

# Phylogeny of New World *Paspalum* (Poaceae, Panicoideae, Paspaleae) based on plastid and nuclear markers

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**Abstract** Phylogenetic analyses of 131 terminals of *Paspalum* and related genera, based on both plastid and nuclear markers, were performed under maximum parsimony and Bayesian methods. The total evidence analyses generated a hypothesis showing that *Paspalum* would be monophyletic if *Spheneria*, *Thrasypopsis* and *Reimarochloa* are included within the genus. *Paspalum inaequivalve* and *P. microstachyum*, two species of the Inaequivalvia group were related to genus *Anthaeantiopsis*, excluded from *Paspalum*, or nested within it by plastid and nuclear markers, respectively. Subgenera *Anachyris* and *Harpostachys* were partially recovered as monophyletic assemblages, while subg. *Ceresia* and *Paspalum* resolved as polyphyletic. Within subgenus *Paspalum*, some informal groups were recovered as monophyletic, while others were resolved as paraphyletic or polyphyletic. Phylogenetic relationships among species of *Paspalum* were partially recovered possibly due to reticulation events among species, autopolyploidization and apomixis; all these processes being common in *Paspalum*, thus obscuring the infragenetic classification.

**Keywords** *Paspalum* · Panicoideae · Poaceae ·  
Phylogeny · Classification

## Introduction

*Paspalum* L. belongs to the grass subfamily Panicoideae, and currently includes nearly 350 species (Zuloaga and Morrone 2005; Rua et al. 2010); most of its species are American, mainly distributed in tropical and subtropical areas, with a few representatives in the Old World (Clayton and Renvoize 1986). The genus is characterized by having racemose, unilateral inflorescences, with spikelets solitary or paired, with the lower glume usually absent, upper glume and lower lemma herbaceous to membranous, the lower palea and lower flower usually absent, and upper antheridium indurate. Physiologically, all species are C<sub>4</sub> of the NADP-ME subtype (Brown 1977), whereas anatomically they belong to the MS type (Hattersley and Watson 1976; Brown 1977).

Most of the species of *Paspalum* have a basic chromosome number of  $x = 10$  (Quarín 1992; Quarín and Burson 1991; Adamowski et al. 2005; Morrone et al. 2006; Pozzobon et al. 2008; Hojsgaard et al. 2009; Sede et al. 2010). Exceptions to this number have been reported: species with  $x = 6$  were reported for *Paspalum alnum* Chase (Quarín 1974), the tetraploid *P. filgueirasi* Morrone and Zuloaga with  $2n = 24$ , and *P. burmanii* Filg., Morrone and Zuloaga an octoploid with  $2n = 48$  (Peñaloza et al. 2008); with  $x = 9$ : *P. lanciflorum* Pilg. (Davidse and Pohl 1974), *P. reduncum* Nees ex Steud. ( $2n = 18$ ), and two tetraploids: *P. ammodes* Trin. ( $2n = 36$ ), and *P. trachycoleon* Steud. ( $2n = 36$ ) (Peñaloza et al. 2008); and with  $x = 16$ : *P. stellatum* Humb. and Bonpl. ex Flügge (Honfi et al. 1990; Pozzobon et al. 2000, and *P. convexum* Humb. and Bonpl. ex Flügge with  $2n = 32$  (Selva 1976; Reeder 1984). The  $x = 10$  basic number is the plesiomorphic state for *Paspalum*, while unusual basic chromosome numbers would be derived states originated by fusion of entire chromosomes or chromosome rearrangements.

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Polyploidy is a common feature in *Paspalum*, being present in nearly 80 % of the species (see Table 1). Among all ploidy levels, tetraploidy is the most frequent condition (ca. 50 %), while most of these tetraploids are apomictic (Quarín 1992; Quarín et al. 2001), an asexual mode of reproduction by seeds. Ployploidy and apomixis are highly associated, since the chances of establishment of new polyploids increase when they present asexual reproduction (Stebbins 1971). In this regard, apomixis plays an important role both in restoring the fertility of allopolyploids (Stebbins 1941; Nogler 1984; Darlington 1939) and in the evolution of agamic complexes in autopolyploids (de Wet and Harlan 1970; Quarín and Norrmann 1987; Norrmann et al. 1989; Quarín 1992; Siena et al. 2008; Ortiz et al. 2013). Most tetraploid species in *Paspalum* are autopolyploids, mainly derived through the fertilization of unreduced gametes (Forbes and Burton 1961; Norrmann et al. 1989; Quarín 1992; Quarín et al. 1998; Stein et al. 2004). The morphological similarity among diploid and tetraploid cytotypes in *Paspalum* and the presence of multivalent chromosome associations in meiosis are consistent with the probable origin of polyploids via autopolyploidy (Quarín and Lombardo 1986; Caponio and Quarín 1987; Quarín and Norrmann 1987; Norrmann et al. 1989; Honfi et al. 1990; Quarín 1992; Quarín et al. 1996, 1998; Hojsgaard et al. 2009).

Recent molecular studies placed *Paspalum* within tribe Paspaleae (Morrone et al. 2012), together with other American genera which share a basic chromosome number of  $x = 10$ ; these genera were previously defined as the  $x = 10$  Paniceae clade (Gómez-Martínez and Culham 2000; Giussani et al. 2001; Duvall et al. 2001; Aliscioni et al. 2003). Morrone et al. (2012) treated *Paspalum* as a monophyletic genus if *Thrasya* Kunth and *Thrasypopsis* Parodi are included in the genus, in agreement with previous results by Giussani et al. (2001), Aliscioni et al. (2003), Denham (2005) and Rua et al. (2010). Additionally, Morrone et al. (2012) stressed that *Reimarochloa* Hitchc. and *Spheneria* Kuhl. should also be treated within the boundaries of *Paspalum* to keep the genus monophyletic.

An infrageneric delimitation of *Paspalum* has been a difficult task, due to the absence of definite morphological characters to define subgenera and other infrageneric categories, i.e., sections or even informal groups (Chase 1929, 1939, unpubl.), in a revision of 140 North American species of the genus, divided *Paspalum* in two subgenera: *Ceresia* (Pers.) Rchb. and *Paspalum*, recognizing within the latter 25 informal groups. Zuloaga and Morrone (2005) analyzed 129 Austral South American species of the genus and accepted three subgenera: *Anachyris* (Nees) Chase, including six species; *Ceresia*, with 21 species, and *Paspalum* with 102 species gathered in 28 informal groups. Denham (2005) included in subgenus *Harpostachys* (Trin.)

**Table 1** Ploidy levels known of *Paspalum* species included in the study

Exclusive diploid species	<b>2x:</b> <i>P. bertonii</i> , <i>P. buchtienii</i> , <i>P. chaseanum</i> , <i>P. chacoense</i> , <i>P. decumbens</i> , <i>P. equitans</i> , <i>P. fimbriatum</i> , <i>P. gardnerianum</i> , <i>P. glabrinode</i> , <i>P. heterotrichon</i> , <i>P. juergensii</i> , <i>P. indecorum</i> , <i>P. malmeanum</i> , <i>P. microstachyum</i> , <i>P. multicaule</i> , <i>P. pallens</i> , <i>P. palustre</i> , <i>P. parviflorum</i> , <i>P. procurrens</i> , <i>P. punilum</i> , <i>P. repens</i> , <i>P. rupestre</i> , <i>P. saccharoides</i> , <i>P. umbrosum</i>
Diploid and polyploid species	<b>2x, 4x:</b> <i>P. alnum</i> , <i>P. atratum</i> , <i>P. caespitosum</i> , <i>P. denticulatum</i> , <i>P. haumanii</i> , <i>P. humboldtianum</i> , <i>P. maculosum</i> , <i>P. malacophyllum</i> , <i>P. modestum</i> , <i>P. orbiculatum</i> , <i>P. regnellii</i> , <i>P. robustum</i> , <i>P. rufum</i> , <i>P. stellatum</i> <b>2x, 8x:</b> <i>P. pilosum</i> <b>2x, 3x, 4x:</b> <i>P. intermedium</i> , <i>P. notatum</i> , <i>P. simplex</i> <b>2x, 4x, 6x:</b> <i>P. candidum</i> , <i>P. conjugatum</i> , <i>P. coryphaeum</i> , <i>P. paniculatum</i> , <i>P. pectinatum</i> , <i>P. plicatum</i> , <i>P. prostratum</i> , <i>P. vaginatum</i> <b>2x, 4x, 5x, 6x:</b> <i>P. minus</i> , <i>P. orbiculare</i> <b>2x, 3x, 4x, 5x, 6x:</b> <i>P. quadrifarium</i> <b>2x, 3x, 4x, 5x, 6x, 12x:</b> <i>P. distichum</i>
Exclusive Polyploid Species	<b>4x:</b> <i>P. acuminatum</i> , <i>P. boscianum</i> , <i>P. campilostachyum</i> , <i>P. commune</i> , <i>P. dasyleurum</i> , <i>P. maritimum</i> , <i>P. pauciciliatum</i> , <i>P. polyphyllum</i> , <i>P. squamulatum</i> , <i>P. unispicatum</i> , <i>P. usteri</i> <b>6x:</b> <i>P. inaequivalve</i> , <i>P. inconstans</i> , <i>P. pubiflorum</i> <b>8x:</b> <i>P. erianthum</i> , <i>P. ovale</i> <b>4x, 5x:</b> <i>P. arundinellum</i> <b>4x, 6x:</b> <i>P. ceresia</i> , <i>P. macrophyllum</i> , <i>P. proliferum</i> , <i>P. virgatum</i> <b>4x, 5x, 6x:</b> <i>P. durifolium</i> <b>4x, 8x:</b> <i>P. ionanthum</i> , <i>P. lineare</i> , <i>P. remotum</i>

Data were obtained from Honfi (2003), Morrone et al. (2006), Pozzobon et al. (2008), Hojsgaard et al. (2009), and Sede et al. (2010)

S. Denham all species of *Thrasya* and those species of the Decumbentes group. A total of nearly 39 informal groups belonging to subgenus *Paspalum* were described in these previous treatments.

Partial phylogenetic studies of *Paspalum* based on morphological characters have been published (Aliscioni 2002; Rua and Aliscioni 2002; Denham et al. 2002, 2010; Denham and Zuloaga 2007); nevertheless, this type of evidence has shed little light in reconstructing the phylogeny of the genus. On the other hand, molecular studies of informal groups were carried out by Vaio et al. (2005), Souza-Chies et al. (2006), Essi and Souza-Chies (2007), and Giussani et al. (2009). Rua et al. (2010) performed a phylogenetic reconstruction of 71 species of *Paspalum* based on four plastid markers (*trnL* intron,

*trnL-trnF* spacer, *atpB-rbcL* spacer, and the *trnG* intron). In order to overcome the difficulty to recognize entities at the infrageneric level, due to the presence of apomictic-polyploid cytotypes, these authors also included a combined molecular and morphological analysis on a subset of 43 diploid taxa; consequently, most infrageneric categories were moderately represented in Rua et al. (2010).

