

Phylogenetic studies favour the unification of *Pennisetum*, *Cenchrus* and *Odontelytrum* (Poaceae): a combined nuclear, plastid and morphological analysis, and nomenclatural combinations in *Cenchrus*

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- **Backgrounds and Aims** Twenty-five genera having sterile inflorescence branches were recognized as the bristle clade within the $x = 9$ Paniceae (Panicoideae). Within the bristle clade, taxonomic circumscription of *Cenchrus* (20–25 species), *Pennisetum* (80–140) and the monotypic *Odontelytrum* is still unclear. Several criteria have been applied to characterize *Cenchrus* and *Pennisetum*, but none of these has proved satisfactory as the diagnostic characters, such as fusion of bristles in the inflorescences, show continuous variation.
- **Methods** A phylogenetic analysis based on morphological, plastid (*trnL-F*, *ndhF*) and nuclear (*knotted*) data is presented for a representative species sampling of the genera. All analyses were conducted under parsimony, using heuristic searches with TBR branch swapping. Branch support was assessed with parsimony jackknifing.
- **Key Results** Based on plastid and morphological data, *Pennisetum*, *Cenchrus* and *Odontelytrum* were supported as a monophyletic group: the PCO clade. Only one section of *Pennisetum* (*Brevivalvula*) was supported as monophyletic. The position of *P. lanatum* differed among data partitions, although the combined plastid and morphology and nuclear analyses showed this species to be a member of the PCO clade. The basic chromosome number $x = 9$ was found to be plesiomorphic, and $x = 5, 7, 8, 10$ and 17 were derived states. The nuclear phylogenetic analysis revealed a reticulate pattern of relationships among *Pennisetum* and *Cenchrus*, suggesting that there are at least three different genomes. Because apomixis can be transferred among species through hybridization, its history most likely reflects crossing relationships, rather than multiple independent appearances.
- **Conclusions** Due to the consistency between the present results and different phylogenetic hypotheses (including morphological, developmental and multilocus approaches), and the high support found for the PCO clade, also including the type species of the three genera, we propose unification of *Pennisetum*, *Cenchrus* and *Odontelytrum*. Species of *Pennisetum* and *Odontelytrum* are here transferred into *Cenchrus*, which has priority. Sixty-six new combinations are made here.

Key words: *Pennisetum*, *Cenchrus*, *Odontelytrum*, Poaceae, phylogenetic analyses, *ndhF*, *trnL-trnF*, *kn1*, apomixis.

INTRODUCTION

Morphological and molecular phylogenetic studies of the grass subfamily Panicoideae have shown that *Pennisetum* Rich. and *Cenchrus* L. are closely related genera within the bristle clade in tribe Paniceae (Gómez-Martínez and Culhman, 2000; Zuloaga *et al.*, 2000; Duvall *et al.*, 2001; Giussani *et al.*, 2001; Kellogg *et al.*, 2004; Bess *et al.*, 2005; Doust *et al.*, 2007; Donadío *et al.*, 2009). This clade includes approximately 25 genera (*Cenchrus*, *Ixophorus* Schltdl., *Paspalidium* Stapf, *Pennisetum* and *Setaria* P.Beauv., among others), and is characterized by the presence of setae or bristles in the inflorescences, derived from inflorescence branch meristems (Doust and Kellogg, 2002; Bess *et al.*, 2005).

Pennisetum and *Cenchrus* are distributed throughout tropical and subtropical regions of the Old and New World and contain 80–140 and 20–25 species, respectively (e.g. DeLisle, 1963; Türpe, 1983; Clayton and Renvoize, 1986; Crins, 1991; Watson and Dallwitz, 1992). Some species of

Pennisetum are cultivated as cereal and forage grasses (e.g. *P. purpureum* Schumach. ‘elephant grass’, *P. glaucum* (L.) R.Br. ‘pearl millet’, *P. clandestinum* Hochst. ex Chiov. ‘kikuyu grass’) or ornamentals (e.g. *P. setaceum* (Forssk.) Chiov. ‘tender fountaingrass’, *P. alopecuroides* (L.) Spreng. ‘foxtail fountaingrass’), and some species of *Cenchrus* and *Pennisetum* are considered important weeds (e.g. *C. ciliaris* L. ‘buffel grass’, *C. echinatus* L. ‘southern sandbur’, *C. myosuroides* Kunth ‘big sandbur’ and *P. polystachion* (L.) Schult. ‘mission grass’) (DeLisle, 1963; Clayton and Renvoize, 1986; Watson and Dallwitz, 1992; Rúgolo de Agrasar and Puglia, 2004).

Pennisetum is not clearly distinguished from *Cenchrus*, and several species that are now included in *Cenchrus* have previously been assigned to *Pennisetum*. For example, *P. ciliaris* is accepted by Chase (1921), Pohl (1980), Judziewicz (1990) and Wipff (2003), whereas it is treated under *Cenchrus* by DeLisle (1963), Clayton (1989), Pohl and Davidse (1994), Zuloaga and Morrone (2003), and Chen and Phillips (2006).

The degree of fusion of the bristles is commonly used to separate these genera (Pilger, 1940; DeLisle, 1963; Clayton, 1972, 1989; Clayton and Renvoize, 1982, 1986; Filgueiras, 1984; Watson and Dallwitz, 1992). In most New World species of *Cenchrus* the degree of fusion is substantial, although there are several Old World species in which this distinction is less obvious (Crins, 1991). Other characters used to distinguish the genera are the presence of pedicellate spikelets and whether the bristles are flattened or stiff. However, none of these characters can be applied effectively to segregate the genera (Webster, 1988). Correll and Johnston (1970) treated *Pennisetum* under *Cenchrus* and presented several diagnostic features for the combined genus.

The species now included in *Pennisetum* (Stapf and Hubbard, 1934; Türpe, 1983; Wipff, 2001, 2003) have been previously placed in the genera *Cenchrus*, *Gymnotrix* P.Beauv., *Holcus* L., *Panicum* L., *Penicillaria* Willd. and *Setaria*. Most members of the genus are perennial. Distinctive characteristics of the genus include the shape and arrangement of the inflorescences, which are paniculate, contracted and spike-like, with fascicles of spikelets on reduced axes that disarticulate at maturity. The rachis terminates in a bristle; the bristles that subtend the spikelets are free, often plumose and disarticulate with the spikelets (Crins, 1991). Wipff (2003) characterized *Pennisetum* as having antrorsely scabrous bristles (not spiny) with fascicle axes that terminate in a bristle, and basic chromosome numbers of $x = 5, 7, 8$ or 9 ; he considered *Cenchrus* as having retrorsely scabrous, spiny bristles, fascicle axes that are terminated in a spikelet and a basic chromosome number of $x = 7$.

Authors have applied different criteria to subdivide *Pennisetum*. Stapf and Hubbard (1934) recognized five sections: *Gymnotrix* (P.Beauv.) Steud. (with two subsections, *Acrostigma* (Leeke) Stapf & C.E.Hubb. and *Pleurostigma* (Leeke) Stapf & C.E.Hubb.), *Pennisetum*, *Penicillaria* (Willd.) Benth & Hook.f. nom. superf., *Heterostachya* Schumach. and *Brevivalvula* Döll. Pilger (1940) recognized three subgenera: *Dactylophora* Leeke, *Eriochaeta* (Fig. & De Not.) Leeke (equivalent to section *Brevivalvula*) and *Pennisetum*; the latter was divided into sections *Cenchropsis* (Leeke) Pilg., *Gymnotrix* (with three subsections: *Acrostigma*, *Beckeropsis* (Fig. & De Not.) Pilg. and *Pleurostigma*) and *Penicillaria*. Brunken (1977) revised section *Pennisetum* and concluded that *Penicillaria* was a synonym of this type section. Finally, Clayton and Renvoize (1986) recognized only sections *Brevivalvula*, *Dactylophora* nom. inval., *Gymnotrix* (with the same subsections as Pilger), *Heterostachya* and *Pennisetum*. The differences between the sections are often weak (Schmelzer, 1997) and are based mainly on morphological characters of the inflorescence. Scholz (2006) established a new monotypic genus, *Kikuyochloa*, based on *Pennisetum clandestinum*. The inflorescences in *Kikuyochloa* are hidden in the leaf sheaths and the spikelets are arranged in simple units without a ring of basal, involucre bristles; the tiny bristles of the individual spikelets never completely encircle the spikelets.

Several cereals or forage species of *Pennisetum* are very important resources for food (e.g. *P. glaucum*). A phylogenetic approach and information relating to the ploidy of the species could prove useful in clarifying the relatedness of species

without the necessity of crossing, in particular when species are difficult to cultivate. Using internal transcribed spacer (ITS) data, Martel et al. (2004) placed two wild forms of *Pennisetum glaucum* (L.) R.Br. subsp. *monodii* (Maire) Brunken in the primary gene pool of domesticated *P. glaucum* (diploid, $x = 7$), where the primary gene pool is defined as taxa that are as easy to cross as within the same species (Harlan and de Wet, 1971). In the secondary gene pool (coenospecies where gene transfer is possible, but fertile hybrids are difficult to obtain) they placed *P. purpureum* (tetraploid, $x = 7$). All remaining species of *Pennisetum* were in the tertiary gene pool (species for which hybrids with the crop would be difficult to obtain and maintain, representing the extreme outer limit of potential genetic exchange; cf. Harlan and de Wet, 1971).

Several basic chromosome numbers have been reported for *Cenchrus* ($x = 9, 10, 17$) and *Pennisetum* ($x = 5, 7, 8, 9, 17$; Table 1). The genera are included in the $x = 9$ Paniceae clade, and the basic chromosome number $x = 9$ is plesiomorphic within the bristle clade and the *Pennisetum*–*Cenchrus* clade (Giussani et al., 2001; Martel et al., 2004; Donadío et al., 2009). Although diploids are not rare among species of *Cenchrus* and *Pennisetum*, polyploid and aneuploid numbers are common within both genera (Table 1). A high frequency of univalents or multivalent associations in metaphase I (e.g. Sisodia, 1970), lagging chromosomes in anaphase I (Dujardin and Hanna, 1984a) and anomalies in meiosis provide evidence of outbreeding in polyploid species of *Pennisetum* (*P. polystachion*, *P. pedicellatum* Trin., *P. squamulatum* Fresen.) and *Cenchrus* (*C. ciliaris*, *C. incertus* M.A.Curtis, *C. setigerus* Vahl.). In this context, consistency and incongruence among plastid (maternal) and nuclear (biparental) phylogenetic analyses should help to elucidate the origin of allopolyploids (e.g. *P. pedicellatum*, *P. polystachion*).

Apospory is a mode of asexual reproduction in which a gametophyte develops directly from diploid cells of the sporophyte without meiosis (Gustafsson, 1946). This apomictic mode is well documented in Panicoideae (Brown and Emery, 1958), being frequent in several species of *Cenchrus* and *Pennisetum* (Ozias-Akins et al., 2003; Ozias-Akins, 2006), and its developmental pattern has been well studied in *Cenchrus ciliaris* and *Pennisetum squamulatum* (Dujardin and Hanna, 1984a; Ozias-Akins et al., 1998; Wen et al., 1998). Evidence for transmission of apomixis by a single chromosome was reported by Ozias-Akins et al. (1993), and subsequent studies have identified molecular markers linked to apomixis (Ozias-Akins et al., 2003). An apospory-specific genomic region (ASGR) (Ozias-Akins et al., 1998), located on a single chromosome, is necessary and sufficient for the expression of apomixis in polyploid taxa (Goel et al., 2003). Also, at least two genes, *Pca21* and *Pca24*, were identified to play a role during apomictic development in *Pennisetum ciliare* or *Cenchrus ciliaris* (Singh et al., 2007), although they can be inherited independently of the ASGR.

Previous phylogenetic studies showed that *Pennisetum* and *Cenchrus* form a strongly supported monophyletic group (Martel et al., 2004; Doust et al., 2007; Donadío et al., 2009). Donadío et al. (2009), using two plastid markers and including almost 20 and seven species of *Pennisetum* and *Cenchrus*, respectively, found the former to be polyphyletic.

TABLE 1. Chromosome numbers and reproductive behaviour reported for the species included in the analyses

