

Morphology and leaf anatomy of *Setaria* s.l. (Poaceae: Panicoideae: Paniceae) and its taxonomic significance

Sandra S. Aliscioni^{1,2} · Juan C. Ospina¹ · Natalia E. Gomiz²

Received: 17 December 2014 / Accepted: 18 September 2015
© Springer-Verlag Wien 2015

Abstract *Setaria* is numerically one of the largest and most taxonomically complex genera of the tribe Paniceae. The currently published phylogenies of *Setaria* are not fully resolved and some of the resulting clades reveal more geographical signals than morphological affinities. Therefore, external similarities among species of *Setaria* do not generally indicate relationships, morphology appears to be highly labile, and few inferences can be made regarding the taxonomy of the genus. In the present study we analyze the variation of the foliar anatomy through a cluster analysis including morphological characters used traditionally to delimit subgenera, sections and species. The aim of this study is to examine whether foliar anatomy, a source of evidence not previously utilized, provides useful information and can be predictive for recognizing homogenous groups of species within *Setaria*. The significance of the characters is evaluated to identify species groups. These groups were compared with infrageneric taxa and monophyletic clades established in previously published molecular phylogenies. Additionally, some foliar anatomical characters associated with the photosynthetic pathway

are also discussed and compared with other members of the tribes Paniceae and Paspaleae.

Keywords Anatomy · Morphology · *Paspalidium* · Photosynthetic type · *Setaria*

Introduction

Setaria P.Beauv. is numerically one of the largest and most taxonomically difficult genera of the tribe Paniceae. This genus comprises about 113 species (Clayton et al. 2002 onwards) widely distributed around the world, concentrated in tropical, subtropical and warm temperate regions. The greatest concentration of species for the Old World is in the tropical regions of Africa (Stapf and Hubbard 1930), and Rominger (1962) established Brazil to be the center of distribution for the species of the New World.

One of the diagnostic characters of the genus is the presence of bristles as sterile branches in the inflorescence, and the number of bristles per spikelet is an attribute often used to identify species. Inflorescences are variable and can be open or spiciform panicles, more or less contracted with spikelets along branches, which can be reduced to racemes. Spikelets, which are a distinctive character for Paniceae, are dorsally compressed and plano-convex with unequal glumes, a male or sterile lower antherium with a membranous lemma and an absent to well-developed palea, and a crustaceous or coriaceous upper antherium, typically elliptical, corrugate, rugose or infrequently smooth.

The genus includes annual or perennial species that grow in very diverse environments from dry, saline, and rocky areas to coastal zones of rivers and streams, in some cases as semi-floating plants such as *S. geminata* (Forssk.) Veldkamp and *S. uda* (S.T.Blake) R.D.Webster. Other

Handling editor: Karol Marhold.

Electronic supplementary material The online version of this article (doi:10.1007/s00606-015-1251-9) contains supplementary material, which is available to authorized users.

✉ Sandra S. Aliscioni
aliscion@agro.uba.ar; saliscioni@darwin.edu.ar

¹ Instituto de Botánica Darwinion, Labardén 200, San Isidro, Buenos Aires, Argentina

² Cátedra de Botánica General, Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martín, 4453 Ciudad Autónoma de Buenos Aires, Argentina

species, such as *S. australiensis* (Scribn. & Merr.) Vickery, prefer semiarid regions or coastal dunes near the sea such as *S. apiculata* (Scribn. & Merr.) K.Schum. Some species are highly invasive weeds in fields and plantations of various crops such as *S. verticillata* (L.) P.Beauv., *S. viridis* (L.) P.Beauv., *S. pumila* (Poir.) Roem. & Schult., and *S. parviflora* (Poir.) Kerguelen (Douglas et al. 1985; Rominger 1962; Prasada Rao et al. 1987; Dekker 2003).

The morphological variation among the species of the genus is large. Regarding leaf morphology, *Setaria* is unusual among genera of Paniceae in the level of variation, because leaves can be flat or folded and sometime plicate such as *S. palmifolia* (J.Koenig) Stapf and *S. barbata* (Lam.) Kunth. The base of the blade can be markedly sagittate such as *S. appendiculata* (Hack.) Stapf and *S. sagittifolia* (Hochst. ex A.Rich.) Walp. Other species exhibit pseudopetioles in all or basal leaves such as *S. petiolata* Stapf & C.E.Hubb.

Setaria is widely studied because several species are of economic importance; some species, such as *S. italica* (L.) P.Beauv. (foxtail millet), are cultivated for grains and are important food sources in some regions of India, China, Korea and Japan (Gao and Chen 1988; Austin 2006). Also, other species provide significant forage, such as *S. magna* Griseb. (Aliscioni et al. 2011; Marinoni et al. 2013).

Although *Setaria* attracts much research, the generic and infrageneric systematic boundaries are still controversial. Based on the classification of Morrone et al. (2012) *Setaria* belongs to the subfamily Panicoideae, tribe Paniceae, subtribe Cenchrinae (Dumort.) Dumort. This subtribe was informally called “bristle clade” because of having species with setae associated with the inflorescence.

Many advances have been made in the phylogeny of the tribe Paniceae and consequently several genera were taxonomically recircumscribed. However, *Setaria*, one of the largest genera of the subtribe Cenchrinae, presents an especially complex taxonomy and still needs a world-wide revision.

The taxonomy of the genus has been confounded by the high degree of overlapping morphological characters within and between species and the diverse polyploidy levels within the genus. For the New World species, Rominger (1962) published a revision of the North American species, considering three subgenera as following: *Setaria* subg. *Ptychophyllum* (A.Braun) Hitchc. with six species, *Setaria* subg. *Paurochaetium* (Hitchc. & Chase) Rominger with 10 species and *Setaria* subg. *Setaria* with 27 species. Later, Fox and Hatch (1999) established the *Setaria* subg. *Reverchoniae* W.E.Fox, including the species *S. reverchonii* (Vasey) Pilg. and *S. variifolia* (Swallen) Davidse. The species from South America were studied by Pensiero (1999) who recognized *Setaria* subg. *Ptychophyllum* with three species, and *Setaria* subg.

Setaria with 39 species, and established *Setaria* subg. *Cernatum* Pensiero, a monotypic subgenus endemic to Ecuador.

The species of the Old World were included in numerous treatments that were published at a regional level. For example, Stapf and Hubbard (1930) recognized *Setaria* sect. *Eusetaria* (invalid), *Setaria* sect. *Panicatrix*, *Setaria* sect. *Ptychophyllum* and *Setaria* sect. *Cymbose-taria* for tropical Africa. A more complete and recent revision of the species from Africa, Asia, Europe and Oceania was published by Morrone et al. (2014) who did not employ infrageneric categories, but identified three major morphological groups based on the type of inflorescence: species with open panicles, species with spiciform panicles, and species with unilateral racemes along the panicle.

Most of the species of *Setaria* that present panicles with unilateral racemes were considered previously by other authors to belong to the genus *Paspalidium* Stapf. Stapf (1920) distinguished this genus based on the presence of bristles terminating the primary branch. Webster (1987) included a total of 28 species in the genus, the majority of which are concentrated in Australia, and only five native to Asia and Africa. Closer examination of the genus shows that some species have bristles subtending more spikelets than just the distal ones. Thus, when the full range of variation in the inflorescences of *Setaria* and *Paspalidium* are considered, both genera are morphologically indistinguishable. Veldkamp (1994) and Webster (1993, 1995) transferred the species from *Paspalidium* to *Setaria*, an opinion followed by Morrone et al. (2014).

There is significant evidence suggesting that *Setaria* is not monophyletic, and neither are some of its subgenera and sections. The phylogeny of Kellogg et al. (2009), based only on the chloroplast gene *ndhF*, showed *Setaria* to be divided into three large well-supported clades, comprising the species of Africa, South America and Eurasia, respectively, and several smaller clades. This phylogeny is not fully resolved and some of these clades reveal more geographical signals than morphological similarities.

The taxonomic importance of leaf blade anatomy has traditionally been recognized in the family Poaceae. In some genera as *Eragrostis* Wolf., anatomical characters seem to be more predictive of relationships than morphological features (Ingram 2010), while in other genera, such as *Puccinellia* Parl., anatomical characters were quite variable and not necessarily more reliable (Davis 1983). However, anatomical characters can be important when they are used together with other characters, such as exomorphology and geographical distribution (Ospina et al. 2015).

Considering this, the aim of this study is to test whether adding information of foliar anatomy to the traditional

morphological characters would be helpful to distinguish homogenous groups of species of *Setaria* and to contrast these groups with traditional classifications and phylogenetic analyses. In the present paper we examine the foliar anatomy, a source of evidence that has been lacking for a significant number of species and species groups of *Setaria*.

