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Five vicarious genera from Gondwana: the Velloziaceae as shown by molecules and morphology

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• *Background and Aims* The amount of data collected previously for Velloziaceae neither clarified relationships within the family nor helped determine an appropriate classification, which has led to huge discordance among treatment by different authors. To achieve an acceptable phylogenetic result and understand the evolution and roles of characters in supporting groups, a total evidence analysis was developed which included approx. 20 % of the species and all recognized genera and sections of Velloziaceae, plus outgroups representatives of related families within Pandanales.
• *Methods* Analyses were undertaken with 48 species of Velloziaceae, representing all ten genera, with DNA sequences from the *atpB-rbcL* spacer, *trnL-trnF* spacer, *trnL* intron, *trnH-psbA* spacer, ITS ribosomal DNA spacers and morphology.
• *Key Results* Four groups consistently emerge from the analyses. Persistent leaves, two phloem strands, stem cortex divided in three regions and violet tepals support *Acanthochlamys* as sister to Velloziaceae *s.s.*, which are supported mainly by leaves with marginal bundles, transfusion tracheids and inflorescence without axis. Within Velloziaceae *s.s.*, an African *Xerophyta* + *Talbotia* clade is uniquely supported by basal loculicidal capsules; an American clade, *Barbacenia s.l.* + *Barbaceniopsis* + *Nanuza* + *Vellozia*, is supported by only homoplastic characters. *Barbacenia s.l.* (= *Aylthonia* + *Barbacenia* + *Burlemarxia* + *Pleurostima*) is supported by a double sheath in leaf vascular bundles and a corona; *Barbaceniopsis* + *Nanuza* + *Vellozia* is not supported by an unambiguous character, but *Barbaceniopsis* is supported by five characters, including diclinous flowers, *Nanuza* + *Vellozia* is supported mainly by horizontal stigma lobes and stem inner cortex cells with secondary walls, and *Vellozia* alone is supported mainly by pollen in tetrads.
• *Conclusions* The results imply recognition of five genera (*Acanthochlamys* (*Xerophyta* (*Barbacenia* (*Barbaceniopsis*, *Vellozia*)))), solving the long-standing controversies among recent classifications of the family. They also suggest a Gondwanan origin for Velloziaceae, with a vicariant pattern of distribution.

Key words: *Acanthochlamys*, *Barbacenia*, *Barbaceniopsis*, Gondwanian origin, morphological and molecular characters, phylogenetic analysis, *Vellozia*, Velloziaceae, vicarious distribution, *Xerophyta*.

INTRODUCTION

Velloziaceae have approx. 250 species (Cronquist, 1981; Dahlgren *et al.*, 1985; Kubitzki, 1998; Mello-Silva, 1991*a*, 2004; Smith and Ayensu, 1974, 1976) of which one occurs in China, another in Yemen and Saudi Arabia, approx. 30 in Africa and Madagascar, and the rest in South America with the exception of one species that reaches Panama in Central America (Mello-Silva, 1995, 2004). Velloziaceae are one of the best examples of a family of consistently heliophile species (Smith, 1962), and the great majority of species are concentrated in the phytochoria *campo rupestre* archipelago (Prance, 1994) of central Brazil.

Since the first Velloziaceae monograph in the 20th century (Smith, 1962), new data for systematics of the family have been produced, including anatomy (Ayensu, 1968, 1969, 1974; Menezes, 1970, 1971*a*, 1973, 1975, 1980, 1984, 1988; Coetzee, 1974; Menezes and Semir, 1990; Mello-Silva, 1990; Sajo *et al.*, 2010), chromosomes (Goldblatt and Poston, 1988; Melo *et al.*, 1997), pollen (Ayensu, 1972; Ayensu and Skvarla, 1974) and phytochemistry (Salatino *et al.*, 1989, 1991; Williams *et al.*, 1991, 1992, 1993, 1994). Nevertheless, species and, more often, generic delimitation within Velloziaceae have often been a source of discordance among authors. One of the reasons for this situation, as in many others in a gradistic context, was the differential emphasis on the various characters used for delimiting groups (Smith and Ayensu, 1974; Menezes, 1980; Mello-Silva, 1991*a*). Morphological (Menezes *et al.*, 1994; Mello-Silva, 2000, 2005) and molecular (Salatino, 1999; Behnke *et al.*, 2000; Salatino *et al.*, 2001) cladistic analyses have cast light on intrafamilial relationships, although the results have been conflicting to some degree, mostly because different sets of taxa and types of characters have been included (Mello-Silva, 2005).

This work is a combined analysis of 67 morphological and five datasets of molecular characters from almost the same

species used by Mello-Silva (2005) in his solely morphological analysis. This represents all genera and sections within the genera of Velloziaceae so far established, together with a number of morphological characters long utilized in the systematics of the family. Groups and characters are discussed, as well as implications for the evolution, classification and biogeography of Velloziaceae.

MATERIALS AND METHODS

Total DNA from samples of Velloziaceae was extracted according to the CTAB method of Doyle and Doyle (1987) from fragments of leaves previously dried on silica gel (Chase and Hills, 1991). Primers used for amplification and thermocycler conditions are presented in Table 1.

The products were purified with GFX PCR purification kit (Amersham Biosciences). The same primers were used for sequencing reactions of the corresponding regions. Sequence analyses were run in automatic sequencers models 3100 and 3700 (Applied Biosystems), using Big Dye 3·0–3·1 and the manufacturer's protocol. Sequences were aligned manually following the guidelines of Kelchner (2000) and the criterion of similarity (Simmons, 2004). Gaps were not treated as characters. Insertions, mainly in ITS, are generally direct repeats of a neighbouring sequence ('type 1a' gap; Golenberg *et al.*, 1993; Kelchner, 2000). Other types of insertions are found, mostly as a result of differences from the outgroup but they are too random for appropriate treatment. A total analysis with the majority of indels removed was performed for comparisons.

Morphological characters are mainly from Mello-Silva (2005), but ptyxis, vessels in leaves, septal nectaries, stigmatic surfaces, nucellus and cyanogenic compounds are not utilized here because they were generalizations and not actual observations in the terminals. Accordingly, chromosome number, epicuticular waxes and flavonoid compounds also could not be analysed for a satisfactory number of terminals. Stomata and outer integument of empty cells in seeds are also not considered here due to new evidence for homology (respectively, Amaral and Mello-Silva, 2009; Sousa 2005). On the other hand, there are nine new characters added from root and stem anatomy, taken from Cattai (2007).

Morphological characters are listed in Table 2 and the matrix in Table 3. Molecular voucher materials are listed in Table 4. Additional morphological vouchers can be found in Mello-Silva (2000, 2005) and in Cattai (2007). Herbaria acronyms follow Thiers (2010). Taxonomic decisions about lumping versus splitting were based on the priorities as discussed in Backlund and Bremer (1998).

Six datasets, plastid *atpB-rbcL* spacer, *trnH-psbA* spacer, *trnL-trnF* spacer, *trnL* intron, ITS nrDNA and morphology, from 48 terminals of Velloziaceae, including *Acanthochlamys bracteata* (APG, 2003, 2009) were analysed. Six sets of analyses were performed. Data from plastid DNA, nuclear ribosomal spacers (ITS nrDNA) and morphology were analysed separately and, then, together in a combined molecular and total evidence analysis. External outgroups are *Encholirium scrutor*, Bromeliaceae (Dahlgren and Rasmussen, 1983; Dahlgren *et al.*, 1985; Gilmartin and Brown, 1987), *Cyclanthus bipartitus* and *Thoracocarpus*

TABLE 1. *Primers used for amplification and thermocycler conditions*

DNA markers	Primers	Reference	Thermocycler conditions
trnL intron, trnL-F spacer	tab c (CGA AAT CGG TAG ACG CTA CG) tab f (ATT TGA ACT GGT GAC ACG AG)	Taberlet *et al.* (1991)	Denaturation at 94 °C 2 min, 33 cycles with denaturation at 94 °C 1 min, annealing 54–62 °C 45 s, extension 72 °C 1 min 20 s and last extension 5 min
atpB-rbcL spacer	atpB-1 (ACA TCK ART ACK GGA CCA ATA A) rbcL-1 (AAC ACC AGC TTT RAA TCC AA)	Chiang *et al.* (1998)	Denaturation at 94 °C 2 min, 28 cycles with denaturation at 94 °C 1 min, annealing at 52 °C 1 min, extension at 72 °C 1 min and last extension 7 min
trnH-psbA spacer	trnHGUG (CGC GCA TGG TGG ATT CAC AAT CC) psbA (GTT ATG CAT GAA CGT AAT GCT C)	Shaw *et al.* (2005)	Denaturation at 80 °C, 33 cycles with denaturation at 94 °C 30 s, annealing at 50–56 °C 30 s, extension at 72 °C 1 min and last extension 10 min
ITS rnDNA	26SE (ACG AAT TCA TGG TCC GGT GAA GTG TTC G) 17SE (TAG AAT TCC CCG GTT CGC TCG CCG TTA C) (internal primers) ITS 2F (GCT GCG TTC TTC ATC GAT GC) ITS 3R (GCA TCG ATG AAG AAC GCA GC)	Sun *et al.* (1994) Kumar and Shukla (2005)	Denaturation at 94 °C 2 min, 28 cycles with denaturation at 94 °C 1 min, annealing at 52 °C 1 min, extension at 72 °C 1 min and last extension 7 min