| Species | <i>n</i> | <i>2n</i> | <i>x</i> | Reproductive behaviour |
|--|--|---|--|---|
| <i>Cenchrus agrimonioides</i> | — | — | — | — |
| <i>C. brownii</i> | 34 ⁽¹⁴⁾ | 34 ⁽⁴⁸⁾ , 36 ⁽²⁶⁾⁽²⁴⁾ , 70 ⁽²³⁾ | 9*, 17 ⁽¹⁴⁾⁽¹⁴⁾ | — |
| <i>C. caliculatus</i> | 34 ⁽³⁰⁾ | — | 17* | — |
| <i>C. ciliaris</i> | 16 ⁽¹⁸⁾ , 17 ⁽⁴³⁾ , 18 ⁽¹⁸⁾⁽²²⁾ | 34 ⁽²³⁾ , 36 ⁽¹⁶⁾⁽²⁴⁾ , 45 ⁽³²⁾ , 54 ⁽²⁶⁾ , 63 ⁽³²⁾ , 78 ⁽²³⁾ , 90 ⁽³²⁾ | 9 ⁽⁴³⁾ | APO, SEX ⁽⁵³⁾ |
| <i>C. echinatus</i> | 34 ⁽¹⁴⁾⁽¹⁸⁾ | 68 ⁽²⁷⁾⁽⁴⁵⁾⁽²⁵⁾⁽²³⁾ , 70 ⁽²³⁾ | 17 ⁽¹⁸⁾⁽²²⁾ | — |
| <i>C. incertus</i> | 16 ⁽²²⁾ , 17 ⁽¹⁸⁾⁽¹⁴⁾ | 30 ⁽²²⁾ , 34 ⁽²⁴⁾⁽¹⁴⁾⁽⁴⁸⁾ | 17 ⁽¹⁴⁾⁽¹⁸⁾ | SEX ⁽⁵⁹⁾ |
| <i>C. myosuroides</i> | 27 ⁽⁶⁾ , 35 ⁽¹⁸⁾ | 54 ⁽⁶⁾ , 70 ⁽³⁾⁽²³⁾⁽⁵⁰⁾ | 9, 10 ⁽¹⁷⁾ | SEX ⁽⁵⁹⁾ |
| <i>C. pilosus</i> | 17 ⁽¹⁴⁾ | 34 ⁽¹⁴⁾⁽⁴⁸⁾ | 17 ⁽¹⁴⁾⁽¹⁴⁾ | — |
| <i>C. setigerus</i> | 17 ⁽⁴³⁾ , 18 ⁽⁴⁷⁾ | 34 ⁽⁴⁷⁾ , 36 ⁽⁴⁶⁾ , 37 ⁽⁴⁷⁾ | 9 ⁽⁴³⁾ , 17 ⁽⁴³⁾ | APO ⁽⁴⁶⁾ |
| <i>Ixophorus unisetus</i> | — | 34 ⁽⁴⁹⁾ | 17* | — |
| <i>Odontelytrum abyssinicum</i> | — | — | — | — |
| <i>Paspalidium geminatum</i> | 9 ⁽⁴³⁾ | 18 ⁽⁵⁶⁾ , 36 ⁽²⁴⁾ | 9 ⁽⁴³⁾ | — |
| <i>Pennisetum</i> sect. <i>Brevivalvula</i> [†] | | | | |
| <i>P. hordeoides</i> | 9 ⁽⁵¹⁾ | 36 ⁽⁵¹⁾ , 54 ⁽⁵¹⁾ | 9 ⁽¹⁾ | APO ⁽⁵³⁾ |
| <i>P. pedicellatum</i> | — | 24 ⁽⁶⁰⁾ , 30 ⁽⁶⁰⁾ , 32 ⁽⁶⁰⁾ , 35 ⁽⁶⁰⁾ , 36 ⁽⁶⁰⁾ , 42 ⁽⁶⁰⁾ , 45 ⁽⁵¹⁾ , 48 ⁽⁶⁰⁾ , 53 ⁽⁶⁰⁾ , 54 ⁽³⁶⁾⁽⁵¹⁾ | 9 ⁽³⁶⁾⁽⁵¹⁾ | APO ⁽³⁶⁾⁽⁵³⁾ |
| <i>P. polystachion</i> subsp. <i>polystachion</i> | 18 ⁽¹³⁾ , 36 ⁽¹²⁾ | 18 ⁽⁵¹⁾ , 24 ⁽⁶⁰⁾ , 32 ⁽⁶⁰⁾ , 36 ⁽⁵¹⁾ , 45 ⁽⁵¹⁾ , 48 ⁽⁶⁰⁾ , 52 ⁽⁶⁰⁾ , 53 ⁽⁴⁸⁾ , 54 ⁽³⁶⁾⁽⁵¹⁾⁽¹³⁾⁽²⁷⁾ , 56 ⁽⁶⁰⁾ , 63 ⁽⁶⁰⁾ , 78 ⁽²³⁾ | 9 ⁽³⁶⁾⁽⁵¹⁾ | APO ⁽²⁰⁾⁽⁵³⁾ |
| <i>P. polystachion</i> subsp. <i>atricum</i> | — | 36 ⁽⁷⁾ | 9* | — |
| <i>Pennisetum</i> sect. <i>Dactylophora</i> [†] | | | | |
| <i>P. lanatum</i> | 18 ⁽⁴³⁾ | — | 9 ⁽⁴³⁾ | — |
| <i>Pennisetum</i> sect. <i>Gymnotrix</i> subsect. <i>Acrostigma</i> [†] | | | | |
| <i>P. alopecuroides</i> | — | 18 ⁽³⁶⁾⁽⁵⁴⁾ | 9 ⁽³⁶⁾ | SEX ⁽³⁶⁾ |
| <i>P. basedowii</i> | — | 54 ⁽²⁰⁾ | 9* | — |
| <i>P. chilense</i> | — | — | — | — |
| <i>P. frutescens</i> | — | 63 ⁽⁴⁵⁾ | 9 ⁽⁴⁵⁾ | APO ⁽⁵³⁾ |
| <i>P. glaucocladum</i> | — | — | — | — |
| <i>P. macrourum</i> | — | 36 ⁽²⁰⁾ | 9 ⁽⁴⁶⁾ | APO ⁽²⁰⁾⁽⁵³⁾ |
| <i>P. massaicum</i> | — | 16 ⁽⁴⁶⁾ , 32 ⁽⁵⁵⁾ | 8 ⁽⁴⁶⁾ | APO, SEX ⁽⁵³⁾ |
| <i>P. meianum</i> | — | 16 ⁽⁵²⁾ , 32 ⁽³⁶⁾ | 8 ^(36, 52) | APO ⁽³⁶⁾ |
| <i>P. natalense</i> | — | — | — | — |
| <i>P. nervosum</i> | — | 36 ⁽⁴⁵⁾ , 72 ⁽⁴⁸⁾ | 9 ⁽⁴⁵⁾ | — |
| <i>P. ramosum</i> | — | 10 ⁽³⁶⁾⁽⁵²⁾⁽⁵⁵⁾ | 5 ⁽³⁶⁾⁽⁵²⁾⁽⁵⁵⁾ | APO, SEX ⁽³⁶⁾ |
| <i>P. sphacelatum</i> | 18 ⁽³²⁾ | — | 9* | — |
| <i>P. thunbergii</i> | — | 18 ⁽¹⁶⁾ | 9* | — |
| <i>Pennisetum</i> sect. <i>Gymnotrix</i> subsect. <i>Beckeropsis</i> [†] | | | | |
| <i>P. montanum</i> | 16 ⁽³¹⁾ | — | 8 ⁽³¹⁾ | — |
| <i>P. unisetum</i> | — | 18 ⁽¹⁶⁾ | 9* | — |
| <i>Pennisetum</i> sect. <i>Gymnotrix</i> subsect. <i>Pleurostigma</i> [†] | | | | |
| <i>P. latifolium</i> | — | 36 ⁽⁴⁵⁾ | 9 ⁽⁴⁵⁾ | APO ⁽⁵³⁾ |
| <i>P. trachyphyllum</i> | — | — | — | — |
| <i>P. tristachyum</i> | — | — | — | — |
| <i>Pennisetum</i> sect. <i>Heterostachya</i> [†] | | | | |
| <i>P. schweinfurthii</i> | — | 14 ⁽³⁶⁾⁽⁵²⁾ | 7 ⁽³⁶⁾⁽⁵²⁾ | SEX ⁽³⁶⁾ |
| <i>P. squamulatum</i> | — | 54 ⁽³⁶⁾ , 56 ⁽²⁾ | 7 ⁽²⁾ , 9 ⁽²⁰⁾⁽³⁶⁾ | APO ⁽²⁰⁾⁽³⁶⁾⁽⁵³⁾ |
| <i>Pennisetum</i> sect. <i>Pennisetum</i> [†] | | | | |
| <i>P. clandestinum</i> | 18 ⁽³⁸⁾ , 27 ⁽³⁴⁾ | 36 ⁽²⁹⁾⁽⁴⁵⁾⁽⁵⁶⁾ | 9 ⁽²⁹⁾ | APO, SEX ⁽⁴⁶⁾ |
| <i>P. flaccidum</i> | 9 ⁽³⁴⁾ , 27 ⁽⁴³⁾ | 18 ⁽⁴⁶⁾ , 36 ⁽⁹⁾⁽⁴⁶⁾ | 9 ⁽⁴³⁾ | APO ⁽⁴⁶⁾ , SEX ⁽⁵³⁾ |
| <i>P. foermeranum</i> | — | — | — | — |
| <i>P. glaucum</i> | 7 ⁽⁴³⁾ | 14 ⁽⁸⁾⁽³⁶⁾ | 7 ⁽³⁶⁾⁽⁴³⁾ | SEX ⁽³⁶⁾ |
| <i>P. orientale</i> | 9, 18 ⁽⁴³⁾ | 36 ⁽³⁶⁾⁽²⁹⁾ , 45 ⁽⁴⁰⁾ | 9 ⁽³⁶⁾ | APO ⁽³⁶⁾⁽⁵³⁾ , SEX ⁽⁵³⁾ |
| <i>P. purpureum</i> | 7 ⁽¹⁾ , 28 ⁽³⁴⁾ | 27 ⁽²⁶⁾ , 28 ⁽³⁶⁾⁽⁸⁾⁽³³⁾ | 7 ⁽³⁶⁾⁽⁴⁵⁾ | APO ⁽⁵⁹⁾ , SEX ⁽³⁶⁾ |
| <i>P. setaceum</i> | — | 27 ⁽³⁶⁾⁽²⁹⁾ , 54 ⁽³⁶⁾ , 68 ⁽⁵⁸⁾ | 9 ⁽³⁶⁾ , 17 ⁽⁵⁸⁾ | APO ⁽⁵³⁾ |
| <i>P. sieberianum</i> | — | — | — | — |
| <i>P. villosum</i> | — | 18 ⁽⁴⁶⁾ , 27 ⁽⁴⁶⁾ , 36 ⁽³⁶⁾ , 45 ⁽⁴⁵⁾ , 54 ⁽⁴⁵⁾ | 9 ⁽³⁶⁾ | APO ⁽³⁶⁾⁽⁵³⁾ |
| <i>P. violaceum</i> | — | 14 ⁽³⁶⁾ | 7 ⁽³⁶⁾ | SEX ⁽³⁶⁾ |
| <i>Pseudoraphis paradoxa</i> | — | — | — | — |
| <i>P. spinescens</i> | — | 32 ⁽²⁷⁾ | 8* | — |
| <i>Rupichloa acuminata</i> | 13 ⁽⁴²⁾ | — | 13 ⁽⁴²⁾ | — |
| <i>Setaria palmifolia</i> | 27 ⁽⁴¹⁾⁽³⁸⁾ | 54 ⁽²⁷⁾ | 9* | — |
| <i>S. sphacelata</i> | 9 ⁽²⁸⁾ , 18 ⁽²⁸⁾ , 27 ⁽²⁸⁾ | 36 ⁽⁵⁾ , 54 ⁽²⁸⁾ | 9* | SEX ⁽⁵⁹⁾ |
| <i>S. parviflora</i> | 18 ⁽²⁸⁾⁽³⁸⁾ | 36 ⁽²⁵⁾⁽¹⁵⁾ | 9* | — |
| <i>Spinifex sericeus</i> | — | 18 ⁽⁵⁷⁾ | 9 ⁽⁵⁷⁾ | — |

Continued

TABLE 1. *Continued*

| Species | <i>n</i> | <i>2n</i> | <i>x</i> | Reproductive behaviour |
|--------------------------------|----------|--|-------------------|------------------------|
| <i>Stenotaphrum secundatum</i> | – | 18 ⁽⁴⁵⁾ (6)(24), 36 ⁽²⁷⁾ | 9 ⁽⁴⁵⁾ | SEX ⁽⁵⁹⁾ |

References: (1) Akenova and Chehheda (1981), (2) Akiyama *et al.* (2006), (3) Avdulov (1931), (4) Bir and Sahni (1986), (5) Bir and Sahni (1987), (6) Brown (1950), (7) Brunken (1979), (8) Burton (1942), (9) Chatterji and Timothy (1969), (10) Chopanov and Yurtsev (1976), (11) Christopher and Abraham (1976), (12) Davidse and Pohl (1972), (13) Davidse and Pohl (1978), (14) Davidse and Pohl (1974), (15) de Wet (1954), (16) de Wet (1960), (17) DeLisle (1963), (18) DeLisle (1964), (19) Dujardin (1979), (20) Dujardin and Hanna (1984b), (21) Emery (1957), (22) Gould (1958), (23) Gould (1965), (24) Gould (1968), (25) Gould and Soderstrom (1967), (26) Gould and Soderstrom (1970), (27) Gould and Soderstrom (1974), (28) Gupta and Singh (1977), (29) Hrishy (1952), (30) Hsu (1972), (31) Hunziker *et al.* (1998), (32) Jensen *et al.* (1989), (33) Kammacher *et al.* (1973), (34) Khosla and Mehra (1973), (35) Khosla and Sharma (1973), (36) Martel *et al.* (1997), (37) Miège (1962), (38) Mehra (1982), (39) Mehra and Rememanandan (1973), (40) Mehra and Sharma (1973), (41) Mehra and Sharma (1975), (42) Morrone *et al.* (1995), (43) Ahsan *et al.* (1994), (44) Norrmann *et al.* (1994), (45) Núñez (1952), (46) Ozias-Akins *et al.* (2003), (47) Crins (1991), (48) Pohl and Davidse (1971), (49) Reeder (1967), (50) Reeder (1968), (51) Renno *et al.* (1995), (52) Rao *et al.* (1989), (53) Schmelzer (1997), (54) Sinha *et al.* (1990), (55) Swaminathan and Nath (1956), (56) Tateoka (1965), (57) Connor (1984), (58) Shanthamma (1979), (59) Brown and Emery (1958), (60) Sisodia (1970).

SEX = sexual; APO = apomictic.

* Inferred basic chromosome number.

† Sectional and subsectional treatment *sensu* Clayton and Renvoize (1986).

However, if *P. lanatum* is excluded, *Pennisetum* is paraphyletic with all the species of *Cenchrus* nested within it. Doust *et al.* (2007) developed primers for a novel single copy nuclear marker that comprises two introns and three exons of the *knotted1* (*kn1*) gene, and also obtained sequences of *ndhF* for four species of *Pennisetum*, six species of *Cenchrus* and some other members of the bristle clade. In their analyses, *Cenchrus* formed a monophyletic group derived from within a paraphyletic *Pennisetum*. Martel *et al.* (2004), using ITS sequences of nuclear ribosomal DNA and including 13 species of *Pennisetum* and only one species of *Cenchrus*, found *C. ciliaris* embedded within *Pennisetum*. Recently, when studying the phylogenetic relationships of *Setaria* and related genera of the bristle clade, Kellogg *et al.* (2009) also placed *Odontelytrum abyssinicum* Hack. in a clade with *Pennisetum* and *Cenchrus*. *Odontelytrum* has only a single species, in which an herbaceous involucre that subtends the spikelet may be homologous to the bract-like cupule in *Cenchrus* and *Pennisetum*.

Based on the resolution previously reported for various genetic markers (Doust *et al.*, 2007; Donadio *et al.*, 2009), two plastid markers (the *trnL-F* region, comprising the *trnL* intron and *trnL-F* spacer, and the *ndhF* gene), and one nuclear marker (*knotted1*) were selected here to study relationships among *Cenchrus*, *Pennisetum* and genera of the bristle clade. In addition, a morphological matrix was used in a combined analysis. Basic chromosome numbers and reproductive characters were optimized on the resulting phylogenetic trees. The present goals were (1) to test the monophyly and circumscription of *Pennisetum*, *Cenchrus* and allied genera; (2) to assess the validity of the traditional taxonomic divisions of the genus *Pennisetum*; and (3) to interpret the role that apomixis, polyploidy and hybridization may have played in the evolution of *Pennisetum* and *Cenchrus*.

MATERIALS AND METHODS

Sampling

For the plastid markers, 51 species (a total of 53 specimens) were sampled, representing nine species of *Cenchrus* (56 % of the

total number of species according to Clayton and Renvoize, 1986), 32 of *Pennisetum* (43 %) and *Odontelytrum abyssinicum*. Species representing different genera of the bristle clade were used as outgroups: *Ixophorus unisetus*, *Paspalidium geminatum*, *Setaria palmifolia*, *S. parviflora*, *S. sphacelata* and *Stenotaphrum secundatum*; *Rupichloa acuminata* was used as functional outgroup (Appendix 1). Of the 49 sequences for the *trnL-F* region, 23 were generated and 26 were downloaded from GenBank. For *ndhF*, 34 sequences of the 53 were generated for this study and 19 were added from GenBank (Appendix 1).

For the nuclear marker (*knotted1*), 42 taxa were studied: eight species belonged to *Cenchrus* and 24 to *Pennisetum*. *Ixophorus unisetus*, *Paspalidium jubiflorum*, *Pseudoraphis paradoxa*, *P. spinescens*, *Setaria palmifolia*, *S. parviflora*, *S. sphacelata*, *Spinifex sericeus* and *Stenotaphrum secundatum* were used as outgroups. Trees were rooted using *Panicum miliaceum*. Seventy-one sequences were generated in this study, and 33 sequences belonging to the ingroup and 27 to the outgroups were obtained from GenBank (Appendix 1).

DNA extraction and sequencing

The material used for DNA extraction was obtained from plants collected in wild populations and dried in silica gel or grown from seeds provided by Plant Gene Resources of Canada (CN), US Department of Agriculture (PI) and the Missouri Botanical Garden (MO) (Appendix 1). DNA extraction was via the modified CTAB protocol of Doyle and Doyle (1987), adapted for small amounts of plant material. When fresh material was not available, DNA was extracted from herbarium specimens using a Dneasy Plant Mini Kit (QIAGEN Inc., Hilden, Germany).

Genomic DNA was used as a template to amplify (by PCR) a plastid fragment containing the *trnL* (UAA) intron and the intergenic spacer between the *trnL* (UAA) 3' exon and the *trnF* (GAA) gene (*trnL-F* region) and the *ndhF* gene encoding a subunit of the respiratory-chain NADH dehydrogenase. A fragment of the single copy nuclear marker *knotted1* (*kn1*), comprising two introns and three exons, was also amplified (see fig. 2 in Doust *et al.*, 2007). *ndhF* was amplified in

three to five overlapping fragments, depending on the difficulty of amplification and the quality of the leaf material, using the following primers: 5F, 536F, 536R, 972F, 972R, 1318F, 1318R and 3R from Olmstead and Sweere (1994), 1660F and 1660R from Aliscioni *et al.* (2003), and 1821F and 1821R from Clark *et al.* (1995). The intron and the intergenic spacer from the *trnL-F* region were amplified using the primers c and f from Taberlet *et al.* (1991) and primers Cii and Fdw from Giussani *et al.* (2009). *kn1* was amplified in one fragment with a nested PCR approach, using as a first set of primers *kn1*-345F and *kn1*-622R from Doust *et al.* (2007) and a second set of primers, designed specifically for this paper, *kn1*-nestF (YGAGTGCCRGAAGGCAAGTA), *kn1*-nest3R (ATRTTGGCGCAGCGATCTG) and *kn1*-nestR (YCTCGTCRGYTCCTCYCTGA).

PCR reactions were performed in a final volume of 25 μ L. Each reaction contained between 50 and 100 ng of DNA, 1.5 units of *Taq* polymerase (Invitrogen Life Technologies, São Paulo, Brazil), 1 \times PCR buffer, 5 mM MgCl₂, 0.2 μ M of each primer and 0.025 mM dNTP each. In species for which these protocols were unsuccessful, 0.4 % bovine serum albumin and 1.6 % dimethyl sulfoxide were included as additives and enhancing agents to increase the yield of PCR reactions. PCR amplifications followed the following programme: a first denaturation period at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 1 min and extension at 72 °C for 90 s. Final extension at 72 °C for 6 min terminated the reactions. The annealing temperature was varied in some cases to improve amplification.

