

## Phylogenetic Relationships in the Genus *Paspalum* (Poaceae: Panicoideae: Paniceae): An Assessment of the Quadrifaria and Virgata Informal Groups

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Communicating Editor: Lena Struwe

**Abstract**—*Paspalum*, an American genus of the  $x = 10$  Paniceae clade, includes about 330 species, four subgenera, and 27 informal groups. Within the genus, the Quadrifaria and Virgata groups are well-represented in South and Central America. Interspecific variability make the delimitation of these groups difficult; hence several species have been included or excluded from Quadrifaria or Virgata depending on the taxonomic treatment. In previous analyses, the Quadrifaria and Virgata groups of *Paspalum* were polyphyletic. Here we present a new appraisal of the classification of both groups based on the phylogenetic analyses of DNA data from the chloroplast: the *rpl16* intron, and the region comprising the *trnL* and *trnF* genes. A monophyletic Virgata clade is recovered, consisting of nearly all the species listed in an unpublished manuscript. The Quadrifaria group is restricted to *P. quadrifarium* and *P. quarinii*. Other clades grouped species traditionally treated within Virgata or Quadrifaria, although their phylogenetic placement needs to be reevaluated. Within most of the clades, diploid and polyploid species seemingly derive from a common ancestor denoting an autopolyploid origin. Allopolyploidy is also possible although reticulate evolution needs to be explored. The wide distribution of the I genome suggests that this is a plesiomorphic state in *Paspalum*.

**Keywords**—chromosome numbers, genome type, phylogeny, *rpl16*, *trnL* – *trnF*.

*Paspalum* L. is primarily an American genus with approximately 330 species distributed in tropical, subtropical and temperate regions (Zuloaga and Morrone 2005). A few species reach cold-temperate regions of northern Patagonia in Argentina (e.g. *P. distichum* L. and *P. vaginatum* Sw.) and central-southern Chile and western Argentina (e.g. *P. dasypleurum* Kunze ex E. Desv.). The species occur in flooded, dry, saline or sandy soils of savannas, coastal dunes, tropical and temperate forests, and prairies. Some species are also found in mountainous regions above 4000 m [e.g. *P. candidum* (Humb. & Bonpl. ex Flügge) Kunth., *P. prostratum* Scribn. & Merr., and *P. pygmaeum* Hack.].

Several species of *Paspalum* are important forage and turf grasses. Dallisgrass (*P. dilatatum* Poir.) and bahiagrass (*P. notatum* Flügge) are the most economically important and widely used for forage production, mainly in the southern U. S. A. (Gates et al. 2004; Evers and Burson 2004). *Paspalum atratum* Swallen has been gaining interest as forage in areas with periodic flooding in Florida, U. S. A., northeastern Argentina, southern Brazil, Thailand, Philippines, and Australia (Evers and Burson 2004). In addition, *P. scrobiculatum* L. is cultivated in India as a cereal crop (Kodo). *Paspalum vaginatum* and *P. notatum* are grown as turf grass, and species of the Quadrifaria group (e.g. *P. exaltatum* J. Presl, *P. haumanii* Parodi, and *P. quadrifarium* Lam.) as ornamentals (Rúgolo de Agrasar and Puglia 2004).

The most common basic chromosome number in *Paspalum* is  $x = 10$ . A few exceptions to this number have been reported for *P. alnum* Chase with  $x = 6$  (Quarín 1974), *P. contractum* Pilg. with  $x = 9$  (Davidse and Pohl 1974), and *P. convexum* Humb. & Bonpl. ex Flügge and *P. stellatum* Humb. & Bonpl. ex Flügge with  $x = 16$  (Selva 1976; Killeen 1990; Honfi et al. 1991). Diploid numbers are well-known for the genus (Norrman et al. 1989; Honfi et al. 1991; Norrman et al. 1994a, 1994b; Zuloaga and Morrone 2005), although most of the investigated species of *Paspalum* (80%) are polyploids, of which 50% are tetraploid and most of these tetraploids are apomictic (Quarín 1992; Quarín et al. 2001).

Based on inbreeding studies, a variety of genome types have been suggested for species of *Paspalum*, showing the complexity of its evolution: the D genome is assigned to *P. vaginatum* (Burson 1981a); the I genome to *P. intermedium* Munro ex Morong & Britton (Burson 1978); the  $I_2$  to *P. conspersum* Schrad. (Burson 1978); the J to *P. juergensii* Hack. (Burson 1979); the  $J_2$  to *P. virgatum* L. (Burson and Quarín 1982); the M to *P. malacophyllum* Trin. (Burson and Hussey 1998); the N to *P. notatum* (Burson 1981b); and the X is an unknown genome cited for the yellow-anthered biotype of *P. dilatatum* (Burson 1983). *Paspalum durifolium* Mez (II,  $J_2$ , XX) is an allopolyploid which shares genomes with *P. virgatum* and *P. dilatatum* (Burson 1983, 1985).

From a taxonomic perspective, *Paspalum* was included in subtribe Paspalinae of the tribe Paniceae, subfamily Panicoideae (Zuloaga et al. 2007). Molecular phylogenetic analyses indicate that this genus belongs to the  $x = 10$  Paniceae clade (Giussani et al. 2001) along with other genera with a basic chromosome number of  $x = 10$  (Gómez-Martínez and Culham 2000; Duvall et al. 2001; Aliscioni et al. 2003). The monophyly of *Paspalum* is well-supported when *Thrasya* Kunth is included in the genus (Giussani et al. 2001; Denham 2005). However, the monophyly of subgenera and informal groups within the genus are still unclear and requires further examination. Infrageneric classification of *Paspalum* is a difficult task. Only a few subgenera and sections have been accepted, and several informal groups have been proposed (Nees ab Esenbeck 1829; Doell 1877; Bentham 1881; Pilger 1929, 1940; Chase 1929; Clayton and Renvoize 1986; Zuloaga and Morrone 2005). To date, two major taxonomic studies have included the taxonomic diversity of *Paspalum* in America. First, Chase (1929) described two subgenera [*Ceresia* (Pers.) Rchb. and *Paspalum*], and 25 informal groups for 140 species in the northern Hemisphere. Later, Zuloaga and Morrone (2005) recognized three subgenera [*Anachyris* (Nees) Chase, *Ceresia*, and *Paspalum*], and 28 informal groups for 129 species in Austral South America. Denham (2005) established a fourth subgenus [*Harpostachys* (Trin.) S. Denham] which

TABLE 1. Main taxonomic treatments of the informal groups *Quadrifaria* and *Virgata* of *Paspalum*. Names between parentheses are the accepted names in Barreto (1966), and Zuloaga and Morrone (2003; 2005). A dash indicates that the species was not treated by the authors; \* indicates that the specimens treated as *Paspalum brunneum* by Chase (1939) and Barreto (1966) were misidentified and correspond to *P. quarinii* not *P. coryphaeum* (Morrone and Zuloaga 2000).

