Systematic Botany (2007), 32(3): pp. 545–559
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A Phylogeny of *Piptochaetium* (Poaceae: Pooideae: Stipeae) and Related Genera Based on a Combined Analysis including *trnL-F, rpl16,* and Morphology

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Communicating Editor: Alan W. Meerow

ABSTRACT. The tribe Stipeae occurs in temperate and warm temperate grasslands of Eurasia, Australia, and America. Although generic circumscription within the tribe has recently undergone significant changes, the American genus *Piptochaetium* has been clearly defined by morphological and anatomical characters. It includes 36 species and 2 varieties, most of them widely distributed in temperate grasslands of South America. Phylogenetic analyses were conducted in *Piptochaetium* and allied genera of the Stipeae, in order to test the monophyly of the genus, re-examine infrageneric taxa, and analyze relationships among species of this genus and allied genera of Stipeae. Two chloroplast molecular markers, *trnL-F* and *rpl16*, as well as morphology were used. Topology between morphological and molecular data mainly differs in the relationships of *Piptochaetium* with *Anatherostipa* and *Piptatherum*. Molecular and combined analyses yielded two major clades in the tribe: the $x = 11$ Clade with *Piptochaetium, Aciachne, Anatherostipa*, and *Jarava vaginata*, and the Aneuploid Clade with *Jarava, Nassella*, and *Piptatherum*. Monophyly of *Piptochaetium* was confirmed by morphological and total evidence (morphology and DNA data) analyses. Monophyly of sect. *Podopogon*, sect. *Piptochaetium* and *Nassella* were only supported by the total evidence analysis. Circumscription of *Jarava* is also discussed.

KEYWORDS: basic chromosome numbers, chloroplast DNA, morphological characters, POY, stipoid phylogeny.

The tribe Stipeae has generally been considered a natural and well-defined entity (Peñailillo 1996; Hsiao et al. 1999). The tribe includes perennial plants, with loose or dense panicles of one-flowered spikelets without a rachilla extension, and lemmas generally with a single, terminal awn or with a sharp point. The Stipeae occurs in temperate and warm temperate grasslands of Eurasia, Australia, and America. Delimitation of tribal boundaries, based on anatomical and morphological characters, has been controversial and with no consensus among taxonomists. As a result, *Ampelodesmos* Link, *Nardus* L., *Amphipogon* R. Br., *Anisopogon* R. Br., and *Milium* L. have been included (Clifford and Watson 1977; Wheeler et al. 1982; Dahlgren et al. 1985; Clayton and Renvoize 1986), or have been excluded from Stipeae (Barkworth and Everett 1987). Moreover, generic circumscription within the tribe has recently undergone changes: many species of *Stipa* L. have been transferred to *Nassella* E. Desv. or *Jarava* Ruiz and Pav., and *Stipa* has been restricted to Eurasia (Barkworth and Everett 1987). American Stipeae are represented by 14 genera (Soreng et al. 2003); *Austrostipa* S.W.L. Jacobs & J. Everett, *Piptochaetium* J. Presl, *Nassella*, and *Piptatherum* P. Beauv. occur in both hemispheres, whereas *Ptilagrostis* Griseb., *Achnatherum* P. Beauv., *Hesperostipa* (M. K. Elias) Barkworth, and *Oryzopsis* Michx. are restricted to North America (Barkworth 1993), and *Anatherostipa* (Hack. ex Kuntze) Peñailillo, *Aciachne* Benth., *Ortachne* Nees ex Steud., and *Jarava* to South America (Peñailillo 1996; 2002). *Stipa* and *Macrochloa* Kunth are cultivated and/or introduced (Soreng et al. 2003).

J. Presl (1830) described *Piptochaetium*, an American genus, based on *P. setifolium*. The genus was then circumscribed to include those species with short and obovoid florets, with a blunt callus, and a weak and deciduous awn. This concept was followed by Hitchcock (1935, 1951). Parodi (1944) expanded the limits of the genus to include species previously treated under *Podopogon* Raf., with long callus and terete florets, and a robust and persistent awn. Hence, Parodi established two sections: sect. *Podopogon* (Raf.) Parodi and sect. *Piptochaetium* (= *Eupiptochaetium*). This classification was applied to the South American species by Burkart (1969), Torres (1970), Petetin (1978), Sánchez Vega (1991), Mujica-Sallés and Marchi (1993), and Cialdella and Arriaga (1998). Thomasson (1978), based on anatomical studies of the lemma, provided additional evidence supporting Parodi's classification, as he demonstrated that the species treated under *Podopogon* also share the presence of regularly dentate fundamental cell sidewalls with species included in sect. *Piptochaetium*. Thereafter, Parodi's concept was also applied to the North American species (Barkworth and Everett 1987; Jacobs et al. 2000).

Piptochaetium as presently circumscribed includes 36 species and 2 varieties, distributed in

temperate regions of America, from the northern United States to central Chile and Argentina. Twenty seven species are found in South America, predominantly in open grasslands of Argentina, southern Brazil, and Uruguay (Cialdella and Arriaga 1998; Cialdella and Giussani 2002).

Jacobs et al. (2000), based on molecular data (ITS) and including an extensive sample of Stipeae, related *Piptochaetium* to *Piptatherum* and several species of *Oryzopsis* now transferred to *Piptatherum*. However, when they used morphological characters, *Piptochaetium* appeared related to *Nassella* and *Hesperostipa*.

As now circumscribed (Cialdella and Giussani 2002), *Piptochaetium* is mainly defined by three characters: florets with involute lemma margins, a boat-shaped bi-keeled palea, and a palea longer than the lemma. Using morphological data, Cialdella and Giussani (2002) analyzed the phylogenetic relationships among species of *Piptochaetium*, and tested the monophyly of the genus in relation to *Nassella* and *Hesperostipa*. In this study, sect. *Podopogon* was paraphyletic whereas sect. *Piptochaetium*, including species of the ''obovoid group'' (Cialdella & Giussani 2002), was monophyletic.

The aims of this study are to test the monophyly of *Piptochaetium*, re-examine infrageneric taxa, and study the phylogenetic relationships among species of this genus and allied genera within the Stipeae, using chloroplast DNA *trnL-F* and *rpl16* sequences and morphological data.

Materials and Methods

Taxon Sampling. Twenty-one species and two varieties of *Piptochaetium* were included as ingroup taxa in all molecular and morphological analyses. A complete sampling of all species of the genus was not possible, due to the lack of material and difficulties in sequencing. Nevertheless, morphological and geographical variability of *Piptochaetium* are well represented by the sampled taxa. Species from five genera of the tribe Stipeae were selected as outgroups: 21 species of *Nassella*, eight species of *Jarava, Piptatherum miliaceum* (L.) Coss., *Anatherostipa brevis* (Torres) Peñailillo, and *Aciachne acicularis* Laegaard. The genus *Hesperostipa* (M.K. Elias) Barkworth could not be considered in this analysis due to the lack of material for sequencing. *Poa holciformis* J. Presl (Pooideae: Poeae) and *Bromus catarthicus* Vahl (Pooideae: Bromeae) were also included to root the tree. *Nassella* and *Jarava* were here circumscribed following Barkworth (1990), Barkworth and Torres (2001), and Peñailillo (2002). A total of 56 taxa were included in the morphological and molecular data matrices. A list of the species and their infrageneric treatment is presented in Appendix 1. Representative specimens from *Piptochaetium* included in the morphological analysis can be found in Cialdella and Giussani (2002). Appendix 1 also includes the voucher information of specimens used here for the molecular analyses. Specimens of *Piptochaetium, Nassella, Jarava, Piptatherum, Anatherostipa, Aciachne, Poa*, and *Bromus* used for the morphological and molecular data sets are presented in Appendix 2.

Morphological Data. A total of 34 morphological characters were included in the data matrix (Appendix 3). Characters used here differ from the characters used by Cialdella and Giussani (2002) by the addition of nine characters (26 to 34), the exclusion of two characters: inflorescence branches (previous character 11), and awn shape (previous character 27), and the re-formulation of two characters related to presence of prickles (previous characters 18 and 19 now combined in character 15; Appendix 4).

DNA Isolation, Amplification and Sequencing. Total DNA was extracted from fresh or silica-gel-dried leaves, using one of several modified CTAB protocols (Doyle and Doyle 1987, Murray and Thompson 1980, and Saghai-Maroof et al. 1984). To extract DNA from a few herbarium specimens the Dneasy Plant Mini kit (Qiagen, Hilden, Germany) was used.

Following extraction, DNA was amplified using the polymerase chain reaction for the *trnL* intron and *trnL-trnF* spacer region (*trnL-F*), and the *rpl16* intron (*rpl16*) of the gene encoding ribosomal protein 16. The *trnL-F* region was amplified using primers C, D, E and F as of Taberlet et al. (1991). For taxa where C and F failed, Cii = 5′ - TAGACGCTACGGACTTGATTG- 3′ and Fdw = 5′-CAGTCCTCTGCTCTACCAGC-3′ were used. Amplification of the *rpl16* intron was accomplished by using primers F71 of Jordan et al. (1996), and R1661 of Kelchner and Clark (1997). When amplification failed, the intron was amplified in two fragments using F584: 5′TTCATTGGGTGGGATGGCG-GAA3′ and R584: 5′TTCCGCCATCCCACCCAATGAA3′. For sequencing reactions, two additional primers were also employed: R270 (Zhang 2000), and F80: 5′-C/ TTATTGCTTCGTATTGTCG-3′.

PCR reaction of 25 or 50 µl contained 20–40 ng/µl of DNA template, and a final concentration of 1× PCR Buffer minus Mg, 5 mM $MgCl_2$, 0.025 mM dNTP each, 0.2 µM each primer, and 1.25–3 units Taq Polymerase, recombinant from Invitrogen life technologies. PCR amplifications were followed under the following conditions for most of the species: 1) *trnL-F*: 1 cycle of 94°C for 5 min, 34 cycles of 94°C for 30 sec, 48°C for 1 min, and 72°C for 1 min 30 sec, and a final extension cycle of 72°C for 7 min; 2) *rpl16*: 1 cycle of 94°C for 4 min, 34 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min 30 sec, and a final extension cycle of 72°C for 7 min. For the species that failed these protocols, variations in the annealing temperature (1–8°C), and number of cycles were followed. In addition, a variety of PCR additives and enhancing agents (bovine serum albumin, dimethyl sulfoxide, formamide) have been used to increase the yield, specificity and consistency of PCR reactions. However, using different combinations of these variations, bad quality or no product was acquired.

Macrogen, Inc. (Seoul, Korea) performed cleaning of PCR products using Montage PCR purification kit from Millipore following manufacturer's protocol. Sequencing reactions were also performed by Macrogen Inc. using a MJ Research PTC-225 Peltier Thermal Cycler and the ABI PRISM BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Foster City, California), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using selected primers sent by us (see above). The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI PRISM 3730XL sequencer (96 capillary type, Applied Biosystems).

To edit and assemble the sequences we used the program Chromas Pro version 1.22 (Technelysium Pty, Ltd., Tewantin, Australia).

A total of 56 taxa were sequenced for *trnL-F* and 50 for *rpl16*. The species that could not be completely sequenced are indicated in Appendix 1. All sequences were submitted to GenBank (http://www.ncbi.nlm.nih.gov); voucher information and accession numbers are provided in Appendix 1.

Phylogenetic Analysis. Parsimony analyses were conducted using three data sets: morphological data, molecular data (*trnL-F* + *rpl16* combined), and a complete combined data set (*trnL-F* + *rpl16* + morphology). Data matrices are deposited in TreeBASE (study number S1720).