For *kn1*, sequences from direct PCR products were used only when they had <3 % polymorphisms as indicated by double peaks in the chromatograms. However, for most species, *kn1* was cloned before sequencing. When possible, at least five clones were sequenced per accession. PCR reactions were run out on a 1 % Tris-borate-EDTA (TBE) agarose gel, the bands of DNA were excised, purified using the QIAquick Gel Extraction Kit Protocol (QIAGEN Inc.) and cloned using the PGEM-T Easy Vector system (Promega Corp., Madison, WI, USA). Colonies were picked and incubated overnight in liquid Luria–Bertani (LB) medium. For checking the insert, plasmids were extracted and incubated with *EcoRI* at 37 °C for 2 h. Digestions were electrophoresed on a 1 % TBE agarose gel stained with ethidium bromide, and colonies that had incorporated the plasmid were re-grown in liquid LB medium. Plasmids for sequencing were extracted using the QIAprep Miniprep protocol (QIAGEN Inc.).

PCR products were sequenced by Macrogen, Inc. (Seoul, Korea). PCR products were cleaned using a Montage PCR purification kit from Millipore following the manufacturer's protocol, and sequencing reactions used ABI PRISM BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Seoul, Korea). Single-pass sequencing was performed on each template using the same primers used for PCR reactions (see above), as well as primers e and d from Taberlet *et al.* (1991) for the *trnL-F* region. Unincorporated terminators were removed by ethanol precipitation. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI PRISM 3730XL sequencer (96-capillary type; Applied Biosystems).

Assembly and editing of sequences used the program Chromas Pro ver. 1.34 (Technelysium Pty, Ltd, Tewantin, Australia). Sequences of *ndhF* and *kn1* were aligned manually following alignments performed by Aliscioni *et al.* (2003) and Doust *et al.* (2007), respectively. Sequences of the *trnL-F* region were aligned using the program DIALIGN at BiBiServ (<http://bibiserv.techfak.uni-bielefeld.de/dialign/>) (Morgenstern, 2004). DIALIGN is a program that compares complete segments of sequences, instead of relying on the sum of individual similarity values or on gap penalties as optimization criteria; it is thus able to establish small conserved regions that cannot be detected by other alignment programs (Morgenstern *et al.*, 1998). The alignment was then adjusted manually. Voucher information and GenBank accession numbers are provided in Appendix 1. Alignments and phylogenetic trees were submitted to TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S10252>).

Morphological characters

Fifty-one morphological characters were scored and used in the phylogenetic analyses (Appendix 2). Characters were taken from direct examination of herbarium specimens (AAU, CAMB, K, PRE and SI; Appendix 3), and in some cases information was obtained from the literature (Türpe, 1983; Clayton *et al.*, 2006). Three to 11 specimens (average, five) were measured per species including, when possible, the specimen used to obtain DNA. The matrix is presented as Supplementary Data, available online, and has been submitted to TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S10252>).

Phylogenetic analyses

Molecular analyses of the plastid markers (*trnL-F* region and *ndhF*) were performed separately and in combination. A morphological analysis was conducted separately, and then a combined analysis of morphology, *trnL-F* and *ndhF* was also performed. The nuclear marker (*kn1*) was analysed independently using several clones per species.

All analyses were conducted using the program TNT version 1.1 (Goloboff *et al.*, 2008), with all characters equally weighted and considered unordered. Gaps were scored as missing data. In all analyses, parsimony-uninformative characters were deactivated. Heuristic searches were performed using 1000 random addition replicates and tree bisection–reconnection (TBR) branch swapping, saving ten trees per replicate. Thereafter, a new search with TBR branch swapping was performed using the shortest trees saved in memory. A strict consensus tree was obtained with all shortest trees found during searches.

Branch support was assessed with 10 000 parsimony jack-knife (JK) replicates (Farris *et al.*, 1996), using ten series of random addition sequences, swapped using TBR and holding two optimal trees per series. Clades were considered to have strong branch support when JK \geq 90 %; moderate support, JK \geq 75 % to <90 %; low support, JK \leq 74 %.

Optimization of morphological, cytological and reproductive characters was performed on all most-parsimonious trees (MPTs) obtained in the plastid analyses and combined

analysis, using the command ‘Common Synapomorphies’ of TNT (Goloboff et al., 2008), by which the optimization shared by all MPTs is represented in the consensus diagram.

RESULTS

Plastid analysis

The total length of the amplified *trnL-F* region ranged from 803 to 888 bp. The aligned matrix consisted of 941 characters, only 39 of which were potentially phylogenetically informative. The matrix included 3.22 % missing data (gaps not included). Four species had >10 % missing data; of these *P. lanatum* had 25 %. When aligning the 53 *ndhF* sequences, only one gap of 6 bp was introduced by *Setaria sphacelata*, producing a matrix length of 2055 characters. The total proportion of missing positions was 2.79 %. Only *Pennisetum glaucocladum* and *P. hordeoides* had >20 % missing data. A total of 116 characters were potentially parsimony-informative.

There was no contradiction in the placement of the ingroup taxa between the *ndhF* and the *trnL-F* datasets when analysed separately, and several moderately and strongly supported clades were recovered in both analyses. Hence, partitions were assumed to be congruent and they were analysed together. The combined data matrix consisted of 53 specimens and 2996 characters in total, with 155 potentially parsimony-informative characters. Only four species could not be amplified for *trnL-F* (*Cenchrus agrimonioides*, *C. caliculatus*, *Pennisetum natalense* and *P. sphacelatum*); however, when excluding these species from the analysis, neither the topology of the consensus tree nor the branch support varied significantly, and hence these species were included in the combined analysis.

The combined plastid analysis produced 2316 trees of 342 steps (CI: 0.53, RI: 0.80); the trees from the combined analysis are congruent with those from the independent analyses (Fig. 1A). Figure 2 shows one of the MPTs with branch lengths drawn to scale. The consensus tree shows *Pennisetum*, *Cenchrus* and *Odontelytrum* in a strongly supported clade, the ‘PCO clade’ with JK = 97 % (Figs 1A and 2). Only *P. lanatum* is excluded from the PCO clade; it is placed in a clade with *Ixophorus unisetus* (Figs 1A and 2) with only weak support (JK = 51 %). This clade does not appear in the *trnL-F* analysis, in which *P. lanatum* is placed in a polytomy outside the PCO clade (tree not shown). Within the PCO clade, *Odontelytrum*, *P. villosum*, *P. trachyphyllum* and a clade of seven species of *Pennisetum* (*P. alopecuroides*, *P. clandestinum*, *P. macrourum*, *P. natalense*, *P. orientale*, *P. sphacelatum* and *P. thunbergii*) form a polytomy with the remaining species of *Pennisetum* and *Cenchrus* (Fig. 1A).

The relationships among the species of *Cenchrus* are weakly supported in clade A (JK = 57 %) together with several species of *Pennisetum*. Clade A includes all sampled species of *Cenchrus*, plus *P. hordeoides*, *P. massaicum*, *P. mezianum*, *P. pedicellatum*, *P. polystachion* subsp. *atrichum*, *P. polystachion* subsp. *polystachion*, *P. purpureum*, *P. ramosum* and *P. setaceum* (Fig. 1A). Within clade A, five species of *Cenchrus* form a clade (subclade Aa) that comprises *C. pilosus* and *C. brownii* (strongly supported as sister taxa:

JK = 99 %), which are sister to a clade with *C. echinatus*, *C. myosuroides* and *C. incertus* (JK = 79 %). *Cenchrus caliculatus* is related to *P. setaceum* (JK = 62 %), and *C. ciliaris* and *C. setigerus* are closely related (JK = 85 %) with *P. purpureum* as their sister group (JK = 93 %). The other strongly supported group within clade A comprises *P. hordeoides*, *P. pedicellatum*, and both subspecies of *P. polystachion* (subclade Ab; JK = 98 %) (Fig. 1A).

Clade B is here represented by *P. glaucum* (both specimens), *P. sieberianum*, *P. squamulatum* and *P. violaceum*; all these species form a strongly supported group (JK = 99 %). Clade C includes *P. chilense*, *P. latifolium*, *P. montanum* and *P. tristachyum* (JK = 95 %).

Morphological analysis

The parsimony analysis of 51 morphological characters yielded 249 trees of 345 steps (CI: 0.27, RI: 0.59). The strict consensus tree showed little resolution, and nodes were poorly supported (tree not shown). However, the PCO clade itself is not strongly contradicted and has moderate support (JK = 81 %). *Pennisetum lanatum*, contrary to the molecular results, is situated in a polytomy near the base of the PCO clade.

Combined analysis of morphology and plastid data

Combining the three datasets (morphology, *trnL-F* and *ndhF*), the analysis yielded 3264 trees of 763 steps with CI = 0.35 and RI = 0.65 (Fig. 1B). When compared with the results from the plastid data alone, support for most of the branches is diminished, probably due to the conflict added by the morphological characters (Fig. 1A, B). The molecular characters dominate the analysis, as the consensus tree of the combined analysis has more nodes in common with the molecular analysis than with the morphological one. However, *P. lanatum*, similar to the morphological analysis, is included as an early branching taxon in the PCO clade (JK = 98 %).

Lettered clades (A, Aa, Ab, etc.) correspond to clades as identified in the combined plastid trees. The combined analysis recovered subclade Aa (JK = 61 %). This subclade is supported by a basic chromosome number of $x = 17$, although this character reversed in *C. brownii* and *C. myosuroides* ($x = 9, 10$; Table 1). Two morphological characters support this clade: the bristles are fused (char 21, $0 > 2$) and the upper glume is almost as long as the spikelet (char 31, $2 > 3$).

Subclade Ab (*P. hordeoides*, *P. pedicellatum* and both subspecies of *P. polystachion*), clade B (both specimens of *Pennisetum glaucum*, *P. sieberianum*, *P. squamulatum* and *P. violaceum*) and clade C (*P. chilense*, *P. latifolium*, *P. montanum* and *P. tristachyum*), as in the combined plastid analysis, are also moderately to strongly supported in the combined analysis (Fig. 1A, B). Subclade Ab is supported by a single morphological synapomorphy which is the coriaceous consistency of the upper lemma (char 38, $1 > 2$). Species of clade B are characterized by eight morphological synapomorphies: the upper glume is vestigial, and consequently is reduced in size (char 30, $2 > 1$; char 31, $1 > 0$), the apex of the lower lemma is scaberulous (char 37, $1 > 2$), the apex of

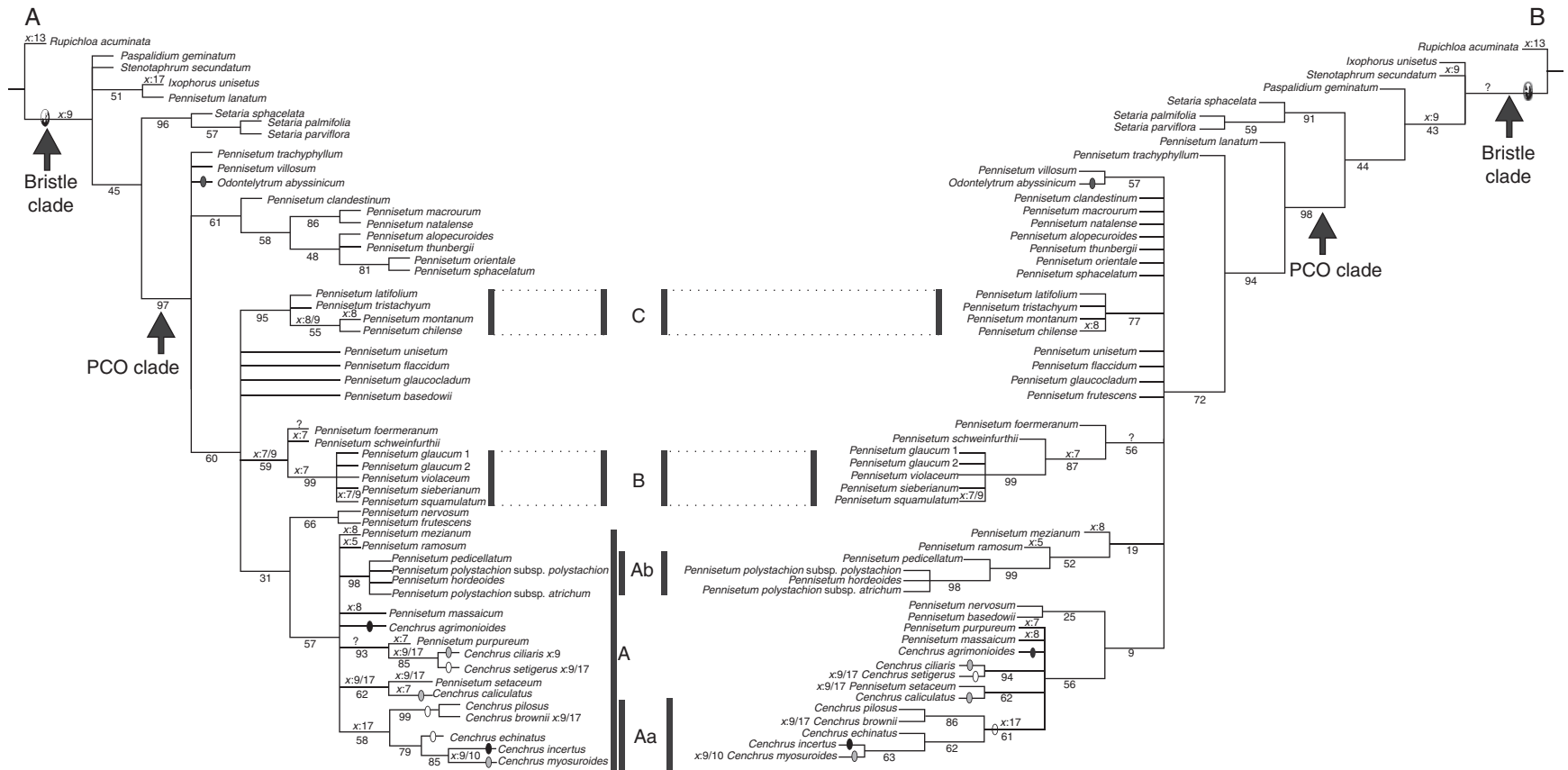


FIG. 1. Phylogenetic relationships of *Pennisetum*, *Cenchrus* and *Odontelytrum*. (A) Strict consensus tree of 2316 MPTs from the combined analysis of the plastid markers (*ndhF* + *trnL-F*). (B) Strict consensus tree of the 3264 MPTs from the combined analysis (*ndhF* + *trnL-F* + cytology and morphology). Numbers below branches represent jackknife branch support. Optimization of the basic chromosome number (Appendix 2, character 1) is shown above the branches and, when necessary, next to the species names. Optimization of the degree of fusion of the bristles (Appendix 2, character 21) is shown as follows: black and white oval, not connate; grey oval, connate below; white oval, connate up to half the total length; black oval, connate up to two-thirds the total length. Bars represent principal clades as discussed in the text. *Pennisetum glaucum* 1 corresponds to voucher PI 326520 (sequences downloaded from GenBank) and *P. glaucum* 2 to voucher *Caxambu* 375 (see Appendix 1).