A comprehensive phylogenetic study with an enlarged sampling of all informal groups and subgenera of *Paspalum*, considering also nuclear markers, is still lacking. In our study, a phylogeny of the American *Paspalum*, based on three different plastid markers (*ndhF*, *rpoA*, *rpl16*) and the nuclear *ETS*, was reconstructed. A total of 109 ingroup taxa, including diploid and polyploid species of *Paspalum* subg. *Anachyris*, *Ceresia*, *Harpostachys*, and *Paspalum* were sampled; 30 of the approximately 39 informal groups of the latter subgenus were represented. The monophyly of *Paspalum* was tested with the inclusion of related genera, accounting for a total of 131 terminals. The monophyly of subgenera and informal groups was herein discussed and related to morphological, cytological and anatomical evidences.

## Materials and methods

### Taxon sampling

A total of 115 specimens of American *Paspalum* were sampled, of which 109 are different species. We analyzed representative specimens for all different subgenera and most informal groups. We also included in the ingroup: *Reimarochloa acuta* (Flüggé) Hitchc., *Spheneria kegelii* (Müll. Hal.) Pilg., *Thrasypopsis juergensii* (Hack.) Soderstr. ex A.G. Burm. and *T. repanda* (Nees) Parodi (see Scatagliini et al. 2007; Morrone et al. 2012; Rua et al. 2010) to test the monophyly of *Paspalum*. In addition, a selection of 12 species belonging to nine closely related genera were included as outgroup taxa based on Aliscioni et al. (2003): *Anthaenantiopsis rojasiana* Parodi, *Axonopus anceps* (Mez) Hitchc., *Echinolaena inflexa* (Poir.) Chase, *Hopia obtusa* (Kunth) Zuloaga and Morrone, *Ichnanthus pallens* (Sw.) Munro ex Benth., *Ophiochloa hydrolithica* Filg., Davidse and Zuloaga, *Ocellochloa chapadensis* (Swallen) Zuloaga and Morrone, *O. piauiensis* (Swallen) Zuloaga and Morrone, *O. stolonifera* (Poir.) Zuloaga and Morrone, *Panicum tuerckheimii* Hack., *P. validum* Mez, and *Strepstachys asperifolia* Desv.; the latter species was used to root the trees.

Detailed information for the 131 samples included voucher information and accession numbers of the new sequences obtained for this work, and of those available in

GenBank are given in Table 2. Voucher specimens are deposited at SI, San Isidro, Buenos Aires, Argentina.

### DNA amplification and sequencing

Most plants were field collected and dried in silica gel, while the remaining samples were obtained from herbarium leaves. Total genomic DNA was extracted using modified CTAB protocols from Doyle and Doyle (1987). Three plastid regions (*rpl16*, *ndhF*, *rpoA*) and the nuclear marker *ETS* were amplified and sequenced for each taxon. Regions with the lowest divergence (*ndhF* and *rpoA*) were selected to resolve the ancestral cladogenetic events, while regions with the greatest divergence (*rpl16* and *ETS*) resolved the more recent ones.

The 3' region of the external transcribed spacer of 18S-26S nuclear ribosomal DNA (*ETS*) was amplified using the primers proposed by Baldwin and Markos (1998).

The *rpl16* intron (approx. 1,400 bp) was amplified using primers RP71F (Jordan et al. 1996) and RP1661R (Kelchner and Clark 1997). In some cases, amplifying the entire region in one reaction was inefficient, so two internal primers RP584F and RP584R designed by Giussani et al. (2009) were also used.

An approximately 1,400 bp fragment containing the entire *rpoA* gene, the 3'-end of the *petD*-encoding subunit IV of the cytochrome b complex, the 3'-end of the *rps11*-encoding ribosomal protein S11 and the two intergenic spacers (IGS) between *rpoA* and *petD* and between *rpoA* and *rps11* were amplified using two pairs of primers RPOA1-RPOA6 and RPOA5-RPOA2 designed by Petersen and Seberg (1997).

Finally, the complete *ndhF* gene, coding NADH dehydrogenase subunit F (approx. 2,100 bp), was amplified in three fragments using the following pairs of primers as specified by Olmstead and Sweere (1994), Clark et al. (1995) and Aliscioni et al. (2003): 5F-972R, 972F-1666R and 1666F-2110R.

*NdhF* outgroup sequences were obtained from Giussani et al. (2001) and Aliscioni et al. (2003) and the same vouchers were used to amplify and sequence the *rpl16*, *rpoA* and *ETS* fragments. Additional 23 *Paspalum* sequences of the *rpl16* were obtained from Giussani et al. (2009), using the same vouchers to amplify *ndhF*, *rpoA* and *ETS* fragments for the present work (Table 2).

PCR reactions were performed in a 25 µl final volume with 50–100 ng of template DNA, 0.2 µM of each primer, 25 mM of each dNTPs, 5 mM MgCl<sub>2</sub>, 1× buffer and 1.5 units of *Taq* polymerase provided by Invitrogen (Brazil). The reaction conditions were: a first period of denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 1 min, and extension at 72 °C for 1 min; a final extension at 72 °C for

**Table 2** List of the 131 terminals analyzed. Infrageneric classification, voucher specimens and Genbank accession numbers are indicated for each taxa

Species	Country of origin	Voucher	Subgenera	Groups	<i>rpoA</i>	<i>ndhF</i>	<i>rpl16</i>	<i>ETS</i>
<i>P. malacophyllum</i> Trin.	Argentina	Morrone 3400	Anachyris		KF810528	AY029671*	EU871050***	KF852783
<i>P. malacophyllum</i>	Brazil	Valls 14411	Anachyris		KF810582	KF852944	KF853044	KF852835
<i>P. procurrens</i> Quarin	Argentina	Quarin 4060	Anachyris		KF810643	KF852996	KF853100	–
<i>P. simplex</i> Morong	Argentina	Morrone 5311	Anachyris		KF810619	KF852974	KF853082	KF852872
<i>P. usteri</i> Hack.	Brazil	Longhi 9614	Anachyris		KF810581	KF852943	KF853043	KF852834
<i>P. volcanense</i> Zuloaga, Morrone & S. Denham	Argentina	Morrone 4374	Anachyris		KF810593	KF852955	KF853055	KF852845
<i>P. biaristatum</i> Filg. & Davidse	Brazil	Rua 636	Ceresia		KF810567	KF852929	KF853031	KF852821
<i>P. buchtienii</i> Hack.	Bolivia	Morrone 4201	Ceresia		KF810642	KF852995	KF853099	–
<i>P. ceresia</i> (Kuntze) Chase	Argentina	Morrone 4386	Ceresia		KF810578	KF852940	KF853040	KF852831
<i>P. cordatum</i> Hack.	Brazil	Longhi-Wagner 9441	Ceresia		KF810577	KF852939	KF853039	KF852830
<i>P. eucomum</i> Nees ex Trin.	Brazil	Longhi-Wagner 9599	Ceresia		KF810616	KF852971	KF853079	KF852869
<i>P. heterotrichon</i> Trin.	Venezuela	Morrone 4739	Ceresia		KF810587	KF852949	KF853049	KF852840
<i>P. humboldtianum</i> Flügge	Argentina	Morrone 5165	Ceresia		KF810618	KF852973	KF853081	KF852871
<i>P. lanciflorum</i> Trin.	Venezuela	Morrone 4760	Ceresia		KF810588	KF852950	KF853050	KF852841
<i>P. longiaristatum</i> Davidse & Filg.	Brazil	Rua 825	Ceresia		KF810566	KF852928	KF853030	KF852820
<i>P. malmeanum</i> Ekman	Bolivia	Morrone 4491b	Ceresia		KF810586	KF852948	KF853048	KF852839
<i>P. pectinatum</i> Nees ex Trin.	Venezuela	Morrone 5375a	Ceresia		KF810641	KF852994	KF853098	–
<i>P. petilum</i> Chase	Venezuela	Morrone 4783	Ceresia		KF810591	KF852953	KF853053	KF852844
<i>P. petrense</i> A.G. Burm.	Brazil	Filgueiras 3510	Ceresia		KF810617	KF852972	KF853080	KF852870
<i>P. polyphyllum</i> Nees ex Trin.	Brazil	Longhi-Wagner 9418	Ceresia		KF810583	KF852945	KF853045	KF852836
<i>P. stellatum</i> Humb. & Bonpl. ex Flügge	Venezuela	Morrone 4745	Ceresia		KF810564	KF852926	KF853028	KF852818
<i>P. alstonii</i> Chase	Venezuela	Morrone 4798	Harpostachys		KF810523	KF852893	KF853004	KF852779
<i>P. campylostachyum</i> (Hack.) S. Denham	Venezuela	Morrone 4702	Harpostachys		KF810590	KF852952	KF853052	KF852843
<i>P. cinerascens</i> (Döll) A.G. Burm. & C.N. Bastos	Paraguay	Zuloaga 7305	Harpostachys		KF810522	KF852997	KF853003	KF852778
<i>P. decumbens</i> Sw.	Bolivia	Morrone 4231	Harpostachys		KF810624	KF852979	KF853086	KF852877
<i>P. foliiforme</i> S. Denham	Venezuela	Morrone 4669	Harpostachys		KF810520	AY029690* Filgueiras 3496	KF853002	KF852776
<i>P. glaziovii</i> (A. G. Burm.) S. Denham	Brazil	Filgueiras 3482	Harpostachys		KF810637	AY029689*	–	KF852890
<i>P. inconstans</i> Chase	Venezuela	Morrone 4738	Harpostachys		KF810521	KF853101	EU871074***	KF852777
<i>P. pilosum</i> Lam.	Venezuela	Morrone 4778	Harpostachys		KF810632	KF852987	KF853093	KF852885
<i>P. procerum</i> S. Denham	Venezuela	Morrone 4740	Harpostachys		KF810589	KF852951	KF853051	KF852842
<i>P. robustum</i> S. Denham	Venezuela	Morrone 4787	Harpostachys		KF810599	KF852961	KF853061	KF852851
<i>P. thrasyoides</i> (Trin.) S. Denham	Brazil	Rua 654	Harpostachys		KF810626	KF852981	KF853088	KF852879
<i>P. trinitense</i> (Mez) S. Denham	Venezuela	Morrone 4776	Harpostachys		KF810638	KF852992	–	KF852891
<i>P. unispicatum</i> (Scribn. & Merr.) Nash	Venezuela	Morrone s/n	Harpostachys		KF810592	KF852954	KF853054	–
<i>P. alnum</i> Chase	Argentina	Morrone 5287	Paspalum	Alma	KF810571	KF852933	KF853035	–
<i>P. bertonii</i> Hack.	Argentina	Zuloaga 7186	Paspalum	Bertoniana	KF810532	KF852997	KF853007	KF852787
<i>P. caespitosum</i> Flügge	Mexico	Morrone 3656	Paspalum	Caespitosa	KF810597	KF852959	KF853059	KF852849
<i>P. chacoense</i> Parodi	Paraguay	Quarin 3630	Paspalum	Caespitosa	KF810547	KF852910	KF853018	KF852802
<i>P. indecorum</i> Mez	Argentina	Zuloaga 7142	Paspalum	Caespitosa	KF810614	KF852969	KF853077	KF852867
<i>P. conjugatum</i> P. J. Bergius	Argentina	Morrone 3339	Paspalum	Conjugata	–	AY029669*	KF853069	KF852859
<i>P. coryphaeum</i> Trin.	Brazil	Zuloaga s/n	Paspalum	Coryphaea	KF810557	KF852919	EU871096***	–
<i>P. dasytrichium</i> Dusén ex Swallen	Brazil	Morrone 5381	Paspalum	Coryphaea	KF810621	KF852976	EU871068***	KF852874