	Chase (1929)	Chase (1939)	Barreto (1954; 1966)	Zuloaga & Morrone (2005)	Molecular phylogeny (clades)
	North America	America	South America	Austral South America	
<b><i>Paspalum</i> - INGROUP</b>					
<i>P. acutum</i> Chase	Virgata	Virgata	-	-	-
<i>P. arechavaletae</i> Hack. ex Arechav. (= <i>P. exaltatum</i> J. Presl)	-	Virgata	-	-	-
<i>P. arundinaceum</i> Poir.	Virgata	Virgata	-	-	sister to Virgata
<i>P. arundinellum</i> Mez	-	-	Quadrifaria	Quadrifaria	Virgata
<i>P. brunneum</i> Mez (= <i>P. coryphaeum</i> Trin.)	-	Quadrifaria*	Quadrifaria*	-	-
<i>P. chaseanum</i> Parodi	-	Plicatula	Virgata	Plicatula	Plicatula + <i>P. rufum</i>
<i>P. commune</i> Lillo	-	Macrophylla	Virgata	Virgata	Macrophylla
<i>P. conspersum</i> Schrad.	Virgata	Virgata	Virgata	Virgata	Virgata
<i>P. coryphaeum</i> Trin.	Coryphaea	Quadrifaria	Quadrifaria	-	Coryphaea
<i>P. dasytrichium</i> Dusén ex Swallen	-	Quadrifaria	-	Quadrifaria	Coryphaea
<i>P. densum</i> Poir.	Virgata	Virgata	Quadrifaria	Quadrifaria	Virgata
<i>P. durifolium</i> Mez	-	Eriantha	Quadrifaria	Ungrouped	Ungrouped
<i>P. exaltatum</i> J. Presl	-	Virgata	Quadrifaria	Quadrifaria	Virgata
<i>P. ferrugineum</i> Trin. (= <i>P. quadrifarium</i> Lam.)	-	Quadrifaria	-	-	-
<i>P. haumanii</i> Parodi	-	Virgata	Quadrifaria	Quadrifaria	Virgata
<i>P. hispidum</i> Swallen	-	Virgata	-	-	-
<i>P. indutum</i> Lucas (= <i>P. coryphaeum</i> )	-	Quadrifaria	-	-	-
<i>P. intermedium</i> Munro ex Morong & Britton	-	Virgata	Quadrifaria	Quadrifaria	Virgata
<i>P. longum</i> Chase	-	Virgata	-	-	-
<i>P. millegrana</i> Schard.	Virgata	Virgata	Quadrifaria	-	Virgata
<i>P. nelsonii</i> Chase	Virgata	Virgata	-	-	-
<i>P. palustre</i> Mez	-	Plicatula	Virgata	Plicatula	Plicatula + <i>P. rufum</i>
<i>P. platyaxis</i> (Döll) Mez (= <i>P. wrightii</i> Hitchc. & Chase)	-	Virgata	-	-	-
<i>P. platyphyllum</i> Schult. (= <i>P. conspersum</i> )	-	Virgata	-	-	-
<i>P. plenum</i> Chase	Virgata	Virgata	-	Quadrifaria	Virgata
<i>P. quadrifarium</i> Lam.	-	Quadrifaria	Quadrifaria	Quadrifaria	Quadrifaria
<i>P. quarinii</i> Morrone & Zuloaga	-	-	-	Quadrifaria	Quadrifaria
<i>P. regnellii</i> Mez	-	Macrophylla	Virgata	Virgata	Macrophylla
<i>P. rufum</i> Nees ex Steud.	-	Eriantha	Virgata	Ungrouped	Ungrouped
<i>P. schreberianum</i> (Flüggé) Nash (= <i>P. millegrana</i> )	-	Virgata	-	-	-
<i>P. secans</i> Hitchc. & Chase (= <i>P. arundinaceum</i> )	Virgata	Virgata (= <i>P. arundinaceum</i> )	-	-	-
<i>P. virgatum</i> L.	Virgata	Virgata	Virgata	Virgata	Virgata
<i>P. wettsteinii</i> Hack. (= <i>P. virgatum</i> )	-	Macrophylla	Virgata	-	-
<b><i>Paspalum</i> - OUTGROUP</b>					
<i>P. distichum</i> L.	Disticha	Disticha	-	Disticha	Disticha
<i>P. erianthum</i> Nees ex Trin.	-	Eriantha	-	Eriantha	Eriantha
<i>P. fimbriatum</i> Kunth	Ungrouped	Fimbriata	-	-	Fimbriata
<i>P. glabrinode</i> (Hack.) Morrone & Zuloaga	-	-	-	Decumbentes	Decumbentes
<i>P. inconstans</i> Chase	-	Decumbentes	-	Decumbentes	Decumbentes
<i>P. macrophyllum</i> Kunth	-	Macrophylla	-	-	Macrophylla
<i>P. malacophyllum</i> Trin.	Malacophylla	Anachyris	-	Subg. Anachyris	Subg. Anachyris
<i>P. plicatulum</i> Michx.	Plicatula	Plicatula	Plicatula	Plicatula	Plicatula + <i>P. rufum</i>
<i>P. remotum</i> Remy	-	Livida	-	Livida	Livida
<i>P. wrightii</i> Hitchc. & Chase	Plicatula	Plicatula	-	Plicatula	Plicatula + <i>P. rufum</i>

includes all species previously considered in *Thrasya* as well as the Decumbentes group of *Paspalum* [excluding *P. glabrinode* (Hack.) Morrone & Zuloaga].

Within subgenus *Paspalum*, the Virgata group was first described by Chase (1929) who included nine species from North America; this group was later expanded to incorporate species of Central and South America (Chase 1939) [Table 1]. Within Virgata, Chase (1929) included tall and robust perennial species with; firm blades and sharp-cutting edges, panicles with few to numerous racemes, and paired spikelets 2–3.9 mm long. Barreto (1954), in a study of the South American species, restricted group Virgata of Chase to species with dark

brown upper anthercia and nonpyramidal panicles. Of these, only two species, *P. conspersum* and *P. virgatum* L., had been previously included in Virgata by Chase (1929, 1939). Barreto (1954) added six other allied species to the group (Table 1). Finally, in the revision of the Austral South American species of *Paspalum*, Zuloaga and Morrone (2005) circumscribed four species under the Virgata group (*P. commune* Lillo, *P. conspersum*, *P. regnellii* Mez, and *P. virgatum*), and considered *P. wettsteinii* Hack. as a synonym of *P. virgatum*. *Paspalum chaseanum* Parodi, and *P. palustre* Mez, were placed in the Virgata group by Barreto (1954) but considered in the Plicatula group by Chase (1939), and Zuloaga and Morrone (2005). *Paspalum*

*rufum* Nees ex Steud., another species included in *Virgata* by Barreto (1954), was regarded as part of the *Eriantha* group by Chase (1939), or as ungrouped within subgenus *Paspalum* by Zuloaga and Morrone (2005).

Chase (1939) listed six species in *Quadrifaria* of subgenus *Paspalum*: *P. brunneum* Mez, *P. coryphaeum* Trin. [the only species previously included in the *Coryphaea* group by Chase (1929)], *P. dasytrichium* Dusén ex Swallen, *P. ferrugineum* Trin., *P. indutum* Luces, and *P. quadrifarium*. Later, Barreto (1966) described group *Quadrifaria* to include ten South American species similar to *P. quadrifarium* (Table 1). All these species share a caespitose growth habit with hard stiff leaves, multiracemose inflorescences with pyramidal-shaped panicles, and pale-stramineous upper antheria. Accordingly, based on the shape of the panicle and the color of the antheria, Barreto (1966) assigned five species of the *Virgata* group sensu Chase (1929, 1939) to the *Quadrifaria* group. Finally, Zuloaga and Morrone (2005) followed Barreto's concept of *Quadrifaria*, adding to this group *P. dasytrichium* (also listed in Chase 1939), and *P. plenum* Chase. The position of *Paspalum durifolium* species was included in the *Quadrifaria* group by Barreto (1966), the *Eriantha* group by Chase (1929, 1939), or ungrouped by Zuloaga and Morrone (2005).

At present, partial phylogenies of *Paspalum* have been published (Aliscioni 2002; Vaio et al. 2005; Essi and Souza-Chies 2007; Souza-Chies et al. 2006); these studies have treated *Quadrifaria* and *Virgata* groups using a reduced sample of species. Using anatomical foliar and exomorphological characters, Aliscioni (2002) found that *Quadrifaria* and *Virgata* are polyphyletic. Similarly, a molecular phylogeny by Vaio et al. (2005) showed the *Quadrifaria* species partitioned in two clades, suggesting that this group is not monophyletic.

Here, we present a molecular phylogeny of the *Quadrifaria* and *Virgata* groups based on DNA sequence data from chloroplast markers, namely the *rpl16* intron, and the region comprising the *trnL* intron, the *trnL* partial exon, plus the intergenic spacer between *trnL* and *trnF* genes. We test the monophyly and the interspecific relationships of *Quadrifaria* and *Virgata* using parsimony. Additionally, several morphological features, the ploidy level and the genome formula are reviewed using the molecular phylogenetic tree to make inferences regarding the evolutionary history of the genus.

## MATERIALS AND METHODS

**Taxonomic Sampling**—We included a total of 50 specimens representing both outgroup and ingroup taxa. A list of specimens analyzed is reported in Appendix 1.

**Outgroup Selection**—We investigated ten species of *Paspalum* representing three subgenera and several informal groups. Classification follows Denham (2005) and Zuloaga and Morrone (2005). Accordingly, eight species were sampled from subg. *Paspalum* [*P. distichum* (*Disticha* group), *P. erianthum* Nees ex Trin. (*Eriantha* group), *P. fimbriatum* Kunth (*Fimbriata* group), *P. glabrinode* (Hack.) Morrone & Zuloaga (ungrouped), *P. macrophyllum* Kunth (*Macrophylla* group, Chase 1939), *P. remotum* J. Rémy (*Livida* group), and *P. plicatulum* Michx. and *P. wrightii* Hitchc. & Chase (*Plicatula* group)], one species from subg. *Harpostachys* (*P. inconstans* Chase), and one species from subg. *Anachyris* (*P. malacophyllum*).