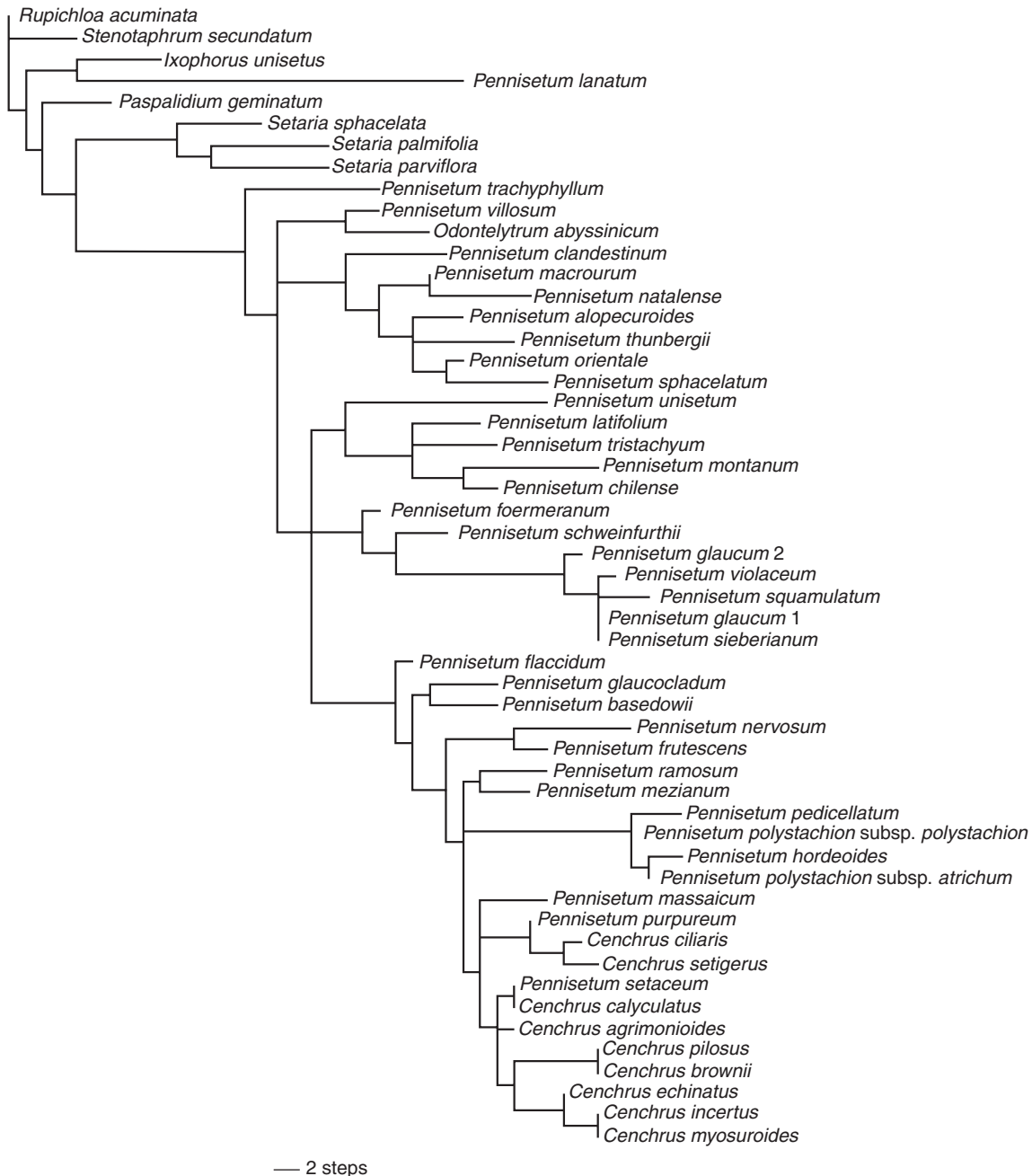


FIG. 2. A single tree depicting relative branch lengths for one of the 2316 MPTs based on a combined analysis using two plastid markers: *ndhF* and *trnL-F*.

the upper lemma is not acuminate (char 39, $1 > 0/2$) and is ciliate (char 40, $1 > 0$), the apex of the upper palea is tridentate (char 44, $1 > 4$) and ciliate (char 45, $1 > 0$), and anther tips are penicillate (char 49, $1 > 0$). *Pennisetum schweinfurthii* is the sister taxon to clade B (JK = 87%); this relationship is supported by a basic chromosome number of $x = 7$, and two morphological characters: a rounded apex of the lower lemma (char 36, $1 > 2$) and connate styles (char 48, $1 > 0$).

Clade C is characterized by two morphological characters: a membranous-ciliate ligule (char 2, $1 > 2$) and an acute apex of the upper palea (char 44, $1 > 0$).

Knotted1 – nuclear data

In all, 131 sequences were used. *Pennisetum clandestinum* and *P. sieberianum* were sequenced directly, whereas for most other species three to five clones were recovered; only one or two clones were obtained for *P. frutescens*, *P. montanum* and *P. violaceum* (Appendix 1). It was not possible to amplify *kn1* from the available herbarium material of *Odontelytrum*, so its placement is unknown. The total length of the aligned matrix was 817 bp; sequences varied from 602 bp in one clone of *Pennisetum chilense* to 747 bp in a clone of *Pseudoraphis spinescens*.

In total, 315 characters were potentially parsimony-informative, and the analysis was stopped when it found 30 000 trees (maximum saved) of 833 steps (CI = 0.53, RI = 0.82); one of the MPTs with branch lengths drawn to scale is shown in Fig. 3. Although the consensus of the 30 000 MPTs revealed several basal polytomies including individual clones, or some clones of the same species were represented in more than one clade, it was possible to recover several major strongly supported clades (Fig. 4).

All species of *Cenchrus* and *Pennisetum*, including *P. lanatum*, form a monophyletic group (JK = 64 %). Other major well-supported clades revealed by the analysis are: clade D (JK = 99 %), which includes three of four clones of *P. thunbergii* (3/4) and all clones of *P. mezianum* (3/3); clade E (JK = 65 %) with all clones of *P. latifolium* (3/3) and *P. macrourum* (3/3) and separate clones of *P. ramosum* (1/3), *P. orientale* (1/4), *P. hordeoides* (1/4) and *P. polystachion* (2/3); clade F (JK = 79 %), which represents a subset of the species included in clade A (Fig. 1A), with *C. caliculatus* (3/4), *C. ciliaris* (4/4), *C. echinatus* (3/3), *C. incertus* (1/4), *C. myosuroides* (2/3), *C. pilosus* (4/4), *C. setigerus* (2/2), *P. frutescens* (2/2) and *P. ramosum* (1/3), but also including clones of *P. chilense* (1/3), *P. glaucocladum* (2/3), *P. orientale* (1/4) and *P. thunbergii* (1/4). Clade G (JK = 94 %) grouped all species included in clade B (Fig. 1A, B): *P. glaucum* (PI 326520, sequences downloaded from GenBank) (2/2) + *P. glaucum* (*Caxambu* 375) (3/3), *P. squamulatum* (4/5), *P. violaceum* (2/2) and *P. sieberianum* (1) and representatives of subclade Ab: *P. hordeoides* (3/4), *P. polystachion* subsp. *atrichum* (4/4), *P. pedicellatum* (1/3) and *P. basedowii* (4/4). A minor clade, but strongly to moderately supported, included only two clones, *P. polystachion* (1/3) and *P. ramosum* (1/3), and will be referred herein as clade H (JK = 88 %).

Comparison of nuclear vs. plastid trees

Pennisetum lanatum. The position of *P. lanatum* was in disagreement among data partitions. When using the morphological partition alone, *P. lanatum* is included in the PCO clade (although with little support), whereas the plastid markers place *P. lanatum* outside. However, the combined analysis included *P. lanatum* within the PCO clade (Fig. 1A, B).

Clades A–F (Figs 1 and 4). Many of the species in the plastid clade A (Fig. 1) appear in the nuclear clade F (Fig. 4). Clade F includes almost all species of *Cenchrus*, except *C. brownii*, and *P. frutescens*, *P. glaucocladum*, *P. thunbergii*, *P. ramosum*, *P. chilense* and *P. orientale*. Clones of these species are also related to other groups: *P. thunbergii* shares at least one genome with species of clade D, whereas *P. ramosum* is allied to species of clades E and H. Meanwhile, clones of *P. chilense* and *P. orientale* fall in a polytomy at the base of the *Pennisetum*–*Cenchrus* clade.

Cenchrus incertus and *C. myosuroides* are part of clade F, although several clones are related to *C. brownii* and *Pennisetum setaceum* in a polytomy separated from clade F. The plastid and combined phylogenetic analyses show four $x = 17$ *Cenchrus* species (*C. pilosus*, *C. brownii*, *C. echinatus* and *C. incertus*) and *C. myosuroides* ($x = 9$,

10) together in subclade Aa. Two clones of *P. setaceum* are closely related to clones of *C. brownii*, *C. incertus* and *C. myosuroides* in the consensus tree of *kn1*.

Four of six taxa of *Pennisetum* section *Brevivalvula* were grouped in subclade Ab in the plastid and combined analyses: *P. polystachion* subsp. *polystachion*, *P. polystachion* subsp. *atrichum*, *P. hordeoides* and *P. pedicellatum* (Fig. 1A, B). The relationship among these species suggests that they share a common genome, as shown by the plastid markers. However, species of subclade Ab present a reticulate pattern of relationships in the nuclear analysis (*kn1*). Different clones of *P. polystachion* subsp. *polystachion* linked this taxon to *P. ramosum* (clade H, Fig. 4) and to species of clade E (*P. hordeoides*, *P. latifolium*, *P. macrourum* and *P. orientale*).

Clade D (Fig. 4). All clones of *Pennisetum mezianum* and most clones of *P. thunbergii* (3/4) are closely related in the nuclear phylogenetic tree in clade D, whereas a single clone of *P. thunbergii* is included in clade F. However, in the plastid analysis, *P. mezianum* and *P. massaicum* are included in clade A and *P. thunbergii* is distantly related and included in a weakly supported clade with *P. alopecuroides*, *P. macrourum*, *P. orientale* and *P. sphacelatum* (Fig. 1A). *Pennisetum mezianum* and *P. massaicum* are morphologically similar and have a basic chromosome count of $x = 8$ (Table 1).

Clades C–E (Figs 1 and 4). All *kn1* clones of *P. macrourum* (3/3), *P. latifolium* (3/3) and the majority of clones of *P. polystachion* subsp. *polystachion* (2/3) are present in clade E together with single clones of *P. hordeoides*, *P. orientale* and *P. ramosum* (Fig. 4). However, these species have apparently acquired their plastids from disparate sources and are distantly related in the plastid phylogenetic tree (Fig. 1A): *P. macrourum* is sister to *P. natalense*, the latter not included in the nuclear tree, and *P. chilense*, *P. latifolium*, *P. montanum*, and *P. tristachyum* (the last of these not included in *kn1*) are closely related in the strongly supported clade C. Meanwhile, *P. polystachion* is included in the strongly supported subclade Ab with *P. polystachion* subsp. *atrichum*, *P. hordeoides* and *P. pedicellatum*.

Clades B–G (Figs 1 and 4). Clade G includes all species of clade B from the plastid and combined phylogenetic analyses: *P. glaucum*, *P. sieberianum*, *P. violaceum* and *P. squamulatum*, plus species corresponding to subclade Ab in part: *P. hordeoides*, *P. pedicellatum*, *P. polystachion* subsp. *atrichum* and *P. basedowii*.

Clade H (Fig. 4). This is a small clade that includes individual *kn1* clones of *P. ramosum* and *P. polystachion* subsp. *polystachion*; other clones of both taxa are also related in clade E. The relationship between these two species is not well resolved by the plastid and morphological data, although they are closely related to species of clade A (Fig. 1A, B).

Ploidy

Most species in the *Pennisetum*/*Cenchrus* clade are polyploid. In the present sample of species, *P. alopecuroides*, *P. glaucum*, *P. ramosum*, *P. schweinfurthii*, *P. thunbergii*, *P. unisetum* and *P. violaceum* are only known as diploids,



FIG. 3. A single tree depicting relative branch lengths for one of the 30 000 MPTs based on the nuclear marker *knotted1*.

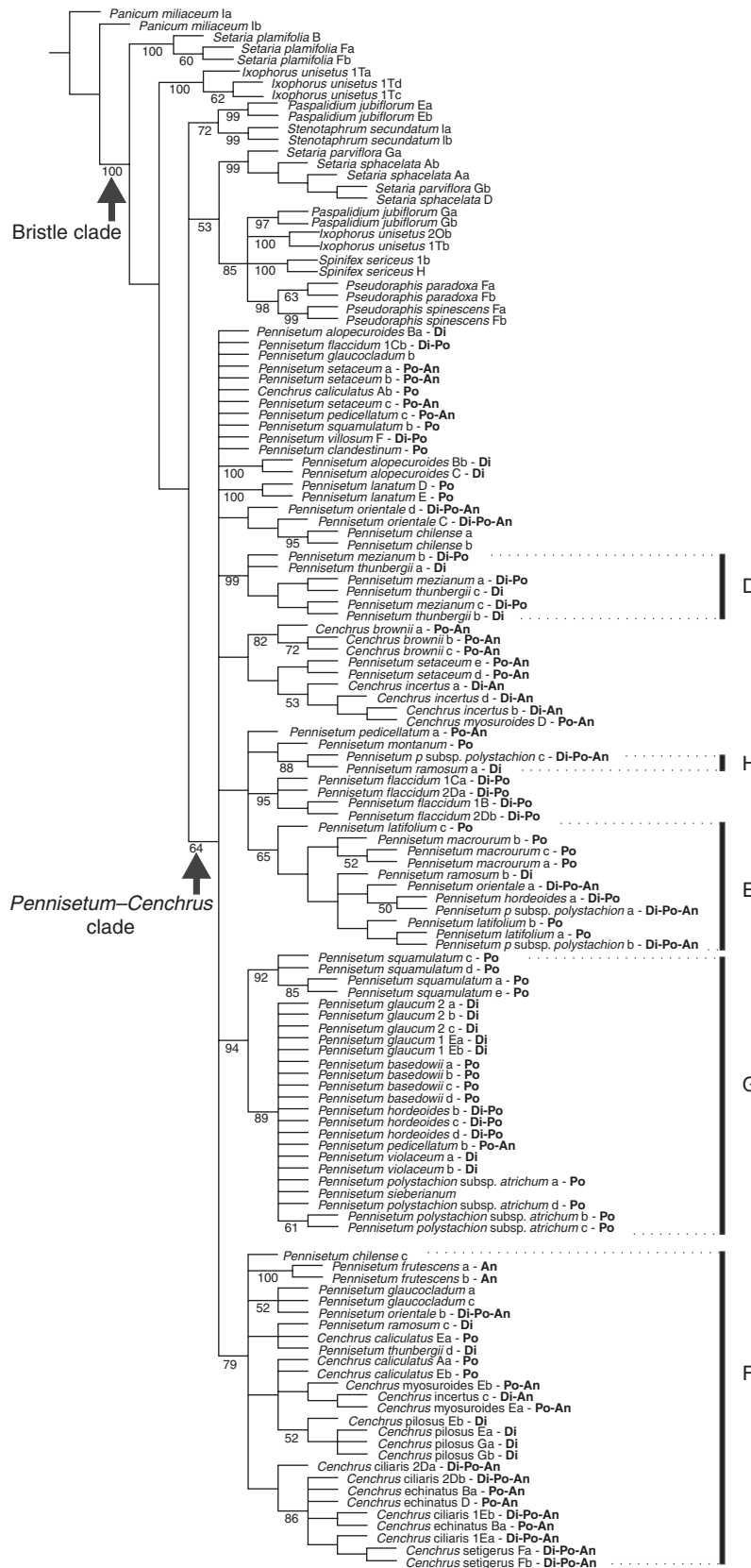


FIG. 4. Strict consensus tree of 30 000 MPTs obtained from parsimony analysis of the nuclear marker *knotted1*. Numbers below the branches represent jackknife branch support. Letters following the species names represent different clones; letters in bold represent the reported ploidy/ies: Di, diploid; An, aneuploid; Po, polyploid. Bars represent principal clades as discussed in the text. *Pennisetum glaucum* 1 corresponds to voucher PI 326520 (sequences downloaded from GenBank) and *P. glaucum* 2 to voucher *Caxambu* 375 (see Appendix 1).

although polyploidy cannot be ruled out. At least two copies of *kn1* were retrieved for *P. alopecuroides*, *P. ramosum* and *P. thunbergii*, indicating polyploidy or duplication of this gene. In addition, diploid, polyploid and aneuploid plants have been reported for *P. flaccidum*, *P. hordeoides*, *P. massaicum*, *P. megianum*, *P. orientale*, *P. polystachion* subsp. *polystachion*, *P. purpureum* and *P. villosum*, although the exact chromosome count for the individual plants sampled here is unknown. Although *P. ramosum* and *P. thunbergii* are reported to be diploids only (Table 1), clones appear in at least two places in the phylogenetic tree (Fig. 4), indicating a polyploid history. Also, *P. ramosum* is reported to be apomictic (Table 1), and apomixis is almost always associated with polyploidy. All species of *Cenchrus* are probably of allopolyploid origin, as the lowest chromosome numbers reported are $n = 17$. All remaining *Pennisetum* species are also polyploid.

DISCUSSION

The monophyly of the *Pennisetum*, *Cenchrus* and *Odontelytrum* clade (PCO clade) is strongly supported by each marker separately or combined (*trnL-F* and *ndhF*), and by the combined analysis (*trnL-F*, *ndhF* and morphology). The position of *P. lanatum* is unclear, depending on the evidence included in the analyses; if considering morphology, or plastid and morphological data together, it is also included in the PCO clade. From the partition analyses, six unambiguous morphological synapomorphies support the group: spikelet not disarticulating from the pedicel and falling together with the bristles as a unit; the pedicel glabrous; the apices of the lemmas acuminate; the margins of the lemma flat; and a membranaceous–cartilaginous upper antherium. Doust and Kellogg (2002) also identified several developmental synapomorphies for the clade: the reduction of the internode on the secondary axis and on other axes, differential elongation of the bristles at maturity, and more bristles than spikelets being initiated in early development. Previous molecular studies proposed the inclusion of *Cenchrus* within *Pennisetum* (Giussani et al., 2001; Doust and Kellogg, 2002; Aliscioni et al., 2003; Bess et al., 2005; Doust et al., 2007; Donadío et al., 2009) and also *Odontelytrum* within *Pennisetum* (Kellogg et al., 2009). The position of *P. clandestinum* within the PCO clade does not support recognition of the genus *Kikuyochloa* proposed by Scholz (2006).