The variation of the foliar anatomy is studied based on a cluster analysis. We also include in the analysis, some of the morphological vegetative and reproductive characters used traditionally to delimit the genus, subgenera, sections and species. The significance of the characters is evaluated to identify species groups, and these groups are compared with infrageneric taxa (Stapf and Hubbard 1930; Rominger 1962; Fox and Hatch 1999; Pensiero 1999) and with monophyletic clades established in published molecular phylogenies (Doust et al. 2007; Kellogg et al. 2009; Kim et al. 2015). Additionally, some foliar anatomical characters associated with the photosynthetic pathway are also discussed and compared with those in other genera of Paniceae and Paspaleae.

Materials and methods

The species studied were selected in an attempt to represent the range of morphological variation and geographical distribution found within the genus. Accordingly, all subgenera or sections are represented, for a total of 93 species. A total of 263 specimens were included because most species were represented by two, or up to seven, accessions to account for infra-specific anatomical variation. However, seven species were represented by single accessions. The studied species and vouchers are listed in Online Resource 1.

To analyze the foliar anatomical structure, transverse sections made of the middle third of the leaf blade were prepared. Some preparations were made from fresh material collected in northeast Argentina. For the majority of species, particularly those from North America and the Old World, samples came from herbarium material. The fresh material was fixed in formalin–acetic acid–alcohol (FAA). The herbarium samples were rehydrated by submerging them in water with commercial detergent and heating them in an oven at 50 °C for 2–4 days, followed by fixing them in FAA. Both fresh and herbarium samples were dehydrated in an ethanol series, transferred to xylene, embedded in paraffin and sectioned on a rotary microtome to a thickness of 10 µm according to standard methods. The histological samples were stained with Safranin-Fast Green and mounted in Canada balsam (D'Ambrogio de Argüeso 1986). Observations were made using a Wild M20 optical microscope. The terminology to the anatomical characters

and descriptions were based on Ellis (1976, 1979). Based on the observations, a total of 24 variable anatomical characters were selected (Online Resource 2).

Morphological observations were based on herbarium specimens from AD, B, BA, BAA, BM, CAMB, G, K, L, LE, M, MA, MO, NBG, NSW, NY, P, PRE, SAM, SI, UPS, US, and W (Thiers 2014). The specimens were analyzed with a Wild M5 stereomicroscope and some data were obtained from literature. A total of 7 vegetative and 16 reproductive informative characters were scored (Online Resource 2).

The data set combines both morphological and anatomical characters for the 263 specimens, with a total of 47 characters (available upon request from the first author).

A cluster analysis was carried out using NTSYS-pc version 2.0 (Rohlf 1998). The standardized matrix was used and distances among OTUs were evaluated based on the Manhattan distance coefficient, using the unweighted pair-group method on the arithmetic average algorithm (UPGMA).

Results

General description of the foliar anatomy

The outline of the blade in the transverse section is usually expanded or opened V-shaped (Fig. 1a), but some species present an in-rolled blade, frequently convolute (Fig. 2c). Exceptionally, the lateral surfaces are highly reduced and there is no distinction between them and the central keel, or blades are semicircular in the transverse section (as in *S. atrata*, *S. paucifolia* and some specimens of *S. restioidea*; Fig. 2e). The adaxial and abaxial surfaces of the blades are generally straight or slightly undulating, without pronounced ribs and furrows (Figs. 1e, 2f). In few species (e.g., *S. fiebrigii*, *S. geminata*, *S. obscura*, *S. pradana*, *S. uda*), the adaxial surface presents pronounced rounded or obtuse ribs associated with first- and second-order vascular bundles, and furrows are located over the third-order vascular bundles (Fig. 1a–d).

The central vascular bundle ranges from scarcely differentiated from the remaining first-order vascular bundles, up to a well developed forming a central keel with several first-order vascular bundles and colorless parenchyma adaxially associated. When the central keel is present, the shape is acute or rounded in the abaxial surface, and flat at the adaxial surface, except in some species where the central keel is adaxially raised (Fig. 2a). The number of first-order vascular bundles per blade varies from 3 up to 21 (*S. magna*), and this character was variable at specific level; the common arrangement of the vascular bundles is one or more than one second-order vascular bundle

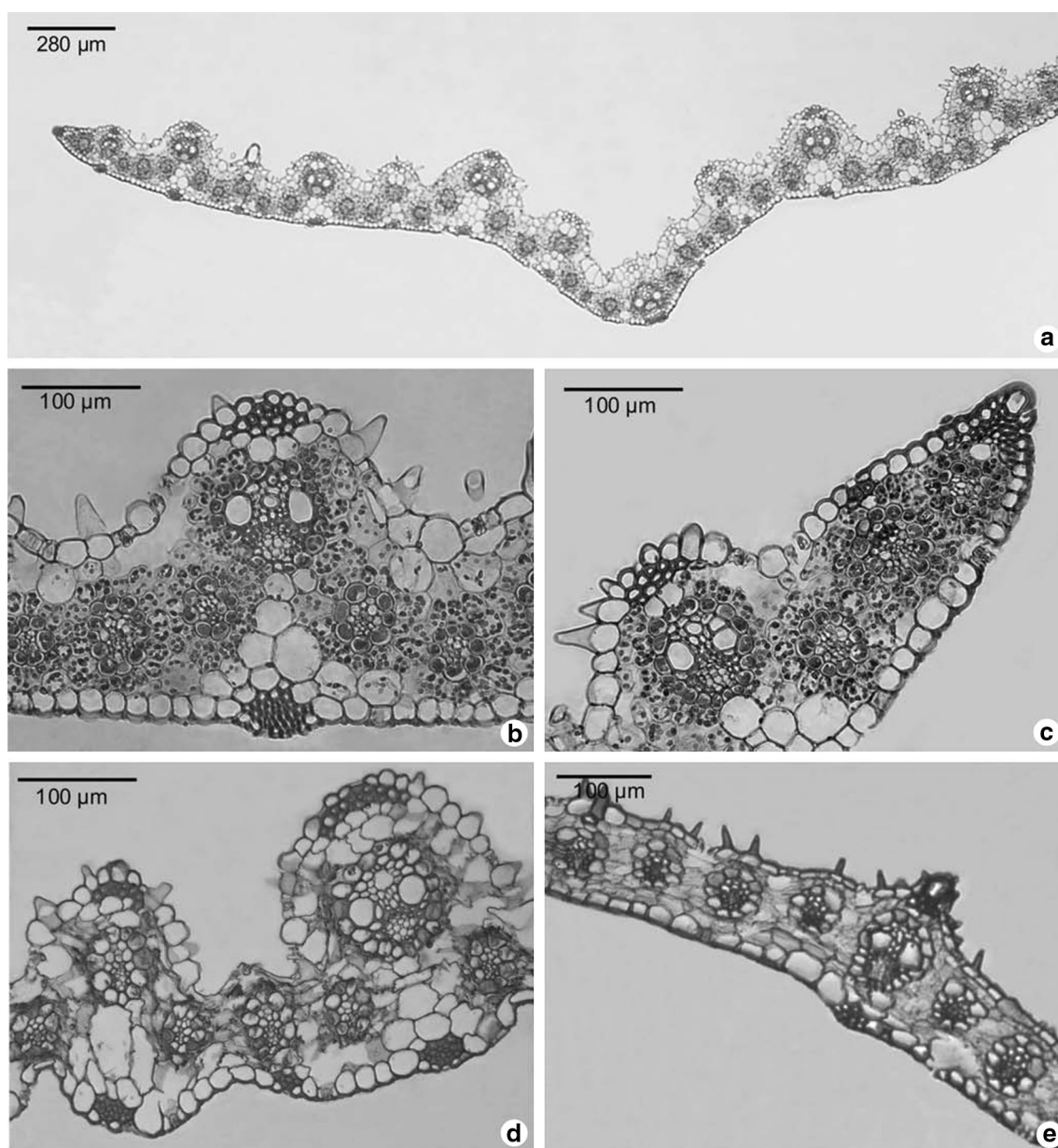


Fig. 1 Light micrographs of *Setaria* leaf blade sections. **a–c** *S. geminata* Aliscioni 717 (BAA, SI). **d** *S. uda* Cowie & Dunlop 7888 (CANB). **e** *S. obtusifolia* Davidse & Ellis 5868 (BAA, MO)

between two consecutive first-order bundles, alternating with third-order bundles.