In addition, four representative genera of the subfamily Panicoideae, used in the molecular phylogenetic study of Giussani et al. (2001), were used as part of the outgroup: *Zea mays* L. (*Andropogoneae*:  $x = 10$ ), *Sacciolepis viloides* (Trin.) Chase (*Paniceae*:  $x = 9$  Clade), *Axonopus anceps* (Mez) Hitchc. (*Paniceae*:  $x = 10$  Clade), and *Chasmanthium latifolium* (Michx.) Link (tribe Centothecae). *Chasmanthium latifolium* was used to root the tree.

**Ingroup Selection**—A total of 20 species from the informal groups *Quadrifaria* and *Virgata* of *Paspalum* (as considered by Chase 1929, 1939;

Barreto 1954, 1966; and Zuloaga and Morrone 2005) were included as ingroup taxa in the molecular analyses (Appendix 1). To represent the cytological, morphological and geographical variability, up to three accessions for each species of the ingroup were sequenced whenever material was available. *Paspalum acutum* Chase, *P. hispidum* Swallen, *P. longum* Chase, and *P. nelsonii* Chase, considered by Chase (1939) in the *Virgata* group were not included in our sampling due to the lack of fresh material.

**DNA Isolation, Amplification and Sequencing**—Total DNA was extracted from fresh or silica-gel-dried leaves, using modified CTAB protocols as described in Giussani et al. (2001). We amplified the *trnL* (UAA) intron, *trnL* (UAA) partial 3' exon, and the intergenic spacer between the *trnL* (UAA) 3' exon and the *trnF* (GAA) gene (*trnL-F* region) using primers C, D, E and F as described in Taberlet et al. (1991); for difficult taxa in which primers C and F failed to amplify, two new primers were designed Cii = 5' TAGACGCTACGGACTTGATTG 3' and Fdw = 5' CAGTCTCTGCTCTACCAGC 3'. The gene encoding the ribosomal protein 16 (*rpl16* intron) was amplified using two shorter modified primers, approximately five base pairs shorter than those originally published by Jordan et al. (1996). These are F71 5' GCTATGCTTAGTGTGTCTC 3' and R1661 5' CCAKATTTTCCACCACGAC 3'. For some species, the region was amplified in two fragments using primers: F584: 5' TTCATTGGGTGCGATGGCGGAA 3' and R584: 5' TTCGCCATCCCAATGAA 3'. For sequencing reactions, one additional internal primer was designed: F80: 5' C/TTATGCTTCGTATTGTCG 3', and the primer R270 (Zhang 2000) was also employed.

The 25 or 50  $\mu$ l Polymerase chain reaction (PCR) contained 20–40 ng/ $\mu$ l of DNA template, and a final concentration of 1 $\times$ PCR Buffer minus MgCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, 0.025 mM each dNTP, 0.2  $\mu$ M each primer, and 1.25–3 units Taq Polymerase (Invitrogen Life Technologies, São Paulo, Brazil). PCR amplifications were performed under the following conditions: 1) *trnL-F*: 1 cycle of 94°C for 5 min, 34 cycles of 94°C for 30s, 48°C for 1 min, and 72°C for 1 min 30s, 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 30s, and a final extension cycle of 72°C for 7 min. In species for which these protocols were unsuccessful, the conditions of the annealing temperature (1–8°C), and number of cycles were modified. In addition, a variety of PCR additives and enhancing agents (bovine serum albumin, dimethyl sulfoxide, formamide) were used to increase the yield, specificity and consistency of PCR reactions.

Cleaning of PCR products was done by Macrogen, Inc. using Montage PCR purification kit from Millipore following the manufacturer's protocol. Sequencing reactions were also performed by Macrogen, Inc. using the ABI PRISM BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Seoul, Korea), following the protocols supplied by the manufacturer. Both forward and reverse strands were sequenced with a minimum overlap of 90% for every taxon. Single-pass sequencing was performed on each template using selected primers to complete a bidirectional contig of the full sequence. Editing and assembling of sequences was conducted using Chromas Pro version 1.34 (Technelysium Pty, Ltd, Tewantin, Australia). Quality of sequences was assessed by visual inspection of the chromatograms. Sequence alignment was made using CLUSTAL W (Thompson et al. 1994) and by eye, manually refined using BioEdit ver. 5.0.9 (Hall 1999). All sequences were submitted to GenBank; voucher information, and GenBank accession numbers, are provided in Appendix 1. Alignments and phylogenetic trees were submitted to TreeBASE (study number S2127).

**Phylogenetic Analysis**—Parsimony-based analyses of sequence data were performed to explore phylogenetic relationships among species. Phylogenetic searches were carried out independently for the region comprising the *trnL* intron, the *trnL* partial exon, plus *trnL-trnF* intergenic spacer (*trnL-F*), and the *rpl16* intron, and for all data sets combined. The analysis of each individual genomic marker was made to explore phylogenetic signal and possible phylogenetic incongruences among the data sets. Because of the maternal inheritance of the chloroplast, the phylogenetic results can be biased. This can be significant when the specimens studied are allopolyploids. Hence, we sequenced different cytotypes for each species including, when possible, a diploid specimen.

Heuristic searches were conducted using TNT ver. 1.1 (Goloboff et al. 2003). During the search we used equal weights and nonadditive characters, and gaps were treated as missing data. Before searches, all uninformative characters were deactivated. The searches involved 1,000 replicates, each of which generated 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 2 trees in each replicate. Subsequently, all optimal trees were swapped using TBR holding a maximum of 100,000 trees. Bremer support (Bremer 1994) was calculated



to find suboptimal trees of one to seven steps longer than the shortest trees, saving a maximum of 100,000 trees in each step. Jackknife support (Farris et al. 1996) was calculated by running 1,000 replicates, starting each replica with a single Wagner tree, and swapped with TBR, and holding a single optimal tree. The resampling probability was set to 0.36.

A literature review was made to compile chromosome numbers and the genome formula in species of *Paspalum* (Appendix 1). These data were used to infer potential trends in genome evolution and to determine whether ploidy levels are correlated with some particular phylogenetic groups. Inflorescence and spikelet diagnostic characters (Barreto, 1954, 1966) of the Virgata and Quadrifaria groups were mapped onto the molecular phylogeny to investigate how many times these characters have independently evolved in these groups.

## RESULTS

**Sequence Data**—The *rpl16* intron sequences varied in length from 1,053 bp in *Chasmanthium latifolium* to 1,097 bp in *Sacciolepis vilvoides*, both species outgroups. Within *Paspalum*, the sequence length for this marker ranged from 1,070 bp in *P. distichum* to 1,086 bp in *P. haumanii* (Morrone s.n.). Missing data for *rpl16* is less than 3%.

The length variation of the *trnL* intron sequence is from 458 bp in *Zea mays* to 543 in all species of *Paspalum* except *P. exaltatum* and both specimens of *P. haumanii*, which have 542 bp. The length of the *trnL-trnF* spacer ranged from 333 bp in *Sacciolepis vilvoides* (outgroup), and 352 bp in *P. malacophyllum* to 370 bp in *P. distichum*. The *trnL* partial exon provided little phylogenetic signal. Missing data for the entire region (*trnL-F*) is 3.7%.

Excluding a poly-T nucleotide region between bases 41 and 56 of the *trnL-trnF* spacer, all the indels are only informative among outgroup taxa, thus gaps were not included in the analyses.

**Phylogenetic Analyses**—The alignment of the *rpl16* intron resulted in 1,178 characters, of which 67 (5.6%) were phylogenetically informative. Parsimony analyses yielded 54 trees of length = 138 (CI = 0.58; RI = 0.79). The consensus retains 25 clades, of which 21 clades have a jackknife value (JK)  $\geq$  50%. The majority of clades were also recovered in the trees of the combined data analyses (Fig. 1). Only the clade including species of the Plicatula group (as defined by Chase 1929; Zuloaga and Morrone 2005), e.g. *P. chaseanum*, *P. palustre*, *P. plicatulum*, and *P. wrightii*, is exclusive of the *rpl16* consensus tree (JK = 68%, figure not shown).

The alignment including the *trnL* intron, *trnL* partial exon and *trnL-trnF* intergenic spacer (*trnL-F*) contains 1,007 positions, of which 60 (6%) are phylogenetically informative. The analyses produced 400 shortest trees of 96 steps (CI = 0.70; RI = 0.88). The consensus retains 19 nodes (18 with JK  $\geq$  50%), which were also recovered in the trees of the total combined analysis (Fig. 1).

The results obtained from the *rpl16* and *trnL-F* data sets are largely congruent, and twelve clades are common to both consensus trees. Topological differences are due to the relatively low resolution for some clades as well as the addition of *Paspalum macrophyllum* into the *P. regnellii-P. commune* clade of the *trnL-F* dataset, which was not included in the *rpl16* consensus tree. Likewise, the position of both specimens of *P. rufum* [Quarín 3754, 3756] (included in a clade with species of the Plicatula group in the *trnL-F* tree) and the relationship of *P. arundinaceum* (as a sister group of a clade with several species of the Virgata group in the *rpl16* tree) are other discrepancies between analyses.