TABLE 2. *Character analysis and coding*

(1) Phyllotaxis: tristichous (0); spirotristichous (1); spirally (2)
(2) Deciduousness of leaves: deciduous (0); persistent, at least the sheath (1)
(3) Abscission line between sheath and lamina: absent (0); present (1)
(4) Distal portion of leaf blade: attenuate (0); truncate (1)
(5) Transverse posture of leaf blade: arcuate (0); plane (1)
(6) Longitudinal posture of dry leaf blade: involute (0); flat (1); revolute (2)
(7) Furrows in leaf blade: absent (0); on abaxial surface only (1); on both surfaces (2)
(8) Papillae in furrows: absent or inconspicuous (0); coronulate (1); finger-like (2)
(9) Leaf trichomes or emergences: absent (0); multicellular base, on lamina (1); multicellular base, on margins and midrib (2); uni- or multicellular with unicellular base (3)
(10) Stomatal distribution in leaves: hypostomatic (0); hypoamphistomatic (1); amphistomatic (2)
(11) Subsidiary cells: smooth (0); ridged (1)
(12) Specialized cells: absent (0); present on adaxial surface only (1); present on both surfaces (2)
(13) Adaxial strands: absent (0); present (1)
(14) Abaxial strands: absent (0); present (1)
(15) Marginal bundle: rounded (0); triangular (1); absent (2)
(16) Conduction tissues in marginal bundle: present (0); absent (1)
(17) Aquiferous hypodermis: extending to bundle sheaths only (0); extending to bundle sheaths and furrows (1); absent (2)
(18) Aquiferous parenchyma between bundles: absent (0); present (1)
(19) Sclerenchyma pattern: *Xerophyta* type (0); *Vellozia* type (1); *Barbacenia* type (2); other types (3)
(20) Phloem strands: two, separated (0); two, united at bottom (1); one (2)
(21) Minor fibro-vascular bundles: absent (0); present (1)
(22) Sheath of leaf vascular bundles (M & *al.*): simple (0); double (1)
(23) Transfusion tracheids (M & *al.*): absent (0); present (1)
(24) Inflorescence (M & *al.*): with major axis (0); suppression of major axis (1)
(25) Flower number: solitary or grouped (0); always solitary (1)
(26) Pedicel position: evident (0); hidden by leaves (1)
(27) Transverse section of pedicel: triangular (0); circular (1)
(28) Vascular bundles in pedicel: six (0); nine (1); 12 (2); 15 (3); 18 (4); 24 (5); 36 (6)
(29) Belt of sclerified cells in pedicel: absent (0); present (1)
(30) Emergence type: capitate or capitate-truncated (0); subulate (1)
(31) Pedicellar emergences: absent (0); laxly disposed (1); densely disposed (2)
(32) Hypanthial emergences: absent (0); laxly disposed (1); densely disposed (2)
(33) Ovary outline: longer than broad (0); ± as long as broad (1); broader than long (2)
(34) Transverse section of ovary: trigonous (0); circular-trilobate (1)
(35) Placentation (S & L): axile (0); parietal (1)
(36) Hypanthial tube: absent or much shorter than ovary (0); shorter than ovary (1); equal to ovary (2); longer than ovary (3)
(37) Perianth (S & L): differentiated (0); undifferentiated (1); absent (2)
(38) Tepal colour: violet (0); white (1); yellow (2); red (3); green (4); orange (5); pink (6)
(39) Corona: absent (0); present (1)
(40) Floral expression: monoclinous (0); diclinous (1)
(41) Stamen number: six (0); nine (1); 12 (2); 15 (3); 18 (4); 24 (5); 30 (6); 36 (7); more (8)
(42) Staminal appendages: absent (0); present (1)
(43) Filaments: cylindrical (0); flat (1); coronoid (2)
(44) Anther attachment: on filament (0); on corona (1); on hypanthium (2)
(45) Anther insertion: basifixed (0); dorsifixed (1)
(46) Apical appendage in anther: absent (0); present (1)
(47) Auricles in anther: absent (0); present (1)
(48) Anther position in antisepalous stamens: extrorse (0); latrorse (1); latero-introrse (2); introrse (3)
(49) Anther position in antipetalous stamens: extrorse (0); latrorse (1); latero-introrse (2); introrse (3)
(50) Anther dehiscence: each pair of microsporangia dehiscing by a single common slit (0); each pair of microsporangia dehiscing by a separated slit (1); bisporangiate (2)
(51) Anther colour: yellow (0); violet (1); white (2); cream (3)
(52) Pollen colour: yellow (0); white (1)
(53) Pollen units: monads (0); tetrads (1)
(54) Relative position of stigma and stamens: stigma above stamens (0); stigma at same level or below stamens (1)
(55) Style × stigma: much longer (0); more or less of the same length (1); much shorter (2)
(56) Stigma lobes: horizontal, fused at centre (0); vertical, fused at apex (1); free (2)
(57) Fruit: loculicidal capsule (0); poricidal capsule (1); intercostal capsule (2); basal loculicidal capsule (3); septicidal capsule (4); other types (5)
(58) Hypanthium in fruit: persistent (0); deciduous (1)
(59) Belt of continuous fibres in root cortex: absent (0); present (1)
(60) Transverse section of adult stem: circular (0); triangular (1)
(61) Bundles of fibres in stem cortex: absent (0); round-shaped (1); continuous belt (2); U-shaped (3)
(62) Compound vascular bundles in stem: absent (0); present (1)
(63) Fibres uniting the stem vascular bundles in maturity: absent (0); present (1)
(64) Central fibrous bundle in stem: absent (0); present (1)
(65) Fibres in stem vascular bundles: absent (0); next to the phloem (1); next to the xylem (2)
(66) Stem cortex: undivided, parenquimatous (0); divided in three regions (1)
(67) Secondary wall in stem inner cortex cells: absent (0); present (1)

M & *al.* = Menezes *et al.* (1994); S & L = Stevenson and Loconte (1995).

bissectus, Cyclanthaceae, and *Pandanus pygmaeus*, Pandanaceae (Chase *et al.*, 1993, 1995, 2006; Clark *et al.*, 1993; Duvall *et al.*, 1993; APG, 2003, 2009). There are in total 52 terminals. Criteria for choosing the Velloziaceae terminals are provided in Mello-Silva (2000, 2005). Trees were arranged with *Encholirium scrutor* as the ultimate outgroup

TABLE 3. *Morphological matrix*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
Acanthochlamys bracteata	1	1	0	0	0	0	0	0	0	0	0	0	1	1	2	-	0	1	3	0	0	0	0	0	0	0	-	?	?	?	-	0	0	0	1	A	3	1	0	0	0	0	0	0	0	1	0	0	2	2	2	?	?	0	-	0	2	0	0	1	0	2	0	1	0	2	1	0
Aylthonia blackii	1	1	0	0	1	0	0	0	1	2	0	0	0	0	0	0	2	0	2	0	0	1	1	1	0	0	1	2	1	0	1	D	0	1	0	3	1	5	1	0	0	0	-	1	1	0	1	3	3	0	2	0	0	0	0	1	0	0	1	0	2	0	1	0	2	1	0
Aylthonia umbrosa	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	2	1	2	0	0	1	1	1	0	0	1	2	0	0	1	D	0	1	0	3	1	3	1	0	0	0	-	1	1	0	1	3	3	0	1	0	0	0	0	1	0	0	1	0	1	0	0	0	2	1	0
Barbacenia flava	1	1	0	0	1	1	0	0	2	2	0	0	0	0	0	0	2	0	2	0	0	1	1	1	0	0	0	2	1	0	2	2	0	0	0	1	1	2	1	0	0	0	-	1	1	0	0	3	3	0	0	0	0	1	0	1	0	0	1	0	2	0	0	0	2	1	0
Barbacenia ignea	1	1	0	0	1	0	0	0	1	2	0	0	0	0	0	0	2	0	2	0	0	1	1	1	0	0	1	2	1	0	2	2	0	0	0	1	1	F	1	0	0	0	-	1	1	0	0	3	3	0	0	0	0	1	0	1	0	0	1	0	2	0	0	0	2	1	0
Barbacenia markgrafii	1	1	0	0	1	1	A	0	1	2	0	0	0	0	0	0	2	1	2	0	0	1	1	1	0	0	1	2	1	0	1	1	0	1	0	3	1	E	1	0	0	0	-	1	1	0	1	3	3	0	0	0	0	1	0	1	0	0	1	0	2	0	0	0	2	1	0
Barbacenia reflexa	1	1	0	0	1	1	0	0	2	2	0	0	1	0	0	1	2	1	2	0	0	1	1	1	1	0	1	2	1	1	2	2	0	0	0	3	1	4	1	0	0	0	-	1	1	0	1	3	3	0	0	0	0	0	0	1	1	0	1	0	2	0	0	0	2	1	0
Barbaceniopsis boliviensis	0	1	0	0	1	0	2	0	2	2	0	0	0	0	0	0	2	0	2	0	0	0	1	1	1	0	1	0	1	1	1	1	0	1	0	0	1	2	0	1	0	0	?	0	?	0	?	1	1	0	0	0	0	1	2	1	1	0	1	0	3	0	0	1	2	1	0
Barbaceniopsis castillonii	0	1	0	0	0	0	2	0	2	2	0	0	0	0	0	0	2	0	2	0	0	0	1	1	0	0	0	0	1	1	2	2	0	0	0	0	1	0	0	1	0	0	1	0	1	1	1	1	1	0	0	0	0	1	2	1	1	0	1	0	3	0	0	1	2	1	0
Barbaceniopsis humahuaquensis	0	1	0	0	0	0	2	0	2	2	0	0	0	0	0	1	2	0	2	0	0	0	1	1	0	0	1	0	1	1	1	1	0	1	0	0	1	2	0	1	0	0	1	0	0	1	1	1	1	0	0	0	0	1	2	1	1	0	1	0	3	0	0	1	2	1	0
Burlemarxia pungens	1	1	0	0	1	1	2	0	1	2	0	0	0	0	0	0	2	0	2	0	0	1	1	1	0	0	0	2	?	1	1	1	0	1	0	2	1	6	1	0	0	0	0	0	0	0	0	2	2	0	3	0	0	0	0	1	1	0	1	0	2	0	0	0	2	1	0
Burlemarxia spiralis	1	1	0	0	1	1	0	0	2	2	0	0	0	0	0	0	2	0	2	0	0	1	1	1	0	0	1	2	1	-	0	0	0	1	0	1	1	6	1	0	0	0	0	0	0	1	0	2	2	0	1	0	0	0	0	1	1	0	1	0	2	0	1	0	2	1	0
Cyclanthus bipartitus	?	0	?	1	?	?	0	0	0	1	0	0	1	0	2	-	2	1	3	2	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	1	1	0	0	1	0	0
Encholirium scrutor	2	0	0	0	0	0	0	0	3	0	0	0	1	0	2	-	2	-	0	2	0	0	0	0	0	0	-	1	1	1	-	0	0	0	1	0	0	0	4	0	0	0	0	1	0	1	0	0	?	?	?	4	0	0	1	1	2	4	-	1	0	0	0	0	0	0	0	0
Nanuza plicata	0	1	1	A	1	0	0	0	2	0	0	0	0	1	1	0	0	0	0	1	0	1	0	1	1	0	0	0	N	0	-	0	0	0	0	0	0	1	A	0	0	0	0	1	0	0	0	1	2	2	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	2	1	1
Pandanus pygmaeus	1	0	0	0	0	B	0	0	2	1	0	0	0	0	0	2	-	0	1	0	2	0	0	0	0	0	-	?	?	?	?	-	-	-	1	1	1	0	2	-	0	1	8	0	?	0	?	1	0	?	?	?	3	?	0	-	?	2	5	-	0	0	1	1	0	0	1	0	0
Pleurostima longiscapa	0	1	0	0	0	0	0	0	2	2	0	2	0	0	0	1	2	0	2	0	0	1	1	1	0	0	0	2	1	0	1	1	0	1	0	1	1	6	1	0	0	0	-	1	0	0	1	3	3	0	1	0	0	0	0	2	2	0	1	1	2	0	1	0	2	1	0
Pleurostima plantaginea	0	1	0	0	0	0	0	0	2	2	0	0	0	0	0	0	2	1	2	0	0	1	1	1	0	0	0	2	1	-	0	0	0	1	0	3	1	3	1	0	0	0	-	1	0	0	1	3	3	0	0	0	0	0	0	2	2	0	1	1	2	0	1	0	2	1	0
Pleurostima purpurea	0	1	0	0	0	2	0	0	2	2	0	0	0	0	0	1	2	0	2	0	0	1	1	1	0	0	0	2	1	0	1	1	0	1	0	1	1	0	1	0	0	0	-	2	0	1	1	3	3	0	1	1	0	1	0	2	2	0	1	0	2	0	1	0	2	1	0
Pleurostima riparia	0	1	0	0	0	2	0	0	2	1	0	0	1	0	0	1	2	1	2	0	0	1	1	1	0	0	1	2	0	-	0	0	0	0	0	0	1	1	1	1	0	0	0	-	2	0	1	1	3	3	0	1	0	0	1	0	2	2	0	1	0	2	0	1	0	2	1	0
Talbotia elegans	0	1	0	0	0	0	0	0	B	1	0	0	0	0	0	0	1	2	0	2	0	0	0	1	1	0	0	0	1	1	-	0	0	0	0	0	0	1	A	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	3	0	1	0	2	0	0	0	2	1	0
Thoracocarpus bissectus	2	0	0	1	1	1	0	0	0	1	0	0	1	1	2	-	2	0	3	2	0	0	0	0	0	0	-	?	?	?	-	-	-	1	?	1	0	1	4	0	1	K	0	?	0	0	0	?	?	?	?	?	?	0	-	-	0	5	-	0	0	1	1	0	0	1	0	0
Vellozia abietina	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	-	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	0	2	0	0	1	2	1	1
Vellozia alata	1	1	0	0	1	0	1	0	2	0	0	0	1	0	0	0	1	0	1	0	0	0	1	1	1	0	0	5	1	-	0	0	0	0	0	1	1	A	0	0	L	1	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	0	1	0	0	1	2	1	1
Vellozia aloifolia	0	1	1	A	1	2	1	2	1	1	0	0	1	1	0	0	1	0	1	0	0	0	1	1	0	0	0	2	1	0	2	2	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	0	0	1	1	1	0	0	1	2	1	1
Vellozia burlemarxii	0	1	0	0	0	0	1	1	2	0	0	D	1	1	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	0	1	2	2	1	0	0	1	0	0	0	4	0	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	1	1	0	0	1	2	1	1
Vellozia campanuloides	0	1	1	0	0	0	1	1	2	0	0	2	1	0	0	0	1	0	1	0	1	0	1	1	1	0	0	0	1	0	1	2	2	1	0	0	1	0	0	0	4	0	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	0	1	0	0	1	2	1	1
Vellozia candida	0	1	1	0	0	2	1	0	2	1	1	0	1	1	0	0	0	0	1	0	0	0	1	1	1	0	0	3	0	0	B	2	0	0	0	0	1	1	0	0	4	0	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	0	0	1	0	1	0	0	1	2	1	1
Vellozia canelinha	0	1	1	0	0	2	1	1	2	0	1	1	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	D	1	0	1	2	2	1	0	0	1	0	0	0	8	1	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	1	1	0	0	1	2	1	1
Vellozia caput-ardeae	1	1	0	0	0	2	1	1	2	1	0	1	1	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	B	1	0	1	1	0	0	0	3	1	A	0	0	4	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	1	0	1	1	1	0	0	1	2	1	1
Vellozia caudata	0	1	1	0	0	2	1	1	2	0	0	D	1	0	0	0	1	0	1	0	1	0	1	1	1	0	0	0	1	0	1	2	2	1	0	0	1	0	0	0	4	0	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	0	1	0	0	1	2	1	1
Vellozia aff. *caudata*	0	1	1	0	0	2	1	1	2	0	0	D	1	1	0	0	1	0	1	0	1	0	1	1	1	0	0	D	1	0	1	2	2	1	0	0	1	0	0	0	4	0	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	0	1	0	0	1	2	1	1
Vellozia compacta	1	1	1	1	1	0	1	1	2	1	0	0	1	1	0	0	1	0	0	0	0	0	0	1	1	1	0	0	C	1	0	1	1	0	0	0	0	1	A	0	0	4	1	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	1	1	0	0	1	2	1	1
Vellozia dasypus	0	1	1	1	1	2	1	0	2	1	1	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0	0	4	0	1	2	2	0	0	0	0	1	0	0	0	4	0	0	0	0	0	0	0	2	2	1	0	0	1	0	0	0	0	0	1	0	1	0	0	1	2	1	1
Vellozia epidendroides	1	1	1	1	1	0	1	1	2	1	0	0	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	1	1	1	1	0	1	1	A	0	0	J	1	0	0	0	0	0	2	2	1	0	0	1	0	0	0	1	0	1	0	1	0	0	1	2	1	1
Vellozia glauca	0	1	1	0	0	B	1	0	2	2	1	1	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	5	0	1	B	2	0	0	0	0	1	0	0	0	G	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	1	0	1	0	0	1	2	1	1
Vellozia hatschbachii	1	1	0	0	1	0	1	1	2	1	0	1	1	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0	0	0	1	1	1	0	0	7	0	0	0	0	0	0	?	?	1	0	0	1	0	0	0	1	0	1	1	1	0	0	1	2	1	1