Most of the species exhibit mesophyll formed of well-developed chlorenchyma, with irregular or incompletely radiate disposition. Highly symmetrical radial arrangements around vascular bundles were not observed in the studied species. In some species, such as *S. apiculata*, *S. faberi*, *S. parviflora* and *S. pumila*, the chlorenchyma of the mesophyll is reduced to a few layers of cells, the blades are very thin, and generally the epidermal cells are large (18–28 µm diam.). Well-developed colorless parenchyma of the adaxial surface of the blade is present in *S. atrata*

and *S. paucifolia* and chlorenchyma is present in the abaxial position (Fig. 2e). Adaxial and/or abaxial extensions of sheaths of colorless parenchyma associated with first-order vascular bundles (Fig. 1b) were observed in some species (*S. fiebrigii*, *S. geminata*, *S. obscura*, *S. punctata*, *S. retiglumis*, *S. uda*). Lacunae with aerenchyma are absent among the species of *Setaria*, including the species related to aquatic habitats; only small and scattered air spaces may be distinguishable in *S. finita*, *S. obscura*, *S. obtusifolia*, *S. punctata* and *S. sagittifolia*.

The sclerenchyma usually forms adaxial and/or abaxial girders associated with the first-order vascular bundles and,

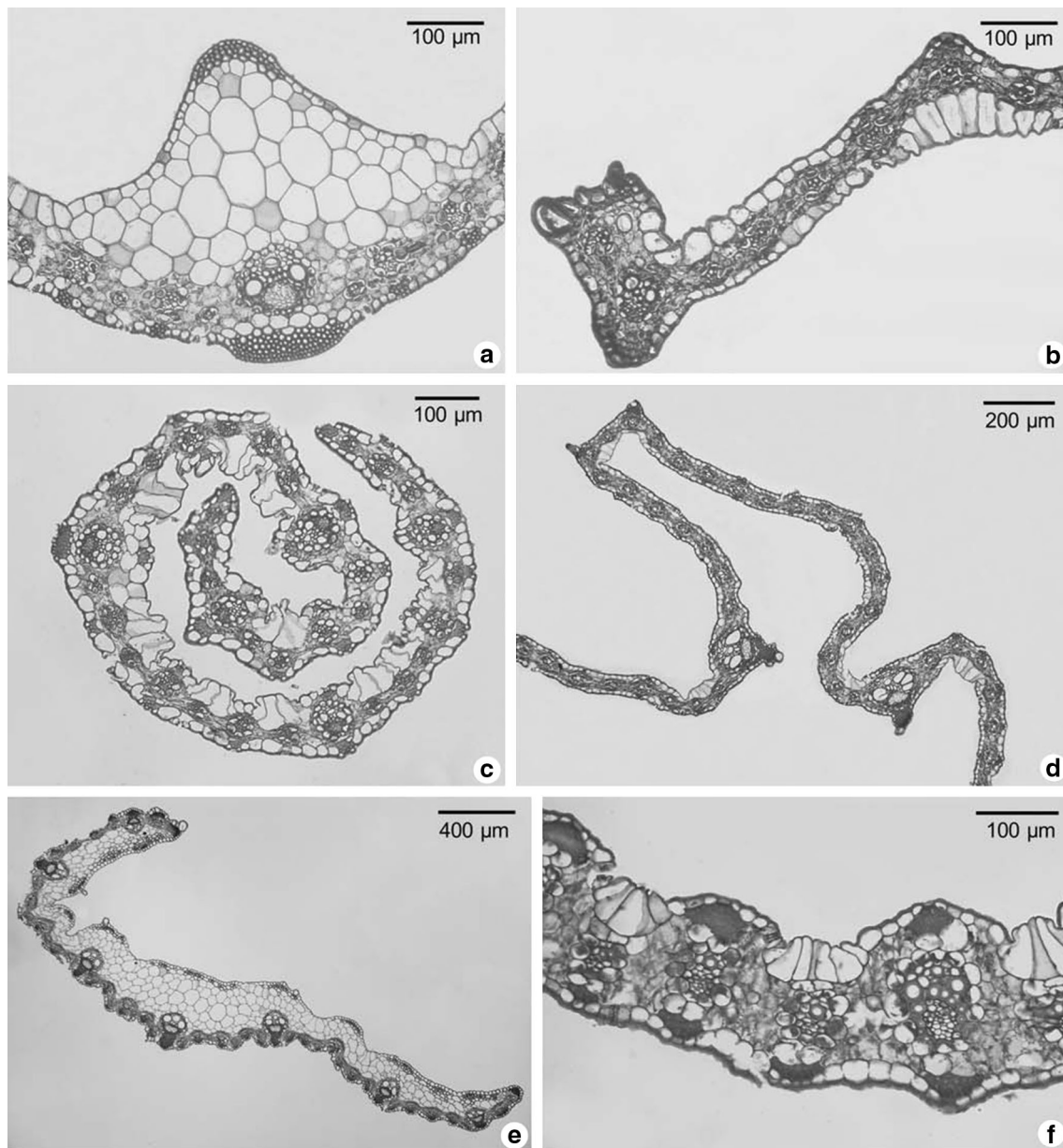


Fig. 2 Light micrographs of *Setaria* leaf blade sections. **a, b, d** *S. sulcata* Smook 5638 (PRE). **c** *S. flavida* Batianoff & Franks 930289 (AD). **e** *S. atrata* Myers 11696 (K). **f** *S. cernua* Jaramillo 7658 (MO)

in some species, the abaxial girders occasionally interrupt the bundle sheaths (e.g. *S. globulifera*, *S. longiseta*, *S. mendocina*, *S. nigrirostris*); second- and third-order vascular bundles and the margins can be associated with epidermal strands of sclerenchyma (Fig. 1c) or unassociated (Fig. 2b).

The most common adaxial and abaxial epidermal cells have flat or slightly convex tangential external walls; papillose epidermis is not common, but when present occurs only on the adaxial surface (*S. geminata*, *S. punctata*, *S. retiglumis*, *S. uda*, *S. obtusifolia*, and some specimens of *S. obscura*, *S. chapmanii*, and *S. reverchonii*) (Fig. 1a–e).

Stomates, unicellular macrohairs and prickles are frequently present in the epidermis and vary at infraspecific level, they are present in adaxial, abaxial or both epidermis. Bulliform cells are adaxially differentiated in regular groups over singular third-order vascular bundles (Fig. 2f), or more than one third-order vascular bundles; but in many species the adaxial bulliform cells are unremarkable compared to other cells of the epidermis; colorless parenchyma cells associated with bulliform cells were not observed in the studied species. The presence of abaxial bulliform cells was observed in all species of *Setaria* subg. *Ptycophyllum*; in this case cells are grouped together in numbers from

around four, up to nine, below one or more minor vascular bundles (Fig. 2b, d).

Anatomical characters related to the photosynthetic type

The vascular bundles in leaf blades of *Setaria* are surrounded completely by a mestome Kranz sheath with rounded cells. They have uniformly thickened walls, centrifugally positioned, specialized chloroplasts and 1–3 chlorenchyma cells between consecutive vascular bundles. *Setaria variifolia* (Fig. 3a, b), *S. utowanaea* (Scribn.) Pilg. (Fig. 3c, d), and the specimen *Aliscioni 777* of *S. vulpiseta* (Lam.) Roem. & Schult. (Fig. 3e, f),

differ from the remaining studied species by presenting vascular bundles surrounded by two sheaths, the typical inner mestome Kranz sheath and an external sheath composed by isodiametric and globose parenchymatous cells with many chloroplasts. This second sheath can be complete or incomplete, and is present in both first-order and minor vascular bundles. Particularly, in the specimen *Aliscioni 777* of *S. vulpiseta*, which was analyzed by fresh material, the cells of this sheath are readily distinguishable from the chlorenchymatous cells of the mesophyll and mestome sheath cells. The walls are thinner, like the cells of the mesophyll, and do not seem to be suberized, but the chloroplasts are distributed toward the periphery of the cell, probably because of the