Table 2 continued

Species	Country of origin	Voucher	Subgenera	Groups	<i>rpoA</i>	<i>ndhF</i>	<i>rpl16</i>	<i>ETS</i>
<i>P. dasyleurum</i> Kunze ex Desv.	Chile	Zuloaga 7241	Paspalum	Dilatata	KF810535	KF852898	KF853010	KF852790
<i>P. pauciciliatum</i> (Parodi) Herter	Argentina	Zuloaga 7191	Paspalum	Dilatata	KF810552	KF852915	KF853023	KF852807
<i>P. acuminatum</i> Raddi	Argentina	Morrone 5367	Paspalum	Dissecta	KF810538	KF852901	KF853013	KF852793
<i>P. repens</i> P.J. Bergius	Argentina	Morrone 5193	Paspalum	Dissecta	KF810534	KF852897	KF853009	KF852789
<i>P. distichum</i> L.	Uruguay	Morrone 5242	Paspalum	Disticha	KF810525	KF852892	EU871051***	KF852780
<i>P. virginatum</i> Sw.	Argentina	Morrone 3393	Paspalum	Disticha	KF810556	AY029665*	KF853027	KF852811
<i>P. erianthum</i> Nees ex Trin.	Bolivia	Morrone 4291	Paspalum	Eriantha	KF810572	KF852934	EU871067***	KF852825
<i>P. fasciculatum</i> Willd. ex Flüggé	Mexico	Zuloaga 7417	Paspalum	Fasciculata	KF810530	KF852895	KF853005	KF852785
<i>P. distortum</i> Chase	Cuba	Zuloaga 9563	Paspalum	Filiformia	KF810610	KF852965	KF853073	KF852863
<i>P. lindenianum</i> A. Rich.	Cuba	Zuloaga 9614	Paspalum	Filiformia	KF810611	KF852966	KF853074	KF852864
<i>P. fimbriatum</i> Kunth	Mexico	Morrone 3651	Paspalum	Fimbriata	KF810543	KF852906	EU871065***	KF852798
<i>P. fimbriatum</i>	Venezuela	Morrone 4664	Paspalum	Fimbriata	KF810615	KF852970	KF853078	KF852868
<i>P. gardnerianum</i> Nees	Brazil	Filgueiras 3480	Paspalum	Gardneriana	KF810576	KF852938	KF853038	KF852829
<i>P. subsesquiglume</i> Döll	Brazil	Rua 777	Paspalum	Gardneriana	KF810574	KF852936	KF853037	KF852827
<i>P. inaequivalve</i> Raddi	Argentina	Zuloaga 7035	Paspalum	Inaequivalvia	KF810595	KF852957	KF853057	KF852847
<i>P. microstachyum</i> J. Presl	Venezuela	Morrone 4683	Paspalum	Inaequivalvia	KF810550	KF852913	KF853021	KF852805
<i>P. ekmanianum</i> Henrard	Bolivia	Morrone 5061	Paspalum	Lachnea	KF810541	KF852904	KF853016	KF852796
<i>P. lineare</i> Trin.	Bolivia	Morrone 4301	Paspalum	Linearia	KF810594	KF852956	KF853056	KF852846
<i>P. pallens</i> Swallen	Bolivia	Morrone 5000	Paspalum	Linearia	KF810580	KF852942	KF853042	KF852833
<i>P. denticulatum</i> Trin.	Paraguay	Zuloaga 7319	Paspalum	Livida	KF810622	KF852977	KF853084	KF852875
<i>P. denticulatum</i>	Argentina	Quarín 4196	Paspalum	Livida	KF810553	KF852916	KF853024	KF852808
<i>P. denticulatum</i>	Argentina	Zuloaga 8607a	Paspalum	Livida	KF810598	KF852960	KF853060	KF852850
<i>P. denticulatum</i>	Argentina	Morrone 5174	Paspalum	Livida	KF810554	KF852917	KF853025	–
<i>P. pubiflorum</i> Rupr. ex E. Fourn.	Mexico	Zuloaga 7379	Paspalum	Livida	KF810555	KF852918	KF853026	KF852810
<i>P. remotum</i> J. Rémy	Argentina	Morrone 3551	Paspalum	Livida	KF810527	AY029668*	EU871052***	KF852782
<i>P. commune</i> Lillo	Argentina	Zuloaga 8480	Paspalum	Macrophylla	KF810558	KF852920	EU871095***	KF852812
<i>P. macrophyllum</i> Kunth	Venezuela	Morrone 4705	Paspalum	Macrophylla	KF810575	KF852937	EU871073***	KF852828
<i>P. regnellii</i> Mez	Argentina	Honfi 130	Paspalum	Macrophylla	KF810545	KF852908	EU871090***	KF852800
<i>P. maculosum</i> Trin.	Argentina	Zuloaga 7216	Paspalum	Maculosa	KF810569	KF852931	KF853033	KF852823
<i>P. ionanthum</i> Chase	Argentina	Zuloaga 7112	Paspalum	Notata	KF810584	KF852946	KF853046	KF852837
<i>P. minus</i> E. Fourn.	Argentina	Zuloaga 7040	Paspalum	Notata	KF810570	KF852932	KF853034	KF852824
<i>P. notatum</i> Alain ex Flüggé	Venezuela	Morrone 4746	Paspalum	Notata	KF810579	KF852941	KF853041	KF852832
<i>P. pumilum</i> Nees	Brazil	Morrone 5383	Paspalum	Notata	KF810585	KF852947	KF853047	KF852838
<i>P. orbiculatum</i> Poir.	Argentina	Zuloaga 7039	Paspalum	Orbiculata	KF810537	KF852900	KF853012	KF852792
<i>P. ovale</i> Nees ex Steud.	Argentina	Zuloaga 7123	Paspalum	Ovalia	KF810568	KF852930	KF853032	KF852822
<i>P. juergensii</i> Hack.	Bolivia	Morrone 4188	Paspalum	Paniculata	KF810620	KF852975	KF853083	KF852873
<i>P. maritimum</i> Trin.	Brazil	Zuloaga 8974	Paspalum	Paniculata	KF810631	KF852986	KF853092	KF852884
<i>P. oligostachyum</i> Salzm. ex Steud.	Brazil	Zuloaga 9043	Paspalum	Paniculata	KF810612	KF852967	KF853075	KF852865
<i>P. paniculatum</i> L.	Mexico	Zuloaga 7398	Paspalum	Paniculata	KF810531	AY029667* Morrone 3354	KF853006	KF852786
<i>P. squamulatum</i> E.Fourn.	Mexico	Zuloaga 7389	Paspalum	Paniculata	KF810536	KF852899	KF853011	KF852791
<i>P. umbrosum</i> Trin.	Argentina	Morrone 5313	Paspalum	Paniculata	KF810551	KF852914	KF853022	KF852806
<i>P. hyalinum</i> Nees ex Trin.	Paraguay	Zuloaga 7279	Paspalum	Parviflora	KF810623	KF852978	KF853085	KF852876
<i>P. multicaule</i> Poir.	Venezuela	Morrone 4686	Paspalum	Parviflora	KF810636	KF852991	–	KF852889
<i>P. parviflorum</i> Rhode ex Flüggé	Brazil	Zuloaga 8997	Paspalum	Parviflora	KF810629	KF852984	KF853090	KF852882
<i>P. atratum</i> Swallen	Bolivia	Morrone 4858	Paspalum	Plicatula	KF810533	KF852896	KF853008	KF852788
<i>P. boscianum</i> Flüggé	Puerto Rico	Axelrod & Stenzel 11108	Paspalum	Plicatula	KF810634	KF852989	KF853095	KF852887
<i>P. chaseanum</i> Parodi	Paraguay	Fortunato 8742	Paspalum	Plicatula	KF810544	KF852907	EU871098***	KF852799
<i>P. geminiflorum</i> Steud.	Bolivia	Morrone 4964	Paspalum	Plicatula	KF810630	KF852985	KF853091	KF852883
<i>P. macranthecium</i> Parodi	Paraguay	Zuloaga 7261	Paspalum	Plicatula	KF810633	KF852988	KF853094	KF852886

