

Phylogenetic Relationships and Evolution in the Strelitziaceae (Zingiberales)

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Abstract—Evolutionary trends and phylogenetic relationships in the Strelitziaceae (Zingiberales) were investigated using sequence data from ten plastid and two nuclear regions and a morphological dataset. The status of species of *Strelitzia* were evaluated in terms of the phylogenetic species concept. Relationships among the genera remain equivocal with two hypotheses emerging: (i) *Strelitzia* sister to a clade comprising *Ravenala* and *Phenakospermum* when indels are included, or (ii) *Ravenala* sister to the remainder of the Strelitziaceae when indels are excluded in/from the combined molecular and ‘total evidence’ analyses. Within *Strelitzia*, *S. nicolai* is sister to the rest of the genus, with *S. alba* sister to *S. caudata*. *Strelitzia reginae* is shown to be paraphyletic as *S. juncea* is nested within it, but more sampling at the population level is needed to confirm the taxonomic status of *S. juncea*. The highly localized and endangered *Strelitzia alba* is confirmed as a distinct species, as are *S. caudata* and *S. nicolai*, despite few morphological differences. Evolutionary trends are linked to changes in habitat and coevolution with pollinators. Climate change in southern Africa is thought to have restricted *Strelitzia nicolai* (or its ancestor) to the eastern coastal region, with subsequent allopatric speciation of *S. alba* and *S. caudata*, and relatively recent parapatric divergence of *S. juncea* from *S. reginae*.

Keywords—Biogeography, molecular phylogenetics, morphology, pollination, *Strelitzia*.

The Strelitziaceae is one of eight families in the Zingiberales, placed loosely within a basal paraphyly known as the ‘banana group’ (Kress 1990; Kress et al. 2001). The family shares with other members of the order an herbaceous habit, large petiolate distichous leaves with transverse venation, and colorful bracteate inflorescences. Features characterising the Strelitziaceae include its woody stem, boat-shaped coriaceous bract enclosing a cincinnus of flowers, three free sepals, two fused petals, and a loculicidal woody capsule containing seeds with brightly colored arils (Wright 1913; Dyer 1976; Kress 1990; Heywood et al. 2007). Despite numerous phylogenetic studies at various levels in the Zingiberales that included members of the Strelitziaceae (e.g. Kress 1990; Kress 1995; Kress et al. 2001; Givnish et al. 2006), to date there has been no thorough investigation of relationships within the Strelitziaceae. The Strelitziaceae are a charismatic family with two of the genera (*Ravenala* Adans. and *Strelitzia* Aiton) widely cultivated as ornamentals and well known throughout the world. *Strelitzia reginae* is emblematic of the South African National Biodiversity Institute (SANBI) and Aloha Airlines, and is currently on the KwaZulu-Natal coats of arms and on coinage in South Africa (50 cent coin), while *Ravenala* symbolizes Air Madagascar.

Ravenala, the ‘traveller’s palm’ (Fig. 1A), grows naturally in primary rainforests of Madagascar (Kress et al. 1994), while *Strelitzia* (the ‘crane flower’ or ‘bird of paradise’) occurs along the east coast and eastern mountains of southern Africa (Dyer 1976; Goldblatt and Manning 2000; Coates Palgrave and Coates Palgrave 2002). The third genus in the family, *Phenakospermum* Endl. (Fig. 1B), grows in transitional or secondary rainforest in tropical northern and central South America (Kress and Stone 1993). Dating of phylogenies and fossils suggest a post-Gondwanan dispersal of the Strelitziaceae (Kress and Specht 2006), but relationships within the family need to be clarified to make meaningful inferences of speciation processes and/or ancestral distributions, as well as evolutionary trends associated with habitat changes and pollination shifts.

Ravenala and *Phenakospermum* are both monotypic, while *Strelitzia* has five currently recognized species (Appendix 1). Confusion regarding the identity and distribution of some of the caulescent (tree-like) species of *Strelitzia* has occurred in the past, e.g. between the widespread *S. nicolai* (Fig. 1C) and restricted *S. alba* (Wright 1913; Dyer 1946a; Moore and Hyypio 1970) and between *S. nicolai* and *S. caudata*, the most northerly species occurring in the Soutpansberg and Barberton mountains of South Africa and the eastern highlands of Zimbabwe (Dyer 1946b, c; van Wyk and van Wyk 1997; Coates Palgrave and Coates Palgrave 2002). These species are difficult to distinguish when not flowering. In addition, there has been considerable discussion regarding the taxonomic status of the two rhizomatous herbaceous species, *S. reginae* (Fig. 1D, E) and *S. juncea* (Moore and Hyypio 1970; Dyer 1975; Dyer 1979), with *S. juncea* sometimes reduced to varietal level within *S. reginae* (Moore and Hyypio 1970). *Strelitzia reginae* has had no less than 22 species or varieties placed in synonymy with it since it was described by Aiton in 1789 (Moore and Hyypio 1970; Archer 2003). Currently two subspecies are recognized in *S. reginae*: *S. reginae* subsp. *mzimvubuensis*, known only from the lower Mzimvubu River in the Eastern Cape, differs from the widespread *S. reginae* subsp. *reginae* in having white petals and a much shorter stigma, as well as leaves with a minutely corrugated texture (van Jaarsveld and Loedolff 2007). As distributions of both *S. alba* and *S. juncea* are restricted and their populations are small, it is important to examine relationships within the genus and to establish whether the currently recognized species are valid in terms of phylogenetic species concepts (PSCs).

Our aim in this study was to construct a phylogeny of the Strelitziaceae using ten plastid DNA regions, two nuclear regions, and morphological data to examine relationships and evolutionary trends within the family. In addition, we aimed to confirm or elucidate the specific status of certain of the *Strelitzia* species, crucial for accurate conservation assessment. The phylogeny also serves as a framework on which to hypothesize the biogeographic history of the group.

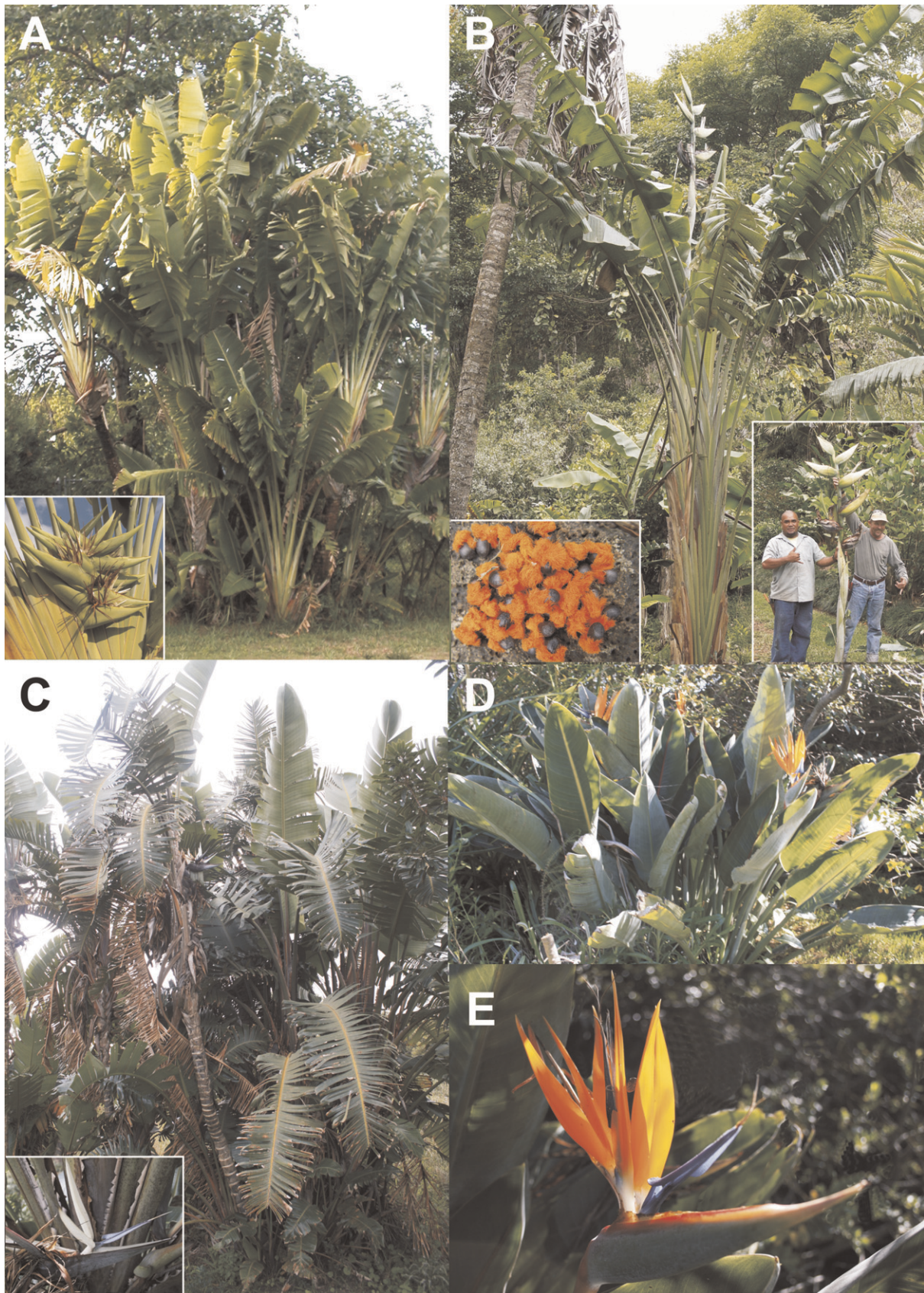


FIG. 1. Habit and inflorescence structure in Strelitziaceae. A. *Ravenala madagascariensis*, the traveller's palm (Kampong Botanical Garden, Miami), inset: compound inflorescence. B. *Phenakospermum guyanense* (McBryde Botanical Garden, Hawaii), insets: seeds with bright orange arils, peduncle with terminal compound inflorescence. C. *Strelitzia nicolai* (Umtamvuna Nature Reserve), inset: compound inflorescence, flowers with white sepals and blue petals. D, E. *S. reginae* (cultivated, Johannesburg garden). D. Habit. E. Simple inflorescence: spathe with flowers, sepals orange, petals blue. Photographs: A = D. Lee, B = D. Lorence, C–E = G. Cron.