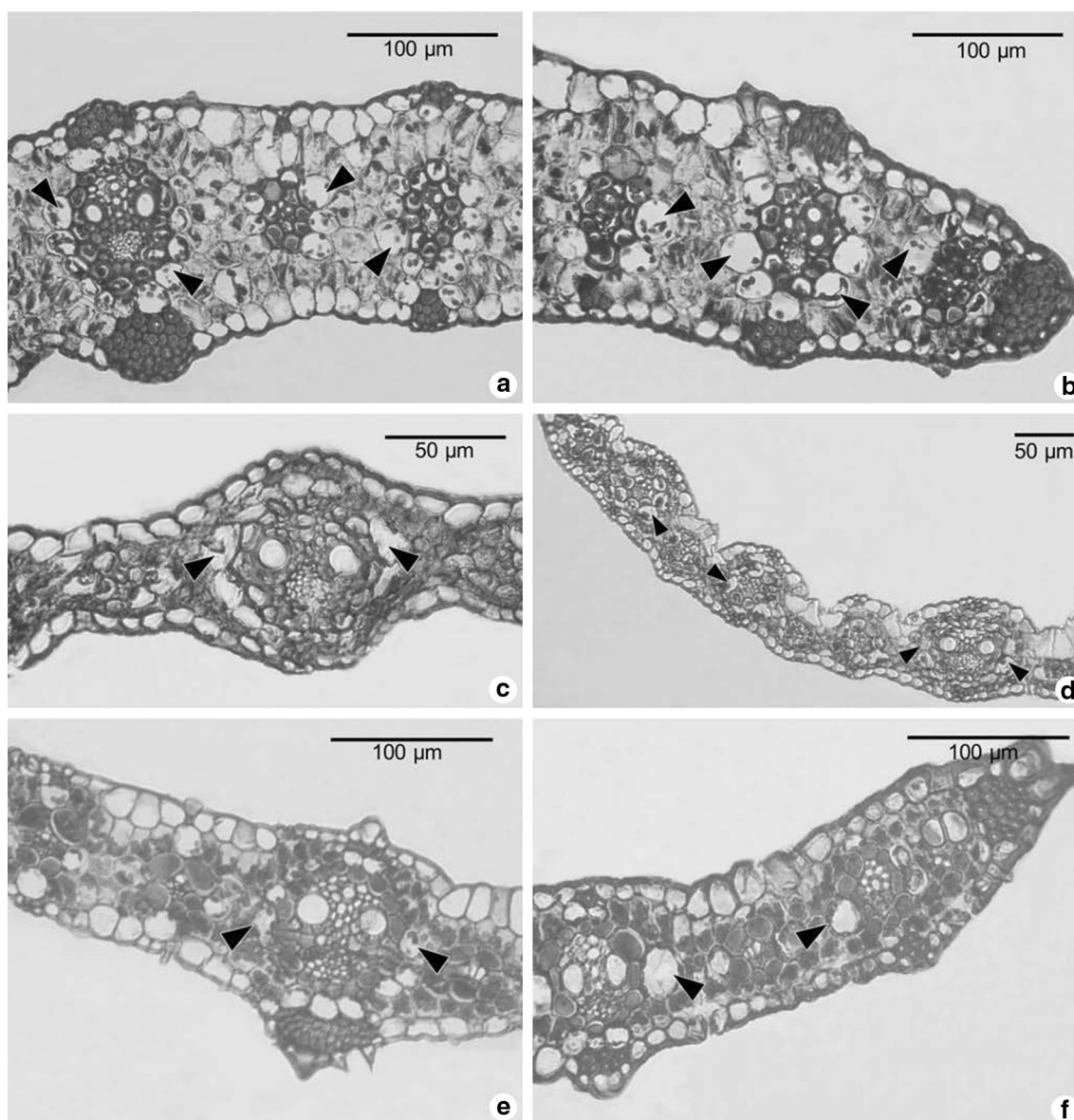


Fig. 3 Light micrographs of *Setaria* leaf blade sections. **a, b** *S. variifolia* Martínez et al. 27089 (MO). **c, d** *S. utowanaea*, **c** Bunch 181 (MO), **d** Steyermark et al. 102307 (MO). **e, f** *S. vulpiseta* Aliscioni 777 (BAA, SI). Arrows indicate external sheaths around vascular bundles

presence of a big central vacuole filling up most of the internal cellular space.

Cluster analysis

The cluster analysis (CA) based on 47 characters from 263 specimens is shown in the Figs. 4 and 5. The correlation of the distance and tree matrix was 0.8 indicating a relative good fit of the phenogram to the distance matrix. The different groups and subgroups are numbered and discussed in the order that appears in the Figs. 4 and 5.

Although some anatomical characters were variable at the intra-specific level in several species, most of the vouchers of each species were included next or near each other in the same clusters. The only exception was *S. restioides* where three specimens are in cluster B2h (Fig. 5), while one specimen (*Fay 6007*) was grouped with all vouchers of *S. paucifolia* and *S. atrata* in the small cluster 1 (Fig. 4).

Cluster 2 included small clusters where the species *S. sagittifolia*, *S. obscura*, and *S. mendocina* emerged independently (Fig. 4). Within cluster 2, two large groups, A (Fig. 4) and B (Fig. 5), can be recognized. All species that were previously considered to belong to the genus *Paspalidium*, *Setaria* subg. *Paurochaetium*, *S. clivalis*, and *S. perrieri* comprised group A (Fig. 4), whereas *Setaria* subg. *Ptycophyllun* and the remaining studied species of *Setaria* are included in the big cluster B (Fig. 5). In cluster A, *S. obtusifolia* was isolated from three recognized sub-clusters; subgroup A1 encompassed species of *Setaria* subg. *Paurochaetium* (*S. chapmanii*, *S. distantiflora*, *S. leonis*, *S. pradana*, *S. reverchonii*, *S. utowanaea*) with *S. clivalis* and *S. perrieri* associated. Another subgroup consisted of species previously treated as *Paspalidium* (excluding *S. obtusifolia*); where the species clearly separated in two associations of species: A2a with *S. geminata*, *S. uda*, *S. retiglumis* and *S. punctata*, and A2b with *S. albobillosa*, *S. rara*, *S. clementii*, *S. basiclada*, *S. flavida*, *S. globoidea*, *S. jubiflora*,

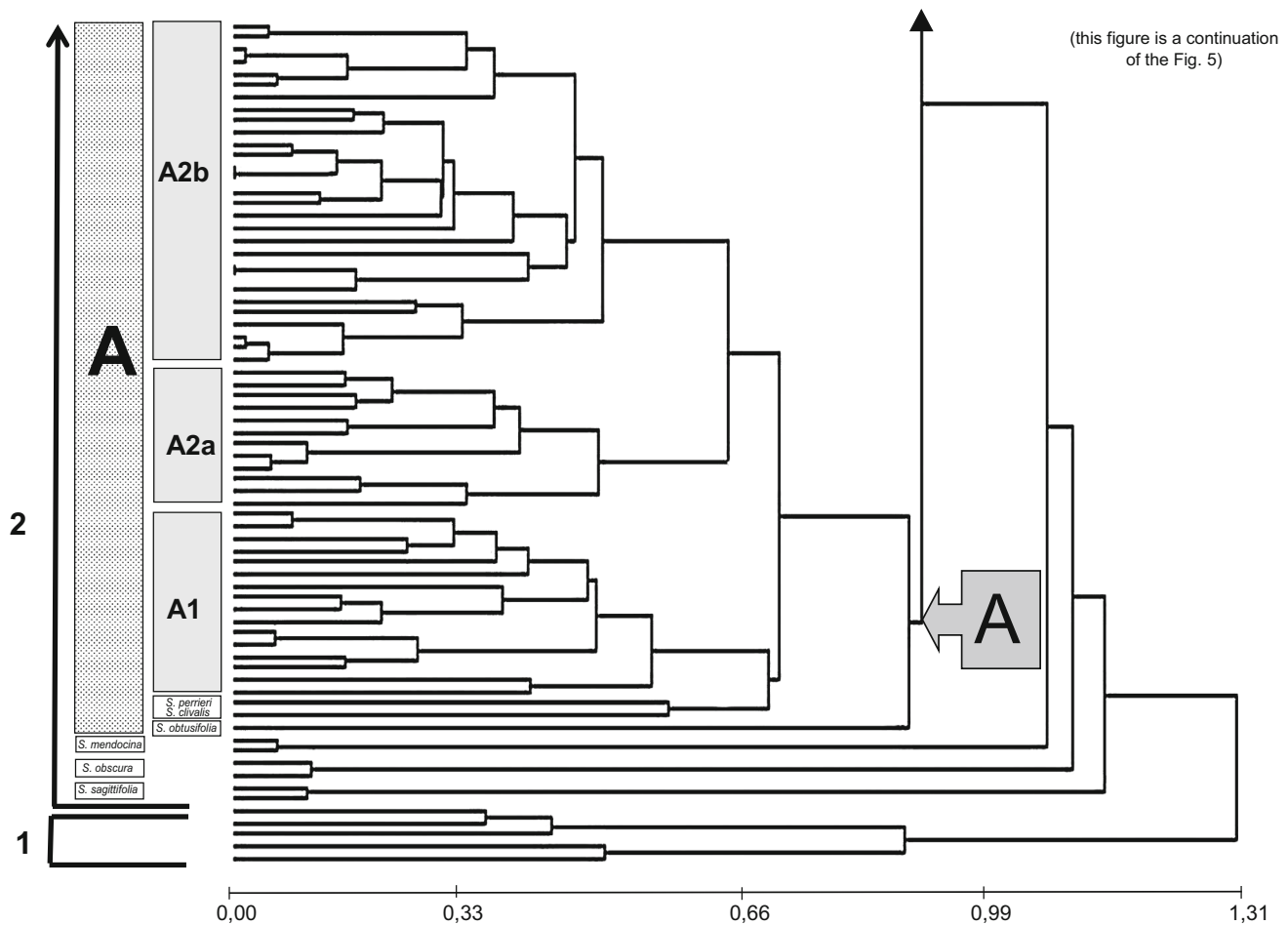


Fig. 4 Part of the phenogram of 263 specimens of *Setaria*, clusters and sub-clusters are discussed in the text (this figure continues in Fig. 5). Cluster 1, *S. paucifolia*, *S. atrata*, *S. restioides* (Fay 6007); cluster A1, *S. chapmanii*, *S. distantiflora*, *S. leonis*, *S. pradana*, *S.*

