

A preliminary approach to the phylogeny of the genus *Paspalum* (Poaceae)

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Abstract

The present work intends to clarify the phylogenetic relationships among the species of *Paspalum* L. belonging to the informal groups Notata/Linearia and Dilatata, and to raise some preliminary hypotheses on the phylogeny of the genus as a whole. A combined dataset including morphological and molecular characters was used to analyze 28 species of *Paspalum* plus some representatives of related genera of the tribe Paniceae. Analyses were performed using both parsimony and maximum likelihood. The monophyly of *Paspalum* is not supported nor contradicted. The circumscription of informal groups of *Paspalum* is discussed, as well as the cladistic treatment of allopolyploid taxa, especially those comprising the Dilatata group. The relationships of members of the Dilatata with their putative progenitors is confirmed, but the monophyly of the group as a whole is not. A close relationship between *P. dilatatum* Poir. and *P. lividum* Trin. ex Schtdl. is shown. Our analysis is consistent with the monophyly of a group comprising Notata + Linearia, with a monophyletic Notata group nested within it. The delimitation of the core Notata is proposed by including *P. conduplicatum* Canto-Dorow, Valls and Longhi-Wagner, *P. notatum* Flüggé, *P. minus* E. Fourn., *P. pumilum* Nees and *P. subciliatum* Chase.

Introduction

The genus *Paspalum* L. comprises about 330 species predominantly from the New World (Clayton & Renvoize, 1986). They are recognized by their plano-convex abaxial spikelets arranged in unilateral raceme-like partial inflorescences, and, with few exceptions, by the lack of the lower glume. *Paspalum*, a member of the tribe Paniceae, is one of the ten largest genera within Poaceae (Watson & Dallwitz, 1994).

Most *Paspalum* species have a chromosome base number $x = 10$, with a few cases reported of $x = 6$ and $x = 9$ (Jarret, Liu & Webster, 1998). Polyploidy is a very common feature among *Paspalum* species, and it is closely related to apomixis (Quarín & Norrmann, 1990); both processes seem to have had a very important evolutionary significance within *Paspalum*. The mean diploid 2CDNA value is 1.6 pg (two species of unknown ploidy, 1.2 and 2.1), and the variation in DNA contents among *Paspalum* species is approximately

3-fold as determined by laser flow cytometry (Jarret et al., 1994; Watson & Dallwitz, 1994).

Recent molecular analyses (Gómez-Martínez & Culham, 2000; Duvall, Noll & Minn, 2001; Giussani et al., 2001) of the tribe Paniceae and the subfamily Panicoideae show that *Paspalum* belongs to a clade characterized by a chromosome base number $x=10$ and is related to other genera having a NADP-ME photosynthetic pathway. Its more closely related taxa are *Anthaenantiopsis* Mez ex Pilg., *Thrasypsis* Parodi, and three species currently included in *Panicum* L. s.l.: *P. obtusum* Kunth, *P. validum* Mez, *P. tuerckheimii* Hack. (Gómez-Martínez & Culham, 2000; Giussani et al., 2001; Aliscioni et al., 2003; Denham & Zuloaga, in press). As currently circumscribed, *Paspalum* is paraphyletic by including *Thrasya* Kunth, a genus which obviously intergrades with the group Decumbentes of *Paspalum* (Burman, 1985; Denham & Zuloaga, in press).

On the basis of exomorphological characters, the genus was subdivided into subgenera, sections, and/or informal groups by different authors (Doell, 1877; Chase, 1929, Pilger, 1929, 1940). The more widely accepted infrageneric classification was proposed by Chase (1929, and unpublished manuscript) and originally comprised two subgenera and 27 informal groups. As a consequence of the problematic circumscription of several groups and the lack of informative characters to diagnose them, Chase's grouping was repeatedly revised and modified by subsequent workers (Parodi & Nicora, unpublished; Barreto, 1974; Cialdella, Morrone & Zuloaga, 1995; Morrone, Zuloaga & Carbonó, 1995; Canto-Dorow, Longhi-Wagner & Valls, 1996; Gomes & Monteiro, 1996; Morrone, Vega & Zuloaga, 1996; Morrone et al., 2000; Denham, Zuloaga & Morrone, 2002; Oliveira & Valls, 2002; Morrone, Denham & Zuloaga, 2004) and no uniform criteria were achieved.

Meanwhile, the informal grouping remains phylogenetically uninformative. The only available studies concerning phylogeny of *Paspalum* are morphology-based and comprise reduced datasets (Aliscioni, 2002) and/or very partial species sampling (Denham et al., 2002; Rua & Aliscioni, 2002; Denham and Zuloaga, in press). Besides these studies, there is poor information about the phylogenetic relationships among *Paspalum* species.

Some informal groups are well studied due to their economic value, as is the case of the groups Dilatata, Notata and Disticha. A large number of *Paspalum* species are valuable as forage grasses (Ramírez, 1954; Bennett, 1962; Allem & Valls, 1987; Filgueiras, 1992; Kretschmer, Kalmbacher & Wilson, 1994), the most important of them in southern South America being *P. dilatatum* Poir. and *P. notatum* Flüggé. The species to which more extensive phylogenetic investigation efforts were devoted is *Paspalum dilatatum*, 'dallisgrass' (Fernandes, Barreto & Salzano, 1968; Bennett, Burson & Bashaw, 1969; Burson, Lee & Bennett, 1973; Burson, 1983, 1991a, b, 1992a, b; Espinoza & Quarín, 2000). It comprises an array of sexual tetraploid and apomictic penta- and hexaploid biotypes, and belongs to the group Dilatata, which also includes other three allopolyploid species. Species of the groups Quadrifaria (*P. intermedium* Munro ex Morong) and Paniculata (*P. juergensii* Hackel) were hypothesized to be parents of this allopolyploid complex (Burson, Lee & Bennett, 1973; Burson, 1983; Espinoza & Quarín, 2000). *Paspalum notatum* belongs to the informal group Notata, which intergrades morphologically with the groups Linearia and Alma and with *P. equitans* Mez, a species currently included in the group Fasciculata. In spite of considerable efforts, the taxonomic boundaries of such groups remain uncertain (Barreto, 1957; Canto-Dorow, Longhi-Wagner & Valu 1996; Oliveira & Valls, 2002).

In our days, molecular data have had a deep impact on the field of plant systematics, and the application of DNA sequence data to phylogenetic problems is now routine. Molecular data have reshaped concepts of relationships and circumscriptions at all levels of the taxonomic hierarchy (Qiu et al., 1999; Soltis, Soltis & Chase, 1999; Small, Cronn & Wendel, 2004). Most molecular data applied to plant systematics come from two main sources: chloroplast DNA (cpDNA) and nuclear ribosomal DNA (rDNA). Molecular phylogenetic studies are not yet available to the genus *Paspalum*, the present work being the first one to present relationships among a set of *Paspalum* species on the base of ITS of the rDNA and *trnL* intron plus the *psbA-trnH* intergenic spacer of the cpDNA. The ITS (Internal Transcribed Spacers of the ribosomal nuclear DNA) have been used in many phylogenetic works to reveal relationships at infrageneric level (Baldwin, 1992;

Baldwin et al., 1995), including the family Poaceae (GPWG, 2001).

