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PHYLOGENY OF BRUNIACEAE BASED ON *mat*K AND ITS SEQUENCE DATA

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Bruniaceae are subendemic to the Cape Floristic Region and represent a characteristic element of the prevalent fynbos vegetation. Their position in the angiosperm system, as well as the intergeneric and infrageneric relationships, has remained unclear. In this study, the phylogeny of Bruniaceae has been reconstructed on the basis of *mat*K and internal transcribed spacer sequences. Molecular evidence clearly places *Linconia* as the sister to the rest of the family. We propose to divide the family into three tribes, the basal Linconieae (with *Linconia* only) and the former two subdivisions of the family, Audouinieae (*Audouinia*, *Thamnea*, *Tittmannia*, including *Pseudobaeckea teres*) and Brunieae (remaining nine genera except *Linconia*). The genera *Berzelia*, *Brunia*, *Pseudobaeckea*, *Raspalia*, *Thamnea*, and *Tittmannia* are not monophyletic and require new taxonomic circumscriptions.

Keywords: Bruniaceae, *mat*K, ITS, molecular systematics.

Introduction

The small southern family Bruniaceae is endemic to the Cape Floristic Region (CFR), with only one species, *Raspalia trigyna*, as an outlier in the province KwaZulu-Natal. In the prevalent fynbos vegetation, Bruniaceae form a characteristic element. Adhering to the taxonomy of the most recent revision of Bruniaceae (Pillans 1947), the family comprises 75 species arranged in 12 genera: *Audouinia* (monotypic), *Berzelia*, *Brunia*, *Linconia*, *Lonchostoma*, *Mniothamnea*, *Nebelia*, *Pseudobaeckea*, *Raspalia*, *Staavia*, *Thamnea*, and *Tittmannia*. Since then, three more species have been discovered: *Lonchostoma esterhuyseniae* (Strid 1968), *Tittmannia esterhuyseniae* (Powrie 1969*a*), and *Linconia ericoides* (Oliver 1999).

Representatives of Bruniaceae are considered long-term "palaeoendemics" (Hall 1987, 1988; Carlquist 1991), i.e., taxonomically isolated descendants from an ancient stock without close relatives in proximity. Their apparent distinctness from other angiosperm taxa has complicated the search for plant groups closely allied to Bruniaceae. To date, the proposed affinities of Bruniaceae within the angiosperms have been varied. Bruniaceae have been placed in Rosales s.l. (Hallier 1912; Cronquist 1981), Hamamelidales (Hutchinson 1969), Saxifragales (Takhtajan 1980), or Pittosporales (Thorne 1976, 1983). Dahlgren and van Wyk (1988) postulated a sister relationship to Grubbiaceae (also endemic to the CFR), while Scott (1999) suggested Epacridaceae (Ericales) as the closest relative to Bruniaceae. Recent molecular data (Savolainen et al. 2000; Soltis et al. 2000; Albach et al. 2001; Bremer et al. 2001, 2002) clearly separate Grubbiaceae and Bruniaceae, as well as Epacridaceae and Bruniaceae. Grubbiaceae are now seen as members of Cornales and Epacridaceae as members of Ericales, while Bruniaceae are clearly placed in the Euasterids II (*sensu* APG II 2003) but remain unassigned to a particular order. In the most comprehensive phylogenetic analysis to date of the Asterids, based on six DNA regions, Bruniaceae are placed as sister to the small South American families Columelliaceae and Desfontainiaceae, basal to Asterales, however, without any significant statistical support (Bremer et al. 2002). Thus, the closest relative to Bruniaceae, which may shed light on the question of their possible Gondwanan origin, is still hard to fathom, although members of Euasterids II are certainly the most likely candidates.

Another unresolved problem concerns the generic relationships in Bruniaceae. As stressed above, the possible old age of the family has led to the evolution of highly differentiated genera, which has resulted in an ongoing debate about their affinities. Classification of the family into Audouinieae (*Audouinia*, *Tittmannia*, *Thamnea*) and Brunieae (remaining genera) was proposed by Niedenzu and Harms (1930) on the basis of anther morphology. In the most recent revision of Bruniaceae (Pillans 1947), systematic weight has been put on ovary structure and flower position (see also Takhtajan 1987). Further morphological treatments have rearranged genera according to pollen morphology (Hall 1988), leaf anatomy (Carlquist 1991), and inflorescence morphology (Classen-Bockhoff 2000). *Audouinia* has consistently been viewed as the most primitive genus of the family, which is also supported by the palaeodiploid chromosome number of n = 11 (Goldblatt 1981). Only Scott (1999) favors *Lonchostoma* as the most primitive genus in the family on the basis of cladistic analysis of morphological and phytochemical characters.