reverchonii, *S. utowanaea*; cluster A2a, *S. geminata*, *S. uda*, *S. retiglumis*, *S. punctata*; cluster A2b, *S. albobillosa*, *S. rara*, *S. clementii*, *S. basiclada*, *S. flavida*, *S. globoidea*, *S. jubiflora*, *S. grandispiculata*, *S. reflexa*, *S. tabulata*, *S. constricta*

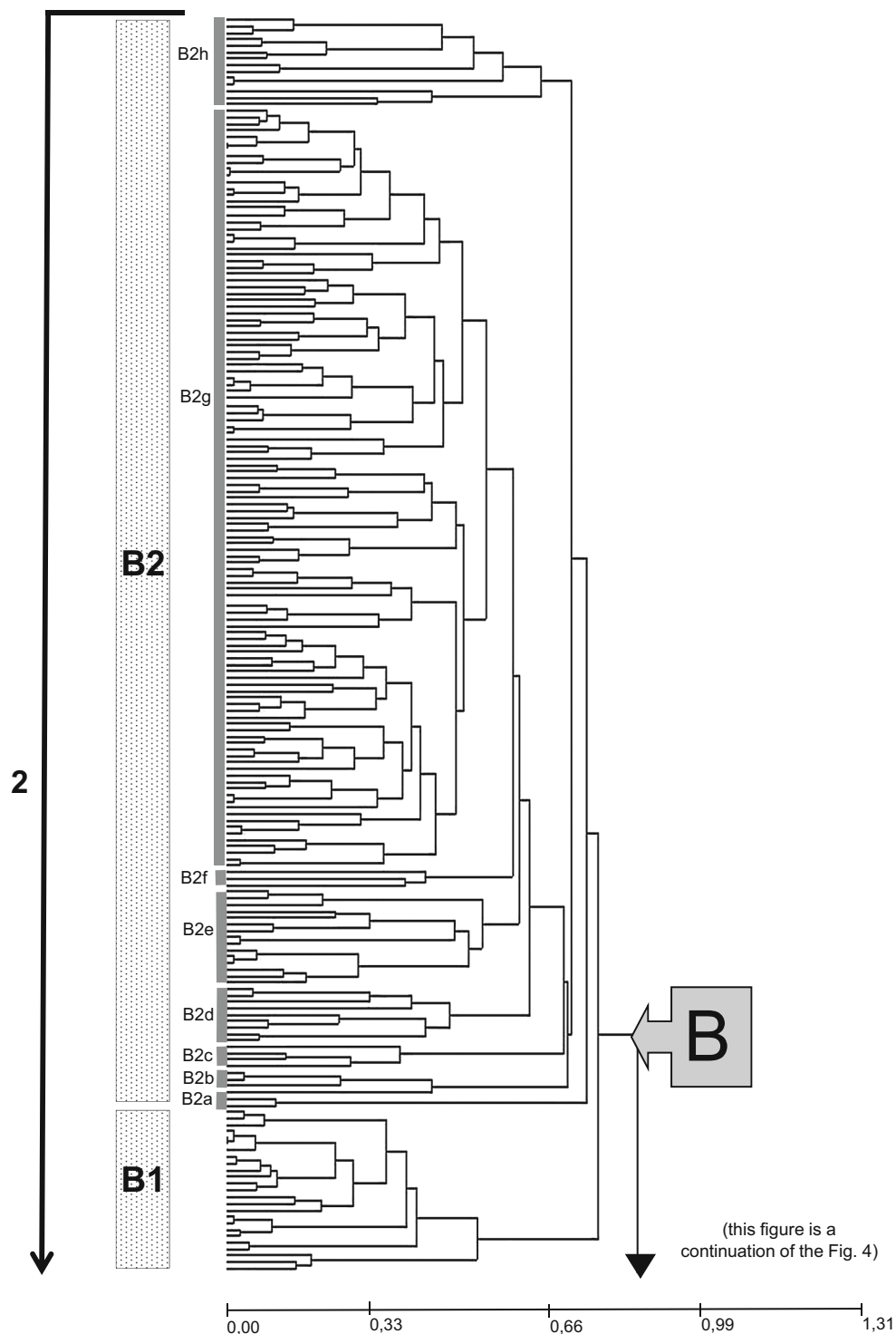


Fig. 5 Part of the phenogram of 263 specimens of *Setaria*, clusters and sub-clusters are discussed in the text (this figure is a continuation of the Fig. 4). Cluster B1, *S. barbata*, *S. kagerensis*, *S. palmifolia*, *S. sulcata*, *S. plicata*, *S. seriata*, *S. homonyma*, *S. lindenbergiana*; cluster B2a, *S. appendiculata*; cluster B2b, *S. longiseta*; cluster B2c, *S. cernua*; cluster B2d, *S. parviflora*, *S. pumila*, *S. ustilata*; cluster B2e, *S. fiebrigii*, *S. globulifera*, *S. variifolia*, *S. parodii*, *S. scabrifolia*; cluster B2f, *S. nicorae*; cluster B2g, *S. adhaerens*, *S. alonsoi*, *S. apiculata*, *S. australiensis*, *S. chondrachne*, *S. cordobensis*, *S. faberi*,

S. finita, *S. forbesiana*, *S. grandis*, *S. grisebachii*, *S. hassleri*, *S. hunzikeri*, *S. intermedia*, *S. italica*, *S. macrostachya*, *S. magna*, *S. lachnea*, *S. longipila*, *S. oblongata*, *S. oplismenoides*, *S. orthosticha*, *S. pampeana*, *S. paspalidioides*, *S. petiolata*, *S. pflanzii*, *S. pseudaris-tata*, *S. rosengurttii*, *S. scandens*, *S. scheelei*, *S. setosa*, *S. stolonifera*, *S. tenacissima*, *S. tenax*, *S. vaginata*, *S. vatkeana*, *S. verticillata*, *S. verticilliformis*, *S. viridis*, *S. vulpiseta*, *S. welwitschii*; cluster B2h, *S. acromelaena*, *S. nigrirostris*, *S. sphacelata*, *S. leucopila*, *S. restioidea*

S. grandispiculata, *S. reflexa*, *S. tabulata*, and *S. constricta* (Fig. 4).

Cluster B is divisible into two groups: B1 containing all species of *Setaria* subg. *Ptycophyllum* (*S. barbata*, *S. kagerensis*, *S. palmifolia*, *S. sulcata*, *S. plicata*, *S. seriata*, *S. homonyma*, *S. lindenbergiana*), and another larger group, B2, that includes several nested sub-clusters of species of *Setaria* (Fig. 5). In cluster B2, the specimens of *S. appendiculata* were the first to emerge independently as branch from the remaining species (B2a). Cluster B2 contains also a small group that included *S. acromelaena*, *S. nigrirostris*, *S. sphacelata*, *S. leucopila*, three specimens of *S. restioidea* (B2h), and a diverse ensemble of other species. Within the later cluster the delimitation of the sub-cluster is less obvious. The specimens of *S. longiseta* (B2b) and *S. cernua* (B2c) emerge as separate branches not closely related to any other species. Then, a group (B2d), which includes *S. parviflora*, *S. pumila* and *S. ustilata*, is recognized, and *S. fiebrigii*, *S. globulifera*, *S. variifolia*, *S. parodii* and *S. scabrifolia* (B2e) appear next. The specimens of *S. nicorae* seem to be unrelated to other species (B2f). Finally, all specimens of the species *S. adhaerens*, *S. alonsoi*, *S. apiculata*, *S. australiensis*, *S. chondrachne*, *S. cordobensis*, *S. faberi*, *S. finita*, *S. forbesiana*, *S. grandis*, *S. grisebachii*, *S. hassleri*, *S. hunzikeri*, *S. intermedia*, *S. italica*, *S. macrostachya*, *S. magna*, *S. lachnea*, *S. longipila*, *S. oblongata*, *S. oplismenoides*, *S. orthosticha*, *S. pampeana*, *S. paspalidioides*, *S. petiolata*, *S. pflanzii*, *S. pseudaristata*, *S. rosenfurtii*, *S. scandens*, *S. scheelei*, *S. setosa*, *S. stolonifera*, *S. tenacissima*, *S. tenax*, *S. vaginata*, *S. vatkeana*, *S. verticillata*, *S. verticilliformis*, *S. viridis*, *S. vulpiseta* and *S. welwitschii* were included in one cluster (B2g).

