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Phylogeny of *Nassella* (Stipeae, Pooideae, Poaceae) Based on Analyses of Chloroplast and Nuclear Ribosomal DNA and Morphology

Ana M. Cialdella,[1,3] Silvana M. Sede,[1] Konstantin Romaschenko,[2] Paul M. Peterson,[2] Robert J. Soreng,[2] Fernando O. Zuloaga,[1] and O. Morrone[1]

[1]Instituto de Botánica Darwinion, Labardén 200, Casilla de Correo 22, B1642HYD San Isidro, Buenos Aires, Argentina.
[2]Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington D. C. 20013-7012, U. S. A.
[3]Author for correspondence (anacialdella@darwin.edu.ar)

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Abstract—The genus *Nassella*, as currently circumscribed, includes 116–117 American species. It is characterized by florets with a strongly convolute lemma, a conspicuous or inconspicuous crown, and a short palea. Using 53 species of *Nassella* and 22 outgroup species we conducted phylogenetic analyses to test the monophyly of *Nassella* and relationships among species. Two plastid (*trnT-trnL* and *rpl32-trnL*) and two nuclear ribosomal (ITS and ETS) regions and morphology were used. Our DNA data alone and combined with morphology showed *Nassella* to be paraphyletic with respect to a monophyletic *Amelichloa*. Two main clades were recovered: one with species of *Nassella* distributed in regions of high elevation from Mexico to northwestern Argentina and one composed of the remaining species of *Nassella* and those of *Amelichloa.* The latter is mainly concentrated in southern South America in a variety of habitats with generally lower elevation than the other clade. The monophyly of the close relative of *Nassella*, the South American genus *Jarava* s. s., was rejected. None of the groups previously circumscribed as subgenera of *Stipa*, that are now considered to be composed of species in *Nassella*, were recovered as monophyletic. The close phylogenetic relationship of *Nassella* and *Amelichloa* is supported by only one morphological synapomorphy: the lemma margins flat and strongly overlapping.

Keywords—ETS, ITS, morphology, phylogeny, plastid DNA, Stipeae.

The tribe Stipeae s. s. (Romaschenko et al. 2012) includes between 572 and 670 species, depending on how the Asian taxa are treated. The species are distributed in temperate and warm temperate grasslands of Africa, Australia, Eurasia, and America (Barkworth 1993). Stipeae includes mostly perennial plants, with loose or dense panicles of one-flowered spikelets, without a rachilla extension, and lemmas usually with a single, terminal awn or with a thickened point, that is entered by the keel and lateral veins. The circumscription of the Stipeae has undergone several changes through the removal of unrelated genera (Barkworth and Everett 1987). Moreover, delimitation of the genera within the tribe has also experienced important changes. The genus *Stipa* L. was, as traditionally circumscribed, the largest genus within the tribe. In South America it was studied by Spegazzini (1901, 1925), who revised the Argentinean species of *Stipa*, including some species from Uruguay and Chile, and recognized several subgenera based on morphological characters of the floret. Some of those subgenera were subsequently removed from *Stipa* and placed into synonymy, such as *S.* subg. *Parastipa* Speg. under *Ortachne* Nees ex Steud. (Soreng et al. 2003), or raised to generic rank, such as *Anatherostipa* (Hack. ex Kuntze) Peñail. (Peñailillo 1996), *Nassella* (Trin.) E. Desv. emend. Barkworth (Barkworth 1990, comprising seven subgenera of *Stipa*), *Jarava* Ruiz et Pav. (including *S.* subg. *Ptilostipa* Speg. and *S.* subg. *Pappostipa* Speg., according to Peñailillo 2002), and *Pappostipa* (Speg.) Romasch., P. M. Peterson & Soreng (Romaschenko et al. 2008, 2011, 2012). As a result of these taxonomic changes, a new delimitation of genera has been suggested, considering morphological and anatomical characters (Romaschenko et al. 2012). Currently, the Stipeae s. s. comprises about 29 genera, 15 or 16 of which are represented by species native to the Americas (Soreng et al. 2003; Arriaga and Barkworth 2006; Romaschenko et al. 2008, 2011, 2012; Cialdella et al. 2010): *Amelichloa* Arriaga et Torres, *Nassella,* and *Piptochaetium* J. Presl, have a wide geographical distribution from Canada to South America; *Achnatherum* sensu Barkworth, *Eriocoma* Nutt., *Hesperostipa* (M. K. Elias) Barkworth, *Oryzopsis* Michx., *Patis* Ohwi, *Piptatheropsis* Romasch., P. M. Peterson & R. J. Soreng, and *Ptilagrostis* Griseb., are restricted to North America or also occur in Eurasia; and *Anatherostipa*, *Aciachne* Benth., *Lorenzochloa* Reeder & C. Reeder, *Ortachne*, *Pappostipa,* and *Jarava* are South American genera. *Aristella* (Trin.) Bertol., *Austrostipa* S. W. L. Jacobs & J. Everett, *Celtica* F. M. Vázquez & Barkworth, *Macrochloa* Kunth, *Oloptum* Röser & H. R. Hamasha, *Stipa* L., and *Stipella* (Tzvelev) Röser & H. R. Hamasha, are cultivated or introduced in the Americas (Soreng et al. 2003; Romaschenko et al. 2011).

Recent studies, based on combined morphological and molecular data, showed that *Achnatherum*, *Anatherostipa, Aciachne*, *Jarava, Nassella*, *Ptilagrostis*, and *Stipa* are polyphyletic groups, while *Aciachne* is polyphyletic or paraphyletic, and *Piptatherum*, *Piptochaetium*, *Austrostipa, Piptatheropsis,* and *Hesperostipa* are monophyletic (Cialdella et al. 2010; Romaschenko et al. 2008, 2011, 2012).

Nassella was originally described as a subgenus of *Stipa* (Trinius 1830), then as a subgenus of *Urachne* (Trinius 1834), and finally raised to generic rank by Desvaux (1854). Later on, Spegazzini (1901, 1925) treated *Nassella* as a subgenus of *Stipa*. Parodi (1947) and Clayton and Renvoize (1986) viewed the genus as containing nine or 15 species with short florets, respectively, but Barkworth (1990) expanded *Nassella*, based on morphological and anatomical characters, to also include species of *Stipa* with long florets. Although some authors (Zanín and Longhi-Wagner 1990; Renvoize 1998) did not agree with this expansion and preferred the traditional treatment of the species under *Stipa*, the new delimitation of *Nassella* has been accepted in several recent treatments (Rojas 1998; Torres 1997; Peñailillo 1998; Jørgensen and León-Yañez 1999; Jacobs et al. 2000; Barkworth and Torres 2001; Soreng et al. 2003; Barkworth et al. 2008; Romaschenko et al. 2012). As a result, *Nassella* is one of the largest genera in the tribe, including, as now

circumscribed, 116–117 species (Barkworth 1990; Jacobs et al. 2000; Barkworth and Torres 2001; Soreng et al. 2003; Romaschenko et al. 2012).

According to Barkworth and Torres (2001), *Nassella* is characterized by a strongly convolute lemma, with a conspicuous or inconspicuous crown at the apex, short palea almost always glabrous and without veins. According to Romaschenko et al. (2012), *Nassella* also has a "ladder-like" lemma epidermal pattern that is believed to be unique within Stipeae. This genus is widely distributed from Canada to Argentina and Chile, also present in Bolivia, Uruguay, Brazil, Colombia, Ecuador, Paraguay, Peru, Venezuela, Guatemala, Costa Rica, Mexico, and U. S. A. Fewer than eight species are native to North America. It is well represented in two South American regions: the central Andean region (Peru, Bolivia, northern and central Chile, and northwestern Argentina), and from northern Patagonia, the Pampas, central and northeastern Argentina to Uruguay, and southern Brazil. Argentina includes 70 species, and more than half of them are concentrated in the northwestern region of the country (Jujuy, Salta, Tucumán, Catamarca, and La Rioja).

Earlier phylogenetic studies rendered *Nassella* monophyletic (Jacobs et al. 2000; Cialdella et al. 2007; Barber et al. 2009), while our recent studies suggested that *Nassella* is polyphyletic (Cialdella et al. 2010), with some species most closely related to *Jarava* and other species most closely related to *Amelichloa* (Romaschenko et al. 2008, 2012). *Nassella, Jarava* s. s. (excluding species now in *Pappostipa*), *Amelichloa,* and the American species of *Achnatherum* are grouped together in a clade called the Major American Clade (MAC) or New World Subclade (Romaschenko et al. 2008; Cialdella et al. 2010; Romaschenko et al. 2012). Based on a chloroplast DNA-derived phylogram, *Jarava* was resolved as sister to a clade of *Nassella, Amelichloa,* and a subset of Mexican species of *Achnatherum* s. l. (Romaschenko et al. 2008, 2012). We chose four regions, two from plastid DNA (*trnT-trnL* and *rpl32-trnL*) and two from nuclear ribosomal DNA (nrDNA), the internal transcribed regions (ITS) and the external transcribed spacer (ETS). Plastid regions and ITS were selected to complete matrices used in previous contributions (*trnT-trnL* in Cialdella et al. 2010; *rpl32-trnL* and ITS in Romaschenko et al. 2012). ETS was chosen to test another highly informative nuclear DNA region.

Potential pitfalls of the ITS region for inferring phylogenies have been widely addressed (Mayol and Roselló 2001; Nieto Feliner et al. 2001; Álvarez and Wendel 2003; Small et al. 2004; Nieto Feliner and Roselló 2007). The ribosomal region is composed of the 18S, 5.8S, and 26S genes, the internal spacers (ITS-1 and ITS-2), and the intergenic spacer (IGS), which includes the external spacer (ETS). This transcriptional unit is in potentially thousands of tandem copies. Although identical sequences in all ribosomal copies are expected (due to concerted evolution process, see Arnheim 1983) the mechanism of gene conversion sometimes fails to homogenize copies in the face of introgression and/or recent interspecific hybridization (Nieto Feliner and Roselló 2007). As a consequence, nonfunctional ribosomal loci (pseudogenes) may lead to wrong phylogenetic inferences. Moreover, the ribosomal ITS regions are prone to evolutionary constraints to maintain the secondary structures for the accurate processing of mature RNAs. As a result, compensatory base mutations could occur, hence violating previous assumptions of neutrality and independence of characters (Liu and Schardl 1994).

Using sequences from four DNA regions and morphological characters from 53 species of *Nassella*, we conducted a phylogenetic analysis using 22 outgroup species to test the monophyly of the genus *Nassella*, to study relationships among *Nassella* and allied genera, especially *Amelichloa* and *Jarava*, and to test the monophyly of the infrageneric taxa previously included in *Stipa* (now under the synonymy of *Nassella*).

Materials and Methods

Taxon Sampling—Fifty-three species of *Nassella* were included in the study, and the samples were either collected in the field or obtained from herbarium material (Appendix 1). All five species of *Amelichloa*, together with *Aristella bromoides* (L.) Bertol., *Achnatherum eminens* (Cav.) Barkworth, *A. inebrians* (Hance) Keng, *A. multimode* (Scribn. ex Beal) Valdés-Reyna & Barkworth, *Aciachne flagellifera* Lægaard, *A. acicularis* Lægaard, *Anatherostipa rigidiseta* (Pilg.) Peñail., *A. obtusa* (Nees & Meyen) Peñail., *Austrostipa campylachne* (Nees) S. W. L. Jacobs & J. Everett , *A. nodosa* (S. T. Blake) S. W. L. Jacobs & J. Everett, *Jarava media* (Speg.) Peñail., *J. ichu* Ruiz & Pav., *J. castellanosii* (F. A. Roig) Peñail., *J. leptostachya* (Griseb.) F. Rojas, *J. plumosula* (Nees ex Steud.) F. Rojas, and *J. scabrifolia* (Torres) Peñail., were also included in the matrix and were selected according to previous phylogenetic analyses to represent the phylogenetic diversity of the tribe as best as possible (Cialdella et al. 2010; Romaschenko et al. 2008, 2012). *Piptochaetium montevidense* was used to root the tree because this taxon is part of a sister clade to the clade that includes MAC in the topology proposed by Cialdella et al. (2010).

Morphological Characters—A total of 14 morphological characters were included in the matrix; they were selected from Cialdella et al. (2007, 2010). These characters were chosen to represent diagnostic features for the genera and to elucidate phylogenetic groups (Table S1, Appendix 2).

