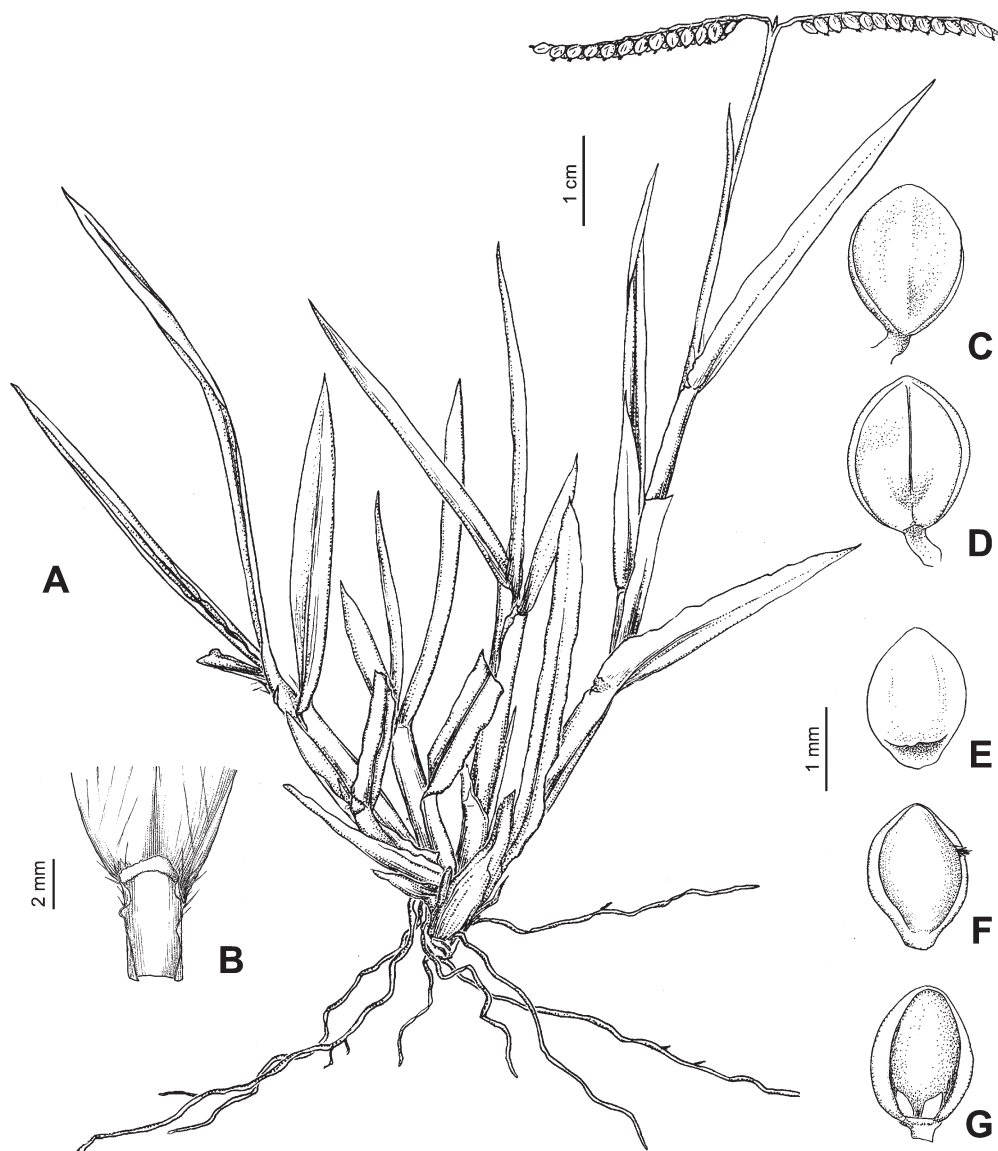
**Habitat and distribution:**—*Paspalum chilense* is known from southern Chile, between ca. 36° and 41° S, in the regions VIII ‘Biobío’, IX ‘Araucanía’ (in both regions known only from ancient collections) and XIV ‘Los Ríos’ (Fig. 3). The only recent collection was made in the vicinity of Lago Ranco (Region XIV), on the slopes of the Calcurrupe river valley, in meadow patches surrounded by woody areas (Fig. 2) covered by a *Nothofagus dombeyi*–*Eucryphia cordifolia* forest, of the type known as ‘Bosque Laurifolio Templado Interior’ (Luebert & Pliscoff 2006). This region has an average annual rainfall of 2500–4000 mm and an annual average temperature of 6–9°C with 50 to 150 days of frost per year (Moreno-González 2011).

**Etymology:**—The epithet refers to the Chilean endemic distribution of the new species.

**Taxonomic observations:**—The new species belongs to the Notata group *sensu stricto* (corresponding to the ‘core Notata’ group of Souza-Chies *et al.* 2006), which comprises perennial grasses with flat leaf blades at least 2 mm wide, racemes 2(3), conjugate or subconjugate, spikelets solitary, green to pale, ovate to suborbicular. Besides *P. chilense*, this group includes *P. barretoii*, *P. bifidifolium*, *P. conduplicatum*, *P. minus*, *P. notatum*, *P. nummularium*, *P. pumilum*, *P. strigosum*, and *P. subciliatum* (the latter two species were not sampled in the phylogenetic analysis).