Vellozia hemisphaerica 1	0 1 0 0 0 2 1 1 2 0 0 D 0 0 0 0 1 0 A 0 1 0 1 1 0 0 0 M 1 0 1 2 2 1 0 0 1 A 0 0 4 0 0 0 0 0 0 2 2 1 0 0 1 0 0 0 1 0 1 1 1 0 0 1 2 1 1
Vellozia hemisphaerica 2	0 1 0 0 0 2 1 1 2 0 0 D 1 0 0 0 1 0 0 0 1 0 1 1 0 0 0 M 1 0 1 2 2 1 0 0 1 A 0 0 4 0 0 0 0 0 0 2 2 1 0 0 1 0 0 0 1 0 1 1 1 0 0 1 2 1 1
Vellozia hirsuta	0 1 A A A B 1 0 1 H 1 A A A 0 0 0 0 1 1 0 0 1 1 1 A 0 O 0 0 B 2 0 0 0 3 1 A 0 0 P 0 0 0 0 0 0 1 1 1 0 0 1 0 0 0 0 1 1 0 1 0 0 1 2 1 1
Vellozia jolyi	1 1 1 1 1 2 0 0 B 0 0 D 1 1 0 0 0 0 0 0 0 0 1 1 1 0 0 0 1 0 1 2 2 1 0 0 1 0 0 0 J 0 0 0 0 0 0 2 2 1 0 0 1 0 0 0 1 0 1 0 1 0 0 1 2 1 1
Vellozia minima	1 1 1 1 1 0 0 0 2 0 0 0 0 0 0 0 0 0 A 0 0 0 1 1 1 0 0 0 0 0 1 1 1 1 0 1 1 A 0 0 0 0 0 0 0 0 0 2 2 1 0 0 1 0 0 0 1 0 1 0 2 0 0 1 2 1 1
Vellozia prolifera	1 1 1 1 1 0 0 0 2 0 0 0 1 0 0 1 0 0 1 0 0 0 1 1 1 1 1 0 0 - 0 0 0 0 0 0 1 0 0 0 2 1 0 0 0 0 0 2 2 1 0 0 1 0 0 0 1 0 1 0 1 0 0 1 2 1 1
Vellozia punctulata	1 1 0 A 1 2 1 1 2 1 0 0 1 1 0 0 1 0 1 0 0 0 1 1 1 0 0 I 1 0 0 A 0 0 0 1 1 A 0 0 4 0 0 0 0 0 0 2 2 1 0 0 1 0 0 0 1 0 1 0 1 0 0 1 2 1 1
Vellozia religiosa	1 1 1 1 1 0 1 0 B 0 0 2 1 0 0 0 1 0 1 0 1 0 1 1 1 0 0 0 1 0 1 2 2 1 0 0 1 0 0 0 2 0 0 0 0 0 0 2 2 1 0 0 1 0 0 0 1 0 1 0 1 0 0 1 2 1 1
Vellozia sessilis	0 1 1 1 0 0 1 0 1 2 0 1 1 0 0 0 1 0 1 0 0 0 1 1 1 1 0 0 0 0 2 2 0 0 0 3 1 1 0 0 0 0 0 B 0 0 0 1 1 1 0 0 1 0 0 0 0 0 1 0 2 0 1 1 2 1 1
Vellozia tubiflora	0 1 1 0 A B 1 0 1 H 0 A 1 1 0 0 1 0 1 0 0 0 1 1 0 0 0 0 1 0 2 2 0 0 0 3 1 A 0 0 J 0 0 0 0 0 0 1 1 1 0 0 1 0 0 0 0 1 1 1 1 0 0 1 2 1 1
Xerophyta dasylirioides	0 1 1 0 0 0 1 0 B 2 0 0 1 1 0 0 0 0 1 0 0 0 1 1 0 0 1 0 1 0 2 2 0 0 0 0 1 0 0 0 0 0 1 0 0 1 1 3 1 0 0 0 0 1 1 1 3 0 1 0 2 0 0 0 2 1 0
Xerophyta eglandulosa	1 1 1 1 1 0 2 0 B 2 0 0 1 1 0 0 1 0 0 0 1 0 1 1 0 1 1 0 0 - 0 0 0 0 0 0 1 0 0 0 0 0 1 0 0 1 0 3 1 0 0 0 0 1 1 1 3 0 1 0 2 0 1 0 2 1 0
Xerophyta equisetoides	0 1 1 0 0 0 2 0 0 2 0 0 1 1 1 0 2 0 2 0 0 0 1 1 0 0 1 2 0 1 2 2 0 1 0 0 1 0 0 0 0 0 2 0 1 1 0 3 1 0 0 0 0 1 1 1 3 0 ? ? ? ? ? ? ? ? ?
Xerophyta pinifolia	1 1 1 1 1 0 1 0 1 2 0 1 1 1 0 0 2 0 0 0 0 0 1 1 0 0 ? 0 1 0 1 1 0 0 0 2 1 0 0 0 0 0 - 2 0 0 1 1 1 0 0 0 0 1 1 1 3 0 1 1 2 0 1 0 2 1 0
Xerophyta retinervis	? 1 1 0 0 0 2 0 2 2 0 0 1 1 1 0 2 0 2 0 0 0 1 1 0 0 0 2 0 1 1 2 0 0 0 0 1 0 0 0 0 0 2 0 1 1 0 3 3 0 0 0 0 1 2 1 3 ? ? ? ? ? ? ? ? ? ?

