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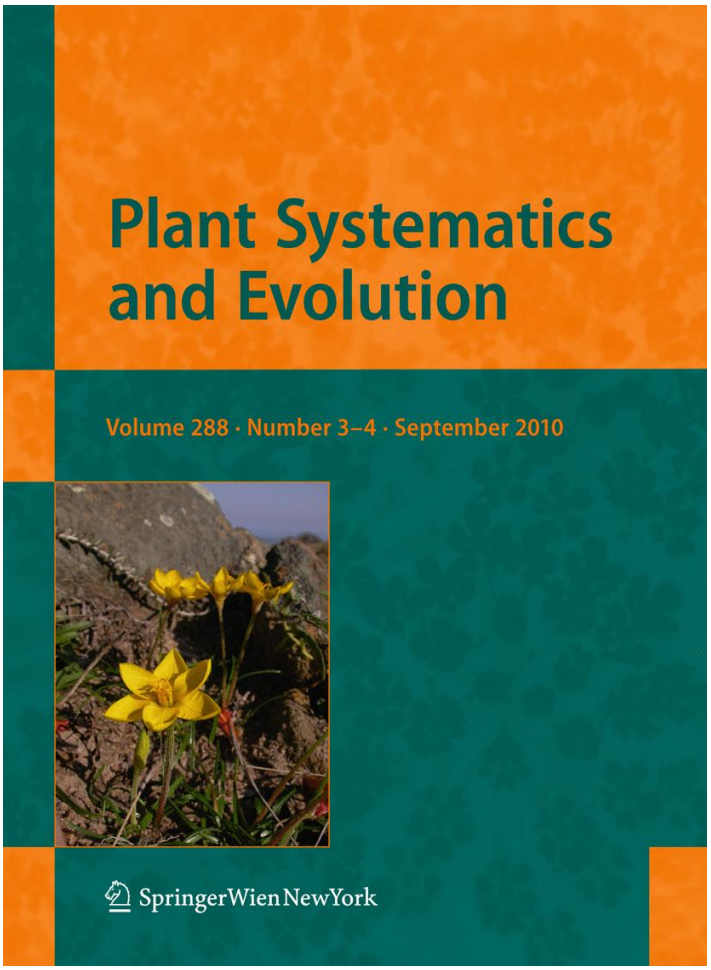

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ORIGINAL ARTICLE

A phylogenetic analysis of the genus *Paspalum* (Poaceae) based on cpDNA and morphology

Gabriel H. Rua · Pablo R. Speranza · Magdalena Vaio · Mónica Arakaki

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Abstract With about 350 species, *Paspalum* is one of the richest genera within the Poaceae. Its species inhabit ecologically diverse areas along the Americas and they are largely responsible for the biodiversity of grassland ecosystems in South America. Despite its size and relevance, no phylogeny of the genus as a whole is currently available and infrageneric relationships remain uncertain. Many *Paspalum* species consist of sexual-diploid and apomictic-polyploid cytotypes, and several have arisen through hybridization. In this paper we explore the phylogenetic structure of *Paspalum* using sequence data of four non-coding cpDNA fragments from a wide array of species which were combined with morphological data for a subset of diploid taxa. Our results confirmed the general monophyly of *Paspalum* if *P. inaequivalve* is excluded and the small genus *Thrasyopsis* is included. Only one of the four currently recognized subgenera was monophyletic but nested within the remainder of the genus. Some informal morphological groups were found to be polyphyletic. The placement of known allopolyploid groups is generally congruent with previously stated hypotheses although some species with shared genomic formulae formed paraphyletic arrangements. Other species formed a basal grade including mostly umbrophilous or hygrophilous species. It is hypothesized that the genus may have diversified as a consequence of the expansion of C4 grass-dominated grasslands in South America.

Keywords *Paspalum* · Polyploidy · cpDNA · Phylogeny · Paniceae

Introduction

With about 350 species (Denham 2005; Zuloaga and Morrone 2005), *Paspalum* L. is one of the richest genera within the Poaceae. Its species inhabit ecologically diverse areas along North, Meso, and South America, and centers of highest diversity have been recognized in the Brazilian Cerrados and the Campos of Argentina, Uruguay, and southern Brazil. A few species are found in Africa, Asia, and Oceania, and three or four can be regarded as cosmopolitan, but the genus is thought to have originated in tropical South America (Chase 1929; Nicora and Rúgolo de Agrasar 1987; Judziewicz 1990). One species, *P. scrobiculatum* L. (Kodo millet), is cultivated as a cereal in Asia, and several others including *P. notatum* Flüggé (bahiagrass) and *P. dilatatum* Poir. (dallisgrass) are regarded as valuable forage grasses (Bennett 1962; Allem and Valls 1987; Filgueiras 1992). These species have been favored by research programs; however, living collections and data on genetics and reproduction for most species are far scarcer. Species of *Paspalum* are largely responsible for the biodiversity of

G. H. Rua and P. R. Speranza contributed equally to this paper.

G. H. Rua (✉)
Cátedra de Botánica Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martín 4453, C1417DSE Buenos Aires, Argentina
e-mail: ruagabri@agro.uba.ar

P. R. Speranza · M. Vaio
Depto. de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Garzón 780, 12900 Montevideo, Uruguay

M. Arakaki
Department of Ecology and Evolutionary Biology, Brown University, 80 Waterman St., Box G-W, Providence, RI 02912, USA

grassland ecosystems in South America, which are severely threatened by the expansion of agriculture. Greater insight into the evolution of the genus as a whole can help understanding the evolution of these rich ecosystems, and consequently help us design better strategies to manage their biodiversity and to facilitate efforts to domesticate those species identified as potential crops.

Morphologically, species of *Paspalum* are characterized by their plano-convex spikelets, probably the only morphological synapomorphy for the genus. They are also recognized by their dorsiventral, raceme-like partial inflorescences and, with few exceptions, by the lack of a lower glume. Molecular analyses of the tribe Paniceae and the subfamily Panicoideae (Gómez-Martínez and Culham 2000; Duvall et al. 2001; Giussani et al. 2001) show that *Paspalum* belongs in a clade whose members share a base chromosome number of $x = 10$ and is related to other genera that have a NADP-ME photosynthetic pathway. The taxa most closely related to *Paspalum* are *Anthaenantiopsis* Mez ex Pilg., the monotypic *Hopia* Zuloaga and Morrone (Zuloaga et al. 2007), and two species currently included in *Panicum* L. s.l.: *P. validum* Mez and *P. tuerckheimii* Hack. (Gómez-Martínez and Culham 2000; Giussani et al. 2001; Aliscioni et al. 2003; Denham and Zuloaga 2007). As currently circumscribed, *Paspalum* includes the former genus *Thrasya* Kunth, which intergrades with the informal group "Decumbentes" of *Paspalum* (Burman1985; Denham 2005; Denham and Zuloaga 2007). Additionally, recent analyses have suggested that *Thrasyopsis* Parodi and *Reimarochloa* Hitchc. are also closely related to *Paspalum* and they should be probably subsumed within it (Rua et al. 2007; Scataglini et al. 2007).

Considerable taxonomic efforts have been devoted to the genus *Paspalum*, especially regarding infrageneric classification (Chase 1929, and unpubl. manuscript; Pilger 1941). The informal grouping originally proposed by Chase (1929), which is based on morphological similarities, is widely accepted. Despite the publication of a number of taxonomic revisions of particular groups in the last 10 years (Oliveira and Valls 2002; Oliveira 2004; Zuloaga and Morrone 2001, 2005; Zuloaga et al. 2004; Denham 2005; and references therein), phylogenetic hypotheses are still fragmentary (Rua and Aliscioni 2002; Denham et al. 2002; Souza-Chies et al. 2006; Denham and Zuloaga 2007; Rua et al. 2007; Scataglini et al. 2007; Giussani et al. 2009). Consequently, no phylogeny of the genus as a whole is currently available and infrageneric relationships remain uncertain and require further studies.

Many *Paspalum* species consist of sexual-diploid and apomictic-polyploid cytotypes, and several have been shown to have arisen through hybridization (Quarin and Norrmann 1990). Furthermore, interspecific hybridization and allopolyploidy may not be morphologically evident in some species complexes in *Paspalum* (Vaio et al. 2005). This fact poses further challenges to the interpretation of molecular and, particularly, morphological data and thus reticulate phylogenetic histories cannot be reconstructed by methods designed to uncover hierarchical relationships (Bachmann 2000; Linder and Riesberg 2004). Moreover, the inclusion of hybrid taxa in a phylogenetic analysis may severely disturb the inference of the relationships among other taxa included in the matrix (McDade 1992; Vriesendorp and Bakker 2005).

Uniparentally transmitted sequences are always expected to evolve hierarchically and their phylogenies can be directly inferred by conventional methods. Because of this, chloroplast DNA sequences from species of different ploidy levels can be included in a phylogenetic study even in the absence of complete information about the allo or autopolyploid nature of the species involved; moreover, as taxon sampling can be increased by using all available material at this stage, the overall phylogenetic hypothesis may even be strengthened. For this reason, although phylogenetic relationships among plastid sequences may have different degrees of incongruence with organismal phylogenies (Rieseberg and Soltis 1991), the use of chloroplast DNA sequences is more convenient and cost effective in exploratory analyses. Putatively more informative sequences such as single-copy nuclear genes (Sang 2002) or ribosomal ITS sequences (Álvarez and Wendel 2003) may involve further technical complications and interpretation, particularly if sequence polymorphism may be expected as in the case of hybrid taxa. In the case of known diploid species, on the other hand, as less incongruence is expected a priori between plastid and nuclear coded morphology, the simultaneous use of both sources of information may be attempted.

