Flora 228 (2017) 50–59

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Inflorescence diversity in subtribe Eleusininae (Poaceae: Chloridoideae: Cynodonteae)

Sebastián E. Muchut[a,b,*], Vanesa Pilatti[a,c], Nora Uberti-Manassero[a,c], Abelardo C. Vegetti[a,d], Renata Reinheimer[d,e,*]

[a] Morfología Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2805, Esperanza, Santa Fe, Argentina
[b] Fellow of Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina
[c] Fellow of Consejo Nacional de Investigaciones Científicas y Técnicas de la República (CONICET), Argentina
[d] Member of Consejo Nacional de Investigaciones Científicas y Técnicas de la República (CONICET), Argentina
[e] Biología Celular, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2805, Esperanza, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form
21 December 2016
Accepted 10 January 2017
Edited by Shahin Zarre
Available online 23 January 2017

Keywords:
Gramineae
Phylogeny
Ancestral state reconstruction
Evolution
Maximum parsimony

ABSTRACT

We studied the inflorescences of 112 members of tribe Chloridoideae subtribe Eleusininae from a morphological and evolutionary perspective to identify the most frequent types and to explore the evolutionary history of selected inflorescence associated characters. Six characters were scored on adult specimens and a principal coordinate analysis was conducted to identify inflorescence types. To investigate the evolution of inflorescences we regenerated the phylogeny of the subtribe and performed ancestral character state reconstructions using Maximum Parsimony. All species have panicles of spikelets with pyramidal, digitate or single-branched appearances. The number of primary branches varies widely among species, although some species have a single primary branch. The lack of terminal spikelet (truncation) and the similarity among primary branches of the inflorescence (homogenization) characterize the majority of the subtribe. In Eleusininae, the spikelet may be uni-, two- or multi-flowered. We found 13 inflorescence types in the group among 72 putative inflorescence forms. About 75% of the species can be divided to five different inflorescence types. Ancestral state reconstruction suggests an evolutionary direction towards simpler inflorescences with spikelets that contain 1–2 florets.

© 2017 Elsevier GmbH. All rights reserved.

1. Introduction

Gramineae tribe Cynodonteae include a large group of tropical and subtropical grasses with a possible center of origin in the African continent arising during the Oligocene (Hartley and Slater, 1960). It includes 839 species (93 genera) divided to 18 subtribes: Aleuropodinae, Boutelouinae, Cteniinae, Eleusininae, Farragininae, Gouiniinae, Gymnopogoninae, Hilariinae, Monanthochloinae, Muhlenbergiinae, Orcuttinae, Pappophorinae, Perotidinae, Scleropogoninae, Traginae, Trichoneuriinae, Tripogoninae and Troidiinae (Soreng et al., 2015). The monophyly of the tribe has already been confirmed in several studies (Columbus et al., 2007; GPWG II, 2012; Peterson et al., 2010, 2012, 2014a, 2014b).

Within Cynodonteae, the Eleusininae is sister to a recently characterized subtribe, the Dactylocteninae (Peterson et al., in press). The statistical support for the monophyly of Eleusininae has grown over the years with the inclusion of more exhaustive sampling and use of more variable molecular markers (Hilu and Alice, 2001; Neves et al., 2005; Peterson et al., 2010, 2012, 2015; Peterson et al., in press; Liu et al., 2011, 2014; Jewell et al., 2012a; Snow et al., 2013; Agrawal et al., 2014). The subtribe Eleusininae include 237 species attributed to 30 genera (Peterson et al., 2015; Peterson et al., in press). The polyphyly of *Chloris* was resolved through the emendation of the genus *Stapfochloa* (Peterson et al., 2015). However, *Coelachyrum* and *Schoenefeldia* remain polyphyletic up to date. *Acrachne racemosa* (B.Heyne ex Roth) Ohwi was recently separated from the subtribe since additional molecular data suggested this species to be sister to *Dactyloctenium* (Peterson et al., in press).

Eleusininae is a large group of annual and perennial grasses found in tropical environments of Africa, Southeast Asia, the Americas, and Australia (Peterson et al., 2010). Despite being widespread and taxonomically variable, the clade appears, at a glance, to have little morphological diversity in its inflorescence structure. Only

* Corresponding authors at: Facultad de Ciencias Agrarias (UNL), Kreder 2805, S3080HOF, Esperanza, Santa Fe, Argentina.
E-mail addresses: sebamuchut@yahoo.com.ar (S.E. Muchut), renatarein@fca.unl.edu.ar (R. Reinheimer).

http://dx.doi.org/10.1016/j.flora.2017.01.004
0367-2530/© 2017 Elsevier GmbH. All rights reserved.