'?', missing data; '–', inapplicable; A, 0 and 1; B, 0 and 2; C, 0 and 4; D, 1 and 2; E, 1 and 4; F, 2 and 5; G, 6 and 7; H, 0, 1 and 2; I, 1, 2 and 3; J, 2, 3 and 4; K, 5, 6 and 7; L, 6, 7 and 8; M, 0, 1, 2 and 3; N, 0, 2, 3 and 4; O, 2, 3, 4 and 5; P, 1, 2, 3, 4 and 5.

(Farris, 1972, 1982; Nixon and Carpenter, 1993; Ferrarezzi and Marques, 1997) based on results of broader analyses conducted for the monocots (e.g. Chase *et al.*, 2006). The combined dataset was analysed using heuristic searches in PAUP 4·0b10 for Macintosh (Swofford, 2002) with 10 000 replicates of randomized taxa entries with tree–bissection–reconnection (TBR) swapping and a tree limit of 100 trees per replicate. Other datasets were analysed using 1000 replicates with ten trees per replicate because of limits on computational memory for performing them. The bootstrap was used to estimate internal support; 1000 replicates of simple-taxon addition, with a limit of ten trees per replicate and TBR swapping were used for the combined data. 'Fast' stepwise addition with 100 000 replicates was used for the other datasets, for the same reason as above. One single tree, the first found, is shown for all analyses; groups not present in the strict consensus tree are marked with an asterisk. Bayesian inference of the combined datasets was conducted in Mr Bayes v.3·1·2 (Huelsenbeck and Ronquist, 2001). The best model of nucleotide substitution for each dataset was determined under AIC criteria using MrModeltest 2·2 (Nylander, 2004). A general time reversible (GTR) model with gamma distribution was selected for *atpB-rbcL* and *trnL-trnF* for total analysis and the Hasegawa, Kishino and Yano (HKY) model of nucleotide distribution with gamma distribution for *atpB-rbcL* in total analysis with indels removed. For ITS nrDNA and *trnH-psbA*, the GTR model followed a gamma distribution combined with a proportion of invariable sites. For morphological data, the standard discrete model was used. Four simultaneous chains were run starting from random trees for 5 million generations, sampling every 100 generations. Examination of the average standard deviation of split frequencies suggested that stationarity was reached. To ensure sampling of topologies after chain convergence, 10 % of generations were discarded as burn-in; posterior probabilities were calculated from the remaining trees and shown as a majority-rule consensus tree. Numbers above branches illustrate branch lengths (DELTRAN optimization), and those below branches are bootstrap percentages (bp) and posterior probability (pp) either equal or greater than 50. MacClade version 4·07 for OS X (Maddison and Maddison, 2005) was used for optimizing morphological characters on trees and presenting results.

RESULTS

The combined matrix has 3614 aligned characters, of which 67 are non-molecular, 2607 are from the plastid genome and 952 from ITS nrDNA. Of these, 542 are variable but uninformative, and 733 are potentially parsimony-informative. As regards the clades, results from different analyses, i.e. morphological, plastid, ITS, combined DNA, total evidence with indels removed and total evidence analyses, do not show strong conflicts. Therefore only detailed information about (*a*) a morphology-based tree, (*b*) a total DNA-based tree (including indels) and (*c*) a total evidence tree (including indels) is presented here. Removing the indels served to diminish resolution and to depress bootstrap values across a subset of the branches.

TABLE 4. *List of plant samples with voucher information and GenBank accession numbers*

Species	*atpB-rbcL*	*trnL-trnF*	*trnH-psbA*	ITS	Voucher and herbaria
Acanthochlamys bracteata P.C.Kao	JN016989	JN016885	JN017041	JN016937	Chase 842 (K)
Aylthonia blackii (L.B.Sm.) N.L.Menezes	JN016990	JN016886	JN017042	JN016938	Mello-Silva 1103 (SPF)
Aylthonia umbrosa (L.B.Sm. & Ayensu)	JN016991	JN016887	JN017043	JN016939	Mello-Silva CFCR9658 (F, K, MBM, RB, SPF)
Barbacenia flava Mart. ex Schult. & Schult.f.	JN016992	JN016888	JN017044	JN016940	Mello-Silva 2662 (SPF)
Barbacenia ignea Mart. ex Schult. & Schult.f.	JN016993	JN016889	JN017045	JN016941	Mello-Silva 2554 (B, K, RB, SPF, US)
Barbacenia markgrafii Schulze-Menz	JN016994	JN016890	JN017046	JN016942	Mello-Silva 1504 (BHCB, K, NY, SPF, W)
Barbacenia reflexa L.B.Sm. & Ayensu	JN016995	JN016891	JN017047	JN016943	Mello-Silva CFCR10793 (F, SPF)
Barbaceniopsis boliviensis (Baker) L.B.Sm.	JN016996	JN016892	JN017048	JN016944	Mello-Silva 2107 (B, BHCB, CDBI, CTES, L, LPB, K, MBM, NY, SI, SPF)
Barbaceniopsis castillonii (Hauman) Ibisch	JN016997	JN016893	JN017049	JN016945	Mello-Silva 1857 (B, CESJ, CTES, L, K, MBM, MCNS, NY, SI, SPF, US)
Barbaceniopsis humahuaquensis Noher	JN016998	JN016894	JN017050	JN016946	Mello-Silva 1872 (B, CESJ, CTES, K, MCNS, SI, SPF, US)
Burlemarxia pungens N.L.Menezes & Semir	JN016999	JN016895	JN017051	JN016947	Mello-Silva 319 (SPF)
Burlemarxia spiralis (L.B.Sm. & Ayensu) N.L.Menezes & Semir	JN017000	JN016896	JN017052	JN016948	Mello-Silva 2548 (SPF)
Cyclanthus bipartitus Poit. ex A.Rich.	JN017001	JN016897	JN017053	JN016949	Mello-Silva 3180 (SPF)
Encholirium scrutor (L.B.Sm.) Rauh	JN017002	JN016898	JN017054	JN016950	Forzza 1488 (BHCB, K, SP, SPF, US)
Nanuza plicata (Mart.) L.B.Sm. & Ayensu	JN017003	JN016899	JN017055	JN016951	Mello-Silva 2133 (SPF)
Pandanus pygmaeus Thouars	JN017004	JN016900	JN017056	JN016952	Pirani 4755 (SPF)
Pleurostima longiscapa (Goethart & Henrard) N.L.Menezes	JN017005	JN016901	JN017057	JN016953	Mello-Silva 2553 (K, SPF)
Pleurostima plantaginea (L.B.Sm.)	JN017006	JN016902	JN017058	JN016954	Salatino CFCR11901 (K, SPF)
Pleurostima purpurea (Hook.) Raf.	JN017007	JN016903	JN017059	JN016955	Menezes 511 (SPF)
Pleurostima riparia N.L.Menezes & Mello-Silva	JN017008	JN016904	JN017060	JN016956	Menezes 1167 (SPF)
Talbotia elegans Balf.	JN017009	JN016905	JN017061	JN016957	Chase 253 (K)
Thoracocarpus bissectus (Vell.) Harling	JN017010	JN016906	JN017062	JN016958	Fiaschi 603 (SPF)
Vellozia abietina Mart.	JN017011	JN016907	JN017063	JN016959	Mello-Silva 1733 (B, K, NY, RB, SPF, US)
Vellozia alata L.B.Sm.	JN017013	JN016909	JN017065	JN016961	Mello-Silva 2368 (K, SPF)
Vellozia aloifolia Mart.	JN017014	JN016910	JN017066	JN016962	Salatino 67 (SPF)
Vellozia burlemarxii L.B.Sm. & Ayensu	JN017015	JN016911	JN017067	JN016963	Mello-Silva 2148 (B, CDBI, CTES, HUEFS, K, M, NY, RB, SPF)
Vellozia campanuloides Mello-Silva	JN017016	JN016912	JN017068	JN016964	Mello-Silva 2770 (K, SPF)
Vellozia candida J.C.Mikan	JN017017	JN016913	JN017069	JN016965	Mello-Silva 2877 (SPF)
Vellozia canelinha Mello-Silva	JN017018	JN016914	JN017070	JN016966	Mello-Silva 2131 (K, SPF)
Vellozia caput-ardeae L.B.Sm. & Ayensu	JN017019	JN016915	JN017071	JN016967	Mello-Silva 1520 (G, NY, SPF, UB)
Vellozia caudata Mello-Silva	JN017020	JN016916	JN017072	JN016968	Mello-Silva 2132 (HUEFS, K, SPF)
Vellozia aff. *caudata* Mello-Silva	JN017012	JN016908	JN017064	JN016960	Mello-Silva 2135 (SPF)
Vellozia compacta Mart. ex Schult. & Schult.f.	JN017021	JN016917	JN017073	JN016969	Mello-Silva 1386 (MBM, MO, SP, SPF)
Vellozia dasypus Seub.	JN017022	JN016918	JN017074	JN016970	Mello-Silva 2578 (SPF)
Vellozia epidendroides Mart. ex Schult. & Schult.f.	JN017023	JN016919	JN017075	JN016971	Mello-Silva 1772 (G, SPF)
Vellozia glauca Pohl	JN017024	JN016920	JN017076	JN016972	Mello-Silva CFCR11585 (BHCB, F, K, MBM, RB, SPF, UEC, US)
Vellozia hatschbachii L.B.Sm. & Ayensu	JN017025	JN016921	JN017077	JN016973	Mello-Silva 2474 (G, SPF)
Vellozia hemisphaerica Seub. 1	JN017026	JN016922	JN017078	JN016974	Mello-Silva 2576 (SPF)
Vellozia hemisphaerica Seub. 2	JN017027	JN016923	JN017079	JN016975	Mello-Silva 2800 (B, HUEFS, K, M, NY, RB, SPF, US)
Vellozia hirsuta Goethart & Henrard	JN017028	JN016924	JN017080	JN016976	Mello-Silva 1503 (SPF)
Vellozia jolyi L.B.Sm.	JN017029	JN016925	JN017081	JN016977	Mello-Silva 2146 (B, K, NY, RB, SPF, US)
Vellozia minima Pohl	JN017030	JN016926	JN017082	JN016978	Mello-Silva 1735 (CTES, K, M, NY, RB, SPF)
Vellozia prolifera Mello-Silva	JN017031	JN016927	JN017083	JN016979	Mello-Silva CFCR10000 (BHCB, CEPEC, NY, SPF)
Vellozia punctulata Seub.	JN017032	JN016928	JN017084	JN016980	Mello-Silva 2587 (HUEFS, K, SPF)
Vellozia religiosa Mello-Silva & D.Sasaki	JN017033	JN016929	JN017085	JN016981	Mello-Silva 2577 (SPF)
Vellozia sessilis L.B.Sm. ex Mello-Silva	JN017034	JN016930	JN017086	JN016982	Mello-Silva 2263 (CTES, K, SPF)
Vellozia tubiflora (A.Rich.) Kunth	JN017035	JN016931	JN017087	JN016983	Mello-Silva 2158 (BHCB, HRCB, HUFU, K, MBM, NY, SP, SPF, SPFR, UEC)
Xerophyta dasylirioides Baker	JN017036	JN016932	JN017088	JN016984	Treutlein 412 (TEX)
Xerophyta eglandulosa H.Perr.	JN017037	JN016933	JN017089	JN016985	Treutlein 410 (TEX)
Xerophyta equisetoides Baker	JN017038	JN016934	JN017090	JN016986	Rodrigues s.n. (SPF 181828)
Xerophyta pinifolia Lam.	JN017039	JN016935	JN017091	JN016987	Treutlein 406 (TEX)
Xerophyta retinervis Baker	JN017040	JN016936	JN017092	JN016988	Cultivated (UConn 199700041)