MATERIALS AND METHODS

Taxon Sampling—All seven currently recognized species comprising the three genera of the Strelitziaceae and two outgroup species, *Orchidantha fimbriata* and *O. siamensis*, were included in this study. *Orchidantha* N. E. Br., the single genus in the Lowiaceae (Lane 1955; Johansen 2005), was selected as the outgroup based on previous phylogenetic analyses. Lowiaceae has repeatedly been recovered as sister to the Strelitziaceae (Chase et al. 2000; Soltis et al. 2000; Kress et al. 2001; Givnish et al. 2006; Soltis et al. 2007), with one exception where Lowiaceae was placed sister to remaining families of the Zingiberales, with Strelitziaceae comprising the next clade-diverging (Johansen 2005). Multiple exemplars (two individuals per species) were used for all *Strelitzia* species to test for monophyly. Appendix 1 lists the specimens with accession numbers and vouchers.

Molecular Characters—Rapidly evolving intron and intergenic spacers were selected from ten plastid regions, viz. *matK -5'trnK*, *psbA-matK*, *psbB-psbH*, *Ycf6-trnC*, *rpl16* intron, *rpoB-trnC*, *trnS-trnfM*, *trnY-trnE* (Shaw et al. 2005), *rpl32-trnL* (Shaw et al. 2007), *rp516* (Levin et al. 2004), and two nuclear regions: the external transcribed spacer (ETS) region of ribosomal DNA and *rpb2*, the second intron of the second subunit of RNA polymerase II. A single accession for each member of the Strelitziaceae was sequenced for all ten plastid regions, and a second accession sequenced for three plastid regions, *matK -5'trnK*, *rp516* and the *rpl16* intron (with the exception of *R. madagascariensis* and *S. alba* for the *rpl16* intron), and the regions not sequenced were coded as missing data.

DNA Extraction, Amplification and Sequence Alignment—DNA was extracted from silica dried or fresh leaf tissue using the DNeasy plant mini kit (Qiagen, Valencia, California) or a modified sodium dodecyl sulfate (SDS) extraction protocol (Edwards et al. 1991; Konieczny and Ausubel 1993). Primers used for amplification of the ten non-coding plastid regions were from references cited above. Polymerase chain reactions were carried out in 25 µl volume using 21.4 µl of 1 × PCR buffer from New England BioLabs (Ipswich, Massachusetts, *Taq* PCR Kit # E5000S), 0.125 µl each of forward and reverse primers at 100 mM, 0.5 µl dNTPs at 10 mM, 0.1 µl *Taq* polymerase (5,000 units/ml), 0.25 µl DNA. For several of the taxa, a 2 × Diamond reaction mix from Bionline (Taunton, Massachusetts) was substituted for the buffer, dNTPs, and *Taq*. Amplification of the cpDNA regions followed Green et al. (2011) and were performed in a BioRad MyCycler, according to the following protocol: denaturation at 95°C for 3 min, 10 cycles beginning at 95°C for 45 sec, followed by a variation in temperature per cycle at a 1°C gradient beginning at 55°C and ending at 45°C, with an extension at 72°C for 45 sec. This was followed by 20 cycles of 95°C for 45 sec, 50°C for 45 sec, and 72°C for 45 sec, and a final extension at 72°C for 5 min. The PCR products were held at 4°C until they were visualized in a 1% agarose gel, and those products yielding a visible band were purified using a MultiScreen PCR₉₆ plate from Millipore (Billerica, Massachusetts). Previously published primers and protocols were used for PCR amplification of the two nuclear regions, ETS and *rpb2* (Baldwin and Markos 1998; Kay et al. 2005; Sass and Specht 2010). Cycle sequencing reactions were performed using Big Dye version 3.1 ABI (Applied Biosystems International, Foster City, California) and sequenced on an ABI 3730 capillary sequencer.

Contigs were assembled and edited using Sequencher v. 4.1 (Genecodes, Ann Arbor, Michigan), and preliminary sequence alignments were performed using the Clustal W algorithm (Thompson et al. 1994) accessed via Geneious Pro 5.0.2 (Drummond et al. 2010). Manual adjustments were made using MacClade v. 4.08 (Maddison and Maddison 2005) or Geneious Pro 5.0.2. Evolutionary events were minimized (Zurawski and Clegg 1987) and alignment of indel events followed recommendations of Kelchner (2000). Areas of ambiguous alignment were excluded from analyses. Total missing data was 31% (25.5% due to the incomplete sequence data for multiple exemplars of species in Strelitziaceae).

Morphological Characters—Thirty-eight morphological characters were selected and coded (Table 1), based on personal observation and the literature (Phillips 1925; Dyer 1946a, b, c; Perrier de la Bathie 1946; Kress and Stone 1993; Kress et al. 1994; Coates Palgrave and Coates Palgrave 2002). Characters were selected to provide resolution at generic and species level within the family, as well as to differentiate between the Lowiaceae and Strelitziaceae.

Orchidantha fimbriata and *O. siamensis* were selected to represent the Lowiaceae. They are members of the Malay Peninsula clade, in which all species are pollinated by fruit beetles of the family Nitidulidae and characterized by a white or cream-colored labellum and dark purple sepals, and are not as modified as the Borneo clade specialized for dung-beetle pollination (Johansen 2005). Species descriptions in Holtum (1970) and Larsen (1961) and anatomical studies by Pederson and Johansen (2004)

TABLE 1. Morphological characters and character states used in phylogenetic analysis of the Strelitziaceae using *Orchidantha* (Lowiaceae) as the outgroup.

1. Habit: Rhizomatous herb, not shrub-like (0); tree-like with woody stem (1); rhizomatous herb, shrub-like (2).	2. Circumference at the base of main stem (cm): 0–20 (0); 30–45 (1); 50–100 (2).	3. Height of plant (m): ≤ 1 m (0); 1–3 (1); 6–14 (2); 20–30 (3).	4. Root stele: Polyarc (0); with medullary vessels and phloem (1).	5. Length of lamina (cm): ≥ 80 cm (0); ≤ 50 cm (1).	6. Width of lamina at widest point (cm): 5–20 (0); 40–125 (1); 1–4 or lamina absent (2).	7. Shape of mature lamina: Elliptic to elliptic-lanceolate (0); Ovate to oblong/oblong-ovate (1); linear/linear-lanceolate to rounded (2).	8. Leaf base shape: Narrowly decurrent (0); cuneate (to rounded) (1); Truncate to cordate (2).	9. Leaf apex: Acuminate (0); acute, mucronate or cuspidate (1); obtuse (2).	10. Leaf venation: Numerous veins not visible parallel to distinct midrib (0); numerous veins visible parallel to distinct midrib (1).	11. Guard cells on leaves: Symmetrical (0); asymmetrical (1).	12. Internal silica cell bodies druse-shaped: Absent (0); present (1).	13. Petiole length (cm): ≤ 30 (0); 100–200 (1); 300 (2).	14. Peduncle length (from stem to base of spathe/inflorescence bract) (cm): Short: 15–45 (0); long: 120–200 (1).	15. Peduncle diameter (immediately below spathe/inflorescence bract) (cm): 0.7–3 (0); 5–18 (1).	16. Position of inflorescence: Axillary (0); terminal (1).	17. Complexity of inflorescence: Polytelic synflorescence (0); compound (1); simple (2).	18. Maximum number of flowers per spathe/main inflorescence bract: > 10 (0); 6–10 (1); 3–6 (2).	19. Length of spathe/main infl. bract (from tip to base) (cm): 25–45 (0); 10–20 (1).	20. Color of spathe/inflorescence bract: Green/yellow-green (0); Green with maroon/purple edges (1); Purple/maroon (2).	21. Keel of lowest sepal: Not tailed (0); tail-like projection present (1).	22. Color of sepals: Dark purple (0); creamy white (1); orange (2).	23. Petals (fusion of): Not fused (0); two lateral petals connate to enclose anthers (but free at base) (1).	24. Middle (adaxial) petal: Not reduced in size (enlarged to form a labellum in most) (0); small and more or less cuculate (1).	25. Color of petals: White/cream or creamy white with green margins (0); blue (1); purple (2).	26. Length of lateral (abaxial) petals from tip to base (cm): 2–4 (0); 7–11 (1); 14–22 (2).	27. Petal lobes: Absent (0); present and rounded (1); present and sagittate (2).	28. Number of fertile stamens: Five (0); six (1).	29. Anther length: Less than 4 cm (0); more than 4 cm (1).	30. Style: Rigid, rod-like (0); flaccid, lax, filiform (1).	31. Style branches/stigma/lobes: Three (0); six (1); one (2).	32. Capsule: Not woody (0); woody (1).	33. Aril (seed hairs) color: White (0); blue (1); orange/red hairs (2).	34. Pollen: Monads (0); polyads (connected by cellulose threads) (1).	35. Chromosome number: $n = 11$ (0); $n = 9$ (1); $n = 7$ (2).	36. Nectar sugars: Hexose-dominant (0); sucrose dominant (1).	37. Time of anthesis: Diurnal (0); nocturnal (1).	38. Anthesis mechanism: Non-explosive (0); explosive (1).
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provided valuable sources of information, and features were confirmed (where possible) by reference to living plants and herbarium specimens. Interpretation of the inflorescence of *Orchidantha* and inference of homologies with the Strelitziaceae were aided by Kirchoff and Kunze (1995) and Kirchoff (2003). Four inflorescence characters were coded as unknown for *Orchidantha* as it was not possible to examine live flowering material.

