

## A phylogenetic study of subtribe *Otachyriinae* (Poaceae, Panicoideae, Paspaleae)

J. M. Acosta · M. A. Scataglini · R. Reinheimer ·  
F. O. Zuloaga

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**Abstract** The subtribe *Otachyriinae* was established, within tribe Paniceae, by Butzin in 1970, originally including 18 genera. Recent phylogenetic studies, based on molecular data, indicated that *Otachyriinae* belongs to tribe Paspaleae and is represented by the genera *Anthaenantia*, *Hymenachne*, *Dallwatsonia*, *Otachyrium*, species of *Panicum* sect. *Laxa*, *Plagiantha*, and *Steinchisma*. These taxa show a peculiar diversity in photosynthetic types, including  $C_4$  species,  $C_3$ – $C_4$  intermediates and  $C_3$  species. The internal evolutionary relationships among members of *Otachyriinae* have not yet been explored in detail. With the aim of providing new evidence to understand phylogenetic relationships of taxa included in *Otachyriinae*, 21 new sequences of the *ndhF* gene from 34 species were generated and analyzed using Bayesian inference and maximum parsimony (MP). The evolution of photosynthetic pathway was studied employing MP, Markov chain Monte Carlo, and maximum likelihood methods. Our results confirm that subtribe *Otachyriinae* is monophyletic. Within the subtribe, *Anthaenantia* is a monophyletic  $C_4$  genus and is sister to the remaining species included in this treatment. *Hymenachne* and *Panicum* sect. *Laxa*, in its traditional concept, are polyphyletic; *Dallwatsonia*, *P. auritum*, and *P. hemitomon* are treated within *Hymenachne*, while three species of *Panicum* sect. *Laxa* are considered a new genus in the subtribe. The genus *Otachyrium* is paraphyletic, while all species of *Steinchisma* are recovered as a monophyletic group with high support. Finally, our data suggest that  $C_4$

and  $C_3$ – $C_4$  photosynthesis evolved just once each during the speciation of the subtribe from a  $C_3$  ancestor.

**Keywords** Poaceae · *Otachyriinae* · Phylogeny · Taxonomy ·  $C_4$  photosynthesis

### Introduction

Subfamily Panicoideae of grasses includes nearly 3,300 species and is larger than most angiosperm families (Giussani et al. 2001). Within this subfamily, two main tribes were distinguished historically, the Andropogoneae (=Sacchareae) and the Paniceae, the latter including a highly diverse and species-rich assemblage, comprising more of 2,000 species of worldwide distribution, mainly distributed in tropical and subtropical regions. Taxa within the Paniceae have a wide variety of morphological, physiological and cytological features. Diversity is expressed in variable floral development, inflorescence types, leaf anatomy and photosynthetic physiology. Pilger (1940) distinguished six subtribes in Paniceae—Antheophorinae, Boivinellinae, Lecomtellinae, Melinidinae, Panicinae and Trachidinae. Butzin (1970) considered 11 subtribes in the Paniceae, among them the *Otachyriinae*, including *Otachyrium* as the most primitive genus, along with 18 additional genera. This subtribe has been recovered, based in a few examined taxa, in subsequent molecular studies (Aliscioni et al. 2003; Duvall et al. 2001; Giussani et al. 2001). Recently, Morrone et al. (2012), proposed a new classification of Panicoideae, dividing Paniceae s.l. into two tribes, the Paspaleae, including all genera with a basic chromosome number of  $x = 10$ , and Paniceae, including genera with a basic chromosome number of  $x = 9$ . Within the Paspaleae, three subtribes are recognized by these

J. M. Acosta (✉) · M. A. Scataglini · R. Reinheimer ·  
F. O. Zuloaga  
Instituto de Botánica Darwinion, Labardén 200,  
Casilla de Correo 22, B1642HYD San Isidro, Buenos Aires,  
Argentina  
e-mail: jacosta@darwin.edu.ar

**Table 1** List of genera considered within the Otachyriinae according to Morrone et al. (2012)

Genera	Species (no.)	Distribution	Main morphological features	Physiological pathway	Historical taxonomic treatment	This treatment no. of species
<i>Anthaenantia</i> P. Beauv.	5	America	Inflorescence contracted, with spikelets pilose, densely arranged; lower glume absent, upper anthercium cartilaginous, with the upper lemma not enclosing the apex of the upper palea	C <sub>4</sub>	Kral (2004)	5
<i>Dallwatsonia</i> B.K. Simon	1	Australia	Inflorescence with spikelets unilaterally arranged on the branches, spikelets glabrous, the lower glume short, and upper anthercium membranous	C <sub>3</sub>	Simon (1992)	0
<i>Hymenachne</i> P. Beauv.	ca. 7	America Asia	Inflorescence open to contracted, spikelets glabrous, arranged unilaterally on the branches, with the upper anthercium membranous, its lemma not covering the palea at the apex	C <sub>3</sub>	–	ca. 10
<i>Otachyrium</i> Nees	8	America	Inflorescence lax and open, spikelets glabrous, with both glumes shorter than the lower lemma and the upper anthercium, the lower palea expanded and indurated at maturity, lower flower staminate, and upper anthercium indurated, smooth, dark and shining	C <sub>3</sub>	Sendulsky and Soderstrom (1984)	8
<i>Panicum</i> sect. <i>Laxa</i> (actually <i>incertae sedis</i> species)	13	America Asia	Inflorescence lax to contracted, with the spikelets unilaterally arranged on the branches, spikelets pilose or glabrous, and upper anthercium membranous to indurate	C <sub>3</sub>	Zuloaga et al. (1992) Hitchcock and Chase (1910, 1915)	5
<i>Plagiantha</i> Renv.	1	America	Inflorescence lax, without spikelets unilaterally arranged, glabrous, the lower palea expanded at maturity with two prominent wings, lower lemma 2-4-nerved, and upper anthercium cartilaginous, covered with compound papillae all over its surface	C <sub>3</sub>	Renvoize (1982)	1
<i>Steinchisma</i> Raf.	7	America	Inflorescence lax to contracted, without spikelets unilaterally arranged, glabrous, lower palea expanded and indurated at maturity, lower lemma 3-5(-7) nerved, and upper anthercium cartilaginous with compound papillae all over its surface	C <sub>3</sub> -C <sub>4</sub>	Zuloaga et al. (1992)	8

Principal morphological features, distribution, number of species and historical taxonomic treatments of each genus are included

authors, Otachyriinae, Arthropogoninae, and Paspalinae. Subtribe Otachyriinae was strongly supported based on analyses of *ndhF* independently or *ndhF* combined with morphology; it was basically defined by a molecular synapomorphy, a 9-bp deletion, and by morphological characters, such as spikelets usually arranged in unilateral branches, with the lower glume shorter than the upper glume and lower lemma, and upper anthercium membranous to indurate.

Morrone et al. (2012) considered within the Otachyriinae nearly 40 species included in the American genera *Steinchisma* Raf., *Otachyrium* Nees, *Plagiantha* Renvoize, *Anthaenantia* P. Beauv., together with *incertae sedis* species of *Panicum* sect. *Laxa*, the pantropical genus *Hymenachne* P. Beauv. and the Australian monotypic genus

*Dallwatsonia*. Information summarizing morphological details, number of species and historical taxonomic treatments of each genus is provided in Table 1.