The goal of the present work is to clarify the phylogenetic relationships among the species of *Paspalum* belonging to the informal groups Notata/Linearia and Dilatata, and to raise some preliminary hypotheses on the phylogeny of the genus as a whole.

Materials and methods

Taxon sampling

A set of 28 species of *Paspalum* were included in our analysis (Table 1), including representatives of the informal groups Dilatata and Notata/Linearia as well as several additional, presumably related species belonging to different groups (Table 1). *Thrasya petrosa* (Trin.) Chase was also included, for the genus *Thrasya* was stated to be nested within *Paspalum* (see Introduction). Five representatives of the genus *Axonopus* P. Beauv. and *Steinchisma laxa* (Sw.) Zuloaga, all members of the $x = 10$ clade of Paniceae, were either simultaneous or alternatively used as outgroup taxa. The molecular data of *Steinchisma laxa* were obtained from Genbank (AY142733 for *trnL* intron and AY129711 for ITS rDNA). Voucher specimens are listed in Table 1, including origin, chromosome counts (when available for the species or the actual accessions used) and informal grouping so far suggested in the literature.

DNA extraction, sequencing, and alignment

DNA was extracted from dehydrated leaves, following a modified CTAB method (Doyle and Doyle, 1987). The *trnL* intron were amplified following (Taberlet et al., (1991)) and for rRNA ITS the amplification followed (Desfeux et al., (1996)), to amplify the *psbA-trnH* spacer (Sang, Crawford & Stuessy, 1997), the protocol was modified using an annealing temperature of 58°C.

The PCR amplified products were purified with shrimp alkaline phosphatase and exonuclease I (Amersham Biosciences) and directly sequenced using automated sequencers ABI Prism 310 (Perkin Elmer), ABI Prism 3100 (Applied Biosystems) and Mega Bace 1000 (Amersham-Pharmacia-Biotech), and their respective recommended kits.

The three fragments were sequenced for almost all species listed in Table 1. The sequences obtained were aligned with Clustal X 1.81 (Thompson et al., 1997) and manually refined. The gaps were treated as fifth state in all described analyses, although their treatment as missing data was also tried.

Morphological characters

Morphological characters were scored mainly from herbarium material at BAA, Buenos Aires, Argentina. A set of 63 characters was defined for use in the analyses (Table 2), including 45 characters of spikelets, 15 of inflorescences, and 3 of vegetative growth. Autapomorphies were not included. All but one (character 28) multistate characters were treated as non-additive. Polymorphic characters were scored as such, as recommended when the polarity of the characters is unknown from previous analyses (Kornet & Turner, 1999). Missing data (including unavailable as well as inapplicable data) represent 6% of the entries in the morphological data matrix.

Phylogenetic analysis

Three independent datasets were used for phylogenetic analyses: morphology, ITS (including ITS1 and ITS2, 508 characters) and cpDNA (including the *trnL* intron and the *psbA-trnH* spacer, 1218 characters). Three different approaches to data analysis were applied to the data matrices: Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). The analyses were performed with the softwares Mr. Bayes 3 (Huelsenbeck & Ronquist, 2001), PAUP* 4.0 (Swofford, 2002) and T. N. T. ver. 1.0 (Goloboff, Farris & Nixon, 2003), respectively.

MP analysis was applied both to separate and combined (total evidence analysis) datasets. A heuristic search strategy was adopted, consisting of 100 random addition sequences plus TBR swapping, using Wagner trees as starting trees and holding a maximum of 10 trees each time. The trees obtained were submitted to an additional round of TBR swapping. In order to detect possible islands, the trees obtained from the above-described analysis were further submitted to 1000 iterations of Parsimony Ratchet (Nixon, 1999). Branches with ambiguous support (min. length=0) were collapsed. Group support was

Table 1. Plant materials used for DNA sequencing in this analysis, including geographic origin, vouchers, genbank accessions, chromosome counts (when available), and suggested allocation of the *Paspalum* species into informal groups

Species	Informal groups according to the literature	Voucher specimen ^{a,b} and germplasm accession code (BRA)	County and Brazilian state (or locality in other countries)	Lat. (S)	Long. (W)	Alt. (m)	Genbank accessions (<i>psbA-trnH</i> , intron <i>trnL</i> , ITS1 and ITS2)	Chromosome number ^c (2n)
<i>Axonopus argentinus</i> Parodi	–	VTsReRm 14421, BRA-20958/002496	Bom Jardim da Serra, SC	27°38'	50°21'	800	AY769117 AY769150 AY771863 AY771896	n.a.
<i>A. brasiliensis</i> (Spreng.) Kuhlms.	–	VTsReRm 14422, BRA-20958/002500	Correia Pinto, SC	27°38'	50°21'	800	AY769118 AY769151 AY771864 AY771897	n.a.
<i>A. compressus</i> (Sw.) P. Beauv.	–	VTsReRm 14428, BRA-20958/002518	Capão Alto, SC	28°12'	50°45'	760	AY769119 AY769152 AY771866 AY771899	60
<i>A. polystachyus</i> G. A. Black	–	VTsReRm 14412, BRA-20958/002470	Correia Pinto, SC	27°38'	50°21'	800	AY769121 AY769154 AY771887 AY771920	60
<i>A. siccus</i> (Nees) Kuhlms.	–	VTsReRm 14413, BRA-20958/002488	Correia Pinto, SC	27°38'	50°21'	800	AY769120 AY769153 AY771890 AY771923	n.a.
<i>Paspalum acuminatum</i> Raddi	Dissecta, Acuminata	VlbDp 14270, BRA-20915/021229	São Gabriel, RS	30°17'	54°23'	250	AY769123 AY769155 AY771861 AY771894	40
<i>P. alnum</i> Chase	Notata, Alma	VTsDp 14231, BRA-20915/020991	São Borja, RS	28°39'	55°58'	120	AY769124 AY769156 AY771862 AY771895	12, 24 (as <i>P. hexastachyum</i> and <i>P. alnum</i>)