Wherever possible, features were examined and measured in the field on living plants of the Strelitziaceae as herbarium specimens are often not representative of the mature plants due to pressing constraints. Living specimens of *Ravenala* and *Phenakospermum* were observed and measured in cultivation at Fairchild Tropical Botanical Garden and the Smithsonian Institute respectively, as well as in the field in Madagascar (*Ravenala*, Kress et al. 1994) and in the Amazon region (*Phenakospermum*, Kress and Stone 1993), respectively. Between three to five mature flowering individuals from each wild population of *Strelitzia* were sampled. (Note: *S. alba* was not flowering at the time.) Voucher specimens were deposited at J, PRE and either NBG or NH. In addition, a representative sample of herbarium specimens (identities confirmed) from BOL, J, NBG, NH, NU, PRE, and SAM were examined and measured. (Herbarium abbreviations as per Holmgren et al. 1990).

The base chromosome number for the Zingiberales is generally accepted as 11, as the Musaceae and most Strelitziaceae are $x = 11$ (Darlinton and Wylie 1955; Small et al. 1980; Manning and Goldblatt 1989; Song et al. 2004)), whereas *Orchidantha* has a base chromosome number of 9 (Larsen 1966; Song et al. 2004). Therefore $x = 11$ was coded as the plesiomorphic state (Table 1), though characters were not ordered in the analysis.

Phylogenetic Analyses—MOLECULAR DATA—Parsimony, maximum likelihood (ML), and Bayesian analyses were performed on the combined data from the plastid regions, the combined nuclear regions, and the combined plastid and nuclear regions. For Bayesian analyses, the data matrix was partitioned according to three partitioning schemes: (1) unpartitioned, (2) a separate partition for each sequenced gene, and (3) separate partitions for markers within each cellular compartment (plastid, nuclear) when relevant. All analyses (parsimony, ML, and Bayesian) were performed on two data sets, one which included all taxa, and one which excluded taxa which were incompletely sequenced for the plastid regions (i.e. the second accession of each of the Strelitziaceae species). Gaps were treated as missing data. In unambiguously aligned regions, parsimony-informative gap characters were scored according to the simple indel ((insertion and deletion) coding method of Simmons and Ochoterena (2000) and added as binary characters to the end of the matrix. All regions were analysed with and without coded indels. The ML analyses were only performed on datasets with indels excluded. Tree topologies and support values from the analyses of the two data sets were compared to evaluate the effect of the missing data. Congruence of the data sets was evaluated using the partition homogeneity test (Farris et al. 1995) performed in PAUP* with heuristic search (1,000 repetitions, 10 random addition sequences) including and excluding indels.

Parsimony and ML analyses were conducted with PAUP* 4.010b (Swofford 2003). Parsimony analyses of separate and combined datasets were performed with characters equally weighted and unordered. Branch and bound searches were performed using the furthest addition sequence. Branch support was evaluated by performing a bootstrap analysis (Felsenstein 1985) using the heuristic search strategy and 1,000 bootstrap replicates, each with 10 random addition replicates. The fit of characters to the trees was measured by the consistency index (Kluge and Farris 1969) and the retention index (Farris 1989) as calculated in PAUP*.

For ML analyses, models were chosen for the plastid data (TVM + G), the nuclear data (HKY + G), and the combined plastid and nuclear data (GTR + I + G) based on the hierarchical likelihood ratio test (hLRT) as implemented in Modeltest v. 3.7 (Posada and Crandall 1998). Models identical to those suggested under the hLRT were identified under the Akaike information criterion (AIC) of Modeltest for the nuclear and the combined plastid-nuclear datasets, but for the plastid regions, the model (GTR + I + G) was identified. Plastid data was analyzed under both suggested models. Heuristic searches with a random order of sequence addition and TBR branch-swapping were repeated 100 times. Bootstrap analyses were performed using 100 replicates, each with 10 random addition sequence addition replicates. Strict consensus trees were computed for maximum parsimony (MP) and ML analyses, rooted for output using *Orchidantha*.

For Bayesian analyses, model selection for each partition was performed using MrModeltest v2 (Nylander 2004). The coded indels were included as a separate data partition, under a modified F81 model (Ronquist and Huelsenbeck 2003). Bayesian inference of phylogeny was performed on the data matrix under each partitioning scheme, with and without indels, using MrBayes v3.2.1 (Huelsenbeck and Ronquist 2001). In each analysis, two analyses of 10 million generations sampled every 1,000 generations were run to completion. In all cases, the two parallel analyses converged (average standard deviation of split frequencies < 0.005). After examining the trace files, the first 10% of the trees were discarded as 'burn in' and a 50% majority rule tree was constructed from the remaining trees.

MORPHOLOGICAL DATA—Fitch parsimony analysis using PAUP* v4.010b (Swofford 2003) was performed on the data matrix (Supplementary Appendix 1) comprising 38 unordered, equally weighted characters. Full heuristic random searches with 100 repetitions, holding one tree each time with TBR swapping, MULPARS and ACCTRAN options in operation. Strict consensus trees for equally most parsimonious (EMP) trees were computed, and bootstrap analysis (1,000 repetitions and 100 random addition sequences) was performed using the same parameters.

COMBINED MOLECULAR AND MORPHOLOGICAL DATA—Parsimony and Bayesian analyses of the combined plastid, nuclear and morphological data matrix (indels included) were performed. A branch and bound maximum parsimony search was performed on the combined (DNA, indels, and morphology) matrix using PAUP* (Swofford 2003). To assess clade support, 1,000 branch and bound bootstrap replicates were performed. The partition homogeneity test (Farris et al. 1995) was performed to assess congruence of these three partitions (heuristic search, 200 repetitions, and 10 random additions). For the Bayesian analysis, the morphological character matrix was included as a separate partition, under the

TABLE 2. Summary of tree statistics for the various data analyses: CI = Consistency Index (excluding uninformative characters), RI = Retention index, EMP = Equally most parsimonious, ML = Maximum likelihood.

Dataset	Number of characters (excl. indels)	Number of parsimony informative characters (excl. indels/incl. indels)	Informative & unambiguous indels	% Missing data	Number of EMP trees (excl. indels/incl. indels)	Tree length (excl. indels/incl. indels)	CI (excl. indels/ incl. indels)	RI (excl. indels/ incl. indels)	Number of ML trees	-Ln
Plastid	5,970	298/340	45	31%	114/114	408/452	0.94/0.95	0.9296/0.9387	6 (both models)	10,317.998
Nuclear	1,845	177/194	17	17%	2/2	253/272	0.92/0.92	0.9281/0.9274	1	3,955.045
Combined plastid & nuclear	7,815	475/534	62	(16.3% for <i>rp(2)</i>) 31%	2/2	662/725	0.93/0.93	0.9274/0.9324	1	14,483.092

Mk1 model of evolution (Nylander 2004). The fully partitioned combined matrix was analyzed using Bayesian methods as described above.

Distribution—A distribution map for *Strelitzia* species was based on wild-collected (i.e. non-cultivated) specimen data from the PRECIS (National Herbarium, Pretoria (PRE) Computerized Information System) data base of the national herbaria PRE, NH, and NBG (Gibbs-Russell and Gonsalves 1984), as well as from wild-collected specimens deposited at BOL, J, and NU and confirmed sightings of *S. juncea* reported by Tony Dold of the Albany Museum herbarium (pers. comm.) and of *S. reginae* by Neil Crouch of SANBI (pers. comm.).