Subtribe Otachyriinae includes C<sub>4</sub> species (i.e., *Anthaenantia*), C<sub>3</sub>-C<sub>4</sub> intermediates (i.e., *Steinchisma*), and C<sub>3</sub> species (i.e., *Dallwatsonia*, *Hymenachne*, *Panicum* sect. *Laxa*, *Plagiantha* and *Otachyrium*). Although the phylogenetic information available for grasses is constantly increasing, the reconstruction of photosynthetic transitions in grasses is problematic. Different studies offered contrasting results according to species sampling and reconstruction methods, ranging from a predominance of C<sub>4</sub> gains over C<sub>4</sub> to C<sub>3</sub> reversals (Christin et al. 2008; Giussani et al. 2001; Grass Phylogeny Working Group II 2012) to a very small number of C<sub>4</sub> origins followed by many losses

(Duvall et al. 2003; Edwards and Still 2008). Other studies inferred a complex history of multiple  $C_4$  appearances, followed by  $C_4$  to  $C_3$  reversals and secondary  $C_4$  reacquisitions (Bouchenak-Khelladi et al. 2009; Vicentini et al. 2008). The evolution of  $C_4$  photosynthesis is best studied using closely related taxa with different photosynthetic types, so subtribe Otachyriinae has a high potential to serve as a model for  $C_4$  grass evolution analyses (Grass Phylogeny Working Group II 2012). However, to make evolutionary inferences, it is necessary to have a well-resolved phylogeny. Such a phylogeny was not available for the Otachyriinae, since only some members of the group have been so far analyzed.

The purpose of our work has three principal aims: (1) to test the monophyly of the Otachyriinae subtribe (2) to establish phylogenetic relationships of species and genera in the Otachyriinae, discuss their affinities and classify them within Otachyriinae and Paspaleae, and (3) to evaluate the evolutionary trends of the photosynthetic pathway in the Otachyriinae. To accomplish these, we sampled *ndhF* sequences of additional species and analyzed them in conjunction with the data published by Morrone et al. (2012).

## Materials and methods

### Morphological analyses

Morphological studies were based on herbarium specimens from B, BAA, BM, CEN, CTES, COL, F, G, IBGE, K, LE, LIL, LPB, MEXU, MO, NY, P, SI, SP, UB, US, VEN, and W [Thiers (2013)]. A list of the species, voucher material and Genbank accession number used in the molecular study is presented in “Appendix”. Comparisons between genera in Otachyriinae based on morphological characters are shown in Table 2.

### Molecular analysis

#### Taxon sampling

The data matrix used in the phylogenetic analyses has a total of 68 sequences. The selection of ingroup and outgroup taxa was based on Morrone et al. (2012). Thirty-four species were included as ingroup, representing all genera of the Otachyriinae (i.e., *Anthaenanthia*, *Dallwatsonia*, *Hymenachne*, *Plagiantha*, *Panicum* sect. *Laxa*, *Otachyrium* and *Steinchisma*). Of these, 21 sequences are new, including three species of *Anthaenatia* (i.e., *A. rufa*, *A. texana* and *A. villosa*), two of *Hymenachne* (i.e., *H. amplexicaulis* and *H. pseudointerrupta*), six of *Otachyrium* (i.e., *O. aquaticum*, *O. grandiflorum*, *O. piligerum*, *O.*

*pterigodium*, *O. seminudum* and *O. succisum*) and three species of *Steinchisma* (i.e., *S. cuprea*, *S. exiguiiflora*, *S. stenophylla*). It should be stressed that two species of *Panicum* sect. *Laxa*. (i.e., *P. hylaeicum* and *P. pilosum*) were previously included in the Otachyriinae by Aliscioni et al. (2003); therefore, we have sequenced the other six species assigned to the *Laxa* group by Zuloaga et al. (1992) and Salaria et al. (2011): *Panicum auritum*, *P. harleyi*, *P. leptachne*, *P. polygonatum*, *P. stagnatile* and *P. stevensianum*. *Panicum hemitomom*, an ungrouped species (Aliscioni et al. 2003), was analyzed in this work since affinities with *Hymenachne* were established by Hsu (1965).

As outgroup, we used three species from subfamily Andropogoneae (i.e., *Zea mays*, *Sorghum bicolor* and *Phacelurus digitatus*) and two other taxa (i.e., *Arundinella nepalensis* and *Arundinella hirta*) from the subfamily Arundinelleae. These taxa were included in the analyses as close relatives of the subfamily Paspaleae (Morrone et al. 2012). In addition, within Paspaleae, 13 species of Paspalinae subtribe and 15 species of Arthropogoninae subtribe were included to test the monophyly of the Otachyriinae subtribe. The 21 sequences obtained for this work must be submitted to GenBank (<http://www.ncbi.nlm.nih.gov>) before the revised version is sent to editor. Voucher information and GenBank accession numbers of the complete data set are listed in “Appendix”.

#### DNA sequencing

Total genomic DNA was extracted from the silica-dried leaves with CTAB protocol (Doyle and Doyle 1987). For herbarium specimens, DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s recommendations. The plastid marker, *ndhF*, was amplified by polymerase chain reaction (PCR). The complete *ndhF* gene (ca. 2100 bp) was amplified using primers specified by Olmstead and Sweere (1994) and Aliscioni et al. (2003). For silica-dried samples, three pairs of primers were used (5F-972R, 972F-1666R and 1666F-3R). For herbarium samples, six smaller fragments were amplified using the pairs of primers 5F-536R, 536F-972R, 972F-1318R, 1318F-1666R, 1666F-1872R and 1872F-3R. For non-overlapping fragments, we filled in missing base pairs with N, although these characters just represent among 20–30 bp of the entire sequence. Percentage of missing data, excluding gaps, was 1.2 %.

PCR reactions were performed in 25  $\mu$ L final volumes with 50–100 ng of template DNA, 0.2  $\mu$ M of each primer, 25  $\mu$ M dNTP, 5 mM  $MgCl_2$  1 $\times$  buffer and 0.3 units of Taq polymerase provided by Invitrogen Life Technologies. For most of the species, PCR was carried out using the following parameters: one cycle of 94  $^{\circ}C$  for 5 min, 39 cycles

**Table 2** Comparison of taxa within subtribe Otachyriinae, based on morphological characters

	<i>Anthraenantia</i>	<i>Hymenachne</i>	<i>Rugoloa</i>	<i>Otachyrium</i>	<i>Plagiantha</i>	<i>Steinchisma</i>
Physiological pathway	C <sub>4</sub>	C <sub>3</sub>	C <sub>3</sub>	C <sub>3</sub>	C <sub>3</sub>	C <sub>3</sub> –C <sub>4</sub>
Spikelets arranged in unilateral branches	No	Yes	Yes	No	No	No
Upper anthercium ornamentation	Glabrous, with simple papillae all over the surface	With prickle hairs and simple papillae	With prickle hairs and simple papillae	Smooth and shining, without hairs and papillae	glabrous, with compound papillae all over the surface	glabrous, with compound papillae all over the surface
Lower palea expanded at maturity	No	No	No	Yes	Yes	Yes
Upper anthercium texture	Indurate	Membranous	Indurate	Indurate	Indurate	Indurate
Upper lemma with margins rolled over the palea	No	No	Yes	Yes	Yes	Yes
Lower glume	Absent	Present	Present	Present	Present	Present

of 94 °C for 30 s, 48 °C for 1 min, and 72 °C for 1 min 30 s, and a final extension cycle of 72 °C for 10 min. For the species that failed this protocol, variations in the annealing temperature (46–53 °C) were followed. In addition, a variety of PCR additives and enhancing agents (bovine serum albumin, dimethyl sulfoxide) have been used to increase the yield, specificity and consistency of PCRs. A negative control with no template was included for each series of amplifications to eliminate the possibility of contamination. PCR products were run out on a 1 % TBE agarose gel stained with SYBR Safe DNA gel stain (Invitrogen) and visualized in a blue-light transilluminator. Automated sequencing was performed by Macrogen, Inc. Forward and Reverse strands were sequenced of each template to reach the complete sequence of each voucher. Sequencing primers were similar to the amplification ones. The presence of a single peak corresponding to each nucleotide base was confirmed in all chromatograms; sequences with multiple peaks were discarded. Alignment was manually performed using BioEdit ver. 5.0.9 (Hall 1999). The aligned matrix was submitted to TreeBASE (<http://www.treebase.org>); study accession URL (<http://purl.org/phylo/treebase/phyloids/study/TB2:S14646>).