Preliminary attempts to establish major evolutionary lineages within *Paspalum* have combined different genomic sources of molecular data and morphology without previously discriminating diploid and polyploid taxa; moreover, partial taxon sampling has emphasized only specific morphological groups (Vaio et al. 2005; Souza-Chies et al. 2006; Giussani et al. 2009). These analyses have shown that sequences with different inheritance patterns clearly affect the placement of known hybrid taxa and that a larger number of species representing the overall diversity of the genus must be sampled to assess the phylogenetic relationships of the genus as a whole (Souza-Chies et al. 2006). In this paper we will explore the phylogenetic structure of the genus *Paspalum* using sequence data of four non-coding cpDNA fragments from a wide array of species and morphological data for a subset of diploid taxa.

Materials and methods

Taxon sampling

Seventy-one species of *Paspalum* were included in our analysis (Table 1), including one species formerly placed in the genus *Thrasya*. Although all members of the *Dilatata*-complex were sequenced, the assemblage was represented in our data matrix by a single sequence, because all sequences were identical (Speranza and Malosetti 2007). The two known species of *Thrasyopsis* and a representative of *Anthaenantiopsis* were also included. Two species of *Axonopus* P. Beauv. were used for the purpose of rooting the cladograms. Voucher specimens are listed in Table 1, including origin, chromosome counts (when available), informal grouping so far suggested in the literature, and GenBank sequence identifiers.

DNA isolation, sequencing, and alignment

For all plant materials, DNA was isolated from fresh leaves or silica-gel-dried leaves using the Sigma Genelute™ kit (Sigma–Aldrich, St Louis, MO, USA) according to the manufacturer's instructions. Four cpDNA regions were amplified and sequenced: the *trnL*(UAA) intron, the *trnL*(UAA)-*trnF*(GAA) spacer, the *atpB-rbcL* spacer, and the *trnG*(UCC) intron. Amplification and sequencing conditions, and primer information are reported in Vaio et al. (2005). All regions were sequenced in both directions on a CEQ 8000 capillary sequencer (Beckman-Coulter, Fullerton, CA, USA). The sequences were edited manually using Sequencher™ (V4.1.4, Genecodes, AnnArbor, MI, USA) and all ambiguous end regions removed. The resulting partial sequences were prealigned with the Clustal-W (Thompson et al. 1994) algorithm implemented in BioEdit (ver. 7.0.9.0, Hall 1999) and the resulting alignments were manually adjusted.

Morphological characters

Morphological characters were scored mainly from herbarium material deposited at BAA, CEN, CTES, MVFA, and the Herbarium of the Universidad Nacional de Misiones, Posadas, Argentina (MNES, not indexed in Holmgren and Holmgren (1998 onwards)). A set of 115 characters was defined for use in the analyses (Appendix), including 63 characters of spikelets, 20 of inflorescences, 31 of vegetative growth, and one of reproduction. Anatomic features of rachises were scored according to Aliscioni and Denham (2008). Obvious autapomorphies were not included. All 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 and Turner 1999). Missing data (including unavailable and inapplicable data) represent 7.6% of the entries in the morphological data matrix.

Phylogenetic analysis

A single matrix containing all four cpDNA sequences was assembled for the entire set of taxa (hereafter referred to as "Matrix A"). This matrix consisted of 2,223 positions 145 of which were phylogenetically informative. Indels that could not be unambiguously aligned were rendered uninformative by introducing gaps in the rest of the taxa. Indels were coded as present/absent only when the alignment of the flanking sequences was unambiguous. A total of 15 indels between 1 and 17 bp were coded and analyzed.

Parsimony analyses were performed using TNT ver. 1.1 (Goloboff et al. 2003b, 2008). Matrix A was analyzed under equal character weights. A heuristic search strategy was adopted, consisting of 10,000 random addition sequences followed by TBR swapping, using Wagner trees as starting trees and holding a maximum of two 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 analysis described above were further submitted to 20,000 iterations of Parsimony Ratchet (Nixon 1999) followed by an additional round of TBR. Branches with ambiguous support (min. length = 0) were collapsed. Group support was quantified through two measures:

1 the decay index of Bremer (BS, Bremer 1994), and
2 the symmetric jackknife frequency (SJF, Goloboff et al. 2003a).

For a model-based approach, Bayesian inference was conducted upon Matrix A using Mr. Bayes 3 (Huelsenbeck and Ronquist 2001). For this analysis, the unequal-frequency General Time Reversible plus Gamma (GTR + I + G) model of evolution was selected with MrModeltest 2.3 (Nylander 2004). Indels were coded as restriction site data following the recommendations in the software documentation. The analysis was performed for two million generations and the chain was sampled every 100 generations. The first 5,000 trees in each run were discarded as burn-in.

A second matrix (hereafter "Matrix B") was assembled combining the cpDNA and morphological data of diploid species. In the case of species where diploids were not available but polyploids had been reported to be morphologically undistinguishable from the corresponding diploids, available polyploid cytotypes were used to represent the species' morphologies. Species known to be allopolyploid, and species for which diploid cytotypes have not been reported (with exception of the two *Thrasyopsis* species, for which no cytogenetic data were available) were

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Table 1 List of the species included in the analysis, voucher specimens, country of origin, chromosome number, informal grouping (sensu Zuloaga and Morrone 2005), and GenBank accession codes

Species	Voucher	Country	2*n*	Group	*atpB–rbcL*	*trnG* intron	*trnL* intron	*trnL–trnF*
Anthaenantiopsis rojasiana Parodi	GG Roitman et al. s.n. (Herb. GH Rua 609) (BAA)	Argentina			EU627205	EU627283	EU627361	EU627439
Axonopus furcatus (Flüggé) Hitchc.	PR Speranza s.n. (CEN)	USA			EU627202	EU627280	EU627358	EU627436
Axonopus rosengurttii G.A. Black	PR Speranza s.n. (MVFA)	Uruguay			EU627206	EU627284	EU627362	EU627440
Paspalum acuminatum Raddi	A Asenjo s.n. (Herb. GH Rua 495) (BAA)	Argentina		Dissecta	EU627200	EU627278	EU627356	EU627434
Paspalum alcalinum Mez	GH Rua et al. 303 (BAA)	Paraguay	60	Livida	EU627203	EU627281	EU627359	EU627437
Paspalum almum Chase	GH Rua and J Fernández 582 (BAA)	Argentina	12	Alma	EU627204	EU627282	EU627360	EU627438
Paspalum arundinaceum Poir.	GH Rua 926 (BAA)	Argentina		Virgata?	EU627207	EU627285	EU627363	EU627441
Paspalum atratum Swallen	GH Rua et al. 261 (BAA)	Brazil	40	Plicatula	EU627208	EU627286	EU627364	EU627442
Paspalum bertonii Hack.	GH Rua et al. 482 (BAA)	Argentina	20	Bertoniana	EU627209	EU627287	EU627365	EU627443
Paspalum bicilium Mez	RC Oliveira and GH Rua 1505 (UB, BAA)	Brazil		Subg. *Ceresia*	EU627210	EU627288	EU627366	EU627444
Paspalum ceresia (Kuntze) Chase	GH Rua and L Aagesen 327 (BAA)	Bolivia	60	Subg. *Ceresia*	EU627211	EU627289	EU627367	EU627445
Paspalum chacoense Parodi	PI 404691 (BAA)	Paraguay	20	Caespitosa	EU627212	EU627290	EU627368	EU627446
Paspalum chaseanum Parodi	Saravia Toledo 13894 (CTES)	Bolivia	40	Plicatula	EU627213	EU627291	EU627369	EU627447
Paspalum commune Lillo	GH Rua and L Aagesen 325 (BAA)	Bolivia	40	Virgata	EU627214	EU627292	EU627370	EU627448
Paspalum compressifolium Swallen	Krapovickas 40758 (CTES)	Brazil	40	Plicatula	EU627215	EU627293	EU627371	EU627449
Paspalum conjugatum P.J. Bergius	GH Rua, MC Gróttola and LG Frank 178 (BAA)	Argentina	40	Conjugata	EU627216	EU627294	EU627372	EU627450
Paspalum conspersum Schrad.	CL Quarin 2319 (CTES)	Argentina	60	Virgata	EU627217	EU627295	EU627373	EU627451
Paspalum cromyorhizon Trin. ex Döll	GG Roitman and al. (Herb. GH Rua 462) (BAA)	Argentina		Notata	EU627219	EU627297	EU627375	EU627453
Paspalum denticulatum Trin.	GH Rua 466 (BAA)	Argentina	40	Livida	EU627220	EU627298	EU627376	EU627454
Paspalum gr. *Dilatata*	MVFA26505	Uruguay		Dilatata	EU627221	EU627299	EU627377	EU627455
Paspalum distichum L.	GH Rua and L Aagesen 323 (BAA)	Bolivia	60	Disticha	EU627222	EU627300	EU627378	EU627456
Paspalum durifolium Mez	GH Rua et al. 492 (BAA)	Argentina		Ungrouped	EU627223	EU627301	EU627379	EU627457
Paspalum ellipticum Döll	GH Rua et al. 472 (BAA)	Argentina		Notata	EU627224	EU627302	EU627380	EU627458
Paspalum equitans Mez	GH Rua et al. 486 (BAA)	Argentina		Ungrouped	EU627225	EU627303	EU627381	EU627459
Paspalum erianthum Nees ex Trin.	RC Oliveira and GH Rua s.n. (Herb. GH Rua 613) (BAA)	Brazil		Eriantha	EU627226	EU627304	EU627382	EU627460
Paspalum exaltatum J. Presl	P Laterra s/n (Herb. GH Rua 915) (BAA)	Argentina	20	Quadrifaria	EU627227	EU627305	EU627383	EU627461
Paspalum falcatum Nees ex Steud.	C Quarin 4052 (CTES)	Brazil	20	Falcata	EU627228	EU627306	EU627384	EU627462
Paspalum fasciculatum Willd. ex Flüggé	C Quarin 3934 (CTES)	Brazil	20	Fasciculata	EU627229	EU627307	EU627385	EU627463
Paspalum flavum J. Presl	M Arakaki and Y Ramírez 1590 (CEN)	Perú		Racemosa	EU627230	EU627308	EU627386	EU627464
Paspalum foliiforme S. Denham	RC Oliveira and GH Rua s.n. (Herb. GH Rua 615) (BAA)	Brazil		Subg. *Harpostachys*	EU627231	EU627309	EU627387	EU627465
Paspalum glabrinode (Hack.) Morrone and Zuloaga	GH Rua et al. 177 (BAA)	Argentina	20	Ungrouped	EU627232	EU627310	EU627388	EU627466
Paspalum guenoarum Arechav.	GH Rua et al. 567 (BAA)	Paraguay	40	Plicatula	EU627233	EU627311	EU627389	EU627467