RESULTS

Molecular Data—Results of the separate and combined analyses of the ten plastid and two nuclear regions are summarized in Table 2. Partition homogeneity tests indicated that plastid and nuclear data compartments were compatible ($p = 0.85$ excluding indels; $p = 1.00$ including indels; $p = 1.00$ including indels and morphology) and therefore these data sets were combined. The inclusion of incomplete plastid data for certain taxa in analyses of the plastid data did not affect resolution or placement of taxa in the ML and Bayesian consensus trees, and the *Strelitzia* clade in the parsimony consensus tree was only slightly less resolved (not shown, see Table 3). Support values were comparable among trees resulting from analyses including or excluding incompletely sampled taxa. For the nuclear data sets, it was not possible to confidently align *rpb2* sequences for the outgroup taxa (*Orchidantha fimbriata* and *O. siamensis*), and these data were excluded from analyses. Matrices and final tree files can be accessed on TreeBASE (study number S12275).

A total of 49 indels was identified for the 10 plastid regions and 17 for the two nuclear regions. The majority of indels in the plastid data set (40/49) supported relationships at family level: 27 for the two *Orchidantha* species included here and 13 for the Strelitziaceae. A 27 base pair (bp) insertion in *matK* supported the relationship between *S. caudata* and *S. alba*. In the nuclear data sets, eight (out of 13) indels in the ETS region were informative at family level and two single bp indels (ETS) and a 193 bp deletion in *rpb2* supported the sister relationship between *Ravenala* and *Phenakospermum*. A four bp deletion in *rpb2* also supported the sister relationship of *S. caudata* and *S. alba*. The remaining indels in both plastid and nuclear data sets were synapomorphic for specimens within a species or occasionally autapomorphic.

Within the Strelitziaceae, placement of *Ravenala* and *Phenakospermum* differed among the various analyses and for the different data sets (trees not shown here, see Table 3 for summaries of trees statistics and topologies). The plastid data alone lacked sufficient information to fully resolve relationships within *Strelitzia* or between the genera in the parsimony analyses. *Strelitzia* is not monophyletic in the Bayesian analyses of the nuclear data set including indels,

possibly because *S. nicolai* is grouped with *Ravenala* and *Phenakospermum* by seven synapomorphic point mutations in the ETS region, while three indels support the sister relationship of *Ravenala* and *Phenakospermum*.

Combined Molecular Analyses—Analyses of the combined molecular data set resulted in two most parsimonious trees and a single most likely tree (Table 2). Bayesian analyses of unpartitioned data and data partitioned between compartments (plastid and nuclear) yielded identical topologies. Bayes factor comparisons favored the fully partitioned analyses (2ln Bayes Factor = 1,145.9). Thus, only the results of the partitioned analyses are presented here (Fig. 2).

Two main topologies reflecting generic relationships in the Strelitziaceae result from the analyses of the combined molecular data sets: (i) *Ravenala* is sister to *Phenakospermum* and *Strelitzia* when indels are excluded (MP, ML, and Bayesian analyses; Fig. 2A), and (ii) *Ravenala* and *Phenakospermum* form a clade sister to *Strelitzia* when indels are included (MP and Bayesian analyses; Fig. 2B). Support for either scenario is weak: < 50% MPBS; 85% MLBS and 0.94/0.89 PP for hypotheses (i)/(ii). Within *Strelitzia* topologies are unaffected by inclusion or exclusion of indels, but branch support for certain relationships increases when indels are included. *Strelitzia nicolai* is placed sister to the remaining *Strelitzia* species which form two strongly supported clades (Fig. 2B, C): one comprising *S. alba* and *S. caudata* (99% MPBS, 1.00 PP, 95.5% MLBS) and the other *S. reginae* and *S. juncea* (98% MPBS, 1.00 PP, 99% MLBS). *Strelitzia juncea* is nested within a paraphyletic *S. reginae*, although in the parsimony consensus tree, relationships between the two *S. reginae* accessions are unresolved (not shown here).

Morphological Data—Of the 38 morphological characters included in the analysis, 30 are parsimony informative. Two trees of 62 steps result from the parsimony analysis of the morphological data set (CI excluding uninformative characters = 0.90, RI = 0.90, RC = 0.82) differing only in the placement of *Ravenala* and *Phenakospermum*. In the first tree (Fig. 3A), they are placed sister to each other in a clade supported by three characters (38, explosive anthesis mechanism; 15, peduncle diameter; 31, style branching). In the second tree (Fig. 3B), *Ravenala* is sister to the remaining Strelitziaceae: (*Phenakospermum* (*Strelitzia* species)), with only two characters supporting the clade comprising the rest of the Strelitziaceae, viz. orange/red aril color (33) and anther length (29).

The Strelitziaceae clade is well supported (100% BS) and included among its many synapomorphies are characters commonly recognized as distinguishing the family, viz. its woody capsule (32), long petioles (13), the presence of druse-shaped silica bodies (12) and a root stele with medullary vessels and phloem (4). In addition, the two fused lateral/abaxial petals (23), reduced middle/adaxial petal (24),

TABLE 3. Summary of generic relationships in the Strelitziaceae for the various analyses and support for the branch comprising either (i) *Phenakospermum* (P) and *Strelitzia* (S), or (ii) *Phenakospermum* and *Ravenala* (R).

Analyses/Data sets	Parsimony		Bayesian		Maximum likelihood (No indels)
	Excl. indels	Incl. indels	Excl. indels	Incl. indels	
Combined plastid, nuclear & morphological data	(R (P, S)) BS < 50%	((R, P) S) BS < 50%	(S(R, P)) PP = 0.97	(S(R, P)) PP = 0.98	-
Combined plastid and nuclear	(R (P, S)) BS < 50%	((R, P) S) BS < 50%	(R (P, S)) PP = 0.89	(S(R, P)) PP = 0.94	(R (P, S)) BS = 85%
Plastid data	Unresolved	Unresolved	(R (P, S)) PP = 0.83	(R(P,S)) PP = 0.84	(R (P, S)) BS = 73%
Nuclear data	(P (R, S)) BS < 50%	(P (R, S)) BS < 50%	(R (P, S)) PP = 0.52	(S(Sn(R,P))) PP = 0.85	(R (P, S)) BS = 59%

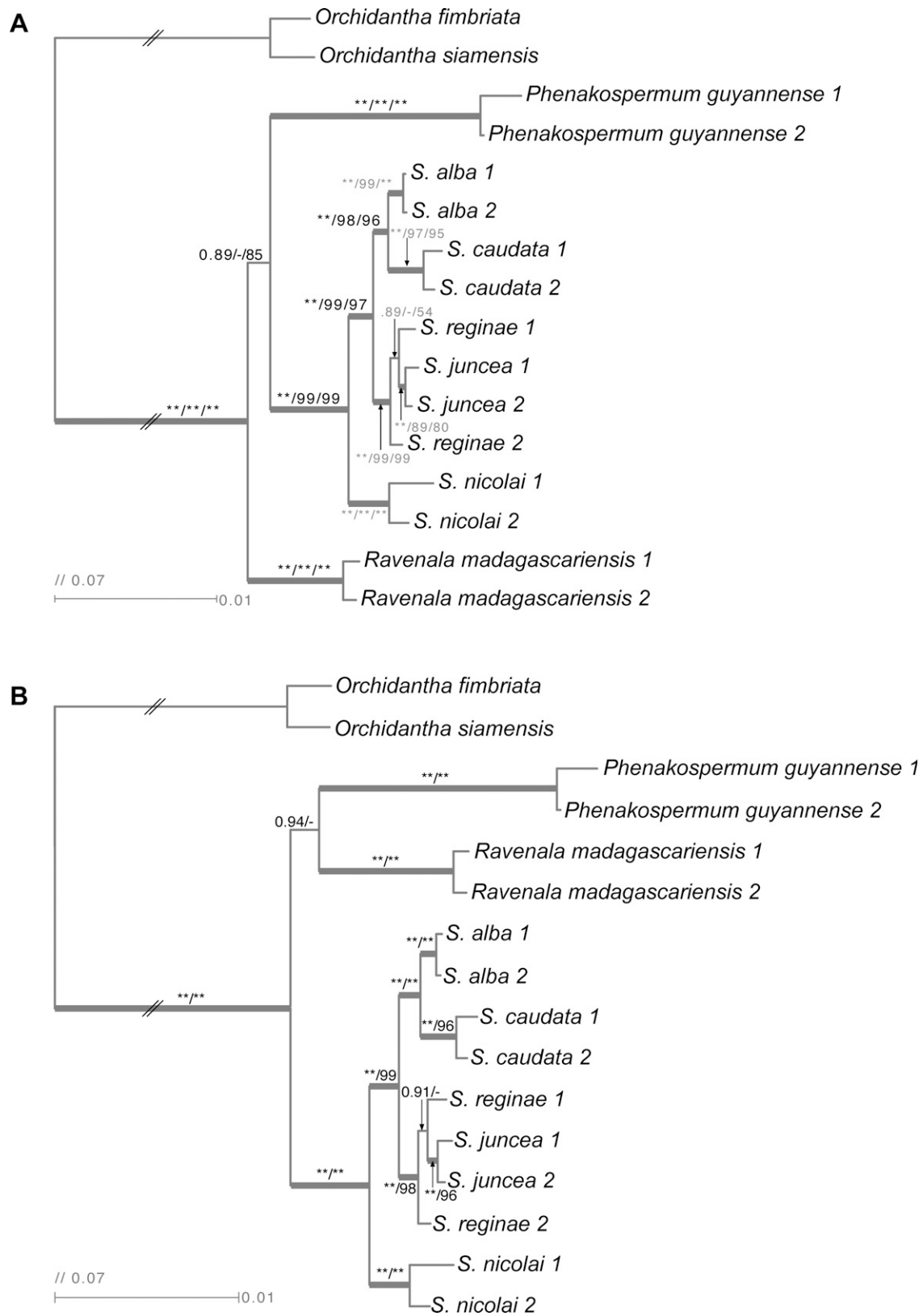


FIG. 2. Phylogenies of the Strelitziaceae resulting from Bayesian analyses of combined chloroplast (10) and nuclear (two) regions/data sets (note *rpb2* only included for *Strelitzia*), (A) excluding and (B) including indels. Support values: (A) Posterior Probability/MP bootstrap/ ML bootstrap; (B) Posterior Probability/MP bootstrap. Note: ** = 1.00/100%.