The combined data set (*rpl16* intron+*trnL-F*), using 126 informative characters from both markers produced three

most parsimonious trees (L = 240; CI = 0.62; RI = 0.82). Figure 1 shows one of the most parsimonious trees; only resolution of basal branches is ambiguous and varies among the three trees (dashed lines in Fig. 1). Figure 2 represents the consensus tree with jackknife and Bremer values shown on branches. The monophyly of *Paspalum* is well supported (JK = 99, Bremer support (BS) > 7). In turn, *Paspalum rufum* (Zuloaga 7110) and *P. durifolium* form a clade (JK = 85, BS = 3) sister to the remaining of the species. In addition, species of the Plicatula group + other accessions of *P. rufum* (Quarín 3754, 3756) form a clade with moderate support (JK = 84, BS = 3). Even though both specimens of *P. rufum* form a well-supported subclade (JK = 99, BS = 6), the relationships with species of the Plicatula group (*P. chaseanum*, *P. palustre*, *P. plicatulum*, and *P. wrightii*) are not resolved (Figs. 1 and 2). Both specimens of *Paspalum coryphaeum* (Zuloaga s.n. and Longhi et al. 9456) and *P. dasytrichium* are united in a clade (BS = 1), with *P. coryphaeum* (Zuloaga s.n.) and *P. dasytrichium* joined in a slightly supported clade (JK = 73, BS = 2; Fig. 2). In addition, in these trees either the Plicatula + *P. rufum* clade or the *P. coryphaeum* + *P. dasytrichium* clade (Fig. 1) can be sister to the remaining species.

As previously circumscribed (Table 1), the Quadrifaria group and the Virgata group are polyphyletic. However, our phylogenetic analyses revealed a clade (Clade 1; JK = 98, BS = 7; Fig. 1) including most of the species of the Virgata group circumscribed in Chase (1939). Clade 1, consisting of *Paspalum arundinellum*, *P. conspersum*, *P. densum*, *P. exaltatum*, *P. haumanii*, *P. intermedium*, *P. millegrana*, *P. plenum*, and *P. virgatum*, is sister to *P. arundinaceum*, although the latter relationship is poorly supported (BS = 1). Within Clade 1, both specimens of *P. exaltatum* form a well-supported subclade (JK = 97, BS = 4) with specimens of *P. haumanii* sister to *P. exaltatum* and highly supported (JK = 95, BS = 3). A second well-supported clade (Clade 2; JK = 84, BS = 2; Fig. 1) including *P. conspersum*, *P. arundinellum*, *P. densum*, *P. millegrana*, *P. plenum*, and *P. virgatum* is sister to *P. intermedium*. Furthermore, a strong relationship is evident among *P. virgatum*, *P. plenum*, *P. millegrana*, and *P. densum* in Clade 3 (JK = 84, BS = 2; Fig. 1).

The remaining analyzed species, previously considered in the Virgata and Quadrifaria groups (Table 1), fall into different clades (Figs. 1 and 2). For instance, the Macrophylla clade includes three species, *Paspalum commune* and *P. regnellii* (JK = 67, BS = 2), which are sister to *P. macrophyllum* (JK = 68, BS = 2). Likewise, the Quadrifaria clade includes two species: *P. quarinii* and *P. quadrifarium* (JK = 92, BS = 3), while the Coryphaea clade departs from Quadrifaria and encompasses two specimens of *P. coryphaeum* and *P. dasytrichium* (BS = 1; Fig. 1). *Paspalum rufum* (Zuloaga 7110) and *P. durifolium*, are part of the "Ungrouped" clade (JK = 85, BS = 3; Fig. 1) suggesting an ambiguous status as reported in Zuloaga and Morrone (2005).

## DISCUSSION

Based on independent and combined analyses of sequence data from the *rpl16* and *trnL-F* chloroplast markers, our study indicates that the informal Quadrifaria and Virgata groups of *Paspalum* are polyphyletic. Species complexes from previous classifications are referred as groups according to the literature references (Table 1), while results from this study are referred as clades. This finding is in agreement with other studies encompassing smaller taxonomic sampling (Aliscioni 2002; Vaio et al. 2005; Souza-Chies et al. 2006). Our molecular

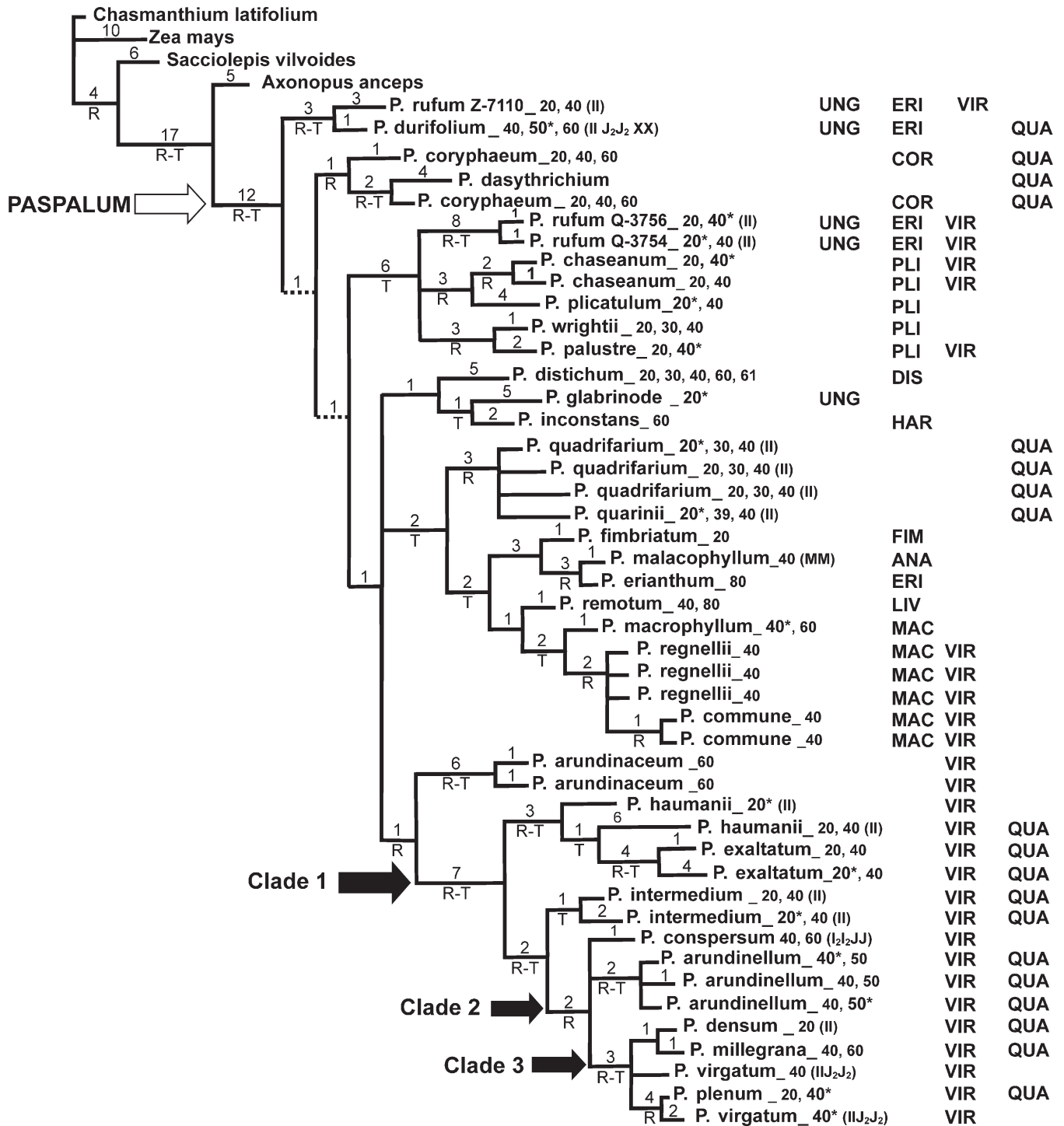


FIG. 1. One of three most parsimonious trees inferred from the *rpl16* + *trnL-F* data sets. Numbers above branches indicate its length, and letters below branches indicate clades that are present in *rpl16* (R) or *trnL-F* (T) trees when analysed alone. Dashed lines correspond to branches that vary between the three most parsimonious trees as explained in the text. Chromosome counts of the species are cited following names ( $2n$ ); \* indicates a chromosome count of the specimen sequenced and references are cited in Appendix 1. Letters between brackets indicate the genome type (see also Appendix 1). Black arrows show principal clades within the Virgata group as discussed in the text. Acronyms to the right correspond to informal groups of *Paspalum* shown in Table 1.

phylogeny provides a new perspective to group the species, and new clades are proposed.