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Table 1 continued

Species	Voucher	Country	2*n*	Group	*atpB–rbcL*	*trnG* intron	*trnL* intron	*trnL–trnF*
Paspalum haumanii Parodi	C Quarin 3860 (CTES)	Argentina	20	Quadrifaria	EU627234	EU627312	EU627390	EU627468
Paspalum humboldtianum Flüggé	GH Rua and L Aagesen 356 (BAA)	Bolivia	20	Subg. *Ceresia*	EU627235	EU627313	EU627391	EU627469
Paspalum inaequivalve Raddi	GH Rua et al. 209 (BAA)	Paraguay	60	Inaequivalvia	EU627236	EU627314	EU627392	EU627470
Paspalum inconstans Chase	GH Rua and L Aagesen 353 (BAA)	Bolivia		Subg. *Harpostachys*	EU627237	EU627315	EU627393	EU627471
Paspalum indecorum Mez	GH Rua et al. 490 (BAA)	Argentina	20	Caespitosa	EU627238	EU627316	EU627394	EU627472
Paspalum intermedium Munro ex Morong and Britton	GH Rua et al. 35 (BAA)	Paraguay	20	Quadrifaria	EU627239	EU627317	EU627395	EU627473
Paspalum ionanthum Chase	GH Rua et al. 309 (BAA)	Argentina	40	Notata	EU627240	EU627318	EU627396	EU627474
Paspalum juergensii Hack.	GH Rua and L Aagesen 354 (BAA)	Bolivia		Paniculata	EU627241	EU627319	EU627397	EU627475
Paspalum aff. jujuyense Zuloaga	GH Rua et al. 507 (BAA)	Paraguay	40	Livida	EU627201	EU627279	EU627357	EU627435
Paspalum lenticulare Kunth	GH Rua et al. 45 (BAA)	Paraguay	40	Plicatula	EU627244	EU627322	EU627400	EU627478
Paspalum lepton Schult.	GH Rua 920 (BAA)	Argentina		Plicatula	EU627242	EU627320	EU627398	EU627476
Paspalum lilloi Hack.	GH Rua et al. 127 (BAA)	Argentina	20	Bertoniana	EU627243	EU627321	EU627399	EU627477
Paspalum lineare Trin.	T Killeen 2218 (CTES)	Bolivia	80	Notata	EU627245	EU627323	EU627401	EU627479
Paspalum maculosum Trin.	GH Rua et al. 487 (BAA)	Argentina	20	Notata	EU627246	EU627324	EU627402	EU627480
Paspalum malacophyllum Trin.	JFM Valls et al. 14855 (CEN)	Brazil	20	Subg. *Anachyris*	EU627247	EU627325	EU627403	EU627481
Paspalum mandiocanum var. subaequiglume I.L. Barreto	GH Rua et al. 301 (BAA)	Paraguay	50	Corcovadensia	EU627248	EU627326	EU627404	EU627482
Paspalum modestum Mez	GH Rua et al. 146 (BAA)	Argentina	20	Plicatula	EU627249	EU627327	EU627405	EU627483
Paspalum notatum Flüggé	GH Rua et al. 296 (BAA)	Brazil	20	Notata	EU627250	EU627328	EU627406	EU627484
Paspalum orbiculatum Poir.	GH Rua et al. 590 (BAA)	Paraguay	20	Orbiculata	EU627251	EU627329	EU627407	EU627485
Paspalum ovale Nees ex Steud.	GH Rua et al. 476 (BAA)	Argentina	80	Ovalia	EU627252	EU627330	EU627408	EU627486
Paspalum palustre Mez	C Quarin 3648 (CTES)	Argentina	20	Plicatula	EU627253	EU627331	EU627409	EU627487
Paspalum paniculatum L.	GH Rua 587 (BAA)	Argentina		Paniculata	EU627254	EU627332	EU627410	EU627488
Paspalum paucifolium Swallen	GH Rua et al. 313 (BAA)	Argentina	40	Eriantha	EU627255	EU627333	EU627411	EU627489
Paspalum pilosum Lam.	GH Rua 528 (BAA)	Argentina		Subg. *Harpostachys*	EU627256	EU627334	EU627412	EU627490
Paspalum plicatulum Michx.	AI Honfi 14 (CTES, MNES)	Argentina	20	Plicatula	EU627257	EU627335	EU627413	EU627491
Paspalum polyphyllum Nees ex Trin.	D Hojsgaard 264 (MNES)	Argentina	40	Subg. *Ceresia*	EU627258	EU627336	EU627414	EU627492
Paspalum pumilum Nees	GH Rua et al. 592 (BAA)	Argentina		Notata	EU627259	EU627337	EU627415	EU627493
Paspalum quadrifarium Lam.	PR Speranza 27 (MVFA)	Uruguay	20	Quadrifaria	EU627260	EU627338	EU627416	EU627494
Paspalum quarinii Morrone and Zuloaga	JFM Valls 11268 (CEN)	Brazil	40	Quadrifaria	EU627261	EU627339	EU627417	EU627495
Paspalum remotum J. Rémy	GH Rua 543 (BAA)	Bolivia	80	Livida	EU627262	EU627340	EU627418	EU627496
Paspalum repens P.J. Bergius	GH Rua et al. (BAA)	Paraguay		Dissecta	EU627263	EU627341	EU627419	EU627497
Paspalum rufum Nees ex Steud.	GH Rua and IB Boccaloni 156 (BAA)	Argentina		Ungrouped	EU627264	EU627342	EU627420	EU627498

Table 1 continued

Species	Voucher	Country	2n	Group	*atpB–rbcL*	*trnG* intron	*trnL* intron	*trnL–trnF*
Paspalum scrobiculatum L.	GH Rua 520 (BAA)	Argentina		Plicatula	EU627265	EU627343	EU627421	EU627499
Paspalum setaceum Michx.	PR Speranza s.n. (CEN)	USA	20	Setacea	EU627267	EU627345	EU627423	EU627501
Paspalum simplex Morong	GH Rua et al. 308 (BAA)	Argentina	40	Subg. *Anachyris*	EU627268	EU627346	EU627424	EU627502
Paspalum stellatum Humb. and Bonpl. ex Flüggé	GH Rua et al. 192 (BAA)	Argentina		Subg. Ceresia	EU627269	EU627347	EU627425	EU627503
Paspalum trichostomum Hack.	RC Oliveira and GH Rua 1502 (UB, BAA)	Brazil		Caespitosa	EU627272	EU627350	EU627428	EU627506
Paspalum unispicatum (Scribn. and Merr.) Nash	I Boccaloni (Herb. GH Rua 506) (BAA)	Argentina	40	Subg. *Harpostachys*	EU627273	EU627351	EU627429	EU627507
Paspalum usteri Hack.	AI Honfi 1175 (MNES)	Argentina	40	Subg. *Anachyris*	EU627274	EU627352	EU627430	EU627508
Paspalum vaginatum Sw.	GH Rua et al. 559 (BAA)	Paraguay		Disticha	EU627275	EU627353	EU627431	EU627509
Paspalum virgatum L.	M Tourn and F Perret s.n. (BAA)	French Guiana	40	Virgata	EU627276	EU627354	EU627432	EU627510
Paspalum wrightii Hitchc. and Chase	C Quarin 4158 (CTES)	Argentina	40	Plicatula	EU627277	EU627355	EU627433	EU627511
Thrasyopsis juergensii (Hack.) Soderstr. and A.G. Burm.	GH Rua and JL Rosa 728 (CEN)	Brazil			EU627270	EU627348	EU627426	EU627504
Thrasyopsis repanda (Nees) Parodi	GH Rua s.n. (CEN)	Brazil			EU627271	EU627349	EU627427	EU627505

excluded from this matrix. The resulting Matrix B contained 3.5% of missing entries, including both unavailable and inapplicable data.

Because of the high levels of homoplasy found in preliminary assessments of the morphological data set, Matrix B was analyzed using implied weights (Goloboff 1993). When using implied weights, TNT downweights homoplastic characters in proportion to their amount of extra steps (homoplasy), and saves trees that minimize "distortion" (*D*), which is an increasing function of homoplasy (Goloboff et al. 2003b).

$$D = e/(e + k),$$

where e = extra steps and k = constant of concavity.