Table 2 continued

Species	Country of origin	Voucher	Subgenera	Groups	<i>rpoA</i>	<i>ndhF</i>	<i>rpl16</i>	<i>ETS</i>
<i>P. modestum</i> Mez	Argentina	Zuloaga 6925	Paspalum	Plicatula	KF810627	KF852982	KF853089	KF852880
<i>P. palustre</i> Mez	Argentina	Bordon 110	Paspalum	Plicatula	KF810559	KF852921	EU871070***	KF852813
<i>P. plicatulum</i> Michx.	Argentina	Zuloaga 8477	Paspalum	Plicatula	KF810628	KF852983	EU871076***	KF852881
<i>P. quadrifarium</i> Lam.	Argentina	Giussani 320	Paspalum	Quadrifaria	KF810542	KF852905	EU871055***	KF852797
<i>P. candidum</i> (Humb. & Bonpl. ex Flügge) Kunth	Mexico	Zuloaga 7396	Paspalum	Racemosa	KF810548	KF852911	KF853019	KF852803
<i>P. penicillatum</i> Hook. f.	Bolivia	Morrone 4808	Paspalum	Racemosa	KF810539	KF852902	KF853014	KF852794
<i>P. prostratum</i> Scribn. & Merr.	Venezuela	Morrone 4709	Paspalum	Racemosa	KF810613	KF852968	KF853076	KF852866
<i>P. pygmaeum</i> Hack.	Venezuela	Morrone 4726	Paspalum	Racemosa	KF810635	KF852990	–	KF852888
<i>P. pulchellum</i> Kunth	Cuba	Zuloaga 9541	Paspalum	Reimaria	KF810625	KF852980	KF853087	KF852878
<i>P. capillifolium</i> Nash	Cuba	Zuloaga 9630	Paspalum	Rupestria	KF810609	KF852964	KF853072	KF852862
<i>P. rupestre</i> Trin.	Cuba	Zuloaga 9588	Paspalum	Rupestria	KF810607	KF852962	KF853070	KF852860
<i>P. saugetii</i> Chase	Cuba	Zuloaga 9610	Paspalum	Rupestria	KF810608	KF852963	KF853071	KF852861
<i>P. saccharoides</i> Nees ex Trin.	Bolivia	Morrone 4211	Paspalum	Saccharoidea	KF810540	KF852903	KF853015	KF852795
<i>P. durifolium</i> Mez	Brazil	Valls 12282	Paspalum	Ungrouped	KF810546	KF852909	KF853017	KF852801
<i>P. equitans</i> Mez	Argentina	Zuloaga 7154	Paspalum	Ungrouped	KF810549	KF852912	KF853020	KF852804
<i>P. glabrinode</i> (Hack.) Morrone & Zuloaga	Argentina	Zuloaga 6809	Paspalum	Ungrouped	KF810524	KF852894	EU871057***	–
<i>P. redondense</i> Swallen	Brazil	Valls 11370	Paspalum	Ungrouped	KF810573	KF852935	KF853036	KF852826
<i>P. restingense</i> Renvoize	Brazil	Zuloaga 9014	Paspalum	Ungrouped	KF810596	KF852958	KF853058	KF852848
<i>P. rufum</i> Nees ex Steud.	Argentina	Zuloaga 7110	Paspalum	Ungrouped	KF810560	KF852922	EU871077***	KF852814
<i>P. rufum</i>	Argentina	Quarín 3756	Paspalum	Ungrouped	KF810561	KF852923	EU871089***	KF852815
<i>P. arundinellum</i> Mez	Argentina	Daviña 479	Paspalum	Virgata	KF810526	AY029663*Zuloaga 6810	EU871086***	KF852781
<i>P. haumanii</i> Parodi	Argentina	Quarín 3854	Paspalum	Virgata	KF810562	KF852924	EU871080***	KF852816
<i>P. intermedium</i> Munro ex Morong & Britton	Argentina	Schinini 28857	Paspalum	Virgata	KF810563	KF852925	EU871081***	KF852817
<i>P. virgatum</i> L.	Argentina	Morrone 3367	Paspalum	Virgata	KF810529	AY029670*	EU871058***	KF852784
<i>Anthaenantiopsis rojasiana</i> Parodi	Argentina	Zuloaga 6747			KF810517	AY029620*	KF852999	KF852773
<i>Axonopus anceps</i> (Mez) Hitchc.	Venezuela	Morrone 4767			KF810516	AY029623* RGM 954	EU871062***	KF852772
<i>Echinolaena inflexa</i> (Poir.) Chase	Brazil	Zuloaga 6943			KF810603	AY029633*	KF853065	KF852856
<i>Hopia obtusa</i> (Kunth) Zuloaga & Morrone	Mexico	Zuloaga 7381			KF810519	AY029659*	KF853001	KF852775
<i>Ichnanthus pallens</i> (Sw.) Munro ex Benth.	Argentina	Morrone 3209			KF810606	AY029638*	KF853068	KF852858
<i>Ophiochloa hydrolythica</i> Filg., Davidse & Zuloaga	Brazil	Filgueiras s/n			KF810601	AY029642*	KF853063	KF852853
<i>Ocellochloa chapadensis</i> (Swalen) Zuloaga & Morrone	Brazil	Longhi s/n			KF810605	AY188486** Zuloaga s/n	KF853067	KF852857
<i>Ocellochloa piauiensis</i> (Swalen) Zuloaga & Morrone	Brazil	Zuloaga s/n			KF810602	AY029656*	KF853064	KF852854
<i>Ocellochloa stolonifera</i> (Poir.) Zuloaga & Morrone	Argentina	Deginani 1806			KF810604	AY188488**	KF853066	KF852855
<i>Panicum tuerckheimii</i> Hack.	Mexico	Stevens & Martínez 25890			KF810639	AY188494**	–	–
<i>Panicum validum</i> Mez	Argentina	Zuloaga 7032			KF810640	AY188495**	KF853097	–
<i>Reimarochloa acuta</i> (Flügge) Hitchc.	Cuba	Zuloaga 9537			KF810518	JN604703****	KF853000	KF852774
<i>Spheneria kegelii</i> (Müll. Hal.) Pilg.	Guyana	Hill & Horn 27203			–	JN604707****	–	–
<i>Streptostachys asperifolia</i> Desv.	Brazil	Zuloaga 6941			KF810600	AY029687*	KF853062	KF852852



**Table 2** continued

Species	Country of origin	Voucher	Subgenera	Groups	<i>rpoA</i>	<i>ndhF</i>	<i>rpl16</i>	<i>ETS</i>
<i>Thrasypopsis juergensi</i> (Hack.)Soderstr. ex A.G.Burm.	Brazil	Rua 728 (CEN)			KF810565	KF852927	KF853029	KF852819
<i>Thrasypopsis repanda</i> (Nees) Parodi	Brazil	Rua s/n (CEN)			–	KF852993	KF853096	–

Sequences obtained from Genbank are marked with asterisks: \* Giussani et al. (2001); \*\* Aliscioni et al. (2003); \*\*\* Giussani et al. (2009); \*\*\*\* Morrone et al. (2012)

6 min terminated the reactions. A negative control with no template was included for each series of amplifications to eliminate any possibility of contamination. PCR products were run out on a 1 % TBE agarose gel stained with ethidium bromide. Automated sequencing was performed by Macrogen, Inc. (Seoul, Korea).

#### Data analysis

Sequence edition, assemblage, and alignment were performed with BioEdit version 5.0.9 (Hall 1999). Alignments were manually refined to reduce the impact of indels with ambiguous lengths.

Three matrices were constructed: the “plastid matrix” combining the three plastid regions (*rpoA* + *rpl16* + *ndhF*), the “nuclear matrix”, including *ETS* sequences and the “total evidence matrix” combining plastid and nuclear matrices. According to Nixon and Carpenter (1996), the combined or total evidence analysis better maximizes cladistic parsimony, allowing secondary signals to emerge producing best-supported hypotheses, while separate analysis is useful to investigate the possibility of reticulation or hybridization events. When amplification failed for part or for a complete sequence, positions were coded as missing data in separated and combined matrices, respectively. *Spheneria kegelli*, which only could be amplified for the *ndhF* region, showed more than 50 % of missing data in the total evidence matrix; hence, it was excluded from this analysis in order to improve resolution of the consensus tree.

Separated and combined parsimony analyses were performed using TNT (Goloboff et al. 2008). The search strategy consisted of heuristic searches performed using 10,000 series of random addition sequences followed by TBR branch rearrangements, and retaining 10 trees per series, using a maximum of 20,000 trees in memory. Thereafter, a new search with TBR branch swapping was performed using the shortest trees saved in memory. Branches with ambiguous length were collapsed, according to collapsing rule 1. A strict consensus tree (Nelson 1979; Rohlf 1982) was generated from the most parsimonious trees.

Initially, gaps were considered as missing data. In the total evidence analysis, indels with unambiguous alignment

were coded as present/absent. When coded, character weighting from 1 to 3 was applied. Informative indels for the ingroup were optimized in all the most parsimonious trees obtained in the total evidence analysis, using the command “Common Synapomorphies” of the TNT, by which the common optimization to every individual tree is represented in the consensus diagram to determine whether they were synapomorphies or homoplasies in the clade of interest.

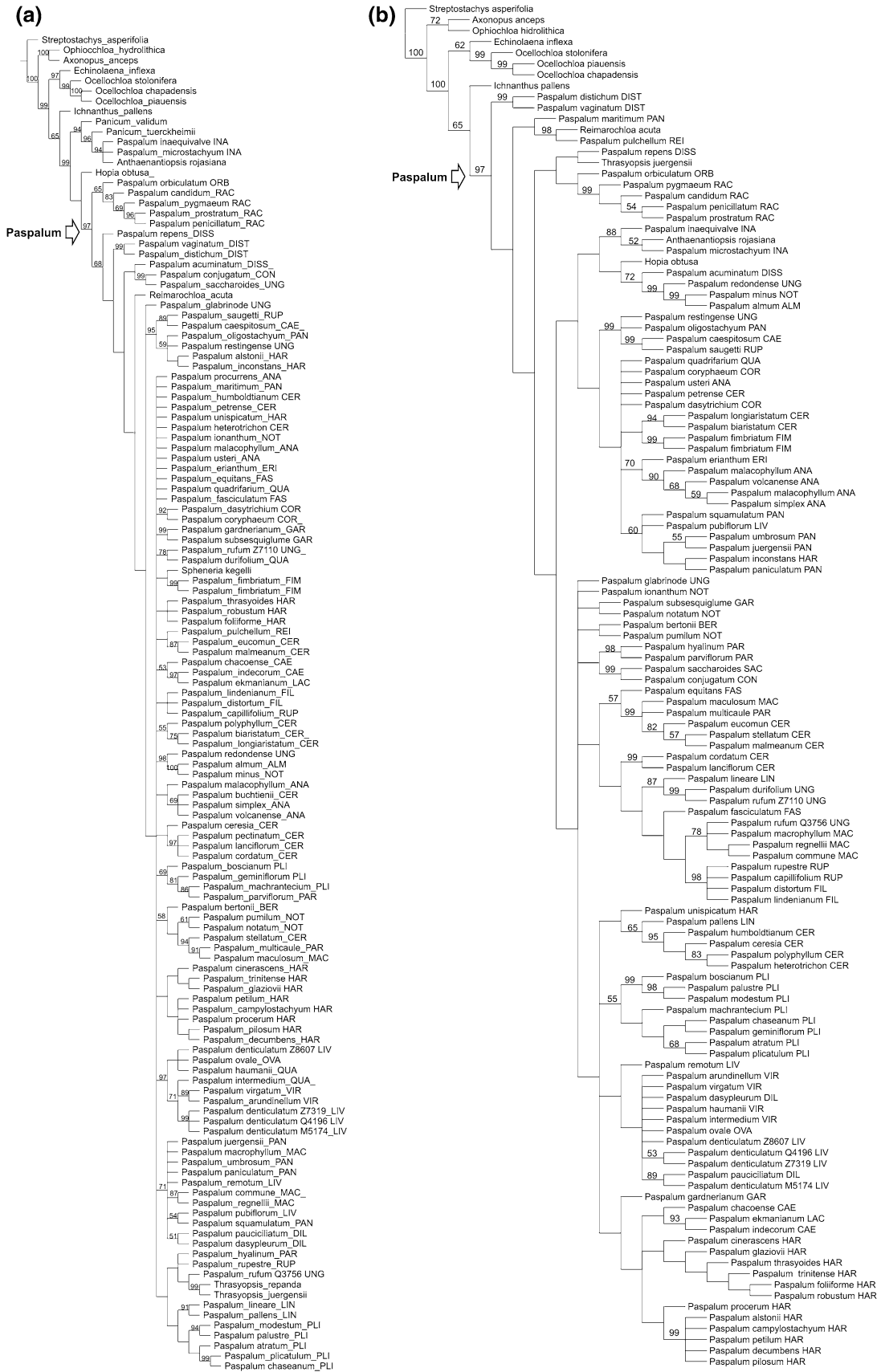
Branch supports were calculated using Jackknife frequencies (JK) for separate analysis and symmetric resampling (SF) not distorted by weights (Goloboff et al. 2003) as implemented in TNT, for the total evidence analysis. Resampling consisted of 10,000 replicates, using heuristic searches of 10 series of addition sequences swapped with TBR, saving 2 trees per series.