The new species has been overlooked since specimens belonging to it had been cited elsewhere under *P. pumilum* (Acevedo de Vargas 1947) or *P. minus* (Zuloaga *et al.* 2004). Nevertheless, *P. chilense* is a morphologically and cytologically distinct species. It is the only species in the group having ligules more than 1 mm long. Furthermore, spikelet shape and size (Fig. 4) distinguish the new species from both *P. pumilum* (broadly ovate, apiculate vs. elliptical to ovate spikelets) and *P. minus* (spikelets up to 2 mm long vs. more than 2.2 mm long). *Paspalum chilense* is most similar to *P. nummularium*, from which it differs by having broadly ovate, apiculate (vs. orbicular, obtuse) spikelets and ligules 1.4

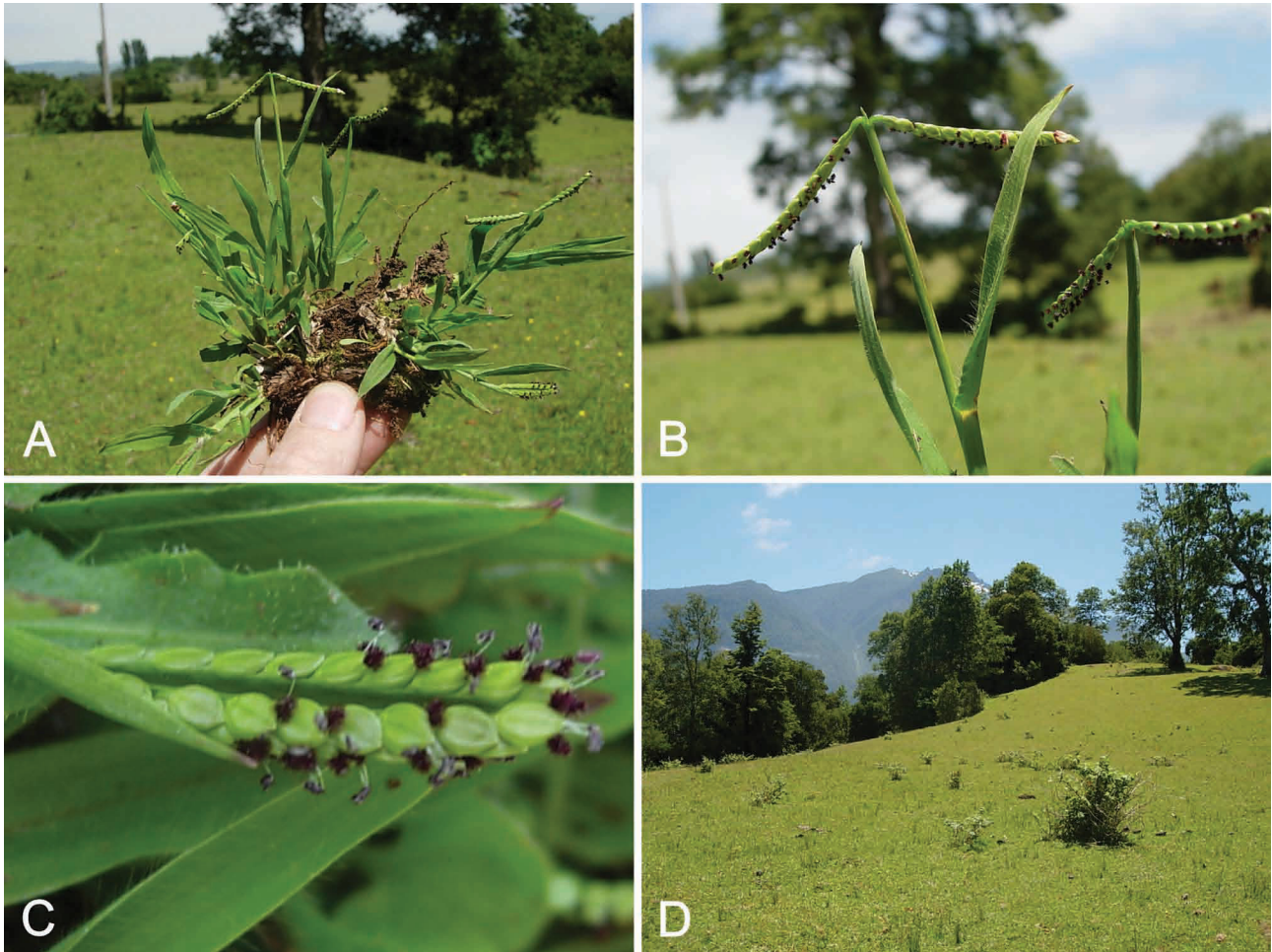
mm long (vs. 0.2–0.4 mm). On the other hand, the spikelets of the new species are very similar in shape to those of *P. barretoii*, but they do not exceed 2 mm in length (vs. 2.2–2.6 mm long in *P. barretoii*). When length/width ratios were plotted against absolute length, the specimens of *P. chilense* were well discriminated from the other species of the core Notata group (Fig. 5). The points representing specimens of *P. chilense* clustered together between *P. nummularium* and *P. pumilum*, the two species of the core Notata group with the smallest spikelets (although *P. pumilum* can also have longer spikelets).



**FIGURE 1.** *Paspalum chilense* sp. nov. A. Habit. B: Detail of ligule. C. Spikelet, abaxial view. D. Spikelet, adaxial view. E. Upper floret, adaxial view. F. Upper floret, abaxial view. G. Upper floret, palea and lodicules. Drawn from the type specimen (*Rua et al. 918*) by Natalia Gómiz.

**Leaf anatomy:**—Leaf blade expanded to open V-shaped in cross section. Midrib conspicuous, with abundant colorless parenchyma. Adaxial epidermis with bulliform cells and common epidermal cells scarcely differentiated. Ab-

axial epidermal cells with inflated external walls. Cuticles thin. Stomata present in both epidermal surfaces. Mesophyll composed of radial chlorenchymatous cells. Three orders of vascular bundles present, but bundles of second and third order obscurely differentiated, all vascular bundles surrounded by a single bundle sheath of parenchymatous cells; the bundles separated by 2–3 parenchyma cells. Abaxial and adaxial sclerenchyma caps associated with first and second order vascular bundles (Fig. 6).