The strength with which a homoplastic character is downweighted depends on the concavity value (k) of the weighting function: the lower the k value the stronger the weighting function. To explore the stability of the results, analyses were performed under 33 different k values. Because distortion is not a linear function of concavity, k values were selected in such a way that they produce regular distortion increments of 1.25%, within a range of 50–90% related to an average non-homoplastic character (Mirande 2009). To test tree stability related to variations of k, comparisons between pairs of contiguous trees (i.e. between trees obtained using k_n and k_{n-1}) were performed, by calculation of

1 SPR-difference, i.e. the number of SPR-swaps required to convert tree n into tree $n - 1$;
2 number of shared taxa (=nodes in agreement subtree); and
3 number of shared groups (=nodes in strict consensus tree).

Calculation of k values, tree searches, and calculation of stability measures were all performed using a TNT script written by J. Marcos Mirande (unpublished), who kindly made it available to us. Because support measures are not comparable when using different weighting functions, BS and SJF values were independently calculated for each concavity. The searching routine was otherwise identical with that described for Matrix A.

Congruence of both data sets was tested through ILD test (Farris et al. 1995) under k values ranging between 3 and 20, using a custom TNT script (Goloboff et al. 2003b; Ramírez 2006).

Results

Matrix A

As a result of the parsimony analysis of Matrix A, 31,080 equally parsimonious trees were found, each 426 steps long

(CI = 0.75, RI = 0.82). The strict consensus obtained with the parsimony analysis is shown in Fig. 1. The trees obtained with parsimony and Bayesian approaches were fully congruent; furthermore, the tree obtained with Mr. Bayes was nearly identical with the majority rule consensus of the trees obtained with parsimony (not shown). Almost all the nodes present in the parsimony consensus tree received 100% posterior probabilities in the Bayesian analysis, the nodes receiving lower posterior probabilities are indicated in Fig. 1. Only one node not present in the parsimony consensus received 100% posterior probability in the Bayesian analysis. This node was included in Fig. 1 and shown as a dashed line.

In all trees (Fig. 1) [*Paspalum* + *Anthaenantiopsis*] formed a strongly supported clade (node 1), and a clade comprising [*Anthaenantiopsis rojasiana* + *P. inaequivalve*] (node 2) was sister to the remaining species of *Paspalum* (node 3).

The basal region of the tree was poorly resolved and mostly included umbrophilous species with more or less plagiotropic culms (*P. orbiculatum*, *P. flavum*, *P. conjugatum*, *P. setaceum*, *P. mandiocanum*, and *P. inconstans*) and floating grasses (*P. repens* and *P. acuminatum*). This poorly resolved portion of the tree also included the rhizomatose-stoloniferous, heliophilous species *P. distichum* and *P. vaginatum*, and *P. glabrinode*, a morphologically very distinct species. The pairs [*P. distichum* + *P. vaginatum*] and [*P. mandiocanum* + *P. inconstans*] were highly supported, whereas [*P. conjugatum* + *P. repens*] formed a moderately supported clade (node 4).

A weakly supported clade (node 5) although with a 100% posterior probability comprised all the remaining species, distributed in two major clades (nodes 6 and 11). These clades appeared consistently, although weakly supported in all analyses. An unresolved region in this clade includes *P. almum*, *P. ceresia*, and three species belonging to subg. *Harpostachys* (Denham 2005). One of the two major clades (node 6, hereafter referred to as the "CQPA" clade) including mostly species with paired spikelets, showed a basal polytomy from which *P. durifolium* and three clades emerge. One of these (node 7) was weakly supported and roughly corresponds to the informal group "Caespitosa" + *P. fasciculatum*. The second clade (node 8) was, in contrast, highly supported, and included *P. intermedium* and allied species, currently members of the "Quadrifaria" group, plus *P. ovale* ("Ovalia" group) and *P. denticulatum* + *P. aff. jujuyense* ("Livida" group). Finally, a third clade (node 9) included the remaining species of the group "Quadrifaria" (*P. quadrifarium* and *P. quarinii*), the group "Paniculata", *P. remotum* ("Livida" group), and a highly supported clade (node 10) containing the group "Humboldtiana" (sensu Parodi and Nicora, unpubl. manuscript, also including *P. paucifolium*), *P. falcatum*, and subg. *Anachyris*.

The other major clade (node 11, hereafter the "NPBT" clade) contained species belonging to groups "Notata" sensu lato (including the former group "Linearia", Zuloaga et al. 2004; Souza-Chies et al. 2006), "Plicatula" (Oliveira 2004; Oliveira and Valls 2008), and "Bertoniana" (Zuloaga and Morrone 2005). It also includes *P. stellatum* (subgen. *Harpostachys*), *P. alcalinum* (group "Livida"), *P. rufum* (ungrouped according to Zuloaga and Morrone 2005), and a highly supported clade comprising the two species of *Thrasyopsis* (node 12). Also well supported were the clades corresponding to group "Bertoniana" (node 13) and to two groups of species belonging to the "Plicatula", whereas the group "Plicatula" itself remained unresolved.

Matrix B

Although morphological and molecular data sets were significantly incongruent at 99% confidence level under all *k* values tried, combined analysis were performed in order to maximize the explanatory power of the available evidence (Nixon and Carpenter 1996). Nevertheless, both data sets were also analyzed separately for comparison.

Analysis of Matrix B yielded eight different trees when analyzed under 33 values of *k* ranging between 2.3 and 20.5 (with a single most parsimonious tree under each *k* value).The most stable topology (Fig. 2) was obtained under *k* ranging between 3.1 and 7.8. When molecular data were analyzed separately, 36 most parsimonious trees were obtained under all concavity values tried (results not shown).

Combined analysis of Matrix B yielded a weakly supported monophyletic genus *Paspalum*, with *Anthenantiopsis* as its sister group. Moreover, *Anthenantiopsis* was nested within *Paspalum* when searches were tried using equal weights (trees not shown). The clade comprising [*Paspalum* + *Anthenantiopsis*] was very strongly supported under all search strategies. The position of *P. inaequivalve* could not be tested further because it is a hexaploid species and polyploids were explicitly excluded from Matrix B. Most groups were consistent with those obtained from Matrix A; however, the species forming the basal grade on analysis of Matrix A were grouped differently, although with no jackknife support (Fig. 2). For the two main internal clades, further resolution was achieved as a consequence of the addition of the morphological signal, especially within the NPBT-clade, where monophyletic groups "Plicatula" (node 14) and "Notata" sensu stricto (node 15) were recovered (Fig. 2). Nevertheless, support for branches was not substantially improved in general and all additional nodes were weakly supported.

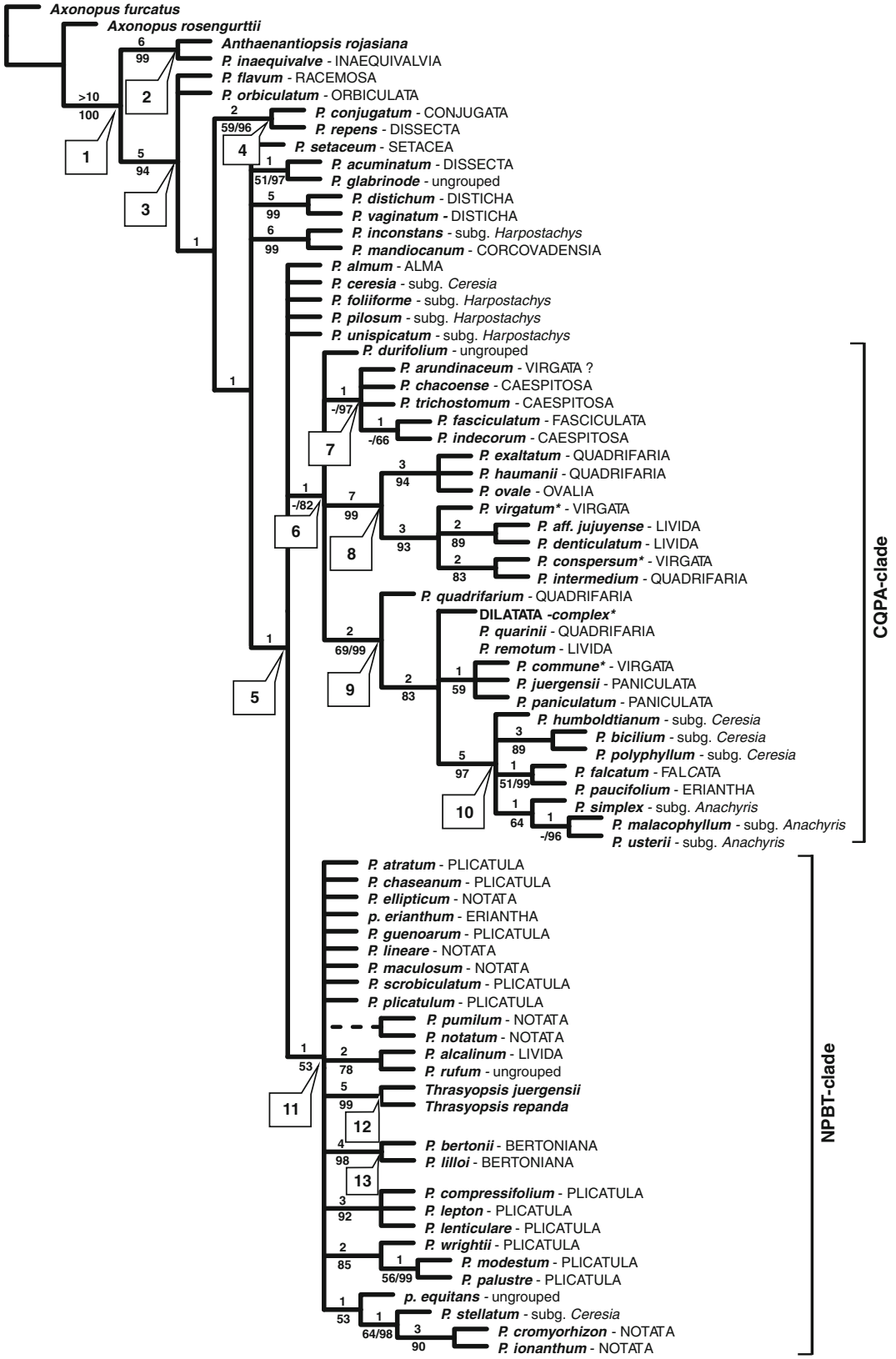

Fig. 1 Strict consensus of 30,080 most parsimonious trees obtained from analysis of Matrix A (all terminals, cpDNA data only) using equal character weighting. *Values above branches*, Bremer supports; *values below branches: left*, symmetric jackknife frequency; *right*, posterior probabilities in Bayesian analysis, only values below 100% are shown; all other nodes received 100% posterior probabilities. The branch shown in *dashed lines* had posterior probability = 100% but did not appear in the MP consensus. *Numbered nodes* are referred to in the main text