### Phylogenetic analyses

Maximum parsimony (MP) analyses were conducted using TNT ver. 1.1 (Goloboff et al. 2008). All characters were equally weighted, treated as unordered, and gaps were scored as missing data. Prior to heuristic searches, all uninformative characters were deactivated. The searches used 1,000 replicates, each of which generated ten Wagner

trees using a random addition sequence of taxa from the data matrix, swapping the initial tree with TBR (tree bisection and reconnection) and retaining a maximum of one tree in each replicate. Subsequently, all optimal trees were swapped using TBR, holding a maximum of 20,000 trees. Branches with ambiguous length of 0 or 1 were collapsed, according to collapsing rule. A strict consensus tree was generated from the MP trees. To evaluate the relative support for individual clades, bootstrap analysis (Felsenstein 1985) was performed using a total of 10,000 replicates. Each replicate was analyzed using a Wagner tree as a starting point followed by TBR branch swapping, saving only one tree per replicate. Bootstrap values (BS) over 90 % are reported.

We also conducted a phylogenetic analysis using Bayesian inference (BI) employing a general time reversible substitution model (GTR+G) chosen using the Akaike information criterion (AIC) as implemented in jModeltest 2.1.1 (Darriba et al. 2012). BI phylogenetic estimates were run for 5 million generations in MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001). Four Markov chains were run simultaneously in two independent runs starting with a random tree. Trees were sampled every 1,000 generations. Convergence diagnostics for log-likelihood values were assessed visually using Tracer v.1.5.0 software (Rambaut and Drummond 2007). Burn-in for each run was determined independently by plotting  $-\log$  likelihood against the number of generations. To calculate the Bayesian posterior probabilities (PP), trees prior to stability of the average standard deviation of split frequencies were excluded, and the remaining trees (7,502 trees) were used to generate a 50 % majority rule consensus tree.

### Ancestral states reconstruction

The photosynthetic pathway was coded as a three state discrete character [photosynthesis C<sub>3</sub> (0), photosynthesis C<sub>3</sub>–C<sub>4</sub> intermediate (1), photosynthesis C<sub>4</sub> (2)]. The character state assignment was based on previous observations reported by Brown (1977), Salariato et al. (2011) and Zuloaga et al. (1992, 1998). The character state dataset used in this study is represented in Fig. 1. Character states were estimated for nodes with a posterior probability equal to/higher than 0.95. Ancestral states of the photosynthetic pathway traits were reconstructed employing MP, Markov chain Monte Carlo (MCMC) and maximum likelihood (ML) methods.

For the MCMC analyses, we used the multistate module in BayesTraits (Pagel et al. 2004; program available at [www.evolution.rdg.ac.uk](http://www.evolution.rdg.ac.uk)) following the methodology as described by Pagel et al. (2004). The range of the hyperprior was set 0–280 based on the preliminary results under ML and the final values of ratedev were 4, since an approximate acceptance rate 20–40 % was achieved (Pagel et al. 2004). Ancestral states were estimated using the most recent common ancestor (MRCA) of selected taxa command. Once these parameters were set, two independent analyses were run for 20 million generations and sampled every 1000th generation to ensure independence. The first 2 million generations were discarded as burn-in (convergence and ESS were checked with Tracer v1.5) and the rest of the samples from the two independent analyses were combined (36,002 samples). Hypothesized character states at internal nodes were tested by estimating Bayes factor (BF). The BF approach was based on smoothed estimates of marginal likelihood analyzed with Tracer v1.5 (Rambaut and Drummond 2007), which applies the method used by Newton and Raftery (1994) with modifications by Suchard et al. (2001). A value between 2 and 5 indicates “positive” support and any value >5 means “strong” support.

For conflicting nodes, ancestral states were reconstructed by ML using the multistate module included in BayesTraits (Pagel et al. 2004) and the 7,502 trees previously calculated. Ancestral states were estimated using the MRCA of selected taxa command, and hypothesized character states at internal nodes were tested by estimating the differences of the log likelihood in which the node in question was constrained to one state versus the other using the fossil command (Pagel et al. 2004). We applied the general rule that two log-likelihood units (using the mean and median of 7,502 tree likelihoods) constitute a significant difference (Pagel et al. 2004). There was no appreciable difference between the mean and the median of the 7,502 tree likelihoods. Consequently, we only calculated the mean.

### Results

The complete *ndhF* data matrix consisted of 68 taxa and 2,068 aligned characters. Of these 2,068 characters, 252 were informative in the MP analysis. Cladistic analysis resulted in 90 MP trees, 565 steps long with CI = 0.55 and RI = 0.83 (Kluge 1989; Kluge and Farris 1969). The MP strict consensus tree was highly congruent with the BI tree, hence only the majority rule tree obtained from BI analysis is presented here (Fig. 1). Branch support obtained under MP and BI analyses are indicated on Fig. 1.

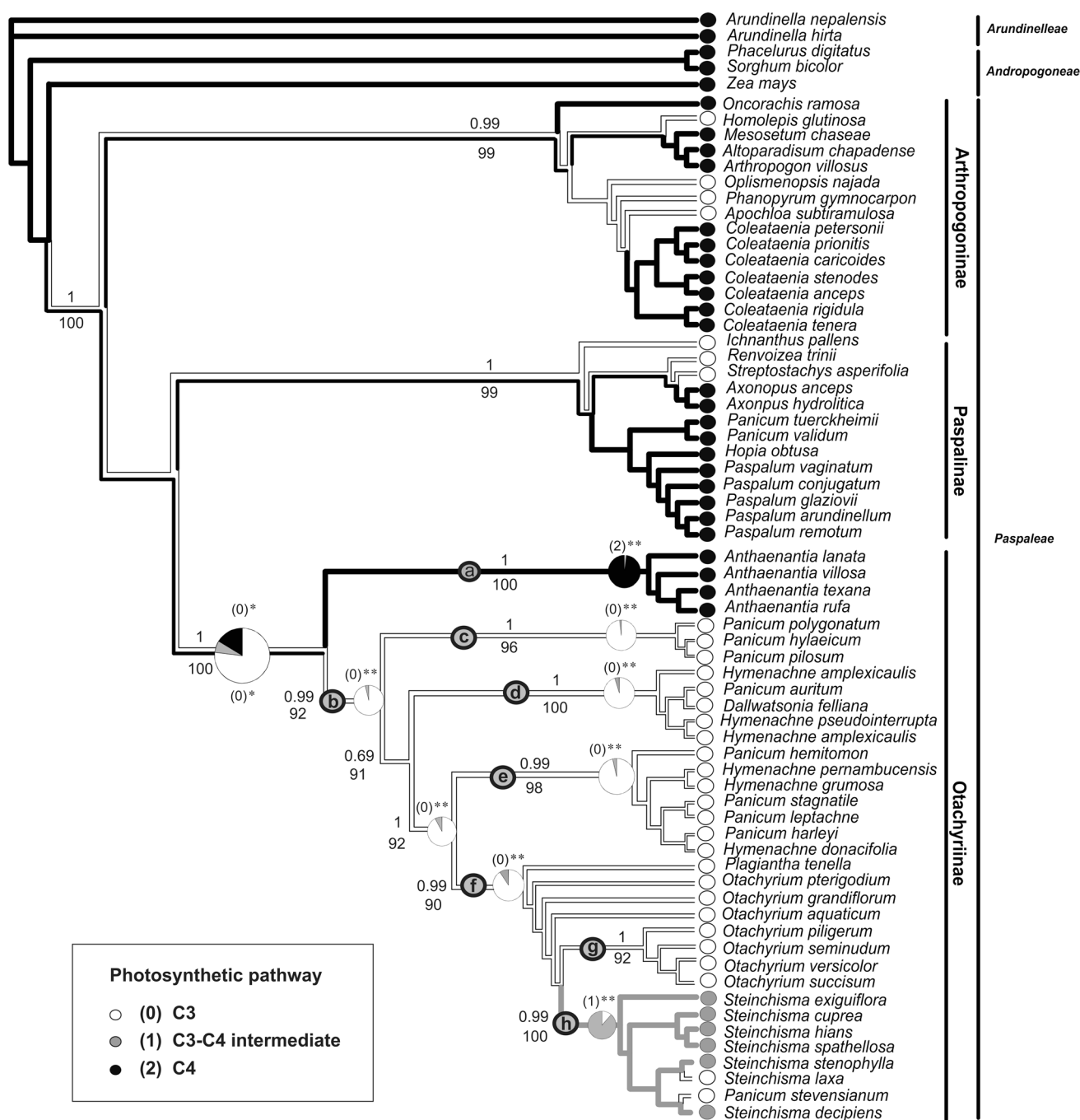
The species included in *Otachyriinae* were resolved in a very well supported group (PP: 1; BS: 100). Several distinct clades were identified within the subtribe. All species of *Anthaenanthia* grouped in the strongly supported clade **a** (PP: 1; BS: 100) indicating that the genus is monophyletic. *Anthaenanthia* appeared as the sister group of a clade **b** (Fig. 1) which included the remaining genera of the subtribe (i.e., *Dallwatsonia*, *Hymenachne*, *Otachyrium*, species of *Panicum* sect. *Laxa*, *Plagiantha* and *Steinchisma*).