Morphological analysis

Morphological analysis found 697 most-parsimonious trees with 464 steps, a consistency index (CI) of 0·44 and a retention index (RI) of 0·70 (Fig. 1A). Velloziaceae emerge as monophyletic (76 bp), with *Acanthochlamys bracteata* as sister of all remaining species. In the 'core' Velloziaceae (66 bp), i.e. Velloziaceae *s.s.* (not including *Acanthochlamys*), *Talbotia* is sister to all other species (bp < 50), which are divided in two major clades. One includes *Barbacenia s.l.* (*Aylthonia* + *Barbacenia s.s.* + *Burlemarxia* + *Pleurostima*; bp < 50) and *Xerophyta* + *Barbaceniopsis* (77 bp). The other (57 bp) includes *Nanuza* sister to *Vellozia* (bp < 50). The morphology consensus tree resulting from the improved matrix with stem characters is almost identical to the one from Mello-Silva (2005). The main differences are, apart from some different terminals, the status of *Aylthonia* and *Barbacenia*, now paraphyletic, and of *Pleurostima*, now monophyletic (54 bp). Nevertheless, conclusions regarding genera and other groups are the same.

Combined DNA analysis

Analysis of the combined DNA matrix found 72 most-parsimonious trees of 2376 steps, CI of 0·70 and RI of 0·79 (Fig. 1B). Velloziaceae are monophyletic (100 bp) with *Acanthochlamys bracteata* as sister of the rest. In Velloziaceae *s.s.* (100 bp), *Talbotia* + *Xerophyta* (91 bp) is sister to American clade (bp < 50). In the latter, *Barbacenia s.l.* (98 bp) is sister to *Barbaceniopsis* + *Nanuza* + *Vellozia* (bp < 50). *Barbaceniopsis* (100 bp) is sister to *Nanuza* + *Vellozia* (99 bp). *Nanuza* is sister to *Vellozia* (96 bp). *Xerophyta* is monophyletic (68 bp).

Total evidence analysis

The total evidence analysis found 48 most-parsimonious trees of 2807 steps, CI of 0·63 and RI of 0·76 (Figs 2 and 3). Velloziaceae are again monophyletic (100 bp/100 pp), with *Acanthochlamys bracteata* as sister of all remaining species. Three non-homoplastic characters, persistent leaves (or at least the sheath; character 2:state 1), two phloem strands (20:0 and 1), and stem cortex divided in three regions (66:1), and one homoplastic character, violet tepals (38:0), support Velloziaceae including *Acanthochlamys*. There are, possibly, four more characters supporting this clade, stem vascular bundles with fibres next to xylem (65:2), *Barbacenia* type of leaf sclerenchyma (19:2), a loculicidal capsule (57:0) and a continuous belt of fibres in stem cortex (61:2). The first and the second are invariable, and the last three present transformations within Velloziaceae. Nevertheless, their optimization is uncertain. *Acanthochlamys* is supported by five homoplastic characters: abaxial strands on leaf blades (14:1), aquiferous hypodermis extending to bundle sheaths only (17:0), hypanthial tube longer than ovary (36:3), cylindrical filaments (43:0) and fibres uniting the stem vascular bundles in maturity (63:1). Perhaps three other homoplastic characters also support it, spiral phyllotaxis (1:2) and dorsifixed, bisporangiate anthers (45:1 and 50:2), all with equivocal optimization.

Velloziaceae *s.s.* (100 bp/100 pp) are supported by non-homoplastic synapomorphies once attributed to the family: leaves with marginal bundles (15:0 and 1; Mello-Silva, 2005), transfusion tracheids (23:1) and inflorescence without major axis (24:1; Menezes *et al.*, 1994). Three more characters, amphistomatic leaves (10:2), aquiferous parenchyma absent between bundles (18:0) and stigma lobes vertical and fused at apex (56:1), are homoplastic. This clade is perhaps also supported by a non-homoplastic, *Barbacenia*-type of sclerenchyma pattern (19:2) and a homoplastic character, tristichous phyllotaxis (character 1:0), both with equivocal optimization.

Within Velloziaceae *s.s.*, there are two major clades. One, *Talbotia* + *Xerophyta* (90 bp/100 pp), is African and supported by trigonous ovary in transverse section (34:0, homoplastic) and basal loculicidal capsules (57:3, non-homoplastic). *Xerophyta* (96 bp/99 pp) is monophyletic and supported by four homoplastic characters, among them style and stigma of the same length (55:1). The African clade is sister to the American clade (59 bp/62pp), which is supported by two homoplastic characters, leaf trichomes or emergences with multicellular base on leaf margins and midrib (9:2) and auriculate anthers (47:1), and includes *Barbacenia s.l.* + *Barbaceniopsis* + *Nanuza* + *Vellozia. Barbacenia s.l.* (100 bp/100 pp) is supported by non-homoplastic double sheath in leaf vascular bundles (22:1), and corona (39:1), and by hypanthial tube shorter than ovary (36:1) and introrse antipetalous anthers (49:3), which are homoplastic. Absence of filaments (44:1 and 2) is also characteristic of this clade, although its transformation series could not be determined. There are also two more homoplastic characters, 12 vascular bundles in pedicel (28:2) and introrse antisepalous anthers (48:3), of uncertain status due to equivocal optimization. *Barbacenia s.l.* is sister to *Barbaceniopsis* + *Nanuza* + *Vellozia* (bp/pp < 50), which is supported only by bundles of fibres in stem cortex not forming a continuous belt (61:1 and 3, state 1 homoplastic, transformation series not determined) and, perhaps, by a central fibrous bundle in stem (64:1, ambiguous). *Barbaceniopsis* (100 bp/100 pp) is supported by a non-homoplastic character within Velloziaceae, diclinous flowers (40:1) and, potentially, by U-shaped bundles of fibres in stem cortex (61:3, ambiguous). There are also four homoplastic ones, leaf blade with furrows in both surfaces (7:2), flowers with subulate emergences (30:1), style much shorter than stigma (55:2) and poricidal capsules (57:1). It is sister to *Nanuza* + *Vellozia* (100 bp/100 pp), which, in its turn, is supported by two non-homoplastic characters within Velloziaceae, stigma lobes horizontal and fused at the centre (56:0) and stem inner cortex cells with secondary walls (67:1), and nine homoplastic characters, leaves with abscission line between sheath and lamina (3:1), with abaxial strands (14:1), with aquiferous hypodermis (17:0 and 1, transformation series not determined), and with *Vellozia* type of sclerenchyma pattern (19:1), plus trigonous pedicel (27:0) and ovary (34:0), each pair of microsporangia in anther dehiscing by a separated slit (50:1), and stigma positioned above stamens (54:0). This clade could also be supported by two ambiguous and homoplastic characters, adaxial strands in leaves (13:1) and bundles of round fibres in stem cortex (61:1). *Nanuza*, supported by three homoplastic autapomorphies, minor fibro-vascular bundles in leaves (21:1), absence of a belt of sclerified cells in pedicel

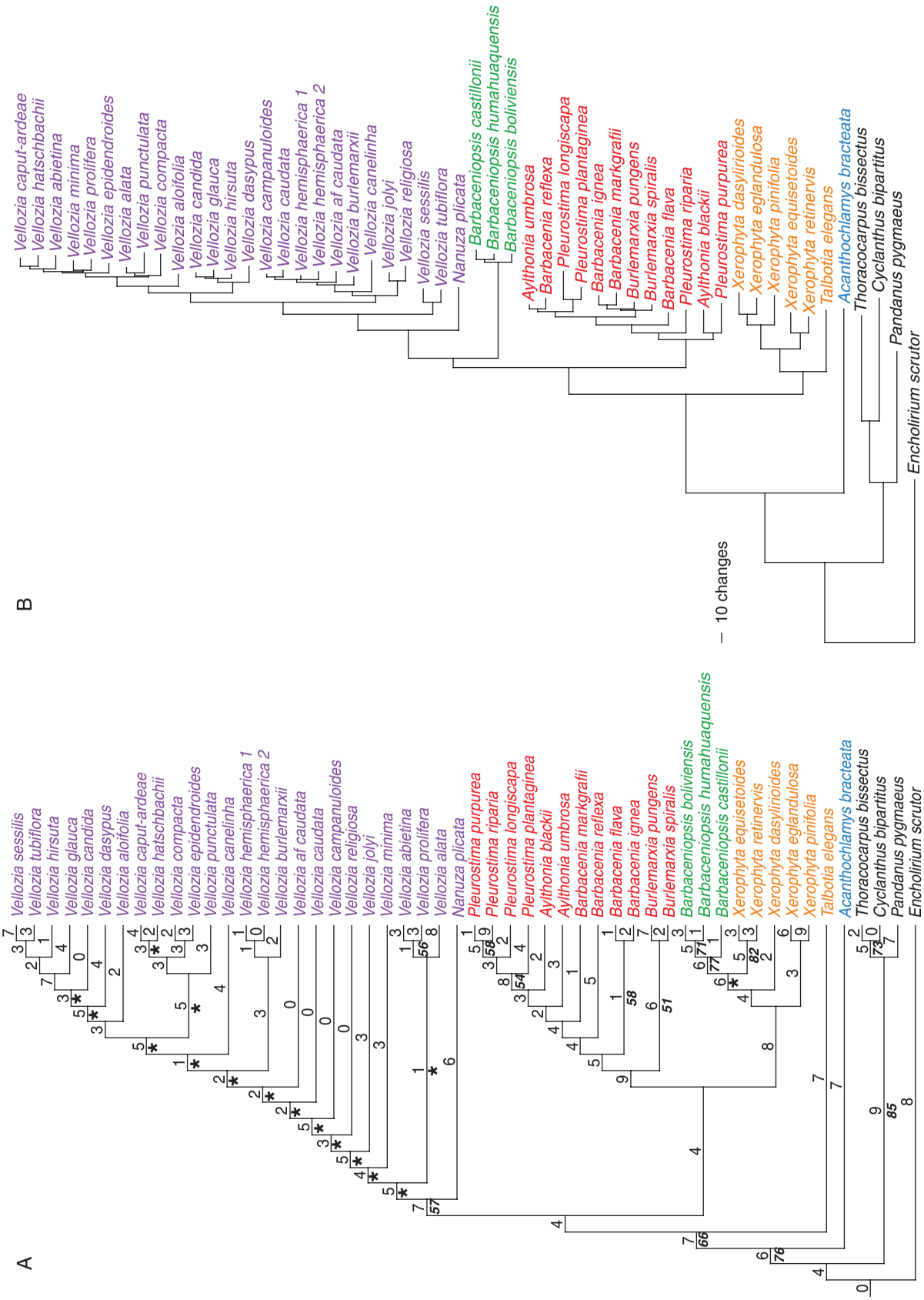

FIG. 1. (A) Single tree selected from the 697 equally most-parsimonious trees produced from analysis of the morphological data matrix. Numbers above branches are estimated substitutions and numbers below branches are bootstrap percentages. Clades not present in all trees are marked with an asterisk. (B) Single tree selected from the 72 equally most-parsimonious trees produced from a combined matrix of all DNA regions. The length of branches is proportional to substitutions.