MORPHOLOGY. We used parsimony to analyze the morphological data set using the program TNT ver. 1.0 (Goloboff et al. 2003). During the search we used equal weights and non-additive characters. The searches involved 1000 replicates each constructing a Wagner tree using a random addition sequence of taxa from the data matrix, swapping the initial tree with TBR (tree bisection and reconnection), and retaining a maximum of two trees in each replicate. Subsequently, all optimal trees were swapped using TBR holding a maximum of 10,000 trees. Bremer supports (Bremer 1994) were calculated to find sub-optimal trees of 1, 2, and 3 steps longer than the optimal trees, saving a maximum of 1000 trees in each step. Jackknife values (Farris et al. 1996) were calculated by running 10,000 replicates using an independent character removal probability of 36% (default). In each replicate a single Wagner tree was constructed and TBR swapped holding a single optimal tree.

DIRECT OPTIMIZATION. DNA data and combined morphology-DNA data were analyzed by parsimony using direct optimization as implemented in the program POY ver. 3.0.11 (Wheeler et at. 2003, documentation by De Laet and Wheeler 2003). POY constructs phylogenetic hypotheses directly without the intervening step of multiple sequence alignment. During the direct optimization procedure (Wheeler 1996) POY incorporates insertion and deletion events in addition to base substitutions. Consequently, base changes and indels are minimized during tree search, and POY selects the tree/trees that requires least changes to fit the input sequences.

When analyzing with POY (or any algorithm implementing sequence alignment), the cost of opening a gap and the cost of a base change must be specified *a priori*. Direct optimization has often been used in combination with sensitivity analysis (sensu Wheeler 1995), where several cost regimes are explored and subsequently evaluated by congruence measures as for example the ILD index (Mickevich and Farris 1981; Farris et al. 1995) to select the cost set that yields the highest congruence between the data partitions.

An alternative approach is to set all transformation costs equal (setting gap cost and base change cost equal), supposedly analogous to standard phylogenetic analyses of morphological data sets (Frost et al. 2001; Grant and Kluge 2003). This cost set has, however, the drawback of counting each position in an indel (each unit gap) separately, hence it does not account properly for indel events that affect several base pairs simultaneously.

De Laet (2005) showed that to accomplish an equal weighting scheme and to apply the same cost to all transformations, including indels of different lengths, the following cost set can be used: opening gap cost = 3, extension gap cost = 1, and base change cost = 2. It is interesting that the cost scheme proposed by De Laet (2005) falls within the zone of highest congruence found empirically in several data sets (Aagesen et al. 2005). Although not exploring the cost set proposed by De Laet (2005), Aagesen et al. (2005) found that the highest congruence (as mentioned by several congruence measures) was obtained when extension gap costs were lower than gap opening costs, and gap opening costs were equal or slightly higher than base change costs – as proposed by De Laet (2005).

Considering the computational effort of direct optimization, a rational criterion for selecting cost set *a priori* is most welcome. Here we chose to apply equal weights to all transformations and consider the cost set presented by De Laet (2005) to be the best heuristic approximation of this weighting scheme currently available (Faivovich et al. 2005; Giannini and Simmons 2005). The cost of a morphological transformation was set to 2 (equal to the base change cost, De Laet pers. comm.). If a regular sensitivity analysis is conducted, as suggested by one anonomyous reviewer (exploring the effect of changing the gap opening costs from 3 to 2, 4 or 8), the ILD index points to a slightly different cost set as the optimal one, selecting a gap opening cost of 2 instead of 3. The topology does, however, not differ from the one discussed here.

MOLECULAR AND COMBINED ANALYSES. Direct optimization is computationally demanding even for a small data set. We used the following search strategy that showed to be efficient for our data set: –replicates 50 –nopr –tbr –stopat 5 –minstop 10 –norandomizeoutgroup –maxtrees 2 –holdmaxtrees 300. This creates a Wagner tree and submits it to TBR swapping holding a maximum of two trees. The procedure was repeated 100 times using the same outgroup. When the optimal length was found five times, the search was abandoned if a minimum of 10 searches had been completed. After the replicates were completed the resulting trees were, by default, submitted to TBR swapping storing a maximum of 300 trees. Bremer supports (BS) were calculated in POY by using the option –bremer and a constrain file. Jackknife values were calculated using the options: –replicates 500 –nospr –tbr –maxtrees 1. This gives 500 replicates each calculating a Wagner tree that is submitted for TBR swapping. The jackknife values (Jk) are obtained by a majority rule consensus tree with a cut-off value of 50%. All matrices and implied alignments (Wheeler et al. 2003) from POY are available upon request from the authors.

RESULTS

Morphological Analysis. The morphological matrix resulted in 54 equal parsimonious trees (length 118, CI=0.36, RI=0.83). The strict consensus tree shows two major clades within Stipeae (Jk = 78): one containing *Aciachne, Piptatherum*, and *Piptochaetium* (BS = 1), and the second clade grouping *Anatherostipa, Jarava*, and *Nassella* (BS = 2; Fig. 1). *Piptochaetium* is recovered as monophyletic (BS = 3), but only section *Podopogon* is supported (BS = 2). The genus *Nassella* is also monophyletic (BS = 2), and is nested within *Jarava*.

Molecular Analysis. The *trnL-F* sequences were divided into intron and spacer before submission to POY. The *trnL* intron sequence length varied from 428 bp in *Nassella cordobensis* to 487 bp in *Piptochaetium calvescens*, and 528 bp in *Bromus catharticus*. The *trnL-trnF* spacer sequence length ranged from 323 bp in *Nassella hyalina* to 345 bp in *Piptochaetium panicoides*. The *rpl16* intron sequence length ranged from 1148 bp in *Piptochaetium seleri* to 1217 bp in *Nassella neesiana, N. curamalalensis*, and *Jarava vaginata*.

The analysis combining the two DNA markers

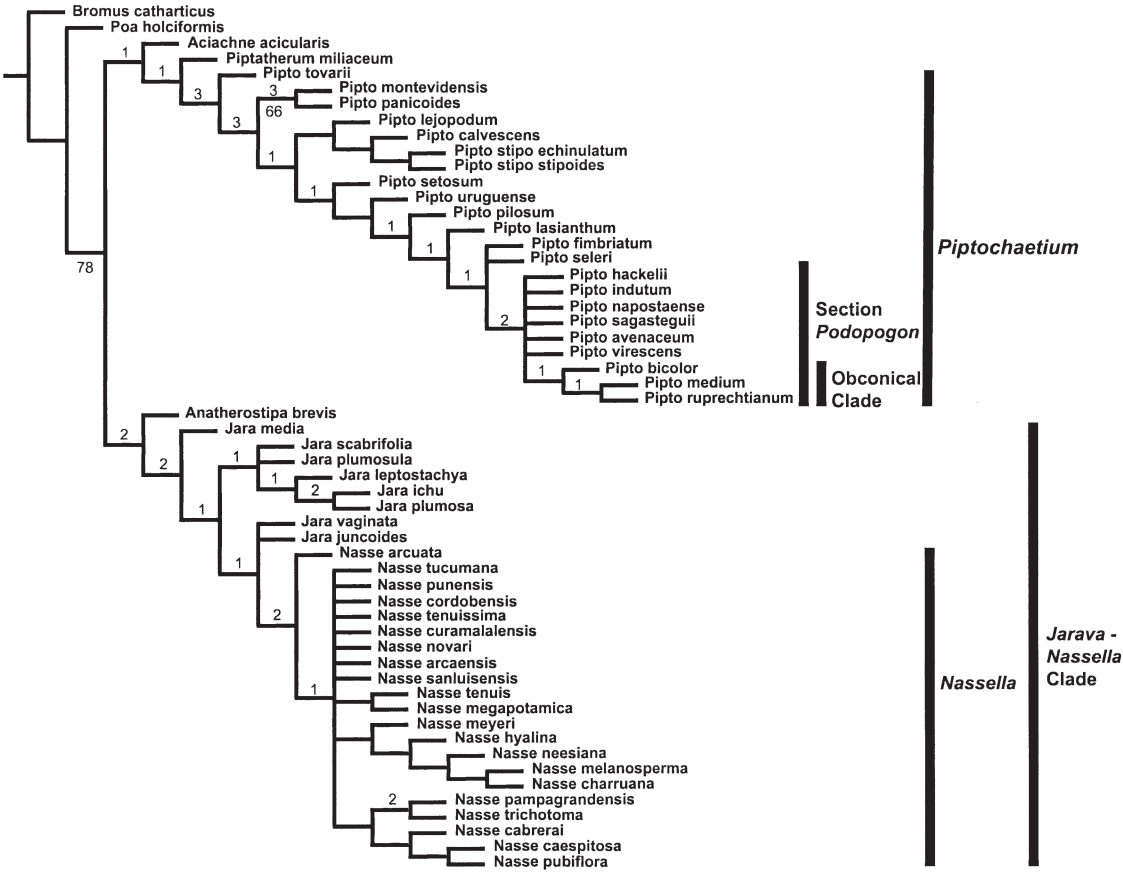

FIG. 1. Strict consensus of 54 most parsimonious trees (L = 118, CI = 0.36, RI = 0.83), using morphological data set. Numbers above and below branches indicate Bremer support values and jackknife percentages, respectively. Bars represent taxonomic groups as discussed in the text.

yielded 300 trees (L=1337), the size of the tree buffer. The consensus tree shows two major clades (Fig. 2): clade 1 comprising species of *Piptochaetium*, the *Aciachne-Anatherostipa* clade, and *Jarava vaginata* (BS = 7); and clade 2 containing species of *Jarava* and *Nassella*, and *Piptatherum* (BS = 8). *Aciachne* and *Anatherostipa* form a well-supported clade (BS = 5), whereas monophyly of *Piptochaetium* is not confirmed.

Piptatherum miliaceum and *Nassella arcaensis* are sister groups of a major clade gathering all other species of *Jarava* and *Nassella* (BS = 2). *Jarava* appears as polyphyletic due to the placement of *J. vaginata* in clade 1 (Fig 2). Relationships between species of *Nassella* and *Jarava* are not resolved; however, some groupings are recovered: *Jarava leptostachya* and *J. scabrifolia*; *Nassella arcuata* and *N. novari*; *N. caespitosa* and *N. punensis*; and clade 3 (BS = 2): *N. cabrerae, N. charruana, N. cordobensis, N. curamalalensis, N. hyalina, N. megapotamica, N. melanosperma, N. neesiana, N. pampagrandensis, N. sanluisensis, N. tenuis, N. tenuissima, N. trichotoma*, and *N. tucumana*.

Morphology and DNA Sequences Combined. When the morphological and molecular data were combined, 18 trees of 1668 steps were found. Figure 3 shows the consensus tree with Bremer and Jackknife support below and above branches, respectively.

The combined analysis showed the tribe Stipeae (BS ≈ 50, Jk = 81) divided in two major clades associated with the basic chromosome number: *Aciachne, Anatherostipa, Jarava vaginata* (unknown number), and *Piptochaetium* are included in clade 1 (BS = 3) or the x = 11 clade; whereas *Piptatherum, Nassella*, and *Jarava* were grouped in clade 2 (BS = 4, Jk = 56) or the Aneuploid clade.