Within the **b** clade, several groups were highly supported in both MP and BI analyses. *Panicum* sect. *Laxa* and *Hymenachne*, as traditionally considered, appeared as polyphyletic groups: clade **c** (PP: 1; BS: 96) comprised *Panicum hylaeicum*, *P. pilosum* and *P. polygonatum*, previously classified in *Panicum* sect. *Laxa*; clade **d** (PP: 1; BS: 100) included *H. amplexicaulis*, *H. pseudointerrupta*, *Panicum auritum* and *Dallwatsonia felliana*. Although the phylogenetic analyses showed that there are some differences among the sequences of *H. amplexicaulis*, one used by Christin et al. (2008) and the other voucher sequenced in our work, both are placed in the same clade. It is worth noting that the sequence generated by us comes from a specimen collected in South America and the sequence used by Christin et al. (2008) comes from a specimen collected in Asia. Also, clade **e** (PP: 0.99; BS: 98) comprised three species of *Panicum* sect. *Laxa* (*Panicum harleyi*, *P. leptachne* and *P. stagnatile*), one *incertae sedis* species of *Panicum*, *P. hemitonon*, and two species of *Hymenachne* (i.e., *H. pernambucensis* and *H. grumosa*).

This study also showed that *Plagiantha* and *Otachyrium* are sisters of *Steinchisma* (clade **f**, PP: 0.99; BS: 90). Even though there is a strongly supported group of *Otachyrium* species, including *O. piligerum*, *O. seminudum*, *O. succisum* and *O. versicolor* (clade **g**; PP: 1; BS: 92), the position of *Otachyrium* species appears resolved as a paraphyletic group in which *Steinchisma* is embedded. All species of *Steinchisma* along with *P. stevensianum* (Fig. 1, clade **h**,) were monophyletic with high support (PP: 0.99; BS: 100).

The ancestral photosynthetic conditions were reconstructed for the strongly supported nodes (Fig. 1). The ancestral-state reconstruction based on MP suggests a C<sub>3</sub> or C<sub>4</sub> photosynthetic pathway for the ancestor of subtribe





**Fig. 1** Majority rule BI consensus tree. The *letters enclosed in circles* indicate the clades referred in the text. The values above and below branches correspond to the Bayesian posterior probability and parsimony bootstrap support, respectively. *Branch shading* indicates maximum parsimony (MP) reconstruction for the photosynthetic pathway. Branch with *two colors* indicates an ambiguous assignment of the ancestral character state obtained under MP reconstruction. *Pie*

*charts* indicate Bayesian ancestral characters posterior probabilities. *Numbers in parenthesis* above and below the pie charts indicate the state with the highest likelihood based on the Bayes factor (BF) and maximum likelihood results, respectively. *Two numbers in parenthesis* indicates an ambiguous assignment of the ancestral character state. *Asterisk* BF between 2 and 5 (positive support); *double asterisk*, BF between 5 and 10 (strong support)

Otachyriinae. However, MCMC and ML methods indicate that the ancestor of Otachyriinae had a C<sub>3</sub> photosynthetic pathway (lnBF ≈ 2; log-likelihood difference 3.02). Under this scenario, the C<sub>4</sub> condition evolved early in the

history of the subtribe from a C<sub>3</sub> ancestor at the base of the *Anthaenantia* clade (lnBF >5), while the C<sub>3</sub>–C<sub>4</sub> intermediate type evolved later, from a C<sub>3</sub> ancestor, before the diversification of the *Steinchisma* clade (lnBF >5).

## Discussion

Subtribe *Otachyriinae* is monophyletic, in concordance with previous published phylogenies (Aliscioni et al. 2003; Morrone et al. 2012; Giussani et al. 2001). This subtribe is represented in our work by the genera *Anthaenantia*, *Hymenachne*, *Dallwatsonia*, *Otachyrium*, species of *Panicum* sect. *Laxa*, *Plagiantha*, and *Steinchisma*, together with ungrouped species *Panicum hemitomom*.

*Anthaenantia* is monophyletic and the sister group of the remaining species included in this subtribe. Several morphological characters define *Anthaenantia* at the generic level, i.e., paniculate inflorescences, lower glume absent, the second glume and lower lemma five-nerved, with at least the median three nerves flanked by narrow longitudinal rows of elongate, often papillose-base (strumose) trichomes, and two florets, the lower antherium with a glumiform lemma and a hyaline, bicarinate palea, enclosing a male floret or sterile, the upper antherium with a cartilaginous lemma and palea, and enclosing a perfect floret.

Accordingly with previous studies (Aliscioni et al. 2003), we conclude that *Panicum* sect. *Laxa* is also polyphyletic. Clade *c* contains only three species previously considered in *Panicum* sect. *Laxa* (i.e., *P. hylaeicum*, *P. pilosum* and *P. polygonatum*) in agreement with previous results (Aliscioni et al. 2003). Therefore, these species should be segregated from *Panicum* and transferred to another genus. *Panicum hylaeicum*, *P. pilosum* and *P. polygonatum* are characterized by having inflorescences with lax panicles, with spikelets  $\pm$  crowded and unilaterally disposed on the branches (*P. pilosum*) or in short branchlets (i.e., *P. hylaeicum*, *P. polygonatum*), spikelets narrowly ellipsoid, hispid with caducous hairs or glabrous, upper antherium ellipsoid, indurate, with stomata, simple papillae, and prickle hairs only present toward the apex, and silica bodies occasionally present at the tip of the lemma. No relationships of these species with other genera of the *Otachyriinae* subtribe are evident. Consequently, this clade is segregated into an independent genus of the subtribe.

Chase (1908) and Pilger (1940, 1954) distinguished *Hymenachne* from *Panicum* sensu lato by the membranous consistency of the upper antherium. Zuloaga and Soderstrom (1985) differentiated *Hymenachne* by the upper antherium texture and the absence of a lower flower; these authors indicated that species of sect. *Laxa* of *Panicum* could be congeneric with *Hymenachne* if the upper antherium texture proved to be a valid diagnostic character. Although *Hymenachne* was considered, in previous works, monophyletic (Aliscioni et al. 2003; Morrone et al. 2012), our results indicate that the genus is polyphyletic and forms two main groups. The first group consists of a strongly

supported clade of Old World species (clade *d*) of *Hymenachne pseudointerrupta* and *H. amplexicaulis*, the latter also present in America, together with another two Old World taxa, *Panicum auritum* and *Dallwatsonia felliana*. These taxa share several morphological characters, such as inflorescence an open or contracted panicle, with the spikelets arranged in unilateral racemes, spikelets ellipsoid, with the lower glume short, three-nerved, upper glume and lower lemma five-nerved, lower palea reduced or absent, lower flower absent, and upper antherium membranous, with the lemma and palea covered by simple papillae and prickle hairs towards the apex. *Panicum auritum* was the only non-American species included in the *Laxa* group by Pilger (1940) or sect. *Laxa* (Zuloaga et al. 1992); this species have been previously transferred to *Hymenachne* (Balansa, 1890), as *H. aurita* (J. S. Presl ex Nees) Balansa. According to our results, we agree with the transfer of *Panicum auritum* to the genus *Hymenachne*. Regarding *Dallwatsonia*, morphological characters of *D. felliana* are similar to those present in Old World species of *Hymenachne*; the genus shares with *Hymenachne* the presence of a lax inflorescence with spikelets unilaterally arranged on the branches, an ellipsoid spikelet, with upper glume and lower lemma subequal, membranous, lower glume shorter than the spikelet, lower antherium neuter, and upper antherium membranous, with prickle hairs and simple papillae toward the apex. Based on molecular and morphological characters, we conclude that *D. felliana* should be transferred to the genus *Hymenachne*.