Relationships among major clades within *Pennisetum*–*Cenchrus*

The ingroup includes a representative sample of the species treated in the sectional treatment (Stapf and Hubbard, 1934; Pilger, 1940; Clayton and Renvoize, 1986), but only *Pennisetum* section *Brevivalvula* is supported as monophyletic by the morphological and plastid analyses (subclade Ab, Fig. 1A, B). Likewise, Martel et al. (2004), in their ITS analysis, only found sections *Brevivalvula* and *Penicillaria* to be monophyletic, although most of the sections were under-represented.

The phylogenetic results from independent data sets (nuclear, plastid and morphology) help to elucidate interspecific relationships within the complex *Pennisetum*–*Cenchrus*–

Odontelytrum. From the plastid phylogenetic trees it was possible to identify monophyletic groups that relate species via maternal inheritance. Evidence of genetic exchange among species via biparental inheritance has been provided by the nuclear marker (*kn1*). The optimization of morphological, cytological and reproductive characters helps to interpret the principal pattern of relationships among species. In most taxa investigated, *kn1* is a single-copy gene. In polyploids, one copy per genome is expected, and the gene is thus useful for dissecting the evolutionary history of polyploid species. Sequences of clones from allopolyploid species are expected to fall in multiple positions in a gene tree, with a set of sequences corresponding to each of the parental genomes.

The position of *P. lanatum* within the *Pennisetum*–*Cenchrus* clade is reinforced by results of the nuclear marker (*kn1*; Fig. 4). Because *P. lanatum* is a tetraploid, it is possible that it represents a particularly wide hybridization event, with the pistillate parent being outside the PCO clade.

Clade A (Fig. 1) was first reported by Donadío et al. (2009), based on data from *trnL-F* and *rpl16*, with minor subclades within it. Both their work and the present study includes *C. brownii*, *C. ciliaris*, *C. echinatus*, *C. incertus*, *C. myosuroides*, *C. pilosus*, *C. setigerus*, *P. ramosum*, *P. polystachion* subsp. *polystachion*, *P. purpureum* and *P. setaceum* within the clade. In addition, the data here place *C. agrimonioides*, *C. caliculatus*, *P. hordeoides*, *P. massaicum*, *P. megianum*, *P. pedicellatum* and *P. polystachion* subsp. *atrichum* within the clade. Donadío et al. (2009) placed *P. frutescens* and *P. flaccidum* within clade A, whereas the here data suggest that they are outside it. Placements of those two species are not strongly supported here, so we cannot rule out the possibility that they belong in the clade. The present results and those from Donadío et al. (2009) use one of the same markers (*trnL-F*); discrepancies may be due to differences in phylogenetic signal between *ndhF* and *rpl16*, and/or to the addition of more species.

Cenchrus brownii is clearly related, in plastid and morphological analyses, to other $x = 17$ *Cenchrus* taxa. The grouping of four $x = 17$ *Cenchrus* species (*C. pilosus*, *C. brownii*, *C. echinatus* and *C. incertus*) has been reported by Donadío et al. (2009). *Pennisetum setaceum* has been reported as having a basic chromosome number of $x = 9$ and 17 (under *P. macrostachyon*, Table 1). The $x = 17$ basic chromosome number and its phylogenetic position revealed that *P. setaceum* shares at least one genome with $x = 17$ species of *Cenchrus*.

The nuclear phylogenetic analysis supports a larger sampling for the secondary gene pool of *P. glaucum* (Martel et al., 2004). Clade G includes all species of clade B from the plastid and combined phylogenetic analyses (Figs 1 and 4) with a basic chromosome number of $x = 7$ (*P. glaucum*, *P. violaceum* and *P. squamulatum*), *P. sieberianum* (basic chromosome number unknown), plus species corresponding to subclade Ab with a basic chromosome number of $x = 9$ (*P. hordeoides*, *P. pedicellatum* and *P. polystachion* subsp. *atrichum*) and *P. basedowii* (Table 1). Following the gene pool classification of Harlan and de Wet (1971), species of clade G would be part of the secondary gene pool of *P. glaucum*, as well as others previously cited for clade B (*P. purpureum* and *P. nervosum*; Donadío et al., 2009).

Reticulation pattern, polyploidy and apomixis

Figures 3 and 4 show that the entire *Pennisetum/Cenchrus* clade is a large polyploid complex. The *kn1* tree does not provide enough resolution to determine the ancestry of all the polyploids, but a few observations can be made. First, the history of allopolyploidization is intricate. *Cenchrus* is probably the result of an ancient cross between an ancestor similar to *P. ramosum* or *P. orientale* and another species of *Pennisetum*. The tree does not identify the other parent with certainty, but one possibility might be a diploid species related to *P. setaceum*.

Speciation has occurred at the polyploid level, as shown by the mix of polyploids in each of the major clades of the phylogenetic tree. It is possible that gene flow is occurring among the species. Because crossing barriers are often reduced in polyploids, this may be expected.

The strongly variable chromosome number found in species of subclade Ab or *Pennisetum* section *Brevivalvula* is a remarkable fact in favour of reticulation among species of *Cenchrus* and *Pennisetum*. Although the basic chromosome number was reported as $x = 9$, *P. polystachion* was found to be a hexaploid with, possibly, three different genomes and little or no pairing among them (Sisodia, 1970). *Pennisetum polystachion* subsp. *polystachion* was reported as $2n = 18, 36, 45, 48, 52, 53, 54$ and $63, 2n = 24$ under *P. subangustum*, and $2n = 32, 56$ and 78 under *P. setosum* (the last two names now synonyms of *P. polystachion*). Two or three distinct *kn1* sequences were found in our accession of *P. polystachion* subsp. *polystachion*, suggesting that the individual plant sequenced was either tetraploid or hexaploid. In addition, sequences of *kn1* for *P. polystachion* subsp. *polystachion* are phylogenetically unrelated to those for *P. polystachion* subsp. *atrichum*, even though their plastid sequences are quite similar. This suggests that the morphological similarity of the two may reflect ancestral gene flow, despite the distinct nuclear genomes.

Similarly, *P. pedicellatum* was found to be an allohexaploid characterized by a low frequency of multivalent formation (tri-, tetra- and hexavalents), a large number of uni- and bivalents, and 75 % anomalies in anaphase I (Naithani and Sisodia, 1966; Sisodia, 1970). Ploidy in *P. pedicellatum* was variable: $2n = 36, 45$ and 54 with several aneuploid numbers: $24, 30, 32, 35, 42, 48, 52$ and 53 . Whereas *P. pedicellatum* is shown to be related by the plastid and combined analyses to subclade Ab (Fig. 1A, B), the nuclear marker (Fig. 4) shows it to be related to clade G (which also includes all $x = 7$ species of clade B and representatives of subclade Ab, together with *P. basedowii*).

When optimizing the basic chromosome number in the plastid and combined consensus trees, $x = 9$ was found to be plesiomorphic and $x = 5, 7, 8, 10$ and 17 were derived (Fig. 1). Similarly, when optimizing the basic chromosome number on the nuclear phylogenetic trees (*kn1*), $x = 9$ is plesiomorphic although several clones of $x = 9$ species were included with $x = 7$ species in clade G.

The nuclear phylogenetic analysis revealed a reticulate pattern of relationships among species of *Pennisetum* and *Cenchrus*, which is also supported by evidence from cytogenetic studies (aneuploids, hexaploids, uni- and multivalent

formations, irregular meiotic behaviour). Hybridization among species is frequent within this group (Dujardin and Hanna, 1984b, 1985, 1989; Jauhar, 1981; Marchais and Tostain, 1997), and sequence relationships suggest that there are at least three different genomes within *Pennisetum–Cenchrus*: the $x = 7$ genome (clade B, *Pennisetum* species), the $x = 9$ genome (most *Pennisetum* and *Cenchrus* species) and the $x = 17$ genome (subclade Aa). The origin of the $x = 17$ genome could be the result of a cross between ancestors of $x = 8$ and $x = 9$, or two $x = 9$ taxa followed by the loss of one chromosome and, in both cases, followed by diploidization of the ancestral polyploid. Other basic chromosome numbers would be reductions from $x = 9$ that appeared independently in *P. ramosum* ($x = 5$), *P. massaicum*, *P. meianum* and *P. montanum* ($x = 8$). To resolve species relationships within *Pennisetum–Cenchrus*, a group of plants in which reticulation and introgression are common processes, would require additional nuclear sequence loci to obtain congruent results and enhance the resolution among taxa.

At least two species of *Cenchrus* (*C. ciliaris* and *C. setigerus*) and 16 species of *Pennisetum* have been reported as facultatively or obligately apomictic (Table 1). Apomixis in *P. squamulatum* and *C. ciliaris* is linked to a single chromosome (Ozias-Akins et al., 1993) that contains a non-recombining ASGR (Ozias-Akins et al., 1998; Roche et al., 1999). The conservation of molecular markers linked to apomixis and the close relationship among species of *Pennisetum* and *Cenchrus* support the view of a single event for the evolution of this trait (Ozias-Akins et al., 2003). It has been suggested that apomixis characterized subfamily Panicoideae before its diversification into the present tribes (Brown and Emery, 1958). Our optimization of the presence of apomixis in the phylogenetic hypotheses is largely uninformative due to insufficient data. If taxa for which reproductive behaviour is not reported or unknown are considered apomictic (Table 1), then apomixis could be considered plesiomorphic, but if those taxa are sexual, then apomictic species have appeared several times during the evolution of the *Pennisetum–Cenchrus* clade. Because apomixis or the molecular markers linked to apomixis can be transferred to different species through hybridization, it seems more likely that apomixis is related to the interbreeding history of the group, rather than appearing independently several times. As a consequence, the acquisition of apomixis through hybridization within *Pennisetum–Cenchrus* would be a common mechanism that allows new genotypes to perpetuate themselves. Facultative apomixis has been shown to stabilize polyploid taxa and to permit limited gene flow in other groups of grasses. In the genera *Dichanthium* Willemet, *Bothriochloa* Kuntze and *Capillipedium* Stapf (tribe Andropogoneae), de Wet and Harlan (1970) documented extensive gene flow at the polyploid level, aided by apomixis. It seems likely that a similar phenomenon is occurring in *Cenchrus/Pennisetum*.

Taxonomic implications

The phylogenetic results presented here strongly support the unification of *Cenchrus* and *Pennisetum*, as previously suggested by Correll and Johnston (1970), and the inclusion of *Odontelytrum*, a monotypic genus occurring in Yemen

and eastern Africa. *Pennisetum* as currently defined is paraphyletic, with *Odontelytrum* and *Cenchrus* embedded within it. Optimization of morphological characters within the PCO clade suggests that no morphological character constitutes a synapomorphy for any of the three genera. Due to the consistency between the present results and different phylogenetic hypotheses (including morphological, developmental and multilocus approaches), and the strong support found for the PCO clade, including the type species of the three genera, we propose unification of *Pennisetum*, *Cenchrus* and *Odontelytrum*. Species of *Pennisetum* and *Odontelytrum* are here transferred to *Cenchrus* (Appendix 4), which has priority (McNeill et al., 2006). In addition to the morphological and developmental synapomorphies of the PCO clade (= *Cenchrus*), *Cenchrus* is here characterized by having one or several spikelets accompanied by one bristle or surrounded by an involucre of multiple bristles, free or moderately to considerably fused, or having bristles fused and forming a cup-like structure (the degree of fusion varying from a small basal disc to a deep cupule), the consistency of the cupule being rigid or herbaceous.

Pennisetum, *Cenchrus* and *Odontelytrum* have been previously included under Cenchrinae by Clayton and Renvoize (1986), together with ten other genera bearing bristles. A comprehensive study including all those genera is being conducted for the tribe Paniceae (Morrone et al., 2008); however, results are too preliminary to reach any decision on the inclusion of other genera within *Cenchrus* as delimited here.

The complex polyploid relationships among the species shown by the nuclear gene phylogenetic analysis (Figs 3 and 4) provide another argument for combining *Pennisetum* and *Cenchrus* in a single genus. Biologically, they are clearly exchanging genes by forming allopolyploids. Their evolution is reticulate, rather than divergent, and hence is inherently difficult to incorporate into a hierarchical classification.

The present study also suggests the need for a comprehensive revision of the group to circumscribe infrageneric taxonomic categories based on monophyly, and the morphological and cytological delimitation of the new groupings. Furthermore, if the intricate pattern of interspecific relationships within the PCO clade is due to hybridization and introgression, determining the evolutionary history will require adding new species and nuclear loci into the analyses.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the matrix of morphological characters that were used in the phylogenetic analyses.

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APPENDIX 1

Taxa studied, voucher information for the specimens sequenced here and GenBank accession numbers. ‘–’ indicates no sequence for that region. Numbers in parentheses indicate published sequences from GenBank and their reference. Material grown from seeds. Letters in bold types represent different clones.

| Taxon | Voucher | GenBank accession | | |
|---|--|-------------------------|-------------------------|---|
| | | <i>ndhF</i> | <i>trnL-F</i> | <i>Kn1</i> |
| <i>Cenchrus agrimonioides</i> Trin. | – | AY623745 ⁽¹⁾ | – | – |
| <i>C. brownii</i> Roem. & Schult. | Venezuela, Ramia & Marrero 9349, SI | GU561510 | EU940005 ⁽²⁾ | a: GU561607 b: GU561608 c: GU561609 |
| <i>C. caliculatus</i> Cav. | – | EF189886 ⁽³⁾ | – | Aa: EF189760 ⁽³⁾ Ab: EF189762 ⁽³⁾ Ea: EF189761 ⁽³⁾ Eb: EF189763 ⁽³⁾ |
| <i>C. ciliaris</i> L. | – | AY029625 ⁽⁴⁾ | EU940006 ⁽²⁾ | 1Ea: EF189764 ⁽³⁾ 1Eb: EF189767 ⁽³⁾ 2Da: EF189765 ⁽³⁾ 2Db: EF189766 ⁽³⁾ |
| <i>C. echinatus</i> L. | – | AF499151 ⁽⁵⁾ | EU940007 ⁽²⁾ | Ba: EF189770 ⁽³⁾ Bb: EF189769 ⁽³⁾ D: EF189768 ⁽³⁾ |
| <i>C. incertus</i> M.A. Curtis | Argentina, Morrone & Giussani 5166, SI | GU561514 | EU940008 ⁽²⁾ | a: GU561598 b: GU561599 c: GU561600 d: GU5616010 D: EF189772 ⁽³⁾ |
| <i>C. myosuroides</i> Kunth | – | AF499152 ⁽⁵⁾ | EU940009 ⁽²⁾ | Ea: EF189771 ⁽³⁾ Eb: EF189773 ⁽³⁾ |
| <i>C. pilosus</i> Kunth | – | EF189887 ⁽³⁾ | EU940010 ⁽²⁾ | Ea: EF189777 ⁽³⁾ Eb: EF189775 ⁽³⁾ Ga: EF189774 ⁽³⁾ Gb: EF189776 ⁽³⁾ |
| <i>C. setigerus</i> Vahl | – | AF499153 ⁽⁵⁾ | EU940011 ⁽²⁾ | Fa: EF189778 ⁽³⁾ Fb: EF189779 ⁽³⁾ |
| <i>Ixophorus unisetus</i> (J. Presl) Schldtl. | – | AY623749 ⁽¹⁾ | EU939980 ⁽²⁾ | 1Ta: EF189883 ⁽³⁾ 1Tb: EF189882 ⁽³⁾ 1Tc: EF189884 ⁽³⁾ 1Td: EF189885 ⁽³⁾ 2Ob: EF189880 ⁽³⁾ |
| <i>Odontelytrum abyssinicum</i> Hack. | Ethiopia, Friis & al. 6699, K | GU561512 | GU561491 | – |
| <i>Panicum miliaceum</i> L. | – | – | – | Ia: EF189758 ⁽³⁾ Ib: EF189759 ⁽³⁾ |
| <i>Paspalidium geminatum</i> (Forssk.) Stapf | – | AY029662 ⁽⁴⁾ | EU939981 ⁽²⁾ | – |
| <i>Paspalidium jubiflorum</i> (Trin.) Hughes | – | – | – | Ea: EF189851 ⁽³⁾ Eb: EF189850 ⁽³⁾ Ga: EF189798 ⁽³⁾ Gb: EF189797 ⁽³⁾ |
| <i>Pennisetum alopecuroides</i> (L.) Spreng. | – | AY029672 ⁽⁴⁾ | EU939986 ⁽²⁾ | Ba: EF189781 ⁽³⁾ Bb: EF189780 ⁽³⁾ C: EF189782 ⁽³⁾ |
| <i>P. basedowii</i> Summerh. & C.E. Hubb. | Australia, Pullen 10417, CANB | GU561515 | GU561495 | a: GU561550 b: GU561551 c: GU561552 d: GU561553 |
| <i>P. chilense</i> (E. Desv.) B.D. Jacks. ex R.E. Fr. | Argentina, Zuloaga & al. 8617, SI | GU561516 | EU939987 ⁽²⁾ | a: GU561554 b: GU561555 c: GU561556 |
| <i>P. clandestinum</i> Hochst. ex Chiov. | Argentina, Morrone s.n., SI | GU561517 | EU939988 ⁽²⁾ | GU561617 |
| <i>P. flaccidum</i> Griseb. | – | AF499150 ⁽⁵⁾ | EU939989 ⁽²⁾ | 1B: EF189786 ⁽³⁾ 1Ca: EF189787 ⁽³⁾ 1Cb: EF189783 ⁽³⁾ 2Da: EF189785 ⁽³⁾ 2Db: EF189784 ⁽³⁾ |
| <i>P. foermeranum</i> Leeke | Namibia, Moss 2017, PRE | GU561511 | GU561496 | – |
| <i>P. frutescens</i> Leeke | Argentina, Deginani 1822, SI | GU561519 | EU939990 ⁽²⁾ | a: GU561557 b: GU561558 |