DNA Isolation, Amplification and Sequencing—Genomic DNA was isolated from silica-dried leaf tissue following a CTAB protocol (Doyle and Doyle 1987) and from herbarium material with the DNeasy plant mini kit (Qiagen, Hilden, Germany). We chose two plastid regions to complete matrices used in previous contributions: the *trnT-trnL* and *rpl32-trnL* intergenic spacers. These non-coding regions were amplified using primers TabA and TabB (Taberlet et al. 1991) and primers $trnL^{(UAG)}$ and rpl32F (Shaw et al. 2007), respectively. Additionally, two nrDNA regions were selected: the ITS from nrDNA, including spacer-1, the 5.8S subunit, and spacer-2, and the ETS. The regions were amplified using primers ITS4 (White et al. 1990) and ITS5A (Stanford et al. 2000) and primers RETS4-F (Gillespie et al. 2010) and 18S-IGS (Baldwin and Markos 1998), respectively. The amplification profile consisted of 94°C for 3 min followed by 30 cycles of 94°C for 1 min, 52°C (58°C for nrDNA) for 1 min, and 72°C for 1 min. The PCR reactions were performed in 25 µl final volume with 50–100 ng of DNA template, 0.2 µM of each primer, 25 µM of dNTPs, 5 mM $MgCl_2$, 1 × Taq buffer, and 1.5 units of *Taq* polymerase (Invitrogen, Life Technologies Sao Paulo, Brazil). Automated sequencing was performed by Macrogen Inc. (Seoul, South Korea). Electropherograms were edited and assembled using BioEdit 5.0.9 (Hall 1999). All sequences were deposited in GenBank (Appendix 1). We combined data from different individuals to represent taxa when it was not possible to obtain sequences from the same individual.

Characterization of ITS Sequences—Evidence for ITS paralogous sequences or pseudogene candidates includes length variation, decreased GC content, low stability of secondary structures and absence of conserved motifs (Mayol and Roselló 2001; Bailey et al. 2003; Nieto Feliner and Roselló 2007). Length variation and GC content was determined using BioEdit version 5.0.9 (Hall 1999). Free energy of RNA transcripts and predicted secondary structures was determined at the DINAMelt web server (http://mfold.rit.albany.edu/?q=DINAMelt/Zipfold) by use of the Zipfold application (Markham and Zuker 2008). The presence of the conserved motif (Liu and Schardl 1994) GGCRY-(4–7n)-GYGYCAAGGAA was searched at the spacer 1 of ITS region.

Sequence Alignment and Phylogenetic Analysis—Sequences were aligned using the program MAFFT version 6 (Katoh and Toh 2008; http://mafft.cbrc.jp/alignment/server/). Indels were coded as binary

characters using simple indel coding (Simmons and Ochoterena 2000) as implemented in SeqState 1.4 (Müller 2005). Both plastid and nuclear regions together with morphological data were concatenated into a single matrix which was deposited in TreeBASE (study number S13590).

The phylogenetic analyses of plastid data, nuclear data, combined DNA data, and combined DNA and morphological data were performed under the parsimony criterion using TNT ver. 1.1 (Goloboff et al. 2008). All characters were considered unordered and parsimony-uninformative characters were excluded from the analyses. We employed a heuristic search strategy. Tree searches were performed with 1,000 random addition sequences (RAS), each followed by tree bisection and reconnection (TBR) branch rearrangements with 10 trees retained from the analysis of each RAS. Trees found were saved in memory and additionally TBR swapped, retaining a maximum of 10,000 total trees. A strict consensus tree was generated from the most parsimonious trees. Branch support was calculated by jackknifing (Farris et al. 1996) with a character removal probability of 36% in each of 10,000 replicates; the heuristic search strategy for each replicate involved five RAS swapped with TBR, with one tree saved per replicate.

Character optimization was performed using TNT ver. 1.1 (Goloboff et al. 2008). Common morphological and molecular synapomorphies of major clades were described for the combined morphological and molecular analysis, although only common morphological synapomorphies, i.e. the morphological characters common to all trees, were displayed on the nodes of the strict consensus tree from that combined analysis. One of the most parsimonious trees obtained in the combined analysis was selected at random to reconstruct morphological character evolution.

Results

Plastid Analysis—The combined *trnT-trnL* and *rpl32-trnL* datasets consisted of 76 taxa and 1,712 characters: 747 aligned positions and six coded indels from *trnT-trnL* and 952 aligned positions and seven coded indels from *rpl32-trnL* (Table 1). Forty-four sequences of *trnT-trnL* (55%) were generated by this study. Of *rpl32-trnL* data set, 46 sequences (69.7%) were new. Of the total characters only 92 (5.4%) were parsimony informative. The analysis of the combined plastid data set yielded more than 10,000 trees (L = 145, CI = 0.697, RI = 0.906; Table 1). The MAC was recovered (jackknife JK = 94%), although relationships within it were unresolved, except for three minor clades: *Nassella novari* Torre/*N. arcuata* (R. E. Fr.) Torres (JK = 92%), *Nassella mucronata* (Kunth) R. W. Pohl/*N. ayacuchensis* (Tovar) Barkworth (JK = 65%), and *Jarava plumosula*/*J. media* (JK = 92%). The strict consensus tree is presented in Fig. S1.

Nuclear Analysis—The length of the sequences obtained in this study is in accordance with the estimated range for angiosperms: 187–298 for ITS1 and 187–252 for ITS2 (Baldwin et al. 1995). The GC content was near 50% for the ITS1 and higher for the ITS2, as expected. The conserved motif was found in all species. The optimal free energy is within the expected range, and the RNA foldings of nearly all sequences are similar to the consensus structure of vascular plants (Baldwin et al. 1995). We thus conclude that there are no pseudogene candidates in the dataset.

The combined ITS and ETS datasets consisted of 72 taxa and 1,020 characters: 597 aligned positions and one coded indel from ITS and 419 aligned positions and three coded indels from ETS, (Table 1). Of the ITS data set, 46 sequences (67.6%) were new. All ETS sequences were generated by this study. Of the total characters, 199 (19.5%) were parsimony informative. The analysis of the combined nuclear data set yielded more than 10,000 trees (L = 425, CI = 0.593, RI = 0.822; Table 1). The strict consensus tree is shown in Fig. 1A. Although the MAC was not recovered, the *Nassella*/*Amelichloa* clade was supported (JK = 54%). Two main clades (A and B) were recognized because they were formed at the basal split of the focal group in the topology obtained using DNA and morphology combined. In this analysis, clade A (JK = 70%) and a clade composed of most members of clade B (JK = ≤ 50%) were recovered. *Amelichloa* (nested within this latter clade composed of most of clade B) was recovered as monophyletic (JK = 66%; Fig. 1A).

Combined Plastid and Nuclear Analysis—The combined four region dataset consisted of 73 taxa and 2,732 characters (Table 1). Of the total characters, 291 were parsimony informative. The analysis of the combined DNA data set yielded more than 10,000 trees (L = 614, CI = 0.577, RI = 0.819; Table 1). The strict consensus tree is shown in Fig. 1B. In the topology obtained from the combined DNA data, the MAC is recovered with high support (JK = 98%), with *Austrostipa*/*Aristella bromoides*/*Achnatherum inebrians* as the sister clade. A polytomy at the base of the MAC involves Clade A, a clade composed of most members of Clade B, and three small clades: (1) one composed of *Jarava plumosula*, *J. media*, and the American *Achnatherum*; (2) one composed of the four other species of *Jarava*; and (3) one composed of *Nassella tenuis* (Phil.) Barkworth and *N. formicarum* (Delile) Barkworth. Within clade A (JK = 67%), only the group *Nassella novari*/*N. arcuata* is strongly supported (JK = 100%). Within most of clade B (JK = 53%), several small clades are recovered and supported. *Amelichloa* monophyly (JK = 87%) is again corroborated on the basis of combined plastid and nuclear DNA evidence (Fig. 1B).

Morphology—The morphological dataset (Table 1, Appendix 2, Table S1) consisted of 73 taxa and 14 characters. The matrix did not contain missing data and all characters were parsimony informative. The analysis of the dataset yielded more than 10,000 trees (L = 53, CI = 0.358, RI = 0.814; Table 1). The strict consensus tree is shown in Fig. S2. The topology is almost completely unresolved and groups A and B were not recovered.

Table 1. Summary information for the data matrices and parsimony analyses; bp: base pairs; CI: consistency index; RI: retention index; MPTs: most parsimonious trees.

	cpDNA (*trnT-trnL*, *rpl32-trnL*)	nrDNA (ETS, ITS)	Molecular combined	Morphology	Combined (molecular and morphology)
Taxa	76	72	73	73	75
Aligned characters	1,712	1,020	2,732	14	2,746
Parsimony informative characters	92	199	291	14	305
Number of coded indels	13	4	17	–	17
Missing data (%)	20.6	19.3	21.5	0	21.5
Number of MPTs / length (steps)	> 10,000 / 145	> 10,000 / 425	> 10,000 / 614	> 10,000 / 53	174 / 719
Main clades recovered	–	A & B (most)	A & B (most)	–	A & B
CI / RI	0.697 / 0.906	0.593 / 0.822	0.577 / 0.819	0.358 / 0.814	0.518 / 0.778

FIG. 1. Strict consensus from the 10,000 most parsimonious trees (the maximum saved) resulting from the analysis of the nuclear ribosomal (ITS and ETS) data alone (A) and the combined plastid (*trnT-trnL* and *rpl32-trnL*) and nuclear ribosomal partitions (B). Numbers above branches are jackknife values. Bars represent membership in the principal clades recognized in Fig. 2, as discussed in the text. MAC: Major American clade.

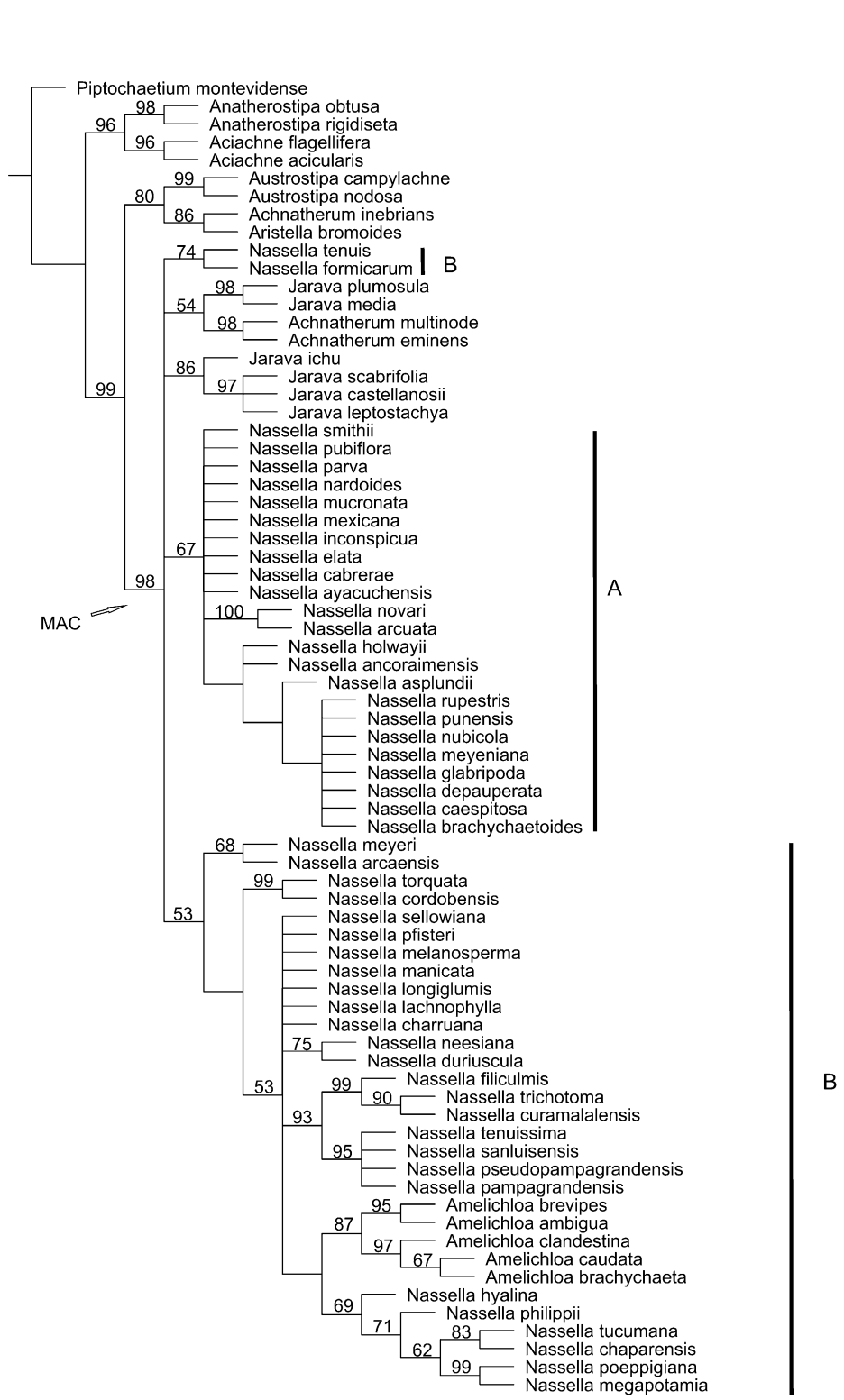

FIG. 1. Continued. See Caption for Fig. 1A.