**Virgata Clade**—The Virgata clade, as referred herein, includes all species of the well-supported Clade 1 (Fig. 2), namely *Paspalum arundinellum*, *P. conspersum*, *P. densum*, *P. exaltatum*, *P. haumanii*, *P. intermedium*, *P. millegrana*, *P. plenum*, and *P. virgatum*. This circumscription is similar to the one

previously proposed by Chase (1939), except for the inclusion of *P. acutum*, *P. hispidum*, *P. longum*, and *P. nelsonii*, here not considered.

Based on Pohl and Davidse (1994) and Zuloaga and Morrone (2005), references from which some morphological measurements were summarized, the Virgata clade is characterized by including tall [1–2 (–3) m], robust, perennial

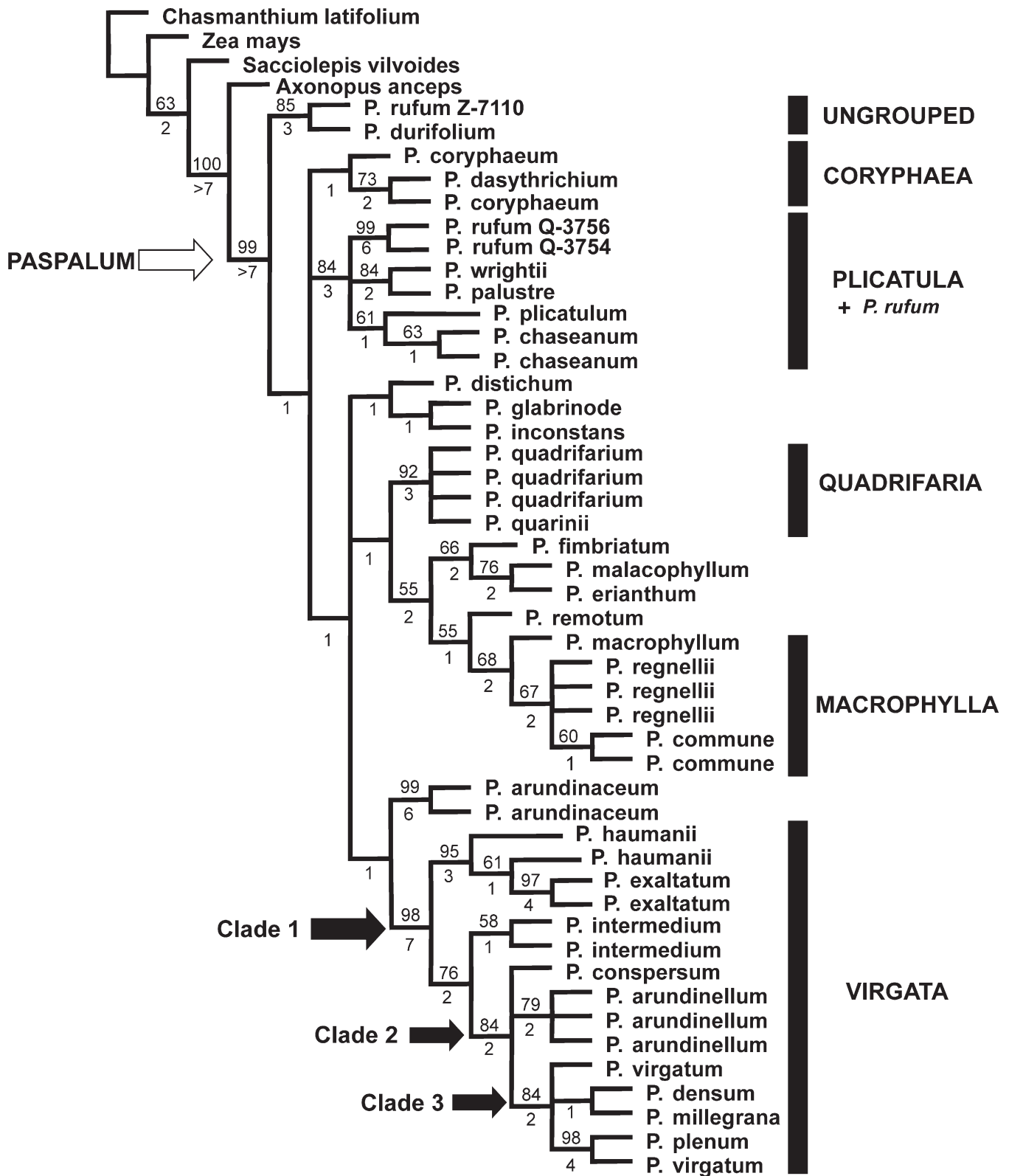


FIG. 2. Consensus of three most parsimonious trees based on the combined *rpl16* + *trnL-F* data sets. Number above branches indicate jackknife support, and number below branches indicate Bremer support. Bars indicate the principal clades recovered by the analyses, while black arrows show principal clades within the Virgata group as discussed in the text.

grasses, leaves with sharp-cutting edges, large panicles [(10–) 20–40 (–50) cm] and numerous racemes [(7–) 20–40 (–100)]. The panicle shape, which depends on the ratio between apical and basal racemes, varies from truncated (4–10/10–20) to pyramidal (0.5–2/7–15). The rachis of the racemes is (0.5–)

1–1.5 (–2) mm in width. The spikelets are ellipsoid or suborbicular to obovoid, (1.6–) 2.2–3.2 (–4) mm long and (1.2–) 1.5–2 (–2.4) mm wide.

Geographically, the species of the Virgata clade are widely distributed in humid grasslands and marshes, ranging from Mexico

and Central America to South America. *Paspalum arundinellum*, *P. exaltatum*, *P. intermedium*, and *P. haumanii* are species restricted to Austral South America, while *P. conspersum*, *P. densum*, *P. millegrana*, *P. plenum*, and *P. virgatum* are widely distributed from Mexico and the Caribbean to Austral South America. *Paspalum conspersum*, *P. virgatum*, and *P. intermedium* have been introduced in North America (Pohl and Davidse 1994; Allen and Hall 2003). In connection with their occurrence in humid habitats, all species of the Virgata clade have air cavities (as seen in transverse cross sections of the leaves) between the first order vascular bundles surrounding the midvein and a group of parenchyma cells above these cavities through the adaxial epidermis. These cavities have been observed in *P. arundinellum*, *P. conspersum*, *P. densum*, *P. exaltatum*, *P. millegrana*, *P. plenum*, and *P. virgatum* (Aliscioni and Arriaga 1998), and in *P. haumanii* and *P. intermedium* (Giussani, pers. obs.), and are more evident in mature leaves. It is feasible that the cavities are genetically controlled and related to gas transport and reduction of stress due to floods (Williams and Barber 1961). These structures also have been reported in the Plicatula and Dissecta groups of *Paspalum* and could represent a phylogenetic informative character in *Paspalum* (Aliscioni 2000). Further morphological analysis of this group would be useful to unveil the interspecific variability of *Paspalum* and to determine new diagnostic characters supporting the monophyly of the Virgata clade and related species.

The panicle shape was usually used to separate species of the Virgata and Quadrifaria groups of *Paspalum* (Barreto 1954, 1966; Zuloaga and Morrone 2005). To make some inferences regarding the evolution of the panicle shape, we optimized this character on the most parsimonious trees and found that the pyramidal shape is plesiomorphic while a truncated panicle has arisen several times in the evolution of *Paspalum* (figure not shown). *Paspalum exaltatum*, *P. haumanii*, and *P. intermedium*, the earliest divergent species within the Virgata clade (Figs. 1 and 2), are characterized by pyramidal panicles, with highly branched secondary paracladia (ramifications of the racemes), a character particularly evident in *Paspalum exaltatum* and *P. haumanii* (Rua 1996); furthermore, *Paspalum exaltatum* and *P. haumanii* form a strongly supported clade (JK = 95; BS = 3; Fig. 2). This relationship is reinforced because both species have the upper glume and lower lemma longer than the upper floret (Zuloaga and Morrone 2005). A novel truncated panicle evolved in members of Clade 2 with two reversals from truncated to pyramidal in *P. densum* and *P. plenum*. Lastly, Clade 3, another well-supported clade, includes species with obovoid spikelets: *P. virgatum*, *P. millegrana*, *P. densum*, and *P. plenum* (Fig. 2). Most of the examined species of *Paspalum plenum* have ellipsoid spikelets; however the specimen included in this analysis (*Maruñak* 539) has suborbicular to obovoid spikelets. This is a derived condition within the Virgata clade, all other species having ellipsoid spikelets.