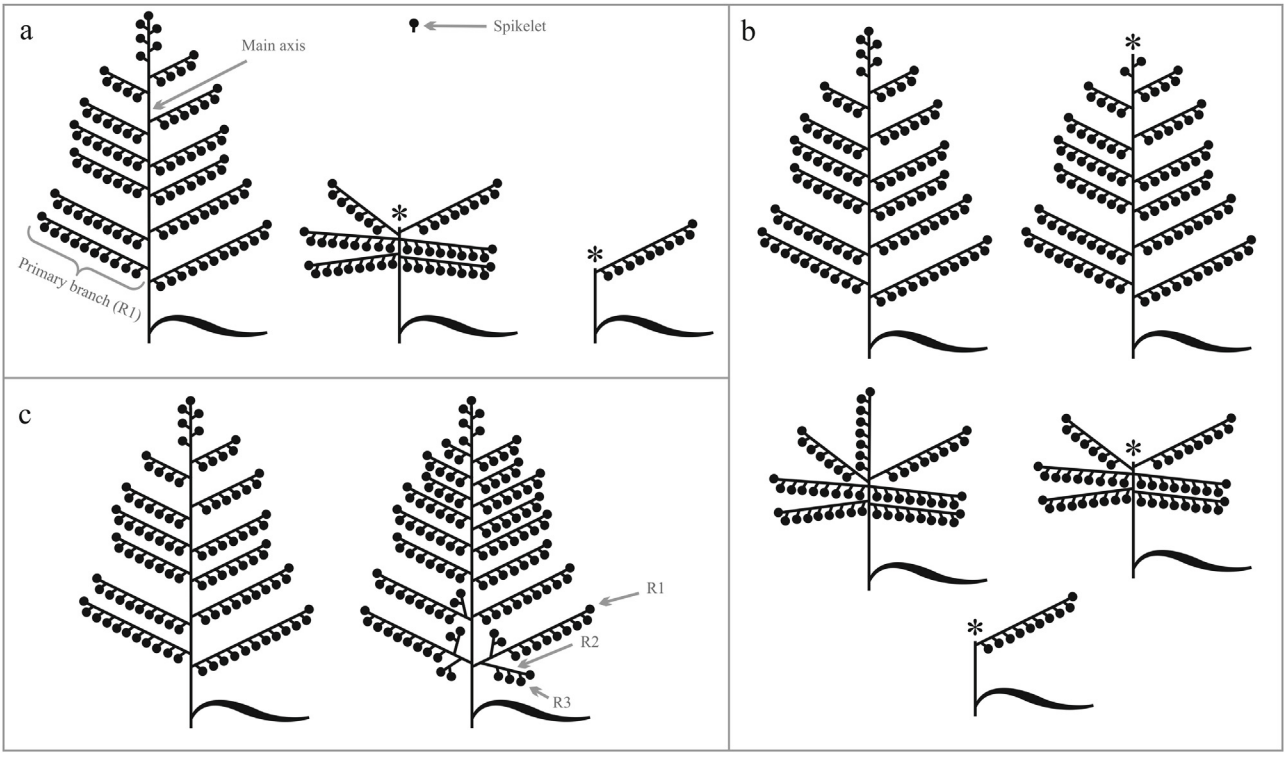

Fig. 1. a–c Simplified diagrams of inflorescences found in subtribe Eleusininae (Poaceae, Cynodonteae). a) Pyramidal, digitate and single-branched inflorescence; b) Non-truncated and truncated inflorescence; c) Fully and partially homogenized inflorescence. R1, primary branch; R2, secondary branch; R3, third branch; *, absence of terminal spikelet (truncation).

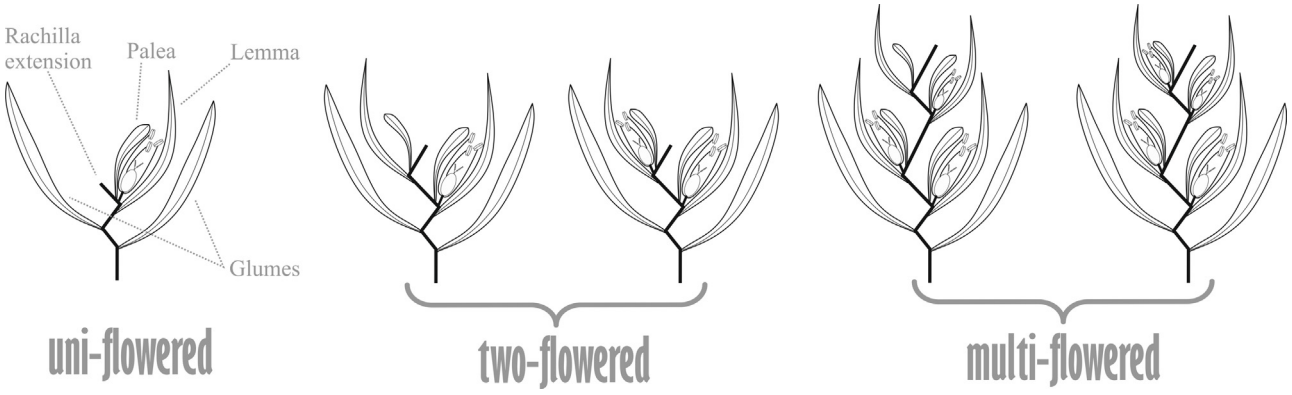

Fig. 2. Simplified diagrams of the different spikelets in subtribe Eleusininae (Poaceae, Cynodonteae).

the general terms raceme, spike and panicle have been used to describe its diversity (De Wet and Harlan, 1970; Hilu and Alice, 2001; Liu et al., 2005; Neves et al., 2005; Jewell et al., 2012b; Snow et al., 2013; Kellogg, 2015a; Peterson et al., 2015); nevertheless, this assumption is based on general descriptions of a small number of species. Previous studies have demonstrated that a better understanding of the diversity of inflorescence types can be achieved when morphological data are analyzed in a comparative manner based on phylogeny (Liu et al., 2005; Reinheimer et al., 2013; Pilatti, 2016). In addition, even with the possibility of finding more types than initially assumed for a group, it has been shown that the frequency of occurrence of the types may vary, where some types are more frequently observed than others (Reinheimer et al., 2013). These observations uncover the existence of constraints (genetic, developmental, or environmental) that direct the evolution of the inflorescence. The type of constraint that prevails may differ among disparate grass lineages, as suggested by Reinheimer et al. (2013). This kind of study has not been undertaken in Eleusininae.

The purpose of this work is to contribute to the knowledge of Eleusininae by studying the inflorescences of its members from a morphological and evolutionary perspective. Our specific goals are: (1) to analyze in detail, the inflorescence morphology of adult specimens, (2) to identify the most frequent inflorescence types in this subtribe, and (3) to explore the evolutionary history of inflorescence characters in order to understand the relationships among different types, illustrating ancestry, and evolutionary processes.

2. Materials and methods

2.1. Morphological studies

Selection of species was based on availability of material. The inflorescence of a total of 112 taxa, representing 23 of the 30 genera

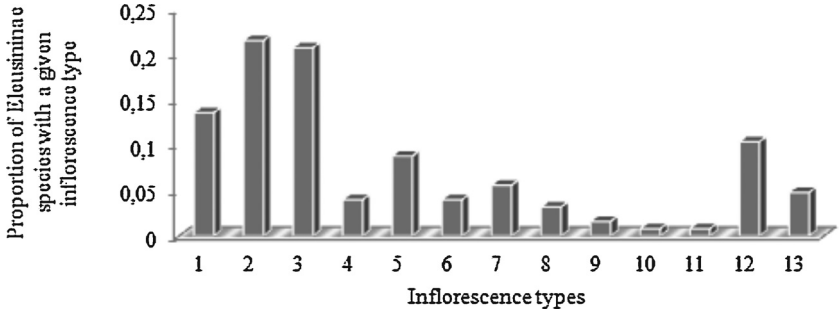

Fig. 3. Proportion of Eleusininae taxa (Poaceae, Cynodonteae) in the 13 inflorescence types recognized in PCO.

Table 1
Inflorescence types in subtribe Eleusininae (Poaceae, Cynodonteae) found by principal coordinates analysis (PCO).