Within the x = 11 clade, *Piptochaetium, Anatherostipa*, and *Aciachne* form a group (Fig. 3, BS = 3) with *Jarava vaginata* as the sister taxon. *Anatherostipa* and *Aciachne* form a well-supported clade (BS = 4; Jk = 70%). *Piptochaetium* is monophyletic (BS = 1) with the *Anatherostipa-Aciachne* clade as sister group. All species of *Piptochaetium* except *P. fimbriatum* are grouped in a well-supported clade (BS = 8), with *P. seleri* as a sister taxon to a clade including the rest of the species (BS = 4). This last

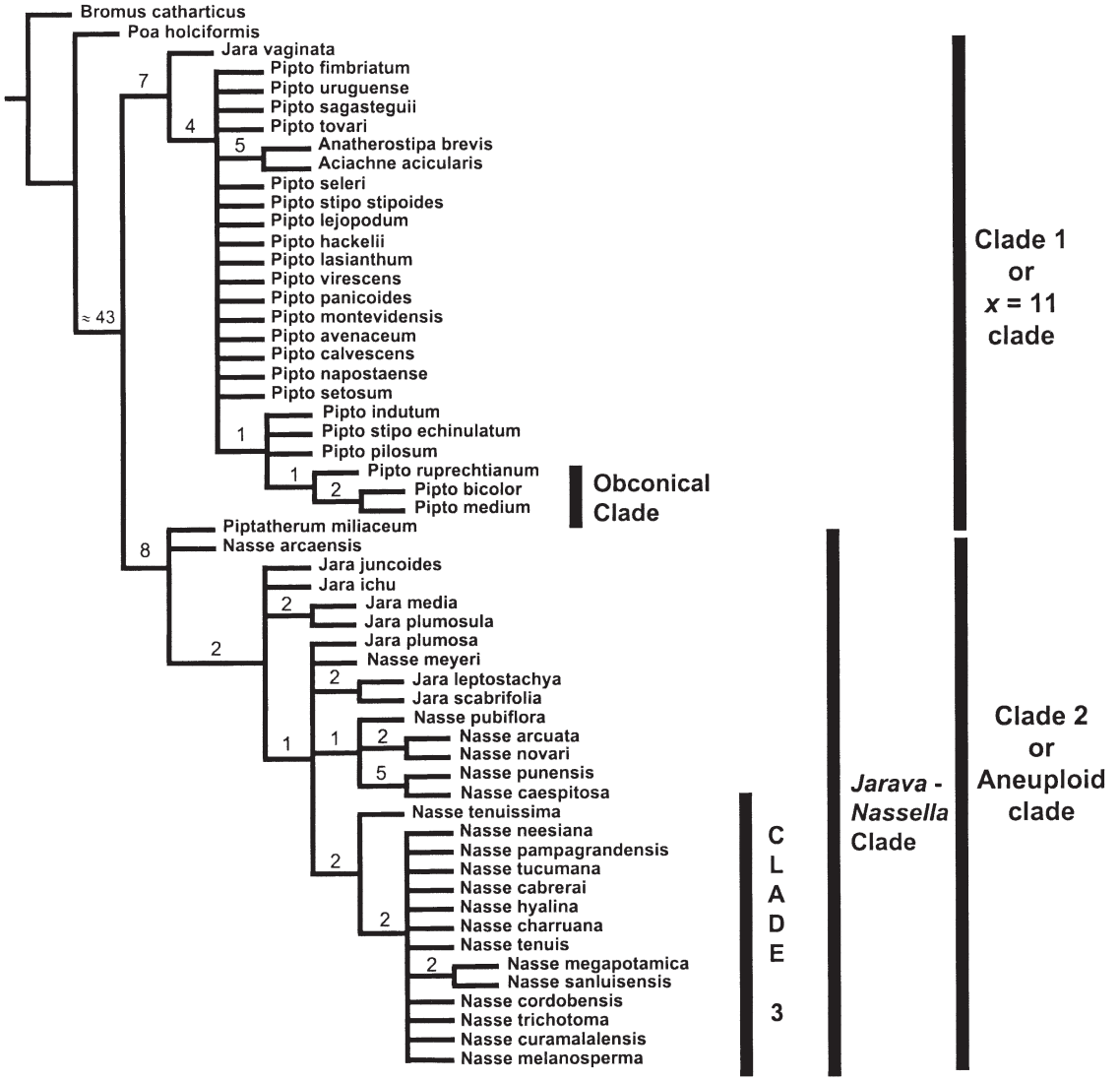

FIG. 2. Strict consensus of 300 most parsimonious trees (L = 1337) using *trnL-F* and *rpl16* combined data set. Numbers above and below branches indicate Bremer support values and jackknife percentages, respectively. Bars represent taxonomic groups as discussed in the text.

clade shows sections *Piptochaetium* (BS = 2) and *Podopogon* (BS = 3) as monophyletic. Within section *Podopogon* only the species with obconical florets (*P. bicolor, P. medium* and *P. ruprechtianum*) are supported as a monophyletic group (BS = 3) and *P. ruprechtianum* is sister to *P. medium-P. bicolor* clade (BS = 7; Jk = 65%). Within section *Piptochaetium*, the relationship between *P. montevidensis* and *P. panicoides* is strongly supported (BS = 6; Jk = 88%).

Jarava vaginata appears in clade 1, rendering *Jarava* polyphyletic. When inspecting POY generated diagnosis and implied alignments of the optimal trees (see De Laet and Wheeler 2003), the relationship between *J. vaginata* and the *Aciachne-Anatherostipa-Piptochaetium* clade appears to be supported by several base changes and small gaps (both diagnosis and implied alignments are available upon requests from the authors). All other species of *Jarava* are joined in a clade, in which species of *Nassella* are nested and form a monophyletic group (BS = 8), principally supported by morphological characters.

The *Jarava-Nassella* clade is well supported (BS = 7, Fig. 3). Some highly supported minor clades are found: *Jarava media* and *J. plumosula* (BS = 4; Jk = 60%), and *J. leptostachya* and *J. scabrifolia* (BS = 3).

Within *Nassella*, three major supported clades are found (Fig. 3): *N. arcuata-N. novari* (BS = 4; Jk = 66%), *N. caespitosa-N. pubiflora -N. punensis* (BS = 4, Jk = 53), and clade 3 (BS = 4).

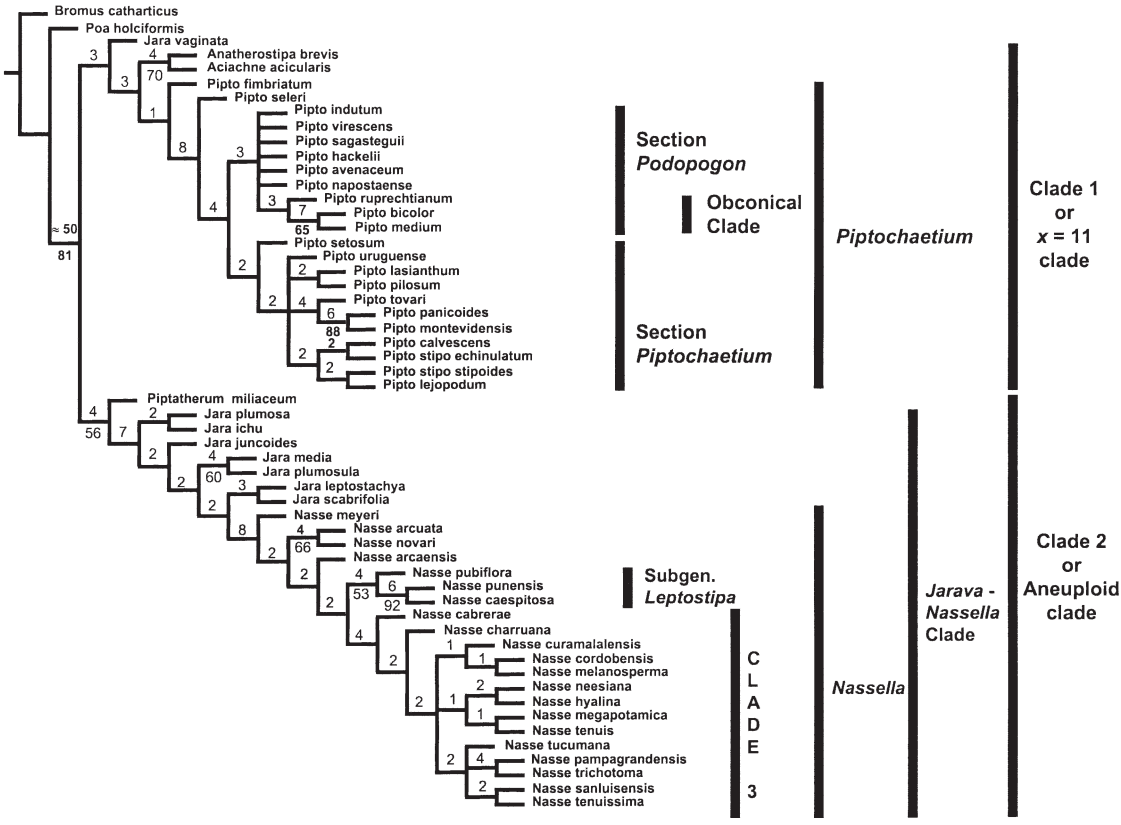

FIG. 3. Strict consensus of 18 most parsimonious trees (L = 1668), using morphological and molecular (*trnL*-F and *rpl16*) combined data set. Numbers above and below branches indicate Bremer support values and jackknife percentages, respectively. Bars represent taxonomic groups as discussed in the text.

DISCUSSION

Monophyly of tribe Stipeae is supported by ITS data (Hsiao et al.1999), but the limits of the tribe are somewhat diffuse, due to the changing position of a few taxa (Jacobs et al. 2000). The presence of one-flowered spikelets, lemma awned or with a sharp firm point and slightly or not compressed florets are generally useful for defining the tribe Stipeae. In the present analyses, including *Bromus* L. and *Poa* L. as outgroups, a flat palea, a densely hairy callus, a pubescent lemma, and a terete to fusiform uncompressed floret are plesiomorphic states that characterize the tribe. Dorsiventral compression of the floret in *Piptatherum* and lateral compression in *Piptochaetium montevidense* and *P. panicoides* (Cialdella and Giussani, 2002) are derived synapomorphies within the Stipeae. Other characters like lemma margins, palea shape, length and venation of the palea, presence of a crown, lemma and palea texture, and epidermal pattern are useful to identify genera within the Stipeae (Barkworth 1990). In this sense, different combinations of these characters are synapomorphies of *Piptochaetium, Nassella, Piptatherum, Aciachne* or *Anatherostipa* when optimized on the DNA-morphology combined tree.

Base Chromosome Number. The base chromosome number is an interesting attribute to consider within the evolution of Stipeae. Mainly due to molecular data, the Stipeae is early divided in two major clades. *Aciachne, Anatherostipa*, and *Piptochaetium* included in the $x = 11$ clade (chromosome numbers according to Covas and Bocklet 1945; Valencia 1946; Parodi 1958; Reeder and Reeder 1968; Valencia and Costas 1968; Davidse and Pohl 1994), although the position of *Jarava vaginata* is in need of verification and its chromosome number assessed. The second clade group *Piptatherum, Nassella*, and *Jarava* in the Aneuploid clade since an aneuploid series of chromosome counts have been reported for these genera, making the basic chromosome number difficult to predict.

Most species of *Piptochaetium* included in the analyses were cited as diploid, with only *P. fimbriatum* known as a tetraploid ($2n = 44$, Brown 1951; Valencia and Costas 1968). The most frequent number in *Piptatherum* is $n = 12$ (Moinuddin et al. 1994); $2n = 20$ was found in *Piptatherum shoshonea-*

num (Curto and Douglass M. Hend.) P. M. Peterson and Soreng, [under *Stipa shoshoneana*, Curto and Henderson 1998]. Species of *Stipa* now included in *Nassella* present: $2n = 28$, 32, 34, 36 or 42 (Myers 1947; Brown 1951; Bowden and Senn 1962); similarly *Jarava* has $2n = 40$, 42 or 44 (Bowden and Senn 1962; Covas and Bocklet 1945).