Most groups recovered in the combined analysis can be recognized by morphological synapomorphies (Fig. 3). Morphological "hard" synapomorphies (i.e. such characters states that are synapomorphic for a group and not represent homoplasies in another part of the tree) were rare and included lack of upper glumes [character 3] and plurinerved upper lemmas [ch. 58] in [*P. malacophyllum* + *P. simplex*] (representatives of subgen. *Anachyris*), multinerved upper glumes [ch. 12] in the *Thrasyopsis* clade, lack of sclerenchyma rings in rachises [ch. 73] in [*P. vaginatum* + *P. conjugatum* + *P. repens*], and air lacunae in midveins [ch. 113] in [*P. modestum* + *P. palustre*]. "Hard" synapomorphies were otherwise molecular.

The most stable characters supporting the main crown clade (node 5, Fig. 1; node 5′, Fig. 2) were outer upper glume veins approximate to margins [ch. 16], palea margins with overlapping wings [ch. 54], and the lack of enrichment branches in the inflorescence [ch. 84]. The CPQA clade was partially supported by single lateral lower lemma veins [ch. 33] and slightly indurate upper florets [ch. 42], and homogeneously or distally paired spikelets [ch. 79]. On the other hand, the NPBT clade did not show any morphological synapomorphy and it is entirely supported by molecular data. Finally, 14 groups were diagnosed exclusively by morphological synapomorphies, of which only two groups [*P. humboldtianum* + *P. paucifolium*] and [*P. cromyorhizon* + *P. equitans*] were reasonably well supported.

Discussion

Monophyly of *Paspalum* and outgroup relationships

Our results confirmed the general monophyly of *Paspalum* as currently circumscribed, i.e. including the species formerly placed in *Thrasya* (Giussani et al. 2001; Denham 2005; Denham and Zuloaga 2007). Nevertheless, the monophyly of *Paspalum* as sampled in our dataset requires either the exclusion of *P. inaequivalve* or the inclusion of *Anthaenantiopsis rojasiana*. *Paspalum inaequivalve* is a rather atypical species which is not obviously related to any other species of *Paspalum* except, perhaps, to *P. microstachyum* J. Presl (Aliscioni and Denham 2009), not included in this analysis. Further sampling of the taxa closely related to *Paspalum* is needed to assess the correct

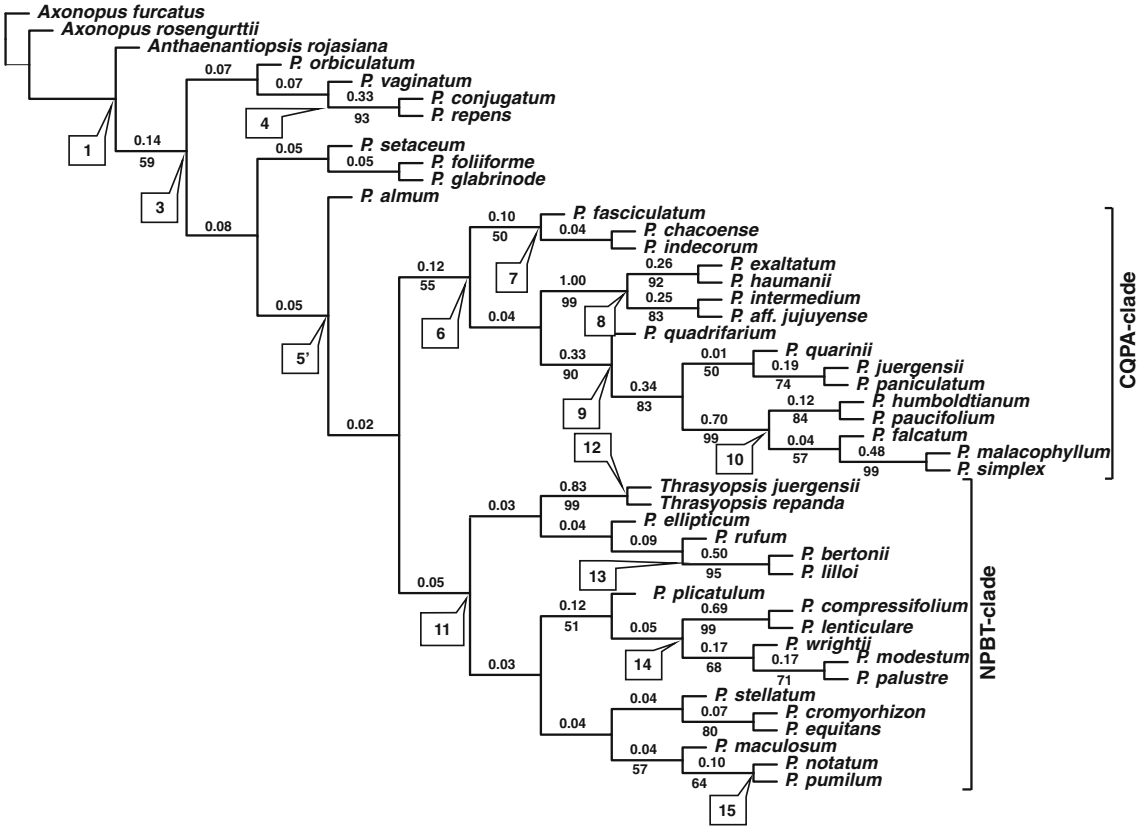

Fig. 2 Most parsimonious tree obtained from analysis of Matrix B (only terminals with known diploid cytotypes, cpDNA and morphology), using implied weights under concavity $k = 6$. *Values above branches*, Bremer supports; *values below branches*, symmetric Jackknife frequency. Nodes 1–13 as in Fig. 1, except for node 5′ which differs from node 5 in Fig. 1 for not including *P. foliiforme*; nodes 14 and 15 do not appear in Fig. 1, and they are referred to in the main text