**FIGURE 2.** *Paspalum chilense* sp. nov. Field photographs from the type collection. A. Plant, showing the cespitose habit. B. Inflorescences, each composed of two subconjugate, divergent racemes. C. Detail of spikelets at anthesis. D. Habitat, in the vicinity of Lago Ranco, Chile. Photographs by G. H. Rua.

**Cytology:**—Chromosome counting of the *typus* material revealed that *P. chilense* is a diploid species with  $2n=20$  chromosomes. Meiotic behavior is normal, with formation of 10 bivalents (Fig. 7). *Paspalum chilense*, here newly recognized as a distinct species, and *P. barretoii*, currently included in the synonymy of *P. minus* (Zuloaga & Morrone 2003, 2005, Zuloaga *et al.* 2004), have been considered conspecific with *P. minus*, an apomictic pentaploid widely distributed in tropical America from Mexico and Cuba to Paraguay (Davidse & Pohl 1978, Honfi *et al.* 1990, Bonilla & Quarín 1997, Honfi 2003). Other ploidy levels have been reported for *P. minus*; however, the diploid cytotypes of *P. minus* reported by Banks (1966) correspond to *P. pumilum*, based on observations of the specimens *Banks 3395* and *Banks 3500* at MO (images available at <http://tropicos.org/Name/25509844>). The identities of the tetraploids reported by Gould (1958) and Davidse & Pohl (1978) under *P. pumilum* could not be confirmed. The specimen *Gould 7568* has not been available to us, whereas the specimen *Davidse 5378* (MO, image available at <http://tropicos.org/Image/100283298>) corresponds to *P. minus*. In addition, the reported chromosome count for *Davidse 5378* was imprecise ( $n=ca. 20$ ) so a pentaploid ( $2n=5x=50$ ) complement cannot be confidently ruled out for this individual. Consequently, besides morphological differentiation, specimens of the closely related *P. chilense* and *P. barretoii*, which inhabit relatively high and cold areas in southern Chile and southern Brazil respectively, exclusively comprise sexual diploids ( $2n=20$ ), a chromosome number not reported with certainty for *P. minus*.

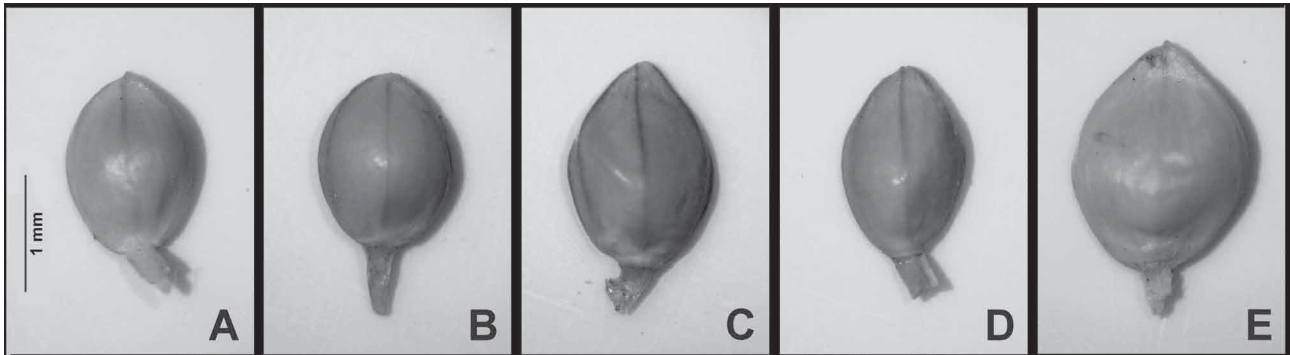


**FIGURE 3.** Distribution of *Paspalum chilense* sp. nov. and the related species *P. barretoii*, *P. pumilum*, *P. nummularium*, *P. conduplicatum*, and *P. minus*. The new species occurs near the southern limit of the generic distribution.