Inflorescence Type	General appearance	Truncation	Degree of homogenization	Degree of branching	Florets per spikelet	Species (example)
1	Digitate	Yes	Full	2°	Uni-flowered	*Schoenefeldia transiens*
2	Digitate	Yes	Full	2°	Two-flowered	*Leptochloa crinita*
3	Digitate	Yes	Full	2°	Multi-flowered	*Tetrapogon cenchriformis*
4	Single-branched	Yes	Full	2°	Uni-flowered	*Microchloa kunthii*
5	Pyramidal	Yes	Full	2°	Multi-flowered	*Leptochloa virgata*
6	Single-branched	Yes	Full	2°	Two-flowered	*Tetrapogon fasciculata*
7	Single-branched	Yes	Full	2°	Multi-flowered	*Tetrapogon villosus*
8	Digitate	No	Full	2°	Uni-flowered	*Cynodon plectostachyus*
9	Digitate	No	Full	2°	Two-flowered	*Eustachys distichophylla*
10	Digitate	No	Full	2°	Multi-flowered	*Eleusine jaegeri*
11	Pyramidal	No	Full	2°	Two-flowered	*Dinebra retroflexa*
12	Pyramidal	No	Full	2°	Multi-flowered	*Leptochloa virgata*
13	Pyramidal	No	Partial	3°	Multi-flowered	*Diplachne fusca var fusca*

currently recognized in the group (Peterson et al., 2015; Peterson et al., in press) was examined under a stereoscopic microscope Nikon SMZ-10. The material studied was obtained from specimens loaned by national and international herbaria (CTES, LP, MO, NY, RSA, SF, SI and US) or collected in the field. A full list of the studied specimens is presented in Appendix A in Supplementary data.

The inflorescence of Eleusininae was characterized using six morphological characters that were chosen based on personal observation and previous studies (Rua, 2003; Kern et al., 2008; Reinheimer and Vegetti, 2008; Reinheimer et al., 2013; Pilatti and Vegetti, 2014; Pilatti, 2016): (1) general appearance of inflorescence (pyramidal/digitate/single-branched), (2) number of primary branches, (3) presence/absence of terminal spikelet at the end of the main axis (non truncated/truncated inflorescence), (4) morphological similarity of the primary branches of the inflorescence (partially homogenized/fully homogenized; Rua and Weberling, 1998), (5) maximum degree of ramification (second order/third order), and (6) number of florets (fertile and sterile) per spikelet.

To explore the morphological variability of the group and to identify representative inflorescence types, we conducted a principal coordinate analysis (PCO), implemented in Infostat (Di Rienzo et al., 2014). The PCO is a multidimensional statistic study that analyses the interdependence among categorical variables and finds a graphical representation of *n* individuals to reflect the similarity between them (Di Rienzo et al., 2014). For this purpose, we constructed a standardized matrix with the morphological characters (see Appendix B in Supplementary data). The results of the Euclidean distance calculations were presented in a two-dimensional plot. As the number of primary branches was highly variable for most of the species, we decided to exclude this character from the statistical analysis. In the cases of polymorphism the corresponding taxa were included as doubled. Once the types were defined, we calculated the proportion of species that display each type.

2.2. Phylogenetic analyses

To investigate the evolution of inflorescence morphology we reconstructed the phylogeny of subtribe Eleusininae mainly based on sequences available in GenBank (www.ncbi.nlm.nih.gov). Plastid markers (*ndhA* intron, *rpl32-trnL*, *rps 16* intron, *rps16-trnK*) and one nuclear marker (ITS) were selected based on previous works (Neves et al., 2005; Roodt-Wilding and Spies, 2006; Columbus et al., 2007; Liu et al., 2007; Liu et al., 2011; Jewell et al., 2012a; Peterson et al., 2010, 2011, 2012, 2014a, 2014b, 2015). To supplement data available in GenBank 43 additional sequences, representing 13 species, were generated in this work. GenBank accession numbers for sequences used in this study are provided in Appendix C in Supplementary data.

2.2.1. DNA extraction, amplification, and sequencing

Total DNA was isolated from silica-dried leaves using modified CTAB protocols (Doyle, 1987) or from herbarium material using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). PCR reactions were performed in a final volume of 25 μL containing 0.15 μL of *Taq* polymerase in a TGradient Thermocycler (Biometra, Göttingen, Germany). The amplification primers and parameters of PCR reactions were established following Peterson et al. (2010). PCR products were cleaned and sequenced by Macrogen (Seoul) using the ABI PRISM BigDye Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Seoul). Single-pass sequencing was performed using the same primers as PCR reactions. The sequences were assembled and edited with BioEdit version 7.2.3 (Hall, 1999) and then aligned using MUSCLE (Edgar, 2004).

2.2.2. Phylogenetic reconstruction

A total of 103 taxa were used as ingroup in the phylogenetic reconstruction. Three outgroup species were selected after

Peterson et al. (2015): *Chasmanthium latifolium* (Michx.) H.O.Yates, *Aristida gypsophilla* Beetle, and *Danthonia compressa* Austin.

The molecular phylogeny based on the five nucleotide markers was estimated using Bayesian Inference using Markov chain Monte Carlo (MCMC) analysis as implemented in MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001) employing the CIPRES Science Gateway (Miller et al., 2010). The model (GTR + G + I) of nucleotide substitution was selected using the Akaike Information Criterion (AIC) implemented in jModeltest version 2.1.6 (Darriba et al., 2012). Four Metropolis-coupled Markov chains were run simultaneously with an incremental heating of 0.2. Two analyses, starting from different random trees, were run for 5 million generations sampling every 1000 to ensure independence of the successive samples. The convergence and effective sample size (ESS) of each replicate were checked using Tracer version 1.5.0 (Rambaut and Drummond, 2007). Twenty-five percent of the total trees were discarded as burn-in. The rest (a total of 7502 trees) were combined to construct a majority-rule consensus tree. The dataset used and the trees generated in this work are deposited at TreeBASE (www.treebase.org).

To investigate the evolutionary history of the inflorescence, we have performed ancestral character state reconstructions using the phylogeny generated above. For that, a parsimony reconstruction

Fig. 4. a–d Reconstruction of the ancestral states for the inflorescence characters in subtribe Eleusininae (Poaceae, Cynodonteae) over the majority-rule consensus tree (generated from 7502 sampled trees) obtained from the Bayesian inference. Branch shading indicates maximum parsimony reconstruction using Mesquite v.3.01. List of characters: a) general appearance, b) truncation, c) degree of homogenization and, d) number of florets per spikelet.