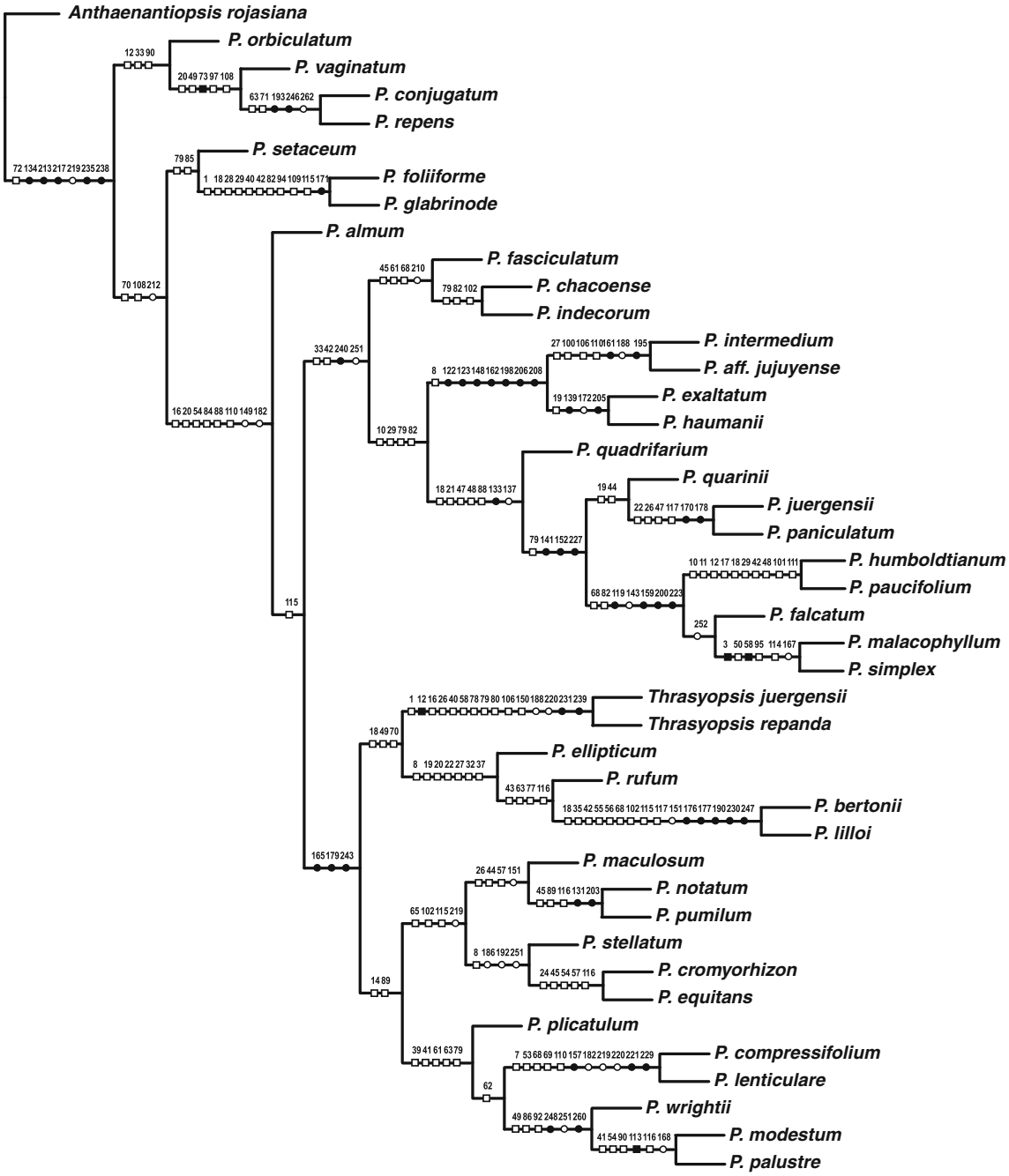

Fig. 3 Synapomorphies mapped onto the tree of Fig. 2. *Shapes on branches* represent synapomorphic changes. Autapomorphic changes are not shown. Ambiguous character reconstructions are not depicted. *Squares*, morphological characters; *circles*, cpDNA characters; *black symbols*, "hard" synapomorphies; *white symbols*, "soft" synapomorphies

placement of these species. Despite this, our data support the view that with few exceptions, the great majority of the species presently included in *Paspalum* form a well supported monophyletic assemblage.

The genus *Thrasyopsis* has been suggested to be phylogenetically related to *Paspalum* (Denham and Zuloaga 2007), and morphologically related to the informal group "Crassa" (Chase, unpubl. manuscript). In fact, in this analysis the two species of *Thrasyopsis* were deeply nested within *Paspalum* and must certainly be transferred to it (new combinations will be formally published in an ongoing paper). Unfortunately, no species of the "Crassa" group were available to assess their phylogenetic affinities with the species of *Thrasyopsis*.

Infrageneric classification

Four subgeneric entities are currently recognized within *Paspalum*: subg. *Ceresia*, subg. *Anachyris*, subg. *Harpostachys*, and subg. *Paspalum* (Denham 2005; Zuloaga and Morrone 2005). Such a subgeneric classification is not supported by our results because it does not reflect natural phylogenetic relationships. Only the species included in subgenus *Anachyris* formed a clearly monophyletic and well supported clade in this study. This assemblage consists of a morphologically distinct alliance of species characterized by having navicular, concavo-convex spikelets with an upper glume reduced or lacking and an upper flower sharply nerved (Morrone et al. 2000). This group is, however, deeply embedded within the genus, so its recognition as a subgenus would render the subgenus *Paspalum* paraphyletic.

A different situation is presented by the species included in subg. *Ceresia*. Our data suggest no close relationships between *P. stellatum* and *P. ceresia*; or between *P. stellatum* and *P. humboldtianum*, *P. polyphyllum*, and *P. bicilium*, a fact already suggested on the basis of morphological evidence (Rua and Aliscioni 2002). The grouping of the last three species together with subg. *Anachyris*, *P. paucifolium* and *P. falcatum* is relatively well supported, whereas *P. ceresia* and *P. stellatum* appear in very distant positions.

Relationships among species of subg. *Harpostachys* were very poorly resolved. These species appear as part of a grade that is basal to the two core clades of the genus. The close relationship between *P. inconstans* (subg. *Harpostachys*) and *P. mandiocanum* (subg. *Paspalum*, "Corcovadensia" group) recovered suggests that, as currently circumscribed (Denham 2005), subg. *Harpostachys* is also not monophyletic.

The taxonomy of *Paspalum* has always been a difficult issue. Delimitation of putative taxa within *Paspalum* has been obscured by frequent overlapping of character distributions probably caused by both homoplasy and hybridization. Because of this, a formal classification has long been eschewed. Indeed, the informal grouping proposed by Chase (1929) on the basis of morphological similarity has taken the place of a formal taxonomy below subgeneric rank (Zuloaga and Morrone 2005), despite the fact that Chase (1929) recognized that only "some groups … are natural aggregations of closely related species" whereas "the constituents of other groups are less obviously allied" (Chase 1929, p. 7).

Our molecular and morphological analysis shows that several currently accepted informal groups are clearly not monophyletic, whereas others probably are. It would be sound to dismiss non-monophyletic groups (see below), or perhaps to re-define them. The convenience of provisionally maintaining informal groups may be questioned, but a new, phylogeny-based circumscription for infrageneric groups is not yet possible. At the current stage of our knowledge about the phylogeny of *Paspalum*, favoring any particular taxonomic grouping seems at least premature.

Polyphyletic morphological groups

Among the morphologically identified groups that were not recovered in our analysis, "Eriantha" and "Livida" appear as particularly polyphyletic. Sampled species belonging to these two groups appear scattered over the entire topology, suggesting that they are highly artificial assemblages. The "Eriantha" group (Morrone et al. 2004) was represented in our analyses by *P. erianthum* and *P. paucifolium* only. Despite the apparent similarity of their spikelets, the former species was included in the NPBT clade, whereas the latter grouped together with the *P. humboldtianum* alliance, deeply nested within the CQPA clade. *P. erianthum* groups together with species belonging to the CQPA clade in the analysis performed by Giussani et al. (2009). High ploidy levels only have been reported for this species (Norrmann et al. 1994) and consequently a complex cytogenetic architecture cannot be ruled out. Resolving the phylogenetic affinities of this kind of species may require more detailed genetic characterization.

Four species currently classified in the "Livida" group (Zuloaga and Morrone 2005; Denham et al. 2010) were included in our analysis. *Paspalum denticulatum*, *P. aff. jujuyense*, and *P. remotum* were placed in the CQPA clade. Nevertheless, *P. denticulatum* and *P. aff. jujuyense* were nested within the *P. intermedium* alliance, whereas the Andean species *P. remotum* was rather related to the "Quadrifaria–Paniculata–Anachyris" alliance. On the other hand, *P. alcalinum* grouped together with *P. rufum*, within the NPBT clade. Despite putatively diagnostic morphological differences, *P. jujuyense* has been synonymized with *P. denticulatum* because intermediate morphologies were found which obscure the limits between both species (Zuloaga and Morrone 2005; Denham et al. 2010). Because available data are currently insufficient, both species were tentatively maintained as separate in our analysis. Nevertheless, the almost identical cpDNA sequences (only one position differs) suggest at least very close affinity between them.

The NPBT clade

This clade supported by molecular characters comprises all the species belonging in the informal groups "Notata", "Plicatula", and "Bertoniana", plus the two species currently included in the genus *Thrasyopsis*, and *P. erianthum* and *P. alcalinum*, which are currently included in groups "Eriantha" and "Livida", respectively (Zuloaga and Morrone 2005).

Monophyly of the group "Bertoniana" was highly supported by both molecular and morphological data. This group comprises only two species, *P. bertonii* and *P. lilloi*, characterized by having pilose spikelets, the upper floret open at the top, and sharply costate leaf blades (Zuloaga and Morrone 2005). Both species share a highly restricted habitat in river margins in the high Paraná basin. Relationships of the group "Bertoniana" within the NPBT-clade were ambiguously resolved.

In this analysis, the species of the "Notata" group sensu lato did not group together; however, a "core-Notata" clade including *P. notatum*, *P. pumilum*, and *P. maculosum* was recovered when morphological and sequence information were both taken into account (Matrix B, Figs. 2 and 3). The "Notata" group comprises species with solitary, mostly glabrous spikelets, and inflorescences composed of two conjugate racemes, sometimes accompanied by one or two additional, more distant ones (Chase 1929; Barreto 1957; Canto-Dorow et al. 1996; Parodi and Nicora, unpubl. manuscript). Recently (Zuloaga et al. 2004), the circumscription of the "Notata" group has been extended to include the species formerly placed in the "Linearia" group (Chase 1929; Parodi and Nicora, unpubl. manuscript; Oliveira and Valls 2002). A preliminary phylogeny based on ITS data and morphology (Souza-Chies et al. 2006) was consistent with merging the two groups, and recovered a monophyletic "core-Notata" group within a clade comprising species of both "Notata" and "Linearia" groups. *P. cromyorhizon* was related to *P. equitans*, a species alternatively excluded (Zuloaga et al. 2004) or included (Souza-Chies et al. 2006) within the "Notata" group. Despite having inflorescences with 4–7 primary branches, *P. equitans* shows clear morphological similarities with species of the "Notata" group, for example *P. ionanthum* and *P. ramboi* (Parodi and Nicora, unpubl. manuscript; Barreto 1983). On the other hand, *P. ellipticum* is currently placed in the "Notata" group sensu lato (Oliveira and Valls 2002; Zuloaga et al. 2004) despite its pilose rather than glabrous spikelets. In our analysis, *P. ellipticum* was undoubtedly placed in the NPBT clade, but its sister relationships were not clearly resolved.