Molecules and Morphology—The combined molecular and morphological data matrix consisted of 75 taxa and 2,746 characters (Table 1). The matrix contained 21.5% missing data excluding gaps. Of the total 2,746 characters, 305 were parsimony informative. The analysis of the combined DNA and morphological data set yielded 174 trees (L = 719, CI = 0.518, RI = 0.778; Table 1). The strict consensus tree is shown in Fig. 2. The monophyly of the MAC was strongly supported (JK = 97%). A clade with two species of the American lineage of *Achnatherum* (*A. multinode* and *A. eminens*) is sister to the MAC core, as defined by Romaschenko et al. (2008, p. 188), which is the *Jarava/Nassella/Amelichloa* clade. This core is supported by a JK value of 60% and three morphological synapomorphies (palea shorter than half the length of the lemma (Figs. 2, 3B), inconspicuous crown (Figs. 2, 3C) and prickles or papillae

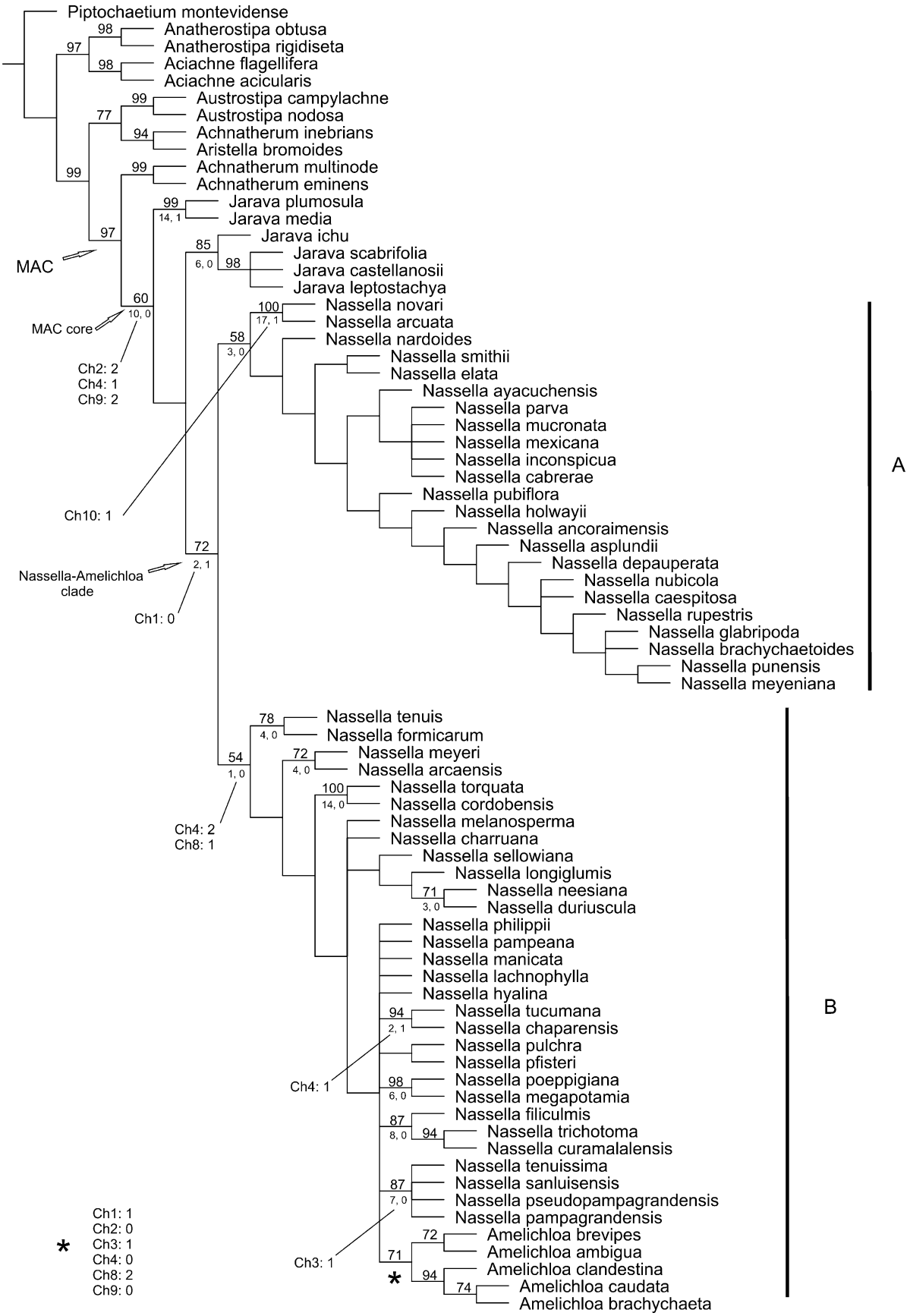

FIG. 2. Strict consensus from the 174 most parsimonious trees resulting from the analysis of the combined molecular and morphological data. Numbers above branches are jackknife values. Numbers below branches refer to molecular synapomorphies: number of base substitutions, number of codified indels. Morphological synapomorphies (character number: state) are shown for selected clades. Bars identify principal clades discussed in the text. MAC: Major American clade.

FIG. 3. Character evolution. A. Lemma margins (character 1; Appendix 2, Table S1). B. Palea length (character 2; Appendix 2, Table E1). C. Lemma crown (character 4; Appendix 2, Table S1). D. Lemma pubescence (character 8; Appendix 2, Table S1).

present in part of the lemma (Fig. 2)) together with 10 base substitutions. Within the MAC core, two independent clades of *Jarava* species were recovered: the clade composed of *Jarava plumosula* and *J. media* (supported by a JK value of 99% and 14 base substitutions plus one codified indel from *trnT-trnL*) and the clade composed of *J. ichu, J. castellanosii, J. leptostachya*, and *J. scabrifolia* (supported by a JK value of 85% and six base substitutions). The latter clade is sister to a clade composed of the remaining genera of MAC core (*Nassella* and *Amelichloa*), and the former is sister to the clade formed by those two clades. The *Nassella*/*Amelichloa* clade is supported by a JK value of 72% and by one morphological synapomorphy (lemma margins flat, strongly overlapping, Fig. 3A), two base substitutions, and one codified indel from ITS. Two main subclades were identified (A and B; Table 1, Fig. 2). All species grouped in clade A (JK = 58%) belong to *Nassella*, and the clade is supported by three base substitutions. Two morphological character states (conspicuous crown and lemma pubescent in part, Figs. 3C and D) and one base substitution are synapomorphies for clade B (JK = 54%).

Within clades A and B various minor groups are well supported. A small group (*N. novari*/*N. arcuata*) is strongly supported (JK = 100%) with 17 base substitutions, one codified indel from *trnT-trnL*, and one morphological character state: the floret length > 7 mm as synapomorphies. *Amelichloa* is monophyletic with moderate support (JK = 71%), including six base substitutions and six morphological characters: lemma margins flat and only slightly overlapping each other, palea half to equal the length of the lemma, crown absent, lemma with hairs covering entire surface (Fig. 3), lemma without prickles or papillae, and callus blunt or truncate.

Morphological Characters Optimization—All morphological characters (Table S1, Appendix 2) were optimized in one of the most parsimonious trees derived from the analysis of the combined data set. The reconstructions of three diagnostic morphological characters for *Nassella* (characters 1, 2, and 4), together with the pubescence on the lemma (character 8) are depicted in Fig. 3. The numbers of steps for each character reconstruction were four, six, 14, and 17, respectively.

Discussion

The hypothesis of monophyly of *Nassella,* as proposed by Cialdella et al. (2007), was rejected in our present analysis. Cialdella et al. (2007) sampled 21 species of *Nassella* but they did not include *Amelichloa*, which we found to be nested in *Nassella* (Figs. 1, 2). This result is in accordance with Barkworth et al. (2008), Romaschenko et al. (2008, 2012), and Cialdella et al. (2010), who sampled both genera. *Jarava* s. s. was resolved as paraphyletic with respect to *Nassella*/*Amelichloa* (Fig. 2) or in a polytomy together with *Nassella*/*Amelichloa* (Fig. 1).

Nassella, as circumscribed by Barkworth (1990) and Barkworth and Torres (2001), is characterized by a combination of diagnostic morphological characteristics of the floret: the strongly overlapping convolute lemma margins; the lemma with a conspicuous or inconspicuous crown; and the reduced (up to 1/3 the length of the lemma, rarely longer) palea without veins and frequently glabrous (Cialdella 2012). In our analysis, the morphological character that provides a synapomorphy for the *Nassella*/*Amelichloa* clade is the lemma margins overlapping (character 1; Figs. 2, 3A). All the species of *Nassella* have strongly overlapping margins, while *Amelichloa* is defined by margins only slightly overlapping or not overlapping at all. Some species of *Austrostipa* also have strongly overlapping lemma margins (in our analysis, *A. nodosa*, Fig. 3A), so this form appears to have evolved independently at least two times.

The length of the palea (character 2) has historically been used to recognize *Nassella* and allied genera (Barkworth 1990; Torres 1997; Peñailillo 2002). In our topology (Fig. 3B), the *Jarava*/*Nassella*/*Amelichloa* clade is defined by a palea shorter than half the length of the lemma, although in some species of *Nassella* and *Amelichloa*, this character is polymorphic and we observed four reversals to the state "half to equal the length of the lemma": in *Nassella inconspicua*, *N. brachychaetoides*, *N. pampeana,* and the *Amelichloa* clade.

Jarava and *Nassella* have a lemma crown (character 4). A crown refers to the fusion of the lemma margins towards the apex, which may or may not be differentiated externally (Jacobs et al. 1995). This fusion of the lemma margins can also be found in other Stipeae, for example in *Piptochaetium* (here represented by *P. montevidense*). In constrast, *Amelichloa* does not have a crown, as the lemma covers only the margins of the palea at maturity (Fig. 3C).

We found two main groups in the *Nassella*/*Amelichloa* clade (A and B). Clade A includes only species of *Nassella,* while clade B comprises species of *Nassella* and species of *Amelichloa* nested in it. Both major clades were recovered when re-running the analyses after removing all the species of *Amelichloa* in both our combined DNA and combined DNA and morphological datasets (unpublished results). Lemma pubescence (character 8) exhibits a noteworthy pattern in the *Nassella*/*Amelichloa* clade. In clade A, the lemma is completely pubescent except in one species: *N. meyeniana*. In species of *Nassella* included in clade B the pubescence of the lemma exhibits all possible states. In species of *Amelichloa* the lemma is always pubescent, although with different density and distribution of hairs (Fig. 3D). Species in clade A are found growing at high elevations, while species in clade B are distributed in different habitats, frequently at lower elevations, so it is possible that this character is environmentally influenced.

Amelichloa was defined by Arriaga and Barkworth (2006) to include five species that were previously treated in other genera (*Achnatherum*, *Jarava,* or *Nassella*). They established this genus based on several morphological characteristics: primarily basal leaf blades rigid and with a sharp tip, caryopses with three longitudinal ribs and persistent stylar bases, and cleistogamous axillary panicles in basal leaf sheaths. All species of *Amelichloa* were included in our analyses and the monophyly of the genus was corroborated with high support for the first time, both in the combined DNA and morphological data and combined DNA data analyses, rejecting the previous hypothesis of Cialdella et al. (2010), who found *A. brevipes* not closely related to the rest of the species of the genus. While the identification of the material used by Cialdella et al. (2010) is now considered dubious, our current results were based on a specimen of *A. brevipes* that was carefully verified. Our present results regarding *Amelichloa,* and its close relationship with *Nassella,* are in accordance with Barkworth et al. (2008) and Romaschenko et al. (2008), although they only included three and two

FIG. 4. Geographical distribution of species in clades A (lines) and B (grey).

species of *Amelichloa*, respectively. In our analysis, six different morphological character states support the *Amelichloa* clade: lemma margins flat, lemma margins not or only slightly overlapping, palea half to equal the length of the lemma, callus blunt or truncate, apex of the lemma without a crown, and lemma completely pubescent without prickles or papillae (Table S1, Appendix 2). Apart from the clade of *Amelichloa*, morphological synapomorphies supported three additional branches in the *Nassella*/*Amelichloa* clade (Fig. 2).

Jarava s. s. (with 32 species presently accepted, after removal of 31 taxa to *Pappostipa* in Romaschenko et al. 2008), represented only by six species in this analysis, is recovered as paraphyletic and closely related to the *Nassella*/*Amelichloa* clade. These results are similar to those of Romaschenko et al. (2008, 2012) and Cialdella et al. (2010), who also resolved *Jarava* as paraphyletic or polyphyletic and related to the *Nassella*/*Amelichloa* clade. In our analyses, the species of *Jarava* are grouped in two clades: *J. media*/*J. plumosula* share the presence of macrohairs on the distal portion of the awn or throughout the awn. The other clade, characterized by a scabrous awn, includes the type of *Jarava*, *J. ichu*, along with *J. scabrifolia*, *J. castellanosii,* and *J. leptostachya* (Table S1, Appendix 2). A similar grouping was found by Cialdella et al. (2007, 2010). Our analyses also give further support for the Major American Clade (Romaschenko et al. 2008, 2011).