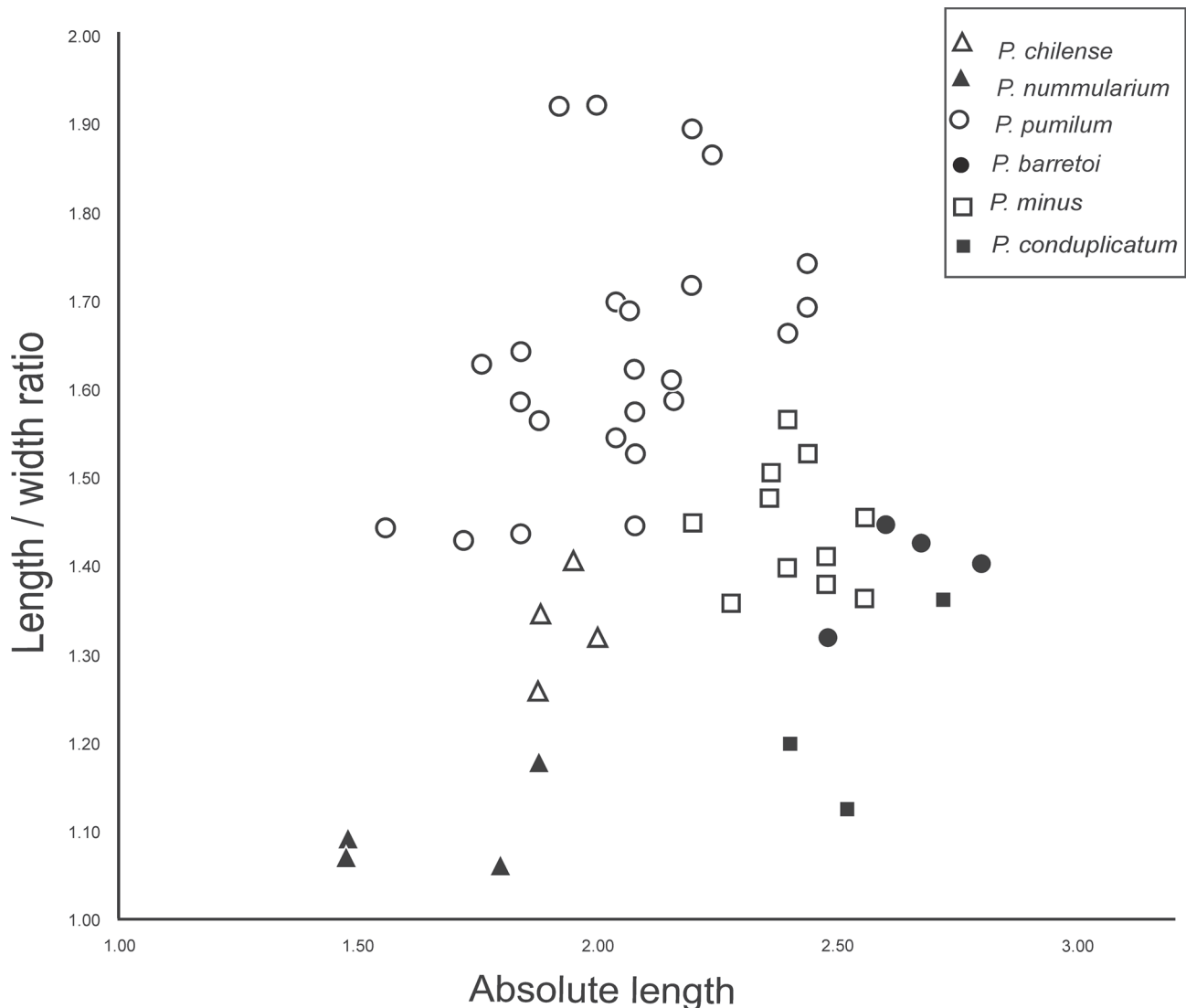
**IUCN Red List category:**—Recent collections of this species are only known from Lago Ranco, Chile. Two specimens conserved in the herbarium M document the occurrence of the species in a much broader area in the last decade of the XIX century, suggesting that the extent of occurrence of the species has been reduced or that it simply has not been re-collected there. In view of this, we consider this a rare and/or inconspicuous species. *Paspalum chilense* should be considered in the category of Endangered (EN) according to IUCN Red List criteria (IUCN 2001), because of its restricted geographical distribution. Further field data are necessary to confirm this conservation status.

Since conservation policies are mostly based on species lists, species usually constitute the unit to be conserved. However, the application of the term ‘species’ to a group of organisms can be considered in dramatically different ways

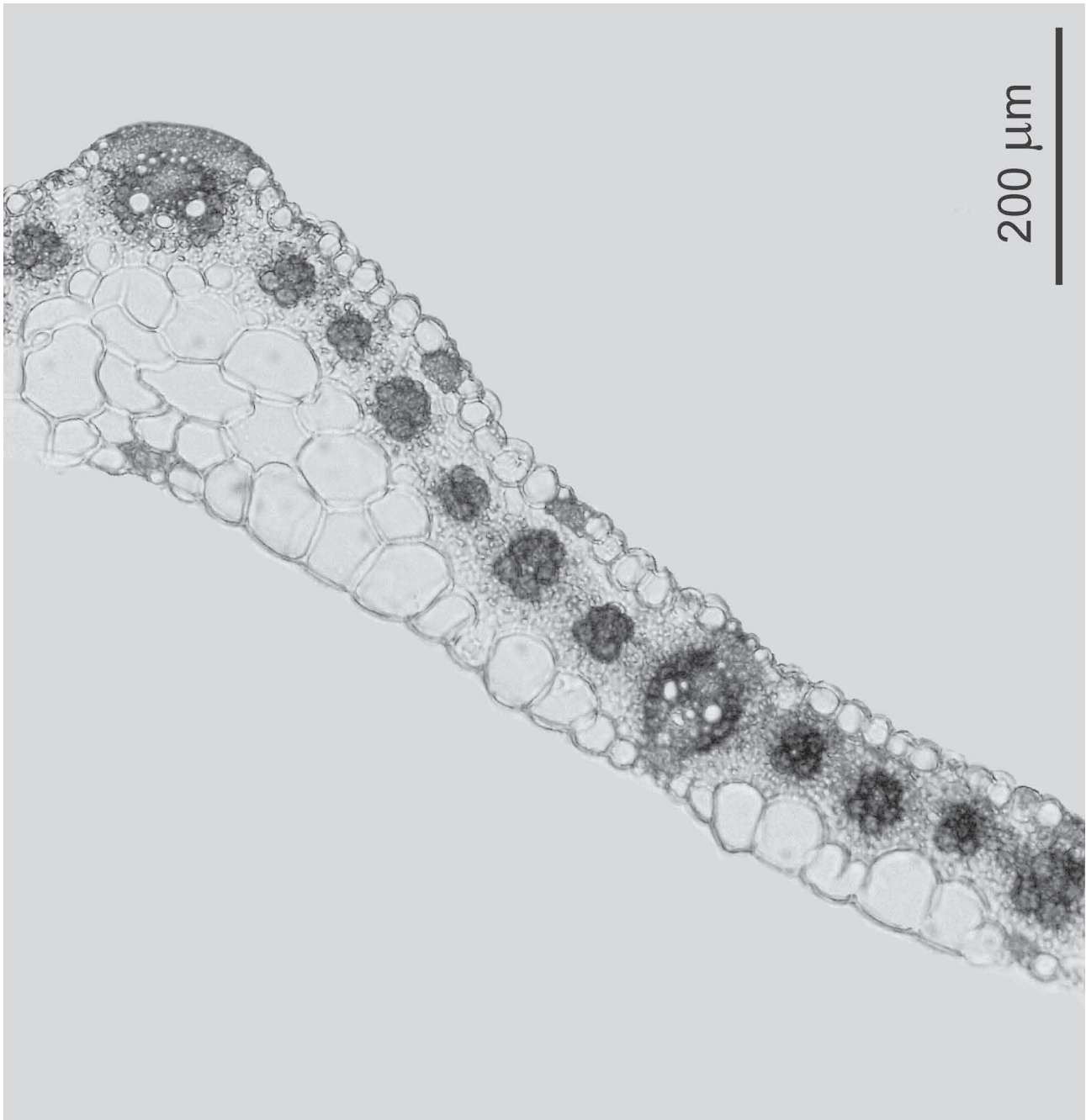
depending on the species concept applied (Agapow *et al.* 2004 and references therein). Cytologically, ecologically, or reproductively differentiated populations that are not easily diagnosable in herbarium specimens, may go unnoticed to conservation policy makers. This seems to be the case of *P. chilense* and *P. barretoii*, two species that are threatened due to the limited size and high specificity of their geographical ranges. Indeed, the identity of both diploid species has been obscured under the synonymy of *P. minus*, and therefore they remained overlooked as threatened taxa.