Aciachne-Anatherostipa-Piptochaetium (*x* = *11 Clade).* The *Anatherostipa-Aciachne* clade is included in a not resolved polytomy with *Piptochaetium* when analyzing DNA on its own, or sister to *Piptochaetium* in the combined analysis. According to the POY-generated diagnoses of the optimal trees the relationship among the three genera is supported by serveral base changes and minor gaps. Based on total evidence, *Piptochaetium* is monophyletic defined by involute lemma margins (0), bi-keeled palea (1), palea longer than the lemma (2), fundamental cell sidewalls regularly dentate (5), and presence of a crown (26). *P. fimbriatum* is clearly circumscribed by these morphological characters. However, when including DNA data, the position of *P. fimbriatum* is controversial and should be further confimed.

Sections within *Piptochaetium* are not supported by molecular or morphological data alone, but both sections are recovered in the combined analyses. Species in sect. *Podopogon* are characterized by long (6), acute or subacute (7) callus and long florets (23), while sect. *Piptochaetium* or the informal ''obovoid'' group (Cialdella and Giussani 2002), present deciduous awns (9) and obovoid to globose florets (11). Evidence from molecular and morphological data supports a common origin for species with obconical florets of sect. *Podopogon*, the Obconical clade: *P. ruprechtianum, P. medium*, and *P. bicolor*.

Piptochaetium fimbriatum, P. seleri, and *P. angustifolium* (the latter not included in these analyses) have been considered as species showing intermediate characters when compared to sections *Podopogon* and *Piptochaetium* (Cialdella and Giussani 2002). The three species did not fit into any of the sections as originally described by Parodi (1944). Due to the simplicity in the spikelets and the difficulty in getting new morphological characters in *Piptochaetium*, only the addition of more molecular data could confirm the monophyly of infrageneric taxa.

Aciachne and *Anatherostipa* are highly supported by molecular and morphological combined analyses. These two genera share a few similar characters like length and shape of fundamental epidermal cells, shape and length of the callus; additionally, all species inhabit high Andean altitudes. Their relationships were previously recognized by Barkworth and Everett (1987) based on the similarity in leaf cross section, lemma epidermis, and floret structure, suggesting the inclusion of *Aciachne* in *Stipa* sect. *Obtusa*, now recognized as *Anatherostipa*.

Jarava-Nassella-Piptatherum *(Aneuploid Clade).* Although *Piptatherum, Nassella* and *Jarava* form a monophyletic clade (Figs. 2–3), there is no morphological evidence for this relationship. *Piptatherum* is clearly distinguished from *Nassella* and *Jarava* in presenting dorsiventrally compressed indurated florets, and open lemma in the apex partially covering the palea, the latter 2-nerved and indurated. Based on morphology, *Aciachne, Piptatherum*, and *Piptochaetium* share indurated lemma and palea, though the texture of the lemma is also similar in *Nassella* (Fig. 1).

The circumscription of *Stipa* and its recent segregates *Nassella, Jarava,* and *Anatherostipa* are still under discussion. Monophyly of *Jarava* should be further studied including an extensive sample of species as well as extra-American species of *Stipa*.

In *Nassella* and *Jarava* the palea is equal to shorter than the lemma (2) and both have shortened fundamental cells in the epidermis of the lemma (4). *Nassella* would be a monophyletic genus, derived from species of *Jarava* (Figs. 1, 3).

Jarava was originally described as a genus (Ruiz and Pavon 1794), then treated as a section of *Stipa* (Trinius and Ruprecht 1842) or a subgenus (Spegazzini 1901; Caro and Sanchez 1973). It is now recognized as a South American genus with 59 species (Peñailillo 2002, 2003). Characters previously used to circumscribe the genus (Peñailillo 2002) like ''macrohairs on the awn'' would have appeared independently in the evolution of the tribe, occurring in species of *Jarava* (*J. media* and *J. plumosula*; Figs. 2, 3), and in species of *Nassella* (*N. arcuata* and *N. novarii*; Figs. 2, 3).

Nassella, traditionally restricted to species with short florets (Parodi 1947), was expanded to include long floret species of *Stipa* s.l. and now comprises 116 species (Barkworth and Torres 2001). In this study, based on a reduced sample of 22 species, synapomorphies for the genus would be: lemma margins strongly overlapping (0), palea nerveless (3), apex of the lemma fusionated in a crown (26), and lemma indurated (33). Additionally, characters that have been considered to support the expansion of *Nassella* are: a strongly convolute lemma, a reduced short palea, lemma epidermal pattern (Thomasson 1978; Barkworth 1990), as well as results from ITS sequence data (Jacobs et al. 2000). Monophyly of *Nassella* is principally supported by morphology although

not contradicted by molecular data. Subgenera of *Stipa* (Spegazzini 1901, 1925) are here recovered in *Nassella* like *Leptostipa* and, within clade 3, several species of *Stipa* subgen. *Nassella*, subgen. *Microstipa*, and subgen. *Stephanostipa*. However, infrageneric taxa need to be reviewed in a more comprehensive study of *Nassella* as currently circumscribed.

ACKNOWLEDGEMENTS. This research was supported by ANPCyT (Agencia Nacional de Promoción Científica y Técnica, Argentina), grants 11739 and 13374, by CONICET grant #5453 and by the Myndel Botanica Foundation. We are also grateful to the curators and staff of the herbaria mentioned in the text, who allowed the study of specimens under their care, and to Prof. Zulma Rugolo de Agrasar, Dr. María Negritto, and Dr. Antenor Sulekic who kindly provided material of different Stipeae for DNA analyses. Finally, we thank the staff of the Darwinion, who helped us throughout.

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APPENDIX 1. Species of Stipeae sequenced for molecular phylogenetic analysis, infrageneric taxa, voucher specimens from which DNA was extracted for sequencing, and GenBank accession numbers: *trnL-F* and *rpl16*. First references of infrageneric taxa of the Stipeae included in the analyses; CA: sections followed by Cialdella & Arriaga (1998); P: sections recognized by Parodi (1944); S1: subgenera designated by Spegazzini (1901); S2: subgenera considered by Spegazzini (1925), SV: sections followed by Sánchez Vega (1991); NI: not formally included in any infrageneric taxon.*: species with half of the *rpl16* intron sequence (550 to 568 pb long) or only the *trnL* intron (482 to 486 pb long); **: species partially incomplete, lacking 150 – 250 bp; -: species not sequenced.

Aciachne acicularis Laegaard; NI; *Sulekic et al. 2782*; DQ887426*; DQ887376.

Anatherostipa brevis (Torres) Peñailillo; NI; *Sulekic et Cano 2554*; DQ887427; DQ887377** .

Bromus catharticus Vahl; NI; *Giussani 354*; DQ887428; DQ887378.

Jarava ichu Ruiz & Pav.; *Jarava* (S1); *Cialdella et al. 536*; DQ887429; DQ887379. *Jarava juncoides* (Speg.) Peñailillo; *Dasystipa* (S1); *Morrone et al. 5120*; DQ887430; DQ887380. *Jarava leptostachya* (Griseb.) F. Rojas; *Jarava* (S1); *Cialdella et al. 417*; DQ887431; DQ887381. *Jarava media* (Speg.) Peñail.; *Ptilostipa* (S1); *Cialdella et al. 242*; DQ887432*; DQ887382**. *Jarava plumosa* (Spreng.) S. W. L. Jacobs & J. Everett; NI; *Morrone et Giussani 5151*; DQ887433*; DQ887383** . *Jarava plumosula* (Nees ex Steud.) F. Rojas; *Ptilostipa* (S1); *Cialdella et al. 258*; DQ887434*; DQ887384** . *Jarava scabrifolia* (Torres) Peñail.; NI; *Cialdella et al. 303*; DQ887435; DQ887385. *Jarava*

vaginata (Phil.) F. Rojas; *Pappostipa* (S1); *Cialdella et al. 264*; DQ887436; DQ887386

Nassella arcaënsis (Speg.) Torres; Dasystipa (S1); *Cialdella et al. 275*; DQ887439; DQ887387**. *Nassella arcuata* (R. E. Fr.) Torres; NI; *Cialdella et al. 183*; DQ887440; DQ887388. *Nassella cabrerae* Torres ; NI; *Cialdella et al. 161*; DQ887441; DQ887389. *Nassella caespitosa* Griseb.; *Dasystipa* (S1), *Leptostipa* (S2); *Cialdella et al. 495;* DQ887442; DQ887390** . *Nassella charruana* (Arechav.) Barkworth; *Stephanostipa* (S1); *Cialdella et al. 12*; DQ887443*; DQ887391*. *Nassella cordobensis* (Speg.) Barkworth; *Microstipa* (S1); *Morrone et Giussani 5147*; DQ887437; DQ887392. *Nassella curamalalensis (Speg.)* Barkworth; *Nassella* (S1), *Microstipa* (S2); *Morrone et al. 5107*; DQ887444; DQ887393. *Nassella hyalina (Nees)* Barkworth; *Stephanostipa* (S1),*Stemmatostipa (S2); Morrone 5168*; DQ887445; DQ887394. *Nassella megapotamica* (Spreng. ex Trinius) Barkworth; *Dasystipa* (S1); *Morrone et al. 5129*; DQ887446; DQ887395. *Nassella melanosperma* (J. Presl) Barkworth; *Stephanostipa* (S1); *Morrone et al. 5139*; DQ887447; DQ887396. *Nassella meyeri* Torres; NI; *Cialdella et al. 488*; DQ887448; DQ887397. *Nassella neesiana* (Trin. & Rupr.) Barkworth; *Stephanostipa* (S1); *Giussani et Morrone 319*; DQ887449; DQ887398. *Nassella novari* Torres; NI; *Cialdella et al. 308*; DQ887450*; DQ887399. *Nassella pampagrandensis (Speg.)* Barkworth; *Nassella* (S1), *Microstipa* (S2); *Cialdella et al. 163*; DQ887451; DQ887400. *Nassella pubiflora* (Trin. & Rupr.) E. Desv. var. *pubiflora*; NI; *Cialdella et al. 168*; DQ887452*; DQ887401** . *Nassella punensis* Torres; NI; *Cialdella et al. 428*; DQ887453; DQ887402. *Nassella sanluisensis* (Speg.) Barkworth; *Nassella* (S1); *Giussani et Morrone 326*; *; -. *Nassella tenuis* (Phil.) Barkworth; *Stephanostipa* (S1); *Morrone et Giussani 5148*; DQ887454*; DQ887403. *Nassella tenuissima* (Trin.) Barkworth; *Nassella* (S1); *Morrone et al. 5148*; DQ887455; DQ887404. *Nassella trichotoma* (Nees) Hack. ex Arechav.; *Nassella* (S1); *Morrone et al. 5102*; DQ887456; DQ887405. *Nassella tucumana* (Parodi) Torres; NI; *Morrone et al. 3774*; DQ887457; DQ887406.

Piptatherum miliaceum (L.) Coss.; NI ; *Rúgolo de Agrasar 2135*; DQ887458*; DQ887407*.