Discussion

Diverse taxonomic treatments have considered *Setaria* to be a morphologically variable ensemble of species (Rominger 1962; Webster 1987; Pensiero 1999; Morrone et al. 2014); additionally the phylogenetic studies demonstrated that the genus is non-monophyletic (Doust et al. 2007; Kellogg et al. 2009). Here we demonstrate that *Setaria* also presents a broad range of variability in the anatomy of the leaf blade.

The different groups recovered in the present analysis are not fully coincident with the clades described in the published phylogenies (Doust et al. 2007; Kellogg et al. 2009; Kim et al. 2015), or with previously proposed infrageneric taxa (Stapf and Hubbard 1930; Rominger 1962; Fox and Hatch 1999; Pensiero 1999). However, some informative comparisons can be made. The major coincidence with some previous taxonomical and phylogenetic studies is that species considered in the past as *Paspalidium*

were included within *Setaria*. Therefore, the early observations about the imprecise boundaries between the two genera, discussed by Veldkamp (1980), Clayton and Renvoize (1986), Webster (1987), and Davidse and Pohl (1992), and the corresponding species transfers into the genus *Setaria* (Veldkamp 1994; Webster 1993, 1995; Morrone et al. 2014), are supported.

Cluster A includes *Setaria obtusifolia* joined with the subgenus *Setaria* subg. *Paurochaetium* and associated *S. clivalis*, *S. perrieri* (A1) along with other species previously treated in *Paspalidium* (A2a and A2b). This group would represent a consistent morphological group if it were not for the peculiar positions of *S. clivalis* and *S. perrieri*, species whose macro-morphology differs markedly, principally in having open and lax, rather than racemose, panicles. *S. perrieri* is an endemic xerophytic species from Madagascar. *S. clivalis* is an Asian species that was described as having slightly plicate blades; however, the specimen studied here lacks this character.

The species previously considered to belong in the genus *Paspalidium*, excluding *S. obtusifolia*, form a cluster that is divisible into two sub-clusters. The sub-cluster A2a is constituted by *S. geminata*, *S. uda*, *S. retiglumis* and *S. punctata*; these species, except the later one, were included in the molecular phylogeny published by Kellogg et al. (2009). In that analysis they constituted a monophyletic group along with *Uranthoecium truncatum* (Maiden & Betche) Stapf, though support for this group was weak. These four species of *Setaria* exhibit a very similar foliar anatomy, having deep furrows in adaxial surface, a papillose adaxial epidermis, macrohairs absent or present only in the adaxial epidermis, extensions sheaths of colorless parenchyma, and the absence of a developed central keel. The species of this sub-cluster are from Australia, except *S. geminata*, which is native to Africa and Asia and introduced in tropical and subtropical areas of other continents. Regarding their habitats, all of the species of this sub-cluster are aquatic or semi-aquatic, frequently with culms leaning or floating and of spongy firmness (but no aerenchyma was observed in the leaves).

The other sub-cluster, A2b, contains 11 species (*S. albobillosa*, *S. basiada*, *S. clementii*, *S. constricta*, *S. flavida*, *S. globoidea*, *S. grandispiculata*, *S. jubiflora*, *S. rara*, *S. reflexa*, and *S. tabulata*), the majority of which were sampled in a molecular phylogeny (Kellogg et al. 2009) where they formed a well-supported monophyletic group along with the Australian xerophytic species *Plagiosetum refractum* Benth. in a Bayesian analysis.

Setaria obtusifolia is isolated within cluster A; this species is morphologically similar to the species of cluster A2a by having papillae in the adaxial epidermis, but differs by lacking adaxial furrows and colorless parenchyma extensions in the sheaths (Fig. 1e).

All the species placed in *Setaria* subg. *Paurochaetium* resolved within cluster (A1). This group historically was connected to the genus *Paspalidium*, an association that is also noted in this analysis. *Setaria* subg. *Paurochaetium* was originally recognized by Hitchcock and Chase (1910) as a subgenus of *Panicum*; then was transferred to *Setaria* at the section rank by Pilger (1940), and later it was elevated to the rank of subgenus by Rominger (1962). Davidse and Pohl (1992) transferred some of the species of *Setaria* subg. *Paurochaetium* to *Paspalidium*; but Webster (1993, 1995) and Veldkamp (1994) transferred all species of *Paspalidium* to *Setaria* based on the fact that some of these species have setae accompanying more spikelets than just the distal one. In the present work, the inclusion of species of *Setaria* subg. *Paurochaetium* in *Setaria* is confirmed. Concerning their foliar anatomy, these species are similar, with few first-order vascular bundles (3–9, rarely 13), minor vascular bundles frequently associated with sclerenchyma, with only one first-order vascular bundle in the central keel, bulliform cells distinguishable over one or more minor bundles and bundles without parenchymatous extensions. These species are distributed around the Caribbean, Mexico, Florida, Colombia and Venezuela.

Rominger (1962) mentioned that *Setaria* subg. *Paurochaetium* may not be a natural group, but in our study these species were grouped in a well-defined cluster. However, it is necessary to include this subgenus in futures phylogenetic analysis to test its monophyly.

Within the large cluster B (Fig. 5), group B1 included species belonging to *Setaria* subg. *Ptycophyllum*, supporting its taxonomic recognition. This group was recognized by Stapf and Hubbard (1930) as a section of *Setaria*. Rominger (1962), in revision of *Setaria* in North America, established this group as a subgenus characterized by perennial plants with plicate blades, a usually loose and open panicle bearing slender spikelets, with a single bristle subtending the terminal spikelet of each ultimate branch, and occasionally a bristle below some of the lower spikelets. The author cited six species in North America, *S. palmifolia* native to India, *S. barbata* apparently native to Africa and three species native to South America that currently are synonyms of *S. sulcata* (Pensiero 2003). Pensiero (1999), in a revision of the genus for South America accepted *S. palmifolia*, *S. barbata* and *S. sulcata*.

Setaria subg. *Ptycophyllum* was resolved as a well-supported monophyletic group in an analysis of the nuclear marker *kn1* (Doust et al. 2007), but was paraphyletic using the plastid marker *ndhF* (Kellogg et al. 2009). Many morphological characters are shared among these species. Furthermore, novel set of anatomical characters were observed to be homogeneous in the present study, including adaxial and abaxial surface without furrows; numerous first-

order vascular bundles per blade (usually more than 11); sclerenchyma in foliar margins and associated with minor vascular bundles absent or scarcely developed; central keel having only one first-order vascular bundle, rounded in abaxial surface; presence of groups of abaxial bulliform cells below the minor vascular bundles (Fig. 2a, b, d).

All species of the *Setaria* subg. *Ptycophyllum* are related to the remaining species of *Setaria* included in this analysis in the large cluster B2, which contains several assemblages of species whose affinities are complex and difficult to interpret. These species constituted very diverse and heterogeneous groups, some of which were treated in *Setaria* subg. *Setaria*, *Setaria* subg. *Cernatum* (Rominger 1962; Pensiero 1999), *Setaria* sect. *Eusetaria*, *Setaria* sect. *Panicatrix* (Stapf and Hubbard 1930), and *Setaria* subg. *Reverchoninae* (Fox and Hatch 1999). In this big group, the cluster B2h (*S. acromelaena*, *S. nigrirostris*, *S. sphacelata*, *S. leucopila*, three specimens of *S. restioidea*) is relatively distinct in the present analysis, but in the phylogeny of Kellogg et al. (2009) these species were dispersed in three different clades. Morphologically they share the presence of spiciform panicles with pilose rachises, but no shared anatomical characters were detected for these species.

The small sub-cluster B2d (*S. parviflora*, *S. pumila* and *S. ustilata*) contains species that have spiciform panicles, with glabrous rachis, spikelets subtended by numerous bristles and a developed lower palea. Regarding the anatomical characters, the leaf blades of these species showed a proportionally reduced mesophyll and both epidermises were composed of large cells. Among these species, *S. parviflora* and *S. pumila* were sampled in the phylogeny of Kellogg et al. (2009), and were resolved as sister taxa.