<i>P. conduplicatum</i> Canto-Dorow, Valls & Longhi-Wagner	Notata	VlbDp 14271 BRA-20915/021237	São Gabriel, RS	30°26'	54°17'	180	AY769126 AY769158 AY771867 AY771900	60*
<i>P. cromyorrhizon</i> Trin. ex Döll	Notata	VTsDp 14258, BRA-20915/021148	Uruguaiiana, RS	29°32'	56°49'	100	AY769127 AY769159 AY771868 AY771901	20, 40*
<i>P. dasyleurum</i> Kunze ex Desv.	Dilatata	V 12112, BRA-20915/020273	Pucón, IX Región, CHILE	n.a.	n.a.	n.a.	AY769128 AY769160 AY771869 AY771902	40*
<i>P. deteccae</i> Quarín	Linearia	Rc 300, BRA-20915/021610	Serranópolis, GO	18°10'	52°00'	570	AY769129 AY769161 AY771870 AY771903	20*, 40
<i>P. dilatatum</i> Poir. Common biotype	Dilatata	VGoHiCm 12030, BRA-20915/015903	Lages, SC	n.a.	n.a.	n.a.	AY769130 AY769162 AY771871 AY771904	50
<i>P. ellipticum</i> Döll	Linearia, Notata, Elliptica	VPoBi 9284, BRA-20915/008168	Itiquira, MT	n.a.	n.a.	n.a.	AY769131 AY769163 AY771872 AY771905	20, 80
<i>P. equitans</i> Mez	Fasciculata	VReMmSv 14581, BRA-20915/023451	Ponta Porã, MS	22°39'	55°37'	650	AY769132 AY769164 AY771873 AY771906	20
<i>P. filifolium</i> Nees ex Steud.	Linearia, Notata	VTsRcRm 14398, BRA-20915/022250	Bom Jardim da Serra, SC	28°23'	49°32'	1400	AY769133 AY769165 AY771874 AY771907	20
<i>P. flaccidum</i> Nees	Recta	V 14879, BRA-20915/024503	Unknown origin. Cultivated at Embrapa Cenargen.	n.a.	n.a.	n.a.	AY769134 AY769166 AY771875 AY771908	n.a.

Table 1. Continued.

Species	Informal groups according to the literature	Voucher specimen ^{ab} and germplasm accession code (BRA)	County and Brazilian state (or locality in other countries)	Lat. (S)	Long. (W)	Alt. (m)	Genbank accessions (<i>psbA-trnH</i> , intron <i>trnL</i> , ITS1 and ITS2)	Chromosome number ^c (2n)
<i>P. intermedium</i> Munro ex Morong & Britton	Quadrifaria	VQFdSv 11840, BRA-20915/012769	Navirai, MS	n.a.	n.a.	270	AY769135 AY769167 AY771876 AY771909	20*, 40
		VTsZi 14288, BRA-20915/021342	Capão da Canoa, RS	29°40'	49°59'	5	AY769136 AY769168 AY771877 AY771910	40*, 80
<i>P. juergensii</i> Hack.	Paniculata	VGoHiCm 11963, BRA-20915/015334	Lages, SC	27°48'	50°17'	830	AY769137 AY769169 AY771878 AY771911	20
<i>P. lineare</i> Trin.	Linearia	Rc 306, BRA-20915/021628	Mineiros, GO	17°35'	52°35'	650	AY769138 AY769170 AY771879 AY771912	20*, 40, 80
<i>P. lividum</i> Trin. ex Schtdl.	Livida	VTsDp 14254, BRA-20915/021113	Uruguaiiana, RS	29°52'	57°13'	100	AY769139 AY769171 AY771880 AY771913	40*
<i>P. maculosum</i> Trin.	Notata, Maculosa	VTsRcRm 14345, BRA-20915/021792	Triunfo, RS	29°45'	51°36'	100	AY769140 AY769172 AY771881 AY771914	20*, 40
<i>P. minus</i> E. Fourn.	Notata	VRcMmsV 14573, BRA-20915/023418	Ponta Porã, MS	22°30'	55°27'	600	AY769141 AY769173 AY771882 AY771915	50*
<i>P. notatum</i> Flüggé	Notata	VRcMmsV 14613, BRA-20915/023558	Japorã, MS	23°29'	53°59'	260	AY769142 AY769174 AY771883 AY771916	20, 40

<i>P. ovale</i> Nees ex Steud.	Linearia, Ovalia	VGoSv 11280, BRA-20915/013871	Ponta Grossa, PR	25°08'	50°09'	980	AY769175	70*
							AY771884	
<i>P. pallens</i> Swallen	Linearia	VSPmSv 13784, BRA-20915/020451	Santo Antonio do Leverger, MT	15°53'	56°02'	90	AY771917	20
							AY769143	
<i>P. pauciciliatum</i> (Parodi) Herter	Dilatata, Livida	VGoSv 11235, BRA-20915/013641	Ponta Grossa, PR	25°08'	50°09'	950	AY769176	40
							AY771885	
<i>P. pumilum</i> Nees	Notata	VTsZi 14280, BRA-20915/021270	Xangri-lá, RS	29°46'	50°02'	10	AY771918	20*
							AY769145	
<i>P. quarinii</i> Morrone & Zuloaga	Quadrifaria	VTsDp 14220, BRA-20915/020923	São Miguel das Missões, RS	28°24'	54°33'	300	AY771919	20*
							AY769178	
<i>P. ramboi</i> Barreto	Notata	VTsZi 14286, BRA-20915/021326	Capão da Canoa, RS	29°46'	50°02'	10	AY771888	60*
							AY769146	
<i>P. subciliatum</i> Chase	Notata	VReMmSv 14488, BRA-20915/022853	Acreúna, GO	17°24'	50°24'	550	AY769179	30, 40
							AY771889	
<i>P. urvillei</i> Steud.	Dilatata	VHiCm 12004, BRA-20915/015679	Alfredo Wagner, SC	27°42'	49°20'	613	AY771922	40*
							AY769147	
<i>P. vaginatum</i> Sw.	Disticha	VTsReRm 14340, BRA-20915/021741	Arroio do Sal, RS	29°28'	49°50'	5	AY769180	20*
							AY771891	
							AY769148	
							AY769181	
							AY771892	
							AY771925	
							AY769149	
							AY769182	
							AY771893	
							AY771926	

Table 1. Continued.

Species	Informal groups according to the literature	Voucher specimen ^{a,b} and germplasm accession code (BRA)	County and Brazilian state (or locality in other countries)	Lat. (S)	Long. (W)	Alt. (m)	Genbank accessions (<i>psbA-trnH</i> , intron <i>trnL</i> , ITS1 and ITS2)	Chromosome number ^c (2n)
<i>Steinchisma laxa</i> (Sw.) Zuloaga	–	n.a.	n.a.	n.a.	n.a.	n.a.	AY142733 AY129711	n.a.
<i>Thrasya petrosa</i> (Trin.) Chase	–	Gr GI, BAA24128	San Javier, Ñuflo de Chávez, Santa Cruz, Bolivia	n.a.	n.a.	n.a.	AY76912	n.a.