Within the Virgata clade the chromosome numbers are variable. The most common number is  $2n = 4x = 40$ . Diploid species include *P. densum*, *P. haumanii*, *P. intermedium*, and *P. plenum*, while pentaploids ( $2n = 5x = 50$ ) were found for *P. arundinellum* (Appendix 1).

Several interspecific crosses have been made between diploid and tetraploid species of *Paspalum* (Burson 1978; Burson and Quarín 1982; Quarín and Norrmann 1990; Caponio and Quarín 1993). The phylogenetic relationships among species of the Virgata clade are supported by results of interspecific crosses, in which hybrids between diploid members of the Virgata clade were characterized by regular meiosis with

high levels of bivalent associations (Quarín and Norrmann 1990; Caponio and Quarín 1993). For instance, Quarín and Norrmann (1990) found that *Paspalum intermedium* was more closely related to *P. haumanii* than to *P. quarinii* (as *P. brunneum*), *P. quadrifarium* (Quarín 3810, included in our analysis), or *P. rufum*. Caponio and Quarín (1993) related *P. densum* to *P. intermedium* as all hybrids had regular meiosis with mostly bivalent associations. In addition, interspecific crossing between a diploid member of *P. intermedium* and the tetraploid *P. conspersum* (Burson 1978), and hybrids between *P. intermedium* ( $2n = 2x = 20$ ) and *P. virgatum* ( $2n = 4x = 40$ ; Burson and Quarín 1982) also revealed the close relationships among species of the Virgata clade.

Even though the position of *Paspalum arundinaceum* indicates that this species is sister to the Virgata clade, further analyses are required to confirm this relationship. The species was reported as hexaploid with multivalent associations and irregular pairing (Davidse and Pohl 1974). Thus, the putative sister relationship with the Virgata clade could reflect a parental linkage to its tentative allopolyploid origin (Davidse and Pohl 1974). *Paspalum arundinaceum* differs from members of the Virgata clade because it has evident adaxial ribs associated with first and second order vascular bundles, with bulliform cells connected to large parenchyma cells displacing the third order vascular bundles to the abaxial epidermis (Aliscioni and Arriaga 1998). *Paspalum arundinaceum* is native to Mesoamerica, the Caribbean, and northern South America (Pohl and Davidse 1994).

**Quadrifaria Clade**—Based on our results, the Quadrifaria clade is here confined to *Paspalum quarinii* and *P. quadrifarium* (Figs. 1 and 2). This clade is made up of only two species of the Quadrifaria group (Table 1), which is strongly supported by our results (JK = 92; BS = 3; Fig. 2). This clade comprises caespitose, tall and robust perennial species with long leaves and pseudo-petiolate basal leaves. Both species have pyramidal panicles 12–30 cm long, with numerous racemes [(10–) 25–40] and narrow rachis (0.4–0.6 mm) with papillose marginal hairs, 1–4 mm long. The spikelets are ellipsoid, 1.6–2.4 mm long and 0.8–1 mm with short and tender hairs with tuberculate base on upper glume and lower lemma (Zuloaga and Morrone 2005). Both *Paspalum quarinii* and *P. quadrifarium* occur in eastern Argentina, southern Brazil, Paraguay and Uruguay, and several chromosome numbers have been reported throughout their distributional range: *P. quadrifarium*  $2n = 20, 30, 40$ , and *P. quarinii*  $2n = 20, 39, 40$  (Appendix 1). The close relationships between these two species have been proposed by Quarín and Norrmann (1990), who reported regular meiotic behavior in hybrids between diploid specimens. Likewise, Vaio et al. (2005) showed a similar pattern in the location of the ribosomal rDNA gene 45S in chromosomes between a diploid specimen of *P. quadrifarium* and a tetraploid specimen of *P. quarinii*. Furthermore, Vaio et al. (2005) reported that *Paspalum quadrifarium* likely hybridizes with species of the Virgata group as they found a tetraploid specimen close related to *P. haumanii*, *P. exaltatum*, and *P. intermedium*.

**Coryphaea Clade**—In the revision of the North American species of *Paspalum*, Chase (1929) proposed the Coryphaea group to include a robust rhizomatous species (*P. coryphaeum*) with hirsute sheaths, long flat blades, and with large panicles with numerous slender racemes. She also indicated that all species related to *P. coryphaeum* were confined to South America, although not revealing which species she referred to. Later, Chase (1939) considered *P. coryphaeum* within the



Quadrifaria group, in which she listed five other species (*P. brunneum*, *P. dasytrichium*, *P. ferrugineum*, *P. indutum*, and *P. quadrifarum*) (Table 1). However, our study did not show a relationship among these species but only weakly supported the close relationship between both specimens of *P. coryphaeum* and *P. dasytrichium*. Both *P. coryphaeum* and *P. dasytrichium* are robust perennial plants with an erect to leaning growth habit, tuberculate hairs on sheaths, and papillose-hirsute leaves (although the hairiness of leaves is variable among specimens in *P. coryphaeum*). Measurements obtained from Chase (1929) and Zuloaga and Morrone (2005) indicate that the panicles are pyramidal, 10–25 cm long, with 10–50 racemes, and the rachis of racemes narrow, 0.3–0.5 mm wide; the spikelets are ellipsoid, 1.8–2.5 mm long and 1 mm wide. Based on the morphological affinities between both species and the phylogenetic placement, we suggest including *P. coryphaeum* and *P. dasytrichium* in the Coryphaea clade analogous to the group of Chase (1929). The geographical distribution of *P. coryphaeum* extends from Panama to the Guianas and northern Brazil, while *P. dasytrichium* is endemic to southern Brazil. *Paspalum coryphaeum* is a polymorphic species with different ploidy levels ( $2n = 20, 40, 60$ ), while the chromosome number is unknown for *P. dasytrichium* (Appendix 1).

**Macrophylla Clade**—This clade consist of all specimens of *Paspalum regnellii* (3) and *P. commune* (2) along with *P. macrophyllum* as the sister to the remainder of the clade. Chase (1939) recognized these species and proposed the informal group Macrophylla in which she also included *P. wetsteinii* and *P. barclayi* Chase. With the exception of *P. wetsteinii* (= *P. virgatum*), we propose reinstating the informal South American group Macrophylla, as proposed by Chase (1939). In addition to the relationships revealed by the phylogenetic analyses, these species are characterized by including perennial plants 0.4–1 (–2) m tall, with wide blades (10–25 mm), rounded at the base; panicles truncate with 3–15 racemes and rachis 0.4–0.8 mm wide. Spikelets are ellipsoid to obovoid, pubescent, with pale or brown anthercia, (2–) 2.2–2.7 (–3) mm long and (1.4–) 1.8–2 mm wide (measurements based on Chase 1927; and Zuloaga and Morrone 2005). Within this clade, tetraploid counts have been cited for *P. commune*, and tetraploid cytotypes were also reported for *P. regnellii* (Appendix 1) The sister species, *P. macrophyllum* includes  $2n = 40$  and 60 cytotypes (Appendix 1). The Macrophylla clade is a South American group of species mainly distributed in moist soils of forest borders or open grounds at middle and low altitudes of Colombia, Ecuador, Peru, Bolivia, and northwestern Argentina. Only *Paspalum regnellii* is restricted to modified soils in forest borders of southeastern South America (northeastern Argentina, eastern Paraguay, and southern Brazil) and Central Brazil.

**Plicatula + *Paspalum rufum* Clade**—The controversial *Paspalum chaseanum* and *P. palustre*, included in the Virgata group by Barreto (1954) and in the Plicatula group by Chase (1939) and Zuloaga and Morrone (2005) (Table 1), were investigated to determine their phylogenetic position. Their alliance with *Paspalum plicatulum* and *P. wrightii* confirms its placement within the Plicatula group. The Plicatula group is easily recognized by the morphology of the spikelet (sterile lemma with transverse wrinkles, the anthercium conspicuously convex, dark brown and shining), although the variability of reproductive and vegetative characters make the identification difficult at the specific level (Zuloaga and Morrone 2005).

Species of the Plicatula group studied here exhibit several ploidy levels:  $2n = 20, 30, 40$  (Appendix 1), and are widespread in America, occupying different environments such as open grounds, wet wood borders, moist sands or margin of streams. Two specimens of *Paspalum rufum*, a diploid (Quarín 3754) and a tetraploid (Quarín 3756) specimen reported as possibly originating via autopolyploidy (Quarín et al. 1998), are related to species of the Plicatula group. *Paspalum rufum* and species of the Plicatula group share a dark brown upper anthercium and occur in low and wet grounds, flooded lands and margins of streams. Nevertheless, additional sampling is needed to corroborate this phylogenetic relationship.