Piptochaetium avenaceum (L.) Parodi; *Podopogon* (P); Henderson 94-29; DQ887459*; DQ887408. *Piptochaetium bicolor* (Vahl) E. Desv.; *Podopogon* (P); *Morrone* 5136; DQ887460; DQ887409. *Piptochaetium calvescens* Parodi; *Piptochaetium* (P); *Morrone 5126 a*; DQ887461; DQ887410. *Piptochaetium fimbriatum* (Kunth) Hitchc.; *Piptochaetium* (P); *Morrone 3616*; DQ887462; DQ887411. *Piptochaetium hackelii* (Arechav.) Parodi; *Podopogon* (P); *Giussani 314*; DQ887463; DQ887412. *Piptochaetium indutum* Parodi; *Podopogon* (P); *Villavicencio 161*; DQ887464*; -. *Piptochaetium lasianthum* Griseb.; *Piptochaetium* (P); *Giussani 318*; DQ887465; DQ887413. *Piptochaetium lejopodum* (Speg.) Henrard; *Piptochaetium* (P); *Morrone 5111*; DQ887466; DQ887414. *Piptochaetium medium* (Speg.) Torres; *Podopogon* (CA); *Giussani 317*; DQ887467; DQ887415. *Piptochaetium montevidense* (Spreng.) Parodi; *Piptochaetium* (P); *Cialdella 16*; DQ887468; DQ887416. *Piptochaetium napostaense* (Speg.) Hack.; *Podopogon* (P); *Cialdella 55*; DQ887469; DQ887417. *Piptochaetium panicoides* (Lam.) E. Desv.; *Piptochaetium* (P); *Cialdella 86*; DQ887470; DQ887418. *Piptochaetium pilosum* (Sánchez Vega) Ciald. & Giussani; *Piptochaetium* (SV); *Sánchez Vega 2459*; DQ887471*; -. *Piptochaetium ruprechtianum* E. Desv.; *Podopogon* (P); *Morrone 5134*; DQ887472; DQ887419. *Piptochaetium sagasteguii* Sánchez Vega; *Podopogon* (SV); *Sánchez Vega 2914*; DQ887473*; -. *Piptochaetium seleri* (Pilg.) Henrard; NI; *Zuloaga 7378*; DQ887474; DQ887420. *Piptochaetium setosum* (Trin.) Arechav.; *Piptochaetium* (P); *Lammers et al. 7763*; DQ887475*; DQ887421*. *Piptochaetium stipoides* (Trin. & Rupr.) Hack. ex Arechav.var. *echinulatum* Parodi; *Piptochaetium* (P); Morrone 5114; DQ887476; DQ887422. *Piptochaetium stipoides* (Trin. & Rupr.) Hack. ex Arechav. var. *stipoides* ; *Piptochaetium* (P); *Giussani 316*; DQ887477; DQ887423. *Piptochaetium tovarii* Sánchez Vega; *Piptochaetium* (SV); *Sánchez Vega 2769*; DQ887478*; -.

Piptochaetium uruguense Griseb.; *Piptochaetium* (P); *Pedersen 5188*; DQ887479*; -. *Piptochaetium virescens* (Kunth) Parodi; *Podopogon* (P); *Morrone 3648*; DQ887480; DQ887424.

Poa holciformis J. Presl; NI *; Soreng 7157;* DQ887481; DQ887425.

APPENDIX 2. List of specimens considered for morphological analyses as outgroups of *Piptochaetium*. Material of *Piptochaetium* considered for morphological matrix can be found in Cialdella & Giussani, 2002. Those cited with (*) were used for DNA sequencing.

Aciachne acicularis Laegaard. ARGENTINA. Salta: Dpto. Orán, Piedra Azul, 3650m s.m., 13-IV-1999, *Sulekic et al. 2782** (SI). ***Anatherostipa brevis*** (Torres) Peñailillo. ARGENTINA. Salta: Dpto. Santa Victoria, entre Ciénaga Abra y Nazareno, 6-III-1999, *Sulekic et Cano 2554** (SI). ***Bromus catharticus*** Vahl. ARGENTINA. Buenos Aires: Pdo. Vicente López, alrededores de Munro, 1-III-2005, *Giussani 354** (SI). ***Jarava ichu*** Ruiz & Pav. ARGENTINA. Córdoba: Dpto. Calamuchita, Villa General Belgrano, cerro de La Virgen, 14-I-2005, *Morrone et Giussani 5149* (SI). Jujuy: Dpto. Dr. Manuel Belgrano, Termas de Reyes, VII-1927, *Burkart 1* (BAA). Dpto. Humahuaca, Iturbe, 8-III-1960, *Meyer et al. 21085* (BAA); Sierra de Zenta, 3500 m s.m., III-1931, *Budin 7433* (BAA). Dpto. Ledesma, Abra entre Iturbe e Iruya, 4000 m s.m., 25-I-1972, *Ruthsatz s.n.* (BAA 14588); Iturbe, 8-III-1960, *Meyer et al. 21805* (BAA). Dpto. Tilcara, Tilcara, Garganta del Diablo, 1-III-1961, *Cabrera et al. 13924* (BAA). Dpto. Tumbaya, Laguna de Volcán, 2100 m s.m., 21-II-2002, *Cialdella et al. 536** (SI). Dpto. Valle Grande, Santa Ana, cerros, 3000 m s.m., 29-II-1940, *Burkart and Troncoso s.n.* (BAA, SI11605). Dpto. Yavi, Quebrada de Toquero, 3600 m s.m., 21-II-1963, *Cabrera et al. 15376* (BAA). ***Jarava juncoides*** (Speg.) Peñailillo. ARGENTINA. Buenos Aires: Pdo. Tornquist, Cerro de la Ventana, ruta nac. 76, camino de Villa La Ventana a Tornquist, borde del cerro Alberto Serrano, 497 m s.m., 13-XI-2004, *Morrone et al. 5120** (SI). ***Jarava leptostachya*** (Griseb.) F. Rojas. ARGENTINA. Jujuy: Dpto. Dr. Manuel Belgrano, entre León y Nevado de Chañi, Pie de la Cuesta, 2600 m s.m., 6-III-1963, *Fabris et al. 3965* (BAA). Dpto. Cochinoca, Abra Pampa, Cerro Huancar, 3500 m s.m., 17-II-1963, *Cabrera et al. 15272* (BAA). Dpto. Humahuaca, ruta 9, de Tres Cruces a Humahuaca, Ayo. Puente del Diablo, a pocos km de Tres Cruces, 22°55′S 65°33′W, 3690 m s.m., 20-II-2002, *Cialdella et al. 486* (SI). Dpto. Susques: ruta 70, cruce con ruta 16, entre Salina de Olaroz y Coranzulí, 4300 m s.m., 23°07′S 66°31′W, 16-III-1994, *Múlgura et al. 1301* (SI). Dpto. Tumbaya, camino de Purmamarca al Abra de Lipán, 3700 m s.m., 14-II-1985, *Kiesling et al. 5205* (SI). Dpto. Yavi, Abra de Yavi, 3400 m s.m., 20-II-1963, *Cabrera et al. 15321* (BAA). Salta. Dpto. Los Andes, ruta 51, de San Antonio de los Cobres a Viaducto La Polvorilla, 4170 m s.m., 18-II-2002, *Cialdella et al. 417** (SI). BOLIVIA. La Paz: Prov. Omasuyos, en la ruta La Paz-Huarina, a la altura de Laja, 3900 m s.m., 28-II-1950, *Krapovickas et al. 7063* (SI). ***Jarava media*** (Speg.) Peñail. ARGENTINA. Jujuy: Dpto. Humahuaca, Humahuaca, falda de los cerros, 3050 m s.m., 16-II-1931, *Parodi 9695* (BAA). Huacalera, 2700 m s.m., 23-II-1955, *Cabrera 11983* (BAA). Dpto. Tumbaya, Ciénaga Grande, 11 km al S de El Moreno, 3500 m s.m., 19-II-1987, *Nicora et al. 8893* (SI); ruta 52, 40 km antes de Purmamarca, 23°41′S 65°42′W, 3870 m s.m., 19-II-2002, *Cialdella et al. 468* (SI). Salta: Dpto. La Poma: Palermo oeste, camino hacia la toma de agua, 2820 m s.m., 13-II-2002, *Cialdella et al. 242** (SI). Tucumán: Dpto. Tafí, ruta 307, camino de El Infiernillo a Amaicha del Valle, 2840 m s.m., 11-II-2002, *Cialdella et al. 179* (SI). ***Jarava plumosa*** (Spreng.) S. W. L. Jacobs & J. Everett. ARGENTINA.