**FIGURE 4.** Comparative view of spikelets of A. *Paspalum chilense* sp. nov. (Rua *et al.* 918). B. *P. barretoii* (Rua *et al.* 736). C. *P. minus* (Valls 15229). D. *P. nummularium* (Rua *et al.* 737). E. *P. pumilum* (Parodi 163).



**FIGURE 5.** Spikelet length/width ratio vs. spikelet length plot showing differences in shape and size between *P. chilense* sp. nov. and the related species *P. nummularium*, *P. barretoii*, *P. pumilum*, *P. minus*, and *P. conduplicatum*.



**FIGURE 6.** *Paspalum chilense* sp. nov. Transverse section of the leaf blade (from the type collection).

**Phylogenetic relationships:**—In the parsimony analysis, two most parsimonious trees were found (17 steps, CI=0.85, RI=0.65). *Paspalum chilense*, *P. minus*, *P. pumilum*, *P. nummularium*, and *P. barretoii* group together into a well-supported clade, sister to *P. notatum*. Phylogenetic relationships within this clade were poorly resolved (Fig. 8).

**Biogeography:**—The discovery of *P. chilense* in southern Chile has interesting biogeographical implications since it provides additional evidence for the relationships between the floras of southern Chile and southern Brazil. Indeed, many floristic elements are shared between both regions, particularly concerning the peat soil area along the humid southeastern edges of the Brazilian ‘Planalto meridional’, in the states of Santa Catarina and Rio Grande do Sul. This region is inhabited by several plant genera clearly related to the Austral–Antarctic flora, such as *Araucaria*, *Drymis*, *Escallonia*, *Podocarpus*, *Gunnera*, *Acaena*, *Fuchsia*, *Griselinia* (Rambo 1953), and *Alstroemeria* (Aagesen & Sanso 2003). It has been postulated (Villagrán & Hinojosa 1997) that there was a geographical continuity of forests across subtropical South America until the Miocene, which would explain the floristic relationships between southern Chilean and southern Brazilian forests. This extensive forest area would have been fragmented as a consequence of the formation of a South American ‘Arid Diagonal’ during the Pliocene. Interestingly, the only other species of *Paspalum*



occurring in southern Chile, the sexual tetraploid *P. dasypleurum* Kunze ex Desvaux (1854: 242), also has a closely related counterpart in the Brazilian Southern Planalto: the also sexual tetraploid *P. dilatatum* biotype 'Vacaria', which may deserve species status (Speranza 2009). Moreover, both the Notata and Dilatata groups include cold-tolerant species from temperate southern regions, and show disjunct distribution patterns with greater diversity in eastern areas and some isolated representatives in the west.