**“Ungrouped” Clade**—This clade includes two species, *Paspalum durifolium* and *P. rufum*. *Paspalum durifolium* is represented here by a pentaploid specimen (Valls 12282) of natural origin (Honfi 2003). Two other cytotypes are also known for this species: an apomictic allohexaploid presumably with three different genomes (II, J<sub>2</sub>, XX) (Burson 1985) and a sexual allotetraploid (Quarín 1994). The strong relationship found between *P. durifolium* (Valls 12282) and *P. rufum* (Zuloaga 7110) (Fig. 2) is congruent with their morphological affinities such as the ellipsoid pubescent spikelets, with an acute apex and with the overlapping distribution (growing in moist grounds or low flooded lands of southeastern South America). We believe that *Paspalum rufum* could represent a parental line of the allopolyploid *P. durifolium*, both of which have at least one genome type in common (Burson and Quarín 1992; Quarín 1994). Unfortunately, there are no cytological studies that confirm the chromosome number and the meiotic behavior of the specimen of *P. rufum*, Zuloaga 7110.

The presence of long hairs on the upper glume and lower lemma of *P. durifolium* and *P. rufum* resemble those of the members of the Eriantha group of Chase (1939), in which Chase included *P. durifolium*, *P. devincenzii* Parodi (synonym of *P. durifolium*), *P. erianthum* Nees, *P. erianthoides* Mez., *P. hassleri* Hack. (synonym of *P. rufum*), and *P. rufum*. The position of these two species within the Eriantha group was tested by adding *P. erianthum* to this analysis. This hypothesis was rejected as our analyses showed that *P. erianthum* is more closely related to *P. malacophyllum* and *P. fimbriatum*, although with moderate support. This finding concurs with that of Morrone et al. (2004), who set apart *P. durifolium* and *P. rufum* from the Eriantha group. Therefore, the taxonomic position of *P. durifolium* (Valls 12282) and *P. rufum* (Zuloaga 7110) will remain in doubt until further studies are conducted.

**Diploid – Polyplod Complexes**—Extensive cytological and inbreeding studies were conducted in species of the Macrophylla, Plicatula, Quadrifaria, and Virgata clades of *Paspalum*, and sufficient data is available to discuss ploidy relationships among species.

The morphological similarity among diploids and tetraploids and the presence of multivalent chromosome associations in polyploid cytotypes suggest the probable origin of polyploids via autopolyploidy; hence, different cytotypes may form part of the same taxonomic complex (Quarín and Lombardo 1986; Caponio and Quarín 1987; Quarín and Norrmann 1987; Norrmann et al. 1989; Honfi et al. 1991; Quarín 1992; Quarín et al. 1996, 1998; Acuña et al. 2005). Diploid and tetraploid specimens were reported for most of the species analyzed in this study while triploid, pentaploid and hexaploid specimens are less frequent (Fig. 1, Appendix 1). When the chromosome numbers of the species are considered for terminal taxa in the most parsimonious trees, diploid



and polyploid taxa merge in most monophyletic clades. For example, the diploid and tetraploid cytotypes of *Paspalum exaltatum* together with the diploid cytotype of *P. haumanii* (Virgata clade); both diploid and tetraploid specimens of *P. rufum*, which are related to the Plicatula clade; and different cytotypes of *P. quarinii* and *P. quadrifarium* (Quadrifaria clade) (Fig. 1). Therefore, polyploidy within monophyletic complexes such as Plicatula + *P. rufum* clade, Quadrifaria clade, and Virgata clade, may have arisen from diploid ancestors via autopolyploidy (Quarín and Lombardo 1986; Quarín et al. 1998). In addition, the morphological homogeneity of the diploid-polyploid monophyletic clades (see above) is consistent with the tentative autopolyploid origin of polyploid races.

Although interspecific hybrids have been obtained from diploids (Quarín and Norrmann 1990), or between polyploids (Burson and Quarín 1982; Burson 1985; Quarín and Norrmann 1987; Burson and Quarín 1992; Caponio and Quarín 1993; Quarín 1994; Burson and Hussey 1998), they are usually highly sterile and rarely found in natural populations. To investigate patterns of reticulate evolution, hybridization of closely related species, and lineage sorting in major groups of *Paspalum*, it is necessary to explore congruence between chloroplast (maternal line) and nuclear phylogenies (both parental lines), to further detect parental origin of diploid – polyploid complexes.

Regarding the genome type variation in *Paspalum*, previous hybridization studies demonstrated that *P. densum* (Caponio and Quarín 1993), *P. durifolium* (Burson 1985), *P. haumanii* (Quarín and Norrmann 1990), *P. quadrifarium* (Quarín and Norrmann 1990), *P. quarinii* (as *P. brunneum* in Quarín and Norrmann 1990; Burson and Quarín 1992), *P. rufum* (Quarín and Norrmann 1990; Burson 1989; Burson and Quarín 1992), and *P. virgatum* (Burson and Quarín 1982) share the I genome. In addition, the I<sub>2</sub> genome of *Paspalum conspersum* would be partially homologous to the I genome (Burson 1978). All these species, which possess the I genome characteristic of *P. intermedium*, are related to different clades in our phylogeny (Fig. 1). Although the genome formula is known for a few species of *Paspalum*, the I genome could be ancestral in the origin of *Paspalum*, appearing as the common basic genome to all species.

In summary, *Paspalum* is a large, polymorphic genus with complex and wide array of geographic, morphological, and taxonomic lineages. Our study provides preliminary insights to circumscribe monophyletic clades and infer interspecific relationships in *Paspalum*. Foremost, a monophyletic Virgata clade, consisting of nearly all the species listed in an unpublished manuscript by Chase, is recovered in our analysis. Also, the Quadrifaria group is restricted to *P. quadrifarium* and *P. quarinii*, and other species traditionally treated within Virgata or Quadrifaria were distantly related. Therefore, the phylogenetic placement of some species is in need of reevaluation within a major taxonomic framework.

**ACKNOWLEDGMENTS.** We are grateful to Amalia Scatagli, and Silvia Denham for helpful discussions and suggestions on the manuscript. To D. Litwiller for comments on the manuscript; H. M. Longhi Wagner, J. F. M. Valls, G. H., Rua, and R. Fortunato for providing some of the samples used in this study. Field and laboratory work was supported in part by Fundación Antorchas grants 4248–73 and 14116–161, Mini-PEET Awards to Enhance Transfer of Taxonomic Knowledge, National Geographic Society research grant 7792–05, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) research grant PIP 5453, Agencia Nacional de Promoción Científica y Técnica (ANPCyT) grants 11739, 13374, 13578, and PAV13714.