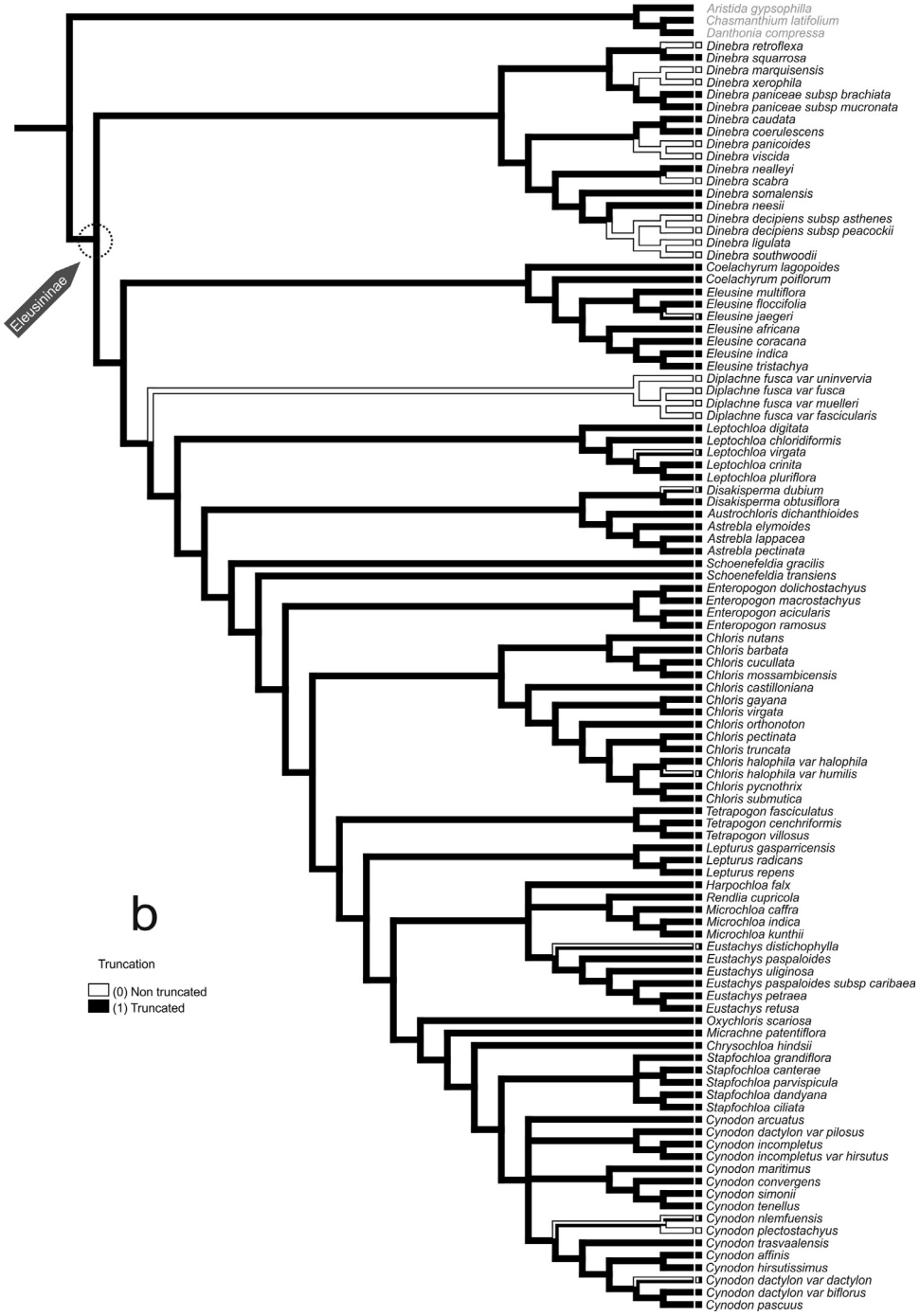

Fig. 4. (*Continued*)

was carried out in Mesquite version 3.01 software (Maddison and Maddison, 2011) using the "trace character history" option. Characters states were treated as un-ordered and we used Markov one-rate (Mk1) model.

3. Results

3.1. Inflorescence morphology

All species of Eleusininae examined in this study have panicles of spikelets. Three different inflorescence appearances were observed among members of the subtribe (Fig. 1a; see Appendix D in Supplementary data): (1) pyramidal, in which primary branches, of decreasing length, borne in nodes separated by long internodes (*Dinebra*, *Diplachne*, *Disakisperma* and *Leptochloa virgata* (L.) P.Beauv.), (2) digitate, in which the primary branches radiate from nodes close to one another (*Austrochloris*, *Chloris*, *Chrysochloa*, *Coelachyrum*, *Cynodon*, *Eleusine*, *Enteropogon*, *Eustachys*, *Leptochloa*, *Oxychloris*, *Schoenefeldia*, *Sclerodactylon*, *Stapfochloa* and *Tetrapogon cenchriformis* (A.Rich.) Clayton) and (3) single-branched, where the inflorescence is composed of a unique primary branch (*Astrebla*, *Lepturus*, *Microchloa* and *Enteropogon macrostachyus* Hochst. ex A. Rich., *Harpochloa falx*, *Micrachne patentiflora*, *Rendlia cupricola*, *Tetrapogon fasciculatus* Hitchc. & Chase and *T. villosus* Desf.).

The number of primary branches per inflorescence is variable among and within species of the subtribe (see Appendix D in

Supplementary data). Inflorescences of genera *Dinebra*, *Diplachne* and *Leptochloa* showed the largest number of primary branches. The inflorescence of *Astrebla*, *Harpochloa*, *Lepturus*, *Microchloa*, and *Enteropogon macrostachyus* K. Schum. ex Engl., *Micrachne patentiflora* (Stent & J.M.Rattray) P.M.Peterson, *Rendlia cupricola* P.A.Duvign., *Tetrapogon fasciculatus* (Hitchc. & Chase) P.M.Peterson and *T. villosus* Desf. are represented only by a single primary branch.

The inflorescence of most of the species of the subtribe lacks the terminal spikelet (Fig. 1b; see Appendix D in Supplementary data). Inflorescences with terminal spikelet at the end of the main axis are found in *Cynodon plectostachyus* (K.Schum.) Pilg., the four varieties of *Diplachne fusca* (L.) P.Beauv. ex Roem. and Schult., and ten species of *Dinebra*. Eight species were registered as polymorphic for this character (see Appendix D in Supplementary data).