The second group of *Hymenachne* includes all American species, *H. donacifolia*, *H. grumosa* and *H. pernambucensis*, together with *Panicum harleyi*, *P. hemitomom*, *P. leptachne* and *P. stagnatile*. These species are similar in overall morphology, being medium to robust plants of stream banks, with membranous ligules, inflorescence an open or contracted panicle, with the spikelets arranged in unilateral racemes, the spikelets with the lower glume short, three-nerved, upper glume and lower lemma five-nerved, lower palea reduced or absent, and upper antherium membranous, with the lemma and palea covered by simple papillae and prickle hairs towards the apex. *Panicum hemitomom*, an endemic species of eastern North America, was classified (Freckmann and Lelong 2002) as the only species of sect. *Hemitoma* Hitchc. and Chase ex Freckmann and Lelong, characterized by being the plants perennial, semi-aquatic, with panicles spiciform and spikelets secund on the inflorescences, spikelets glabrous, with the upper lemma thin, flexible, rolled over the palea only near the base. *Panicum hemitomom* differs from species of *Panicum* s. str. by the inflorescence type, upper antherium consistency, and by being a non-Kranz species with a basic chromosome number of  $x = 10$ . When treating North American species of *Panicum*, Hitchcock and Chase (1910)

mentioned that this species departs, from the typical species of *Panicum*, by its less rigid upper anthercium and by the tip of the palea not entirely inclosed by the upper lemma, defining characters of the genus *Hymenachne*. Therefore, molecular and morphological data agree with the transfer of *P. hemitomom* to *Hymenachne* by Hsu (1965). *Panicum harleyi*, *P. leptachne* and *P. stagnatile*, are three *incertae sedis* species that are placed in clade *e* together with species of *Hymenachne*, Nevertheless, and until further studies can resolve the Old World and the New World clades of *Hymenachne*, no transfer of these species to the latter genus is done in this study.

Also, it should be mentioned that three species of *Panicum* sect. *Laxa*, *P. bresolini*, *P. condensatum* and *P. longum* (Zuloaga et al. 1992), were not analyzed in this work since it was not possible to leaf material for DNA extraction. It is worth mentioning that *P. longum* is only known from the type material, while there are very few collections of *P. bresolini* and *P. condensatum*. Morphologically, these species are similar to the previous taxa mentioned, of the American clade; nevertheless, its phylogenetic position would eventually be confirmed by additional studies.

*Plagiantha* and *Otachyrium* are placed, within the highly supported clade *f*, together with *Steinchisma*. The relationship between these genera was already stressed in previous studies (Aliscioni et al. 2003; Morrone et al. 2012; Zuloaga et al. 1998), although taxon sampling has been scarce. The *Plagiantha-Otachyrium-Steinchisma* clade is morphologically characterized by the presence of an expanded, firm and thick at maturity, lower palea, and a five-nerved upper glume. Within this clade, *Plagiantha* and *Steinchisma* differ from *Otachyrium* by having the upper anthercium covered with compound papillae that are uniformly distributed over the lemma and palea; *Plagiantha* departs from *Steinchisma* by the presence of spikelets obliquely arranged on the pedicels, lower lemma two-four-nerved, being *P. tenella* a non-Kranz species.

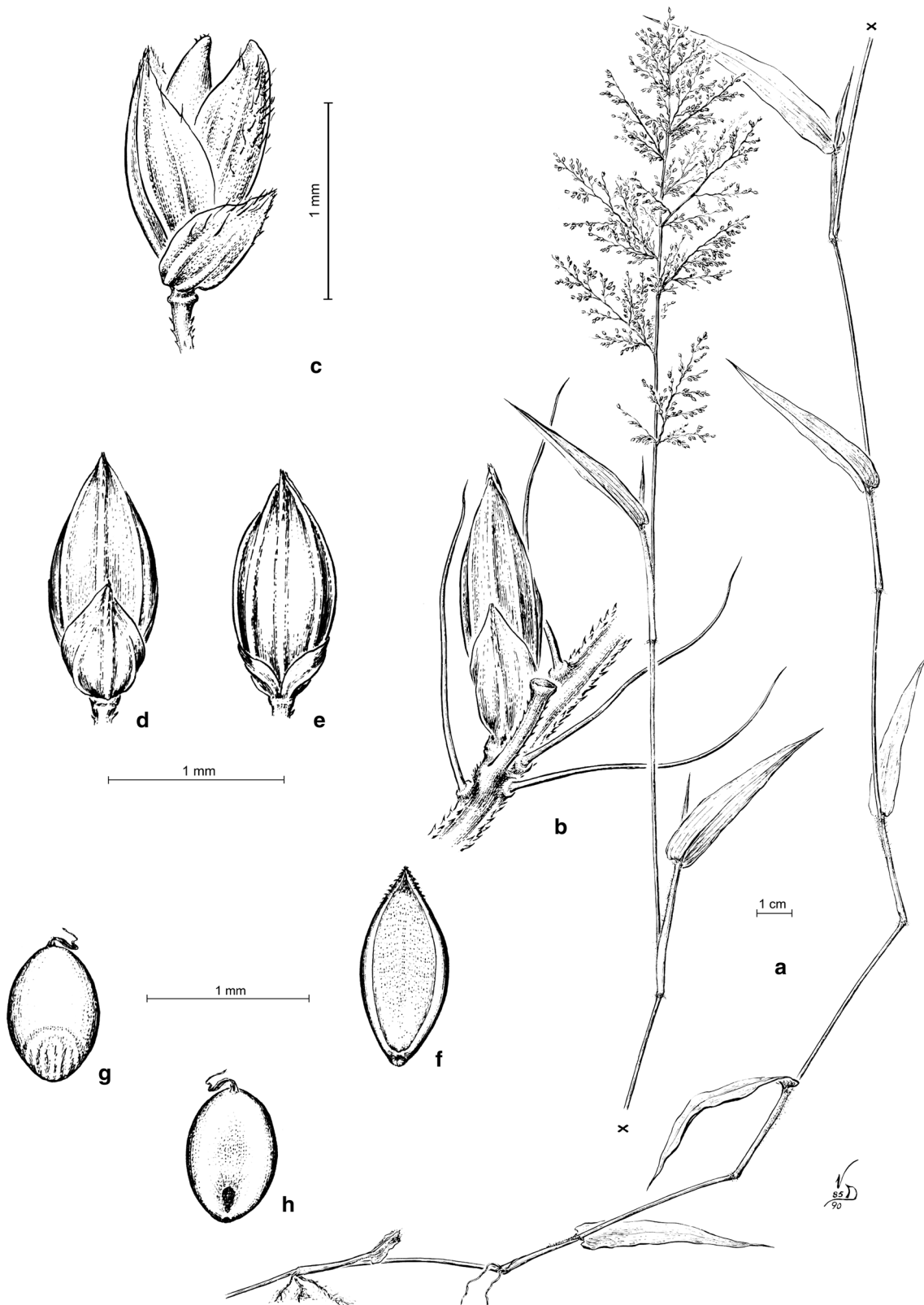
*Otachyrium* is in our analyses a paraphyletic genus. Species of this genus are widely distributed in South America, where they exhibit the highest diversity in central and northern Brazil. All species of *Otachyrium* share an expanded lower palea, and a chartaceous, dark and shining, sometimes opaque and greenish, upper anthercium. Sendulsky and Soderstrom (1984) mentioned two morphological groups within the genus, the first one integrated by *O. succisum*, *O. piligerum*, *O. seminudum* and *O. versicolor*, all taxa with a hard, gibbous, beaklike upper lemma, which is usually dark and shining. It is noteworthy to mention that these species formed a well-supported lineage in our study, (Fig. 1, clade *g*). The second group defined by Sendulsky and Soderstrom (1984), includes *O. aquaticum*, *O. grandiflorum* and *O. pterigodium*. The present study

indicates that these taxa are not monophyletic, but instead form a grade at the base of clade *g*. Consequently, monophyly of the genus and relationships of *Otachyrium* species are not fully solved here and in need of further studies.