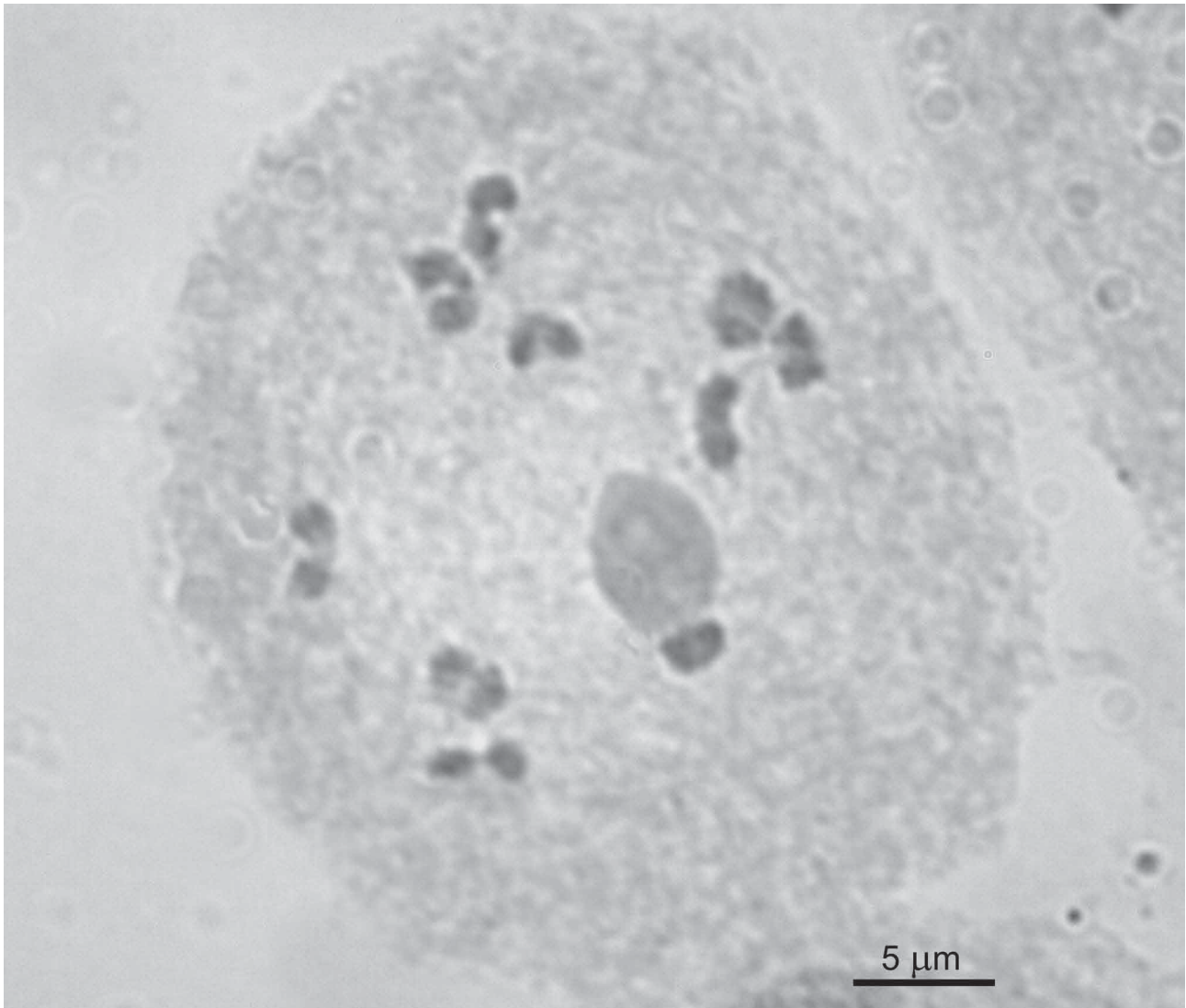


FIGURE 7. Meiotic microsporocyte of *Paspalum chilense* sp. nov. (Rua et al. 918) at diakinesis showing 10II.

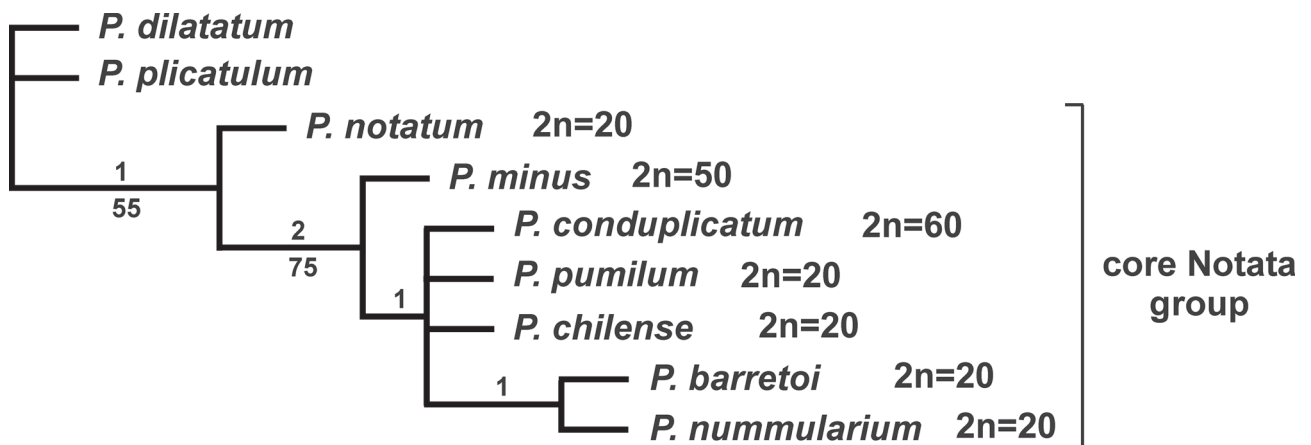


FIGURE 8. Phylogenetic relationships of *P. chilense* sp. nov. and related species in the Notata group s.s., obtained from four cp-DNA markers. Strict consensus of two most parsimonious trees (length=17). Numbers above branches indicate Bremer support, those below branches represent symmetric jackknifing frequencies. Chromosome numbers are indicated beside each species name.

**Additional specimens examined:**—CHILE. Región de la Araucanía: provincia de Cautín, Villarrica, 1897, *F.W. Neger s.n.* (M). Región del Biobío: provincia Concepción, Concepción, 1893–96, *F.W. Neger s.n.* (M). Región de los Ríos: provincia del Ranco, Lago Ranco, río Calcarrupe, 19 December 1944, *O. Boelcke 324* (BAA, SI).

## Key to the species of *Paspalum*, core Notata group

1. Spikelets (2.8–)3–4 mm long; plants creeping ..... *P. notatum*
- Spikelets up to 2.8 mm long; plants cespitose ..... 2
2. Spikelets pilose, at least on the upper glume ..... 3
- Spikelets glabrous ..... 4
3. Spikelets 2.4–2.7 mm long, ovate ..... *P. subciliatum*
- Spikelets ca. 2 mm long, broadly ovate ..... *P. strigosum*
4. Proximal leaf sheaths keeled, conduplicate; spikelets ca. 2 mm wide ..... *P. conduplicatum*
- Proximal leaf sheaths not keeled, convolute; spikelets usually up to 1.9 mm wide (occasionally up to 2 mm in *P. barretoii*). .....5
5. Spikelets more than 2.2 mm long and 1.5 mm width .....6
- Spikelets generally up to 2.1 mm long, width 1–1.7 mm (up to 2.4 mm long in *P. pumilum*, but then less than 1.5 mm width) ... 7
6. Racemes ascending at maturity, conjugate to approximate, the internode between them rarely reaching 5 mm long; spikelets acute ..... *P. minus*
- Racemes reflexed at maturity, subconjugate, separated by an internode more than 5 mm long; spikelets shortly apiculate ..... *P. barretoii*
7. Spikelets elliptical to ovate, length/width ratio greater than or equal to 1.4. .... 8
- Spikelets broadly ovate to orbicular, length/width ratio up to 1.4 ..... 9
8. Leaf blades conspicuously bifid at apex ..... *P. bifidifolium*
- Leaf blades not bifid at apex ..... *P. pumilum*
9. Spikelets obtuse, length/width ratio up to 1.2; ligule 0.2–0.4 mm long. .... *P. nummularium*
9. Spikelets apiculate, length/width ratio greater than 1.2; ligule ca. 1.4 mm long. .... *P. chilense*