The majority of the studied species have fully homogenized inflorescences bearing up to second order of branching (Fig. 1c; see Appendix D in Supplementary data). Partially homogenized inflorescences were reported in *Dinebra marquisensis* (F. Br.) P.M.Peterson & N.Snow and *D. scabra* (Nees) P.M.Peterson and N.Snow. In these two cases a few proximal branches display up to third order of branching (Fig. 1c; see Appendix D in Supplementary data), while the rest of the inflorescence has a branching of second order. Polymorphism for this character was registered for *Dinebra panicoides* (J.Presl) P.M.Peterson and N.Snow, *D. viscida* (Scribn.) P.M.Peterson and N. Snow, *Diplachne fusca* (L.) P.Beauv. ex Roem.

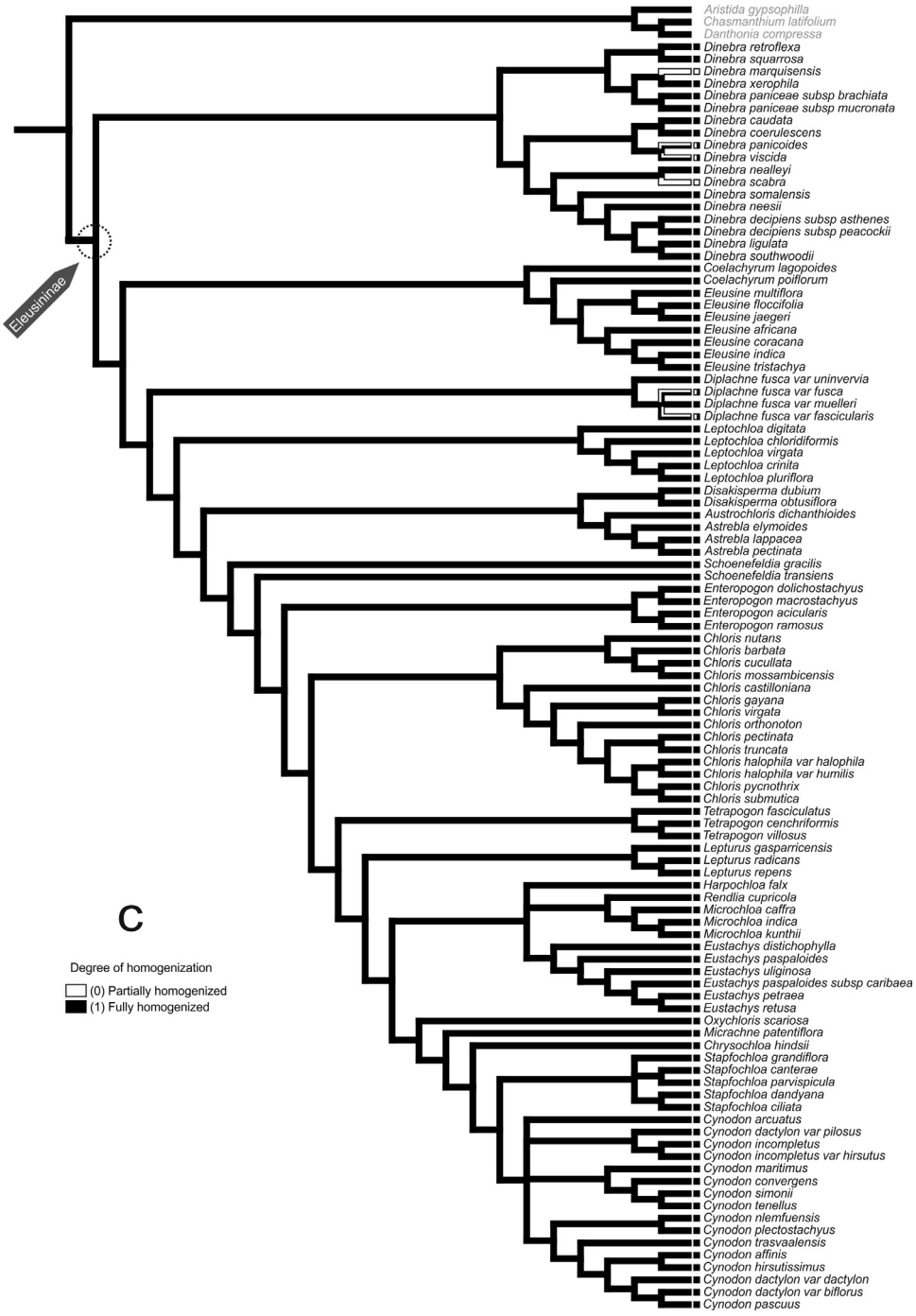

Fig. 4. (*Continued*)

Fig. 4. (*Continued*)

& Schult. subsp. *fascicularis* (Lam.) P.M.Peterson & N.Snow and *D. fusca* var. *fusca* (see Appendix D in Supplementary data).

All species belonging to Eleusininae have spikelets enclosing hermaphrodite florets (Fig. 2). The existence of sterile florets is also common in the group. When present, sterile florets bear lemma and/or palea rudiments and develop above the fertile florets. Spikelets with only one floret (uni-flowered) were found in *Lepturus radicans* (Steud.) A. Camus, *Micrachne patentiflora*, and for genera *Microchloa*, *Schoenefeldia* and *Cynodon* (see Appendix D in Supplementary data). Two-flowered spikelets (enclosing two hermaphrodite florets or one sterile) were observed in *Chrysochloa*, *Eustachys*, the monotypic genus *Austrochloris*, *Cynodon dactylon* (L.) Pers. var. *biflorus* Merino and in most species of *Chloris* (12/17) (see Appendix D in Supplementary data). Multi-flowered spikelets (enclosing all hermaphrodite florets or with a few sterile) were observed in *Astrebla*, *Coelachyrum*, *Dinebra* (except *D. retroflexa*), *Diplachne*, *Disakisperma*, *Eleusine*, *Stapfochloa*, *Harpochloa*, *Oxychloris*, and *Sclerodactylon* (see Appendix D in Supplementary data).

3.1.1. Inflorescence categories

The principal coordinates analysis (PCO) placed all taxa in 13 different categories of inflorescences (Table 1) according to the 72 theoretical possibilities, i.e. general appearance × truncation × ° of homogenization × ° of branching × florets per spikelet ($3 \times 3 \times 2 \times 2 \times 2 = 72$). Seventy percent of the variation

is shown by the two principal axes (see Appendix E in Supplementary data).