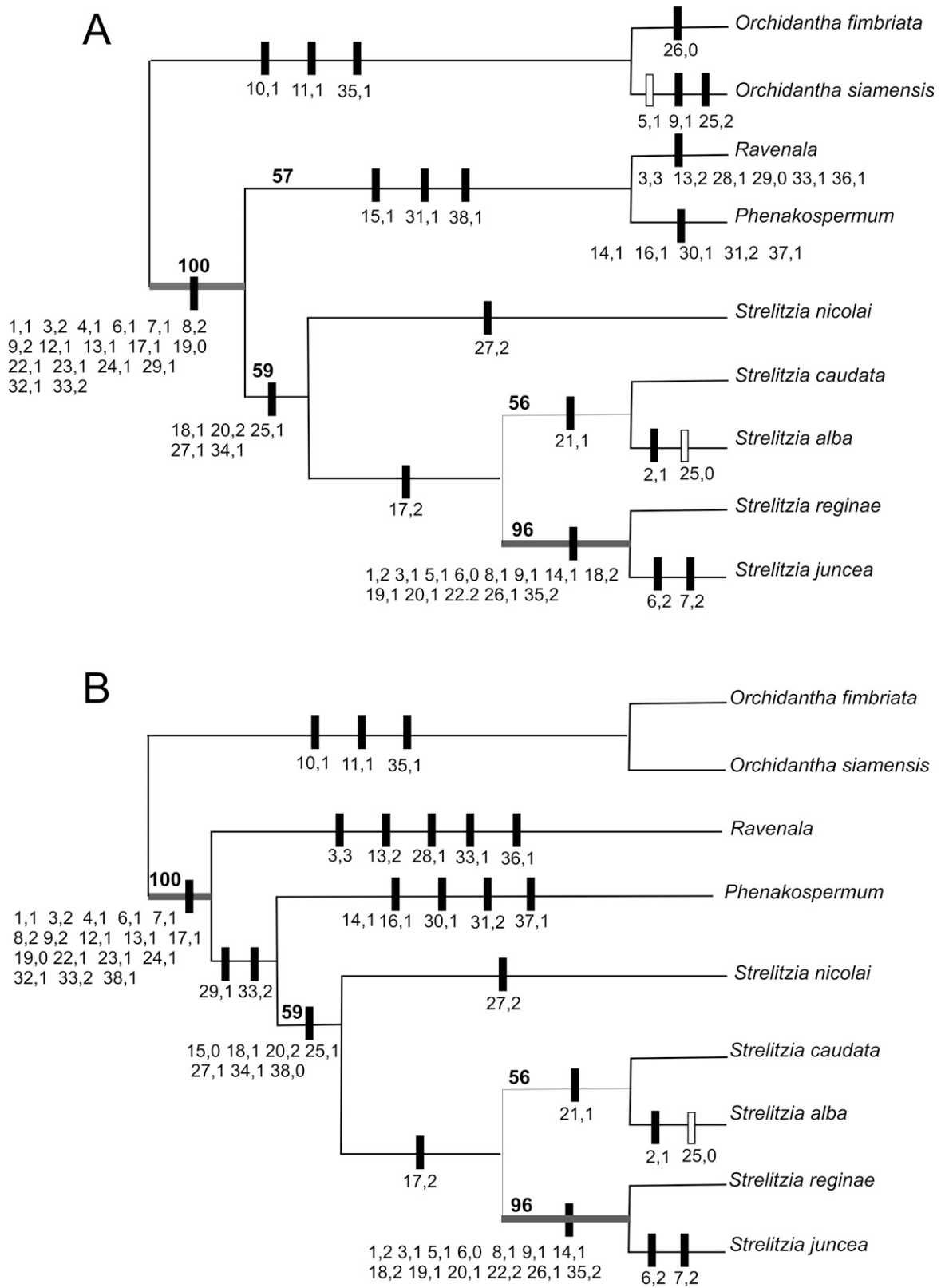


FIG. 3. Two EMP trees resulting from parsimony analysis of 38 morphological characters in the Strelitziaceae, with *Orchidantha* (Lowiaceae) as outgroup (62 steps, CI = 0.90, RI = 0.90, RC = 0.82). Solid bars = non-homoplasious apomorphies, unfilled bars = homoplasious apomorphies. Bootstrap support above branches.

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and the presence of rounded or sagittate lobes near the base of the abaxial petals (27) are shown to be diagnostic characters for the group.

Within the Strelitziaceae, *Ravenala* has six (Fig. 3A) or five (Fig. 3B) autapomorphies and *Phenakospermum* has five autapomorphies (Fig. 3A, B). The genus *Strelitzia* is relatively well supported (78% BS) with five (Fig. 3A) or six (Fig. 3B) characters distinguishing the genus. The sister relationship of *S. caudata* and *S. alba* is only weakly supported (56% BS), united by the presence of a tail-like projection on the keel of the lowest sepal (21,1). The clade comprising the remaining species of *Strelitzia* is only weakly supported (BS < 50%) by the possession of a simple inflorescence (vs. compound in *S. nicolai*). No morphological autapomorphies are evident in *S. caudata* in this analysis, and only one and two autapomorphies in *S. nicolai* and *S. alba*, respectively. *Strelitzia reginae* and *S. juncea* are strongly supported as sister species (BS 96%). Characters supporting this relationship include those related to its shrub-like habit (characters 1 and 3), number of flowers per spathe (18), length and color of spathe bracts (19 and 20), the color of sepals (22), chromosome number (35), and shorter lateral petals (26). Only lamina shape and width (characters 6 and 7) distinguish *S. reginae* and *S. juncea*.

Combined Molecular and Morphological Data—Analysis of the morphological data with the molecular data favored

the topology in which *Strelitzia* is placed sister to *Ravenala* and *Phenakospermum* as this resulted from Bayesian analyses including and excluding indels, as well as from the parsimony analysis including indels (Fig. 4). Support for these relationships is weak (< 50% MPBS) to moderate (PP = 0.99 excluding indels and 0.98 including indels). In contrast, *Ravenala* was placed sister to *Phenakospermum* and *Strelitzia* in the parsimony analysis excluding indels (not shown here), as was the case in the molecular analysis excluding indels. Support for all clades within *Strelitzia* is strong: 1.0 (PP) or 100% (MPBS) (Fig. 4). As in previous analyses, *Strelitzia nicolai* is sister to a clade comprising the other *Strelitzia* species. *Strelitzia alba* and *S. caudata* are resolved as sister species in a strongly supported clade, although the only morphological synapomorphy supporting this relationship is the tail-like projection on the keel of the lowest sepal (Fig. 3), noted to be larger in *S. caudata* but present in both species (Dyer 1946b, c). Nonetheless, 12 non-homoplasious molecular synapomorphies support their sister relationship, including two indels.

DISCUSSION

Relationships Within the Strelitziaceae—Generic relationships within the Strelitziaceae remain equivocal: *Strelitzia* is sister to a clade comprising *Ravenala* and *Phenakospermum* in