Bayesian analysis was conducted in the total evidence matrix using MrBayes version 3.1.2 (Ronquist and Huelssenbeck 2003). Modeltest 3.7 (Posada and Crandall 1998) was employed to determine the sequence evolution model that best fits the data of each marker, using Akaike information criterion (Akaike 1974): GTR+G for *rpoA*, TPM3uf+I+G for *ETS*, GTR+I+G for *rpl16* and TVM+G for *ndhF*. We carried out two independent runs of 4,000,000 generations using four Markov chains (one cold and three heated chains), sampling every 1,000 generations. Posteriors were analyzed after a burn-in of 1,000 trees. The program Tracer v.1.4 (Rambaut and Drummond 2007) was used to examine Bayesian parameters to determine stationarity.

The total evidence matrix is available from the corresponding author or can be found at <http://purl.org/phylo/treebase/phyloids/study/TB2:S12574>.

#### Results

A total of 131 terminals were considered in the plastid matrix. Sequences of *ndhF* + *rpl16* + *rpoA* markers resulted in 4,858 aligned positions, 496 of them being phylogenetically informative. A total of 124 terminals were considered in the “nuclear matrix”; *ETS* failed to amplify for six taxa (*Paspalum buchtienii*, *P. pectinatum*, *P.*





**Fig. 1** Maximum parsimony analysis of separate data. **a** Strict consensus tree obtained from plastid data. **b** Strict consensus tree obtained from nuclear data. Jackknife values are showed above branches

*procurrens*, *Panicum tuerckheimii*, *P. validum*, and *Thrasypsis repanda*). The *ETS* matrix provided 431 positions, of which 275 were informative.

Separate analyses were performed under parsimony (Fig. 1). For the plastid matrix up to 20,000 most parsimonious trees of 1,387 step-long were obtained (CI = 0.45, RI = 0.69), and the strict consensus tree was poorly resolved (Fig. 1a). Analyzing the *ETS* dataset yielded up to 20,000 trees of 1,863 steps (CI = 0.31, RI = 0.60), and a moderately resolved strict consensus tree was obtained with low branch support in basal nodes (Fig. 1b).

The total evidence matrix (plastid + *ETS*) comprised 130 terminals, excluding *Spheneria*, with 5,289 characters, of which, 771 had informative positions. The six taxa that failed in amplifying *ETS* were coded as missing data. The total percentage of missing data was 5.7 % (indels, not included). Nine informative unambiguous indels were found for the ingroup and listed in Table 3.

When analyzing the total evidence matrix under parsimony, up to 20,000 shortest trees of 3,474 steps were found (CI = 0.35, RI = 0.60), resulting in a well resolved strict consensus tree. When analyzing the matrix by coding indels as presence/absence under weight = 1 and weight = 2, both analyses resulted in identical strict consensus trees, equal to that obtained when considering gaps as missing data. When assigning weight = 3, a similar topology was obtained, except for *Paspalum lineare*, species that grouped with other species of *Linearia* group, and the inclusion of *P. unispicatum* and *P. cinerascens* within subg. *Harpostachys*. Under weight = 3, the number of most parsimonious trees decreased drastically to 3,264 trees (3,566 step-long with CI = 0.35 and RI = 0.60). The total evidence consensus tree, including optimizations of indels is shown in Fig. 2.

Under Bayesian inference, the total evidence tree was mostly congruent with the parsimony tree if applying weight = 3, shown in Fig. 2; posterior probabilities (pp) are showed in this cladogram. Only three positions, with low posterior probabilities ( $\leq 0.77$ ), were incongruent between both approaches, which are indicated with broad lines in the cladogram.

Parsimony and Bayesian total evidence analyses (Fig. 2) resolve *Paspalum* as paraphyletic (SF = 86 %, pp = 1) since *Spheneria kegelli* (based on plastid analysis), *Reimarochloa acuta*, and both species of *Thrasypsis* are nested within the genus. *Paspalum inaequivale* and *P. microstachyum* are related to *Anthaenantiopsis rojasiana*

and two “incertae sedis” species of *Panicum*: *P. tuerckheimii* and *P. validum* in a highly supported clade, sister to *Paspalum*, and should be considered apart from *Paspalum*.

Racemosa group, with four out of five species, resolves clearly monophyletic (SF = 100 %, pp = 1) with *Paspalum orbiculatum* (Orbiculata group), as its sister species (SF = 84 %, pp = 1). This group is sister to a clade with *P. conjugatum* (Conjugata group) and *P. saccharoides* (Saccharoidea group) (SF = 100 %, pp = 1), and *P. repens* (Dissecta group) as its sister species. Then, *Disticha* group, which was monophyletic (SF = 100 %, pp = 1), appears to be sister of the remaining species of *Paspalum*, which are grouped in a large polytomy.

Within this polytomy, two minor and three major clades resolve in the consensus tree, but not in the Bayesian analysis. The first minor clade groups three species: *Reimarochloa acuta* with *P. pulchellum* (Reimaria group) (SF = 86 %), and *P. maritimum* (Paniculata group) as its sister species. A consistent second minor group is composed by three species: *P. minus* (Notata group), *P. alnum*, the single species of the Alma group, (SF = 100 %, pp = 1), together with *P. redondense*, an ungrouped species (SF = 99 %, pp = 1). *Paspalum acuminatum* (Dissecta group) appears related to this clade in the parsimony analysis with low support values (SF = 55 %), but related to *Disticha* in the Bayesian tree (pp = 0.75).

The first major clade, similar to the NPBT clade sensu Rua et al. (2010), comprises several groups of *Paspalum* (Fig. 2): Notata, Bertoniana, Linearia, and Plicatula plus both species of *Thrasypsis* (SF = 99 %, pp = 1), along with *P. hyalinum* and *P. parviflorum*, species of the Parviflora group. This clade presents a basal polytomy with *P. ionanthum* (Notata group) and three subclades: Notata group (*P. notatum* and *P. pumilum*) together with Bertoniana group (*P. bertonii*), (pp = 0.99), then, the ungrouped species *P. equitans* + Subg. *Ceresia* p.p + Parviflora (*P. multicaule*) and Maculosa (*P. maculosum*) groups (SF = 78 %, pp = 1), and a third subclade with all species of Plicatula + Parviflora p.p. groups (*P. hyalinum* and *P. parvifolium*; SF = 98 %, pp = 1), sister to the Linearia group (*P. lineare* and *P. pallens*; pp = 0.67). All species of the third subclade share an insertion of an adenine in the position 1,160 of the *rpoA* alignment (Table 3) being an homoplastic indel as it is also present in *P. rufum* (Q3756), and *P. stellatum*, the latter a species of subg. *Ceresia* p.p.

The second major clade includes a non-definite group of species, the Uncertain subclade recovered in both nuclear and plastid analyses independently, and also in the total evidence analyses (SF = 83 %, pp = 1). All species in this subclade share a 5 bp insertion from position 15 to 19 of the *rpl16* matrix (Table 3); this insertion is also present in *P. alstonii*, a species classified within subg. *Harpostachys*

**Table 3** Description of the indels found in the alignment

Character	Marker	Indel and position	Species with state = 1 (presence)
1	<i>rpoA</i>	Insertion 828–863	<i>P. maculosum</i> – <i>P. multicaule</i>
2		Deletion 1,022–1,024	<i>P. fasciculatum</i> – <i>P. ekmanianum</i> – <i>P. chacoense</i> – <i>P. indecorum</i>
9		Insertion 1,160	Plicatula-Linearia– <i>P. hyalinum</i> – <i>P. parviflorum</i> – <i>P. stellatum</i> – <i>P. rufum</i> Q3756
6	<i>rp16</i>	Insertion 15–19	Uncertain clade– <i>P. alstonii</i>
5		Insertion 244–249	<i>P. sauetii</i> – <i>P. caespitosum</i>
3		Insertion 349–352	<i>P. ekmanianum</i> – <i>P. indecorum</i>
4		Insertion 472–510	<i>P. thrasyoides</i> – <i>P. foliiforme</i>
7	<i>ETS</i>	Insertion 325–326	<i>P. minus</i> – <i>P. alnum</i>
8		Insertion 264–265	<i>P. cordatum</i> – <i>P. lanciflorum</i>

The positions correspond to the matrix of each individual marker. Clades or species with presence of each indel are indicated

(Denham 2005). The Uncertain subclade is sister to a group of species of groups Coryphaea (*P. coryphaeum* and *P. dasytrichium*; SF = 97 %, pp = 1 as defined by Giussani et al. 2009), Fimbriata (both specimens of *P. fimbriatum*, including also the genus *Spheneria*), Paniculata and Livida, together with another clade which includes all species of subg. *Anachyris*, three species previously assigned to subg. *Ceresia*: *P. petrense*, *P. biaristatum*, and *P. longiaristatum* (Denham et al. 2002), and *P. erianthum* of the Eriantha group (Morrone et al. 2004) (Fig. 2).

The third major clade is composed of two major arrangements: one, including *P. fasciculatum* of the Fasciculata group, together with species of group Macrophylla (SF = 98 %, pp = 1) and the clade *P. rufum* (Q3756) + Filiformia + Rupestris (this clade appears in the Plicatula group in the Bayesian tree). This clade is related to another one consisting of the ungrouped *P. rufum* (Z7110) and *P. durifolium* (SF = 100 %, pp = 1), merged with species of Sect. Pectinata of subgen. *Ceresia*. The second major arrangements gathers group Gardneriana (SF = 97 %, pp = 1) sister to a clade including species of groups Dilatata, Livida p.p., Ovalia, and Virgata (SF = 99 %; pp = 1), plus a clade including *Ceresia* p.p. and subg. *Harpostachys* (excluding *P. inconstans*, pp = 0.97), with Caespitosa + Lachnea (SF = 99 %; pp = 1) as its sister group (pp = 0.98).

## Discussion

Bayesian and parsimony approaches showed *Paspalum* monophyletic if *Spheneria*, *Thrasyopsis* and *Reimarochloa* are eventually included within the genus (see discussion of *Spheneria*). *Paspalum inaequivalve* and *P. microstachyum*, two species of the Inaequivalvia group, are here related to *Anthaenantiopsis* and excluded from the genus by the plastid and combined data, although nested within *Paspalum* by the *ETS* nuclear marker. The latter result shows a conflictive and, up to now, unresolved position for these taxa.

*Thrasyopsis* is a small genus with two South American species, *T. repanda* and *T. juergensii*, which grow in open areas of southern Brazil. The genus, established by Parodi (1946), has been related to *Paspalum* based on morphological (Denham and Zuloaga 2007) and molecular characters (Rua et al. 2010). The present work further confirms this relationship, since both species of *Thrasyopsis* were nested within *Paspalum*, a result based independently on both nuclear and plastid data. While this contribution was being reviewed, Rua and Valls (2012) transferred both species of *Thrasyopsis* to *Paspalum*.