Among the 13 inflorescence types found in PCO, 56% of the studied taxa groups within types 1 (14%), 2 (22%), and 3 (21%) (Fig. 3; Table 1). Inflorescences types 1, 2, and 3 are digitate, truncated, fully homogenized, and bear a 2° order of branching. Type 1 inflorescence has uni-flowered spikelets, whereas type 2 has two-flowered spikelets, and type 3 presents the multi-flowered spikelets. Type 12 (10%) corresponds to taxa with pyramidal, non-truncated, fully homogenized inflorescences, that bear 2° order of branching and multi-flowered spikelets (Fig. 3; Table 1). Type 5 (9%) represent pyramidal inflorescences, truncated, fully homogenized, with 2° order of branching that bear multi-flowered spikelets (Fig. 3; Table 1). Types 7, 4, and 6 characterize 14% of the studied species. These three types have single-branched inflorescences that are truncated and fully homogenized, bearing 2° order of branching. Particularly, type 7 inflorescence bears multi-flowered spikelets, whereas type 4 has uni-flowered spikelets, and type 6 includes two-flowered spikelets (Fig. 3; Table 1). Type 13 (5%) represents non-truncated inflorescences with pyramidal appearance, partially homogenized with up to 3° order of branching, bearing multi-flowered spikelets (Fig. 3; Table 1). Types 8 (3%), 9 (2%), and 10 (1%) are digitate inflorescences, non-truncated and fully homogenized (Fig. 3; Table 1). In particular, type 8 inflorescence has uni-flowered spikelets, whereas type 9 has two-flowered spikelets, and type 3 includes multi-flowered spikelets. Type 11 (1%) represents inflorescences with a pyramidal appearance, truncated, fully homogenized, with 2° order of branching, and bearing two-flowered spikelets (Fig. 3; Table 1).

3.2. Phylogenetic analysis

The topology of the Bayesian majority-rule consensus tree (based on 7502 samples) is similar to that recovered by Peterson et al. (2015). Eleusininae is monophyletic with *Dinebra* sister to the remaining taxa (see Appendix F in Supplementary data). Our analysis recovered 14 well-supported genera (PP = 1.00). The genera *Coelachyrum* (sister group to *Eleusine*) and *Schoenefeldia* (sister group to *Enteropogon*, *Chloris*, *Tetrapogon*, *Lepturus*, *Harpochloa*, *Rendlia*, *Microchloa*, *Eustachys*, *Oxychloris*, *Micrachne*, *Chrysochloa*, *Stapfochloa* + *Cynodon* clade) formed a paraphyletic assemblage.

3.2.1. Ancestral state reconstruction

General appearance (Fig. 4a). The general appearance of the ancestor of Eleusininae may have been either pyramidal or digitate. Deep nodes as reconstructed contain digitate inflorescences. This indicates that single-branched inflorescences are derived character states. Single-branched inflorescences evolved exclusively from digitate ones at least three times, e.g. in *Astrebla* ancestor, in *Enteropogon macrostachyus* and before the divergence of *Tetrapogon spp*. and its sister group.

Truncation (Fig. 4b). The ancestor of Eleusininae may have had a truncated inflorescence. The non-truncated inflorescence appears to be a derived state having evolved independently 13 times. Reversion to the ancestral state has not been detected.

Degree of homogenization (Fig. 4c). The reconstruction shows an ancestor of Eleusininae with a fully homogenized inflorescence. The evolution of partially homogenized inflorescence is only seen in some species of *Dinebra* and *Diplachne fusca*. These results indicate that partially homogenized inflorescence is a derived character state.

Number of florets per spikelet (Fig. 4d). Parsimony reconstruction shows that multi-flowered spikelets represent the most likely basal state in Eleusininae. Both two-flowered and uni-flowered spikelets are derived character states that evolved several times in the subtribe. Reversion to the ancestral state is seen in *Enteropogon macrostachyus*, *Chloris nutans* (Stapf) P.M. Peterson, *C. barbata* Sw., *C. gayana* Kunth, *Tetrapogon cenchriformis*, *T. villosus*, *Harpochloa falx* (L. f.) Kuntze, *Oxychloris scariosa* (F. Muell.) Lazarides and in the genus *Stapfochloa*.

4. Discussion

The importance of studying inflorescence variations resides in understanding that its branching pattern influences the number of seeds that the inflorescences would bear (Doust, 2007). The grasses clade owes its vast taxonomic diversity to the alteration in this branching architecture (Doust and Kellogg, 2002). In Eleusininae, all members have panicles of spikelets that differ in their appearance determined by the arrangement, length of internodes and/or number of primary branches. Pyramidal panicles present primary branches separated for long internodes and with a decreasing length towards the apex. The digitate inflorescence may be result of the shortening of the main axis of the internode (Vegetti and Anton, 1995). On the other hand, the often confused single-branched inflorescence found in Eleusininae corresponds to an inflorescence bearing a unique primary branch that may appear to be the main axis extension because of its vertical position (Perreta et al., 2009).

The processes of truncation and homogenization characterize most species in this subtribe. In grasses, the absence of terminal structures is a common phenomenon which is frequently associated with homogenization (Vegetti and Anton, 2000; Reinheimer and Vegetti, 2008; Perreta et al., 2009; Reinheimer et al., 2013; Pilatti, 2016). Our results support the previously conceived idea that every truncated inflorescence is fully homogenized (Vegetti and Anton, 2000; Perreta et al., 2009). These results, combined with the high level of polymorphism we found for both processes, offer additional support for the hypothesis that homogenization precedes the loss of distal reproductive structures.

In Eleusininae, the spikelet may be uni-, two- or multi-flowered. It has been shown that the variation in number of florets per spikelet is regulated by the precise timing of expression of miR172 family that controls the activity of AP2-like genes (Lee et al., 2007; Chuck et al., 2007, 2008; Lee and An, 2012 Ren et al., 2013; Kellogg, 2015b). Since Eleusininae shows evolution from multi-flowered to uni-flowered spikelets, one might assume that changes in the regulation of miR172 expression accompany this evolutionary trend.

The PCO analysis performed here separated the 112 studied taxa in 13 inflorescence groups associated with different character states. The finding of a small fraction of forms among a wide theoretical range can be explained not only by the interaction between organism and environment, but by developmental restrictions at genetic levels (Prusinkiewicz et al., 2007). Similar results were described for panicoid grasses (Reinheimer et al., 2013). Liu et al. (2005) studied inflorescences of chloridoid grasses, describing twelve processes responsible for its diversity, and accounting for just four basic inflorescence types. Nevertheless, they failed at discerning truncated main axes and did not register polymorphisms, at least for Eleusininae species.

Our phylogenetic reconstruction of the Eleusininae corroborates almost entirely with the tree obtained by Peterson et al. (2015). The position of *Rendlia cupricola* J.Divgn. supports its systematic placement already stated in that work. However, there are a few differences regarding the sister relationships among *Harpochloa*, *Microchloa*, *Eustachys*, *Oxychloris*, and *Micrachne*. The discrepancies may be the consequence of the 13 species and 43 new sequences added in our analysis.