^aVouchers deposited at: BAA, Herbario Gaspar Suarez, Buenos Aires, Argentina (Bolivian specimen); CEN, Cenargen Herbarium, Brasilia, Brazil (Brazilian and Chilean specimens).

^bPlant collectors: Bi, L. Bianchetti; Cm, S.Cavalli-Molina; Dp, D.A.Palmieri; Fd, M.S.França Dantas; GI, G.I. Lavia Go, K.E.Gomes; Hi, M.C.M.Hickenbick; Ib, I. L.Barreto; K, Mm, M.D.Moraes; Pm, R.N.Pittman; Po, A.Pott; Q, C.L.Quarin; Rc, R.C.Oliveira; Rm, R.Miz; S, C.E.Simpson; Sv, G.P.Silva; Ts, T.Souza-Chies; V, J.F.M.Valls; Zi, F.Zilio.

^cSources of information on chromosome numbers (a few yet unpublished informed by J.F.M. Valls): Barreto (1983), Fernandes et al. (1974), Hickenbick et al. (1975), Honfi, Quarin and Valls (1990), Normann, Quarin and Killeen (1994), Pozzobon, Valls and Santos (2000), Rodrigues et al. (2001), Quarin (1974), Quarin and Caponio (1995) and Quarin and Normann (1987).

*Chromosome counts obtained from the same accessions used here.
n.a., not available.

Table 2. Morphological characters and state assignments used in the cladistic analysis

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1. Upper glume: not edge-forming [0], forming an edge around the lower lemma [1].
 2. Upper glume distal portion: rounded [0], obtuse [1], acute [2], acuminate [3].
 3. Upper glume apex: rounded [0], obtuse [1], apiculate [2], acute [3], acuminate [4], truncate [5].
 4. Upper glume dorsum: flat [0], bowed [1], rolled [2].
 5. Upper glume consistency: hyaline, tiny [0], membranous [1].
 6. Upper glume number of lateral veins on each glume half: none [0], one [1], two [2], three [3], four or more [4].
 7. Upper glume abaxial vein protrusion: not prominent [0], prominent [1].
 8. Upper glume distal convergence of inner lateral veins: not convergent [0], convergent [1].
 9. Upper glume vein distribution: more or less equidistant [0], lateral veins approximate towards the margins [1].
 10. Upper glume outer vein position: sub-marginal [0], not marginal [1].
 11. Upper glume marginal region: folded [0], flat [1].
 12. Upper glume between-vein indumentum: glabrous to scabrous [0], pubescent at the base [1], fully pubescent [2], pubescent towards the apex [3].
 13. Upper glume marginal region (beyond outer veins) indumentum: glabrous to scabrous [0], scarcely pubescent at base [1], pubescent [2], distally ciliate [3].
 14. Upper glume apex: navicular or cucullate [0], flat [1], folded but distally open [2].
 15. Upper glume surface: smooth [0], transversely crumpled or wrinkled [1].
 16. Upper glume whether “flabby” or not: tight [0], “flabby” [1].
 17. Upper glume symmetry: symmetrical [0], asymmetrical [1].
 18. Lower lemma distal portion: rounded [0], obtuse [1], acute [2], acuminate [3].
 19. Lower lemma apex: rounded [0], obtuse [1], apiculate [2], acute [3], acuminate [4], truncate [5].
 20. Lower lemma consistency: hyaline, tiny [0], membranous [1], laterally indurate [2], fully indurate [3].
 21. Lower lemma between-vein indumentum: glabrous [0], distally pubescent [1], basally pubescent [2], fully pubescent [3].
 22. Lower lemma number of lateral veins on each side: none [0], one [1], two [2], three [3], four or more [4].
 23. Lower lemma midvein: lacking [0], present [1].
 24. Lower lemma distal convergence of inner lateral veins: not convergent [0], convergent [1].
 25. Lower lemma marginal region: folded [0], flat [1].
 26. Lower lemma marginal region (beyond outer veins) indumentum: glabrous [0], pubescent [1].
 27. Lower lemma axillary flower: wanting [0], reduced to a palea [1], well developed [2].
 28. Upper floret pigmentation: whitish [0], stramineous [1], yellow/ochraceous [2], brown [3], dark brown, shining [4].
 29. Upper floret induration: not indurate [0], slightly indurate [1], strongly indurate [2].
 30. Upper floret length relative to the lower lemma: nearly equal [0], conspicuously shorter [1].
 31. Upper floret shape: elliptical [0], ovate [1], obovate [2], rhomboid [3], orbicular [4].
 32. Upper floret base: sessile [0], “pseudo-stipitate” [1].
 33. Upper floret lemma dorsum: more or less flattened [0], bowed towards the base [1], sharply bowed [2], gibbose [3], rolled, cylindrical [4].
 34. Upper floret palea dorsum: convex [0], flat [1], concave [2].
 35. Upper floret lemma apex: acute, pointed [0], acute but blunt at the very apex [1], rounded [2], acuminate [3], obtuse, blunt [4].
 36. Upper floret epidermal papillae on the lemma: wanting [0], present [1].
 37. Upper floret apex indumentum: wanting [0], present [1].
 38. Upper floret palea margins: not winged [0], with non-overlapping wings [1], with overlapping wings [2].
 39. Upper floret palea adaxial surface: smooth [0], papillose to prickled [1].
 40. Upper floret lemma apex: open [0], cucullate [1].
 41. Upper floret lemma margins: not thickened [0], slightly thickened [1], sharply thickened [2].
 42. Upper floret number of lateral veins on each lemma half: none [0], one [1], two [2], three or more [3].
 43. Upper floret midvein: lacking [0], present [1].
 44. Upper floret anther pigmentation: yellow [0], purple-tinged [1], deep purple [2], ferruginous [3].
 45. Pigmentation of stigmas: whitish [0], lila [1], purple [2], yellow [3].
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Table 2. Continued.