The "Plicatula" group includes species characterized by a sharply plano-convex spikelet and a dark brown, shiny upper floret (Chase 1929; Oliveira 2004). Analysis of Matrix B yielded a monophyletic "Plicatula" group with a clade containing *P. wrightii*, *P. modestum*, and *P. palustre* nested within it. These three species are hydrophytic grasses sharing spongy leaf sheaths, a whitish midvein in the leaf blades, an upper floret neither so dark nor so bowed as those of typical "Plicatula" species (Parodi and Nicora, unpubl. manuscript; Barreto 1974; Oliveira 2004), and chromosome pairing affinity (Martínez and Quarin 1999). They were segregated as a different group by Barreto (1974), together with the presumably annual *P. boscianum*. Our analysis thus supports the inclusion of these species within the "Plicatula" group, as currently accepted by most authors (Oliveira 2004; Zuloaga and Morrone 2005; Oliveira and Valls 2008).

Our analysis placed *Paspalum rufum* in the NPBT clade, but its relationships with other taxa within the clade were not clear. Some accessions of this species were also allied to the "Plicatula" group in other molecular analysis (Giussani et al. 2009). This species has morphological similarities with species of the groups "Eriantha" and "Virgata" (Barreto 1954; Zuloaga and Morrone 2005) and a degree of chromosome pairing with species of the "Quadrifaria" group (Quarin and Norrmann 1990); it also shares with the "Plicatula" group the occurrence of a dark brown upper floret. As reconstructed on our phylogenetic hypothesis, chromosome pairing affinity with the "Quadrifaria" group seems to be the result of synplesiomorphy.

The placement of *P. stellatum* near *P. cromyorhizon*, and *P. equitans* was challenging. *P. stellatum* is a very distinct species, morphologically related to *P. ceresia* and other species with a dilated, membranaceous rachis currently placed in the subgenus *Ceresia* (Denham et al. 2002). Known polyploids of *P. stellatum* have unusual chromosome numbers ($2n = 32$ and 52; Honfi et al. 1990) suggesting an allopolyploid origin including at least one genome with a base chromosome number not equal to 10. Because no confirmed diploid material of this species was available, the hypothesis of allopolyploidy involving a maternal chromosome donor belonging to the NPBT clade is to be further explored. On the other hand, inclusion of diploid material of this species would be necessary to analyze its phylogenetic relationships.

The CQPA clade

The CQPA clade includes, distributed in three subclades, representatives of informal groups "Caespitosa", "Quadrifaria", and "Paniculata", and of subgenus *Anachyris*, along with several species currently placed in different groups. One of these clades corresponds to the "Caespitosa" group and it also includes *P. arundinaceum*, a species currently assigned to the "Virgata" group (Chase 1929; Judziewicz 1990) or at least related to it (Giussani et al. 2009). This clade further includes *P. fasciculatum*, a morphologically distinct, creeping, paludose grass with flabellate inflorescences, which is currently ascribed to the monotypic "Fasciculata" group (Zuloaga and Morrone 2005). However, *P. fasciculatum* shares with the other species in this clade the occurrence of solid culms and basally constricted upper florets and the occasional occurrence of a lower glume.

The other two clades were both highly supported. Species of the informal group "Quadrifaria", comprising

robust tussock grasses with multi-racemed inflorescences (Barreto 1966, 1974; Gomes and Monteiro 1996; Zuloaga and Morrone 2005), appeared partitioned between these two groups, a result also found in a cpDNA-based analysis of the groups "Quadrifaria" and "Virgata" (Giussani et al. 2009), and supported by fluorescent in situ hybridization (FISH) data of the 45S rDNA (Vaio et al. 2005). Indeed, an alliance of species related to *P. intermedium* was placed in one clade, whereas *P. quadrifarium* and *P. quarinii* were placed in the other. Interestingly, known allopolyploid species of groups "Virgata" and "Dilatata" were also scattered in both clades, suggesting different maternal affinities (Vaio et al. 2005, see below).

The clade of *P. quadrifarium* and allied species is characterized by having upper florets with a bowed lemma and a more or less concave palea, an upper glume without folded margins, rhizomes with cataphylls, and dorsally rounded leaf sheaths, although all characters show further reversions and no one is exclusive. This clade includes the "Paniculata" group, represented by *P. paniculatum* and *P. juergensii*, and, unexpectedly, a clade comprising a group of species morphologically related to *P. humboldtianum* which includes *P. paucifolium* (currently in the "Eriantha" group), the odd *P. falcatum*, and the species included in the subgenus *Anachyris*. The morphological relationship among *P. humboldtianum*, *P. polyphyllum*, and *P. paucifolium* was already shown by Parodi and Nicora (unpubl. manuscript) who included all these species in the informal group "Humboldtiana", no longer recognized by later authors. All these species are rhizomatous grasses and have an upper glume with pilose to ciliate margins. Additionally, the sampled cpDNA fragments of *P. polyphyllum* and *P. bicilium* differ in eight positions (two positions autamorphic of *P. bicilium* and six autapomorphic of *P. polyphyllum*) and a 19 bp insertion in the *trn*L-*trn*F spacer, suggesting these entities are not conspecific as currently recognized (Zuloaga and Morrone 2005). Nevertheless, this fact needs corroboration by further sampling at both the species and population levels.

Paspalum falcatum is a very distinct species with no obvious morphological affinities, so that it has been alternatively placed in groups "Lachnea" (Chase, unpubl. manuscript), "Stellata" (Barreto 1974), or the monotypic "Falcata" (Zuloaga and Morrone 2005; Parodi and Nicora, unpubl. manuscript). The phylogenetic relationships resulting from our analysis were, however, not completely unpredictable, because *P. falcatum* shares with *P. polyphyllum* its growth habit and vegetative morphology, and with *P. malacophyllum* (subgenus *Anachyris*) its concavo-convex spikelets and ciliate rachis.

Finally *P. durifolium*, a species included in the group Quadrifaria by Barreto (1966) and Gomes and Monteiro (1996), and left ungrouped by Zuloaga and Morrone (2005), remained ungrouped within the CQPA clade. The situation of this known allopolyploid species is discussed below.

Allopolyploid groups

As stated above, the cladistic treatment of allopolyploid taxa implies several difficulties. The informal groups "Dilatata" and "Virgata" have been extensively studied and they have been shown to be entirely composed of allopolyploid species, on the basis of cytogenetic evidence (Fernandes et al. 1968; Burson et al. 1973; Burson 1978, 1979, 1983, 1991, 1995; Burson and Bennett 1976; Burson and Quarin 1982; Caponio and Quarin 1990; Honfi et al. 1990; Quarin and Caponio 1995). Chromosome-pairing data have shown that members of the groups "Dilatata" and "Virgata" have partially homologous genomes. The tetraploid members of the Dilatata group have been assigned the IIJJ genomic formula (reviewed in Speranza 2009) whereas the species included in the "Virgata" group, *P. virgatum* and *P. conspersum* have been assigned the genomic formulae I_2I_2JJ and IIJ_2J_2, respectively (Burson 1978; Burson and Quarin 1982). The I and J genomes were originally named after *P. **i**ntermedium* and *P. **j**uergensii* belonging in the groups "Quadrifaria" and "Paniculata", respectively. On the basis of the assigned genomic denominations it is clear that the genome donors for the Dilatata and Virgata groups may have been different. The apomictic members of the Dilatata group include a third genome designated X for which no sound phylogenetic hypotheses are available. This genome is thought to have been contributed to the group by a paternal progenitor and consequently no information about its origin is expected to derive from the analysis of cpDNA data (Speranza 2009).

Interestingly, in our analysis, these two allopolyploid groups with partially homologous genomes were placed differently within the CQPA clade. Whereas the two well characterized species belonging to the "Virgata" group, *P. conspersum* and *P. virgatum*, were nested within the *P. intermedium* alliance, which is thought to contribute I-type genomes, *P. commune* and the entire "Dilatata" complex were grouped within the clade containing *P. quadrifarium* nearer the "Paniculata" group, a proposed source of J-type genomes. While cpDNA sequence identity within the Dilatata group suggests a narrow genetic base for the origin of all of its member species, the members of the Virgata group seem to have originated independently with maternal contributions of different members of the *P. intermedium* alliance. Another species in which cytogenetic affinities with the IIJJ genomic combination have been found is *P. durifolium* (IIJJXX) (Burson 1985). The position of the maternal genome donor for this species remained unresolved.

Whereas the J genomes have been reported for species of the "Paniculata" group only, the I-type genomes occur in species of at least the two clades into which the group "Quadrifaria" was split, and also in *P. rufum*, a species of the NPBT clade. Thus, it becomes clear from the phylogenetic relationships obtained from our data that the definitive identification of the genomic sources for the species of the "Dilatata" and "Virgata" groups is far from being achieved. The currently proposed sources of I genomes form a paraphyletic/polyphyletic assemblage, whereas the proposed donor of the J genome (presumably *P. juergensii* or *P. paniculatum*) is nested within a part of this assemblage. According to this, the ability to pair with the I-type genomes seems to be a plesiomorphic condition at least within the crown *Paspalum* species. The current assignment of the I and J genomes may have been biased by the diploid materials and knowledge available when the original crosses were made. A general understanding of the relationships among the main clades within the genus will provide the basis for a more systematic analysis of genomic relationships between the polyploid groups and diploid species in the future.