Reconstruction of the ancestral state suggests digitate or pyramidal inflorescence, bearing multi-flowered spikelets, with fully homogenized and truncated structures to represent the most prim-

itive states. Interestingly, these character states correlate with our inflorescence types 3 and 5. These two types correspond to 30% of the species in our study, and even if we did not perform divergence time estimation, it is possible to infer an apparent stability within the state changes for this subtribe. In contrast, the single-branched form, the spikelets bearing few florets (one or two), the partially homogenized inflorescences, and the loss of structures in the main axis, were the most derived states. The derivation of a smaller inflorescence (truncated and single-branched) with less florets per spikelets in the advanced taxa in our group, suggests a reductive trend regarding the evolution of inflorescence in Eleusininae. Liu et al. (2005) also had shown homogenization and truncation to be the main responsible events leading to reductive evolution in chloridoid inflorescences. Homogenization has also simplified the inflorescence morphology in Melinidinae (Panicoideae) (Salariato et al., 2010).

5. Conclusions

This work constitutes the first step to unraveling the inflorescence evolution in Cynodontoid grasses. The lack of these kinds of studies in the tribe makes it hard to establish comparative hypotheses. In addition, the few former morphological studies conducted, do not focus on phylogenetically supported groups. The simple methods used in our work helped us the better understanding of the morphological diversity in Eleusininae and the possible evolutionary history of its inflorescences. Despite the relevant number of species that may have conserved the ancestral inflorescence morphology, the subtribe seems to present an evolutionary trend towards reduced inflorescences with spikelets enclosing few florets. Nevertheless, new studies on Cynodontoid grasses should explore the inflorescence characters with more exhaustive techniques, such as genetic and developmental approaches, in order to get more insights into the evolution of inflorescence forms.

Acknowledgments

Authors thank the curators of the herbaria CTES, LP, MO, NY, RSA, SF, SI, and US for providing plant material. We would also like to thank Dr. Juan Carlos Tivano for accompanying the authors on numerous field expeditions and Dr. Paul M. Peterson for reviewing an earlier draft of our manuscript. Financial support was provided by PICT2011-0590 (to A.V.), PICT 2013-0757 (to R.R.), and UNL-CAID+D 2011 (50120110100213LI) (to R.R.).

Appendices A–F Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.flora.2017.01.004.

References

Agrawal, R., Agrawal, N., Tandon, R., Raina, S.N., 2014. Chloroplast genes as genetic markers for inferring patterns of change: maternal ancestry and phylogenetic relationships among *Eleusine* species. AoB Plants 6, 1–17.

Chuck, G., Meeley, R., Irish, E., Sakai, H., Hake, S., 2007. The maize tasselseed4 microRNA controls sex determination and meristem cell fate by targeting Tasselseed6/indeterminate spikelet1. Nat. Genet. 39 (12), 1517–1521.

Chuck, G., Meeley, R., Hake, S., 2008. Floral meristem initiation and meristem cell fate are regulated by the maize AP2 genes ids1 and sid1. Development 135 (18), 3013–3019.

Columbus, J.T., Cerros-Tlatilpa, R., Kinney, M.S., Siqueiros-Delgado, M.E., Bell, H.L., Griffith, M.P., Refulio-Rodriguez, N.F., 2007. Phylogenetics of Chloridoideae (Gramineae): a preliminary study based on nuclear ribosomal internal transcribed spacer and chloroplast trnL–F sequences. Aliso: J. Syst. Evol. Bot. 23 (1), 565–579.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9 (8), 772.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2014. InfoStat Versión. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina URL http://www.infostat.com.ar.

Doyle, J.J., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Doust, A., 2007. Architectural evolution and its implications for domestication in grasses. Ann. Bot. 100 (5), 941–950.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32 (5), 1792–1797.

GPWG II (Grass Phylogeny Working Group II), 2012. New grass phylogeny resolves deep evolutionary relationships and discovers C4 origins. New Phytol. 193, 304–312.

Hall, T.A., 1999. BioEdit Software, Version 7.2.3. North Carolina State University, Raleigh, NC.

Hartley, W., Slater, C., 1960. Studies on the origin, evolution, and distribution of the Gramineae. III. The tribes of the subfamily Eragrostoideae. Aust. J. Bot. 8 (3), 256–276.

Hilu, K.W., Alice, L.A., 2001. A phylogeny of Chloridoideae (Poaceae) based on matK sequences. Syst. Bot. 26 (2), 386–405.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17 (8), 754–755.

Jewell, M., Frere, C.H., Harris-Shultz, K., Anderson, W.F., Godwin, I.D., Lambrides, C.J., 2012a. Phylogenetic analysis reveals multiple introductions of Cynodon species in Australia. Mol. Phylogenet. Evol. 65 (2), 390–396.

Jewell, M., Zhou, Y., Loch, D.S., Godwin, I.D., Lambrides, C.J., 2012b. Maximizing genetic, morphological, and geographic diversity in a core collection of Australian bermudagrass. Crop Sci. 52 (2), 879–889.

Kellogg, E.A., 2015a. Subfamily chloridoideae kunth ex beilschm (1833). In: Flowering Plants. Monocots. Springer International Publishing, pp. 353–397.

Kellogg, E.A., 2015b. Inflorescence structure. In: Flowering Plants. Monocots. Springer International Publishing, pp. 25–38.

Kern, V.G., Guarise, N.J., Vegetti, A.C., 2008. Inflorescence structure in species of *Spartina* schreb. (Poaceae: chloridoideae: cynodonteae). Plant Syst. Evol. 273 (1–2), 51–61.

Lee, D.Y., An, G., 2012. Two AP2 family genes, supernumerary bract (SNB) and Osindeterminate spikelet 1 (OsIDS1), synergistically control inflorescence architecture and floral meristem establishment in rice. Plant J. 69 (3), 445–461.

Lee, D.Y., Lee, J., Moon, S., Park, S.Y., An, G., 2007. The rice heterochronic gene SUPERNUMERARY BRACT regulates the transition from spikelet meristem to floral meristem. Plant J. 49 (1), 64–78.

Liu, Q., Zhao, N.X., Hao, G., 2005. Inflorescence structures and evolution in subfamily Chloridoideae (Gramineae). Plant Syst. Evol. 251 (2–4), 183–198.