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46. Inflorescence terminal spikelet: wanting [0], present [1].
47. Inflorescence arrangement of primary branches: several branches along an axis with conspicuous internodia [0], several branches the two distal ones conjugate [1], only two conjugate primary branches [2], one branch alone [3].
48. Maximum number of orthostichies: distichous [0], polystichous [1].
49. Inflorescence pubescence on pulvinula: glabrous [0], shortly pubescent [1].
50. Inflorescence long cilia arising from pulvinula: wanting [0], present [1].
51. Inflorescence a spikelet ending each primary branch: present [0], lacking, lateral spikelets becoming rudimentary towards apex [1].
52. Inflorescence rachis cross section [0], trigonous [1], laterally expanded into narrow wings having chlorenchyma: laterally expanded into membranous epidermal wings [2].
53. Inflorescence rachis venation: a midnerve thick and prominent [0], several parallel equal-range nerves [1].
54. Inflorescence rachis margin: smooth [0], scabrous [1], with more or less scattered cilia [2], with a dense fringe of tuberculate cilia [3].
55. Inflorescence rachis surface indumentum: glabrous [0], scabrous [1], pubescent [2].
56. Inflorescence arrangement of spikelets: solitary [0], homogeneously paired [1], proximally paniculate, distally paired [2].
57. Inflorescence “pedicells” cross section: terete [0], trigonous/tetragonous [1], flattened [2].
58. Inflorescence setae on “pedicells”: wanting [0], present [1].
59. Inflorescence enrichment shoots: lacking [0], present [1].
60. Inflorescence enrichment branching pattern: paniculate [0], cymose [1], monophyadic [2].
61. Growth habit growth direction of rhizomes: orthotropous forming tussocks [0], shortly plagiotropous, culms bunched anyway [1], plainly plagiotropous [2].
62. Growth habit growth direction of the culm: orthotropous to geniculate [0], proximally plagiotropous [1], fully plagiotropous [2].
63. Leaf blade reduction in cross section: none leaf blade expanded throughout [0], proximally elliptical to semicircular [1], elliptical to semicircular throughout [2].
-

quantified through two measures: (1) the decay index of Bremer (DB, Bremer, 1994), and (2) the absolute Bootstrap Frequency (BF, Felsenstein, 1985), calculated from 1000 resampling iterations using Poisson independent re-weighting (Farris et al. in Horovitz, 1999). Morphological synapomorphies (MS) diagnosing each clade are reported when relevant.

The appropriate model of nucleotide substitution for ML and BI analyses were determined using the software Modeltest 3.06 (Posada & Crandall, 1998) using the LTR criterion. The models selected were: K80+G, to ITS matrix, HKY85+G, to chloroplast matrix, and TrN+I+G to the combined matrix. The BI was undertaken through 1,000,000 replications and ML analysis was undertaken through heuristic search, as-is option. Consistency, Retention, Rescaled Consistency and Homoplasy indexes were also calculated (respectively: CI, RI, RC, HI).

Results

Systematic relationships inferred from the combined analysis (total evidence)

The parsimony analysis of the combined data produces two equally parsimonious trees, each 1550 steps long (1206 if only informative characters are used). Searches using parsimony ratchet yielded no additional trees. In all trees (Figure 1) *Paspalum* results monophyletic (DB=5; BF=79). *Thrasya petrosa* appears as sister taxon of *Axonopus*, with low support.

Within *Paspalum*, three major clades are recovered in the strict consensus tree.

Clade I (DB=1; no bootstrap support; MS: characters 4, 6, 14, 22, 33, 44, and 54) links *P. flaccidum* Nees to a well-supported clade (DB=5; BF=83) formed by *P. juergensii* and *P. quarinii* Morrone and Zuloaga.

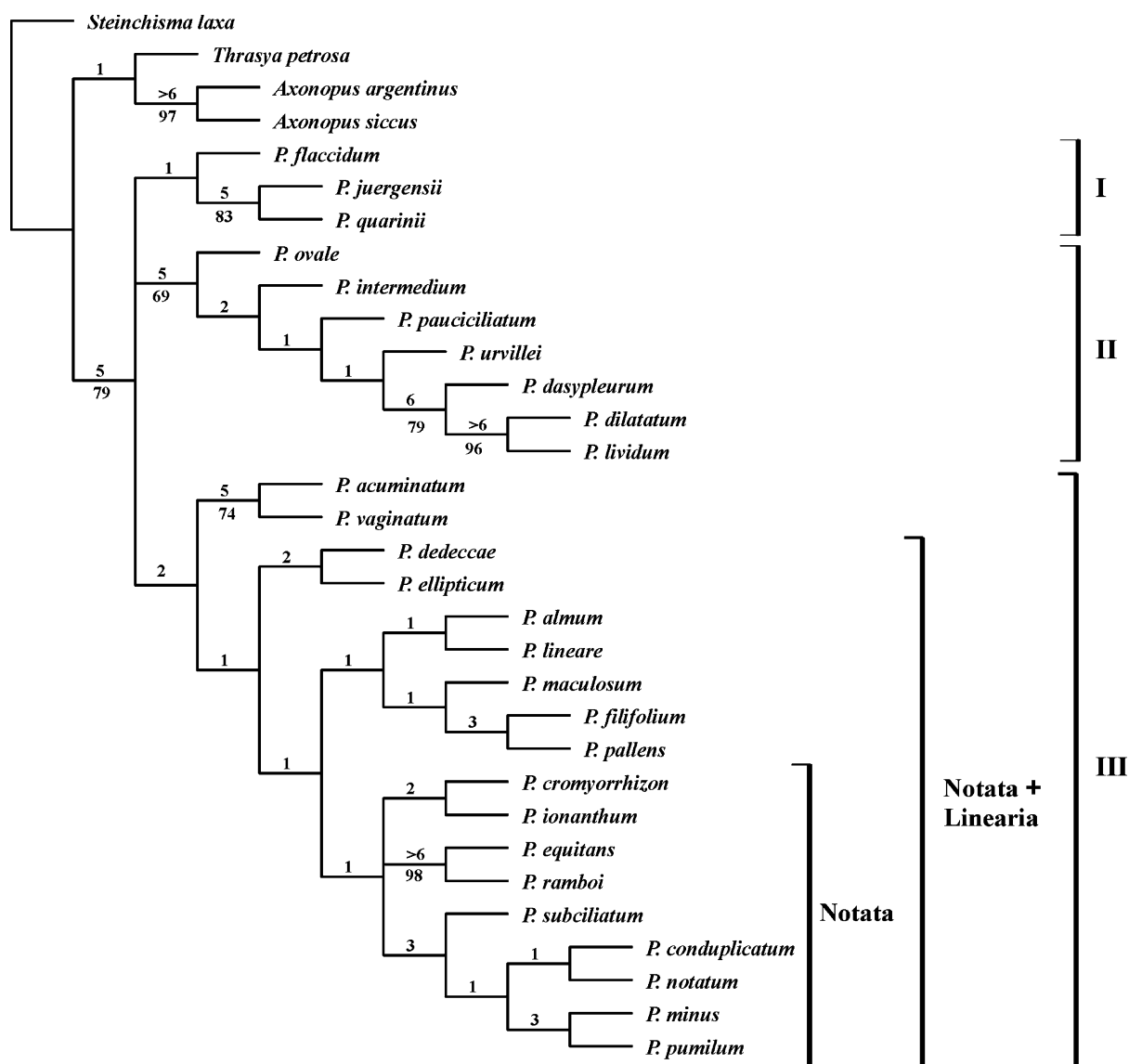


Figure 1. Strict consensus of 2 most parsimonious trees (length = 1550 steps) obtained from the combined (morphological, ITS, and cp-DNA) datasets. Figures above branches represent decay index of Bremer (BS), below branches are Bootstrap frequencies (BF). Square brackets indicate the clades discussed in the text.