*Reimarochloa* comprises three species distributed from North America and the Caribbean to Argentina. The genus has been distinguished by lacking both glumes, with the upper antherium membranous and open at the apex, the upper flower with 1 or 2 stamens. Scataglini et al. (2007) proposed the inclusion of *Reimarochloa* in *Paspalum* based on plastid sequences of *Reimarochloa acuta*. Here we conclude, based on plastid and nuclear evidence, that *Reimarochloa* has to be considered within *Paspalum*, being related to *P. pulchellum* (Reimaria group) by *ETS* and combined parsimony analyses, or sister to a large polytomy in plastid parsimony and Bayesian analyses.

*Spheneria* is a monotypic genus with *S. kegelli* growing in “Cerrados” of northern Brazil. Since no other sequence of the genus could be obtained, *Spheneria* was analyzed with the *ndhF* sequence published by Morrone et al. (2012); when this sequence was included in the plastid matrix, the genus appeared nested within *Paspalum* as the sister group of *P. fimbriatum*. Nevertheless, and taking into account that the genus was only studied with one sequence, the inclusion of *Spheneria* in *Paspalum* needs to be confirmed by further molecular studies; also, *Spheneria* morphologically differs from *Paspalum* by the presence of a long pungent callus at the base of the spikelet, obliquely articulated to the pedicel, and by the beaked upper antherium. Finally, species of the Fimbriata group of *Paspalum* differ from *Spheneria* by having conspicuously winged spikelets.

### Incertae sedis species (Inaequalvalvia group)

*Paspalum inaequalvalve* and *P. microstachyum* were either excluded or included in the genus based on plastid or nuclear markers, respectively. Chase (1939, unpubl.) classified *P. inaequalvalve* and *P. microstachyum* in the informal group Inaequalvalvia. Zuloaga and Morrone (2005), in their revision of Austral South American species of *Paspalum*, treated *P. inaequalvalve* within the Inaequalvalvia group. Rua et al. (2010), using different plastid markers, found *Anthraenantiopsis rojasiana* as the closest related taxon to *Paspalum inaequalvalve*, with both species in a clade sister to *Paspalum*. Relationships among species of this group and other species of *Paspalum* should be further investigated.

Although the total evidence analyses support *Paspalum* as monophyletic if both *P. inaequalvalve* and *P. microstachyum* are excluded, the independent analysis of the nuclear *ETS* showed both species of Inaequalvalvia group included within *Paspalum*. Therefore, the exclusion of this group is not conclusive. Aliscioni and Denham (2009) found atypical features, related to C<sub>4</sub> photosynthesis, in *P. inaequalvalve* and *P. microstachyum*: besides the typical mesophyll cells (PCA) and mestome sheath cells (PCR), a third type of cells, the ‘globose parenchymatous cells’, were observed in both taxa, an autapomorphy for *Paspalum*. On the other hand, similar globose parenchymatous cells, as remnants of the outer parenchymatous sheath, were previously described for *Anthraenantiopsis* (Morrone et al. 1993), and *Panicum validum* and *P. tuerckheimii* (Zuloaga et al. 1989); therefore, this character might have taxonomic significance for the clade Inaequalvalvia–*Anthraenantiopsis*–*Panicum validum*–*P. tuerckheimii*.

The inclusion or not of the group Inaequalvalvia within *Paspalum* is not definitive: while *ETS* and morphology support keeping the species within the genus, plastid data and anatomical features relate *P. inaequalvalve* and *P. microstachyum* to *Anthraenantiopsis* and species of *Panicum* without a clear taxonomic position.

### Infrageneric classification

Informal groups and subgenera of *Paspalum* were recovered partially in monophyletic assemblages. Subgenera *Anachyris* and *Harpostachys* are monophyletic, although the phylogenetic position of *P. usteri* and *P. inconstans* remain dubious in relation to both subgenera, respectively. Subg. *Ceresia* was recovered as polyphyletic, with species distributed in each of four major clades described in this analysis. Subg. *Paspalum* is also polyphyletic, with some informal groups or sections monophyletic.

Subg. *Anachyris*: *Anachyris* was established at the generic level by Nees Von Esenbeck (1850) on the basis of

*A. paspaloides*. Later, Chase (1927) treated *Anachyris* as a subgenus of *Paspalum*. According to Morrone et al. (2000), the subgenus includes a total of six American species, with *P. malacophyllum* widely distributed from Mexico to Argentina, while the remnant five taxa are restricted to South America. Subg. *Anachyris* is characterized by including species having boat-shaped spikelets, with the upper lemma having prominent longitudinal nerves on the abaxial surface. Also, the upper glume is typically missing in the subgenus, although it is occasionally present, and incompletely developed in two species, *P. volcanense* and *P. usteri*. Nevertheless, the latter character does not constitute a synapomorphy for the subgenus, since it is also absent in other species and informal groups of *Paspalum*, such as *P. candidum* (Racemosa group), *P. gardnerianum* (Gardneriana group) and *P. pulchellum* (Reimaria group).

In our work, four species of *Anachyris* appear in a clade sister to the Eriantha group (Morrone et al. 2004), a relationship that agrees with previous results of Giussani et al. (2009), and to three species previously assigned to subg. *Ceresia*: *P. petrense*, *P. biaristatum*, and *P. longiaristatum* (Denham et al. 2002). *Paspalum biaristatum* and *P. longiaristatum* share spikelets with the upper glume and the lower lemma awned; these two species were defined as sect. Biaristata of subg. *Ceresia* by Filgueiras and Davidse (1994). Although the association *Anachyris*–Eriantha–*Ceresia* does not have a morphological concordance, similar results were obtained by Rua et al. (2010) using four different plastid markers. The fifth species of *Anachyris*, *P. usteri*, appears, without support, in both total evidence analyses as the sister species of the clade *Anachyris*–Eriantha–*Ceresia* p.p.

Subg. *Ceresia*: *Ceresia* includes, according to Denham et al. (2002), and Zuloaga and Morrone (2005), a total of 25 species distributed from Mexico to Argentina. Denham et al. (2002), in a morphological phylogenetic study of the subgenus, proposed the monophyly of *Ceresia* and considered synapomorphies of the subgenus a membranous upper anthercium with the upper palea gaping at the apex. On the contrary, our analysis shows that subg. *Ceresia* is polyphyletic, with species disposed in four different groups: the first one, which includes *P. pectinatum*, *P. lanciflorum*, and *P. cordatum*, agrees with the previous circumscription of sect. Pectinata (Rua and Aliscioni 2002; Denham et al. 2002). Here, species of Pectinata are related to the *P. rufum* (Z7110)–*P. durifolium* ungrouped complex. On the other hand, *P. stellatum*, *P. eucomum*, and *P. malmeanum* appear in a clade with *P. multicaule*, species of group Parviflora, and *P. maculosum* of the Maculosa group. According to Denham et al. (2002), these three species of *Ceresia* constitute a clade supported by three morphological synapomorphies: two racemes per inflorescence, and obovoid upper anthercium without macro or microhairs. The inclusion of species of





◀ **Fig. 2** Maximum parsimony analysis of the total evidence matrix. Strict consensus of the 3,264 trees resulting of weighting gaps = 3. Branch supports are indicated below branches as symmetric frequencies. Incongruent positions with the Bayesian analysis are indicated with broad lines. Posterior probabilities are listed above branches. Optimizations of indels are indicated with a *broad single bar* (synapomorphic) and *narrow double bars* (homoplastic), the indel numbers correspond to those indicated in Table 3

*Ceresia*, and in particular *P. stellatum*, in the NPBT clade has already been proposed by Rua et al. (2010). This work reinforces the position of *P. stellatum* in the NPBT clade together with other two species of *Ceresia*: *P. malmeanum* and *P. eucomum*.

The third group of *Ceresia* is composed of five species: *P. polyphyllum*, *P. buchtienii*, *P. heterotrichon*, *P. humboldtianum*, and *P. ceresia*. This group agrees, except *P. ceresia*, with clade B of Denham et al. (2002). All these species share a rhizomatous habit and have an upper glume with pilose to ciliate margins. Parodi (unpubl.) placed *P. humboldtianum* and *P. polyphyllum* in group Humboldtiana. According to Zuloaga and Morrone (2005), *P. humboldtianum* is morphologically similar to *P. polyphyllum* and *P. buchtienii*. We found that the five species of *Ceresia* mentioned above, are grouped in a clade close to species of subg. *Harpostachys* and species of groups Lachnea and Caespitosa; nevertheless, posterior probabilities and support are low in this clade.

Subg. *Harpostachys*: *Thrasya*, as traditionally circumscribed, was an American genus with nearly 22 species (Burman 1987; Watson and Dallwitz 1992). Burman (1985) distinguished *Thrasya* by having racemes with a wide winged rachis, carrying adaxial and abaxial solitary spikelets distributed in a row. Burman (1985) pointed out that the distinction between *Thrasya* and *Paspalum* was difficult because of the presence of several species with intermediate characteristics and mentioned its close relationship with species of the Decumbentes group of *Paspalum*. Different authors (Burman 1980, 1985; Denham 2005; Denham and Zuloaga 2007) have stressed the relationship of species of group Decumbentes of *Paspalum* with *Thrasya*, on the basis of morphological characters such as having terminal and axillary inflorescences in the upper foliar sheaths, solitary racemes, the presence of lower glumes, and a staminate lower flower. As a result, Denham (2005) transferred 22 species of *Thrasya* to *Paspalum*, including them in subg. *Harpostachys* together with species previously included in Decumbentes; several phylogenetic studies, based on morphological and anatomical (Denham and Zuloaga 2007) or molecular characters (Giussani et al. 2001; Aliscioni et al. 2003; Duvall et al. 2001; Rua et al. 2010), confirmed that species of the Decumbentes group and species of genus *Thrasya* constitute a monophyletic group and corroborated the inclusion

of *Thrasya* in *Paspalum*. Aliscioni and Denham (2008) concluded, in an anatomical study of rachises of racemes in *Paspalum*, that an exclusive type of flat rachis is present in the majority of species assigned to subg. *Harpostachys* where racemes are solitary and spikelets not paired.

The present work is the first study that analyzes molecular data including a representative number of species (13) assigned to subg. *Harpostachys*. The results not only confirmed the inclusion of *Thrasya* in *Paspalum*, but also corroborated the *Thrasya*–Decumbentes relationship, since all examined species belonging to both groups, with the exception of *P. inconstans*, were grouped in the same clade, corresponding to subg. *Harpostachys* as proposed by Denham (2005). The *Harpostachys* clade had no support in the parsimony strict consensus tree, but showed a posterior probability of 0.97 in the Bayesian analysis. *Paspalum inconstans* was reported as a hexaploid species (Morrone et al. 2006); this ploidy level could be an evidence of a possible hybrid origin, so a definite position for this species should be confirmed with cytogenetic studies.

Subg. *Paspalum*: The subg. *Paspalum* was not recovered as a monophyletic group, but some of its informal groups were.

Racemosa: This group was recovered as monophyletic, with high support in all the analyses performed. The four analyzed species appear, together with *P. orbiculatum*, as sister to the remainder of *Paspalum*. These results are in concordance with those obtained with different markers, by Rua et al. (2010), who included in their study a single species of Racemosa, *P. flavum*, which was located together with *P. orbiculatum* as sister to the remaining species of *Paspalum*.