One of the aims of our study was to test the monophyly of *Stipa* s. l. subgenera (Spegazzini 1901, 1925) that are now included in *Nassella* (Barkworth 1990; Torres 1997). Spegazzini based these groups on different characteristics of the floret: awn length and vestiture, callus shape, callus length/floret diameter ratio, conspicuous crown, lemma vestiture, floret shape and length of the glume length/floret length ratio. Barkworth (1990) and Torres (1997) included several subgenera of *Stipa* under the synonymy of *Nassella*, without recognizing any infrageneric taxa. In all our analyses, those subgenera were not recovered as monophyletic, not even the monophyly of *S*. subg. *Leptostipa* Speg., which had been previously recovered by Cialdella et al. (2007). We only noticed a trend in some morphological characters: within clade A, there are several species with a sharp callus, a conspicuous crown, and a pubescent lemma, while in clade B several species show florets with a glabrous lemma (sometimes with hairs only on the base of the dorsal nerve) and a conspicuous crown.

The main groups resolved within the *Nassella*/*Amelichloa* clade are geographically structured (Fig. 4). Species in clade A are distributed in high elevation regions from Bolivia and northwestern Argentina to northern South America and Mexico. Species of clade B are mainly concentrated in Argentina, southern Bolivia, southern Brazil, Chile, Paraguay and Uruguay, although some species are also present in Peru, Ecuador, Colombia, Mexico, and U. S. A. Species in clade B are distributed in several ecological regions, but in general the elevation is lower when compared to group A.

Our analyses, using combined DNA data and combined DNA and morphological data, showed that *Amelichloa* is nested within *Nassella*. This close relationship is not consistent with the morphological differences between both genera (as seen by the many character state changes on the branch leading to the former in Fig. 2). Additionally, previous analyses showed that there are anatomical characters related to the lemma micromorphology that distinguish *Amelichloa* from *Nassella* (Romaschenko et al. 2012). *Amelichloa*, here represented by all species of the genus, is strongly supported as monophyletic, but the position of *Amelichloa* makes *Nassella* paraphyletic. However, combining them would result in a poorly circumscribed *Nassella*, considering that there are strong morphological differences between the genera. We hope to further investigate the possibility of a reticulate origin of *Amelichloa* using low-copy nuclear genes. Clarifying the relationship of *Nassella* and *Amelichloa* will require additional analyses, based on more intensive sampling, and the addition of other morphological and molecular data.

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Appendix 1. List of specimens considered for morphological and molecular analyses and GenBank accession numbers of *trnT-trnL, rpl32-trnL*, ITS, and ETS. -, sequence not obtained.

Achnatherum eminens (Cav.) Barkworth. MEXICO. State Coahuila: Bosques de la montaña, cerca de casa J. Valdés, camino de Saltillo a Los Lirios, 25° 23′ N, 100° 42′ W, 2,115 m, *Zuloaga et al. 9639* (SI); GU192062,-, -, -. MEXICO. State Zacatecas: 45.1 km E of Sombrerete on Hwy 45 towards Fresnillo, *Peterson & Annable 10952* (US); -, JF697923, JF697693, -. ***A. inebrians*** (Hance) Keng. Without data, *Ho et al.230* (MO); GU192043, -, -, -. CHINA. Gansu: Hezuozhen, N end of town, ca. 130 km SSW Lanzhou, *Soreng et al. 5393* (US); -, JF697926, GU254626, -. ***A. multinode*** (Scribn. ex Beal) Valdés-Reyna & Barkworth. MEXICO. State Coahuila: Bosques de la montaña, cerca de casa J. Valdés, camino de Saltillo a Los Lirios, 25° 23′ N, 100° 42′ W, 2,115 m, *Zuloaga et al. 9646* (SI); GU192061, -, -, KC904275. Without data, *Hoge 264* (US); -, JF697932, JF697699, -.

Aciachne acicularis Laegaard. ARGENTINA. Prov. Salta: Orán, Piedra Azul, 3,650 m, *Sulekic et al. 2782* (SI); GU192038, -, -, -. PERÚ. Dept. Ancash: 14 km NE of Pampas and 4 km E of Central Office of La Mina, *Peterson & Rodriguez 13931* (US); -, JF697948, GU254625, -. ***A. flagellifera*** Laegaard. ECUADOR. Prov. Imbabura: 4,150 m, *Laegaard 54503* (AAU); GU192042, -, -, -. Prov. Tungurahua: *Laegaard 19436* (AAU); -, JF697949, GU254654, -.

Amelichloa ambigua (Speg.) Arriaga & Barkworth. ARGENTINA. Prov. Río Negro: Gral. Roca, ruta nac. 22, km 1,065, a 111 km de Gral. Roca camino a Choele Choel, 39°05′S, 66° 21′ W, *Morrone et al. 5762* (SI); GU192063, KF294085, KF294127, KC904276. ***A. brachychaeta*** (Godr.) Arriaga & Barkworth. ARGENTINA. Prov. Buenos Aires: Reserva

Otamendi, *Morrone 5509* (SI); GU192051, -, -, KC904277. Prov. Río Negro: Gral. Roca, ruta nacional 22, km 1,065, a 111 km de Gral. Roca, camino a Choele Choel, *Morrone et al. 5764* (SI). ***A. brevipes*** (E. Desv.) Arriaga & Barkworth. ARGENTINA. Prov. Neuquén: Catán Lil, ruta nacional 40, km 2,303, pasando la Bomba camino a Zapala, *Morrone et al. 6185* (SI); KC904332, KF294086, KF294128, KC904278. ***A. caudata*** (Trin.) Arriaga & Barkworth. ARGENTINA. Prov. Buenos Aires: Gral. Pueyrredón, Sierra de los Padres, *Giussani et Morrone 356* (SI); Tornquist, Parque Provincial Sierra de la Ventana, base del Cerro Bahía Blanca, 38° 12′ S, 62° 16′ W, *Morrone et al. 5461a* (SI); GU192052, -, EU489095, KC904279. ***A. clandestina*** (Hack.) Arriaga & Barkworth. MEXICO. State Coahuila: Parque Recreativo El Chorro, 25° 23′ N, 100° 47′ W, *Zuloaga et al. 9637* (SI); GU192058, KF294087, KF294129, KC904280.

Anatherostipa obtusa (Nees & Meyen) Peñail. PERÚ. Dept. Ancash: Cordillera Blanca, 37 km E of Raquia on Route 02-014 on road towards Huarez, *Peterson et Rodriguez 13811* (US); -, JF697954, JF697708, -. ***A. rigidiseta*** (Pilg.) Peñail. BOLIVIA. Dept. La Paz: *Beck s. n.* (LPB); -, JF697955, GU254612, -.

Aristella bromoides (L.) Bertol. Without data, *Ivanina s / n* (MO); GU192048, -, -, -. UKRAINE. Crimea, *Romaschenko et Didukh 439* (KW); -, JF697915, GU254624, -. .***Austrostipa campilachne*** (Nees) S. W. L. Jacobs & J. Everett. AUSTRALIA. Western Australia: 10 km NE of Bindoon on HWY 95 (Great Northern) and 3 km E on Dewars Pool Rd., *Peterson et al. 14267* (US); -, JF697960, GU254627, -. ***A. nodosa*** (S. T. Blake) S. W. L. Jacobs & J. Everett. Without data, *Hill 5099* (MO); GU192055, -, -, -. AUSTRALIA. New South Wales: *Ward 203* (KW); -, JF697966, JF697715, -.

Jarava castellanosii (F. A. Roig) Peñail. ARGENTINA. Prov. Jujuy: 21 km S of Cieneguillas at 0.2 km N of Rodeo on road to Abra Pampa, *Peterson et Annable 10336* (US); -, JF697983, EU489112, -. ***J. ichu*** Ruiz & Pav. ARGENTINA. Prov. Córdoba: Calamuchita, Villa General Belgrano, cerro de La Virgen, *Morrone et Giussani 5149* (SI). Prov. Jujuy: Tilcara, Garganta del Diablo, *Cabrera et al. 13924* (BAA); Tumbaya, Laguna de Volcán, 2,100 m, *Cialdella et al. 536* (SI). PERU. Dept. Puno: NW end of Laguna Lagunillas, 20 air km W of Santa Lucia, N and S sides of new Hwy 30 to Arequipa, 74 air km WSW of Juliaca, *Peterson et al. 20745* (US); -, JF697984, EU489124, -. ***J. leptostachya*** (Griseb.) F. Rojas. ARGENTINA. Prov. Jujuy: Cochinoca, Abra Pampa, Cerro Huancar, 3,500 m, *Cabrera et al. 15272* (BAA). Prov. Salta: Los Andes, ruta 51, de San Antonio de los Cobres a Viaducto La Polvorilla, 4,170 m, *Cialdella et al. 417* (SI); GU192034, -, KF294130, KC904281. ***J. media*** (Speg.) Peñail. ARGENTINA. Prov. Jujuy: Humahuaca, falda de los cerros, 3,050 m, *Parodi 9695* (BAA). Prov. Salta: La Poma: Palermo oeste, camino hacia la toma de agua, 2,820 m, *Cialdella et al. 242* (SI); GU192035, -, -, KC904282. ARGENTINA. Prov. La Rioja: Sierra de Sanogasta, Hwy 40, 43 air km due E of jct Villa Union jct with Hwy 76, 1 rd km W of Cuesta Miranda, *Peterson et al. 19337* (US); -, JF697985, EU489129, -. ***J. plumosula*** (Nees ex Steud.) F. Rojas. ARGENTINA. Prov. Jujuy: Tilcara, 2,600 m, Cabrera 7713 (BAA). Prov. Salta: Cachi, ruta 42, Parque Nacional Los Cardones, camino hacia Seclantas, 2,850 m, *Cialdella et al. 258* (SI); GU192036, -, -, KC904283. PERU. Dept. Ayacucho: S of Ayacucho ca. 4 km on Hwy 3 toward Abancay, *Peterson et al. 20471* (US); -, JF697986, EU489133, -. ***J. scabrifolia*** (Torres) Peñail. ARGENTINA. Prov. Jujuy: Cochinoca, Pozuelos, Río Cincel, 22° 28′ S, 65° 59′ W, 3,660 m, *Deginani et al. 552* (SI). Prov. Salta: Chicoana, ruta 33, de Cachi a Ciudad de Salta, 3,210 m, *Cialdella et al. 303* (SI). ARGENTINA. Prov. Salta: 40 km N of Amblayo on spur road off Hwy 33 between Cachi and El Carril, *Peterson 11712* (US); -, JF697988, EU489136, -.