Catamarca: Dpto. Paclin, Gracián, 3-I-1940, *Castellanos s.n.*, BAA 33463. Córdoba: Dpto. Punilla, ruta nac. 38, km 63, entre Villa Giardino y La Cumbre camino a Capilla del Monte, 15-I-2005, *Morrone and Giussani 5151** (SI). ***Jarava plumosula*** (Nees ex Steud.) F. Rojas. PERÚ. Lima: Prov. Canta, Road to Canta, km 74, near Yasao to outside of Canta, 18-V-1981, *Sullivan et al. 967* (SI). ARGENTINA. Jujuy: Dpto. Humahuaca, Mina Aguilar, Espinazo del Diablo, 3800 m s.m., 21-III-1973, *Ruthsatz s.n*. (BAA 14563). Dpto. Tilcara, Tilcara, 2600 m s.m., 28-I-1943, *Cabrera 7713* (BAA); Laguna de Volcán, 23°55′S 65°28′W, 2100 m s.m., 21-II-2002, *Cialdella et al. 534* (SI). Dpto.Yavi, Abra de Yavi, 3400 m s.m., 20-II-1963, *Cabrera et al. 15333* (BAA). Salta. Dpto. Cachi, ruta 42, Parque Nacional Los Cardones, camino hacia Seclantás, 2850 m s.m., 14-II-2002, *Cialdella et al. 258** (SI). ***Jarava scabrifolia*** (Torres) Peñail. ARGENTINA. Jujuy: Cochinoca, Pozuelos, Río Cincel, 22° 28′ S 65° 59′ W, 3660 m s.m., 9-II-1995, *Deginani et al. 552* (SI). Yavi, Yoscaba, 3670 m s.m., 6-II-1973, *Ruthsatz s.n.* (BAA 14605, 14607). Salta: Dpto. Chicoana, ruta 33, de Cachi a Ciudad de Salta, pasando desvío a ruta 42, 3210 m s.m., 15-II-2002, *Cialdella et al. 303** (SI). ***Jarava vaginata*** (Phil.) F. Rojas. ARGENTINA. Jujuy: Dpto. Cochinoca, Abra Pampa, Cerro Huancar, 3500 m s.m., 22-II-1963, *Cabrera et al. 15406* (BAA). Dpto. Humahuaca, Huacalera, 2700 m s.m., 23-II-1955, *Cabrera 11987* (BAA). Dpto. Susques, subida a Alto Los Chorrillos, 4100 m s.m., 16-II-1980, *Cabrera et al. 31759* (SI). Dpto. Tilcara, Posta de Hornillos, 2400 m s.m., 10-II-1959, *Cabrera 13180* (BAA). Dpto. Tumbaya, subida de Purmamarca a Abra de Pives, 3700 m s.m., 24-IV-1975, *Cabrera et al. 26354* (SI); ruta 52, 40 km antes de Purmamarca, 23°41′S 65°42′ W, 3870 m s.m., 19-II-2002, *Cialdella et al. 464* (SI); Tumbaya, 2100 m s.m., 15-III-1973, *Ruthsatz s.n.* (BAA 14638); ruta 52, 40 km antes de Purmamarca, 3870 m s.m., 19-II-2002, *Cialdella et al. 464* (SI). Dpto. Yavi, Abra de Yavi, 3400 m s.m., 20-II-1963, *Cabrera et al. 15335* (BAA). Salta. Dpto. Cachi, ruta 42, Parque Nacional Los Cardones, camino hacia Seclantás, 2730 m s.m., 14-II-2002, *Cialdella et al. 264** (SI). ***Nassella arcaënsis*** (Speg.) Torres. ARGENTINA. Jujuy: Humahuaca, Iturbe, 8-III-1960, *Meyer et al. 21073* (BAA). Tumbaya, Volcán, 2200 m s.m., 4/6-III-1965, *Cabrera et al. 16866* (LP). Salta: Dpto. Cachi, desde ruta 40, camino interno hacia Las Paylas, 3030 m s.m., 14-II-2002, *Cialdella et al. 275** (SI). ***Nassella arcuata*** (R. E. Fr.) Torres. ARGENTINA. Jujuy: Dpto. Cochinoca, Abra Pampa, Cerro Huancar, 3500 m s.m., 17-II-1963, *Cabrera et al. 15274* (BAA);entre Río Doncellas y Casabindo, 3540 m s.m., 8-II-1995, *Deginani et al. 512* (SI). Dpto. Humahuaca, Ruta 9, de Tres Cruces a Humahuaca, Ayo. Puente del Diablo, 3690 m s.m., 20-II-2002, *Cialdella et al. 490* (SI). Dpto. Tilcara, Huacalera, Pampa Corral, 4000 m s.m., 2-III-1955, *Cabrera 12133* (BAA). Dpto. Yavi, Yavi Chico, 3420 m s.m., 6-III-1940, *Meyer s.n. LIL 14931* (BAA, SI). Salta: Dpto. Rosario de Lerma, desvío de Ruta 51, entre Santa Rosa de Tastil y Alfarcito, 3380 m s.m., 21-II-1987, *Nicora et al. 9002* (SI). Tucumán: Dpto. Tafí, ruta 307, camino de El Infiernillo a Amaicha del Valle, 2840 m s.m., 11-II-2002, *Cialdella et al. 183** (SI). ***Nassella cabrerae*** Torres. ARGENTINA. Jujuy: Dpto. Cochinoca, Queta, 3570 m s.m., 8-II-1995, *Deginani et al. 501* (SI). Dpto. Rinconada, Ruta nac. 9, 8 km pasando Puesto del Marqués, 3690 m s. m., 19-II-1997, *Zuloaga et al. 6031* (SI). Tucumán: Dpto. Tafí, ruta 307, camino de Tafí del Valle a Amaicha del Valle, 1 km antes de El Infiernillo, 2790 m s.m., 10-II-2002, *Cialdella et al. 161** (SI). BOLIVIA. La Paz: Prov. Loayza, 12.1 mi NW of Villa Loza on road towards Urmiri and Sapahaqui, 4-III-1993, *Peterson et al. 12649* (SI). ***Nassella caespitosa*** Griseb. ARGENTINA. Jujuy: Dpto. Humahuaca, Palca de Aparzo, 3480 m s.m., 6-II-1995, *Deginani et al. 421* (SI); Esquinas Blancas, 7-III-1965, *J. Fernández s.n.* (BAA 4899); Mina Aguilar, 4200 m s.m., 23-II-1963, *Cabrera et al. 15469* (BAA); Mina Aguilar, 4450 m s.m., 21-II-1972, *Ruthsatz s.n.* (BAA 14569); Ruta 9, de Tres Cruces a Humahuaca, Esquinas Blancas, 3630 m s.m., 20-II-2002, *Cialdella et al. 495** (SI). Dpto. Rinconada, Laguna de Pozuelos, 3690 m s.m., 10-II-1995, *Deginani et al. 609* (SI). Dpto. Tumbaya, Ruta 52, 40 km antes de Purmamarca, 3870 m s.m., 19-II-2002, *Cialdella et al. 466* (SI). Salta. Dpto. Iruya, Iruya, 3180 m s.m., 7-II-1995, *Deginani et al. 469* (SI). ***Nassella charruana*** (Arechav.) Barkworth. ARGENTINA. Entre Ríos: Dpto. Gualeguaychú, Ruta 12, km 25, Perdices, frente a Destacamento de Policía, 5-11-2001, *Cialdella et al. 12** (SI). Dpto. Paraná, Paraná a Gob. Racedo, 19-XII-1962, *Burkart 23935* (SI). URUGUAY. Colonia: Ayo. de las Víboras, 17-I-1962, *Rosengurtt et al. 8562* (SI). Soriano: Juan Jackson, Ea. Santa Elena, XII-1937, *Gallinal et al. 727* (SI). ***Nassella cordobensis*** (Speg.) Barkworth. ARGENTINA. Córdoba: Dpto. Calamuchita, Villa General Belgrano, Cerro de la Virgen, 14-1-2005, *Morrone and Giussani 5147** (SI). Dpto. Colón, Ascochinga, ''La Quebrada'', 20-I-1938, *Giardelli 1041* (SI). La Pampa: Laguna Amarga, monte, 17-II-1948, *Burkart 15959* (SI). Cerros Lihuel Calel, 18-II-1948, *Burkart 15961* (SI). ***Nassella curamalalensis*** (Speg.) Barkworth. ARGENTINA. Buenos Aires: Pdo. Tornquist, Villa Arcadia, Cerro del Amor, 460 m s.m., 12-XI-2004, *Morrone et al. 5107** (SI); Sierra de la Ventana, sin fecha, *Spegazzini s.n*., BAA 49 a (SI). ***Nassella hyalina*** (Nees) Barkworth. ARGENTINA. Buenos Aires: Pdo. Zárate, Isla Talavera, a orillas del Río Paraná Guazú, 19-II-2005, *Morrone 5168** (SI). Entre Ríos: Dpto. La Paz, Ayo. Quebracho, cerca del Ayo. Feliciano, 15-I-1960, *Burkart and Bacigalupo 21111* (SI). Dpto. Islas del Ibicuy, ruta nac. 12, 3 km de Ceibas camino a Médanos, 10–20 m s.m., 30-III-2005, *Morrone et al. 5203* (SI). URUGUAY. Paysandú: some 20 km W of Quebracho, Ea. Las Correntinas, 15-I-1994, *Pedersen 15868* (SI). ***Nassella megapotamica*** (Spreng. ex Trinius) Barkworth. ARGENTINA. Buenos Aires: Delta, Caraguatá Superior, 4-XII-1931, *Burkart 3918* (SI). Pdo. Tandil, alrededores de Tandil, a 3 km al SE del centro, 250 m s.m., 14-XI-2004, *Morrone et al. 5129** (SI). Entre Ríos: Delta del Paraná, Ayo. Martínez, X-1044, *Burkart 15037* (SI). ***Nassella melanosperma*** (J. Presl) Barkworth. ARGENTINA. Buenos Aires: Pdo. Tandil, Sierra de las Ánimas, La Cascada, 281 m s.m., 15-XI-2004, *Morrone et al. 5139** (SI). BRAZIL. Rio Grande do Sul: Pelotas, Beira de estrada, 10-XI-1954, *da Costa Sacco 217* (SI). ***Nassella mexicana*** (Hitchc.) R. W. Pohl. ARGENTINA. Jujuy: Dpto. Ledesma, Parque Nacional Calilegua, Aguas Blancas, 25-II-1986, *Iudica and Ramadori 170* (SI). Dpto. Dr. Manuel Belgrano, entre León y Nevado de Chañi, Pie de la Cuesta, 2600 m s.m., 6-III-1963, *Fabris et al. 3949, 3964, 3971* (LP); Rodeo Grande, III-1963, *Fabris et al. 4178* (LP). Dpto. Tumbaya, Volcán, subida al Abra Morada, 9-III-1967, *Cabrera 18417* (LP); Abra Morada, 3200 m s.m., 10-III-1967, *Cabrera et al. 18390* (LP). BOLIVIA. La Paz: Prov. Omasuyos, Camino de Achacachi a Sorata, 2 km antes de Curupampa, 3713 m s.m., 23-III-2004, *Morrone and Belgrano 4910** (SI). VENEZUELA. Mérida: Parque Nac. Sierra Nevada, 3 km del desvío de Apostadero, 60 km de Mérica, Páramo de Mucubají, 3600 m s.m., 23-IX-2003, *Morrone et al. 4727* (SI). ***Nassella meyeri*** Torres. ARGENTINA. Jujuy: Dpto. Humahuaca, Ruta 9, de Tres Cruces a Humahuaca, Ayo. Puente del Diablo, 3690 m s.m., *Cialdella et al. 488** (SI). Dpto. Tilcara, al este de Huacalera, 2950 m s.m., 28-III-1967, *Werner 678* (BAA ex LIL); al este de Huacalera, 24-III-1967, *Werner 518* (BAA ex LIL); Huacalera, 14-II-1969, *Cabrera et al. 19761* (LP). ***Nassella neesiana*** (Trin. & Rupr.) Barkworth. ARGENTINA. Buenos Aires: Pdo. Tandil, ruta prov. 74, Sierra del Tigre, campos junto a la Reserva del Tigre, 31-XII-2002, *Giussani and Morrone 319** (SI). Pdo. Tornquist, Villa Arcadia, Cerro del Amor, 460 m s.m., 12-XI-2004, *Morrone et al. 5104* (SI). Pdo. Zárate, Isla Talavera, a orillas del Río Paraná Guazú, 19-II-2005, *Morrone 5170* (SI). Jujuy: Dpto. Dr. Manuel Belgrano, La