Clade II (DB=5; BF=69; MS: ch 8, 11, and 41) includes the species of the Dilatata group, as well as *P. intermedium*, *P. lividum* Trin. ex Schldl., and *P. ovale* Nees ex Steud. Within this group, sister relationships of *P. dilatatum* with *P. lividum* (DB>6; BF=96; MS: ch 73 and 81) and of both with *P. dasypleurum* Kunze ex Desv. (DB=6; BF=79; MS: ch 41) are highly supported by the combined data.

Clade III (DB=2; no BF support; MS: ch 47, 56, and 57) includes two subclades, one of them (DB=5; BF=74; MS: ch 3, 30, 37, 53, 59, 62) formed by *P. acuminatum* Raddi and *P. vaginatum* Sw., the other one (DB=1; no BF support; MS: ch 39) containing all representatives of the Notata/Linearia group, including *P. alnum* Chase and *P. equitans*. Within the last clade, *P. dedecae* Quarín and *P. ellipticum* Döll are

linked together to form a moderately supported clade (DB=2; no bootstrap support; MS: ch 44 and 63) which is sister to another clade including the remaining species of the group (DB=1; no bootstrap support; MS: ch 35). They are further grouped into two clades with weak support, one of them (MS: ch 3) including *P. alnum*, *P. lineare* Trin., *P. maculosum* Trin., *P. filifolium* Nees ex Steud., and *P. pallens* Swallen, the other one (MS: ch 8, 16, 32, and 41) comprising most species currently assigned to the Notata group plus *P. equitans*; both clades are only supported by morphological characters. The phylogenetic relationship of *P. equitans* with *P. ramboi* Barreto is strongly supported (DB > 6; BF = 98; MS: ch 35).

Systematic relationships supported by separate datasets

The analysis of ITS data (without *Thrasya petrosa*, for which no ITS data has been available) also yielded a monophyletic *Paspalum* (DB > 6; BF = 99) with *Axonopus* as sister group (Figure 2). The Dilatata-clade (clade II) is supported (DB = 3; BF = 74) by the ITS analysis. The sister relationship of *P. juergensii* and *P. quarinii* is well supported by ITS data (DB = 2; BF = 79), as well as that of *P. equitans* and *P. ramboi* (DB = 4; BF = 96). This clade appears as sister group of *P. filifolium* (DB = 2; BF = 80), thus it contradicts the close relationship of this species with *P. pallens* recovered by the combined analysis.

The analysis of cpDNA data yields a poorly resolved consensus tree (not shown). The *Thrasya* + *Paspalum* clade is very strongly supported (DB > 6; BF = 100) by this dataset, but the monophyly of *Paspalum* itself is not supported (nor contradicted). The plastid DNA data contribute substantially to support the closest relationship of *P. dilatatum* and *P. lividum* (DB = 3; BF = 98).

The ML analysis of the combined molecular matrix yielded a general topology (Figure 3) similar to that illustrated in the Figure 1 based on parsimony. As expected, the Bayesian analysis yields a tree identical to that obtained by the ML method (not shown). The Notata/Linearia clade is recovered with minor alterations by the ML-analysis, as well as a subclade corresponding to the Notata group (*sensu* Canto-Dorow,

Longhi-Wagner & Valls 1996). *Paspalum pallens* is however not included in the Notata clade nor is sister of *P. filifolium*; it appears instead as sister taxon of *P. dedecae* + *P. lineare*. The position of *P. vaginatum* as sister of the remaining *Paspalum* species may be artificial because of its incomplete plastid sequences.

In the separate analysis of morphological data (not shown) *Paspalum* appears as a weakly supported clade (DB = 1; no bootstrap support; MS: characters 6, 16, 28, 29, 31, 40, 50, and 52), which is sister to either *Thrasya petrosa* or *T. petrosa* + *Axonopus siccus*. Within *Paspalum*, a poorly supported major clade is recovered which includes all terminals of clade II (cf. Figure 1) plus *P. ovale*, *P. intermedium* and *P. lividum*; the rest of species remain as an unresolved basal polytomy. Within the major clade, which was also highly unresolved, three small sub-clades were recovered by the analysis: *P. filifolium* + *P. pallens* (DB = 3; BF = 62, the only reasonably supported sub-clade), *P. conduplicatum* + *P. maculosum*, and *P. acuminatum* + *P. vaginatum*.

Finally, it is remarkable that the clade corresponding to the Notata/Linearia groups (clade III) is not individually supported by any separate dataset, and only becomes evident (although weakly supported) when the datasets are combined.

Discussion

Monophyly of the genus Paspalum

The monophyly of the genus *Paspalum* has been questioned on the basis of recent analyses based on molecular (Duvall et al., 2001; Giussani et al., 2001) and morphological (Denham & Zuloaga, in press) evidence. In all these analyses *Paspalum* is paraphyletic because *Thrasya* appears embedded within it. *Thrasya* seems to constitute a highly apomorphic group of species morphologically related to the informal group Decumbentes (*sensu* Chase, 1929) of *Paspalum* (Burman, 1985). The Decumbentes group is characterized by the frequent presence of a lower glume in at least one spikelet of each pair, the rare development of a male lower floret (both characters otherwise lacking in *Paspalum*), and the occurrence of a cymose-branched system of inflorescences at the axil of the 'flag leaf'.