Diversification of the genus and origin of the major clades

Among the species diverging early in our phylogeny, *Paspalum inaequivalve*, *P. flavum, P. orbiculatum*, and *P. conjugatum* are creeping species inhabiting shaded forest areas or forest margins, and *P. repens* is a freely floating grass. Hence, most basally diverging lineages within *Paspalum* include hygrophytic grasses with plagiotropic culms. These species are sister to a poorly resolved backbone while branch lengths are relatively longer within the rest of the tree and autapomorphies are relatively abundant. This fact suggests an understory origin of the genus and subsequent relatively rapid radiation into open grassland habitats that gave rise to the groups containing most of the species of the genus. Interestingly, if such a scenario were correct, the evolutionary history of the genus *Paspalum* would have recapitulated the history of the family *Poaceae* as a whole (GPWG 2001).

Major diversification and expansion of C_4 grass-dominated ecosystems both globally and in South America is thought to have taken place during the Miocene–Pliocene boundary (Cerling et al. 1997; Jacobs et al. 1999). It can be hypothesized that the radiation that gave rise to the major clades of *Paspalum* may be chronologically linked to the same climatic repatterning and biological events that promoted the expansion of C_4 grasses in South America and globally. The divergence of shade-tolerant species appearing in the basal grade may have taken place before the widespread availability of open grassland habitats. Such a diversification timing is generally congruent with the chronological framework presented by Vicentini et al. (2008) where the phylogenetic origin of C_4 grass clades precedes their expansion and dominance of grassland communities. Because of the abundance, species richness, and widespread distribution of *Paspalum* in South America, further analysis of ecological and biogeographical patterns within the genus on a sound phylogenetic context may potentially provide invaluable information of the recent biogeographical history of the continent.

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Appendix

Morphological characters used for phylogenetic analysis.

1. Lower glume, presence: lacking [0], present [1]. **2.** Lower glume, position: on the medial plane of the spikelet [0], turned to one side of the spikelet [1]. **3.** Upper glume, presence: lacking [0], present [1]. **4.** Upper glume, length relative to the lower lemma: nearly equal [0], conspicuously shorter [1]. **5.** Upper glume, edge: not edge-forming [0], forming an edge around the lower lemma [1]. **6.** Upper glume, distal portion: rounded [0], obtuse [1], acute [2], acuminate [3]. **7.** Upper glume, apex: rounded [0], obtuse [1], apiculate [2], acute [3], acuminate [4], truncate [5]. **8.** Upper glume, dorsum: flat [0], bowed [1]. **9.** Upper glume, a tuft of long hairs at the base: lacking [0], present [1]. **10.** Upper glume, consistency: hyaline, tiny [0], membranous [1]. **11.** Upper glume, a marginal fringe of tuberculate hairs: lacking [0], present [1]. **12.** Upper glume, number of lateral veins on each glume half: none [0], one [1], two [2], three [3], four or more [4]. **13.** Upper glume, abaxial vein protrusion: not prominent [0], prominent [1]. **14.** Upper glume, distal convergence of inner lateral veins: not convergent [0], convergent [1]. **15.** Upper glume, midrib: lacking [0], present [1]. **16.** Upper glume, vein distribution: equidistant, no veins approximate towards

margins [0], only outer lateral veins approximate towards margins [1], all lateral veins approximate towards margins [2]. **17.** Upper glume, outer vein position: marginal [0], not marginal [1]. **18.** Upper glume, marginal region: folded [0], flat [1]. **19.** Upper glume, between-vein indumentum: glabrous to scabrous [0], pubescent at the base [1], fully pubescent [2], pubescent towards the apex [3]. **20.** Upper glume, marginal region (beyond outer veins) indumentum: glabrous to scabrous [0], scarcely pubescent at base [1], pubescent [2]. **21.** Upper glume, hair base: simple [0], with [tinged] cushions [1], both [2]. **22.** Upper glume, apex: navicular or cucullate [0], flat [1]. **23.** Upper glume, surface: smooth [0], transversely crumpled or wrinkled [1]. **24.** Upper glume, whether flabby or not: tight [0], flabby [1]. **25.** Upper glume, symmetry: symmetrical [0], asymmetrical [1]. **26.** Lower lemma, distal portion: rounded [0], obtuse [1], acute [2], acuminate [3]. **27.** Lower lemma, apex: rounded [0], obtuse [1], apiculate [2], acute [3], acuminate [4], truncate [5], emarginate [6]. **28.** Lower lemma, dorsum: flat [0], bowed [1], concave [2], sulcate [3]. **29.** Lower lemma, consistency: hyaline, tiny [0], membranous [1], laterally indurate [2]. **30.** Lower lemma, a basal tuft of hairs: wanting [0], present [1]. **31.** Lower lemma, a marginal fringe of tuberculate hairs: wanting [0], present [1]. **32.** Lower lemma, between-vein indumentum: glabrous [0], distally pubescent [1], basally pubescent [2], fully pubescent [3]. **33.** Lower lemma, number of lateral veins on each side: none [0], one [1], two [2], three [3]. **34.** Lower lemma, midvein: lacking [0], present [1]. **35.** Lower lemma, distal convergence of inner lateral veins: not convergent [0], convergent [1]. **36.** Lower lemma, marginal region: folded [0], flat [1]. **37.** Lower lemma, marginal region (beyond outer veins) indumentum: glabrous [0], pubescent [1]. **38.** Lower lemma, apex: navicular to cucullate [0], flat [1], folded [2]. **39.** Lower lemma, internerval space: smooth [0], wrinkled [1]. **40.** Lower lemma, axillary flower: wanting [0], reduced to a palea [1], well developed [2]. **41.** Upper floret, pigmentation: pale to stramineous [0], brown [1], dark brown, shining [2], purplish [3]. **42.** Upper floret, induration: not indurate [0], slightly indurate [1], strongly indurate [2]. **43.** Upper floret, length relative to the lower lemma: nearly equal [0], conspicuously shorter [1]. **44.** Upper floret, shape: elliptical [0], ovate [1], obovate [2], rhomboid [3], orbicular [4]. **45.** Upper floret, basal constriction: lacking [0], present [1]. **46.** Upper floret, callus: glabrous [0], laterally tufted [1], tufted around [2]. **47.** Upper floret, lemma dorsum: more or less flattened [0], bowed towards the base [1], sharply bowed [2], gibbose [3]. **48.** Upper floret, palea dorsum: convex [0], flat [1], concave [2]. **49.** Upper floret, lemma apex: acute, pointed [0], acute but blunt at the very apex [1], rounded [2], acuminate [3], obtuse [4]. **50.** Upper floret, lemma nerves: not prominent [0], sharply prominent [1]. **51.** Upper floret, epidermal papillae on the lemma: wanting [0], present [1]. **52.** Upper floret, abscission at maturity: none [0], occurring [1]. **53.** Upper floret, apex indumentum: wanting [0], present [1]. **54.** Upper floret, palea margins: not winged [0], with non-overlapping wings [1], with overlapping wings [2]. **55.** Upper floret, palea adaxial surface: smooth [0], papillose [1]. **56.** Upper floret, lemma apex: open [0], cucullate [1]. **57.** Upper floret, lemma margins: not thickened [0], slightly thickened [1], sharply thickened [2]. **58.** Upper floret, number of lateral veins on each lemma half: none [0], one [1], two [2], three or more [3]. **59.** Upper floret, midvein: lacking [0], present [1]. **60.** Upper floret, lemma [inner]-nerves: distant from margins [0], submarginal [1]. **61.** Upper floret, anther pigmentation: yellow [0], purple-tinged [1], deep purple [2]. **62.** Pigmentation of stigmas: whitish [0], lila [1], purple [2], yellow [3]. **63.** Upper floret, caryopsis hilum: punctiform [0], elliptical [1], linear [2]. **64.** Inflorescence, terminal spikelet: wanting [0], present [1]. **65.** 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]. **66.** Inflorescence, arrangement of primary branches II: all alternate [0], verticillate at least in the lower nodes [1]. **67.** Maximum number of orthostichies: none [0], one [1], two [2], three [3], four [4]. five [5], six [6]. **68.** Inflorescence, main axis cross section: polygonal [0], flattened [1]. **69.** Inflorescence, pubescence on pulvinula: glabrous [0], [shortly] pubescent [1]. **70.** Inflorescence, long cilia arising from pulvinula: wanting [0], present [1]. **71.** Inflorescence, a spikelet ending each primary branch: present [0], lacking, lateral spikelets becoming rudimentary towards apex [1]. **72.** Inflorescence, rachis cross section: trigonous, not expanded [0], laterally expanded into wings having chlorenchyma [1], laterally expanded into membranous epidermal wings [2]. **73.** Inflorescence, rachis, sclerenchima ring: lacking [0], present [1]. **74.** Inflorescence, rachis, medullar lacunae: lacking [0], present [1]. **75.** Inflorescence, rachis venation: a midnerve thick and prominent [0], several parallel equal-range nerves [1]. **76.** Inflorescence, rachis margin: smooth [0], scabrous [1], with more or less scattered cilia [2], with a dense fringe of tuberculate cilia [3]. **77.** Inflorescence, rachis: straight to slightly sinuous [0], sharply sinuous [1]. **78.** Inflorescence, rachis surface indumentum: glabrous [0], scabrous [1], pubescent [2]. **79.** Inflorescence, arrangement of spikelets: solitary [0], homogeneously paired [1], proximally paniculate, distally paired [2]. **80.** Inflorescence, concrescence of sPc branchlets with the rachis: (nearly) free, the spikelets appear pedicellate [0], concrescent, the spikelets appear subsessile [1]. **81.** Inflorescence, a crown of hairs at the top of pedicells: lacking [0], present [1]. **82.** Inflorescence, pedicells

cross section: terete [0], trigonous/tetragonous [1], flattened [2]. **83.**

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