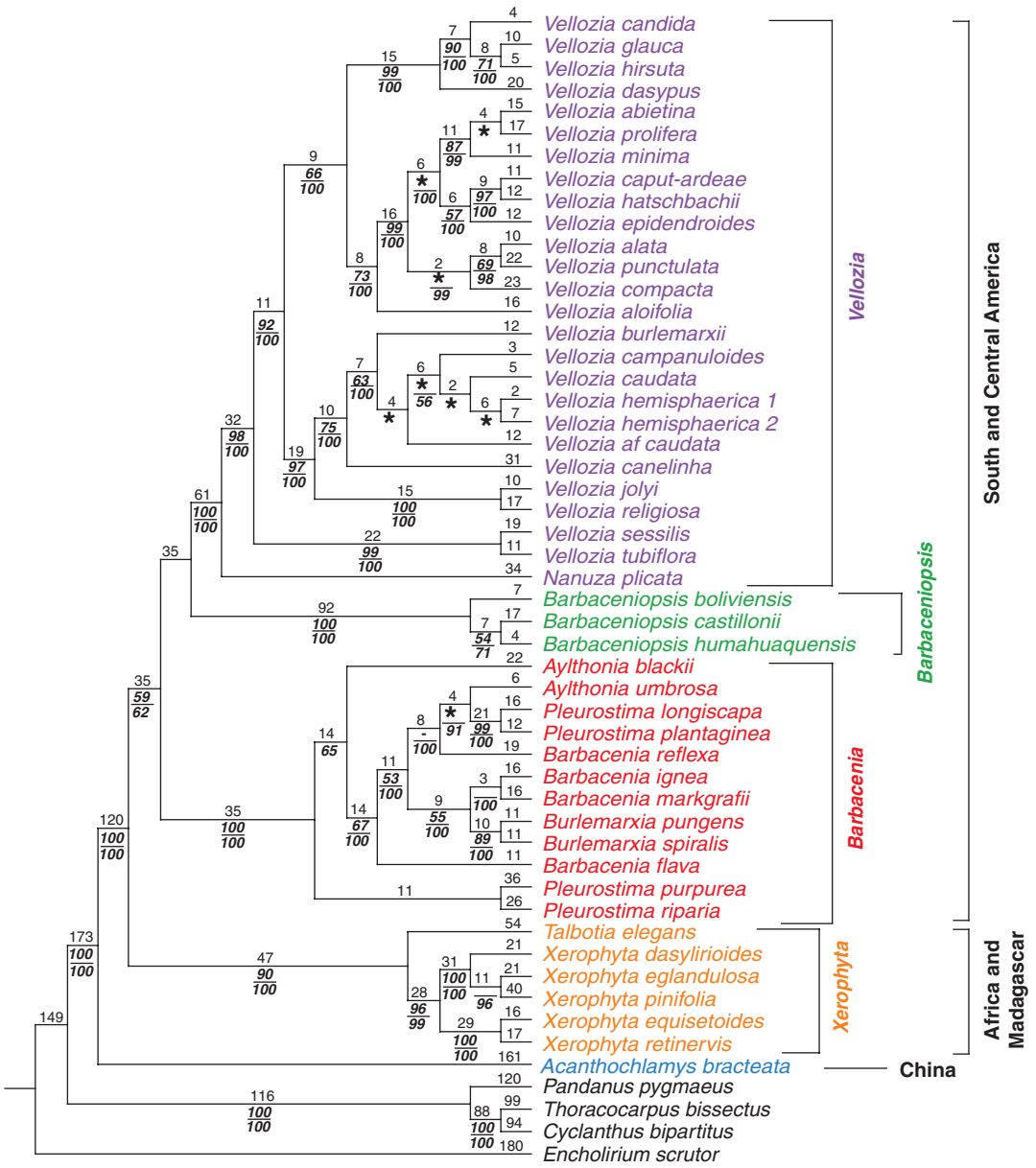

FIG. 2. Single tree selected from the 48 equally most-parsimonious trees produced from the combined matrix of all data. Numbers above branches are estimated substitutions and numbers below branches are bootstrap percentages/posterior probability. Clades not present in all trees are marked with an asterisk.

(29:0) and trigonous stem (60:1), is sister to *Vellozia* (98 bp/100 pp), which is supported by a non-homoplastic character, pollen in tetrads (53:1), and three homoplastic ones, leaf blade with furrows only on abaxial surface (7:1), filaments cylindrical (43:0) and anthers non-auriculate (47:0). Within *Vellozia*, the *V. sessilis* + *V. tubiflora* clade (99 bp/100 pp) is sister to all other species. It is supported by two homoplastic characters, leaf trichomes or emergences with multicellular bases disposed on lamina (9:1) and hypanthial tube longerthan ovary (36:3). In that main clade, (((*V. burlemarxii*, *V. campanuloides*, *V. caudata*, *V.* aff. *caudata*, *V. hemisphaerica* 1, *V. hemisphaerica* 2), *V. canelinha*) (*V. jolyi*, *V. religiosa;* 97 bp/100 pp))) is supported by a non-homoplastic character, hemispheric ovary (33:2), and by four homoplastic characters, specialized cells on both surfaces of lamina (12:2), minor vascular bundles in leaf blade (21:1), circular-trilobate ovary (34:1) and poricidal capsules (57:1). A second group, composed of nine species, ((*V. abietina*, *V. minima*, *V. prolifera*) (*V. alata*, *V. punctulata*) ((*V. caput-ardeae*, *V. hatschbachii*) *V. epidendroides*) *V. compacta*; 99 bp/100 pp), has almost no internal resolution. Five homoplastic characters, spirotristichous phyllotaxis (1:1), leaf blade involute when dry (6:0), hypanthial emergences absent to laxly disposed (32:0 and 1), staminal appendages (42:1) and poricidal capsules (57:1), support it. A third and final group (99 bp/100 pp) is composed of four species, (((*V. hirsuta*, *V. glauca*) *V. candida*) *V. dasypus*), and supported by two homoplastic characters, stomata with ridged subsidiary cells (11:1) and pedicel without a belt of sclerified

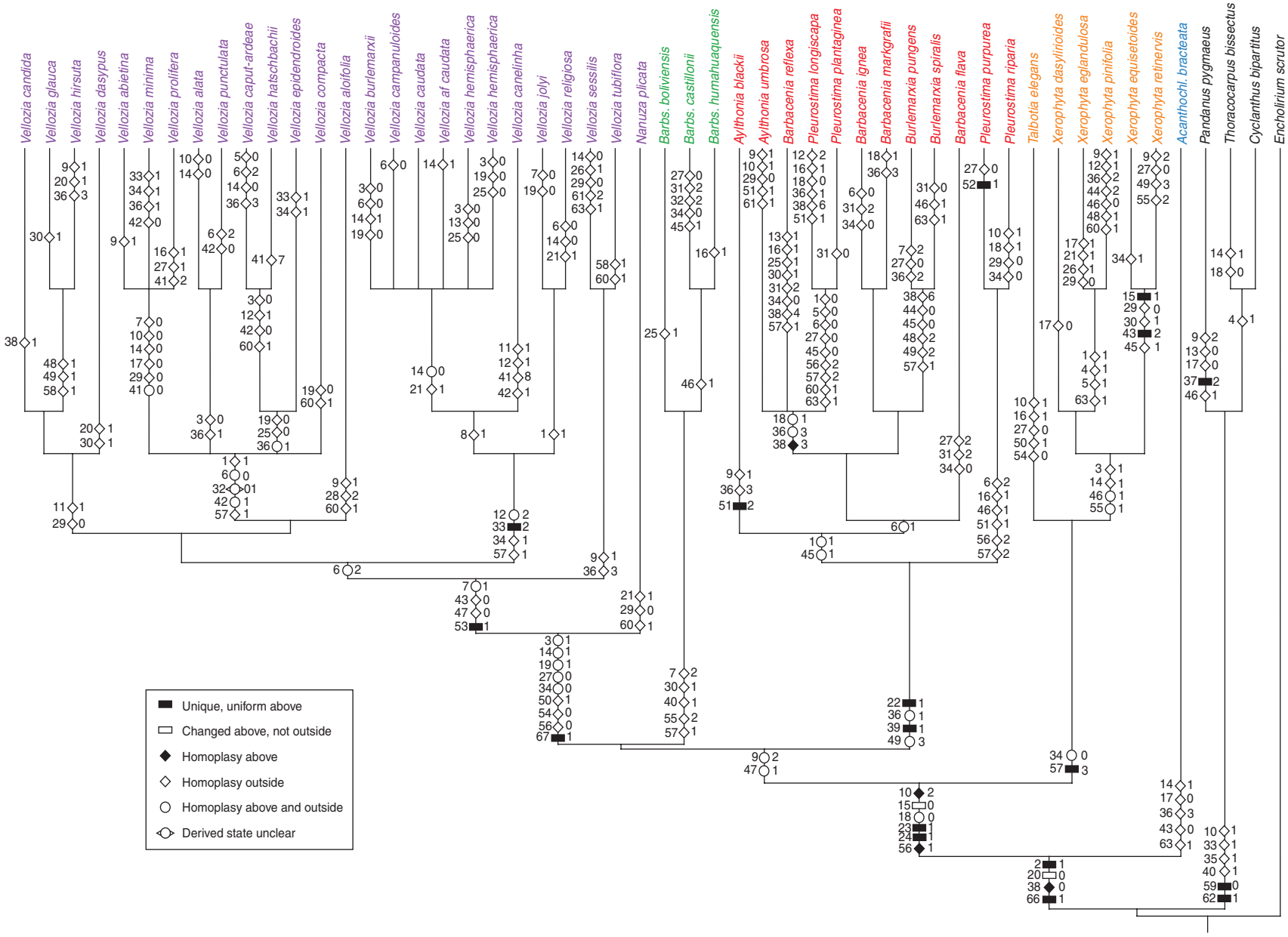

FIG. 3. Strict consensus tree generated from the 48 equally most-parsimonious trees produced from the combined matrix of all data. Character bars are shown in the key (unambiguous changes only). Numbers at the left side of character bars refer to character numbers, and those at the right indicate the character state.

cells (29:0), and perhaps, also by aquiferous hypodermis extending to bundle sheaths only (17:0, ambiguous).