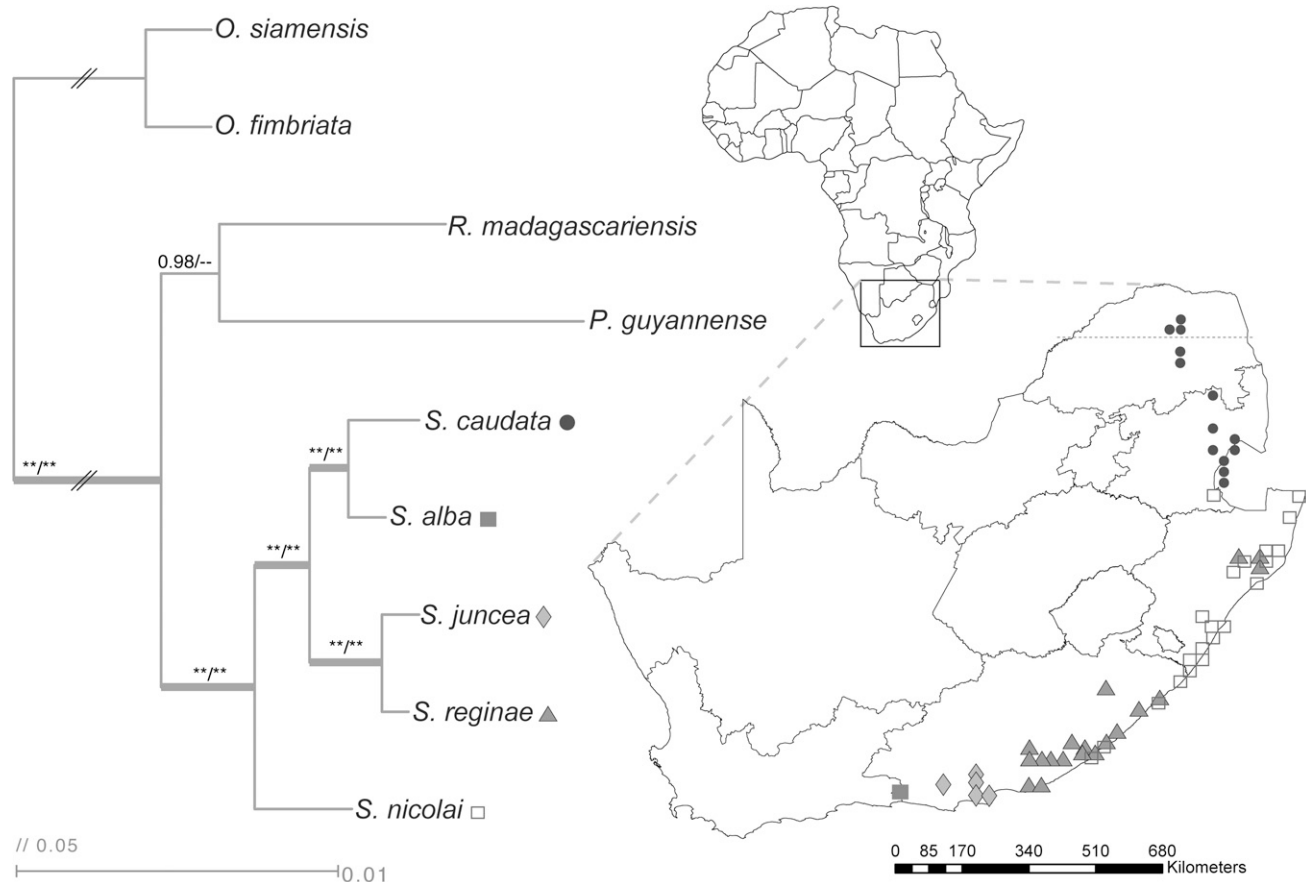


FIG. 4. Single most parsimonious tree and Bayesian consensus tree resulting from phylogenetic analysis of combined molecular (10 chloroplast and two nuclear regions; *rpb2* only included for *Strelitzia*; including indels) and morphological data for the Strelitziaceae with *Orchidantha* (Lowiaceae) as outgroup. Numbers above branches: Bayesian posterior probability/maximum parsimony bootstrap values (** = 1.00/100%). Distribution of *Strelitzia* species (in South Africa) shown alongside. Dashed gray line indicates the Tropic of Capricorn. (Note: Occurrence of *S. caudata* in the eastern highlands of Zimbabwe not shown here.)

the phylogenies resulting from the combined plastid and nuclear analyses including indels (Fig. 2B) and in the 'total evidence' analysis (Fig. 4), with statistical support stronger when morphological data are included. It is also one of two topologies resulting from the analysis based on morphological data (Fig. 3A). This is consistent with the phylogeny based on the plastid gene *ndhF* (Givnish et al. 2006). Two single bp indels in the ETS region and a large deletion (193 bp) in the *rpb2* region support the sister relationship of *Ravenala* and *Phenakospermum*. Although Simmons et al. (2001) found that longer deletions were not always better phylogenetic characters than shorter deletions, it seems unlikely that two such closely related genera would have obtained such a large deletion independently.

In contrast, *Ravenala* is sister to the remaining Strelitziaceae in the combined molecular analysis excluding indels (Fig. 2A), although bootstrap and posterior probability values are relatively low for the relationship here. This is similar to most previous studies, which also placed *Ravenala* sister to the rest of the Strelitziaceae (Smith et al. 1993; Chase et al. 2000; Soltis et al. 2000; Kress et al. 2001; Soltis et al. 2007), an hypothesis that also agrees with the alternate topology resulting from parsimony analysis of the morphological data (Fig. 3B). In previous analyses, *rbcl* and 18S have proven to be uninformative in resolving generic relationships among Strelitziaceae (Kress et al. 2001) and signal from the morphology partition appears to have played a key role in placing *Ravenala* sister to the rest of Strelitziaceae in the combined analysis of Kress et al. (2001). In contrast, *atpB* weakly supports *Phenakospermum* as sister to a clade comprising *Ravenala* and *Strelitzia* (Kress et al. 2001), as in the consensus tree resulting from the parsimony analyses based on nuclear data only in this study (not shown, Table 3). The molecular markers selected for this analysis are more rapidly evolving than those used in previous studies, yet there is lack of congruence when indels are included or excluded. Inclusion of coded indels and morphological characters with the sequence data supports the hypothesis that *Strelitzia* is sister to a clade comprising the remaining two genera (Fig. 4). Indels have been shown to provide valuable additional data when coded accurately (e.g. Hilu and Lawrence 1999; Simmons et al. 2001; Ingvarsson et al. 2003; Lang et al. 2006), although they may be prone to homoplasy in certain regions and/or groups of plants (e.g. the *trnL* intron of the Pomaderreae, Rhamnaceae, Kellermann and Udovicic 2006). Nonetheless, indels have been found to have significantly less homoplasy than base characters in a survey of 38 sequence-based phylogenetic analyses (Simmons et al. 2001) and are considered here to be useful characters.

Strelitzia is confirmed as a monophyletic group and the caulescent species appear to be good species (Figs. 2–4) according to the phylogenetic species concept (PSC) of Cracraft (1983: 170), where a species is 'the smallest diagnosable cluster of individuals within which there is a parental pattern of ancestry and descent.' Despite few morphological differences between *S. nicolai*, *S. alba*, and *S. caudata*, we found high molecular support (posterior probabilities of 1.00 and MP bootstrap values of 98–100%) for the relationships and taxonomic status of these taxa (Figs. 2, 4), although additional sampling within species is desirable to confirm these results. *Strelitzia alba* and *S. caudata* are separated geographically (Fig. 4) and it is apparent that they have diverged at a molecular level with minimal reproductive isolation via morphological change.

The specific status of *Strelitzia juncea* remains uncertain. It is nested within *S. reginae* (Fig. 2) and the lack of a morphological synapomorphy for *S. reginae* (Fig. 3) suggests a paraphyletic *S. reginae* from which *S. juncea* arose. This could support the contention that *S. juncea* may simply be a geographically restricted lineage of *S. reginae* and should be reduced to subspecies or varietal status. However, as *S. juncea* appears to be monophyletic within *S. reginae*, it could have recently diverged from *S. reginae*, which has not as yet evolved a distinct apomorphy. This paraphyletic relationship is not uncommon (Kornet and McAllister 1993; Rieseberg and Brouillet 1994; Crisp and Chandler 1996; Brummit and Sosef 1998; Brummitt 2002; Zander 2007) and this is a classic example of a division into descendants with asymmetric population sizes, and geographic proximity (Fig. 4). As noted previously, the taxonomic status of these species has been extensively debated. *Strelitzia juncea* was originally described as a distinct species by Link in 1821, but Moore and Hyypio (1970) proposed that *S. juncea* should be reduced to a cultivar of *S. reginae*. However, Van de Venter observed developmental differences in the leaves of seedlings of *S. reginae* and *S. juncea* grown in a controlled environment (Van de Venter et al. 1975; Dyer 1975). Based on the assumption that genetic differences governed leaf development, and on their distinct geographic locales and habitats, the retention of the taxa as distinct species was supported (Dyer 1975) but is not universally accepted today. Van de Venter's suggestion that *S. juncea* arose by mutation from *S. reginae* (Dyer 1975) appears to be supported by the results here. It should also be noted that hybrids between the species have also been observed and artificially created (Small et al. 1980). A more detailed population level study is necessary to assess any ongoing gene flow among populations, as *S. juncea* could represent a case of incipient speciation.

Evolutionary Trends in the Strelitziaceae—CHANGE IN HABIT—There has been a change from a pachycaul or arborescent to a shrub-like herbaceous habit within the Strelitziaceae that is likely linked to habitat change. *Phenakospermum* and *Ravenala* both grow principally in secondary rainforest, to which a pachycaul habit seems suited, as it does to the coastal subtropical or temperate forests of South Africa where *Strelitzia nicolai* and *S. alba* occur, respectively. The pachycaul habit also seems suited to the montane forests occupied by *S. caudata*. The major phenological shift evident in the growth form of *S. reginae* and *S. juncea* (Fig. 3A,B), i.e. the change to an herbaceous (yet shrub-like) habit, with underground rhizome and no main erect stem, was likely linked to a change in habitat. *Strelitzia reginae* grows along river banks, in coastal bush and thicket mainly in the Eastern Cape (Goldblatt and Manning 2000) with disjunct populations known from Zululand (N. Crouch pers comm.) and the Transkei region of South Africa (Van Jaarsveld and Loedolff 2007). *Strelitzia juncea* grows in association with drought resistant plants like *Encephalartos horrida* Lehm. and a variety of succulents, such as *Euphorbia* L., *Crassula* L., and *Cotyledon* L. (Dyer 1979). The loss of lamina would likely have adapted *S. juncea* to drought conditions (Cowling et al. 2005) and possibly reduced its sensitivity to frost.