Nassella ancoraimensis F. Rojas. BOLIVIA. Dept. Cochabamba: Ayapaya, cuenca Río Tambillo, Estancia Linco, 2,980 m, *Baar 93a* (LPB). Dept. La Paz: South of La Paz on Hwy 1 towards Oruro, 6.7 mi NW of Villa Loza on road to Urmiri and Sapahaqui, *Peterson et al. 12634* (US); KC904333, KF294088, KF294131, KC904284. ***N. arcaensis*** (Speg.) Torres. ARGENTINA. Prov. Catamarca: Belén: Culampajá (Sierra de Culampajá), *Peirano 1014 b* (LIL). Prov. Jujuy: Humahuaca, Iturbe, *Meyer et al. 21073* (BAA, LIL). Prov. Salta: Dept. Cachi, desde ruta 40, camino interno hacia Las Paylas, 25° 01′ S, 63° 13 W, *Cialdella et al. 275* (SI); KC904334, -, -, KC904285; 4 km N of Saladillo on Hwy 40, N of La Poma, *Peterson et Annable 11751* (US); -, KF294089; KF294132, -. Prov. Tucumán: Tafí del Valle, entre Amaicha del Valle y El Infiernillo, *Zuloaga 9509* (SI). ***N. arcuata*** (R.E. Fr.) Torres. ARGENTINA. Prov. Catamarca: Santa María, Sierra de Aconquija, 3,100 m, *Peirano s. n.* (LIL 271). Prov. Jujuy: Cochinoca, Abra pueblo de Cochinoca, 22° 44′ S 65° 53′ W, 3,690 m, *Deginani et al. 493* (SI). Prov. Salta: Rosario de Lerma, Ruta 51, de Salta a San Antonio de Los Cobres, 57 km pasando el Puente de Integración argentino-chilena, Esquina Negra, 3,240 m, *Cialdella et al. 409* (SI). Prov. Tucumán: Tafí, ruta 307, camino de El Infiernillo a Amaicha del Valle, 2,840 m s.m, *Cialdella et al. 183* (SI); GU192032, KF294090, KF294133, KC904286. BOLIVIA. Dept. Tarija: José María Aviléz, Pampa de Tajzara, Arenales, 3,820 m, *Beck et Paniagua 27076* (LPB, SI). CHILE. I Region: Tarapacá, Prov. Arica, Putre, *Niemeyer H - Fernández C 4-93* (SGO 140706). 92 km E of Arica on HWY 11 towards Putre, 3,100 m, *Peterson et Soreng 15695* (SI, US). ***N. asplundii*** Hitchc. BOLIVIA. Dept. Cochabamba: Tapacarí, Phia kala, Aynoka de Quinua, km 125 en la carretera Cba-Oruro, 4,200 m, *Pestalozzi 291* (LPB). Dept. La Paz: José M. Camacho, G. V. Puni, *Cocarico 700* (LPB). Dept. Oruro: S. Pagador, 4 mi. E of Urmiri, 3,770 m, *Peterson et al. 12785* (LPB); Sajama, Curahuara de Carangas, *Renvoize 5232* (US); KC904335, KF294091, KF294134, KC904287. Dept. Potosí: Quijarro, 9 mi N of Rio Mulatos on road towards Challapata, 3,650 m, *Peterson et al. 12828* (LPB). Sud Chichas, al N de San Vicente al S de Atocha, 4,040 m, *Peterson et al. 12879* (LPB). CHILE. I Region: Collahuasi-Copaquiri, 3,800 msnm, *Teillier 3069* (SGO 140841). ***N. ayacuchensis*** (Tovar) Barkworth. PERÚ. Dept. La Libertad: Santiago de Chuco, 23 km SW of Huamachuco on road towards Alto de Tamboras and Pampas, 3,540 m, *Peterson 13967* (MO, US); KC904336, KF294092, KF294135, KC904288. ***N. brachychaetoides*** (Speg.) Barkworth. ARGENTINA. Prov. Catamarca: Ambato, Cumbre de Humaya y quebradas vecinas, 2,000 m, *Saravia Toledo et al. 13034* (SI). Prov. Salta: 4 km N of Saladillo on Hwy 40, N of La Poma, *Peterson et Annable 11748* (US); KC904337, KF294093, EU489144, KC904337. Prov. Tucumán: Trancas, La Queñoa, *Rodríguez 555 p. p.* (BA). ***N. cabrerae*** Torres. ARGENTINA. Prov. Jujuy: Valle Grande, de San Francisco a Alto de Calilegua, 23° 37′ S 64° 54′ W, *Zuloaga et al. 10314* (SI); KC904338, -, -, KC904290. Prov. Tucumán: Tafí, Ruta 307, camino de Tafí del Valle a Amaicha del Valle, 2,790 m, *Cialdella et al. 161 p. p.* (SI). BOLIVIA. Dept. Cochabamba: Vacas, 3,500 m, *Cárdenas 467* (BAA). Dept. La Paz: Murillo, La Paz Autopista, Curva Bartos, *Valenzuela 246* (LPB, SI). Dept. Oruro: Cercado, 14 mi S of Oruro on HWY towards Challapata, 3,580 m, *Peterson et al. 12700* (LPB). Dept. Potosí: 8 km N of Villazón on Hwy 702 towards Tupiza, 3,580 m, *Peterson et Annable 11790* (SI, US). ***N. caespitosa*** Griseb. ARGENTINA. Prov. Catamarca: Ambato, Sierra de Ambato, falda E, alrededores de Casa de Cubas, 3,000 m, *Hunziker 19839* (CORD). Prov. Jujuy: Humahuaca, Ruta 9, de Tres Cruces a Humahuaca, Esquinas Blancas, 3,630 m, *Cialdella et al. 495* (SI); KC904339, -, -, KC904291; 4 km E of Tres Cruces on road to Humahuaca, *Peterson et Annable 10288* (US); -, KF294094, KF294136, -. Prov. La Rioja: Famatina, Cueva de Pérez, 3,800 m, *Krapovickas 6247* (BAA, CORD). Prov. Salta: Iruya, 22°50′ S 65°15′ W, 3,180 m, *Deginani et al. 469* (SI). Prov. Tucumán: Tafí del Valle, Entre Amaicha del Valle y El Infiernillo, 2,930 m, *Zuloaga et al. 9504* (SI). BOLIVIA. Dept. Oruro: S. Pagador, 2 mi W of Urmiri, 3,620 m, *Peterson et al. 12753* (LPB). CHILE. II Region: Antofagasta, km 33,5, East from San Pedro de Atacama, on paved HWY towards Argentine border at Paso de Jama, 4,120 m, *Peterson et al. 15516* (CONC). ***N. chaparensis*** F. Rojas. BOLIVIA. Dept. Chuquisaca: Belisario Boeto, Chapas, *Murguia 122* (MO, US); KC904340, KF294095, -, KC904292. Dept. Cochabamba: Chapare, Buena Vista, 3,800 m, *Aleman 3000* (LPB). ***N. charruana*** (Arechav.) Barkworth. ARGENTINA. Prov. Buenos Aires: Campana, Reserva Natural Otamendi, *Morrone et al. 5506* (SI). Prov. Corrientes: Curuzú Cuatiá, Sin localidad, *Castellanos s. n.* (BA). Prov. Entre Ríos: Gualeguaychú, Ruta 12, km 25, Perdices, frente a Destacamento de Policía, *Cialdella et al. 12* (SI); KC904341, KF294096, KF294137, KC904293. URUGUAY. Dept. Colonia: La Estanzuela, *Burkart 15790* (SI). Dept. Soriano: Juan Jackson, Estancia Sta. Elena, parcela 4B, *Gallinal 727* (SI). ***N. cordobensis*** (Speg.) Barkworth. ARGENTINA. Prov. Buenos Aires: Maipú, Camino de Madariaga a Las Arenas, *Cabrera 8581* (SI). Prov. Catamarca: Ambato, El Rodeo, Sierra de Ambato, *Calderón 1343* (BAA). Prov. Córdoba: Calamuchita, Villa General Belgrano, Cerro de la Virgen, *Morrone et Giussani 5147* (SI); KC904342, KF294097, KF294138, KC904294. Prov. La Rioja: Chilecito, Cuesta de Guachín, *Cabrera 24589* (LP). Prov. Mendoza: Malargüe, Ruta provincial 180, Cerrito de Las Lajas, *Prina 2645* (SI). Prov. San Juan: Valle Fértil, De Sierra Elizondo a Sierra Chávez, *Kiesling et al. 6618* (SI). Prov. San Luis: Pedernera, Sierra de Yulto, Estancia El Quebrachal, potr. 24, 1 km del puesto, *Anderson 1590* (SI). Prov. Santiago del Estero: Ojo de Agua, Sierra de Sumampa, *Molina 1975* (BAB). ***N. curamalalensis*** (Speg.) Barkworth. ARGENTINA. Prov. Buenos Aires: Tornquist, Villa Arcadia, Cerro del Amor, 460 m, *Morrone et al. 5107* (SI); KC904343, KF294098, KF294139, KC904295. Prov. La Pampa: Chapaleufú, I. Alvear, *Steibel et al. 10728* (SRFA). URUGUAY. Dept. Soriano: Juan Jackson, *Gallinal et al. 5526* (SI). ***N. depauperata*** (Pilg.) Barkworth. ARGENTINA. Prov. Catamarca: Ambato, Sierra de Ambato, falda E, subiendo desde El Rodeo hacia el Cerro Manchado, rumbo a Casa de