DISCUSSION

Characters and classification in total evidence analysis

In both total analyses, monophyly of Velloziaceae and the position of *Acanthochlamys bracteata* as sister to all other members of the family is in accordance with Chase *et al.* (1995, 2006), Behnke *et al.* (2000), Salatino *et al.* (2001) and Mello-Silva (2005). Thus, it supports sinking of Acanthochlamydaceae into Velloziaceae (Wu, 1988; APG, 1998, 2003, 2009; contra Kao and Kubitzki, 1998), maximizing phylogenetic information of classification. In Mello-Silva (2005), synapomorphies of the family including *Acanthochlamys* were only perianth violet and tenuinucellate ovules, the latter from Stevenson and Loconte (1995) and not analysed here. Velloziaceae are now supported by at least four character states, three of them non-homoplastic. Persistent leaves, or at least the sheath (2:1), are easily observable and a good diagnostic characteristic for the family (Martius and Zuccarini, 1823; Seubert, 1847; Smith, 1962; Menezes, 1976; Kubitzki, 1998). This character has been misinterpreted in Mello-Silva (2000, 2005), who associated it with presence of an abscission zone (character 3). Two phloem strands (20:0) characterize all Velloziaceae (contra McPherson *et al.*, 1997), although in some species strands are united at the bottom (20:1; see Mello-Silva, 2000, fig. 1M, N). This character was also misinterpreted in Mello-Silva (2000, 2005), who assigned state 2 (two strands united at bottom) as homologous to state 3 (one strand), which they are not. Stem cortex divided in three regions (66:1) is a new synapomorphy of Velloziaceae. Finally, violet tepals (38:0) still support Velloziaceae as in Mello-Silva (2005), although the transformation series for this character within the family is complex.

In the African clade of Velloziaceae *s.s.*, *Talbotia* + *Xerophyta*, recognition of monospecific *Talbotia*, although possible because of its position, would imply disregarding an easily observable fruit character, the basal loculicidal dehiscence (57:3), for delimiting the group. Besides, *Talbotia elegans* and all studied species of *Xerophyta* are hexaploid (Goldblatt and Poston, 1988; Melo *et al.*, 1997). For maximizing phylogenetic information and ease of identification, *Talbotia* should be transferred to *Xerophyta*. The American clade, including *Barbacenia s.l.*, *Barbaceniopsis*, *Nanuza* and *Vellozia*, is a new result among Velloziaceae phylogenetic studies, although it has low support. Its two supporting homoplastic characters, leaf trichomes or emergences with multicellular base on leaf margins and midrib (9:2) and auriculate anthers (47:1), have been used by Menezes (1980, 1981) to characterize *Pleurostima*.

Within American Velloziaceae, the same characters used by Menezes (1971*b*, 1980) to define subfamily Barbacenioideae, double sheath in leaf vascular bundles (22:1) and corona (39:1), support the *Barbacenia s.l.* clade. The relationships among genera involved, in both total analyses, show them all polyphyletic except *Burlemarxia*, but even that, if maintained, would leave *Barbacenia* paraphyletic. Similar conclusions could be obtained from Behnke *et al.* (2000) and Salatino *et al.* (2001). Thus, with respect to the criterion of monophyly, all genera in that clade should be considered synonymous with *Barbacenia*, with the same circumscription as subfamily Barbacenioideae *sensu* Menezes (1971*b*, 1980). This result also renders Menezes' subfamily Vellozioideae paraphyletic.

The clade of *Barbaceniopsis* + *Nanuza* + *Vellozia*, present in the total analyses, is also a new result for Velloziaceae phylogenetic studies, but because of its weak support, both by clearly optimized morphological characters and DNA, it is better to recognize at least the Andean *Barbaceniopsis* as a separate genus. This would be also a reasonable conclusion from the total analysis with indels removed, in which *Barbaceniopsis* is in a basal trichotomy within the American clade. Within *Barbaceniopsis*, the analyses support recognition of *Barbaceniopsis castillonii*, as did Ibisch *et al.* (2001), and it appears more closely related to *B. humahuaquensis* than to *B. boliviensis*, in which synonymy it had been placed since Smith (1962).

The *Nanuza* + *Vellozia* clade corresponds to subfamily Vellozioideae *sensu* Smith and Ayensu (1976), thus rendering their subfamily Barbacenioideae paraphyletic. Sinking *Nanuza* in *Vellozia*, as proposed by Mello-Silva (2005), would leave *Vellozia* characterized by stigma lobes (Seubert, 1847; Smith, 1962), an easily observed character. On the other hand, *Vellozia s.s.* is characterized mainly by pollen in tetrads, and *Nanuza* alone is only supported by homoplastic, mostly anatomical characters. *Nanuza* is monospecific (contra Alves, 2002), and merging it with *Vellozia* would maximize phylogenetic information and ease of identification.

Within *Vellozia*, the analysis without indels fails to establish relationships among the main groups, which are almost the same as in the total analysis with indels. In this analysis, *V. sessilis* + *V. tubiflora* are sister to all other species. *Vellozia tubiflora* is the most widespread species of Velloziaceae. It is highly variable (Mello-Silva, 2011), and several of its morphological characters are coded as polymorphic. An analysis of its populations could reveal a paraphyletic taxon in relation to *V. sessilis*, a narrow endemic species with several autapomorphies (Mello-Silva, 1997, 2000). Nine terminals, *V. burlemarxii*, *V. campanuloides*, *V. caudata*, *V.* aff. *caudata*, *V. canelinha*, *V. hemisphaerica* 1 and 2, *V. jolyi* and *V. religiosa* belong to the *V. hemisphaerica* group, representing its five species (R. Mello-Silva and D. Sasaki, unpubl. res.). Smith and Ayensu (1976) informally defined the group by placing *V. burlemarxii* near *V. hemisphaerica* in a separate group in their identification key, characterized by a hemispherical ovary (33:2) and minor vascular bundles in leaf blade (21:1). The first character proved to be a non-homoplastic synapomorphy of the group, but the second appears to have originated twice within the group and again in *Nanuza*. Another group is composed of nine species, *V. abietina*, *V. alata*, *V. caput-ardeae*, *V. compacta*, *V. epidendroides*, *V. hatschbachii*, *V. minima*, *V. prolifera* and *V. punctulata*. This group also occurs in the analyses of Mello-Silva (2000), but in no other Velloziaceae phylogenetic analyses or classifications. Despite that, Smith and Ayensu (1976) used the hypanthial emergences absent to laxly disposed (32:0 and 1) to link together a large group. Nonetheless, that group did not include species of sect. *Xerophytoides* (Smith and

Ayensu, 1976; Mello-Silva, 1991*b*), represented here by *V. abietina*, *V. minima* and *V. prolifera*. A final group (((*V. hirsuta*, *V. glauca*) *V. candida*) *V. dasypus*) is also present in Behnke *et al.* (2000) as ((*V. crassicaulis*, *V. glochidea*) *V. hirsuta*) and in Salatino *et al.* (2001) as (*V. candida*, *V. hirsuta*). The placement of *V. caput-ardeae*, *V. hirsuta* and *V. tubiflora* completely apart from one another reinforces the polyphyletic condition of *Vellozia* section *Radia* (Smith and Ayensu, 1976), which had previously been merged with *Vellozia* (Mello-Silva, 2000).

Historical taxonomic characters and their evolution

The taxonomic history of Velloziaceae, as of many other families under gradistic concepts, is linked mainly to floral characters. When describing the family, Vandelli (1788) also set up two genera distinguished by stamen number and stigma form. Jussieu (1789) followed him, describing a third genus also based on a combination of those characters. Such combinations are still in evidence in most recent systems (Mello-Silva, 1991*a*; Kubitzki, 1998).

Six stamens (41:0) is the ancestral condition in Velloziaceae (contra Menezes, 1980), and it is constant in all genera, except for *Vellozia s.s.* (i.e. excluding *Nanuza*). It is also an odd characteristic within Pandanales, in which stamen number is variable. Within *Vellozia s.l.* (i.e. including *Nanuza*) the ancestral condition is also six stamens. However, the most common situation, flowers with 18 stamens, cannot be established as primitive in *Vellozia s.s.* The sister clade of all other *Vellozia s.s.*, *V. sessilis* + *V. tubiflora*, brings together a species with six stamens and another with 12, 15 or 18 stamens, rendering equivocal this character optimization. Analysis of representative populations of *V. tubiflora* will cast light on polarization of transformations within that species, as well as their relationship with *V. sessilis*, thus defining the basal situation within *Vellozia s.s.* Similar situations mask evolution of stamen number in less inclusive clades. Within the *V. hemisphaerica* group, 12 stamens could be a synapomorphy of clade, *V. jolyi* + *V. religiosa*, that reverted to 18 in some populations of *V. jolyi*, or a derived situation independently acquired by *V. religiosa* and some populations of *V. jolyi*. Within clade *V. abietina*–*V. compacta*, a reduction to 12 and 6 stamens has occurred in *V. prolifera* and in *V. abietina* plus *V. minima*, respectively, the primitive condition of which is undetermined. Six stamens have lead Sprengel (1827), Schultes and Schultes (1829), Baker (1875) and Menezes (1980) to classify as *Xerophyta* those species and their relatives later placed together in *Vellozia* sect. *Xerophytoides* (Smith and Ayensu, 1976; Mello-Silva, 1991*b*). It has also helped to make those species sister to the rest of the *Vellozia* clade in Mello-Silva (2005), a situation that also occurs in the present morphological analysis (Fig. 1A). Other reductions took place within species, such as in *V. epidendroides*, with 18–12 stamens. However, not only reductions but also multiplications of stamens have occurred within *Vellozia*, sometimes dramatically, as in *V. canelinha*, which has 48–66 (Mello-Silva, 1993), and *V. alata* with 30–72 stamens (Sazima, 1978).