## Acknowledgments

We are indebted to the following persons and institutions: Eduardo Piel-Bohmwald for his invaluable assistance in field work, Natalia Gomiz for her excellent illustrations, Sandra Aliscioni for help on the anatomical description, Máximo Gauto Acosta for revision of the Latin diagnose, Jeffery M. Saarela and two anonymous reviewers, and CONICET (‘Consejo Nacional de Investigaciones Científicas y Técnicas’), ANPCyT (‘Agencia Nacional de Promoción Científica y Tecnológica’) and Universidad de Buenos Aires (Argentina) for financial support.

## References

- Aagesen, L. & Sanso, A.M. (2003) The phylogeny of the Alstroemeriaceae, based on morphology, *rps16* intron, and *rbcL* sequence data. *Systematic Botany* 28: 47–69.
- Acevedo de Vargas, R. (1947) Sobre *Paspalum pumilum* Nees en Chile. *Boletín Museo Nacional Historia Natural* 23: 43–46.
- Agapow, P.M., Bininda-Emons, O.R.P., Crandall, K.A., Gittleman, J.L., Mace, G.M., Marshall, J.C. & Purvis, A. (2004) The impact of species concept on biodiversity studies. *The Quarterly Review of Biology* 79: 161–179.  
<http://dx.doi.org/10.1086/383542>
- Banks, D.J. (1966) *Paspalum minus* (Gramineae) in Louisiana and Mississippi. *Rhodora* 68: 94–96.
- Barreto, I.L. (1974) *O gênero Paspalum (Gramineae) no Rio Grande do Sul. Dissertação (Livro Docência-Fitotecnia)*. Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, 258 pp.
- Bonilla, J.R. & Quarin, C.L. (1997) Diplosporous and aposporous apomixis in a pentaploid race of *Paspalum minus*. *Plant Science* 127: 97–104.  
[http://dx.doi.org/10.1016/S0168-9452\(97\)00111-8](http://dx.doi.org/10.1016/S0168-9452(97)00111-8)
- Bremer, K. (1994) Branch support and tree stability. *Cladistics* 10: 295–304.  
<http://dx.doi.org/10.1111/j.1096-0031.1994.tb00179.x>
- Burson, B.L. (1992) Cytogenetic relationships between *Paspalum dilatatum* and *P. cromyorrhizon*, *P. indecorum*, and *P. laxum*. *Plant Science* 153: 244–249.