The sub-cluster B2e (*S. fiebrigii*, *S. globulifera*, *S. variifolia*, *S. parodii* and *S. scabrifolia*) contains similar species, which have pseudopetiolate leaf blades, and also spikelets with a developed lower palea. However, the spiciform panicles vary from strict to lax. Regarding foliar anatomical characters, these are similar and species have a central keel with only one first-order vascular bundle and an adaxial surface with prominent furrows. In this sub-cluster, *S. fiebrigii*, *S. globulifera*, *S. parodii* and *S. scabrifolia* are from South American and, with the exception of *S. parodii* that was not sampled in the molecular phylogeny, these species are phylogenetically related in a strongly supported monophyletic clade (Kellogg et al. 2009). *Setaria variifolia* also was placed in this cluster. It is from Mesoamerica and was said to be allied to *S. reverchonii* by Fox and Hatch (1999) and placed in *Setaria* subg. *Reverchoninae*. However, in the present work *S. variifolia* was not related to *S. reverchonii*, which was placed in cluster A1.

The cluster B2g included a large ensemble of species (*S. adhaerens*, *S. alonsoi*, *S. apiculata*, *S. australiensis*, *S. chondrachne*, *S. cordobensis*, *S. faberi*, *S. finita*, *S. forbesiana*, *S. grandis*, *S. grisebachii*, *S. hassleri*, *S. hunzikeri*, *S. intermedia*, *S. italica*, *S. macrostachya*, *S. magna*, *S. lachnea*, *S. longipila*, *S. oblongata*, *S. oplismenoides*, *S. orthosticha*, *S. pampeana*, *S. paspalidioides*, *S. petiolata*, *S. pflanzii*, *S. pseudaristata*, *S. rosenfurtii*, *S. scandens*, *S. scheelei*, *S. setosa*, *S. stolonifera*, *S. tenacissima*, *S. tenax*, *S. vaginata*, *S. vakeana*, *S. verticillata*, *S. verticilliformis*, *S. viridis*, *S. vulpiseta* and *S. welwitschii*) with minimal morphological, anatomical and geographical distinctions. *Setaria adhaerens* was found in this group and was treated in our analysis as a separate species; however, its position near *S. verticillata* in the same cluster confirmed the synonymy established by Morrone et al. (2014). Furthermore, *S. viridis* (the type of the genus), is located in this group along with *S. faberi*, to which it is morphologically similar and phylogenetically related (Kellogg et al. 2009; Kim et al. 2015).

Concerning the photosynthetic pathway, we demonstrate with numerous examples that the arrangement of the vascular bundles and the type of vascular sheath observed in the studied species in *Setaria* is a C₄; “panicoid type”, NADP-me biochemical type, with Kranz anatomy of the MS (X and MS-) subtype (Brown 1977; Ellis 1977; Hattersley and Watson 1976). This was reported previously based on a much smaller number of species (Metcalf 1960; Ellis 1977; Carraro et al. 1991; Watson and Dallwitz 1992 onwards). However, we also observed some variation, *S. variifolia*, *S. utowanaea*, and one specimens of *S. vulpiseta* showed a second external complete or incomplete sheath.

Different genera of the tribes Paspaleae and Paniceae with C₄, and NADP-me biochemical type, include some species with the same unusual external sheaths that was observed here in some *Setaria*. Within the tribe Paspaleae, this condition was described in *Coleataenia petersonii* (Hitchc. & Ekman) Soreng, *C. prionitis* (Nees) Soreng and *C. scabrida* (Döll) Zuloaga (Scataglini et al. 2014), and *Cyphonanthus discrepans* (Döll) Zuloaga & Morrone (Zuloaga et al. 1989; Morrone et al. 2007) of the subtribe Arthropogoninae. It was also found in *Paspalum inaequivalve* Raddi and *P. microstachyum* J.Presl (Aliscioni and Denham 2009); *Aaika tuerckheimii* (Hack.) J.R.Grande and *Oswaldoa valida* (Mez) J.R.Grande (Zuloaga et al. 1989; Lizarazu et al. 2014); *Anthaenantiopsis fiebrigii* Parodi, *A. perforata* (Nees) Parodi and *A. rojasiana* Parodi (Morrone et al. 1993) of the subtribe Paspalinae. Regarding the tribe Paniceae, these unusual external sheaths were described in *Alloteropsis semialata* (R.Br.) Hitchc. (Brown, 1975) of the subtribe Boivinellinae; in *Neurachne* R.Br. and *Paraneurachne* S.T. Blake (Hattersley et al. 1982) of the subtribe Neurachninae. Two additional species

with external sheaths, *Chaetium cubanum* (C.Wright) Hitchc. and *C. festucoides* Nees., are C₄ NADP-me (Morrone et al. 1998), but belong to the subtribe Melinidinae that is otherwise characterized as having PEP-ck photosynthesis.

In all these works, the authors described the external sheath and in the majority of the cases assumed that these species share the C₄ photosynthetic pathway typical of the genus. However, very few biochemical or physiological studies were carried out to check this presumption, and furthermore, the real function of the external sheath is still unknown for the mentioned species. On the other hand, when this character was explored in an evolutionary context of the tribes Paniceae and Paspaleae (Morrone et al. 2012), it appeared to be restricted to diverse and unrelated clades. However, we found this attribute to be relatively frequent because it is present in most subtribes. Lastly, we also observed an external sheath of C₄ NADP-me type in *Setaria*, this being the first report of this condition for the subtribe Cenchrinae.

As a final conclusion, we consider that the revision of the taxonomy of *Setaria* must be based on a strongly supported phylogeny, but unfortunately the phylogenies published currently are not well resolved and reveal *Setaria* as a collection of unrelated groups of species, including other small genera of the subtribe Cenchrinae. At one extreme, these genera that are now taxonomically recognized could be subsumed in a broadly construed *Setaria*. However, it seems more probable to us that the genus will ultimately be split into several genera. Some of the clusters described here as A2a, A2b and B1, which are also monophyletic with relatively strong support in different phylogenetic analyses, could tentatively be considered to represent the cores or precursors of future new genera.

Acknowledgments We are grateful to the anonymous referee for comments on the manuscript, Gabriela Zarlavsky for technical assistance and the Myndel Botanica Foundation for the grants.

References

- Aliscioni SS, Denham SS (2009) Atypical foliar anatomy related to Kranz syndrome in *Paspalum inaequivalve* and *Paspalum microstachyum* (Poaceae: Panicoideae: Paniceae). *Flora* 204:718–729. doi:10.1016/j.flora.2008.09.010
- Aliscioni SS, Gomiz NE, Torretta JP, Pensiero JF (2011) Reproductive biology of *Setaria magna* Griseb. (Poaceae: Panicoideae: Paniceae). *Pl Syst Evol* 293:111–118. doi:10.1007/s00606-011-0428-0
- Austin DF (2006) Fox-tail millets (*Setaria*: Poaceae), abandoned food in two hemispheres. *Econ Bot* 60:143–158. doi:10.1663/0013-0001(2006)60
- Brown WV (1975) Variations in anatomy, associations, and origins of Kranz tissue. *Amer J Bot* 62:395–402. doi:10.2307/2442093