Almona, 3-III-1963, *Fabris et al. 3807* (LP). Dpto. Tilcara, Alrededores de Tilcara, 2500 m s.m., 11-II-1959, *Cabrera et al. 13363* (LP). Dpto. Tumbaya, Volcán, 2000 m s.m., 22-II-1960, *J. Hunziker 7116* (BAA). URUGUAY. Colonia: Ruta 12, km 48 camino de Nueva Palmira a Cardona, pasando Palo Solo, 70–80 m s.m., 31-III-2005, *Morrone et al. 5234* (SI). ***Nassella novari*** Torres. ARGENTINA. Jujuy: Dr. Manuel Belgrano, entreLeón y Nevado de Chañi, Las Cuevas, 3000 m s.m., III-1963, *Fabris et al. 4043* (LP). Tumbaya, subida de Purmamarca a Abra de Pives, 3000 m s.m., 24-IV-1975, *Cabrera et al. 26350* (LP, SI); al N del volcán, 2150 m s.m., 18-III-1973, *Ruthsatz 14629* (BAA); Purmamarca, Tascal, 3400 m s.m., 15-II-1963, *Cabrera et al. 15155* (LP). Salta: Dpto. Cachi, 46 km de Cachi, camino a Salta, 3060 m s.m., 19-III-1972, *Krapovickas et al. 21976* (SI). Dpto. Chicoana, ruta 33, de Cachi a Ciudad de Salta, pasando desvío a ruta 42, 3210 m s.m., 15-II-2002, *Cialdella et al. 308** (SI). ***Nassella pampagrandensis*** (Speg.) Barkworth. ARGENTINA. Jujuy: Santa Bárbara, Cachipunco, 13-II-1964, *Fabris et al. 5222* (LP). Tilcara, Yala de Monte Carmelo, 2900 m s.m., 19/21-I-1966, *Fabris et al. 6482* (LP). Tumbaya, Chilcayo, 2300 m s.m., 5-I-1966, *Fabris et al. 6102* (LP); Volcán, cerros, 10-III-1958, *Cabrera and Marchionni 12935* (BAA, LP); Chilcayo, Finca del Dr. Gronda, 2800 m s.m., *Deginani et al. 363* (SI). Tucumán: Dpto. Tafí, ruta 307, camino de Tafí del Valle a Amaicha del Valle, 1 km antes de El Infiernillo, 2790 m s.m., 10-II-2002, *Cialdella et al. 163** (SI). ***Nassella pubiflora*** (Trin. & Rupr.) E. Desv. var. ***pubiflora.*** ARGENTINA. Jujuy: Cochinoca, Cochinoca, I-1946, Cabezas s.n. herb. *Parodi 15149* (BAA), *15150* (BAA). Valle Grande, Santa Ana, 3200 m s.m., 1-III-1940, *Burkart and Troncoso s.n.* SI 11756 p.p. (BAA, SI), SI 11759 (BAA). Tucumán: Dpto. Tafí, ruta 307, camino de Tafí del Valle a Amaicha del Valle, 1 km antes de El Infiernillo, 2790 m s.m., 10-II-2002, *Cialdella et al. 168** (SI). BOLIVIA. La Paz: Prov. Omasuyos, Camino de Achacachi a Sorata, 2 km antes de Curupampa, 3713 m s.m., 23-III-2004, *Morrone and Belgrano 4908* (SI). ***Nassella punensis*** Torres. ARGENTINA. Jujuy: Dpto. Cochinoca, Abra de Rachaite, 4140 m s.m., 22° 53′ S 66° 13′ W, *Múlgura et al. 1315* (SI). Dpto. Humahuaca, Ruta 9, de Tres Cruces a Humahuaca, Ayo Puente del Diablo, 3690 m s.m., 20-II-2002, *Cialdella et al. 482* (SI); Tres Cruces, 2-II-1944, *Soriano 628* (BAA); Mina Aguilar, 4200 m s.m., 23-II-1963, *Cabrera et al. 15469* (LP). Rinconada, Mina Pirquitas, 4300 m s.m., 1-III-1964, *Schwabe et al. 848* (LP), 1132 (LP). Valle Grande, Caspalá, cumbres en las ciénagas a 4500 m s.m., 3-III-1940, *Burkart and Troncoso s.n.* (BAA ex SI 11841). Salta: Dpto. Los Andes, ruta 51, de San Antonio de los Cobres a Viaducto La Polvorilla, 4170 m s.m., 18-II-2002, *Cialdella et al. 428** (SI). ***Nassella sanluisensis*** (Speg.) Barkworth. ARGENTINA. Córdoba: Dpto. Calamuchita, cercanías de Villa General Belgrano, I-2003, *Giussani and Morrone 326** (SI). Jujuy: Cochinoca, sin localidad, verano de 1959, *Cabezas s.n.* (SI 23175). Santa Bárbara, Abra de los Morteros, 26-I-1975, *Zuloaga and Deginani 269* (LP). Dpto. Tumbaya, Ruta nac. 9, 4 km de Volcán, camino a San Salvador de Jujuy, cantera sobre el Río Grande, 2040 m s.m., 10-XII-1998, *Morrone et al. 3160* (SI). ***Nassella tenuis*** (Phil.) Barkworth. ARGENTINA. Buenos Aires: Pdo. Tornquist, Abra de la Ventana, Cerro Bahía Blanca, 400 m s.m., 13-XI-2004, *Morrone et al. 5116** (SI). La Pampa: Dpto. Utracán, Gral. Acha, 5-XI-1953, *Burkart 19210* (SI). ***Nassella tenuissima*** (Trin.) Barkworth. ARGENTINA. Buenos Aires: Pdo. Villarino, ca. 60 km E of town of Río Colorado, 50 m s.m., 16-XII-1938, *Eyerdam et al. 23745* (SI). Catamarca: Summit Cuesta El Portezuelo, 5-II-1943, *Barlett 19605* (SI). Córdoba: Dpto. Calamuchita, Villa Gral. Belgrano, cerro de La Virgen, 14-I-2005, *Morrone and Giussani 5144* (SI), *5148** (SI). ***Nassella trichotoma*** (Nees) Hack. ex Arechav. ARGENTINA. Buenos Aires: Pdo. Gral. Alvear, ruta nac. 205, entre Saladillo y S. C. Bolívar, km 276, 12-XI-2004, *Morrone et al. 5099* (SI). Pdo. Guaminí, Ruta prov. 65, 77 km de Daireaux camino a Guaminí, Laguna Alsina, 12-XI-2004, *Morrone et al. 5102** (SI). ***Nassella tucumana*** (Parodi) Torres. ARGENTINA. Jujuy: Dpto. Dr. Manuel Belgrano, Lagunas de Yala, próximo a Laguna El Rodeo, 2060 m s.m., 15-XII-1992, *Pensiero & Marino 4636* (SI); Amancay, 1500 m s.m., 11-II-1943, *Parodi 14724*, *14728* (BAA); Lagunas de Yala, 2200 m s.m., 5-II-1943, *Parodi 14501*, *14506*, *14521* (BAA); Cerros de Yala, 19-II-1940, *Burkart & Troncoso s.n.* (BAA, SI 11305); El Cucho, 11-II-1943, *Parodi 14715* (BAA). Salta: Dpto. Santa Victoria, ruta prov. 19, 10 km de Los Toldos camino a Lipeo, 19-XI-2001, *Morrone et al. 3774** (SI). ***Piptatherum miliaceum*** (L.) Coss. ARGENTINA. Buenos Aires: Distrito Federal, Quinta de Lezica, Caballito, 24-XII-1910, *Hicken s.n.*, SI 13458 (SI). La Pampa: Dpto. Santa Rosa, naturalizada en la calle Argentino Valle 555, 18-I 2003, *Rúgolo de Agrasar 2135** (SI). ***Piptochaetium avenaceum*** (L.) Parodi. U.S.A. Florida: Wakulla, 07/04/1994, *Henderson 94-29** (US). ***Piptochaetium bicolor*** (Vahl) E. Desv. ARGENTINA. Buenos Aires: Tandil, Sierra de las Animas, La Cascada, 281 m, 15/11/2004, *Morrone 5136** (SI). ***Piptochaetium calvescens*** Parodi. ARGENTINA. Buenos Aires: Coronel Suárez, Ruta Prov. 76, Abra de Pantanoso Viejo, 19 km de Sierra de la Ventana camino a Olavarria, 350 m, 14/11/2004, *Morrone 5126 a** (SI). ***Piptochaetium fimbriatum*** (Kunth) Hitchc. MEXICO. Michoacán: Camino de Maravatio a Zirapecuaro, Ruta 15d, km 193, 8 km antes del desvío a Acambaro-Zinapecuaro, 06/10/2001, *Morrone 3616** (SI). ***Piptochaetium hackelii*** (Arechav.) Parodi. ARGENTINA. Buenos Aires: Tandil Rta prov. 74, Sierra del Tigre, campos junto a la Reserva del Tigre, 31/12/2002, *Giussani 314** (SI). ***Piptochaetium indutum*** Parodi. BOLIVIA. La Paz: Ingavi Cantón Jesús de Machaca, 4120 m, 15/03/1989, *Villavicencio 161** (LPB). ***Piptochaetium lasianthum*** Griseb. ARGENTINA. Buenos Aires: Tandil, Rta prov. 74, Sierra del Tigre, campos junto a la Reserva del Tigre, 31/12/2002, *Giussani 318** (SI). ***Piptochaetium lejopodum*** (Speg.) Henrard. ARGENTINA. Buenos Aires: Tornquist, Villa Arcadia, Cerro del Amor, 1 km de Sierra de la Ventana pasando por V. Arcadia,460 m, 12/11/2004, *Morrone 5111** (SI). ***Piptochaetium medium*** (Speg.) Torres. ARGENTINA. Buenos Aires: Tandil, Rta prov. 74, Sierra del Tigre, campos junto a la Reserva del Tigre, 31/12/2002, *Giussani 317** (SI). ***Piptochaetium montevidense*** (Spreng.) Parodi. ARGENTINA. Entre Ríos: Gualeguaychú ruta 16, km 58, de Gualeguaychú a Gualeguay 05/11/2001, *Cialdella 16** (SI). ***Piptochaetium napostaense*** (Speg.) Hack. ARGENTINA. Tucumán: Tafí, camino desde Tafí del Valle hacia Las Carreras, a 2 km de Tafí del Valle, Cerro El Pelado, 2130 m, 09/02/2002, *Cialdella 55** (SI). ***Piptochaetium panicoides*** (Lam.) E. Desv. ARGENTINA. Tucumán: Tafí camino desde Las Carreras hacia El Mollar, 16 km antes de El Mollar, 2370 m, 09/02/2002, *Cialdella 86** (SI). ***Piptochaetium pilosum*** (Sánchez Vega) Ciald. & Giussani. Perú. Cajamarca: Entre Llacanora y Namora, 2750 m, 11/04/1981, *Sánchez Vega 2459** (MO). ***Piptochaetium ruprechtianum*** E. Desv. ARGENTINA. Buenos Aires: Tandil, Sierra de las Animas, La Cascada, 281 m, 15/11/2004, *Morrone 5134** (SI). ***Piptochaetium sagasteguii*** Sánchez Vega. PERÚ. Cajamarca: Cerro El Guitarrero, ladera occidental del valle de Cajamarca, 2800 m, 15/01/1983, *Sánchez Vega 2914** (MO). ***Piptochaetium seleri*** (Pilg.) Henrard. México. México: Carretera 190 del Distrito Federal a Puebla, km 55, 3100 m, 27/09/2001, *Zuloaga 7378** (SI). ***Piptochaetium setosum*** (Trin.) Arechav. CHILE. VI Region: Colchagua, 2 km W of Poblacion on the highway from Nancagua to Pichilemu, 460 m, 15/11/1990, *Lammers et al. 7763** (CONC). ***Piptochaetium stipoides*** var. ***echinulatum*** Parodi. ARGENTINA. Buenos Aires: Tornquist Villa Arcadia, Cerro del Amor, 1 km de Sierra de la Ventana pasando por V. Arcadia, 460 m, 12/11/2004, *Morrone 5114** (SI). ***Piptochaetium stipoides*** (Trin. & Rupr.) Hack. ex Arechav. var. ***stipoides.*** ARGENTINA. Buenos Aires: Tandil, Rta prov.

74, Sierra del Tigre, campos junto a la Reserva del Tigre, 31/12/2002, *Giussani 316** (SI). ***Piptochaetium tovarii*** Sánchez Vega. Perú. Cajamarca: Dist. Encañada El Usnio, carretera Cajamarca-Celendín, 3120 m, 06/04/1982, *Sánchez Vega 2769** (MO). ***Piptochaetium uruguense*** Griseb. ARGENTINA. Misiones: Estancia La Soledad, 22/10/1959, *Pedersen 5188** (MO). ***Piptochaetium virescens*** (Kunth) Parodi. México. México: Ruta 15, km 61, camino entre Zitacuaro y Toluca, 08/10/2001, *Morrone 3648** (SI). ***Poa holciformis*** J. Presl. CHILE. Region Metropolitana. Santiago: M. Rio Yeso, above Embalse del Yeso and up to and around Termas del Plomo, 13-I-2002, *Soreng 7157** (US).

APPENDIX 4. List of morphological characters and character states.