<http://dx.doi.org/10.1086/297028>

- Canto-Dorow, T.S., Valls, J.F.M., & Longhi-Wagner, H.M. (1995) Espécies novas de *Paspalum* L. (Poaceae) do sul do Brasil. *Bradea* 6: 331–336.
- Canto-Dorow, T.S., Longhi-Wagner, H.M. & Valls, J.F.M. (1996) Revisão taxonômica das espécies de *Paspalum* L., grupo Notata (Poaceae–Paniceae) do Rio Grande do Sul, Brasil. *Iheringia* 47: 3–44.
- Chase, A. (1929) The North American species of *Paspalum*. *Contributions from the United States National Herbarium* 28: 1–310.
- D'Ambrogio de Argüeso, A.C. (1986) Manual de técnicas en histología vegetal. Hemisferio Sur, Buenos Aires.
- Davidse, G. & Pohl, R.W. (1978) Chromosome numbers of tropical American grasses (Gramineae). *Annals of the Missouri Botanical Garden* 65: 637–649.  
<http://dx.doi.org/10.2307/2398863>
- Desvaux, E. (1854) CXLVI. Gramíneas. In: Gay, C. *Flora Chilena* 6, Fain & Thunot, Paris, pp. 233–469, pl. 74–83.
- Ellis, R.P. (1976) A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section. *Bothalia* 12: 65–109.
- Flüggé, J. (1810) *Graminum Monographiae. Pars I. Paspalum. Reimaria*. F. Perthes & J. H. Besser, Hamburg, pp. 1–224.
- Fournier, E.P.N. (1886) *Mexicanas Plantas. Pars secunda Gramineae*. Typographeo Reipublicae, Paris, pp. 1–160.
- Gould, F.W. (1958) Chromosome numbers in Southwestern grasses. *American Journal of Botany* 45: 757–767.  
<http://dx.doi.org/10.2307/2439737>
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98
- Honfi, A.I. (2003) Citoembriología de poliploides impares en el género *Paspalum* L. (Panicoideae: Gramineae). Doctoral Dissertation, Universidad Nacional de Córdoba, Argentina, pp. 1–203.
- Honfi, A.I., Quarin, C.L. & Valls, J.F.M. (1990) Estudios cariológicos en gramíneas sudamericanas. *Darwiniana* 30: 87–94.
- IUCN (2001) *The IUCN red list categories and criteria*, Version 3.1. IUCN Red List Unit, Cambridge U.K. Available from: <http://www.iucnredlist.org/> (accessed 5 March 2014).
- Linnaeus, C. (1759) *Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus II. Editio decima, reformata*. Laurentius Salvius, Stockholm, pp. 825–1384.
- Luebert, F. & Pliscoff, P. (2006) *Sinopsis bioclimática y vegetal de Chile*. Editorial Universitaria, Santiago.
- Moreno-Gonzalez, R.A. (2011) *Flora del Parque Futangue, Lago Ranco, Región de los Ríos*. Universidad Austral de Chile, Valdivia.
- Nees von Esenbeck, C.G. (1829) *Agrostologia Brasiliensis, seu, Descriptio graminum in imperio Brasiliensi huc usque detectorum*. In: Martius, C.F.P. (Ed.) *Flora Brasiliensis seu Enumeratio Plantarum* 2(1). J.G. Cotta, Stuttgart and Tübingen, 608 pp.
- Poiret, J.L.M. (1804) *Encyclopédie Méthodique, Botanique* 5. H. Agasse, Paris, pp. 1–748.
- Rambo, S.J.B. (1953) História da flora do Planalto Rio-Grandense. *Anais Botânico do Herbário Barbosa Rodrigues, Itajaí* 5: 1–50.
- Rua, G.H., Speranza, P.R., Vaio, M. & Arakaki, M. (2010) A phylogenetic analysis of the genus *Paspalum* (Poaceae) based on cpDNA and morphology. *Plant Systematics and Evolution* 288: 227–243.  
<http://dx.doi.org/10.1007/s00606-010-0327-9>
- Sendlusky, T., Burman, A.G. (1980) A new species of *Paspalum* (Gramineae) from Brazil. *Brittonia* 32(4): 487–489.  
<http://dx.doi.org/10.2307/2806154>
- Souza-Chies, T.T., Essi, L., Rua, G.H., Valls, J.F.M. & Miz, R. (2006) A preliminary approach to the phylogeny of the genus *Paspalum* (Poaceae). *Genetica* 126: 15–32.  
<http://dx.doi.org/10.1007/s10709-005-1428-1>
- Soderstrom, T.R. (1965) Gramineae. In: Maguire, B. *et al.*, The botany of the Guayana Highland-Part VI. *Memoirs of the New York Botanical Garden* 12: 1–7.
- Speranza, P.R. (2009) Evolutionary patterns in the Dilatata group (*Paspalum*, Poaceae). *Plant Systematics and Evolution* 282: 43–56.  
<http://dx.doi.org/10.1007/s00606-009-0205-5>
- Studel, E.G. (1853) *Synopsis Plantarum Glumacearum* 1. Stuttgartiae, J.B. Metzler.
- Thompson, J.D., Higgins, D.G., & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.
- Villagrán, C. & Hinojosa, L.F. (1997) Historia de los bosques del sur de Sudamérica, II: Análisis fitogeográfico. *Revista Chilena de Historia Natural* 70: 241–267.
- Zarlavsky, G.E. (2014) *Histología Vegetal: técnicas simples y complejas*. 1a ed. Buenos Aires: Sociedad Argentina de Botánica, pp 1–198.
- Zuloaga, F.O. & Morrone, O. (2003) *Paspalum*. In: Zuloaga, F.O., Morrone, O., Davidse, G., Filgueiras, T.S., Peterson, P.M., Soreng, R.J. & Judziewicz, E.J. (Eds.) *Catalogue of New World Grasses (Poaceae): III. Subfamilies Panicoideae, Aristidoideae, Arundinoideae, and Danthoioideae*. *Contributions from the United States National Herbarium* 46: 1–662.

- Zuloaga, F.O. & Morrone, O. (2005) Revisión de las especies de *Paspalum* para América del Sur Austral (Argentina, Bolivia, sur de Brasil, Chile, Paraguay y Uruguay). *Monographs in Systematic Botany from the Missouri Botanical Garden* 102: 1–297.
- Zuloaga, F.O., Pensiero, J. & Morrone, O. (2004) Systematics of *Paspalum* Group Notata (Poaceae–Panicoideae–Paniceae). *Systematic Botany Monographs* 71: 1–75.  
<http://dx.doi.org/10.2307/25027926>

## Appendix 1.

### Specimens analyzed for spikelet allometry and geographical distribution.

***Paspalum barretoii*:** Boldrini & Eggers 1345 (ICN), Longhi-Wagner et al. 2290 (ICN), Rua & Córdova 731, 736 (BAA), Zanin et al. 917 (ICN). ***P. chilense*:** Neger s.n. (M), Neger s.n. (M), Boelcke 324 (BAA, SI), Rua et al. 918 (BAA). ***P. conduplicatum*:** Valls 1912, Valls et al. 6913, 14838 (CEN). ***P. minus*:** Buchtien 86 (BAA), Ciuffi 4 (BAA), Hitchcock s.n. (BAA), Killeen 1521 (SI), 1584 (SI), Zuloaga et al. 9569 (SI), Norrmann et al. 169 (BAA), Hitchcock s.n. (Amer. Gr. Nat. Herb. 915, BAA), Hassler 12546 (BAA), Bourgeau s.n. (P, photograph), Davidse 5378 (MO, photograph), Valls et al. 7652 (CEN), 15229 (BAA), Vázquez & Rojas 22691 (SI). ***P. nummularium*:** Chase 8436, 9326 (US), Rua & Córdova 737 (BAA), Silva 331, 1003 (BAA). ***P. pumilum*:** Black 54–18159 (BAA), Hatschbach 3009, 3713 (BAA), Hitchcock 10350 (BAA), Parodi 12210 (BAA), Rambo 55070 (BAA), Ramirez 413 (BAA), Rojas 14528 (BAA), Rosengurtt B1696, PE–4622, B5092, 11292 (BAA), Rua et al. 592, 716, 749 (BAA), Rua & Córdova 735, 738, Sacco 106 (BAA), Sarmiento 142 (BAA), Silva & Correo 234 (BAA), Tourn s.n. (BAA 23015), without collector 163, 295, 1242 (BAA).