Cubas, 3,300-3,400 m, *Hunziker 19791 bis* (CORD). Prov. Jujuy: Sierra de Santa Victoria, basin 6 rd km SW of Abra de Lizoite, 38 air km ESE La Quiaca on Hwy 5, *Peterson et al. 19582*, *19589* (SI, US); KC904344, KF294099, KF294140, KC904296. Prov. Salta: Los Andes, San Antonio, Alto Chorrillos, 4,300 m, *Cabrera & Schwabe 146* (BAA, LP 76676). Prov. San Juan: Iglesia, Bajando de Ojo de Agua, *Dalmasso 1599* (MERL). Prov. Tucumán: Tafí Viejo, Lara, 3,200 m, *Rodríguez 321 p. p.* (BA). BOLIVIA. Dept. Cochabamba: Ayopaya, 10 km al NW de Independencia, 3,000 m, *Beck et Seidel 14516* (LPB, SI). Dept. La Paz: Aromo, S Lahuachaca, 3,720 m, *Ruthsatz 78* (LPB). ***N. duriuscula*** (Pilg.) Barkworth. CHILE. IV Region: Cuesta Cavilolén, *Jiles 2670* (SGO 59835). VI Region: O'Higgins, Cerro La Leona, Rinconada de Chancón, *Muñoz C. & Johnson 2646* (SGO 113137). XIV Region: Valdivia, Llancacura, 150 m, *Gunckel 17405* (US); -, -, -, KC904297. ***N. elata*** (Speg.) Torres. ARGENTINA. Prov. Juyuy: 34 km S of La Quiaca on Ruta Nacional 9 towards Abra Pampa, at "Demostrativo La Intermedia", *Peterson et Annable 10306* (US); -, KF294100, -, -. Prov. Salta. Santa Victoria, ruta provincial 145, de Abra de Fundición a El Cóndor, 22° 26′ S, 65° 10′ W, 4,530 m, *Zuloaga et al.10888* (SI); KC904345, -, -, KC904298. ***N. filiculmis*** (Delile) Barkworth. ARGENTINA. Prov. Buenos Aires: Bahía Blanca, Camino a Nueva Roma, *Cabrera 6792* (SI). Prov. Chubut: Cushamen, El Hoyo, subida a La Cascada, 1.5 km al E del pueblo, *Morrone et al. 5673* (SI), KC904346, KF294101, KF294141, KC904299. Prov. Córdoba: Punilla, Copina, *Burkart 7203* (SI). Prov. Corrientes: Ituzaingó, Isla Apipé Grande, Pto. San Antonio, *Krapovickas et al. 24108* (SI). Prov. Entre Ríos: Concordia, 9 km al S de la represa, *Zucol 165* (SI). Prov. La Pampa: Chapaleufú, I. Alvear, *Steibel et al. 10931* (SRFA). Prov. Misiones: Apóstoles, San José, Escuela Agrotécnica Salesiana P. Gentilini, *Cabrera et al. 28589* (SI). Prov. Neuquén: Lácar, Est. F. Pucará, *Rúgolo 475* (BAA). Prov. Río Negro: Bariloche, Base N del Cerro Ventana, *Morrone et al. 6132* (SI). Prov. San Luis: Pedernera, San José del Morro, Estancia La Morena, Cerro El Morro, *Boelcke et al. 16651* (SI). Prov. Santa Fé: Gral. López, cerca de Laguna Dicasa, *Lewis 4100* (SI). CHILE. VIII Region: La Laja, Malacura, en lomajes al lado S del Río Cascajo, 820 m, *Muñoz C - Schick 1497* (SGO 111595). URUGUAY. Dept. Lavalleja: Intersección rutas 60-12, *Bonifacino et al. 1840* (SI). Dept. Montevideo: Cerro, *Rosengurtt B 5239* (SI). Dept. Paysandú: Chapicuy, orillas del Río Uruguay, *Rosengurtt B 4900* (SI). ***N. formicarum*** (Delile) Barkworth. ARGENTINA. Prov. Buenos Aires: Gral. Lavalle, Cari Lauquen, Talares, *Cabrera 8500* (SI). Prov. Entre Ríos: Gualeguay: Sin localidad, *Zucol 270* (SI). Prov. La Pampa: 69 km S of Santa Rosa on Hwy 35, *Peterson 11225* (US); KC904347, KF294102, KF294142, KC904300. Prov. Río Negro: Avellaneda, Isla Choele Choel, Luis Beltrán, *Burkart s. n.* (SI 15888). ***N. glabripoda*** Torres. ARGENTINA. Prov. Jujuy: Sierra de Quichagua, Hwy 74, 3.7 rd km W Rio Rachayte xing, 7 rd km W of Rachayte, 53 air km, 22° 51′ S, 66° 11′ W, 3,940 m, *Peterson et al. 19563* (SI, US); KC904348, -, -, KC904301. Prov. La Rioja: Famatina, Cuevas de Medina, Sierra de Famatina, 3,300 m, *Hunziker 1969* (BAA, CORD). Prov. San Juan: Iglesia, Reserva de San Guillermo, camino a Los Caserones, *Nicora et al. 8310* (SI). ***N. holwayii*** (Hitchc.) Barkworth. ARGENTINA. Prov. Jujuy. W slope Sierra de Santa Victoria, basin 6 rd km SW of Abra Lizoite, 38 air km ESE La Quiaca on Hwy 5, 22° 14′ S, 65° 15′ W, 4,172 m, *Peterson et al.19583* (SI, US); KC904349, -, -, KC904302; 2.4 km NW of Cieneguillas at jct. of road to Santa Catalina and Casira/Piscuno, *Peterson et Annable 10324* (US); -, KF294103, KF294143, -. BOLIVIA. Dept. La Paz: Loayza, 12.1 mi NW of Villa Loza on road towards Urmiri and Sapahaqui, 3,850 m, *Peterson et al. 12650* (LPB). Dept. Oruro: Pantaleón Dalence, desvío de Vinto, 21 km hacia Machacamarca, subiendo hasta la Mina de San Luis, 3,900 m, *Beck 17962* (LPB, SI). ***N. hyalina*** (Nees) Barkworth. ARGENTINA. Prov. Buenos Aires: Zárate, Isla Talavera, a orillas del Río Paraná Guazú, *Morrone 5168* (SI); KC904350, KF294104, -, KC904303. Prov. Catamarca: Ambato, Los Varela, 1 km W, camino Humaya, *Saravia Toledo 13023* (SI). Prov. Córdoba: Colón: Ascochinga, *Giardelli 610* (SI). Prov. Corrientes: Ituzaingó, Camino del Buen Ayre, Parque Las Malvinas, *Morrone et Giussani 3598* (SI). Prov. Entre Ríos: Concordia, Arroyo Yuquerí Chico, *Lorentz s. n.* (BAF). Prov. Jujuy: Tumbaya, El Moreno, *Breglia 195* (SI). Prov. La Pampa: Quemú Quemú, Colonia Barón, *Steibel et Martínez 6073* (SRFA). Prov. La Rioja: Gdor. Gordillo, Chamical, Villa Paula de Sarmiento, a orillas del canal Cordero, *Ariza Espinar 165* (CORD). Prov. Prov. Mendoza; 26 km NW of San Jose on road towards Potrerillos, *Peterson et Annable 11402* (US); -, -, KF294144, -. Prov. San Luis: Pedernera, Mercedes, *Rosa 116* (SI). Prov. Santa Fé: Gral. López, Estancia La Santa Fé, *Lewis 1459* (SI). Prov. Tucumán: Capital, *Lillo s. n.* (LIL 40101). ***N. inconspicua*** (J. Presl) Barkworth. ARGENTINA. Prov. Jujuy: Dr. Manuel Belgrano, Yala, cerros, *Burkart et Troncoso s. n.*, SI 11281 (SI, BAA). Prov. Tucumán: Tafí, ruta 307, camino de Tafí del Valle a Amaicha del valle, 1 km antes de El Infiernillo, 26° 46′ S, 65° 43′ W, 2,790 m, *Cialdella et al. 169* (SI); KC904351, -, KF294145, KC904304. BOLIVIA. Dept. Cochabamba: Chapare, Cantón Colomi, 8 km al NW de Colomi, Candelaria Pie de Gallo, zona Chimparancho, *Beck 18106* (SI). Dept. La Paz: Ingaví, Cantón Jesús de Machaca, Comunidad Titicani-Tacaca, a 20 km de Guaqui, *Villavicencio 293* (SI). Dept. Potosí: José M. Linares Lizarazu, Comunidad Alkatuyo, Estancia Chulipa, 53 km SE de Potosí, 4.5 km al SW de la escuela de Alkatuyo, 3,510 m, *Marino 30* (LPB). ***N. lachnophylla*** (Trin.) Barkworth. CHILE. Región Metropolitana: Santiago, Valle del Río Clavillo, Cordillera de Santiago, *Grandjot 4657* (SI); Santiago, Cerro lo Chena, 700 m, *Gunckel 18262* (US); KC904352, -, -, KC904305. Región VI: Colchagua, San Fernando, Centinela, 350 m, *Montero 1357* (CONC 85412). ***N. longiglumis*** (Phil.) Barkworth. ARGENTINA. Prov. Buenos Aires: Tornquist, Tornquist, *Cabrera 8064* (SI). Prov. Catamarca: 18 km E of El Portezuelo at Cuesta del Portezuelo and 38 km E of Catamarca on Hwy 42, *Peterson 11651* (US); -, JF697994, EU489147, -. Prov. Córdoba: Calamuchita, Villa Gral. M. Belgrano, alrededores de la ciudad, *Morrone et Giussani 5784* (SI); KC904353, -, -, KC904306. Prov. Jujuy: Tumbaya, Chilcayo, Finca del Dr. Gronda, 23° 56′S 65° 29′W, 2,800 m, *Deginani et al. 368* (SI). Prov. La Pampa: Utracán, Gral. Acha, *Burkart 19214* (SI). Prov. Río Negro: Avellaneda, Ruta Nacional 22, a 73 km de Choele Choel, camino a Río Colorado, *Morrone et al. 5772* (SI). Prov. Salta: San Carlos, *Hunziker 2625* (BAA). Prov. San Luis: La Capital, Entre Potrero de Funes y El Volcán, *H. A. L. 7359* (SI). Prov. Santiago del Estero: Pellegrini, Salliqueló, *Cabrera 8038* (SI). Prov. Tucumán: Tafí del Valle, *Parodi 11074* (BAA). CHILE. VIII Region: Ñuble, camino Chillán-Bulnes, *Marticorena 466* (CONC 29357). ***N. manicata*** (E. Desv.) Barkworth. CHILE. IV Region: Coquimbo, Bosque de Talinay, *Muñoz-Coronel 1274* (SGO 57866). CHILE. Bio Bio: E of Concepcion provice line, between Copiulemu and Tomeco, on Ruta O-50, at km 32, *Soreng 7000* (US); KC904354, KF294105, EU489152, KC904307. ***N. megapotamia*** (Spreng. ex Trinius) Barkworth. ARGENTINA. Prov. Buenos Aires: Tandil, alrededores de Tandil, a 3 km al SE del centro, 250 m, *Morrone et al. 5129* (SI); KC904355, KF294106, KF294146, KC904308. Prov. Córdoba: Colón, Río Corapel, *Nicora 1187* (SI). Prov. Entre Rios: Gualeguaychú, Arroyo Seibo (Delta inferior), *Hunziker 4627* (BAB, SI). Prov. San Luis: Gral. Pedernera, Sierra El Morro, cuenca inferior, *Anderson 3433* (CORD, VMSL). Prov. Tucumán: Burruyacú, Sierra de Medina, *Venturi 2719* (LIL). BRASIL. State Rio Grande do Sul: Lagoa Vermelha, *Rosengurtt et Del Puerto 9091* (SI). State Santa Catarina: Bom Jardim da Serra, ruta BR-438, Km 37, *Rúgolo de Agrasar et al. 1503* (SI). URUGUAY. Dept. Soriano: Juan Jackson, *Gallinal et al. 4465P* (SI). ***N. melanosperma*** (J. Presl) Barkworth. ARGENTINA. Prov. Buenos Aires: Tandil, Sierra de las Ánimas, La Cascada, 281 m s.m, *Morrone et al. 5139* (SI); GU192031, KF294107, KF294147, KC904309. Prov. Chaco: Maipú, 40 km al S de J. J. Castelli, *Molina 2506* (BAB). Prov. Córdoba: Marcos Juárez, sin localidad, *Stuckert 14797* (CORD). Prov. Corrientes: Bella Vista, *Boelcke 1592* (SI). Prov. Entre Ríos: Federación, Salto Grande del Río Uruguay, *Hunziker 4525* (BAB, SI). Prov. La Pampa: Chapaleufú, I. Alvear, *Steibel et Troiani 6217* (SRFA). Prov. Misiones: Apóstoles, camino de Cerro Azul a Apóstoles, *Zuloaga 524* (SI). ***N. mexicana*** (Hitchc.) R. W. Pohl. VENEZUELA. State Mérida: Parque Nac. Sierra Nevada, 3 km del desvío de Apostadero, 60 km de Mérica, Páramo de Mucubají 3,600 m, *Morrone et al. 4727* (SI); KC904356, -, -, -. ARGENTINA. Prov. Jujuy: Tumbaya, Volcán, subida al Abra Morada, *Cabrera 18417* (LP). Prov. La Rioja: Famatina, La Vega de La Hoyada, 2,700-2,800 m, *Jiménez s. n.* ex Herb. *Kurtz 15151* (CORD). Prov. Salta: La Caldera, Cuesta del Carancho, arriba del Potrero del castillo, 2,900 m, *Sleumer et Vervoorst 2584 p. p.* (LIL). Prov. Tucumán: Tafí, ruta provincial 307, entre Carapunco y La Bolsa, 26° 45′ S, 65° 44′ W, 2,920 m, *Zuloaga et al. 9517* *(SI); 17 km N of Tafi on Hwy 307 to Amaicha del Valle, *Peterson et al. 10141* (US); -, KF294108, KF294148, -. BOLIVIA. Dept. Cochabamba: Punata, camino a Malga-Punata, Chaki ghocha, 3,500 m, *Hensen 12508* (LPB). Dept. La Paz: Omasuyos, camino de Achacachi a Sorata, 2 km antes de Curupampa, *Morrone et Belgrano 4910b* (CTES, LPB, SI); -, -, -, KC904310. Dept. Tarija: Límite Prov. Cercado, cerranía El Cóndor, 48 km de Tarija, 2,830 m, *Beck & Alzérreca 32217* (LPB, SI). ***N. meyeniana*** (Trin. & Rupr.) Parodi. ARGENTINA. Prov. Jujuy: 34 km S of La Quiaca on Ruta Nacional 9 towards Abra Pampa, at "Demostrativo La Intermedia", *Peterson et Annable 10301* (US); -, KF294109, EU489153, -. Prov. Salta: Valles Calchaquíes, ca 69 air km due N of cachi, Hwy 40, 72 rd km N of jct Hwy 38, N La Quesera, 24° 30′ S, 66° 10′ W, 3,854 m, *Peterson et al. 19520* (SI, US); KC904357, -, -, KC904311. BOLIVIA. Dept. La Paz: Aroma, Huaraco, 3,700 m, *Beck 8817* (LPB, SI). Dept. Potosí: 10 km N of Villazon on Hwy 702 towards Tupiza, 3,500 m, *Peterson et Annable 11871* (SI, US). Dept. Tarija: Avilés, Iscayachi, 24 km hacia Villazón, 3,610 m, *Beck 11042* (LPB). CHILE. XV Region: Termas de Jurasi, 4,070 m, *Moreira et al. 1654* (SGO). ***N. meyeri*** Torres. ARGENTINA. Prov. Catamarca: Belén,