Saccharoidea: This group includes a peculiar single species, *P. saccharoides*, characterized by having a subflabellate inflorescence with many racemes hanging downwards, and spikelets with the upper glume 2-nerved and covered by long, pale hairs, the upper lemma 2-nerved. None of these characters relates the species to *P. conjugatum*, of the Conjugata group, or *P. repens* (of the Dissecta group).

Dissecta: Group Dissecta was related to group Disticha by being the species aquatic or subaquatic plants with decumbent to prostrated culms and broad rachises (Morrone et al. 1996). Our study indicates that Dissecta is not monophyletic, since the two sampled species, *P. repens* and *P. acuminatum*, are located in different clades. The position of *Paspalum acuminatum* is uncertain, it appears as the sister species of *P. redondense*–*P. minus*–*P. alnum* in the parsimony analysis, but related to Disticha in the Bayesian tree. *Paspalum repens* is grouped with *P. saccharoides*–*P. conjugatum*. The relationship between *P. repens* and *P. conjugatum* had already been proposed by Rua et al. (2010), based on different plastid markers.

**Disticha:** Both species of this group are gathered with high support in all the analyses performed, thus resulting in a monophyletic group. This clade was located as sister group of the basal polytomy of the genus and have morphological affinities with *Conjugata*, by including perennial and stoloniferous species with the inflorescence with two conjugate racemes.

Groups *Racemosa*, *Conjugata*, *Dissecta*, and *Disticha* share foliaceous rachises. The last three sections present the type II rachises described by Aliscioni and Denham (2008). This type of rachis is characterized by the obligate presence of a cavity or spongy tissue at the medulla; this condition is seen in rachises with narrow wings (*Disticha* and *Conjugata*) or in species of *Dissecta* having wide wings with many vascular bundles. These three sections also include species that are aquatic or grow in wet places, such as ditches or near rivers or costal areas; so, rachises of type II may have arisen independently in species or group of species adapted to wet places (Aliscioni and Denham 2008). According to the phylogenetic results obtained in the present work, and those of Rua et al. (2010), these diverging lineages within *Paspalum* include hygrophytic grasses with plagiotropic culms, yielding the type II of rachises, a morphological characteristic restricted to basal groups of the genus.

**Reimaria:** *P. pulchellum* is the only member of group *Reimaria*; Chase (1939), characterized this species with inflorescences having usually two, occasionally up to four racemes, the spikelets with both glumes wanting and placed on the racemes showing its alternate dark and pale backs. The fact that spikelets of this species lack both glumes clearly relates *P. pulchellum* with *Reimarochloa*, with which it appears in a strongly supported clade in the *ETS* and total evidence parsimony analyses; alternately *P. pulchellum* is related to species of NPBT clade by plastid and Bayesian analyses but without posterior probabilities or jackknife support.

**Alma:** *Paspalum alnum* has been previously placed in group *Notata* (Chase 1939, unpublished; Canto-Dorow et al. 1996) or *Alma* (Quarín 1974; Zuloaga et al. 2004). Quarín (1974) segregated this species in the monotypic group *Alma*, and indicated that *P. alnum* has a unique basic chromosome number in *Paspalum* with  $x = 6$  (Quarín 1974; Quarín and Hanna 1980). The association of *P. alnum* with *P. minus* has strong support in both the plastid and nuclear analyses. Both species share, as a synapomorphy, an insertion of an adenine and a thymine at positions 325 and 326 of the *ETS* marker. The pair *P. alnum*–*P. minus* appeared related to the ungrouped species *P. redondense*; this relationship displays high values of support in all performed analyses.

**Parviflora:** Chase (1939, unpubl.) included 14 species in group *Parviflora*, characterized by including annual or

perennial plants, with culms usually simple, inflorescences with one to four racemes, and spikelets minute. Examined species of this group are located within the NPBT clade in different positions, stressing the polyphyly of the group. *Paspalum parviflorum* and *P. hyalinum* are included in the *Plicatula* clade, while *P. multicaule* forms a well-supported pair with *P. maculosum* (see *Maculosa*, below). Species of *Plicatula* differ from *P. parviflorum* and *P. hyalinum* by the presence of plano-convex spikelets, the lower lemma usually wrinkled, with the upper antheridium dark brown to black. Both *P. hyalinum* and *P. parviflorum* have small spikelets with the upper glume and lower lemma hyaline, 2-nerved. The position of these two species of *Parviflora* group within *Plicatula* clade is recovered in both total evidence analyses, but is variable in the separate approach.

**Plicatula:** This group encompasses a complex arrangement of species of difficult delimitation. It was established by Chase (1929) and includes around 30 species, characterized by its spikelets conspicuously plano-convex, lower lemma usually with transverse wrinkles, and upper antheridium dark brown to black. All analyzed species assigned to *Plicatula* were here gathered in one clade; however, *Plicatula* could be paraphyletic since this clade also includes, as previously mentioned, two species of group *Parviflora*, *P. hyalinum* and *P. parviflorum*. The *Plicatula*–*Parviflora* p.p. clade, share, along with the sister clade consisting of *P. pallens* and *P. lineare*, the insertion of one adenine at position 1,160 of the *rpoA* gene. It should be stressed that this insertion is also present in *P. rufum* Q3756, which appears included in this clade in the Bayesian tree.

**Notata:** Nash (1912) and Chase (1929) recognized *Notata* and defined the group by having their species a perennial habit, culms compressed, mostly basal blades, inflorescences with conjugate racemes and solitary spikelets. Zuloaga et al. (2004) expanded Chase delimitation of *Notata* by including in this group species originally classified in group *Linearia* (Chase 1929); Zuloaga et al. (2004) recognized 17 species in *Notata*, 15 of which are restricted to South America. Essi and Souza-Chies (2007) performed a molecular phylogeny of *Linearia*, *Notata* and related groups, and concluded that only a small core of eight species of *Notata* could be accepted as a good formal clade; similarly, Souza-Chies et al. (2006) and Rua et al. (2010) postulated a core-*Notata* clade including only three species. Of the five species of group *Notata* included in our phylogenetic study, four are located in the NPBT clade, while *P. minus* constitutes, as previously mentioned, a very well-supported clade with *P. alnum* (of group *Alma*). Within the NPBT clade, *P. notatum* and *P. pumilum* group with *P. bertonii* (*Bertoniana*), whereas *P. ionanthum* remains in an unresolved position.

**Maculosa:** *P. maculosum*, the unique species of *Maculosa* group, according to Chase (1939, unpubl.) groups in

a strongly supported clade with *P. multicaule* (Parviflora), related to *Ceresia* species of the NPBT clade: *Paspalum eucomum*, *P. malmeanum*, and *P. stellatum*. This result was obtained in both separate and total evidence analyses. *Paspalum maculosum* and *P. multicaule* share an insertion of 36 bp in the *rpoA* alignment. Both species present solitary spikelets arranged in conjugate racemes.

**Linearia:** This group was characterized by Chase (1929) by including perennial plants with cespitose culms, narrow blades, conjugate inflorescences and narrow ellipsoid spikelets. In our total evidence analysis, the two studied species of Linearia, *P. pallens* and *P. lineare*, grouped together in the NPBT clade, where they are close to Notata species but forming the sister group of Plicatula, sharing with this section the insertion of an adenine at position 1,160 of the *rpoA* gene. However, the position of *P. lineare* was variable since it also appeared related to *P. durifolium*–*P. rufum* when gaps were considered missing or weighted = 1 and = 2.

**Uncertain clade:** This clade consists of *P. inconstans* (subg. *Harpostachys*), *P. oligostachyum* (group Paniculata), *P. restingense* (ungrouped), *P. sauetii* (group Rupetria) and *P. caespitosum* (group Caespitosa). Although these species do not share morphological characters, the clade is well-supported and the species do share an insertion in a plastid marker. The position of *P. inconstans* is variable in the separate analysis, while the remnant four species are recovered together in both the plastid and in the nuclear phylogeny. This uncertain position of *P. inconstans* could be an evidence of its possible hybrid origin, according to its exclusive hexaploid status.

**Coryphaea:** 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 large panicles with numerous slender racemes. Chase (1929) also indicated that other species, related to *P. coryphaeum*, were confined to South America. Later, Chase (1939, unpubl.) arranged *P. coryphaeum* within the Quadrifaria Group. Giussani et al. (2009) pointed out that Quadrifaria should be restricted to two species, *P. quadrifarium* and *P. quarinii*, and placed *P. coryphaeum* and *P. dasytrichium* in group Coryphaea. Both *P. coryphaeum* and *P. dasytrichium* are robust perennial plants with an erect to leaning growth habit, tuberculate hairs on sheaths, papillose-hirsute leaves, and spikelets glabrous. Our study confirms the monophyly of Coryphaea group in a strongly supported clade.

**Quadrifaria:** According to Giussani et al. (2009) this group is restricted to two species, *Paspalum quadrifarium* and *P. quarinii*. The close relationship between these two species was already proposed by Quarín and Norrmann (1990), who reported regular meiotic behavior in hybrids

between diploid specimens. In the present work, *P. quadrifarium* appears in the CQPA clade (sensu Rua et al. 2010), close to Coryphaea, Paniculata and the *Anachyris*–*Eriantha*–*Ceresia* clade, corroborating the position already published by Rua et al. (2010) based on different plastid markers.

**Paniculata:** This group includes approximately ten species. A total of seven species of Paniculata were examined; of these *P. paniculatum*, *P. juergensii*, *P. umbrosum* and *P. squamulatum* formed a moderately supported monophyletic group placed in the CPQA clade with *P. pubiflorum* of group Livida as sister species. As previously mentioned, *P. oligostachyum* is located in the Uncertain clade, while *P. maritimum*, a species from northern Brazil, is related to *Reimarochloa acuta* and *P. pulchellum* (of group Reimaria) or remains in an unresolved position in the Bayesian tree.

**Livida:** Denham et al. (2010) reviewed species traditionally placed in group Livida and indicated that Livida is an artificial group, without characters that delimit it from the remaining groups within *Paspalum*. The present work showed Livida as a polyphyletic group: *P. denticulatum* is placed in a strongly supported clade related to species of group Virgata. *Paspalum remotum* and *P. pubiflorum* were differentiated of the remaining species of Livida by Zuloaga and Morrone (2005) and Denham et al. (2010), based on the type of the inflorescence and the shape and indumentum of the spikelet. In the present work, they appeared not related to each other or to the rest of Livida taxa; *Paspalum remotum* is a sister species of the Dilatata group, while *P. pubiflorum* is included in a well-supported clade with species of Paniculata.

**Fasciculata:** (Chase 1929, 1939, unpubl.) and Zuloaga and Morrone (2005) classified *P. fasciculatum* within the monotypic group Fasciculata. *Paspalum fasciculatum* is distinguished by its creeping and aquatic habit, solid culms, flabellate inflorescences carrying long-ellipsoid spikelets with the lower glume present or absent. Although *P. fasciculatum* shares a deletion with *P. caespitosum*, *P. indecorum*, and *P. ekmanianum*, the topology obtained relates the species to group Macrophylla.