There has also been a decrease in size and complexity of the inflorescence in the Strelitziaceae: the multiple-tiered (compound) inflorescence seen in *Ravenala*, *Phenakospermum*, and *Strelitzia nicolai* is reduced to a 'simple' inflorescence with a single spathe in the other four *Strelitzia* species (Figs. 1, 3).

Peduncle length has increased together with the change in growth form in *S. reginae* and *S. juncea* (Fig. 3). The inflorescence has been thus raised to vegetation height, which is necessary, as it is no longer emerging from a leaf axil on a tall, erect stem. There is also a reduction in the number of flowers produced per cincinnus (Fig. 3), possibly to reduce the weight on the lengthened peduncle.

POLLINATION SYNDROMES—Our results suggest that pollination features associated with an unspecialized pollinator, or possibly birds, are plesiomorphic in the Strelitziaceae, with subsequent specialization to prosimians (lemurs) in *Ravenala*, to bats in *Phenakospermum*, and further specialization to birds in *Strelitzia*. According to the hypothesis that *Phenakospermum* and *Ravenala* form a clade sister to *Strelitzia*, the common ancestor of *Phenakospermum* and *Ravenala* could possibly have been less specialized with subsequent modification in the separate lineages as they evolved. Certainly bats are also able to utilize and pollinate *Ravenala madagascariensis* in cultivation outside of Madagascar (Calley et al. 1993), and sunbirds have also been observed to visit *Ravenala* in Madagascar (Kress et al. 1994; Birkinshaw and Colquhoun 1998). Birkinshaw and Colquhoun (1998) argue that bats could be effective pollinators of *Ravenala*, but their numbers have been reduced in Madagascar and their feeding behavior modified by the advent of fruit tree plantations. The creamy white flowers suggest a nocturnal pollinator and maximum nectar production occurs during the first night of anthesis (Calley et al. 1993), although it is subsequently secreted both night and day (Kress et al. 1994).

Kress et al. (1994) considered the pollination features of *Ravenala* to be plesiomorphic in this lineage (as did Lane 1955), a notion consistent with the hypothesis of Sussman and Raven (1978) that pollination by non-flying mammals is archaic and has apparently persisted in this case in Madagascar, an isolated region that is depauperate in flying vertebrate floral visitors of any significance. This hypothesis of archaic non-flying vertebrate pollinators is also consistent with the topology resulting from combined phylogenetic analyses in Kress et al. (2001) with the topologies resulting from combined molecular analyses excluding indels here (Fig. 2A), and one of two the morphological trees (Fig. 3B).

Phenakospermum and *Ravenala* share features such as large inflorescences, dull green bracts, a creamy white perianth, explosive floral mechanism, and relatively short-lived flowers. Subsequently unique features characteristic of each genus may have co-evolved with their animal visitors in situ (Kress and Stone 1993). The terminal inflorescence held above the leaves on a long peduncle (Fig. 1B), and the flaccid style with a single stigma lobe are autapomorphies for *Phenakospermum* (Fig. 3). In contrast, *Ravenala*'s large inflorescences are borne in the axils of the leaves (i.e. below the crown, Fig. 1A) and are therefore accessible to arboreal animals (lemurs) that visit for the copious sucrose-dominant nectar (Kress et al. 1994). The creamy-white flowers with their stiff, rod-like styles are protected by the tough bracts and require a strong pollinator to open them (Kress et al. 1994) or they may open by touch or even unaided (Calley et al. 1993).

Bird pollination is common to all strelitzias and is linked to the hexose-dominant nectar and the blue petal color [due to anthocyanins in the petal epidermal cells (Kronstedt and Walles 1986)] seen in all but *S. alba*, where there has been a reversal to white petals (Fig. 3). Both features are associated

with flowers pollinated by passerine perching birds (Cronk and Ojeda 2008). Within *Strelitzia*, however, there have been some changes in the pollination syndrome and the specific pollinator responding to it. Sunbirds are known to pollinate the widespread *Strelitzia nicolai* (Gibson 1975; Frost and Frost 1981; Nichols 2007) and possibly also white eyes (Gibson 1975). Sunbirds have been observed sitting on the portion of the blue petals enclosing the anthers in contact with the sticky pollen (Nichols 2007). In contrast, weaver birds have been shown to be the pollinators of *S. reginae* (Rowan 1974; Skead 1975; Coombs and Peter 2009). Weavers alight on the fused blue petals and make contact with the anthers and the stigma (Rowan 1974; Skead 1975; Coombs et al. 2007; Coombs and Peter 2009), and although sunbirds frequently visit the flowers of *S. reginae*, they are nectar thieves and seldom come into contact with the pollen on the anthers as they sit on the spathe (Coombs and Peter 2009). The change from white to orange sepal color in the clade comprising *S. reginae* and *S. juncea* (Fig. 3) may be the attractant for the weaver birds, where the orange color is mainly due to a high concentration of carotenoids in elongated chromoplasts (Simpson et al. 1975; Kronstedt and Walles 1986). This change could be related to the change in habitat, where orange is more noticeable in a thicket than white, which would be highly visible in a forest. The flowers on their long peduncles are also presented more conspicuously in *S. reginae* and *S. juncea* than in the arborescent species.

DISPERSAL—Arils appear to have evolved a number of times to be instrumental in attracting frugivores in the Zingiberales, most notably in the 'ginger group': Costaceae, Zingiberaceae, and Marantaceae (Heywood et al. 2007) where myrmecochory is common (Andersson 1998; Larsen et al. 1998; Lengyel et al. 2010). The brightly colored aril of the Strelitziaceae serves to attract birds (e.g. tinkerbirds, doves, bulbuls, barbets; Frost and Frost 1981; Nichols 2007) for seed dispersal and its appeal is linked to its high energy food value. The high lipid content of the arils makes the seeds attractive to frugivores: the dry mass of the aril of *Strelitzia nicolai* is 67% lipid (Frost and Frost 1980), while that of *Phenakospermum* and *Ravenala* is over 50% (C. Pirone, unpubl. data). The aril is bright orange in *Strelitzia* and *Phenakospermum* (Fig. 1B), but blue in *Ravenala* (Fig. 3). The orange color has recently been shown to be primarily due to bilirubin, a compound previously known only in animals as the breakdown product of haem (Pirone et al. 2009, 2010), and it persists for years after cell death (D. Lee and C. Pirone, pers. obs.). Although bilirubin is found at low concentrations among other angiosperms (Pirone et al. 2010), it is not known to contribute to color in any species outside of the Strelitziaceae. In contrast to the Strelitziaceae, the aril of the Lowiaceae seems to play no role in dispersal of the seeds. It is fragile and not well attached to the large seeds, which are therefore not well adapted for long distance dispersal by the small rodents that possibly collect them (Johansen 2005).

Biogeography—Hypotheses concerning the likely drivers of speciation in *Strelitzia* require consideration of the vegetation changes over time in southern Africa. At the time of the initial divergence into the ancestor of *Ravenala* and *Phenakospermum* and the ancestor of *Strelitzia* (during the Eocene epoch 58–40 mya, Kress and Specht 2006), the mixed gymnosperm-angiosperm forest (Scott et al. 1997) associated with the warm, temperate, moist climate of the Mid-Cretaceous was replaced by one more suited to the cooler and drier

conditions of south-central Africa at that time (Scotese 2001). This was either a dry forest with an understory of Cape elements (Scholtz 1985) or possibly an early thicket mosaic comprising an araucarian woodland with patches of closed forest/thicket in a matrix of fynbos (Cowling et al. 2005).

In addition to being pachycaulous (and well suited to the forests described above), the ancestor of *Strelitzia* was likely more widespread than the current distribution of the genus suggests. All species of *Strelitzia* are currently distributed along the eastern seaboard and/or mountains of southern Africa (Fig. 4), similar to the distribution pattern of four species of *Clivia* (Amaryllidaceae). The discovery of a fifth, disjunct species, *C. mirabilis*, in an isolated canyon in the arid Northern Cape (Rourke 2002), and sister to the summer rainfall species of *Clivia* in the east/northeast of South Africa (i.e. similar distribution pattern to *Strelitzia*), supports a hypothesis that the genus previously occupied a wider range spanning the Eastern and Western Cape during a moister climatic regime (Conrad et al. 2003). We hypothesize a similar scenario for *Strelitzia*.

We propose that climate change in southern Africa restricted the ancestral *Strelitzia* species to the eastern coastal regions of South Africa. From the late Miocene onwards, the climate of southern Africa became rapidly colder and drier and by end of the Pliocene, dense woodlands (or thicket) in the interior were replaced by more open savanna and grassland (Vrba 1985). Climatic fluctuations of the Pleistocene resulted in extended cold and dry conditions alternating with briefer warmer and wetter spells, and the contraction (glacials) and expansion (interglacials) of thicket vegetation (Cowling et al. 2005). The east-west climatic gradient, likely to have been first established across southern Africa during the latter part of the Miocene, apparently persisted through much of the Pliocene (Partridge 1997). The late Pliocene cooling resulted in greater aridity. This would have resulted in retreat of the dry forest or forest/thicket mix towards the east of the subcontinent, where all species of *Strelitzia* are currently found. *Strelitzia nicolai*, for example, currently grows naturally in coastal forest and/or thicket in the north eastern part of South Africa (Fig. 4), in regions where subtropical evergreen forest once dominated (Axelrod and Raven 1978).