Liu, Q., Peterson, P.M., Columbus, J.T., Zhao, N., Hao, G., Zhang, D., 2007. Inflorescence diversification in the finger millet clade(Chloridoideae, Poaceae): a comparison of molecular phylogeny and developmental morphology. Am. J. Bot. 94 (7), 1230–1247.

Liu, Q., Triplett, J.K., Wen, J., Peterson, P.M., 2011. Allotetraploid origin and divergence in Eleusine (Chloridoideae, Poaceae): evidence from low-copy nuclear gene phylogenies and a plastid gene chronogram. Ann. Bot. 108 (7), 1287–1298.

Liu, Q., Jiang, B., Wen, J., Peterson, P.M., 2014. Low-copy nuclear gene and McGISH resolves polyploid history of *Eleusine coracana* and morphological character evolution in *Eleusine*. Turk. J. Bot. 38, 1–12.

Maddison, W.P., Maddison, D.R., 2011. Mesquite: a Modular System for Evolutionary Analysis. Version 3.01, Available at http://www.mesquiteproject.org/packages/hypha/manual/index.html.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, pp. 1–8.

Neves, S.S., Swire-Clark, G., Hilu, K.W., Baird, W.V., 2005. Phylogeny of Eleusine (Poaceae: chloridoideae) based on nuclear ITS and plastid trnT–trnF sequences. Mol. Phylogenet. Evol. 35 (2), 395–419.

Perreta, M.G., Ramos, J.C., Vegetti, A.C., 2009. Development and structure of the grass inflorescence. Bot. Rev. 75, 377–396.

Peterson, P.M., Romaschenko, K., Johnson, G., 2010. A classification of the Chloridoideae (Poaceae) based on multi-gene phylogenetics trees. Mol. Phylogen. Evol. 55, 580–598.

Peterson, P.M., Romaschenko, K., Barker, N.P., Linder, H.P., 2011. Centropodieae and *Ellisochloa*, a new tribe and genus in Chloridoideae (Poaceae). Taxon 60 (4), 1113–1122.

Peterson, P.M., Romaschenko, K., Snow, N., Johnson, G., 2012. A molecular phylogeny and classification of *Leptochloa* (Poaceae: chloridoideae: Chlorideae) sensu lato and related genera. Ann. Bot. (Oxf.) 109, 1317–1329.

Peterson, P.M., Romaschenko, K., Herrera Arrieta, Y., 2014a. A molecular phylogeny and classification of the cteniinae, farragininae, gouiniinae, gymnopogoninae, perotidinae, and trichoneurinae (Poaceae: chloridoideae: cynodonteae). Taxon 63 (2), 275–286.

Peterson, P.M., Romaschenko, K., Soreng, R.J., 2014b. A laboratory guide for generating DNA barcodes in grasses: a case study of *Leptochloa* (Poaceae: chloridoideae). Webbia 69 (1), 1–12.

Peterson, P.M., Romaschenko, K., Herrera Arrieta, Y., 2015. A molecular phylogeny and classification of the Eleusininae with a new genus, *Micrachne* (Poaceae: chloridoideae: Cynodonteae). Taxon 64 (3), 445–467.

Peterson, P.M., Romaschenko, K., Herrera Arrieta, Y., 2017. A Molecular Phylogeny and Classification of the Cynodonteae (Poaceae: Chloridoideae). Taxon.

Pilatti, V., Vegetti, A., 2014. Diversity of inflorescences in the boutelouinae subtribe (Poaceae: chloridoideae: cynodonteae). Flora 209 (8), 426–434.
Pilatti, V., 2016. Diversidad Y Evolución De Las Inflorescencias En Las Subtribus más Derivadas De Cynodonteae (Chloridoideae-Poaceae). Tesis Doctoral, Facultad De Bioquímicas Y Ciencias Biológicas. Universidad Nacional del Litoral, pp. 1–189.
Prusinkiewicz, P., Erasmus, Y., Lane, B., Harder, L.D., Coen, E., 2007. Evolution and development of inflorescence architectures. Science 316 (5830), 1452–1456.
Rambaut, A., Drummond, A.J. 2007. Tracer v1.5. http://beast.bio.ed.ac.uk/tracer.
Reinheimer, R., Vegetti, A.C., 2008. Inflorescence diversity and evolution in the PCK clade (Poaceae: panicoideae: Paniceae). Plant Syst. Evol. 275 (3–4), 133–167.
Reinheimer, R., Amsler, A., Vegetti, A.C., 2013. Insights into panicoid inflorescence evolution. Organ. Divers. Evol. 13 (2), 97–110.
Ren, D., Li, Y., Zhao, F., Sang, X., Shi, J., Wang, N., Guo, S., Ling, Y., Zhang, C., Yang, Z., He, G., 2013. MULTI-FLORET SPIKELET1, which encodes an AP2/ERF protein, determines spikelet meristem fate and sterile lemma identity in rice. Plant Physiol. 162 (2), 872–884.
Roodt-Wilding, R., Spies, J.J., 2006. Phylogenetic relationships in southern African chloridoid grasses (Poaceae) based on nuclear and chloroplast sequence data. Syst. Biodivers. 4 (04), 401–415.
Rua, G.H., Weberling, F., 1998. Growth form and inflorescence structure of *Paspalum* L. (Poaceae, Paniceae): a comparative morphological approach. Beitr. Biol. Pfl 69 (3), 363–431.
Rua, G.H., 2003. Growth forms, branching patterns, and inflorescence structure in *Digitaria* sect. *Trichachne* (Poaceae, Paniceae). Flora 198 (3), 178–187.
Salariato, D.L., Zuloaga, F.O., Giussani, L.M., Morrone, O., 2010. Molecular phylogeny of the subtribe Melinidinae (Poaceae: panicoideae: Paniceae) and evolutionary trends in the homogenization of inflorescences. Mol. Phylogenet. Evol. 56 (1), 355–369.
Snow, N., Peterson, P.M., Romaschenko, K., 2013. Systematics of *Disakisperma* (Poaceae, chloridoideae, chlorideae). PhytoKeys 26, 21–70.
Soreng, R.J., Peterson, P.M., Romaschenko, K., Davidse, G., Zuloaga, F.O., Judziewicz, E.J., Morrone, O., 2015. A worldwide phylogenetic classification of the Poaceae (Gramineae). J. Syst. Evol. 53 (2), 117–137.
Vegetti, A.C., Anton, A.M., 1995. Some evolution trends in the inflorescence of Poaceae. Flora 190, 225–228.
Vegetti, A.C., Anton, A.M., 2000. The grass inflorescence. Grasses: Syst. Evol., 29–31.