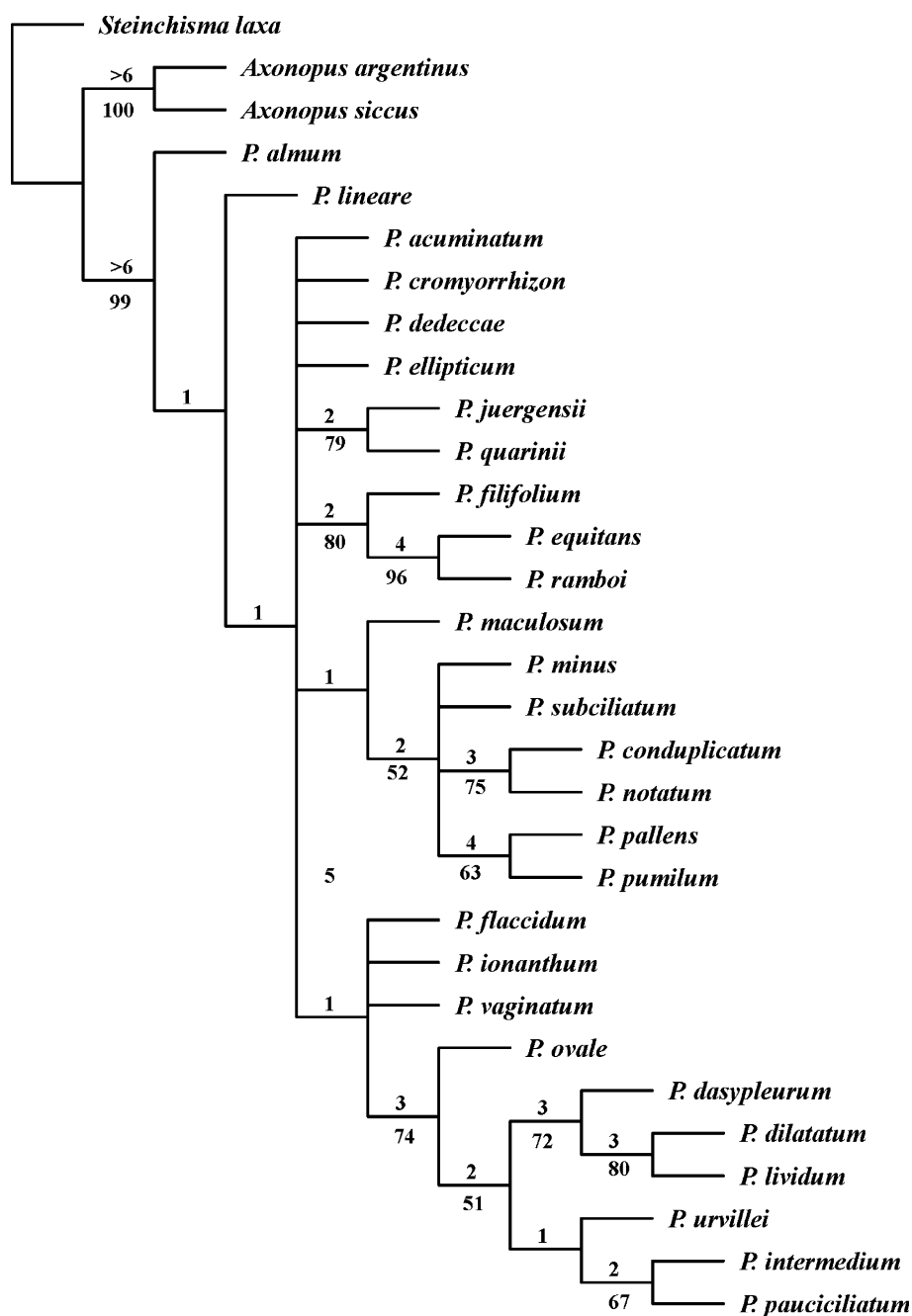


Figure 2. Strict consensus of 411 most parsimonious trees (length = 709 steps) obtained from the ITS data. Figures above branches represent decay index of Bremer (BS), below branches are Bootstrap frequencies (BF).

Our analysis included no species of the Decumbentes group and only one species of *Thrasya*. Thus, our taxon sampling does not make possible to test the hypothesis of paraphyly of *Paspalum*. The placement of *Thrasya* as sister taxon of *Paspalum* supported by the cpDNA data

is consistent with the analyses mentioned above, but the combined analysis suggests an alternative placement of *Thrasya* as sister of *Axonopus*. Thus, the question of whether the species of *Thrasya* are nested within *Paspalum* rendering it paraphyletic, or *Thrasya* plus the Decumbentes group form a

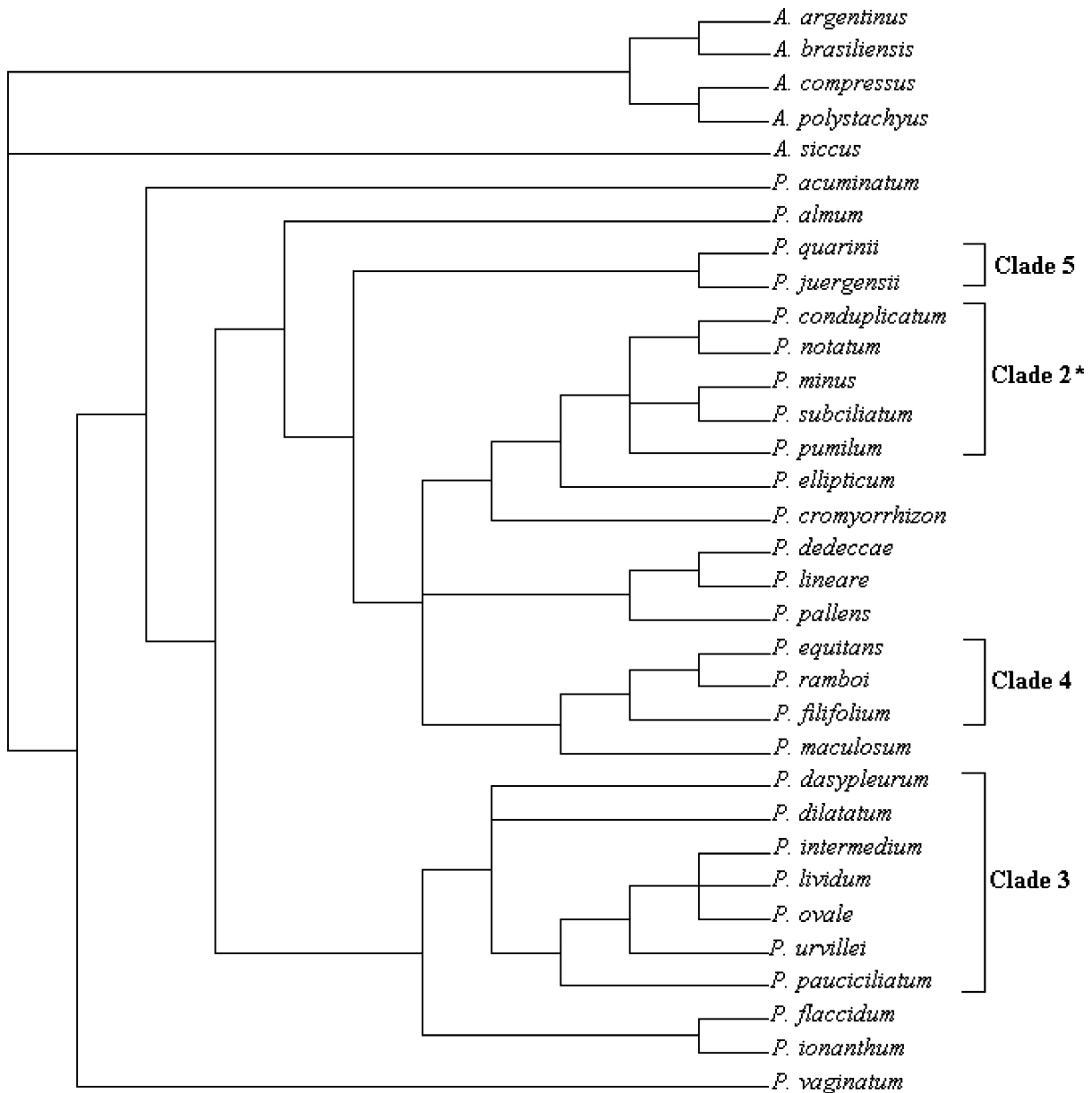


Figure 3. Maximum Likelihood tree for the combined datasets of *Paspalum* and outgroups. Model TrN+I+G.

clade to be excluded from a monophyletic *Paspalum* remains open.