Farallón Negro, *Cámara Hernández 130* (BAA). Prov. Jujuy: Humahuaca, Ruta 9, de Tres Cruces a Humahuaca, Ayo. Puente del Diablo, 3,690 m, *Cialdella et al. 488* (SI); KC904358, KF294110, KF294149, KC904312. Prov. La Rioja: Gral. Lamadrid, Los Molles, entre Jagüé y cerro El Leoncito, *Krapovickas et Hunziker 5535* (CORD). Prov. Salta: San Carlos, Amblayo, *Hunziker 2635* (BAA). Prov. San Juan: Iglesia, Quebrada de Aguas Negras, *Cabrera et al. 24377* (LP). Prov. Tucumán: Tafí del Valle, El Molle, en el camino entre Tafí del Valle y Amaicha, km 91/92, *Hunziker et al. 24894b* (CORD). ***N. mucronata*** (Kunth) R. W. Pohl. ARGENTINA. Prov. Salta: Cachi, ruta 33, de Piedra del Molino a El Carril, Herradura, *Zuloaga et al. 11261* (SI). Prov. Tucumán: Tafí Viejo, La Ciénaga, *Lillo 2785* (LP). BOLIVIA. Dept. Cochabamba: Mizque, Canton Molinero, Rakaypampa, 2,800 m, *Sigle 195* (LPB). Dept. La Paz: Los Andes, a 81 km del Puesto carretero, carretera La Paz-Tiquina, 3,860 m, *Rúgolo et Villavicencio 1818, 1821* (SI). Dept. Oruro: Sebastián Pagador, Cantón Huari, Ayllu Mallkoca, 3,825 m, *Beck 29966* (LPB, SI). CHILE. VIII Region: Bío Bío, Ñuble, *Grandjot 2795* (SI). PERÚ. Dept. Ancash: Recuay, *Peterson 13799* (MO); KC904359, KF294111, KF294150, KC904313. ***N. nardoides*** (Phil.) Barkworth. ARGENTINA. Prov. Catamarca: Belén, Reserva de la biósfera "Laguna Blanca," *Reca et Ramadori 150* (SI). Prov. Jujuy: approximately 12 km SE of Tres Cruces on Hwy 9 towards Humahuaca, *Peterson et Annable 11773* (US); KC904360, KF294112, EU489154, KC904314. Prov. La Rioja: Gral. Sarmiento, Quebrada de Machaco, Piedra Parada, *Hunziker et Caso 4130* (CORD). Prov. Salta: Hwy 40, 7.7 air km N of Abra de Acay, 16 rd km S of jct Hwy 51, SSE of San Antonio de los Cobres, 18 air km, 24° 22′ S, 66° 14′ W, 4,251 m, *Peterson et al. 19539* (SI, US). Prov. San Juan: Iglesia, Reserva de San Guillermo, al oeste del Refugio de Agua del Godo, *Kiesling et al. 4580* (SI). BOLIVIA. Dept. Cochabamba: Tapacarí, Comunidad de Japo (km 125 Cochabamba-Oruro), Waillara, 4,370 m, *Pestalozzi 1028* (LPB). Dept. La Paz: Murillo, La Paz-El Alto, 4,020 m, *Beck 3975* (LPB). Dept. Potosí: Daniel Campos, Uyuni, *Hicken 1* (SI). Dept. Tarija: Avilez, Cuenca de Tajsara, 3,560-3,660 m, *Campero Meyer 58* (LPB). CHILE. I Región: Tarapacá, Cordillera de Tarapacá, *Philippi s. n.* (CORD). Región II: Antofagasta, ruta nacional 27, de Paso de Jama a San Pedro de Atacama, *Zuloaga et al. 11148* (SI). ***N. neesiana*** (Trin. & Rupr.) Barkworth. ARGENTINA. Prov. Buenos Aires: Tandil, ruta prov. 74, Sierra del Tigre, campos junto a la Reserva del Tigre, *Giussani et Morrone 319* (SI); KC904361, -, -, KC904315. Prov. Catamarca: Ambato, 23 km de Catamarca, rumbo a El Rodeo, *Saravia Toledo et al. 13370* (SI). Prov. Chaco: Primero de Mayo, Colonia Benítez, *Schulz 3975* (BAB). Prov. Córdoba: Colón, Ascochinga, *Nicora 970* (SI). Prov. Corrientes: Capital, Riachuelo, arenal alto de Yatay-poñy, *Schinini 12358* (SI). Prov. Entre Ríos: La Paz, Santa Elena, *Burkart et al. 23259* (SI). Prov. Juyuy: 58 km N of San Salvador de Juyuy on Hwy 9 and ll km on road to Tiraxi, *Peterson et Annable 10258* (US); -, EU489155, JF697996, -. Prov. La Pampa: Capital, Santa Rosa, *Steibel 10969* (SRFA). Prov. La Rioja: Sarmiento, Jagüé, patio alrededores del destacamento de guardafaunas, *Biurrun et Molina 4495* (SI). Prov. Mendoza: Capital, Ciudad de Mendoza, *Covas 15013* (SI). Prov. Misiones: San Ignacio, Camino de Loreto a San Ignacio, *Zuloaga et Deginani 435* (SI). Prov. Salta: Rosario de Lerma, Campo Quijano, 1,500 m, *Parodi 13538* (BAA). Prov. San Luis: Gral. Pedernera, José del Morro, Estancia La Morena, Cerro El Morro, *Boelcke et al. 16650* (SI). Prov. San Juan: Calingasta, Reserva Natural Estricta El Leoncito, *Haene 1952* (SI). Prov. Santa Fé: Castellanos, Rafaela, *Pensiero 1190* (SI). Prov. Santiago del Estero: Belgrano, 10 km NO de Mojón de Hierro, próximo Fortín Inca, *Pensiero et Faurie 3471* (SI). Prov. Tucumán: Burruyacú, El Puestito, *Venturi 7449* (SI). BOLIVIA. Dept. Cochabamba: Sin localidad, *Parodi 10179* (SI). Dept. Santa Cruz: Valle Grande, Clausura Cochabambita, 1,650 m, *Joaquín et Martínez 11312* (SI). Dept. Tarija: Arce, Viniendo desde Tarija rumbo a Bermejo, en Rumi Cancha, 3,782 m, *Negritto et al. 468* (CORD, SI). BRASIL. State Santa Catarina: São Joaquim, Chapada Bonita, 6 Km W of São Joaquim toward Estancia, *Smith 15873* (SI). CHILE. Región VIII: Bío Bío, Concepción, *Kunkel 2027* (SI). PARAGUAY. Dept. Cordillera: Barretiro, orilla del monte, galería Arroyo Yaguarý, *Burkart 18867* (SI). Dept. Paraguarí: Estación Barrerito-Caapucú, *Ramírez 1150* (SI). URUGUAY. Dept. Colonia: Ruta 12, Km 48, camino de Nueva Palmira a Cardona, pasando a Palo Solo, *Morrone et al. 5234* (SI). Dept. Tacuarembó: Ruta 5, Km 399, Arroyo Tres Cruces, *Seijo et al. 2533* (CTES, SI). ***N. novari*** Torres. ARGENTINA. Prov. Jujuy: Dr. Manuel Belgrano, entre León y Nevado de Chañi, Las Cuevas, 3,000 m, *Fabris et al. 4043* (LP). Prov. Salta: Chicoana, ruta 33, de Cachi a Ciudad de Salta, pasando desvío a ruta 42, 3,210 m, *Cialdella et al. 308* (SI); GU192033, KF294113, KF294151, KC904316; 50 km E of Cachi on Hwy 40 towards Salta at jct. of road to Amblayo and Iszona, *Peterson et Annable 10222* (US). ***N. nubicola*** (Speg.) Torres. ARGENTINA. Prov. Catamarca: Ambato, Sierra de Ambato, falda este, subiendo desde El Rodeo hacia el Cerro Manchado, rumbo a Casa de Cubas, *Hunziker et al. 19728* (CORD). Prov. Jujuy: Tumbaya, Volcán, Chilcayo, camino a Abra Morada, 2,800-3,000 m, *Kiesling et al. 5784* (SI). Prov. Tucumán: Tafí, ruta 307, camino de El Infiernillo a Amaicha del Valle, 26° 40′ S, 65° 48′ W, 2,840 m, *Cialdella et al. 181* (SI); KC904362, KF294115, KF294152, KC904317. BOLIVIA. Dept. La Paz: Larecaja, camino de Achacachi a Sorata, 3 km antes de Sorata, *Morrone et Belgrano 4915* (CTES, LP, SI). ***N. pampagrandensis*** (Speg.) Barkworth. ARGENTINA. Prov. Catamarca: Belén, Sierra de Belén, NW of Condor Huasi, ca. 4 km, 14 air km NW of La Puerta de San José, jct Hwy 40 N of Belén 15 km, *Peterson et al. 19412* (SI, US). Prov. Jujuy: Tumbaya: Volcán, cerros, *Cabrera et Marchionni 12935* (BAA, LP). Prov. La Rioja: Quebrada del Portezuelo, camino de la Mina Esperanza, *Hunziker 5109* (MERL, SI). Prov. Salta: Chicoana, Cuesta del Obispo, *Nicora et al. 9215* (SI). Prov. San Luis: Junín, Merlo, *Baez 121* (SI). Prov. Tucumán: Tafí, ruta 307, camino de Tafí del Valle a Amaicha del Valle, 1 km antes de El Infiernillo, 2,790 m, *Cialdella et al. 163* (SI); GU192028, KF294116, KF294153, KC904318. ***N. pampeana*** (Speg.) Barkworth. ARGENTINA. Prov. Buenos Aires: Tornquist, Parque Provincial Sierra de la Ventana, subida al mirador de la Ventana, 38° 12′ S, 62° 16′ W, *Morrone et al. 5438* (SI); KC904363, KF294117, -, KC904320. Prov. Chubut: Florentino Ameghino, Lochiel, cerca de Camarones, *Soriano 1989* (BAA, SI). Prov. La Pampa: Lihuel Calel, Sierra de Lihuel Calel, *De Azcue s. n.* (BAB 91224). Prov. Mendoza: San Rafael, Sierra del Nevado, Arroyo Chacy-co, Puesto Barroso, *Boelcke et al. 15563* (SI). Prov. Río Negro: San Antonio, Sierra Grande, unos 3 km sobre ruta provincial 9 hacia Punta Colorada, serranías al NO, *Cocucci et al. 3790* (CORD, SI). Prov. San Juan: Calingasta, Quebrada de Las Burras, Vega del Mal Paso, *Kiesling et al. 7449* (SI). Prov. San Luis: Coronel Pringles, Sierra de Pillahuincó, *Cabrera 7370* (SI). Prov. Santa Cruz: Deseado, 10 km al NO de Puerto Deseado, camino a Tellier, Co. Dujón, *Correa 2624* (BAA). ***N. parva*** Torres. ARGENTINA. Prov. Tucumán: Trancas, ruta de Hualinchay a Tolombón, 26° 19′ S, 65° 39′ W, 2,820 m, *Zuloaga et al. 10051* (SI); -, -, -, KC904319. Prov. Catamarca: Ambato, Cerro El Manchado, *Türpe 1148* (LIL, SI). Prov. Jujuy: Valle Grande, Santa Ana, praderas, 3,000 m, *Burkart et Troncoso s. n.* (BAA ex SI 11689). Prov. Tucumán: Trancas, Ruta de Hualinchay a Tolombón, *Zuloaga et al. 10051* (SI). ***N. pfisteri*** (Matthei) Barkworth. CHILE. VII Region: Maule, Camino de Parral a Cauquenes, km 36, *Marticorena et Matthei 488* (CONC). VIII Region: Ñuble: 20 km south of Chillan, and 1.4 km north of Puente Larqui, on the Panamerican Hwy, *Lammers et al. 7943* (CONC). CHILE. VIII Region: Bio Bio: S of Chillan, S of Nebuco on Ruta 5S, ca 2 km S of exit to Concepcion, *Soreng 7017b* (US); -, JF697997, EU489157, KC904321. ***N. philippi*** (Steud.) Barkworth. ARGENTINA. Prov. Buenos Aires: La Plata, Villa Elisa, *Cabrera 9817* (SI). Prov. Entre ríos: Gualeguaychú, camino a Ibicuy, a 3 km del cruce con ruta nacional 12, 33° S, 58° 48′ W, *Morrone 6200* (SI); KC904364, KF294118, KF294154, -. Prov. Corrientes: Monte Caseros, Estancia La Pelota, Bañados del Timboi, *Nicora 5097* (BAA). Prov. Santa Fé: San Cristóbal, Ruta 39, a 16 km del cruce con Ruta 4, *Stofella 258* (SI). CHILE. IX Region: Araucanía, Cautín, Temuco, *Acevedo 71* (SI). URUGUAY. Dept. Cerro Largo: Bañado Medusa, *Montoro 317* (SI). Dept. Rivera: Río Negro y Arroyo Hospital, Vichadero, *Rosengurtt 6963b* (SI). ***N. poeppigiana*** (Trin. & Rupr.) Barkworth. ARGENTINA. Prov. Buenos Aires: Tornquist, Parque Provincial Sierra de la Ventana, subida al mirador de la Ventana, entre la base y la parada "6", 38° 12′ S, 62° 16′ W, *Morrone et al. 5449* (SI); KC904365, -, -, KC904322. Prov. Chubut: Futaleufú, Región del Corcovado, *Illin 76* (SI). Prov. Córdoba: Presidente Roque Sáenz Peña, sin localidad, *Hunziker 12862* (CORD). Prov. La Pampa: 69 km S of Santa Rosa on Hwy 35, *Peterson et Annable 11221* (US); -, KF294119, KF294155, -. Prov. Neuquén: Lácar, San Martín de Los Andes, Parque Nacional Lanín, sector Lácar, *Vanni et al. 4478* (CTES, SI). Prov. Río Negro: Bariloche, Parque Nacional Nahuel Huapi, Lago Perito Moreno, angostura este-oeste, *Morrone et Giussani 5824* (SI). CHILE. Región VIII: Bío Bío, Ruta nacional 181, Km 77, camino de Malalcahuelo a Curacautín, *Morrone et al. 5991* (SI). Región IX: Araucanía, Malleco, Curacautín, *Burkart 9458* (SI). X Region: De Los Lagos, 17 Km de Curacautín, camino a Conguillo, Km 25 de la ruta interlagos, *Morrone et al. 5548* (SI). ***N. pseudopampagrandesis*** (Caro) Barkworth. ARGENTINA. Prov. Córdoba: Calamuchita, Cerro Áspero, sendero de Cumbrecita al Cerro Champaquí, *Krapovickas 7810* (SI). Prov. Salta: Santa Victoria, Nazareno, 22° 30′ S, 65° 06′ W, 3,050 m, *Zuloaga et al.10853* (SI); KC904366, -, -, KC904323. Prov. Tucumán: Tafí del Valle: Ruta provincial 307, 11 km S de Tafí del Valle, paraje La Angostura, *Pensiero et Marino 4173* (SI). ***N. pubiflora*** (Trin. & Rupr.) E. Desv. var. ***pubiflora***. ARGENTINA. Prov. Catamarca: Pomán, Sierra de Ambato, falda oeste subiendo desde El Rincón hacia Las casitas, rumbo al Cerro Manchado, *Hunziker et Ariza Espinar 20541* (CORD). Prov. Jujuy:

Cochinoca, Abra pueblo de Cochinoca, 22° 44′ S 65° 53′ W, 3,690 m, *Deginani et al. 488* (SI). Prov. La Rioja: Sanagasta, Sierra de Velasco, subiendo el Cerro Yacuchiri desde Pampa de La Viuda, cerca de Quebrada El Lampazo, *Biurrun et al. 6899* (SI). Prov. Salta: Los Andes, Camino a Abra del Acay, ruta 40, Las Pircas, *Nicora et al. 8961* (SI). Prov. San Juan: Caucete: Mogote Corralitos, Sierra de Pie de Palo, *Haene 1085* (MERL, SI). Prov. Tucumán: Tafí, ruta 307, camino de Tafídel Valle a Amaicha del Valle, 1 km antes de El Infiernillo, 2,790 m, *Cialdella et al. 168* (SI); KC904367, -, -, KC904324; 28 km SE of Amaicha del Valle on Hwy 307 towards Tafi del Valle, *Peterson et Annable 11618* (US); -, KF294120, EU489158, -. BOLIVIA. Dept. La Paz: Loayza, 12.1 mi NW of Villa Loza on road towards Urmiri and Sapahaqui, 3,850 m, *Peterson et al. 12652* (LPB). Dept. Oruro. Sebastián Pagador, Cantón Huan, Ayllu Malkoca, 3,825 m, *Beck 29967* (LPB, SI). Dept. Potosí. Nor Chichas, 29 mi SE of Uyuni on road towards Atocha, 3,780 m, *Peterson et al. 12838* (LPB). CHILE. I Region: 70 km NE of Huara on Hwy A-55 towards Colchane, *Peterson & Soreng 15616* (CONC).***N. pulchra*** (Hitchc.) Barkworth. U. S. A. California: San Luis Range, Montana de Oro State Park, N slopes of Valencia Pk., *Soreng et al. 7407* (US); KC904368, -, -, KC904325. ***N. punensis*** Torres. ARGENTINA. Prov. Jujuy: Cochinoca, Abra de Rachaite, 4,140 m, 22° 53′ S 66° 13′ W, *Múlgura et al. 1315* (SI). Prov. Salta: Los Andes, ruta 51, de San Antonio de los Cobres a Viaducto La Polvorilla, 4,170 m, *Cialdella et al. 428* (SI); KC904369, -, KF294156, -. BOLIVIA. Dept. Potosí: Approximately 28 km NW of Salo and 46 km E of Atocha, *Peterson et Annable 11844* (SI, US). ***N. rupestris*** (Phil.) Torres. ARGENTINA. Prov. Salta: Los Andes, de San Antonio de los Cobres a Abra de Acay, 24° 25′ S, 66° 14′ W, 4,600 m, *Zuloaga et al. 11191* (SI); 39 km W of Purmamarca on Hwy 52 and 70 km NW of Tumbaya, *Peterson et Annable 10347* (US); KC904370, KF294121, EU489160, KC904326. Prov. Tucumán: Tafí Viejo, Cumbres Calchaquíes, Cerro Negrito, 4,000 m, *Türpe 419* (LIL). BOLIVIA. Dept. Cochabamba: Tapacarí, Patakaluyo, al E de Japo K'asa (km 125 en la carretera Cochabamba-Oruro), 4,160 m, *Pestalozzi 250* (LPB). Dept. La Paz: Ingavi, cantón Jesús de Machaca, comunidad Titicani-Tacaca, 20 km de Guaqui, 3,850 m, *Villavicencio 825* (LPB). Dpto Oruro: Ladislao Cabrera, De Salinas de G. Mendoza hacia el oeste, vía Iswaya, 3,600 m, *Beck 11810* (LPB, SI). Dept. Tarija: Avilez, cerca Passajes, 3,600 m, *Bastió 674* (LPB). CHILE. II Region: Steep slopes along road from Caspana to El Tatio, 3,800 m, *Peterson et al. 15564* (SI, US). ***N. sanluisensis*** (Speg.) Barkworth. ARGENTINA. Prov. Buenos Aires: Coronel Suárez, Sierra de las Tunas, *Pertusi 54* (LP). Prov. Catamarca: Ambato, Sierra de Ambato, camino a El Rodeo, 1,300 m, *Parodi 14013* (LP). Córdoba: Calamuchita, cercanías de Villa General Belgrano, *Giussani et Morrone 326* (SI); KC904371, -, -, KC904326. Prov. Jujuy: Tumbaya, ruta nac. 9, 4 km de Volcán, camino a S. S. de Jujuy, cantera sobre el Río Grande, 2,040 m, *Morrone et al. 3160* (SI). Prov. La Pampa: Chical Co, Lomas de Jagüel del Moro, *Prina et al. 2975* (SI). Prov. La Rioja: Chilecito, Cuesta de La Miranda, *Calderón 1063* (SI). Prov. Mendoza: San Rafael, Sierra Pintada, Valle Grande, *Prina et al. 1951* (SI). Prov. San Luis: Cerro la Aguada, *Burkart 10927* (SI). Prov. San Juan: Valle Fértil, Sierra de Valle Fértil, Los Bretes, *Kiesling et al. 4981* (SI). Prov. Santiago del Estero: Ojo de Agua, Sierras de Sumampa, Ojo de Agua a Sumampa, 7 km de la primera por ruta 13, *Piccinini 3781* (BAB). ARGENTINA. Without data, *Roig 3293* (US); -, KF294122, EU489161, -. ***N. sellowiana*** (Nees ex Trin. & Rupr.) Peñail. BRASIL. State Paraná: Ponta Grossa, "ribas in campo," *Dusén 12102* (SI). BRASIL. Without data, *Reitz et Klein 5314* (US); -, KF294123, EU489162, KC904328. ***N. smithii*** (Hitchc.) Barkworth. PERÚ. Dept. Puno: El Collao, 10 km NW of Pomata on road towards Ilave, *Peterson et al.14627* (MO, US); -, KF294124, KF294157, KC904329. ***N. tenuis*** (Phil.) Barkworth. ARGENTINA. Prov. Buenos Aires: Tornquist, Abra de la Ventana, Cerro Bahía Blanca, 400 m, *Morrone et al. 5116* (SI); KC904372, -, -, -. Prov. Córdoba: Tercero Arriba, Río Tercero, *Burkart 10187* (SI). Prov. Chubut: Rawson, 36 km al S de Trelew, *Ruiz Leal 14808* (BAA). Prov. La Pampa: Guatraché, Estancia La Julia, *Rúgolo de Agrasar 1273* (SI). Prov. La Rioja: Famatina, Sierra de Famatina, Mina El Oro, *Calderón 1087* (SI). Prov. Mendoza: approximately 16 km SW of Potrerillos on road towards San Jose, *Peterson et Annable 11412* (US); -, KF294125, KF294158, -. Prov. Neuquén: Los Lagos, Estancia Fortín Chacabuco, *Boelcke 10601* (BAA). Prov. Río Negro: Pichi Mahuida, Ruta 22, al E de Río Colorado, *Burkart 15821* (BAA). Prov. San Juan: Zonda, Estancia Maradona, Agua Pinto, *Kiesling et al. 6043* (SI). Prov. San Luis: General Pedernera, Nueva Escocia, *Burkart 10838* (SI). Prov. Santa Cruz: Deseado, camino de Fitz Roy a Caleta Olivia, *Nicora 7597* (BAA). CHILE. VIII Region: Bío Bío, ruta 160, camino de Cañete a Tirúa, a 4 Km de Cañete, *Morrone et al. 6085* (SI). Región X: Los Lagos, Villarrica, Ruta interlagos, alrededores de Villarrica, *Morrone et al. 5552* (SI). ***N. tenuissima*** (Trin.) Barkworth. ARGENTINA. Prov. Buenos Aires: Villarino, Balneario Chapelcó, *Villamil et Nicora 2161* (SI). Prov. Catamarca: Ambato, El Rodeo, Ruta Provincial 4, entre Las Juntas y Piedras Blancas, *Donadío et al. 210* (SI). Prov. Córdoba: Calamuchita, Villa Gral. Belgrano, Cerro de La Virgen, *Morrone et Giussani 5148* (SI); KC904373, -, KF294159, -. Prov. La Pampa: Guatraché, Laguna La Tigra, *De Aracama s. n.* (SI 90486). Prov. La Rioja: Famatina, Sierra de Famatina, Mina El Oro, *Calderón 1183* (SI). Prov. Mendoza: Malargüe: Sierras de Borborán, *Prina et al. 2949* (SI). Prov. Río Negro: San Antonio: Sierras Grandes (Yacimiento de hierro Fabricaciones Militares), cercano a ruta nacional 3, *Piccinini et García 1308* (BAB). Prov. San Juan: Zonda, Estancia Maradona, Agua Pinto, *Kiesling 6071* (SI). Prov. San Luis: Chacabuco, Sierra de la Estanzuela, *Ezcurra 341* (SI). Prov. Santa Cruz: Río Chico, Lago Pueyrredón, *Boelcke 12844* (BAA, BAB, SI). Prov. Santa Fé: Vera, La Loca, *Pire et al. 441* (SI). Prov. Santiago del Estero: Ojo de Agua, a la salida de Ojo de Agua hacia, Pozo Grande, *Sayago 3490* (BAA). Prov. Tucumán: Tafí del Valle, ruta provincial 307 *Renvoize et al. 3392* (SI). ***N. torquata*** (Speg.) Barkworth. ARGENTINA. Prov. Buenos Aires: Tornquist, Sierra de la Ventana, ruta 76, cerro frente a la entrada al Parque Provincial Sierra de la Ventana, 38° 04′ S, 62° 01′ W, *Morrone et al. 5481* (SI); KC904374, -, KF294160, KC904330; Coronel Suárez: "Hab. In graminosis prope Sierra de Curá-malal, Dic 1899," *Spegazzini s. n.* ex LPS 2430 (LP). URUGUAY. Dept. Salto: Termas de Arapey, *Arrillaga de Maffei 1522* (SI). ***N. trichotoma*** (Nees) Hack. ex Arechav. ARGENTINA. Prov. Buenos Aires: Pdo. Guaminí, Ruta prov. 65, 77 km de Daireaux camino a Guaminí, Laguna Alsina, *Morrone et al. 5102* (SI); KC904375, -, -, KC904331. Prov. Catamarca: Ancasti, Cumbre de Ancasti, falda E, El Taco (ruta provincial 2), *Hunziker 15700* (CORD). Prov. Córdoba: Santa María, Alta Gracia, *Burkart 10136* (SI). Prov. Entre Ríos: Paraná, *López s. n.* (BAB 13073). Prov. La Pampa: Guatraché, Estancia La Julia, potrero 1, *Rúgolo de Agrasar 1269* (SI). Prov. Mendoza: Parque del Oeste, *Sanzin 146* (SI). Prov. San Juan: 40 km SW of Zonda at Agua Pinto (Estancia Maradona), *Peterson et Annable 11506* (US); -, JF697998, EU489164, -. Prov. San Luis: Coronel Pringles, Sierra de Pillahuincó, *Cabrera 7307* (SI). Prov. Santa Fé: General López, Castellanos, *Lewis 1405* (SI). Patagonia. Sin provincia ni localidad, *Ameghino s. n.* (SI 85612). URUGUAY. Dept. Soriano: Juan Jackson, *Rosengurtt 227b* (SI). ***N. tucumana*** (Parodi) Torres. ARGENTINA. Prov. Juyuy: 58 km N of San Salvador de Juyuy on Hwy 9 and ll km on road to Tiraxi, *Peterson et Annable 10255* (US); -, KF294126, KF294161, -. Prov. Salta: Santa Victoria, ruta prov. 19, 10 km de Los Toldos camino a Lipeo, *Morrone et al. 3774** (SI). Prov. Tucumán: Tafí Viejo: Siambón, 1,300 m, *Parodi 10663* (BAA).

Piptochaetium montevidense (Spreng.) Parodi. ARGENTINA. Prov. Entre Ríos: Gualeguaychú, ruta 16, km 58, de Gualeguaychú a Gualeguay, *Cialdella et al. 16* (SI); GU192029, -, KF294162, -. Prov. Jujuy: Valle Grande, de San Francisco a Alto de Calilegua, *Zuloaga et al. 10321* (SI).

APPENDIX 2. List of characters and character states.

1. Lemma margins: flat, strongly overlapping (0) / flat, not or only slightly overlapping (1) / slightly involute (2) / conspicuously involute (3). 2. Palea length: half to equal the length of the lemma (0) / longer than the lemma (1) / shorter than half the length of the lemma (2). 3. Callus shape: acute or subacute (0) / blunt or truncate (1). 4. Lemma crown: absent (0) / inconspicuous (1) / conspicuous (2). 5. Palea texture: membranous (0) / indurate (1). 6. Lemma texture: membranous (0) / indurate (1). 7. Lemma apex: minutely 2-toothed or without any tooth (0) / with 2 conspicuous teeth (1). 8. Lemma pubescence: absent (0) / present in part of the lemma (1) / present all over the lemma (2). 9. Prickles and/or papillae of the lemma: absent (0) / present all over the lemma (1) / present in part of the lemma (2). 10. Floret length: < or equal to 7 mm (0) / > 7 mm (1). 11. Floret width: < or equal to 1 mm / > 1 mm. 12. Glume texture: membranous (0) / indurate (1). 13. Disarticulation of the awn or awnlike tip and the lemma: absent (0) / present (1). 14. Glume length: longer than the floret (0) / shorter than the floret (1).