Subsequent divergence from the common ancestor with *Strelitzia nicolai* resulted in two lineages (Figs. 2–4) one comprising *S. alba* and *S. caudata*, where the pachycaul/arborescent habit was retained, and the other the herbaceous *S. reginae* and *S. juncea* form. The temperate forest in which *S. alba* occurs near Plettenberg Bay and Knysna in the Western Cape is also a remnant of a much more widespread forest in more mesic conditions (Axelrod and Raven 1978), as inferred from late Pleistocene pollen records studied by Schalke (1973) and reviewed by Livingstone (1975) and van Zinderen Bakker (1976). The increased cooling and aridity during the late Pliocene may have resulted in the restriction of temperate forest to the southern region in the Western Cape where *S. alba* is found (Fig. 4). This could reflect a vicariant event, in which the ancestor of *S. alba* and *S. caudata* was separated from that of *S. nicolai* as the forest retreated. Subsequent allopatric speciation likely occurred via dispersal northwards by the ancestor of *S. caudata* to the northeastern mountains of South Africa and ultimately as far north as the eastern highlands of Zimbabwe. The montane regions in which *S. caudata* occurs are associated with the afro-

forest patches in the Chimanimani-Nyanga/Vumba mountains of Zimbabwe, as well as the Soutpansberg (Limpopo Province) and the mountains around Barberton in Mpumalanga, South Africa (Fig. 4). These forests may have retreated to these montane refugia as the climate over southern Africa became more arid. These hypotheses could be tested by estimating divergence times within *Strelitzia* in the context of a broader phylogenetic analysis.

The herbaceous lineage comprising *Strelitzia reginae* and *S. juncea* occurs mainly in the Albany region of the Eastern Cape (Fig. 4). *Strelitzia reginae* is commonly found along river banks in the Valley Bushveld (Acocks 1988) or more specifically in the Great Fish Noorsveld, Kowie Thicket, and Albany Coastal Belt (Hoare et al. 2006). Thicket, especially the non-succulent forms that include *S. reginae*, appears to have strong floristic affinities with the Tongaland-Pondoland subtropical forests along the eastern coast of southern Africa (White and Moll 1978; Cowling 1983). This thus includes the area where *S. nicolai* currently occurs. Although Cowling et al. (2005) argue for an ancient (Eocene) origin of the thicket biome, the ancestor of *S. reginae* probably originated at a later date, either through dispersal or vicariance as the thicket was replaced by fynbos to the southwest and savanna to the north (Cowling et al. 2005). *Strelitzia juncea* is likely a recent parapatric (or possibly sympatric) divergence from *S. reginae*, slightly inland to the drier succulent thicket habitat.

Conservation—Four species of *Strelitzia* are currently listed as ‘Least Concern’ in the recent assessment of threat levels to the South African flora (Raimondo et al. 2009), but we here note our concern for *S. alba*. *Strelitzia alba* is currently confined to two (or possibly three) small populations in the Garden Route region of the Western Cape, and neither mature flowers nor evidence of past flowering have been observed for a number of years (D. McCallum pers. comm.). The health of the plants in the populations is also in question, as they seem moribund and the populations’ size is likely declining. Our concern for these wild populations of *S. alba* is mirrored by Xaba (2010) who notes that many of the suckers and any seeds produced are harvested by collectors before being able to germinate and/or establish themselves. This species urgently needs to be reassessed in terms of threats to its continued existence, and efforts to cultivate it more widely should be pursued.

Strelitzia juncea has been assessed as vulnerable [VU B1ab (ii,iii,v)] as it is known from only six populations in the wild (Schutte-Vlok et al. 2009), and only one of these (from near Uitenhage) is extensive (Dyer 1979). Although widely cultivated, the wild populations of *S. juncea* are declining because of quarrying and illegal horticultural trade, and are threatened by invasive alien plants (Schutte-Vlok et al. 2009). It is therefore important to establish the status of *S. juncea* more conclusively with more intensive sampling at the population level and to increase levels of protection for the wild populations.

In summary, generic relationships within the Strelitziaceae remain equivocal with two fairly weakly supported hypotheses: *Strelitzia* as sister to *Ravenala* and *Phenakospermum* or *Ravenala* sister to *Phenakospermum* and *Strelitzia*. Within *Strelitzia*, relationships are clearly resolved. Of taxonomic importance is the conclusion that *Strelitzia reginae* is paraphyletic with *S. juncea* and this could lead to it being considered a subspecies or variety thereof, maintaining its conservation status. Further work is needed to establish levels of gene flow between populations of *S. reginae* and *S. juncea*.

The highly localized *Strelitzia alba* is shown to be sister to *S. caudata* in a clade sister to *S. reginae*, with *S. nicolai* sister to the rest of the genus. The ancestor of *Strelitzia* is hypothesized to have been more widespread than the current distribution of the genus suggests, with climatic and associated vegetation changes during the Miocene and/or Pliocene resulting in a retraction of range to the eastern seaboard of southern Africa, with subsequent recent diversification into different habitats of local regions.

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- APPENDIX 1. List of specimens investigated: Taxa, voucher, locality information, and Genbank numbers: *matK-5'trnK*, *psbA-matK*, *psbB-psbH*, *rp132-trnL*, *rpL16* intron, *rpoB-trnC*, *rps16* intron, *trnS-trnFM*, *Ycf6-trnC*, *ETS*, *rpb2*. (SA = South Africa.)
- Orchidantha fimbriata* Holtum, *Jestrow s. n.* FTG 144233, Malaysia, JQ027153, JQ027171, JQ027180, JQ027216, JQ027189, JQ027198, JQ027162, JQ027225, JQ027207, -, -; *Orchidantha siamensis* K. Larsen, W. J. Kress 3406798 (US), southern Thailand, JQ027154, JQ027172, JQ027181, JQ027217, JQ027190, JQ027199, JQ027163, JQ027226, JQ027208, -, -; *Phenakospermum guyanense* (L. C. Rich) Endl. ex Miq, W. J. Kress & D. E. Stone 86-2099 (DUKE, SEL), French Guiana, en route to Montsinery, JQ027155, JQ027174, JQ027183, JQ027219, JQ027191, JQ027201, JQ027164, JQ027228, JQ027210, JQ027236, JQ027255; *Lorence & Bartlett* 047865 (PTBG), ex hort., JQ027248, -, -, -, JQ027277, -, JQ027267, -, -, JQ027242, JQ027260; *Ravenala madagascariensis* Sonn., W. T. Gillis 32480 (FTG), ex hort., JQ027156, JQ027173, JQ027182, JQ0272218, JQ027192, JQ027200, JQ027165, JQ027227, JQ027209, JQ027237, JQ027256; *Driscoll* 85.0756 (UCBG), ex hort., JQ02747, -, -, -, -, JQ027266, -, -, JQ027241, JQ027259; *Strelitzia alba* (L. f.) Skeels, *Chetty & Haw* K19A (J), SA, Western Cape, Nature's Valley, JQ027159, JQ027178, JQ027188, JQ027224, JQ027197, JQ027206, JQ027170, JQ027233, JQ027215, JQ027239, JQ027252; *Chetty & Haw* 20B (J), SA, Western Cape, Nature's Valley, JQ027249, -, -, -, -, JQ027268, -, -, JQ027243, JQ027261; *Strelitzia caudata* R. A. Dyer; *Chetty* 01A (J), SA, Mpumalanga, Nature's Rift, JQ027161, JQ027179, JQ027187, JQ027223, JQ027196, JQ027205, JQ027269, JQ027232, JQ027214, JQ027240, JQ027253; *K. sBalkwill* 12512 (J), SA, Mpumalanga, Moederlief, JQ027274, -, -, -, JQ027276, -, -, -, JQ027273, JQ027164; *Strelitzia juncea* Link, *Jestrow* 144319 (FTG), ex hort., JQ027158, JQ027175, JQ027186, JQ027222, JQ027195, JQ027204, JQ027167, JQ027231, JQ027213, JQ027234, JQ027258, JQ027244; *Chetty & Haw* 15A (J), SA, Eastern Cape, Uitenhage, JQ027250, -, -, -, JQ027279, -, JQ027270, -, -, JQ027244, JQ027263; *Strelitzia nicolai* Regel & Körn, *Jestrow s. n.* FTG 100573, ex hort., JQ027157, JQ027177, JQ027185, JQ027220, JQ027194, JQ027202, JQ027168, JQ027229, JQ027211, JQ027235, JQ027254; *Chetty & Haw* 11B (J), SA, KwaZulu-Natal, Port Edward, -, -, -, JQ027281, -, JQ027272, -, -, JQ027246, JQ027265; *Strelitzia reginae* Aiton, *Jestrow s. n.* FTG 144320, ex hort., JQ027160, JQ027176, JQ027184, JQ027221, JQ027193, JQ027203, JQ027166, JQ027230, JQ027212, JQ027238, JQ027257; *Chetty & Haw* 13A (J), SA, Eastern Cape, East London, JQ027251, -, -, -, JQ027280, -, JQ027271, -, -, JQ027245, JQ027264.