Continued

APPENDIX *Continued*

| Taxon | Voucher | GenBank accession | | |
|--|---|---|---|---|
| | | <i>ndhF</i> | <i>trnL-F</i> | <i>Knl</i> |
| <i>P. glaucocladum</i> Stapf & C.E. Hubb. | Botswana, <i>Smith 2403</i> , PRE | GU561520 | GU561497 | a: GU561569 b: GU561570 c: GU561571 |
| <i>P. glaucum</i> (L.) R. Br. (1) | – | AF499149 ⁽⁵⁾ | EU939991 ⁽²⁾ | Ea: EF189789 ⁽³⁾ Eb: EF189788 ⁽³⁾ |
| <i>P. glaucum</i> (L.) R. Br. (2) | Brasil, <i>Caxambu 375</i> , MBM | GU561521 | GU561498 | a: GU561547 b: GU561548 c: GU561549 |
| <i>P. hordeoides</i> (Lam.) Steud. | Nepal, <i>Staiton & al. 8844</i> , K | GU561522 | GU561499 | a: GU561565 b: GU561566 c: GU561567 d: GU561568 |
| <i>P. lanatum</i> Klotzsch | India, <i>Bohr 9207</i> , K India, <i>Siddigi 1053</i> , K | GU561523 – | – GU561500 | E: EF189790 ⁽³⁾ |
| <i>P. latifolium</i> Spreng. | Uruguay, <i>Morrone 5231</i> , SI | GU561524 | EU939993 ⁽²⁾ | a: GU561559 b: GU561560 c: GU561561 |
| <i>P. macrourum</i> Trin. | CN 87800* | GU561525 | AY116266 ⁽⁷⁾ | a: GU561562, (6) b: GU561563 c: GU561564 |
| <i>P. massaicum</i> Stapf <i>P. mejianum</i> Leeke | Kenya, <i>Greenway & Kanuri 12834</i> , K PI 214061* | GU561526 GU561527 | GU561501 GU561502 | – a: GU561572 b: GU561573 c: GU561574 |
| <i>P. montanum</i> (Griseb.) Hack. <i>P. natalense</i> Stapf <i>P. nervosum</i> (Nees) Trin. <i>P. orientale</i> Rich. | – South Africa, <i>Strey 10968</i> , K Argentina, <i>Morrone 5329</i> , SI CN 84066* | AY188498 ⁽⁶⁾ GU561528 GU561529 GU561530 | EU939994 ⁽²⁾ – EU939996 ⁽²⁾ GU561503 | GU561579 – – a: GU561580 b: GU561581 c: GU561582 d: GU561583 |
| <i>P. pedicellatum</i> Trin. | CN 87902* | GU561531 | GU561504 | a: GU561584 b: GU561585 c: GU561586 |
| <i>P. polystachion</i> subsp. <i>polystachion</i> (L.) Schult. | Bolivia, <i>Morrone & Belgrano 5060</i> , SI | GU561533 | EU939997 ⁽²⁾ | a: GU561587 b: GU561588 c: GU561589 |
| <i>P. polystachion</i> subsp. <i>atrichum</i> (Stapf & C.E. Hubb.) Brunken | Tanzania, <i>Bjornstad 1704</i> , K | GU561532 | GU561505 | a: GU561612 b: GU561613 c: GU561614 d: GU561615 |
| <i>P. purpureum</i> Schumach. <i>P. ramosum</i> (Hochst.) Schweinf. | Argentina, <i>Morrone & al. 4473</i> , SI CN84079* | GU561534 GU561535 | EU939999 ⁽²⁾ EU929056 | – a: GU561590 b: GU561591 c: GU561592 |
| <i>P. schweinfurthii</i> Pilg. <i>P. setaceum</i> (Forssk.) Chiov. | Ethiopia, <i>Friis & al. 7745</i> , K Argentina, <i>Morrone 5373</i> , SI | GU561536 GU561537 | GU561506 EU940000 ⁽²⁾ | – a: GU561593 b: GU561594 c: GU561595 d: GU561597 e: GU561596 |
| <i>P. sieberianum</i> (Schltdl.) Stapf & C.E. Hubb. <i>P. sphacelatum</i> (Schumach.) T. Durand & Schinz <i>P. squamulatum</i> Fresen. | PI 532675* South Africa, <i>Smook 5934</i> , PRE PI 248534* | GU561538 GU561539 GU561540 | EU940001 ⁽²⁾ – EU929057 | GU561616 – a: GU561602 b: GU561603 c: GU561604 d: GU561605 e: GU561606 |
| <i>P. thunbergii</i> Kunth | CN 87791* | GU561541 | GU561507 | a: GU561575 b: GU561576 c: GU561577 d: GU561578 |

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APPENDIX Continued

| Taxon | Voucher | GenBank accession | | |
|--|--|-------------------------|-------------------------|---|
| | | <i>ndhF</i> | <i>trnL-F</i> | <i>Kn1</i> |
| <i>P. trachypyllum</i> Pilg. | Kenya, <i>Bogdan 1151</i> , K | GU561542 | GU561508 | – |
| <i>P. tristachyum</i> (Kunth) Spreng. | Bolivia, <i>Morrone & al. 4234</i> , SI | GU561543 | EU940002 ⁽²⁾ | – |
| <i>P. unisetum</i> (Nees) Benth. | Sudan, <i>Friis & Vollesen 129</i> , K | GU561544 | EU929058 | – |
| <i>P. villosum</i> R. Br. ex Fresen. | – | EF189888 ⁽³⁾ | EU940004 ⁽²⁾ | F: EF189792 ⁽³⁾ |
| <i>P. violaceum</i> (Lam.) Rich. ex Pers. | CN 88058* | GU561545 | GU561509 | a: GU561610 b: GU561611 Fa: EF189807 ⁽³⁾ Fb: EF189808 ⁽³⁾ Fa: EF189809 ⁽³⁾ Fb: EF189810 ⁽³⁾ |
| <i>Pseudoraphis paradoxa</i> Pilg. | – | – | – | Fa: EF189807 ⁽³⁾ Fb: EF189808 ⁽³⁾ Fa: EF189809 ⁽³⁾ Fb: EF189810 ⁽³⁾ |
| <i>P. spinescens</i> (R. Br.) Vickery | – | – | – | Fa: EF189809 ⁽³⁾ Fb: EF189810 ⁽³⁾ |
| <i>Rupichloa acuminata</i> (Renvoize) Salariato & Morrone | Brazil, <i>Zuloaga & Morrone s.n.</i> , SI | AY029692 ⁽⁴⁾ | GU561490 | – |
| <i>Setaria palmifolia</i> (J. König) Stapf | MO 801593–2 | AY029680 ⁽⁴⁾ | GU561492 | B: EF189833 ⁽³⁾ Fa: EF189832 ⁽³⁾ Fb: EF189834 ⁽³⁾ Ga: EF189813 ⁽³⁾ Gb: EF189814 ⁽³⁾ Aa: EF189815 ⁽³⁾ |
| <i>S. parviflora</i> (Poir.) Kerguelén | PI 316422 | AY029682 ⁽⁴⁾ | GU561493 | Ab: EF189817 ⁽³⁾ D: EF189816 ⁽³⁾ H: EF189822 ⁽³⁾ Ib: EF189824 ⁽³⁾ |
| <i>S. sphacelata</i> (Schumach.) Stapf & C.E. Hubb. ex M.B. Moss | PI 268145 | AY029681 ⁽⁴⁾ | GU561494 | Ia: EF189854 ⁽³⁾ Ib: EF189855 ⁽³⁾ |
| <i>Spinifex sericeus</i> R. Br. | – | – | – | |
| <i>Stenotaphrum secundatum</i> (Walter) Kuntze | – | AY029684 ⁽⁴⁾ | EU939985 ⁽²⁾ | |

References: (1) Kellogg et al. (2004), (2) Donadio et al. (2009), (3) Doust et al. (2007), (4) Giussani et al. (2001), (5) Doust and Kellogg (2002), (6) Aliscioni et al. (2003), (7) Hodkinson et al. (2002).

APPENDIX 2

Cytological and morphological characters used in the cladistic analyses and coding states.

1. Chromosome basic number: $x = 5$ (0), $x = 7$ (1), $x = 8$ (2), $x = 9$ (3), $x = 17$ (4), $x = 13$ (5), $x = 10$ (6). **2. Ligule:** membranous (0), ciliate (1), membranous–ciliate (2). **3. Contra-ligule:** absent (0), present (1). **4. Leaf blade:** flat (0), convolute (1). **5. Spikelets subtended by an involucre composed of bristles:** absent (0), present (1). **6. Inflorescence:** terminal (0), axillary (1). **7. Inflorescence-type:** contracted to spiciform (0), open (1). **8. Panicle axis:** scaberulous (0), glabrous (1), pubescent (2). **9. Involucre:** pedicellate (0), sessile (1). **10. Pedicel of the involucre:** glabrous (0), pubescent (1), scaberulous (2). **11. Spikelet:** pedicellate (0), sessile (1). **12. Disarticulation at the base of the spikelet:** absent (0), present (1). **13. Disarticulation at the base of the involucre:** absent (0), present (1). **14. Disarticulation at the base of the upper anthecium:** absent (0), present (1). **15. Disarticulation at the base of the pedicel:** absent (0), present (1). This character applies to the species in which the spikelet or the involucre is pedicellate and the disarticulation point is between the pedicel and the rachis. **16. Pedicel of the spikelet:** glabrous (0), pubescent (1), scaberulous (2). **17. Number of bristles:** less than 20 (0), more than 21 (1). **18. Bristles:** antrorsely scaberulous (0), retrorsely scaberulous (1). **19. Bristles:** all the bristles plumose (0), some bristles plumose (1), without plumose

bristles (2). **20. Disposition of the bristles:** one whorl (0), two or more whorls (1). **21. Bristles:** free (0), connate below (1), connate up to half the total length (2), connate up to two-thirds of the total length (3). **22. Length of the bristles:** one conspicuously longer bristle (0), two or more conspicuously longer bristles (1), all the bristles as long as the spikelet (2), all the bristles longer than the spikelet (3). **23. Fertile spikelets per involucre:** one (0), two or more (1). **24. Spikelets:** isomorphous (0), heteromorphous (1). **25. Lower floret:** sterile (0), male (1). **26. Lower glume:** absent (0), vestigial (1), complete (2). **27. Length of the lower glume:** less than 1/3 of the spikelet (0), between 1/3 and half the spikelet (1), between half the spikelet and 2/3 (2), more than 2/3 of the spikelet (3), same length as the spikelet (4). **28. Consistency of the lower glume:** hyaline (0), membranous (1). **29. Apex of the lower glume:** acute (0), acuminate (1), rounded (2). **30. Upper glume:** absent (0), vestigial (1), complete (2). **31. Length of the upper glume:** less than 1/3 of the spikelet (0), between 1/3 and half the spikelet (1), between half the spikelet and 2/3 (2), more than 2/3 of the spikelet (3), same length as the spikelet (4). **32. Consistency of the upper glume:** hyaline (0), membranous (1), chartaceous (2). **33. Apex of the upper glume:** acute (0), acuminate (1), rounded (2). **34. Length of the lower lemma:** between 1/3 and half the spikelet (0), between half the spikelet and its total length (1), same length as the spikelet (2). **35. Consistency of the lower lemma:** hyaline (0), membranous (1), chartaceous (2). **36. Apex of the lower lemma:** acute (0), acuminate (1), rounded (2), bidentate (3), tridentate (4). **37. Margin or**

apex of the lower lemma: ciliate (0), glabrous (1), scaberulous (2). **38. Consistency of the upper lemma:** membranous (0), chartaceous (1), coriaceous (2). **39. Apex of the upper lemma:** acute (0), acuminate (1), rounded (2), bidentate (3), tridentate (4). **40. Margin or apex of the upper lemma:** ciliate (0), glabrous (1). **41. Lower palea:** absent (0), vestigial (1), complete (2). **42. Apex of the lower palea:** acute (0), acuminate (1), rounded (2), bidentate (3), tridentate (4). **43. Margin or apex of the lower palea:** ciliate (0), glabrous (1). **44. Apex of the upper palea:** acute (0), acuminate (1), rounded (2), bidentate (3), tridentate (4). **45. Margin or apex of the upper palea:** ciliate (0), glabrous (1). **46. Lemma margin:** flat (0), involute (1). **47. Lodicules:** absent (0), present (1). **48. Styles:** connate (0), free (1). **49. Anther tip:** glabrous (0), penicillate (1). **50. Upper anthercium texture:** smooth (0), rugose (1). **51. Consistency of the upper anthercium when mature** (with caryopsis): crustaceous (0), membranous–cartilaginous (1).

APPENDIX 3

Taxa studied in the morphological phylogeny and voucher information.

Cenchrus brownii: Honduras, *Montoya* 28, SI. Mexico, *Ku and Yam* 410, SI. Thailand, *Laegaard and Norsangsi* 21870, SI. **C. ciliaris:** Argentina, *Cabrera et al.* 31024, SI; *Cabrera et al.* 29829, SI. Ecuador, *Laegaard* 53064, SI. Mexico, *Lizama* 1456, SI. **C. echinatus:** Argentina, *Burkart and Gamero* 21614, SI; *Hicken* 12972, SI; *Venturi* 5509, SI. Thailand, *Laegaard* 21791, SI. **C. incertus:** Argentina, *Pozner and Belgrano* 173, SI. Brazil, *Conrad and Dietrich* 2141, SI. **C. myosuroides:** Argentina, *Morrone and Giussani* 5162, SI; *Burkart* 20289, SI; *Burkart* 22133, SI; *Guaglianone and Tur* 2458, SI. **C. pilosus:** Peru, *Sánchez Vega and Guevara* 6217, SI. **C. setigerus:** Kenya, *Verdcourt* 2628, SI.

Ixophorus unisetus: Bolivia, *Vargas* 2101, SI. Mexico, *Zuloaga et al.* 7360, SI.

Odontelytrum abyssinicum: Ethiopia, *Ash* 2595, K; *Friis et al.* 6699, K. Tanzania, *Greenway and Kanuri* 12617, K. Yemen, *Bisset* 281, K; *Wood* 1945, K.

Paspalidium geminatum: Eritrea, *Pappi* 6829, SI. Ethiopia, *Burger* 1135, SI. Tanzania, *Dunipes and Jefford s.n.*, SI.