0. Lemma margins: flat, strongly overlapping = 0; flat, not or only slightly overlapping 0 1; involute = 2. **1. Palea shape:** flat on the abaxial surface = 0; bikeeled on the abaxial surface = 1. **2. Palea length:** half the length of the lemma to equal the length of the lemma = 0; longer than the lemma = 1; shorter than half the length of the lemma = 2. **3. Palea venation:** nerveless = 0; 2-nerved = 1. **4. Long fundamental cells of the lemma epidermis (more than 3 times the length of the silica cells):** absent = 0; present = 1. **5. Fundamental cell sidewalls:** irregularly sinuate or not sinuate = 0; regularly dentate = 1; regularly sinuate = 2. **6. Callus length:** shorter than 0.8 mm = 0; equal to or longer than 0.8 mm = 1. **7. Callus shape:** acute or subacute = 0; blunt or truncate = 1. **8. Callus pubescence:** glabrous or slightly hairy = 0; densely hairy = 1. **9 Awn:** persistent = 0; deciduous = 1. **10. Upper glume length:** longer than the floret = 0; equal to shorter than the floret = 1. **11. Floret shape:** terete to fusiform = 0; obconical = 1; obovoid to globose = 2; lens-shaped = 3; lanceolate = 4. **12. Compression of the floret:** absent or slightly compressed = 0; laterally and conspicuously compressed = 1; dorsally and conspicuously compressed = 2. **13. Lemma pubescence:** absent = 0; present all over the lemma = 1; present only along the dorsal nerve = 2. **14. Papillae of the lemma:** absent = 0; present = 1. **15. Prickles of the lemma:** absent = 0; present over almost all the lemma surface = 1; present over the distal third of the lemma = 2. **16. Base of prickles:** surrounded but nor covered by adjacent epidermal cells = 0; partially covered by adjacent epidermal cells = 1. **17. Lemma width just below the crown:** as wide as the crown = 0; narrower than the crown = 1. **18. Crown contracted to the base of the awn:** absent = 0; present = 1. **19. Crown shape:** straigth, not revolute = 0; revolute toward the outside = 1. **20. Macrohairs in the crown:** absent = 0; present = 1. **21. Papillae in the crown:** absent = 0; present = 1. **22. Prickles in the crown:** absent = 0; present = 1. **23. Floret length/width ratio:** more or equal than 3.6 = 0; less or equal than 3.5 = 1. **24. Crown width:** narrow [0.2–0.6 (−0.9)] mm = 0; wide (1–1.8 mm) = 1. **25. Awn:** straight, not geniculate = 0; geniculate or bigeniculate = 1. **26. Crown, that is the lemma apex fusionated in a solid cylinder differentiated or not differentiated externally:** absent = 0; present = 1. **27. Macrohairs on the awn:** absent = 0; present along the proximal portion of the awn (column) = 1; present along the distal protion of the awn (subula) = 1; present along the whole awn = 2. **28. Papus:** absent = 0; present = 1. **29. Glumes texture:** membranous = 0; indurated = 1. **30. Disarticulation line between the awn and the lemma:** absent = 0; present = 1. **31. Length of the awn:** shorter than the floret = 0; longer than the floret = 1. **32. Palea texture:** membranous = 0; indurated = 1. **33. Lemma texture:** membranous = 0; indurated = 1.

APPENDIX 3. Data matrix used in the morphological analyses for *Piptochaetium* and outgroups. Numbers in the first row represent the characters described in the text, together with their codification. Numbers within brackets show a polymorphism for the particular character of the species involved. Dashes indicate character inapplicable for a taxon.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Aciachne acicularis	1	0	0	1	1	2	0	1	0	0	2	0	0	0	0	0	–	–	–	–	–	–	–	1	–	0	0	0	0	1	0	0	1	1
Anatherostipa brevis	1	0	0	1	1	2	0	0	1	0	0	0	0	1	0	0	–	–	–	–	–	–	–	1	–	1	0	0	0	0	1	1	0	0
Bromus catarthicus	0	1	0	1	1	[02]	–	–	–	0	0	3	1	0	0	0	–	–	–	–	–	–	–	0	–	0	0	–	0	0	1	0	0	0
Jarava ichu	1	0	2	1	0	0	0	1	1	[01]	0	0	0	1	0	2	0	–	–	–	–	–	–	0	–	1	0	0	1	0	1	1	0	0
J. juncoides	1	0	2	1	0	0	1	0	1	0	0	0	0	1	0	2	0	–	–	–	–	–	–	0	–	1	0	0	0	0	1	1	0	0
J. leptostachya	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	2	0	–	–	–	–	–	–	0	–	1	0	0	1	0	1	1	0	0
J. media	1	0	0	1	0	0	0	0	1	0	0	0	0	1	0	2	0	–	–	–	–	–	–	0	–	1	0	3	0	0	1	1	0	0
J. plumosa	1	0	2	1	0	0	0	1	1	1	2	0	0	1	0	2	0	–	–	–	–	–	–	0	–	1	0	0	1	0	1	1	0	0
J. plumosula	1	0	2	1	0	0	0	0	1	1	0	0	0	1	0	2	0	–	–	–	–	–	–	0	–	1	0	[23]	0	0	1	1	0	0
J. scabrifolia	1	0	2	1	0	0	0	0	1	1	0	0	0	1	0	2	0	–	–	–	–	–	–	0	–	1	0	0	0	0	1	1	0	0
J. vaginata	1	0	[02]	1	0	0	1	0	1	0	0	0	0	1	0	2	0	–	–	–	–	–	–	0	–	1	0	1	1	0	1	1	0	0
Nassella arcaensis	0	0	2	0	0	0	1	0	1	0	0	0	0	1	0	2	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. arcuata	0	0	2	0	0	0	1	0	1	[01]	0	0	0	1	0	2	0	0	1	0	0	0	0	0	[01]	1	1	3	0	0	1	1	0	1
N. cabrerae	0	0	0	0	0	0	[01]	0	1	1	0	[02]	0	1	0	1	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. caespitosa	0	0	[02]	0	0	0	0	[01]	1	1	0	[02]	0	1	0	0	–	1	0	0	[01]	0	0	0	0	1	1	0	0	0	1	1	0	1
N. charruana	0	0	2	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	0	1	1	0	1
N. cordobensis	0	0	2	0	0	0	1	0	1	0	0	0	0	0	1	2	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. curamalalensis	0	0	[02]	0	0	0	[01]	1	1	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. hyalina	0	0	2	0	0	0	0	0	1	0	0	0	0	2	0	1	0	1	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. megapotamica	0	0	2	0	0	0	1	0	1	0	0	0	0	2	1	0	–	0	1	0	1	0	0	0	[01]	1	1	0	0	0	1	1	0	1
N. melanosperma	0	0	2	0	0	0	1	0	1	0	0	0	0	0	1	0	–	1	1	0	1	0	0	0	1	1	1	0	0	0	1	1	0	1
N. mexicana	0	0	2	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. meyeri	0	0	2	0	0	0	1	0	1	1	0	0	0	2	0	2	0	1	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. neesiana	0	0	2	0	0	0	1	0	1	0	0	0	0	2	0	1	0	1	1	0	1	0	0	0	1	1	1	0	0	0	1	1	0	1
N. novari	0	0	2	0	0	0	1	0	1	1	0	0	0	1	0	2	0	0	1	0	1	0	0	0	0	1	1	1	0	0	1	1	0	1
N. pampagrandensis	0	0	0	0	0	0	0	1	1	1	0	2	0	0	0	1	0	0	1	0	1	0	0	1	0	1	1	0	0	0	1	1	0	1
N. pubiflora	0	0	[02]	0	0	0	0	1	[01]	1	0	2	0	1	0	0	–	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	1
N. punensis	0	0	2	0	0	0	0	0	1	[01]	0	0	0	1	0	0	–	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. sanluisensis	0	0	2	0	0	0	0	1	1	0	0	0	0	2	0	2	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	0	1
N. tenuis	0	0	2	0	0	0	1	0	1	0	0	0	0	2	1	0	–	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
N. tenuissima	0	0	2	0	0	0	0	1	1	0	0	0	0	0	1	2	0	0	1	0	1	0	0	[01]	0	1	1	0	0	0	1	1	0	1
N. trichotoma	0	0	0	0	0	0	0	1	1	[01]	0	2	0	0	0	1	0	0	1	0	1	0	0	1	0	1	1	0	0	0	1	1	0	1
N. tucumana	0	0	2	0	0	0	0	1	1	0	0	[02]	0	1	0	1	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1
Piptatherum miliaceum	1	0	0	1	1	0	0	1	0	1	0	2	2	0	0	0	–	–	–	–	–	–	–	1	–	0	0	0	0	0	1	1	1	1
Poa holciformis	1	1	0	1	1	2	0	1	0	–	2	3	1	0	0	0	–	–	–	–	–	–	–	0	–	–	0	–	0	0	–	–	0	0
Piptochaetium avenaceum	2	1	1	1	1	1	1	0	1	0	0	0	0	0	0	1	0	1	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1
P. bicolor	2	1	1	1	1	1	1	0	1	0	0	1	0	0	0	1	0	1	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1
P. calvescens	2	1	1	1	1	1	0	1	0	1	0	2	0	0	[01]	1	0	1	0	1	0	1	1	1	1	1	1	0	0	0	1	1	1	1
P. fimbriatum	2	1	1	1	1	1	0	1	1	0	0	0	0	1	0	0	–	1	1	0	[01]	0	[01]	1	0	1	1	0	0	0	1	1	1	1
P. hackelii	2	1	1	1	1	1	1	0	1	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1
P. indutum	2	1	1	1	1	1	1	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	1	1	1	1

APPENDIX 3. Continued.

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
P. lasianthum	2	1	1	1	1	1	0	1	1	1	0	2	0	1	0	1	0	1	1	0	1	0	1	1	0	1	1	0	0	0	1	1	1	1
P. leiopodum	2	1	1	1	1	1	0	1	0	1	0	2	0	0	0	2	0	1	0	1	0	0	1	1	1	1	1	0	0	0	1	1	1	1
P. medium	2	1	1	1	1	1	1	0	1	0	0	1	0	0	0	2	0	1	1	0	0	0	1	[01]	0	1	1	0	0	0	1	1	1	1
P. montevidense	2	1	1	1	1	1	0	1	0	1	0	3	1	0	1	0	–	1	1	0	0	1	1	1	0	1	1	0	0	0	1	1	1	1
P. napostaense	2	1	1	1	1	1	1	0	1	0	0	0	0	0	0	2	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1
P. panicoides	2	1	1	1	1	1	0	1	0	1	0	3	1	0	[01]	0	–	1	1	0	0	1	1	1	0	1	1	0	0	0	1	1	1	1
P. pilosum	2	1	1	1	1	1	0	1	1	1	0	2	0	1	0	1	0	0	1	0	0	0	1	1	0	1	1	0	0	0	1	1	1	1
P. ruprechtianum	2	1	1	1	1	1	1	0	1	0	0	1	0	0	0	2	0	1	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1
P. sagastegui	2	1	1	1	1	1	1	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1
P. seleri	2	1	1	1	1	1	0	1	1	0	0	0	0	1	0	1	0	1	1	0	1	0	1	1	0	1	1	0	0	0	1	1	1	1
P. setosum	2	1	1	1	1	1	0	1	1	1	0	2	0	0	0	2	0	1	1	0	0	0	1	1	0	1	1	0	0	0	1	1	1	1
P. stipoides echinulatum	2	1	1	1	1	1	0	1	1	1	0	2	0	0	0	1	1	1	0	1	0	1	1	1	1	1	1	0	0	0	1	1	1	1
P. stipoides stipoides	2	1	1	1	1	1	0	1	1	1	0	2	0	0	0	[012]	0	1	[01]	1	[01]	[01]	[01]	1	[01]	1	1	0	0	0	1	1	1	1
P. tovarii	2	1	1	1	1	1	0	1	0	1	0	2	0	0	0	0	–	0	1	0	0	0	0	1	0	1	1	0	0	0	1	1	1	1
P. uruguense	2	1	1	1	1	1	0	1	1	1	0	2	0	0	0	1	0	1	1	0	0	0	1	1	0	1	1	0	0	0	1	1	1	1
P. virescens	2	1	1	1	1	1	1	0	1	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	1