All species of *Steinchisma* were recovered as a group, with high support (clade *h*). This result is in agreement with previous morphological and molecular studies (Aliscioni et al. 2003; Zuloaga et al. 1998) that treated *Steinchisma* as a distinct genus. *Panicum stevensianum*, previously classified in *Panicum* sect. *Laxa* (Zuloaga et al. 1992) was phylogenetically positioned in our study within the *Steinchisma* clade. This species shares with *Steinchisma* several morphological characters, such as the presence of a lower palea expanded at maturity, upper anthercium cartilaginous, covered with compound papillae, and spikelets arranged in open panicles, not in unilateral racemes. Therefore, considering these morphological similarities, and the phylogenetic position displayed in our molecular analyses, we proposed the exclusion of *P. stevensianum* from *Panicum* sect. *Laxa* and its inclusion in *Steinchisma*.

Subtribe Otachyriinae is well known by its diversity in photosynthetic types. Members of the Otachyriinae can be C<sub>3</sub>, C<sub>4</sub> or C<sub>3</sub>-C<sub>4</sub> intermediates. Based on leaf anatomy studies, species of *Anthaenantia* have C<sub>4</sub> photosynthesis; in this genus, the Kranz sheath has developed from a mestome sheath (Brown 1977). Most *Steinchisma* species have a C<sub>3</sub>-C<sub>4</sub> intermediate photosynthetic pathway. Brown et al. (1985) showed that the leaves of *S. hians* (= *Panicum milioides*) and *S. spathellosa* (= *Panicum spathellosum*), C<sub>3</sub>-C<sub>4</sub> intermediate species, are characterized by the presence of a parenchymatous vascular bundle with specialized centripetal chloroplasts (although fewer chloroplasts than those present in Kranz species), surrounded by a compact mesophyll, with small amounts of air space. Also, the number of mesophyll cells, between contiguous vascular bundles, was intermediate between C<sub>3</sub> and C<sub>4</sub> species, and this mesophyll was more radially arranged around vascular tissues than in C<sub>3</sub> species (Zuloaga et al. 1998). *Panicum laxum*, transferred to *Steinchisma* by Aliscioni et al. (2003), was considered a C<sub>3</sub> species (Brown et al. 1985; Brown 1977). However, Zuloaga et al. (1992) noted that, in some examined specimens of *S. laxa*, the anatomy tends to be C<sub>3</sub>-C<sub>4</sub> intermediate, because of the presence of specialized chloroplasts in the outer bundle sheath cells; regarding the external morphology, these specimens also have compound papillae over the upper lemma and palea, a distinguishing character of *Steinchisma*. We can conclude that the majority of species of *Steinchisma* are C<sub>3</sub>-C<sub>4</sub> intermediate, but also that this genus includes some C<sub>3</sub> species, such as *S. laxa* and *P. stevensianum*. Finally, the rest of the members of the Otachyriinae, i.e., *Hymenachne*, *Otachyrium*, *Plagiantha* and *Panicum* sect. *Laxa*, were considered as a





**Fig. 2** *Rugolola polygonata*: **a** habit, **b** branch of the panicle with a spikelet. *Rugolola hylaeica*: **c** spikelet, lateral view. *Rugolola pilosa*: **d** spikelet, ventral view; **e** spikelet, dorsal view; **f** upper anthercium, ventral view; **g** caryopsis, embryo view; **h** caryopsis, hilum view

typical C<sub>3</sub> species (Brown 1977; Zuloaga et al. 1992). Our data suggest that C<sub>4</sub> and C<sub>3</sub>–C<sub>4</sub> photosynthesis evolved just once each during the diversification of the subtribe from a C<sub>3</sub> ancestor. These results are in agreement with Morrone et al. (2012) and the Grass Phylogeny Working Group II (2012), who suggested that the plesiomorphic state in the Otachyriinae, as well as the Paniceae s.s., would be the C<sub>3</sub> photosynthetic pathway.

### Taxonomic treatment

***Hymenachne aurita*** (J. Presl ex Nees) Balansa, J. Bot. (Morot) 4: 144. 1890. *Panicum auritum* J. Presl ex Nees, Fl. Bras. Enum. Pl. 2(1): 176. 1829.—TYPE: Philippines. Luzon, *T. Haenke s.n.* (lectotype, PR!), designated by Veldkamp, Blumea 41: 187. 1996; isolectotypes, LE, MO-1837624!, MO-1837639!, W!).

***Hymenachne felliana*** (B.K. Simon) Zuloaga, comb. nov. *Dallwatsonia felliana* B.K. Simon, Austrobaileya 3(4): 678, fig. 5. 1992.—TYPE: Australia. Queensland: Cook District, Rokeby National Park, Langi Lagoon, 25 km N of Ranger Station, 30 Apr 1991, *D.G. Fell 2295* (holotype, BRI-AQ540192; isotypes, CANB, K!).

***Hymenachne hemitomom*** (Schult.) C.C. Hsu, J. Fac. Sci. Univ. Tokyo, Sect. 3, Bot. 9(3): 90. 1965. *Panicum hemitomom* Schult., Mant. 2: 227. 1824. *Panicum walteri* Muhl., Descr. Gram.: 108. 1817, nom. illeg. hom., not Pursh, 1814.—TYPE: United States of America. Georgia. “*Panicum dimidiatum* Walter. Ell. 478.” (isotype, PH-00031491!).

***Rugoloo*** Zuloaga, gen. nov.—TYPE SPECIES: *Rugoloo pilosa* (Sw.) Zuloaga (= *Panicum pilosum* Sw.).

Rhizomatous perennials, culms rooting and branching at the lower nodes, then erect; ligules membranous; blades ovate-lanceolate to lanceolate, rounded to subcordate or cordate at base. Inflorescence a terminal panicle; main axis scabrous, branches ascending, alternate or opposite, axis of the branches and pedicels scabrous or pilose, spikelets unilateral on short first- or second-order appressed branches, paired on short pedicels. Spikelets narrowly ellipsoid, pilose to glabrous; lower glume less than half the length of the spikelet, three-nerved; upper glume and lower lemma subequal, five-nerved; lower palea and lower flower present or absent; upper antherium ellipsoid, indurate; caryopsis ellipsoid, hilum punctiform.

**Etymology**—*Rugoloo* is named in honor of Prof. Zulma E. Rúgolo de Agrasar, a renowned argentinean agrostologist and our colleague at the Darwinion Institute.

1. ***Rugoloo hylaeica*** (Mez) Zuloaga, comb. nov. *Panicum hylaeicum* Mez, Notizbl. Bot. Gart. Berlin-Dahlem 7: 75. 1917. *Panicum laxum* Sw. var. *pubescens* Döll, in

C. Martius, Fl. Bras. 2(2): 213. 1877, pro parte.—TYPE: Brazil. Pará: in vicinibus Santarem, Aug 1850, *R. Spruce 10612 (Panicum 26)* (holotype, M!; isotypes, K!, P!, US-Z0080752!, fragment ex M) Fig. 2.