Vertical, fused at apex stigma lobes (56:1) appears as a synapomorphic condition of Velloziaceae *s.s.* (i.e. excluding *Acanthochlamys*), with one later transformation into horizontal, centrally fused stigmas in *Vellozia s.l.* (i.e. including *Nanuza*), and two independent transformations into free, lateral stigmas in species of *Pleurostima*. Those species (*P. longiscapa*, *P. plantaginea*) and (*P. purpurea*, *P. riparia*) have been classified as *P.* sects. *Graziela* and *Pleurostima*, respectively (Menezes, 1981). These transformations are congruent with basal attachment of anthers, another attribute used by Menezes (1981) to define *Pleurostima*. Although it is a synapomorphy of *P.* sect. *Graziela*, it is a symplesiomorphy in *P.* sect. *Pleurostima*. Some other attributes, such as a hypanthial tube, auricles in anthers and anther position, that have been used to justify the splitting of *Barbacenia s.l.* into four genera (Menezes, 1971*b*, 1980, 1981; Menezes and Semir, 1991) are homoplastic and could perhaps be explained by shifts in pollination strategies.

Characters and outgroups

As in most analyses focusing on Velloziaceae, some questions (mostly minor) regarding evolution of some morphological characters remain open due to the absence of representatives from Stemonaceae, Triuridaceae and Dioscoreales in the present study. These problems do not affect the topology of the trees, just interpretation of morphological change. Stemonaceae are closely related to Velloziaceae as they are the sister family of the sister clade of Velloziaceae, which is, in its turn, the sister of the rest of Pandanales, the sister-order of Dioscoreales (Chase *et al.*, 2006). Triuridaceae have been absent from most analyses due to their modified (reduced) plastid genomes, but now they are positioned within Pandanales (Chase *et al.*, 2000; Stevens, 2001). For these families, assessing morphological data similar to those here analysed is not an easy task, although necessary for better evaluation of homologies and, thus, character evolution. Tenuinucellate ovules are not found in Stemonaceae or Dioscoreales (Cronquist, 1981; Stevenson and Loconte, 1995; Kubitzki, 1998), but they do occur in Triuridaceae (Maas-van de Kamer and Weustenfeld, 1998). Leaves with a sheath seem to be deciduous in all taxa of Dioscoreales and Pandanales except Velloziaceae. A violet perianth, together with other colours, occurs in Burmanniaceae (Maas-van de Kamer, 1998), *Aletris* (Nartheciaceae) (Tamura, 1998; Tamura *et al.*, 2004), Stemonaceae (Kubitzki, 1998) and Triuridaceae (Maas-van de Kamer and Weustenfeld, 1998). Six stamens are here a symplesiomorphy shared by Velloziaceae and the outgroup, but the situation could be different as stamen number is variable and something other than six in many members of Dioscoreales and Pandanales. Loculicidal capsules are present in some Burmanniaceae (Maas-van de Kamer, 1998), Nartheciaceae subfamily Narthecioideae (Tamura, 1998) and some Dioscoreaceae (e.g. the former Taccaceae; Kubitzki, 1998). Anatomical characters, phloem strands, fibres in stem vascular bundles, divisions of and bundles of fibres in the stem cortex have never been investigated in the other groups. The same situation applies to phyllotaxis (1), sclerenchyma pattern (19), and anther insertion and dehiscence (45 and 50), which could be synapomorphies of *Acanthochlamys* or Velloziaceae *s.s.*, depending on the optimization provided by the inclusion

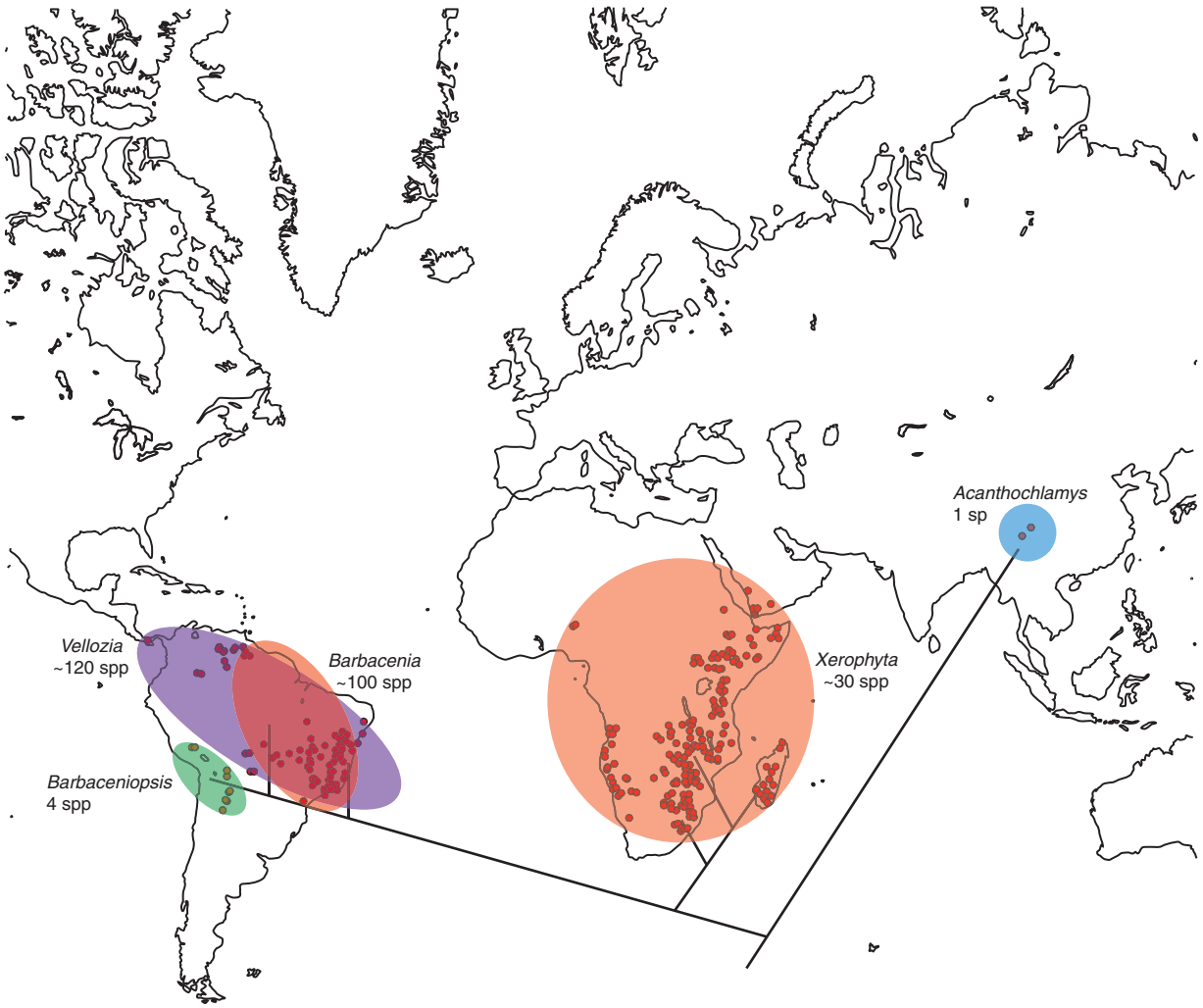

FIG. 4. Correlations between phylogenetic relationship of the five accepted genera of Velloziaceae and their geographical distribution.

of other outgroups. Two floral structures of Stemonaceae (Kubitzki, 1998), not analysed here, could be parallel with those in Velloziaceae and deserve investigation. The anthers are apiculate, as in species of '*Pleurostima*' (Menezes, 1981; Kubitzki, 1998), and the connectives are also long and resemble the corona of *Barbacenia* or the enlarged filaments of several species of *Xerophyta*. A search for more outgroup data is one of the investigative efforts to be accomplished by future students of Velloziaceae.

Biogeography

The distribution of Velloziaceae (Fig. 4) suggests a Gondwanan origin of the family and a possible vicarious splitting of main clades. In this scenario, long-distance dispersal as claimed by Ayensu (1973) and Menezes (1980) is not necessary to explain the geographical distribution of Velloziaceae, although it is the main explanation for other amphi-Atlantic taxa (e.g. Givnish *et al.*, 2004; Renner, 2004). The first cladogenetic event in the family, the splitting of *Acanthochlamys* from remaining genera, should have been consequence of the separation of Indian Plate from Gondwanaland, as first suggested by Wu (1988), which began about 115 Mya. The next split, between African and South American species, could correspond to the splitting of those two continents, which took place around 100 Mya (Scotese *et al.*, 1988). These ages are compatible with the age of the stem group of Velloziaceae stated by Janssen and Bremer (2004), although they did not include *Acanthochlamys* in their analysis. Nevertheless, the results are more compatible with Velloziaceae sister to the remaining Pandanales (Chase *et al.*, 2006) than to Velloziaceae sister to Stemonaceae (Bremer and Janssen, 2006). The absence of Velloziaceae fossils impedes a concrete evaluation of these estimated dates but fossils from Triuridaceae (Gandolfo *et al.*, 2002) and Pandanaceae (Kvacek and Herman, 2004) do suggest that Pandanales were well diversified by the late Cretaceous. Subsequent cladogenesis within both African and American clades could be explained also by events other than vicariance. In Africa, results point to dispersal of *Xerophyta* into Madagascar, as continental species form a clade in which the Madagascan taxa are embedded. In South America, *Barbacenia* and *Vellozia* are largely sympatric, and the history of their distribution must be related to several minor factors. However, the split of *Barbaceniopsis* could be

related to the Andean orogeny, as it is endemic to that mountain range, with allopatric distribution. This uplift occurred in the last 20–15 Mya (Burnham and Graham, 1993), during the time of establishment of the crown group of Velloziaceae (Janssen and Bremer, 2004).

Conclusions

Results from all analyses reinforce inclusion of *Acanthochlamys bracteata* in Velloziaceae and its position as sister to the rest of the family. Total analysis adds also at least three non-homoplastic characters, viz., persistent leaves, two phloem strands, and stem cortex divided in three regions, to the tenuinucellate nucellus (Stevenson and Loconte, 1995) and violet perianth (Mello-Silva, 2005) as synapomorphies of the family.

The American clade and its subclade *Barbaceniopsis* + *Nanuza* + *Vellozia* are not well supported, neither by non-homoplastic, conspicuous characters nor bootstrap percentages. On the other hand, these are groups that more or less correspond to accepted genera and are well established.

Xerophyta, including *Talbotia* or not, and *Vellozia*, including *Nanuza* or not, are well supported. However, for reasons of maximizing phylogenetic information and ease of identification (Backlund and Bremer, 1998), *Talbotia* should be transferred to *Xerophyta* and *Nanuza* to *Vellozia. Barbacenia s.l.*, encompassing *Aylthonia*, *Burlemarxia* and *Pleurostima*, is also well circumscribed, and it is impossible to recognize the smaller genera without violating the principle of monophyly.

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