Pennisetum alopecuroides: Argentina, *Rúgolo de Agrasar* 2144, SI. **P. basedowii:** Australia, *Pullen* 10417, CANB; *Wolfe and Martin* 144, CANB; *Paijmans* 2513, CANB; *Jacobs* 1322, CANB; *Perry* 201, CANB. **P. chilense:** Argentina, *Kiesling et al.* 9467, SI; *Krapovickas* 3182, SI; *Zuloaga and Deginani* 3772, SI; *Correa et al.* 4477, SI; *Múlgura et al.* 1250, SI; *Venturi* 4891, SI. **P. clandestinum:** Argentina, *Burkart* 18503, SI; *Nicora* 9248, SI; *Villar* 26077, SI. **P. flaccidum:** Pakistan, *Duthie* 12666, K; *Hartman* 167, K; *Norris* 85, K; *Stewart* 10010, K; *Winterbottom* 202, K. **P. foermeranum:** Namibia, *Ellis* 1069, PRE; *Moss and Jacobsen* 45, PRE; *Sittman* 9, PRE; *Smook* 5182, PRE; *Smook* 5231, PRE. **P. frutescens:** Argentina, *Burkart* 20204, SI; *Cardini* 89, SI; *Job* 1175, SI; *Jørgensen* 2891, SI. Paraguay, *Arenas* 1745, SI. **P. glaucocladum:** Botswana, *Gibbs Russell* 2811, PRE; *Smith* 1694, PRE;

Smith 2403, PRE. Namibia, *de Winter and Marais* 4761, PRE. **P. glaucum:** Argentina, *Burkart* 514, SI; *Burkart* 18479, SI. **P. hordeoides:** India, *Adams* 3887, K; *van der Maesen* 5033, K. Liberia, *Baldwin* 9942, K. Nepal, *Stainton et al.* 8844, K. **P. lanatum:** India, *Bor* 9207, K; *Duthie s.n.*, K; *Wingate s.n.*, BAA 13627. Pakistan, *Siddigi et al.* 1053, K; *Stewart* 8830A, K; *Webster and Nasir* 6491, K. **P. latifolium:** Argentina, *Burkart and Troncoso* 26263, SI; *Cabrera et al.* 26474, SI; *Porta* 209, SI; *Schwarz* 7647, SI; *Zuloaga et al.* 5054, SI. **P. macrourum:** Kenya, *Bogdan* 3514, K; *Bogdan* 3637, K. South Africa, *Acocks* 18652, PRE; *Barker* 588, PRE; *Fugler* 105, PRE; *Taylor* 9939, PRE; *Victor* 954, PRE. Tanzania, *Wingfield* 59, K; *Wingfield* 1004, K; *Wingfield* 914, K. **P. massaicum:** Kenya, *Ament* 799, K; *Bogdan* 896, K; *Bogdan* 3614, K; *Bogdan* 2409, K; *Edwards* 2987, K; *Greenway and Kanuri* 12834, K. **P. mezianum:** Namibia, *Acocks* 18046, PRE; *Smook* 5117, PRE. Tanzania, *Greenway* 9836, K; *Greenway and Kanuri* 11773, K; *Raynal* 19340, K; *Richards* 23694, K; *Richards* 25202, K. **P. montanum:** Argentina, *Cabrera et al.* 20643, SI; *Cabrera et al.* 34738, SI; *Giardelli* 998, SI; *Zuloaga* 3767, SI. Bolivia, *Morrone and Belgrano* 4931, SI. **P. natalense:** South Africa, *Acocks* 10122, PRE; *Codd* 1367, PRE; *Edwards* 2031, PRE; *Strey* 10968, K; *Ward* 4200, PRE. **P. nervosum:** Argentina, *Burkart et al.* 26853, SI; *Burkart* 21094, SI; *Jørgensen* 2406, SI; *Pedersen* 8316, SI; *Zuloaga et al.* 844, SI. **P. orientale:** Argentina, *Rúgolo de Agrasar* 2188, SI. **P. pedicellatum:** India, *Bor* 9207, K. Kenya, *Jeffery* 538, K. Sudan, *Beshir Eff.* 429, K; *Daws* 906, K; *Harrison* 87, K; *Simpson* 7295, K. **P. polystachion subsp. polystachion:** Bolivia, *Laegaard* 22323, AAU. Costa Rica, *Herrera* 1544, SI. Ecuador, *Laegaard* 71230, AAU; *Laegaard* 71338, AAU; *Laegaard* 71517, AAU. Tanzania, *Southon* 224, SI. Sri Lanka, *Comanor* 734, SI; *Cooray* 69091411R, SI. **P. polystachion subsp. atrichum:** Kenya, *Grant* 878, K. Tanzania, *Bjornstad* 1704, K; *Ngoundai* 31, K. **P. purpureum:** Argentina, *Burkart* 18502a, SI. Bolivia, *Zuloaga et al.* 1444, SI. Costa Rica, *Grayum* 3433, SI. Puerto Rico, *Nee* 44104, SI. **P. ramosum:** Tanzania, *Leippert* 5628, SI. **P. schweinfurthii:** Ethiopia, *Friis et al.* 7745, K. Sudan, *Jalen* 19, K; *Sherif* 4028, K; *Wickens* 854, K. **P. setaceum:** Argentina, *Morrone* 5373, SI; *Rúgolo de Agrasar* 2145, SI; *Rúgolo de Agrasar* 2183, SI; *Hurrell and Bazzano* 5646, SI. Venezuela, *Ramía and Grande* 9341, SI. **P. sieberianum:** Saudi Arabia, *Collenette* 7909, K; *Cope* 166, K; *Fernandez* 82, K; *Fernandez* 1276, K. Yemen, *Wood* 3428, K. **P. sphacelatum:** Kenya, *Stewart* 364, K; *Thulin and Tidigs* 277, K; *Wesche* 1685, K. Tanzania, *Renvoize and Abdallah* 2403, K; *Taylor* 10316, K. South Africa, *Mohle* 234, PRE; *Sheepers* 1395, PRE; *Smook* 5803, SI; *Smook* 5934, PRE; *Smook* 6682, SI; *Victor* 1782, PRE. **P. squamulatum:** Kenya, *Bogdan* 1863, K; *Bogdan* 2418, K; *Bogdan* 3833, K; *Glover and Samuel* 2733, K. Tanzania, *Greenway et al.* 13173, K. **P. thunbergii:** South Africa, *Drews* 157, PRE; *Du Toit* 2491, PRE; *Liebenberg* 7305, PRE; *Loxton* 238, PRE; *Pappi* 241, SI; *Roberts* 3290, PRE; *Smook* 5028, PRE. **P. trachypyllum:** Kenya, *Bogdan* 1151, K; *Faden and Evans* 74/710, K. Uganda, *Maitland* 1390, K; *Snowden* 1445, K; *Thomas* 1155, K. **P. tristachyum:** Argentina, *Schreiter* 4039, SI; *Venturi* 1349, SI; *Williulz*

225, SI. Bolivia, *Buchtien 457*, SI. ***P. unisetum***: Ethiopia, *Friis et al. 593*, K. Sudan, *Friis and Vollesen 129*, K. Uganda, *Katende 677*, K. Tanzania, *Greenway and Kanuri 15170*, K. ***P. villosum***: Argentina, *Crespo 33*, SI; *Hicken 12965*, SI; *Rúgolo de Agrasar 2141*, SI. Eritrea, *Pappi 1987*, SI.

Rupichloa acuminata: Brazil, *Zuloaga et al. 4843*, SI; *Zuloaga et al. 4766*, SI.

Setaria palmifolia: Guatemala, *Türckheim 1450*, SI. Philippines, *Fénix 117*, SI. Venezuela, *Zuloaga and Ortiz 4527*, SI. ***S. parviflora***: Argentina, *Ragonese 2344*, SI; *Tivano 394*, SI; *Vegeti 361*, SI. Bolivia, *Morrone and Belgrano 4935*, SI. ***S. sphacelata***: Argentina, *Morrone et al., 649*, SI; *Morrone et al. 5109*, SI; *Zuloaga and Morrone 7222*, SI. Paraguay, *Morrone and Pensiero, 565*, SI.

Stenotaphrum secundatum: Argentina, *Burkart 274*, SI; *Burkart 1494*, SI; *Lanfranchi 74*, SI; *Rúgolo de Agrasar 1033*, SI.

APPENDIX 4.

Nomenclatural changes

Cenchrus americanus (L.) Morrone, **comb. nov.** Basionym: *Panicum americanum* L., Sp. Pl. 1: 56. 1753. LECTOTYPE. Illustration in Clusius, Rar. Pl. Hist 2: 215. 1601 (lectotype, designated by Clayton & Renvoize, in Polhill (ed.), Fl. Trop. E. Africa, Gramineae 3: 672-1982).

Panicum glaucum L., Sp. Pl.: 56. 1753, non *Cenchrus glaucus* Mudaliar & Sudaraj, 1957. *Pennisetum glaucum* (L.) R. Br., Prodr. 1: 195. 1810. LECTOTYPE: Sri Lanka, *Hermann s.n.* (lectotype, BM, designated as holotype by Rauschert, Feddes Repert 83(9–10): 662, 1973).

Cenchrus abyssinicus (Hack.) Morrone, **comb. nov.** Basionym: *Odontelytrum abyssinicum* Hack., Oesterr. Bot. Z. 48: 86. 1898. TYPE: Ethiopia. Gaffat to Debra Tabor, 2700 m, 1863, *Shimper 1121* (holotype, B; isotype, K).

Cenchrus advena (Wipff & Veldkamp) Morrone, **comb. nov.** Basionym: *Pennisetum advena* Wipff & Veldkamp, Sida 18(4): 1033, f. 1. 1999. TYPE: United States. Texas, Brazos Co., cultivated at Texas A&M University, 18 Sep 1990, *J.K. Wipff 1723* (holotype, L; isotypes, K, MO, US, UTC).

Cenchrus annuus (Mez) Morrone, **comb. nov.** Basionym: *Pennisetum annuum* Mez, Bot. Jahrb. Syst. 56 (Beibl. 125): 7. 1921. TYPE: Peru. Lima-Oroya, 17 Apr 1910, *A. Weberbauer 5354* (holotype, B; isotype, US).

Cenchrus arnhemicus (F. Muell.) Morrone, **comb. nov.** Basionym: *Pennisetum arnhemicum* F. Muell., Fragm. 7: 109. 1873. TYPE: Australia. Upper river Victoria River, *F. Mueller s.n.* (holotype, MEL).

Cenchrus bambusiformis (E. Fourn.) Morrone, **comb. nov.** Basionym: *Gymnotrix bambusiformis* E. Fourn., Mexic. Pl. 2: 48. 1886. *Pennisetum bambusiforme* (E. Fourn.) Hemsl. ex B.D. Jacks., Index Kew. 2: 458. 1895. TYPE: Mexico. Mirador, Mar 1842, *J.G. Schaffner 338* (holotype, P; isotypes, P, US-207605).

Cenchrus basedowii (Summerh. & C.E. Hubb.) Morrone, **comb. nov.** Basionym: *Pennisetum basedowii* Summerh. & C.E. Hubb., Bull. Misc. Inform. Kew 1926: 440. 1926. TYPE: Australia. King Sound, May River, *Basedow 13* (holotype, K).

Cenchrus caninus (Reinw. ex Blume) Morrone, **comb. nov.** Basionym: *Saccharum caninum* Reinw. ex Blume, Catal. Hort. Bogor.: 38. 1823. *Pennisetum caninum* (Reinw. ex Blume) Koord., Exkurs.-Fl. Java 1: 140. 1911. SYNTYPES. Indonesia. Java, *Reinwardt s.n.*, *Junghuhn s.n.* and *Zollinger s.n.* (types not located).

Gymnotrix macrostachys Brongn., Voy. Monde 2(2): 104, t. 11. 1830, non *Cenchrus macrostachys* Hochst. ex Steud., 1854. *Pennisetum macrostachys* (Brongn.) Trin., Mém. Acad. Imp. Sci. Saint-Petersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat. 3, 1(2–3): 177. 1834. TYPE: ‘Moluccas’ (type not located).

Cenchrus chilensis (E. Desv.) Morrone, **comb. nov.** Basionym: *Gymnotrix chilensis* E. Desv., Fl. Chile 6: 251, t. 74. 1853. *Pennisetum chilense* (E. Desv.) B.D. Jacks. ex R.E. Fr., Nova Acta Regiae Soc. Sci. Upsal. 1: 172. 1905. TYPE: Chile, *C. Gay s.n.* (holotype, P; isotypes, K, W).

Cenchrus clandestinus (Hochst. ex Chiov.) Morrone, **comb. nov.** Basionym: *Pennisetum clandestinum* Hochst. ex Chiov., Annuario Reale Ist. Bot. Roma 8: 41, pl. 5, fig. 2. 1903. TYPE: Ethiopia, *Schimper 2084* (holotype, FI; isotypes, G, K, TUB).

Cenchrus complanatus (Nees) Morrone, **comb. nov.** Basionym: *Gymnotrix complanata* Nees, Bonplandia (Hanover) 3: 83. 1855. *Pennisetum complanatum* (Nees) Hemsl., Biol. Cent.-Amer., Bot. 3(19): 507. 1885. TYPE: Panama, *Seemann 1560* (holotype, BM; isotype, US-0093598).

Cenchrus compressus (R. Br.) Morrone, **comb. nov.** Basionym: *Pennisetum compressum* R. Br., Prodr.: 195. 1810. TYPE: Australia. New South Wales, *R. Brown 6139* (holotype, K).

Panicum alopecuroides L., Sp. Pl. 1: 55. 1753, non *Cenchrus alopecuroides* Thunb., 1794. *Pennisetum alopecuroides* (L.) Spreng., Syst. Veg. 1: 303. 1825. LECTOTYPE: China, *without collector* (lectotype, LINN-80-1, designated by Veldkamp in Cafferty, Jarvis & Turland, Taxon 49(2): 253, 2000).

Alopecurus hordeiformis L., Sp. Pl. 1: 60. 1753. *Pennisetum hordeiforme* (L.) Spreng., Syst. Veg. 1: 302. 1825, non *Cenchrus hordeiformis* Thunb., 1794. LECTOTYPE: India, *Hudson 29* (lectotype, LINN-82-2, designated by Cope in Cafferty, Jarvis & Turland, Taxon 49(2): 245, 2000).

Cenchrus crinitus (Kunth) Morrone, **comb. nov.** Basionym: *Gymnotrix crinita* Kunth, Nov. Gen. Sp. (quarto ed.) 1: 112. 1815(1816). *Pennisetum crinitum* (Kunth) Spreng., Syst. Veg. 1: 302. 1825. TYPE: Mexico. Michoacán, *F.W.H.A. von Humboldt & A.J.A. Bonpland, s.n.* (holotype, P).

Cenchrus distachyus (E. Fourn.) Morrone, **comb. nov.** Basionym: *Gymnotrix distachya* E. Fourn., Mexic. Pl. 2: 48. 1886. *Pennisetum distachyum* (E. Fourn.) Rupr. ex Chase, Contr. U.S. Natl. Herb. 22(4): 229. 1921. LECTOTYPE: Mexico. Barranca de San Martin prope Zacuapan, *Galeotti 5680* (lectotype, BR, designated by Chase, Contr. U.S. Natl. Herb. 22(4): 230, 1921).

Cenchrus domingensis (Spreng. ex Schult.) Morrone, **comb. nov.** Basionym: *Gymnotrix domingensis* Spreng. ex Schult., Mant. 2: 284. 1824. *Pennisetum domingense* (Spreng. ex Schult.) Spreng., Syst. Veg. 1: 302. 1825. TYPE: Santo Domingo, *Bertero s.n.* (type not located).

Cenchrus dowsonii (Stapf & C.E. Hubb.) Morrone, **comb. nov.** Basionym: *Pennisetum dowsonii* Stapf & C.E. Hubb., Bull. Misc. Inform. Kew 1933: 279. 1933. TYPE: Kenya. Naivasha District, Aberdares Range, plain of Lake O'Boლოსat, 2100 m, W.J. Dowson 562 (holotype, K; isotype, EA).

Cenchrus durus (Beal) Morrone, **comb. nov.** Basionym: *Pennisetum durum* Beal, Grass. N. Amer. 2: 163. 1896. LECTOTYPE: Mexico. Chihuahua, Potrero Mts., 12 Oct 1886, C.G. Pringle 817 (lectotype MSC, designated by Chase, Contr. U.S. Natl. Herb. 22(4): 229, 1921; isolectotypes, CM, MO-2977366, MO-3727999, US-691229).

Cenchrus flaccidus (Griseb.) Morrone, **comb. nov.** Basionym: *Pennisetum flaccidum* Griseb., Gött. Nach. 1868: 86. 1868. TYPE: India. Kashmir, Ladak, 1900–1300, Nubra s.n. [Thomson] (holotype, GOET?).

Cenchrus flexilis (Mez) Morrone, **comb. nov.** Basionym: *Pennisetum flexile* Mez, Notizbl. Bot. Gard. Berlin-Dahlem 7: 51. 1917. TYPE: India. Kaschmir, Scinujpur, Clarke 29026 (holotype, B).

Cenchrus foermeranus (Leeke) Morrone, **comb. nov.** Basionym: *Pennisetum foermeranum* Leeke, Z. Naturwiss. 79: 26. 1907. SINTYPE: Namibia. Herero-land, Windhoek, 1897, I. Fischer 77; Windhoek, R. Foermer 46 (syntypes, B; isosyntypes, K).

Cenchrus glaucocladus (Stapf & C.E. Hubb.) Morrone, **comb. nov.** Basionym: *Pennisetum glaucocladum* Stapf & C.E. Hubb., Bull. Misc. Inform. Kew 1933: 276. 1933. TYPE: Zimbabwe. Hunyani River, 1410 m, F. Eyles 4903 (holotype, K).

Cenchrus hohenackeri (Hochst. ex Steud.) Morrone, **comb. nov.** Basionym: *Pennisetum hohenackeri* Hochst. ex Steud., Syn. Pl. glum. 1: 103. 1854. TYPE: India. Nilgiri Hills, R.F. Hohenacker 930 (holotype, K; isotypes, L, M, US-978422, US-1127293, US-3243707).

Cenchrus hordeoides (Lam.) Morrone, **comb. nov.** Basionym: *Panicum hordeoides* Lam., Tabl. Encycl. 1: 170. 1791. *Pennisetum hordeoides* (Lam.) Steud., Syn. Pl. Glumac. 1: 103. 1854. TYPE: Sierra Leone, Smeathman s.n. (holotype, P).