- Brown WV (1977) The Kranz syndrome and its subtypes in grass systematics. *Mem Torrey Bot Club* 23:1–97
- Carraro L, Patrignania G, Orsernigoa M (1991) *Avena sativa* and *Setaria glauca*: an anatomical and ultrastructural comparison between C₃ and C₄ plants. *Caryologia* 44:361–374. doi:10.1080/00087114.1991.10797203
- Clayton WD, Renvoize SA (1986) *Genera Graminum: grasses of the World*. Her Majesty's Stationery Office, London
- Clayton WD, Vorontsova MS, Harman KT, Williamson T (2002) GrassBase-The online World Grass Flora. CVHW02. <http://www.kew.org/data/grasses-db/cite.htm>. Accessed 27 May 2015
- D'Ambrogio de Argüeso AC (1986) *Manual de Técnicas En Histología Vegetal*. Hemisferio Sur, Buenos Aires
- Davidse G, Pohl RW (1992) New taxa and nomenclatural combinations of Mesoamerican grasses (Poaceae). *Novon* 2:81–110. doi:10.2307/3391667
- Davis JI (1983) Phenotypic plasticity and the selection of taxonomic characters in *Puccinellia* (Poaceae). *Syst Bot* 8:341–353. doi:10.2307/2418354
- Dekker J (2003) The foxtail (*Setaria*) species-group. *Weed Sci* 51:641–656. doi:10.1614/P2002-IR
- Douglas BJ, Thomas AG, Morrison IN, Maw MG (1985) The biology of Canadian weeds. 70. *Setaria viridis* (L.) P.Beauv. *Canad J Pl Sci* 65:669–690. doi:10.4141/cjps85-089
- Doust AN, Penly AM, Jacobs SWL, Kellogg EA (2007) Congruence, conflict, and polyploidization shown by nuclear and chloroplast markers in the monophyletic “bristle clade” (Panicoideae, Panicoideae, Poaceae). *Syst Bot* 32:531–544. doi:10.1600/036364407782250670
- Ellis RP (1976) A procedure for standardizing comparative leaf anatomy in the Poaceae. I. The leaf-blade as viewed in transverse section. *Bothalia* 12:65–109. doi:10.4102/abc.v12i1.1382
- Ellis RP (1977) Distribution of the Kranz syndrome in the southern African Eragrostoideae and Panicoideae according to bundle sheath anatomy and cytology. *Agroplanta* 9:73–110
- Ellis RP (1979) A procedure for standardizing comparative leaf anatomy in the Poaceae. II. The epidermis as seen in surface view. *Bothalia* 12:641–671. doi:10.4102/abc.v12i4.1441
- Fox WEL, Hatch S (1999) New combinations in *Setaria* (Poaceae: Paniceae). *Sida* 18:1037–1047
- Gao MJ, Chen JJ (1988) Isozymic studies on the origin of cultivated foxtail millet. *Acta Agron Sin* 14:131–136
- Hattersley PW, Watson L (1976) C₄ grasses: an anatomical criterion for distinguishing between NADP-malic enzyme species and PCK or NAD-malic enzyme species. *Austral J Bot* 24:297–308. doi:10.1071/BT9760297
- Hattersley PW, Watson L, Johnston CR (1982) Remarkable leaf anatomical variations in *Neurachne* and its allies (Poaceae) in relation to C₃ and C₄ photosynthesis. *Bot J Linn Soc* 84:265–272. doi:10.1111/j.1095-8339.1982.tb00364.x
- Hitchcock A, Chase A (1910) The North American species of *Panicum*. *Contr U S Natl Herb* 15:1–396
- Ingram AL (2010) Evolution of leaf blade anatomy in *Eragrostis* (Poaceae). *Syst Bot* 35:755–765. doi:10.1600/036364410X539844
- Kellogg EA, Aliscioni SS, Morrone O, Pensiero JF, Zuloaga FO (2009) A phylogeny of *Setaria* (Poaceae, Panicoideae, Paniceae) and related genera based on the chloroplast gene *ndhF*. *Int J Pl Sci* 170:117–131. doi:10.1086/593043
- Kim S, Kim Ch-S, Lee J, Lee I-Y, Chung Y-J, Cho M-S, Kim S-C (2015) Phylogenetic relationships among species of *Setaria* (Paniceae; Panicoideae; Poaceae) in Korea: insights from nuclear (ITS and *kn1*) and chloroplast DNA sequence data. *Pl Syst Evol* 301:725–736. doi:10.1007/s00606-014-1111-z
- Lizarazu MA, Nicola MV, Salariao DL (2014) Taxonomic re-evaluation of *Panicum* sections *Tuerckheimiana* and *Valida* (Poaceae: Panicoideae) using morphological and molecular data. *Taxon* 63:265–274. doi:10.12705/632.34
- Marinoni LD, Zabala JM, Exner E, Pensiero JF (2013) Comportamiento germinativo y potencial forrajero de *Setaria magna* (Poaceae). *Bol Soc Argent Bot* 48:261–270
- Metcalfe CR (1960) *Anatomy of the monocotyledons I GRAMINEAE*. Oxford University Press, London
- Morrone O, Filgueiras TS, Zuloaga FO, Dubcovsky J (1993) Revision of *Anthraenantiopsis* (Poaceae: Panicoideae: Paniceae). *Syst Bot* 18:434–453. doi:10.2307/2419418
- Morrone O, Zuloaga FO, Arriaga MR, Pozner R, Aliscioni SS (1998) Revisión sistemática y análisis cladístico del género *Chaetium* (Poaceae: Panicoideae: Paniceae). *Ann Missouri Bot Gard* 85:404–424. doi:10.2307/2992040
- Morrone O, Scataglini MA, Zuloaga FO (2007) *Cyphonanthus*, a new genus segregated from *Panicum* (Poaceae: Panicoideae: Paniceae) based on morphological, anatomical and molecular data. *Taxon* 56:521–532
- Morrone O, Aagesen L, Scataglini MA, Salariao DL, Denham SS, Chemisquy MA, Sede SM, Giussani L, Kellogg EA, Zuloaga FO (2012) Phylogeny of the Paniceae (Poaceae: Panicoideae): integrating plastid DNA sequences and morphology into a new classification. *Cladistics* 28:333–356. doi:10.1111/j.1096-0031.2011.00384.x
- Morrone O, Aliscioni SS, Veldkamp JF, Pensiero JF, Zuloaga FO, Kellogg EA (2014) Revision of the Old World species of *Setaria* (Poaceae: Panicoideae: Paniceae). *Syst Bot Monogr* 96:1–161
- Ospina JC, Aliscioni SS, Denham SS (2015) A revision of *Festuca* (Loliinae, Pooideae, Poaceae) in Chile. *Phytotaxa* 223:1–66. doi:10.11646/phytotaxa.223.1.1
- Pensiero JF (1999) Las especies sudamericanas del género *Setaria* (Poaceae, Paniceae). *Darwiniana* 37:37–151
- Pensiero JF (2003) *Setaria*. In: Zuloaga FO, Morrone O, Davidse G, Filgueiras TS, Peterson PM, Soreng RJ, Judziewicz EJ (eds) *Catalogue of New World Grasses (Poaceae): III. Subfamily Panicoideae, Aristidoideae, Arundinoideae, and Danthoioideae*. *Contr U S Natl Herb* vol 46, pp 569–539
- Pilger R (1940) Gramineae III. In: Engler A, Prantl K (eds) *Die Natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten, insbesondere den Nutzpflanzen, unter Mitwirkung zahlreicher hervorragender Fachgelehrten begründet*. W Engelmann, Leipzig
- Prasad Rao KE, Wet JMJ, Brink DE, Megesha MH (1987) Intraspecific variation and systematics of cultivated *Setaria italica*, foxtail millet (Poaceae). *Econ Bot* 41:108–116
- Rohlf F (1998) NTSYS-Pc: numeral taxonomy and multivariate analysis system versión 20. Department of Ecology and Evolution State University of New York, Stony Brook, New York
- Rominger JM (1962) Taxonomy of *Setaria* (Gramineae) in North America. *Illinois Biol Monogr* 29:1–132
- Scataglini MA, Aliscioni SS, Zuloaga FO (2014) On the taxonomic of *Panicum scabridum* (Poaceae, Panicoideae, Paspaleae). *Phytotaxa* 163:01–015. doi:10.11646/phytotaxa.163.1.1
- Stapf O (1920) *Paspalidium*. In: Prain D (ed) *Flora of Tropical Africa, Gramineae (Maydeae-Paniceae)* vol 9. Reeve & Co, Ashford (Kent), UK, pp 582–586
- Stapf O, Hubbard CE (1930) *Setaria*. In: Prain D (ed) *Flora of Tropical Africa, Gramineae (Maydeae-Paniceae)*, vol 9. Reeve & Co, Ashford (Kent), UK, pp 769–866
- Thiers B (2014) *Index Herbariorum: a global directory of public herbaria and associated staff*. N Y Bot Gard Virtual Herb Thie14. <http://sweetgum.nybg.org/ih>. Accessed 27 Aug 2014
- Veldkamp JF (1980) *Setaria clivalis* (Ridl.) Veldkamp, comb. nov. (Gramineae). *Misc Pap Landbouwhoogeschool* 19:315–320
- Veldkamp JF (1994) Miscellaneous notes on Southeast Asian Gramineae. IX *Setaria* and *Paspalidium*. *Blumea* 39:373–384

- Watson L, Dallwitz MJ (1992) The grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. <http://delta-intkey.com>. Accessed 12 Aug 2014
- Webster RD (1987) The Australian Paniceae (Poaceae). J. Cramer, Berlin and Stuttgart
- Webster RD (1993) Nomenclature of *Setaria* (Poaceae: Paniceae). Sida 15:447–489
- Webster RD (1995) Nomenclatural changes in *Setaria* and *Paspalidium* (Poaceae: Paniceae). Sida 16:439–446
- Zuloaga FO, Morrone O, Dubcovsky J (1989) Exomorphological, anatomical, and cytological studies in *Panicum validum* (Poaceae: Panicoideae: Paniceae): its systematic position within the genus. Syst Bot 14:220–320. doi:[10.2307/2418908](https://doi.org/10.2307/2418908)