The Notata/Linearia group

The groups Notata and Linearia comprise perennial species with two conjugate racemes and solitary spikelets (Chase, 1929). The affinity between both groups was discussed elsewhere

(Valls & Pozzobon, 1987; Oliveira & Valls, 2002). A common situation in *Paspalum* is that a given informal group consists of a set of 'core' species plus some 'peripheral' species which intergrade morphologically with other groups. After morphological analysis, the groups Notata and Linearia seem to agree with this pattern. Indeed, the limit between both groups is far from being clear (Oliveira & Valls, 2002).

Nevertheless, the trees yielded by the combined analysis of morphology, ITS, and cpDNA data here presented are consistent with a (weakly supported) monophyletic group (hereafter the ‘Notata/Linearia clade’) comprising all the Notata/Linearia species included in the analysis. This group is sister to the clade consisting of *P. acuminatum* and *P. vaginatum*, two species with subconjugate racemes and solitary spikelets, but plagiotropous growth of the culms, which are currently placed in groups Dissecta or Acuminata and Disticha, respectively. A clade corresponding almost exactly to the Notata group of most authors (hereafter the ‘Notata clade’) is nested within the Notata/Linearia, whereas the Linearia group remains paraphyletic. The delimitation of a “core” Notata group would closely link *P. conduplicatum*, Canto-Dorow, Valls and Longhi-Wagner, *P. notatum*, *P. minus* E. Fourn., *P. pumilum* Nees and *P. subciliatum* Chase.

Paspalum alnum, a species alternatively considered a member of the Notata (Barreto, 1957, 1974; Canto-Dorow, Longhi-Wagner & Valls 1996) or proposed as a distinct group, the Alma group (Parodi & Nicora, unpublished; Quarín, 1974), was placed in our analysis as sister to *P. lineare*, thus it is not consistent with its inclusion within Notata. *Paspalum alnum* differs from all other species of *Paspalum* by its unusual chromosome base number $x=6$. Its placement within *Paspalum*, and more generally within the $x=10$ clade of Paniceae, corroborates the hypothesis that such chromosome base number is autoapomorphic.

Paspalum maculosum, another species alternatively included in the Notata group (e.g. Chase, unpublished manuscript; Barreto, 1957, 1974; Parodi & Nicora, unpublished) or segregated, together with *P. serpentinum* Hochst., as the distinct group Maculosa (Judziewicz, 1990), resulted also placed outside the Notata clade, as sister of *P. filifolium*–*P. pallens*.

On the other hand, *P. equitans*, a species currently related to *P. fasciculatum* Willd. because of its flabellate inflorescences composed of several racemes (Chase, unpublished manuscript; Barreto, 1974), was consistently placed within the Notata clade and strongly supported as sister of *P. ramboi*. Remarkably, the close affinity between *P. equitans* and *P. ramboi* had been already pointed out by Barreto (1983), who indicated that both species share compression of basal leaf sheaths and

spikelet shape. Moreover, *P. equitans* strongly resembles other species of the Notata/Linearia group, e.g. *P. ionanthum* Chase, because of their glabrous, solitary spikelets, and their closely packed orthotropous culms. On the other hand, more than two racemes scattered along the inflorescence axis occasionally occur in other species of the Notata group, as *P. ramboi*, *P. notatum*, *P. minus*, and *P. pumilum*. Quarín and Norrmann (1987) report a high degree of meiotic chromosome pairing in hybrids of *P. equitans* \times *P. cromyorrhizon* Trin. ex Döll, indicating they share the same N genome of *P. notatum*, in spite of their full F_1 sterility.

The phylogenetic relationships of the Recta group, here represented by *P. flaccidum*, with the Notata/Linearia is not supported by our data. Its proper placement must be further explored.

The Dilatata group

A cladistic treatment of the species belonging to the Dilatata group is especially problematic because they are allopolyploids (Burson, 1991a, Souza-Chies & Cavalli-Molina, 1995, Casa et al., 2002, Machado et al., 2005), thus their evolutionary history includes at least a reticulation event.

This informal group was subject of interest since the 1950s (Bashaw & Forbes, 1958; Bashaw & Holt, 1958), particularly regarding the origin of the common dallisgrass. Previous cytogenetic studies (Burson et al., 1973; Burson, 1983, Caponio & Quarín, 1990) suggested that *P. juergensii* and *P. intermedium* (or related species belonging to groups Paniculata and Quadrifaria, respectively) can be progenitors of the tetraploid *P. dilatatum*, but the genomic formula of pentaploid and hexaploid biotypes includes a third genome. In spite of considerable research effort, the affinities of this genome remain obscure. Additionally, *P. lividum* was hypothesized to have contributed to the genomic formula of *P. pauciciliatum* (Parodi) Herter, another species of the Dilatata group (Valls, 2000).

The inclusion in a cladistic analysis of taxa of known hybrid origin can be questioned in several ways (Bachmann, 2000; Chase et al., 2003). Nevertheless, we included representatives of the Dilatata group in the present analysis with the purpose of testing the monophyly of the group as a whole, i.e. the diversification of the group after a unique

hybridization event, as well as their relationships with the putative parental species.

Our ITS and combined analyses place the species of the Dilatata group within a clade including also *P. intermedium*, *P. lividum*, and *P. ovale*. However, separate analysis of cpDNA data does not support this placement, but suggests some species of the Dilatata to be placed near *P. juergensii* and *P. quarinii*. These results are consistent with the hypothesis that the allopolyploid Dilatata group is paternally linked with *P. intermedium* (or a related species), and maternally linked to *P. juergensii* (or a related species). This is also consistent with the fact that ITS sequences of hybrids are typically homogenized by concerted evolution (Small et al., 2004) and tend to evolve through conversion to one of the parental copies (Bachmann, 2000), usually the paternal one.

Surprisingly, *P. dilatatum* and *P. lividum* form a clade strongly supported both by ITS and cpDNA datasets. This clade maintains its identity independently of the alternative placements of the species of Dilatata by the different datasets. Such relationship deserves further investigation, including a more comprehensive dataset.

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