2. ***Rugoloo pilosa*** (Sw.) Zuloaga, comb. nov. *Panicum pilosum* Sw., Prodr.: 22. 1788. *Setaria pilosa* (Sw.) Kunth, Revis. Gramin. 1: 47. 1829. *Panicum distichum* Lam. var. *pilosum* (Sw.) Griseb., Fl. Brit. W. Ind.: 548. 1864.—TYPE. Jamaica, without locality, *O.P. Swartz s.n.* (holotype, S!; isotypes, LD-1266585!, M!, US-80916!, fragment ex S) Fig. 2.

3. ***Rugoloo polygonata*** (Schrad.) Zuloaga, comb. nov. *Panicum polygonatum* Schrad., Mant. 2: 256. 1824. *Setaria polygonata* (Schrad.) Kunth, Revis. Gram. 1: 47. 1829. *Panicum pilosum* Sw. var. *polygonatum* (Schrad.) Döll, in C. Martius, Fl. Bras. 2(2): 211. 1877.—TYPE. Brazil. Bahia: Ilheus, 1816, *Prince Maximilian s.n.* (holotype, LE!; isotypes, B, BAA-1935 !, fragment ex B, US-80925 !, fragment ex LE) Fig. 2.

***Steinchisma stevensiana*** (Hitchc. & Chase) Zuloaga, comb. nov. *Panicum stevensianum* Hitchc. & Chase, Contr. U.S. Natl. Herb. 17: 498, fig. 77. 1915.—TYPE: Puerto Rico. Campo Alegre, near Laguna del Tortuguero, 25 Nov 1913, *A. Chase 6616* (holotype, US-693323!; isotypes, NY!, US!).

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### Appendix

Sampled taxa, voucher specimens from which DNA was extracted for sequencing, and GenBank accession numbers. Specimens sequenced for this paper are in bold and underlined

#### Tribe Andropogoneae

*Phacelurus digitatus* (Sibth. & Sm.) Griseb., AF117418

*Sorghum bicolor* (L.) Moench, U21981

*Zea mays* L., U21985

#### Tribe Arundinelleae

*Arundinella hirta* (Thunb.) Tanaka, AF117393

*Arundinella nepalensis* Trin., AF117394

#### Tribe Paspaleae

##### Subtribe Arthropogoninae

*Altoparadisium chapadense* Filg. & Al., AY029619

*Apochloa subtiramulosa* (Renvoize & Zuloaga) Zuloaga & Morrone, AY188490

*Arthropogon villosus* Nees, AY029622

*Coleataenia anceps* (Michx.) Soreng, AY188455  
*Coleataenia caricoides* Nees ex Trin. Morrone 4969, GU253330  
*Coleataenia petersonii* (Hitchc. and Ekman) Soreng, AY188479  
*Coleataenia prionitis* (Nees) Soreng, AY029652  
*Coleataenia rigidula* Bosc ex Nees, AY188482  
*Coleataenia stenodes* Griseb. Zuloaga 9540, GU253333  
*Coleataenia tenera* (Beyr. ex Trin.) Soreng, AY188491  
*Homolepis glutinosa* (Sw.) Zuloaga & Soderstr., AY029637  
*Mesosetum chaseae* Luces, AY029641 (Giussani & al. 2001)  
*Oncorachis ramosa* (Zuloaga & Soderstr.) Morrone & Zuloaga, AY029686  
*Oplismenopsis najada* (Hack. & Arechav.) Parodi, AY188453  
*Phanopyrum gymnocarpon* (Elliott) Nash, AY188469  
**Subtribe Otachyriinae.**  
*Anthaenantia lanata* (Kunth) Benth., AY029640  
*Anthaenantia rufa* (Elliott) Schult., Henderson 96820; Dale Thomas 158388  
*Anthaenantia texana* Kral, Kral 93287  
*Anthaenantia villosa* (Michx.) P. Beauv., Kral 93319; Atha 3578  
*Dallwatsonia felliana* B.K. Simon, JN604685.  
*Hymenachne amplexicaulis* (Rudge) Nees, Morrone 5190  
*Hymenachne amplexicaulis* (Rudge) Nees AM849158  
*Hymenachne donacifolia* (Raddi) Chase, AY029635  
*Hymenachne grumosa* (Nees) Zuloaga, AY188468  
*Hymenachne pernambucense* (Spreng.) Zuloaga, AY188478  
*Hymenachne pseudointerrupta* Müll. Hal., Beusehom 4230  
*Otachyrium aquaticum* Send. & Soderstr., Mori 12348  
*Otachyrium grandiflorum* Send. & Soderstr., Fonseca & Alvarenga 2206  
*Otachyrium piligerum* Send. & Soderstr., Rodriguez 157  
*Otachyrium pterigodium* (Trin.) Pilg., Andersson 8880  
*Otachyrium seminudum* Hack. ex Send. & Soderstr., Walter 904; Dias 82  
*Otachyrium succisum* (Swallen) Send. & Soderstr., Jansen-Jacobs 2723; Jansen-Jacobs 2816  
*Otachyrium versicolor* (Döll) Henrard, AY029643  
*Plagiantha tenella* Renvoize, AY029674  
*Panicum auritum* J. Presl ex Nees, Olsen 144  
*Panicum harleyi* D. Salariato, Morrone & Zuloaga, Harley 5480  
*Panicum hemitomon* Schult., Jones 23315  
*Panicum hylaeicum* Mez, AY188470  
*Panicum leptachne* Döll, Longhi-Wagner 9511

*Panicum pilosum* Sw., AY188480  
*Panicum polygonatum* Schrad., Morrone 4269  
*Panicum stagnatile* Hitchc. & Chase, Harmel 8404  
*Panicum stevensianum* Hitchc. & Chase, Ekman 1088  
*Steinchisma cuprea* (Hitchc. & Chase) W.V. Br., Beetle 7708  
*Steinchisma decipiens* (Nees ex Trin.) W.V. Br., AY188499  
*Steinchisma exiguiflora* (Griseb.) W.V. Br., Zuloaga 9580  
*Steinchisma hians* (Elliot) Nash, AY029685  
*Steinchisma laxa* (Sw.) Zuloaga, AY029655  
*Steinchisma spathellosa* (Döll) Renvoize, AY188500  
*Steinchisma stenophylla* (Hack.) Zuloaga & Morrone, Veredao da Silva 3459, Mendonca 3192  
**Subtribe Paspalinae**  
*Axonopus anceps* (Mez) Hitchc., AY029623  
*Axonopus hydrolithica* Davidse and Zuloaga, AY029642  
*Hopia obtusa* (Kunth) Zuloaga & Morrone, AY029659  
*Ichnanthus pallens* (Sw.) Munro ex Benth., AY029638  
*Panicum tuerckheimii* Hack., AY188494  
*Panicum validum* Mez, AY188495  
*Paspalum arundinellum* Mez, AY029663  
*Paspalum conjugatum* Bergius, AY029669  
*Paspalum glaziovii* (A.G. Burm.) S. Denham, AY029689  
*Paspalum remotum* J. Remy, AY029668  
*Paspalum vaginatum* Sw., AY029665  
*Renvoizea trinii* (Kunth) Zuloaga & Morrone, EU107781  
*Streptostachys asperifolia* Desv., AY029687