**Macrophylla:** This clade consists of *Paspalum regnellii* and *P. commune* along with *P. macrophyllum* as its sister species. Chase (1939, unpubl.) included these species in the informal group Macrophylla in which she also included *P. wettsteinii* and *P. barclayi* Chase. Giussani et al. (2009), when reinstating group Macrophylla, treated in this group the species mentioned above with the exception of *P. wettsteinii* (= *P. virgatum*). Species of group Macrophylla are characterized by including perennial plants, with wide blades, rounded at the base and panicles truncate with 3–15 racemes; spikelets are ellipsoid to obovoid, pubescent, with a pale or brown antherium (Zuloaga and Morrone 2005). In our study, group Macrophylla was recovered and is strongly supported;



it appears related to *P. fasciculatum* and to the clade *P. rufum* Q3756–Rupestria–Filiformia in the parsimony analysis.

Filiformia and Rupestria: Species assigned to Filiformia and Rupestria grouped in the same clade with *P. rufum* Q3756 as sister species, except *P. saugetii* (Rupestria), which is located in the uncertain clade. Both groups are restricted to the Caribbean, and include tufted perennials with slender culms, narrow blades and usually solitary spikelets. This clade appears related to Macrophylla group and *P. fasciculatum* in the parsimony analysis, but included in the Plicatula clade in the Bayesian tree.

Gardneriana: Both analyzed species of this group, *P. gardnerianum* and *P. subsesquiglume*, are gathered in a strong monophyletic clade. Species of Gardneriana have terminal inflorescences, rachis of racemes triquetrous to broadly winged, and glabrous spikelets, with the upper glume absent or reduced, lower lemma hyaline and upper anthercium plano-convex, indurate and strongly papillose.

Dilatata: This group comprises four species, distinguished by being perennial plants, cespitose to stoloniferous, characterized by having paired, pilose and plano-convex spikelets, with ciliate margins and upper glume and lower lemma 3–5 nerved. Both analyzed species of Dilatata, *P. pauciculatum* and *P. dasypleurum*, are in a well-supported, monophyletic clade with *P. remotum* as its sister species, and related to the Virgata clade.

Virgata: This group was first described by Chase (1929), who initially included in it nine species from North America; later, Virgata was expanded with the inclusion of Central and South American species (Chase 1939, unpubl.). In a study of the South American species of Virgata, Barreto (1954) restricted the group to species with dark-brown upper anthercia and non-pyramidal panicles. Zuloaga and Morrone (2005) classified within the Virgata group four species: *P. commune*, *P. conspersum*, *P. regnellii*, and *P. virgatum*, and treated *P. wettsteinii* Hack. as a synonym of *P. virgatum*. A monophyletic Virgata clade, consisting of nearly all the species listed by Chase and *P. ovale* as its sister group, and a Quadrifaria group restricted to *P. quadrifarium* and *P. quarinii* were proposed by Giussani et al. (2009). In our study, Virgata, as proposed by Giussani et al. (2009), is recovered, together with *P. ovale* (Ovalia group) and *P. denticulatum*, a species originally pertaining to the Livida group; the latter is not included in Giussani et al. (2009) treatment. Although the Virgata–*P. denticulatum* relationship does not seem to have a morphological correlation, the same association has been already postulated by Rua et al. (2010). Within the Livida clade here obtained, there is a well-supported group of Virgata, including *P. arundinellum* and *P. virgatum*, related to *P. denticulatum* (Q4196, Z7319, and M5174); there are also species of both groups, *P. intermedium*, *P. haumanii*, and

*P. denticulatum* (Z8607a), whose positions remain unresolved within the clade.

Caespitosa: This heterogeneous group was established by Chase (1929), in which she initially included a total of seven species and later expanded it to 14 species (Chase 1939, unpubl.). In our analysis Caespitosa is polyphyletic: *Paspalum caespitosum* was located in the Uncertain clade, while the other two studied species, *P. chacoense* and *P. indecorum*, grouped together in a strongly supported clade with *P. ekmanianum* of group Lachnea, all these species gathered in a clade that constitutes the sister group of subg. *Harpostachys*. These three species share a deletion of three base pairs at positions 1,022–1,024 of the *rpoA* gene, a character also present in *P. fasciculatum*. The relationship Caespitosa–Lachnea as the sister group of subg. *Harpostachys* shows low support in the strict consensus of parsimony analysis, but displays 0.98 of posterior probabilities in the Bayesian tree.

#### Ungrouped species

*Paspalum equitans*: This is a species alternatively excluded (Zuloaga et al. 2004; Zuloaga and Morrone 2005) or included within group Notata (Souza-Chies et al. 2006). *Paspalum equitans* is characterized by having fasciculate inflorescence with 4–7 primary branches. In the present work, and in agreement with Rua et al. (2010), this species is located in the NPBT clade, where it is the sister species of a clade including *P. malmeanum*, *P. eucomum*, *P. stellatum*, and the pair *P. maculosum*–*P. multicaule*.

*Paspalum glabrinode*: Morrone and Zuloaga (1989) included *P. glabrinode* in group Decumbentes, by having a dimorphic lower glume in the paired spikelets. Later, the species was excluded by Denham et al. (2010) from subg. *Harpostachys* on the basis of the number of racemes per inflorescence (12–40 racemes), the absence of axillary inflorescences in the last leaf sheath and the presence of stinging hairs on the distal portion of the leaf sheaths. The data presented in this work do not relate *P. glabrinode* with species of *Harpostachys*, or with species of other groups, since it remains in an unresolved position, forming part of the basal polytomy of the genus.

*Paspalum redondense*: This species is characterized by including conspicuously stoloniferous plants, inflorescences with 6–8 racemes, and spikelets with upper glume and lower lemma corrugated. Zuloaga and Morrone (2005) related *P. redondense* with *P. indecorum* and *P. caespitosum*, species which are not related in our analysis. On the contrary, *P. redondense* appears, in a strongly supported clade, with the pair *P. alnum*–*P. minus*, both species with geminate racemes.

*Paspalum rufum*/*P. durifolium*: Two vouchers of *P. rufum* were analyzed: Z7110 and Q3756. The first

appeared related to a pentaploid *P. durifolium* specimen (V12282) of natural origin (Honfi 2003). Unfortunately, there are no cytological studies that confirm the chromosome number and the meiotic behavior of the specimen Z7110 (Giussani et al. 2009). The strong relationship found between *P. durifolium* (V12282) and *P. rufum* (Z7110) had already been obtained and discussed in the *rp116/trnL-F* analyses of Giussani et al. (2009). The second voucher of *P. rufum* (Q3756) is a tetraploid specimen that appears as a sister species to a clade of groups Filiformia and Rupestris, related to Macrophylla and *P. fasciculatum*. When analyzing this same voucher, Giussani et al. (2009) related it to species of the Plicatula group, the latter also sharing the insertion of an adenine at position 1,160 of the *rpoA* gene. In our analyses, the entire clade appeared related to Plicatula in the Bayesian tree, but remains sister to Macrophylla group in the parsimony tree, even when the information from the indels was included and weighted in the analysis.

#### Diploids, polyploids and apomixis in *Paspalum*

*Paspalum* species consist of sexual-diploid and apomictic-polyploid cytotypes of which few have been shown to have arisen through hybridization (Quarín and Norrmann 1990), while most polyploids were reported as autopolyploids (Quarín and Lombardo 1986; Quarín and Norrmann 1987; Norrmann et al. 1989; Quarín et al. 1996, 1998; Hojsgaard et al. 2009; Ortiz et al. 2013). In our study, highly supported clades were obtained for some diploid–polyploid groups, such as Macrophylla, Racemosa, Plicatula, and Virgata, thus confirming the tentative autopolyploid origin of polyploid races in these groups.

It is well known that within *Paspalum* aneuploid chromosome numbers are infrequent (Table 1), while triploid, pentaploid, or hexaploid chromosome numbers reveal an irregular segregation or the interchange of an entire genome by allopolyploidy. Moreover, apomixis accompanies both polyploidy and hybridization events helping the fixation of genome rearrangement; all these processes could obscure the infrageneric classification. Therefore, the taxonomic positions obtained in our work of the pentaploid of *P. durifolium* (related to *P. rufum*) and of the hexaploid *P. inconstans* (excluded of subg. *Harpostachys*) must be confirmed. In order to carry out studies in tentative allopolyploid species and to investigate patterns of reticulate evolution by hybridization, it would be necessary to explore congruence between data from maternal line (plastidial) and from both parental lines (nuclear), amplifying and cloning fragments of single-copy nuclear genes where both genomes are represented.

#### Taxonomic treatment

*Paspalum aberrans* (Döll) Morrone and Zuloaga, comb. nov. *Reimaria aberrans* Döll, Fl. Bras. 2(2): 38, pl. 13. 1877. *Reimarochloa aberrans* (Döll) Chase, Proc. Biol. Soc. Wash. 24: 137. 1911. *Reimarochloa aberrans* (Döll) Kuhlm., Comm. Lin. Telegr., Bot. 67(11): 91. 1922, nom. illeg. -TYPE: BRAZIL. Pará: Habitat prope Santarem, R. Spruce 851 (lectotype, M, designated by Chase, Proc. Biol. Soc. Wash. 24: 137. 1911, probable isoelectotype, NY-00431444!).

*Paspalum stagnophilum* Morrone and Zuloaga, nom. nov., non *Paspalum acutum* Chase. *Reimaria acuta* Flügge, Gram. Monogr., Paspalum: 217. 1810. *Reimarochloa acuta* (Flügge) Hitchc., Contr. U.S. Natl. Herb. 12(6): 198. 1909. -TYPE: VENEZUELA: Río Orinoco, F.W.H.A. von Humboldt & A.J.A. Bonpland s.n. (holotype, B!; isotypes, HAL-0063536!, P-HUM-00669329!, US!).

=*Agrostis brasiliensis* Spreng., Novi Provent.: 45. 1818. *Reimaria brasiliensis* (Spreng.) Schltr., Bot. Zeitung (Berlin) 10:17 1852. *Reimarochloa brasiliensis* (Spreng.) Hitchc., Contr. U.S. Natl. Herb. 12(6): 198. 1909. -TYPE: BRAZIL: without locality, *Otto* s.n. (not located).

The new name makes reference to the growing habitat of the species.

*Paspalum eglume* Morrone and Zuloaga, nom. nov., non *Paspalum oligostachyum* Salzm. ex Steud. *Reimaria oligostachya* Munro ex Benth., J. Linn. Soc., Bot. 19: 34. 1881. *Reimarochloa oligostachya* (Munro ex Benth.) Hitchc., Contr. U.S. Natl. Herb. 12(6): 199. 1909. -TYPE: UNITED STATES OF AMERICA. Florida: without locality, Curtiss 3566 (lectotype, US, designated by Hitchcock, Contr. U.S. Natl. Herb. 12: 199. 1909).

The new name makes reference to the spikelet without glumes.

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