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- APPENDIX 1. Voucher information and GenBank accession numbers. For species of *Paspalum*, the chromosome counts, type of genome, and corresponding references are cited following species names in square brackets. Voucher specimens are deposited in: BAA, BAB, CTES, SI (Holmgren et al. 1990). GenBank accession numbers are cited in this order: *trnL-F* and *rpl16* intron. \* denotes the chromosome count of the voucher specimen cited, followed by the corresponding reference.
- Axonopus anceps* (Mez) Hitchc.—Venezuela, Bolívar, Gran Sabana, Morrone O. et al. 4767 (SI), EU871111, EU871062.
- Chasmanthium latifolium* (Michx.) H.O. Yates.—Argentina, Buenos Aires, cultivated, *Giussani L. M.* 353 (SI), EU871113, EU871064.
- Paspalum arundinaceum* Poir.— [2n = 60 (Davidse and Pohl. 1974)]. Argentina, Facultad de Agronomía, cultivated from seeds CIAT # 16081, *Rua G.* 23963 (BAA), EU871121, EU871072; Venezuela, Miranda, Morrone O. et al. 4661 (SI), EU871120, EU871071. *Paspalum arundinellum* Mez— [2n = 40, 50 (Honfi et al., 1991; Honfi, 2003; Sede, Escobar, Morrone, and Zuloaga (unpublished data)]. Argentina, Corrientes, *Quarín C. L.* 4113 (CTES), EU871134, EU871085; 2n = 50\* (Honfi, 2003); Formosa, *Daviñaf. R. and A. I. Honfi* 479 (CTES), EU871135, EU871086, 2n = 40\* (Honfi, 2003); Misiones, *Zuloaga F. O. and Morrone O.* 6790 (SI), EU871115, EU871066. *Paspalum chaseanum* Parodi— [2n = 20, 40 (Espinoza and Quarín, 1997; Quarín, unpublished data)]. Bolivia, Santa Cruz, *Toledo C. S.* 13894 (CTES), EU871136, EU871087, 2n = 40\* (Quarín, unpublished data); Paraguay, Boquerón, *Fortunato R.* 8742 (BAB), EU871147, EU871098; *Paspalum commune* Lillo— [2n = 40 (Quarín, 1977; Hunziker et al., 1998; Zuloaga and Morrone, 2005; Morrone et al., 2006; Sede, Escobar, Morrone, and Zuloaga (unpublished data)]. Argentina, Tucumán, *Cialdella A. M. et al.* 35 (SI), EU871110, EU871061; Salta, *Zuloaga F. O.* 8480 (SI), EU871144, EU871095, 2n = 40\* (Sede, Escobar, Morrone, and Zuloaga, unpublished data). *Paspalum conspersum* Schrad.— [2n = 40, 60 (Quarín, 1977; Quarín and Hanna, 1980; Dandin and Chennaveeraiah, 1983; Honfi, et al. 1991; Adamowski et al., 2005; Zuloaga and Morrone, 2005); Genome type I<sub>1</sub>, J<sub>1</sub> (Burson, 1978). Argentina, Corrientes, *Quarín C. L.* 4292 (CTES), EU871118, EU871069. *Paspalum coryphaeum* Trin.— [2n = 20, 40, 60 (Davidse and Pohl, 1974; Burson, 1975; Honfi et al., 1991, Morrone et al., 1995; Adamowski et al., 2005)]. Brazil, Biri Biri, *Zuloaga F. O. s.n.* (SI), EU871145, EU871096; Serra do Caraça, *Longhi-Wagner H. M. et al.* 9654 (SI), EU871141, EU871092. *Paspalum dasytrichum* Dusén ex Swallen— Brazil, Paraná, *Morrone O. and Zuloaga F. O.* 5381 (SI), EU871117, EU871068. *Paspalum densum* Poir.— [2n = 20 (Caponio and Quarín, 1993); Genome type II (Caponio and Quarín, 1993)]. Bolivia, Santa Cruz, *Morrone O. and Giussani L. M.* 4298 (SI), EU871108, EU871059. *Paspalum distichum* L.— [2n = 20, 30, 40, 60, 61 (Davidse and Pohl, 1974; Quarín, 1977; Quarín and Burson, 1991; Honfi et al., 1991)]. Argentina, Entre Ríos, *Zuloaga F. O. and Morrone O.* 6935 (SI), EU871100, EU871051. *Paspalum durifolium* Mez— [2n = 40, 50, 60 (Burson, 1985, Quarín, 1994; Honfi, 2003); Genome types II, J<sub>2</sub>, XX (Burson, 1983; 1985)]. Argentina, Corrientes, *Quarín C. L.* 4110 (CTES), EU871140, EU871091, 2n = 50\* (Honfi, 2003). *Paspalum erianthum* Nees ex Trin.— [2n = 80 (Norrman et al., 1994b)]. Bolivia, Santa Cruz, *Morrone O. & Giussani L. M.* 4291 (SI), EU871116, EU871067. *Paspalum exaltatum* J. Presl— [2n = 20, 40 (Burson and Bennett, 1971; de Moraes Fernandes et al. 1974; Quarín, unpublished data)]. Argentina, Buenos Aires, *Giussani L. M. and Morrone O.* 321 (SI), EU871105, EU871056; Buenos Aires, Balcarce, *Laterra s.n.* (CTES), EU871127, EU871078, 2n = 20\* (Quarín, unpublished data). *Paspalum fimbriatum* Kunth— [2n = 20 (Davidse and Pohl, 1974)]. Mexico, Quintana Roo, *Morrone O. and Giussani L. M.* 3651 (SI), EU871114, EU871065. *Paspalum glabrinode* (Hack.) Morrone & Zuloaga— [2n = 20 (Morrone et al., 2006)]. Argentina, Misiones, *Zuloaga F. O. and O. Morrone* 6809 (SI), EU871106, EU871057, 2n = 20\*. *Paspalum haumanii* Parodi— [2n = 20, 40 (Burson, 1975; Norrmann et al., 1989); Genome type II (Quarín and Norrmann, 1990)]. Argentina, Buenos Aires, *Morrone O. s.n.* (SI), EU871102, EU871053; Santa Fe, *Quarín C. L.* 3854 (CTES), EU871129, EU871080, 2n = 20\* (Norrmann et al., 1989). *Paspalum inconstans* Chase— [2n = ca. 60, 60 (Sede, Escobar, Morrone, and Zuloaga, unpublished data; Morrone et al., 2006)]. Venezuela, Trujillo, *Morrone O. et al.* 4732 (SI), EU871123, EU871074. *Paspalum intermedium* Munro ex Morong & Britton— [2n = 20, 40 (Burson, 1978; Burson and Quarín, 1982; Honfi et al., 1991; Caponio and Quarín, 1993; Norrmann et al., 1989; 1994a; 1994b; Quarín, 1994); Genome type II (Burson, 1978)]. Argentina, Corrientes, *Zuloaga F. O. and Morrone O.* 7122 (SI), EU871103, EU871054; Chaco, *Schinini A.* 28857 (CTES), EU871130, EU871081, 2n = 20\* (Norrmann et al., 1989). *Paspalum macrophyllum* Kunth— [2n = 40, 60 (Davidse and Pohl, 1974; Sede, Escobar, Morrone, and Zuloaga (unpublished data)]. Venezuela, Táchira, *Morrone O. et al.* 4705 (SI), EU871122, EU871073, 2n = 40\* (Sede, Escobar, Morrone, and Zuloaga, unpublished data). *Paspalum malacophyllum* Trin.— [2n = 40 (Honfi et al., 1991; Norrmann et al., 1994b; Burson and Hussey, 1998; Hunziker et al., 1998), Genome type MM (Burson and Hussey, 1998)]. Argentina, Salta, *Morrone O. et al.* 3400 (SI), EU871099, EU871050. *Paspalum millegrana* Schard.— [2n = 40, 60 (Davidse and Pohl, 1972; Morrone et al., 1995)]. Mexico, Veracruz, *Zuloaga F. O. et al.* 7420 (SI), EU871109, EU871060. *Paspalum palustre* Mez — [2n = 20, 40 (Quarín and Burson, 1991; Martínez and Quarín, 1999)]. Argentina, Corrientes, *Bordón A.* 110 (CTES), EU871119, EU871070, 2n = 40\* (Quarín, unpublished data). *Paspalum plenum* Chase— [2n = 20, 40 (Davidse and Pohl, 1972; Norrmann et al., 1994b)]. Argentina, Salta, *Maruñak* 539 (CTES), EU871133, EU871084, 2n = 40\* (Quarín, unpublished data). *Paspalum plicatum* Michx.— [2n = 20, 40 (Davidse and Pohl, 1972; 1974; de Moraes Fernandes et al., 1974; Hunziker et al., 1998; Honfi et al., 1991; Norrmann et al., 1994b; Sede, Escobar, Morrone, and Zuloaga, unpublished data)]. Argentina, Salta, *Zuloaga et al.* 8477 (SI), EU871125, EU871076, 2n = 20\* (Sede, Escobar, Morrone, and Zuloaga, unpublished data). *Paspalum quadrifarium* Lam.— [2n = 20, 30, 40 (Quarín and Lombardo, 1986; Norrmann et al., 1989; Norrmann et al., 1994a; Speranza et al., 2003), Genome type II (Quarín and Norrmann, 1990)]. Argentina, Buenos Aires, *Giussani L. M. and Morrone O.* 320 (SI), EU871104, EU871055; *Morrone O. s.n.* (SI), EU871128, EU871079; Corrientes, *Quarín C. L.* 3810 (CTES), EU871131, EU871082, 2n = 20\* (Norrmann et al., 1989). *Paspalum quarinii* Morrone & Zuloaga— [2n = 20, 39, 40 (as *P. brunneum* in Burson, 1975; Norrmann et al., 1989; Honfi et al., 1991; Burson and Quarín, 1992), Genome type II (as *P. brunneum* in Quarín and Norrmann, 1990; Burson and Quarín, 1992)]. Argentina, Santa Fe, *Quarín C. L.* 3755 (CTES), EU871132, EU871083, 2n = 20\* (Norrmann et al., 1989). *Paspalum regnellii* Mez— [2n = 40 (Norrmann, 1981; Honfi et al., 1991)]. Argentina, Misiones, *Honfi A. I.* 130 (CTES), EU871139, EU871090; Brazil, Paraná, *Longhi Wagner H. M. et al.* 9507 (SI), EU871146, EU871097; *Longhi Wagner H. M. et al.* 9447 (SI), EU871143, EU871094. *Paspalum remotum* Remy— 2n = 40, 80 (Hunziker et al., 1998; Sede, Escobar, Morrone, and Zuloaga, unpublished data)]. 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*Zea mays* L.— X86563, AF133477.