## References

- Aliscioni SS, Giussani LM, Zuloaga FO, Kellogg EA (2003) A molecular phylogeny of *Panicum* (Poaceae: Paniceae): tests of monophyly and phylogenetic placement within the Panicoideae. *Am J Bot* 90(5):796–821
- Bouchenak-Khelladi Y, Anthony Verboom G, Hodgkinson TR, Salamin N, Francois O, Ní Chonghaile G, Savolainen V (2009) The origins and diversification of C4 grasses and savanna-adapted ungulates. *Glob Chang Biol* 15(10):2397–2417. doi:10.1111/j.1365-2486.2009.01860.x
- Brown WV (1977) The Kranz syndrome and its subtypes in grass systematics. *Mem Torrey Bot Club* 23(3):1–97
- Brown RH, Bouton JH, Evans PT, Malter HE, Rigsby LL (1985) Photosynthesis, morphology, leaf anatomy, and cytogenetics of hybrids between C3 and C3/C4 *Panicum* species. *Plant Physiol* 77(3):653–658. doi:10.1104/pp.77.3.653
- Butzin F (1970) Die systematische Gliederung der Paniceae (the systematic arrangement of the Paniceae). *Willdenowia* 6(1):179–192
- Chase A (1908) Notes on genera of Paniceae, III. *Proc Biol Soc Wash* 21:175–188

- Christin P-A, Besnard G, Samaritani E, Duvall MR, Hodkinson TR, Savolainen V, Salamin N (2008) Oligocene CO<sub>2</sub> decline promoted C<sub>4</sub> photosynthesis in grasses. *Curr Biol* 18(1):37–43. doi:[10.1016/j.cub.2007.11.058](https://doi.org/10.1016/j.cub.2007.11.058)
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Meth* 9(8):772. doi:[10.1038/nmeth.2109](https://doi.org/10.1038/nmeth.2109)
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19(1):11–15
- Duvall MR, Noll JD, Minn AH (2001) Phylogenetics of Paniceae (Poaceae). *Am J Bot* 88(11):1988–1992
- Duvall MR, Saar DE, Grayburn WS, Holbrook GP (2003) Complex transitions between C<sub>3</sub> and C<sub>4</sub> photosynthesis during the evolution of Paniceae: a phylogenetic case study emphasizing the position of *Steinchisma hians* (Poaceae), a C<sub>3</sub>–C<sub>4</sub> intermediate. *Int J Plant Sci* 164(6):949–958
- Edwards EJ, Still CJ (2008) Climate, phylogeny and the ecological distribution of C<sub>4</sub> grasses. *Eco Lett* 11(3):266–276. doi:[10.1111/j.1461-0248.2007.01144.x](https://doi.org/10.1111/j.1461-0248.2007.01144.x)
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39(4):783–791. doi:[10.2307/2408678](https://doi.org/10.2307/2408678)
- Freckmann RW, Lelong MG (2002) Nomenclatural changes and innovations in *Panicum* and *Dichantheium* (Poaceae: Paniceae). *SIDA* 20:161–174
- Giussani LM, Cota-Sanchez JH, Zuloaga FO, Kellogg EA (2001) A molecular phylogeny of the grass subfamily Panicoideae (Poaceae) shows multiple origins of C<sub>4</sub> photosynthesis. *Am J Bot* 88(11):1993–2012
- Goloboff PA, Farris JS, Nixon KC (2008) TNT, a free program for phylogenetic analysis. *Cladistics* 24(5):774–786
- Grass Phylogeny Working Group II (2012) New grass phylogeny resolves deep evolutionary relationships and discovers C<sub>4</sub> origins. *New Phytol* 193(2):304–312
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Series* 41:95–98
- Hitchcock AS, Chase A (1910) The North American species of *Panicum*. *Contr US Natl Herb* 15:1–396
- Hitchcock AS, Chase A (1915) Tropical North American species of *Panicum*. *Contr US Natl Herb* 17:459–539
- Hsu CC (1965) The classification of *Panicum* (Gramineae) and its allies with special reference to the characters of lodicule, style-base and lemma. *J Fac Sci Univ Tokyo, Sect* 3(9):43–150
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17(8):754–755
- Kluge AG (1989) A concern for evidence and a phylogenetic hypothesis of relationships among epicrates (Boidae, Serpentes). *Syst Biol* 38(1):7–25. doi:[10.1093/sysbio/38.1.7](https://doi.org/10.1093/sysbio/38.1.7)
- Kluge AG, Farris JS (1969) Quantitative phyletics and the evolution of anurans. *Syst Biol* 18(1):1–32. doi:[10.1093/sysbio/18.1.1](https://doi.org/10.1093/sysbio/18.1.1)
- Kral R (2004) An evaluation of *Anthenantia* (Poaceae). *Sida* 21:293–310
- Morrone O, Aagesen L, Scataglini MA, Salaricato DL, Denham SS, Chemsiquy MA, Sede SM, Giussani LM, Kellogg EA, Zuloaga FO (2012) Phylogeny of the Paniceae (Poaceae: Panicoideae): integrating plastid DNA sequences and morphology into a new classification. *Cladistics* 28(4):333–356. doi:[10.1111/j.1096-0031.2011.00384.x](https://doi.org/10.1111/j.1096-0031.2011.00384.x)
- Newton MA, Raftery AE (1994) Approximate bayesian inference with the weighted likelihood bootstrap. *J Royal Stat Soc Ser B (Methodological)* 56:3–48
- Olmstead RG, Sweere JA (1994) Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Syst Biol* 43(4):467–481. doi:[10.1093/sysbio/43.4.467](https://doi.org/10.1093/sysbio/43.4.467)
- Pagel M, Meade A, Barker D (2004) Bayesian estimation of ancestral character states on phylogenies. *Syst Biol* 53(5):673–684. doi:[10.1080/10635150490522232](https://doi.org/10.1080/10635150490522232)
- Pilger R (1940) Gramineae III: Unterfam. Panicoideae. In: Engler, Prantl (eds) *Die Natürlichen Pflanzenfamilien*, vol 14. 2nd edn. Leipzig, p 208
- Pilger R (1954) Das System der Gramineae unter Ausschluß der Bambusoideae. *Bot Jahrb Syst* 76(3):281–384
- Rambaut A, Drummond A (2007) Tracer, version 1.4. Computer program and documentation distributed by the author, website <http://beast.bio.ed.ac.uk/Tracer>. Accessed April 2013
- Renvoize SA (1982) A new genus and several new species of grasses from Bahia (Brazil). *Kew Bull* 37(2):323–333
- Salaricato DL, Morrone O, Zuloaga FO (2011) New species of Paniceae (Poaceae, Panicoideae) from Brazil. *Syst Bot* 36(1):53–58. doi:[10.1600/036364411x553126](https://doi.org/10.1600/036364411x553126)
- Sendulsky T, Soderstrom TR (1984) Revision of the south American genus *Otachyrium* (Poaceae: Panicoideae). *Smithsonian Cont Bot* 57:1–24
- Simon BK (1992) Studies in Australian grasses 6, *Alexfloydia*, *Cliffordiochloa* and *Dallwatsonia*, three new panicoid grass genera from Australia. *Austrobaileya* 3:669–681
- Suchard MA, Weiss RE, Sinsheimer JS (2001) Bayesian selection of continuous-time Markov chain evolutionary models. *Mol Biol Evol* 18:1001–1013
- Thiers B (2013) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>. Accessed 10 Aug 2013
- Vicentini A, Barber JC, Aliscioni SS, Giussani LM, Kellogg EA (2008) The age of the grasses and clusters of origins of C<sub>4</sub> photosynthesis. *Glob Chang Biol* 14(12):2963–2977. doi:[10.1111/j.1365-2486.2008.01688.x](https://doi.org/10.1111/j.1365-2486.2008.01688.x)
- Zuloaga FO, Soderstrom TR (1985) Classification of the outlying species of New World *Panicum* (Poaceae: Paniceae). *Smithsonian Cont Bot* 59:1–63
- Zuloaga FO, Ellis RP, Morrone O (1992) A revision of *Panicum* subgenus *Phanopyrum* section *Laxa* (Poaceae: Panicoideae: Paniceae). *Ann Mo Bot Gard* 79(4):770–818. doi:[10.2307/2399720](https://doi.org/10.2307/2399720)
- Zuloaga FO, Morrone O, Vega AS, Giussani LM (1998) Revisión y análisis cladístico de *Steinchisma* (Poaceae: Panicoideae: Paniceae). *Ann Mo Bot Gard* 85:631–656