Cenchrus intectus (Chase) Morrone, **comb. nov.** Basionym: *Pennisetum intectum* Chase, Contr. U.S. Natl. Herb. 24(8): 485. 1927. TYPE: Ecuador. Loja: between Loja and San Lucas, ca. 2500 m, 6 Sep 1923, A.S. Hitchcock 21477 (holotype, US-1163845).

Cenchrus lanatus (Klotzsch) Morrone, **comb. nov.** Basionym: *Pennisetum lanatum* Klotzsch, Bot. Ergebn. Reise Waldemar: 65, fig. 99. 1862. TYPE: India, Hoffmeister s.n. (holotype, C?).

Cenchrus latifolius (Spreng.) Morrone, **comb. nov.** Basionym: *Pennisetum latifolium* Spreng., Syst. Veg. 1: 302. 1825. TYPE: Uruguay. Montevideo, F. Sellow s.n. (holotype, B?).

Cenchrus longissimus (S.L. Chen & Y.X. Jin) Morrone, **comb. nov.** Basionym: *Pennisetum longissimum* S.L. Chen & Y.X. Jin, Bull. Bot. Res., Harbin 4(1) 65, fig. 2. 1984. TYPE: China. Guizhou, Duyun Xian, 23 Aug 1930, Y. Tsiang 6040 (holotype, JSBI).

Cenchrus macrourus (Trin.) Morrone, **comb. nov.** Basionym: *Pennisetum macrourum* Trin., Gram. Panic.: 64.

1826. SINTYPE: South Africa, Link s.n.; Cape of Good Hope, Swartz s.n. (sintype of Swartz s.n., LE).

Cenchrus massaicus (Stapf) Morrone, **comb. nov.** Basionym: *Pennisetum massaicum* Stapf, Bull. Misc. Inform. Kew 1906: 82. 1906. LECTOTYPE: Kenya. Machakos District, Makindu, Linton 72 (lectotype, K, designated by Stapf & Hubbard, Bull. Misc. Inform. Kew 1933: 273, 1933).

Cenchrus mezianus (Leeke) Morrone, **comb. nov.** Basionym: *Pennisetum mezianum* Leeke, Z. Naturwiss. 79: 39. 1907. SYNTYPES: Tanzania, Arusha-Moshi, Uhlig 1076, Tanzania/Kenya, Burraberger, Uhlig 35; Kenya, Makinde River, Hässner 584 (isosyntype of Uhlig 1076, K; sintype of Uhlig 35, B, isosintype, EA; sintype of Hässner 584, B).

Cenchrus mildbraedii (Mez) Morrone, **comb. nov.** Basionym: *Pennisetum mildbraedii* Mez, Notizbl. Bot. Gart. Berlin-Dahlem 7: 52. 1917. TYPE: Rwanda. Sabinio to Mgahinga, Mildbraed 1763 (holotype, B).

Cenchrus monostigma (Pilg.) Morrone, **comb. nov.** Basionym: *Pennisetum monostigma* Pilg., Bot. Jahrb. Syst. 30(1): 120. 1901. SYNTYPES: Cameroon. Zwischen Manus-Quelle und Kamewrum-Pic, 2800 m, Feb 1891, Preuss 822; Cameroon. Manus-Quelle, 1891, Preuss 984 (syntypes, B).

Cenchrus occidentalis (Chase) Morrone, **comb. nov.** Basionym: *Pennisetum occidentale* Chase, Contr. U.S. Natl. Herb. 24(8): 483. 1927. TYPE: Ecuador. Guayas, west of Guayaquil, 20 Jun 1923, A.S. Hitchcock 19953 (holotype, US-1163831).

Cenchrus orientalis (Rich.) Morrone, **comb. nov.** Basionym: *Pennisetum orientale* Rich., in Persoon, Syn. Pl. 1: 72. 1805. TYPE: 'Cenchrus orientalis Willd. (ined.) Hab. in Oriente.' (type not located).

Cenchrus pauperus (Nees ex Steud.) Morrone, **comb. nov.** Basionym: *Pennisetum pauperum* Nees ex Steud., Syn. Pl. Glumac. 1: 102. 1854. TYPE: Ecuador. Galapagos Islands. Anon. s.n. (holotype, P; isotype, K).

Cenchrus pedicellatus (Trin.) Morrone, **comb. nov.** Basionym: *Pennisetum pedicellatum* Trin., Mem. Acad. Imp. Sci. Saint-Petersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat. 3,1(2–3): 184. 1834. TYPE: Cape Verde Islands, St. Iago, D. Peters s.n. (holotype, LE-TRIN-1101-01).

Cenchrus pedicellatus subsp. *unispiculus* (Brunken) Morrone, **comb. nov.** Basionym: *Pennisetum pedicellatum* Trin. subsp. *unispiculum* Brunken, Bot. J. Linn. Soc. 79(1): 62. 1979. TYPE: Ghana. Cape Verde Islands, R. Innes 30227 (holotype, PRE; isotype, K).

Cenchrus peruvianus (Trin.) Morrone, **comb. nov.** Basionym: *Pennisetum peruvianum* Trin., Linnaea 10(3): 295. 1836. TYPE: Peru: Andes Peruviae, 1834, E.F. Poeppig (holotype, LE; isotypes, BM, US).

Cenchrus petiolaris (Hochst.) Morrone, **comb. nov.** Basionym: *Gymnotrix petiolaris* Hochst., Flora 27: 250. 1844. *Pennisetum petiolare* (Hochst.) Chiov., Annuario Reale Ist. Bot. Roma 8(3): 324. 1908. TYPE: Ethiopia. Mt. Scholoda, Schimper 136 (isotype, K).

Cenchrus pilcomayensis (Mez) Morrone, **comb. nov.** Basionym: *Pennisetum pilcomayense* Mez, Bot. Jahrb. Syst. 56(Beibl. 125): 7. 1921. TYPE: Paraguay. In regione cursus

inferioris fluminis Pilcomayo, May 1906, *T. Rojas 61* (holotype, B; isotypes, P, US-978374).

Pennisetum frutescens Leeke, *Z. Naturwiss.* 79: 35. 1907, non *Cenchrus frutescens* L., 1753. TYPE: Argentina. Chaco, Fuerte Sarmiento, Dragones, *P.G. Lorentz & G.H.E.W. Hieronymus 584* (holotype, B; isotype, GOET).

Cenchrus polystachios (L.) Morrone, **comb. nov.** Basionym: *Panicum polystachion* L., *Syst. Nat.* (ed. 10) 2: 870. 1759. *Pennisetum polystachion* (L.) Schult., *Mant.* 2: 146. 1824. LECTOTYPE: India, without collector (lectotype, LINN-80-4, designated by van der Zon, Wageningen Agric. Univ. Pap. 92-1: 335, 1992).

Cenchrus polystachios subsp. *atrichus* (Stapf & C.E. Hubb.) Morrone, **comb. nov.** Basionym: *Pennisetum atrichum* Stapf & C.E. Hubb., *Bull. Misc. Inform. Kew* 1933: 282. 1933. *Pennisetum polystachion* subsp. *atrichum* (Stapf & C.E. Hubb.) Brunken, *Bot. J. Linn. Soc.* 79(1): 63. 1979. TYPE: Malawi. Zomba, 1170 m, *Manning 4* (holotype, K).

Cenchrus procerus (Stapf) Morrone, **comb. nov.** Basionym: *Beckeropsis procera* Stapf, *Bull. Misc. Inform. Kew* 1933: 272. 1933. *Pennisetum procerum* (Stapf). W.D. Clayton, *Kew Bull.* 32(3): 580. 1978. TYPE: Kenya. Nakuru, *Hitchcock 25117* (holotype, K; isotype, BR).

Cenchrus prolificus (Chase), Morrone, **comb. nov.** Basionym: *Pennisetum prolificum* Chase, *Contr. U.S. Natl. Herb.* 22(4): 231, fig. 75. 1921. TYPE: Mexico. Veracruz, Barranca of Metlac, ca. 900 m, 29 Jan 1895, *C. G. Pringle 6075* (holotype, US-250836; isotype, MO-2977369).

Cenchrus purpureus (Schumach.) Morrone, **comb. nov.** Basionym: *Pennisetum purpureum* Schumach., *Beskr. Guin. Pl.*: 44. 1827. TYPE: Ghana, *Thonning 355* (holotype, C; isotype, BM).

Cenchrus quianningensis (S.L. Zhong) Morrone, **comb. nov.** Basionym: *Pennisetum quianningense* S.L. Zhong, *J.S. SouthW. Agricv. Coll.* 1982(4): 75, pl. 1. 1982. TYPE: China. Sichuan, Qian'ning, 12 Aug 1974, *West Sichuan Veget. Exped. 05820* (holotype, SWAU).

Cenchrus ramosus (Hochst.) Morrone, **comb. nov.** Basionym: *Gymnotrix ramosa* Hochst., *Flora* 27(16): 252. 1844. *Pennisetum ramosum* (Hochst.) Schweinf., *Beitr. Fl. Aethiop.*: 301. 1867. TYPE: Sudan. Sennaar, *T. Kotschy 199* (isotypes, BM, G, K, L, MO).

Cenchrus rigidus (Griseb.) Morrone, **comb. nov.** Basionym: *Gymnotrix rigida* Griseb., *Abh. Königl. Ges. Wiss. Göttingen* 19: 263. 1874. *Pennisetum rigidum* (Griseb.) Hack., *Anales Mus. Nac. Buenos Aires* 11: 84. 1904. TYPE: Argentina. Córdoba: Ascochinga, Apr 1871, *P.G. Lorentz 47* (holotype, GOET; isotypes, CORD, W).

Cenchrus riparius (Hochst. ex A. Rich.) Morrone, **comb. nov.** Basionym: *Pennisetum riparium* Hochst. ex A. Rich., *Tent. Fl. Abyss.* 2: 381. 1851. TYPE: Ethiopia. Adua (Adoa), *G.H.W. Schimper 84* (holotype, P; isotypes, B, BR, G, K, M, US-1061597).

Cenchrus rupestris (Chase) Morrone, **comb. nov.** Basionym: *Pennisetum rupestre* Chase, *Contr. U.S. Natl. Herb.* 24(8): 484. 1927. TYPE: Peru: Matucana, alt. 2400 m, 12 Apr-3 May 1922, *J.F. MacBride & W. Featherstone 453* (holotype, US-1161395).

Cenchrus sagittatus (Henrard) Morrone, **comb. nov.** Basionym: *Pennisetum sagittatum* Henrard, *Blumea Suppl.* 1: 229, tab. 16, fig. 26. 1937. TYPE: Bolivia. Sur Yungas, La Florida, 1700 m, 4 Feb 1932, *L.R. Parodi 10069* (holotype, L; isotypes, BAA, K, US-1539315).

Cenchrus setaceus (Forssk.) Morrone, **comb. nov.** Basionym: *Phalaris setacea* Forssk., *Fl. Aegypt.-Arab.*: 17. 1775. *Pennisetum setaceum* (Forssk.) Chiov., *Boll. Soc. Bot. Ital.* 1923: 113. 1923. TYPE: Egypt, *P. Forsskål s.n.* (isotype, BM).

Cenchrus shaanxiensis (S.L. Chen & Y.X. Jin) Morrone, **comb. nov.** Basionym: *Pennisetum shaanxiense* S.L. Chen & Y.X. Jin, *Bull. Bot. Res., Harbin* 4(1): 68, fig. 3. 1984. TYPE: China. Shaanxi, Luoyang Xian, 870 m, 2 Nov 1958, *C.L. Tang 957* (holotype, JSBI).

Cenchrus sichuanensis (S.L. Chen & Y.X. Jin) Morrone, **comb. nov.** Basionym: *Pennisetum sichuanense* S.L. Chen & Y.X. Jin, *Bull. Nanjing Bot. Gard.* 1988-1989: 5. 1990. TYPE: China. Sichuan, Derong Xian, 2000-3000 m, *c.i.* 3366 (type not located).

Cenchrus sphacelatus (Nees) Morrone, **comb. nov.** Basionym: *Gymnotrix sphacelata* Nees, *Fl. Afr. Austral. Ill.*: 68. 1841. *Pennisetum sphacelatum* (Nees.) T. Durand & Schinz, *Consp. Fl. Afr.* 5: 784. 1894. SYNTYPES: South Africa. Stormberg, *J.F. Drège s.n.*; Gekau to Mbashe (Basche), *J.F. Drège s.n.*; Gekau, *J.F. Drège s.n.* (isosyntypes, K).

Cenchrus squamulatus (Fresen.) Morrone, **comb. nov.** Basionym: *Pennisetum squamulatum* Fresen., *Mus. Senckenberg.* 2: 137. 1837. TYPE: Ethiopia. Semien (Simen), *Rüppell s.n.* (holotype, FR).

Cenchrus stramineus (Peter) Morrone, **comb. nov.** Basionym: *Pennisetum stramineum* Peter, *Repert. Spec. Nov. Regni Veg. Beih.* 40(1): 71, fig. 37. 1930. TYPE: Tanzania. Masai District, Ngorongoro, *Peter 43215* (holotype, B).

Cenchrus subangustus (Schumach.) Morrone, **comb. nov.** Basionym: *Panicum subangustum* Schumach., *Beskr. Guin. Pl.*: 59. 1827. *Pennisetum subangustum* (Schumach.) Stapf & C.E. Hubb., *Bull. Misc. Inform. Kew* 1933: 271. 1933. TYPE: Ghana. *Thonning s.n.* (holotype, C).

Cenchrus tempisqueusis (R.W. Pohl) Morrone, **comb. nov.** Basionym: *Pennisetum tempisqueense* R.W. Pohl, *Fieldiana, Bot.* 38(2): 6, fig. 2. 1976. TYPE: Costa Rica. Guanacaste, 8 km N of Hacienda Palo Verde, 14 km WSW of Bagaces, 10 m, 20 Feb 1969, *R.W. Pohl & G. Davidse 11725* (holotype, ISC; isotypes, CR-47189, F, K, UC, US-3055850).

Cenchrus thulinii (S.M. Phillips) Morrone, **comb. nov.** Basionym: *Pennisetum thulinii* S.M. Phillips, *Kew Bull.* 46(3): 535. 1991. TYPE: Ethiopia. Arussi Prov., Chilalo awraja, Katar river, ca. 20 km SW of Asella, 2200 m, *M. Thulin 1541* (holotype, K; isotypes, EA, UPS).

Cenchrus thunbergii (Kunth) Morrone, **comb. nov.** Basionym: *Pennisetum thunbergii* Kunth, *Révis. Gramin.* 1: 50. 1829. TYPE: South Africa, *Thunberg s.n.* (holotype, UPS).

Cenchrus trachyphyllus (Pilg.) Morrone, **comb. nov.** Basionym: *Pennisetum trachyphyllum* Pilg., *Bot. Jahrb. Syst.* 30(1): 122. 1901. SINTYPES: Tanzania. Usambara, Lutindi, Jul 1893, *C. Holst 3253*; Bulua, Sep 12893, *C. Holst 5003*; Usambara, Kwai, Oct 1899, 4600 m, *Albers 170*; Wegen, Sep 1899, *Albers 363*; Usagara, W.-Uluguru, 4700 m, *Stuhlmann 9087* (isosintype of *C. Holst 3253*, K, M; sintype of *Albers 170*, B).

Cenchrus trisetus (Leeke) Morrone, **comb. nov.** Basionym: *Pennisetum trisetum* Leeke, Z. Naturwiss. 79: 30. 1907. TYPE: Ethiopia, Begemeder, Efak, *Schimper 1411* (holotype, B; isotype, K).

Cenchrus unisetus (Nees) Morrone, **comb. nov.** Basionym: *Gymnotrix uniseta* Nees, Fl. Afr. Austral. III.: 66. 1841. *Pennisetum unisetum* (Nees) Benth., J. Linn. Soc., Bot. 19: 47. 1881. TYPE: South Africa, Durban (Port Natal), *J.F. Drège s.n.* (isotypes, K, L, S).

Cenchrus violaceus (Lam.) Morrone, **comb. nov.** Basionym: *Panicum violaceum* Lam., Tabl. Encycl. 1: 169. 1791. *Pennisetum violaceum* (Lam.) Rich. ex Pers., Syn. Pl. 1: 72. 1805. TYPE: Senegal, *D. Rousillon s.n.* (holotype, P).

Cenchrus weberbaueri (Mez) Morrone, **comb. nov.** Basionym: *Pennisetum weberbaueri* Mez, Notizbl. Bot. Gart. Berlin-Dahlem 7: 50. 1917. TYPE: Peru: Dept. Junin, Tarma, 3000–3300 m, 10 Feb 1903, *A. Weberbauer 2393* (holotype, B).