Agreement prevails concerning a close relationship between *Audouinia*, *Tittmannia*, and *Thamnea*, but there is no concordance on the affinities of other genera. While Pillans (1947) and Takhtajan (1987) favor *Berzelia* as the most derived genus in the family, Takhtajan (1987) further groups *Berzelia* and *Mniothamnea* because of their unilocular,

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uniovulate ovaries. Carlquist (1991) found that *Berzelia* and *Nebelia* agree in leaf anatomy with the presumably basal *Audouinia*. Using inflorescence morphological data, Classen-Bockhoff (2000) distinguished four groups with *Audouinia*, *Tittmannia*, *Pseudobaeckea teres*, *Linconia*, *Thamnea*, and *Berzelia* possessing primitive features and *Nebelia*, *Pseudobaeckea*, *Lonchostoma*, and part of *Raspalia* having derived inflorescences. Regarding the rather conflicting conclusions based on morphological or phytochemical data, additional information from molecular data is urgently warranted. This is provided for by this study.

Material and Methods

Plants

Material of 62 species and the variety *Pseudobaeckea cordata* var. *monostyla* was collected in the wild and stored in silica gel. DNA extraction from herbarium material proved to be unsuccessful except in *Raspalia stokoei* and *Thamnea diosmoides*. The 65 sampled taxa (~82% of the family) include all biogeographically disjunct and ecologically divergent species (table A1). *Thamnea thesioides* (Esterhuysen 35408) cited by Hall (1988) is identical to *Thamnea uniflora* (MJG 040290) because both specimens are collected from the same population on the small summit plateau of Blokkop and show the characteristic ovary structure of *T. uniflora* (Pillans 1947).

Methods

Markers and primer combinations. The chloroplast marker *mat*K was selected for the analysis of intrafamilial relationships. Sequences were acquired from 65 taxa covering all genera, three of them completely (*Audouinia*, *Lonchostoma*, *Mniothamnea*). The coding region of *mat*K and the flanking introns were sampled. To design Bruniaceae-specific primers, the complete *trn*K intron region including *mat*K was initially amplified using primers *trn*K-3914F and *trn*K-2R (Johnson and Soltis 1994; Steele and Vilgalys 1994). Conserved sections of sequence fragments obtained by this means were used to identify ca. 20 bp as a basis for four new Bruniaceae-specific primers: 389 F (TAC GAT CAA TTC ATT CAA TAT TTC C), 1120 F (CCT CTG ATT GGA TCA TTG GCT), 664 R (GAC GAA GAT GGA TTC GTA TTC), and 1304 R (AGC ACA AGA AAG TCG AAG TA). Suitability of the primers was tested with the shareware program Primers! for the Mac (http://iubio.bio.indiana.edu:7780/archive/00000398/). The four new *mat*K primers proved to be applicable for all examined Bruniaceae species and allowed the whole *trn*K intron region to be sequenced (*mat*K and flanking introns, ca. 2500 bp). The *mat*K was sequenced in three portions, with *trn*K-3914F/664R, 389F/1304R, and 1120F/*trn*K-2R functioning as primer combinations.

To add phylogenetic information from the nuclear genome and to clarify relationships predominantly on the species level, we applied the widely used internal transcribed spacer (ITS) regions (Baldwin et al. 1995). For all analyses, ITS 1, 5.8 S, and ITS 2 (called ITS in the following) were sequenced and included in phylogenetic analyses. ITS sampling was limited by amplification difficulties. Forty ITS sequences were finally obtained, again covering all genera (table A1). The initial PCR and sequencing primers ITS A, ITS B, ITS C, and ITS D described by White et al. (1990) were used successfully in a subset of taxa only (*Berzelia cordata*, *Berzelia rubra*, *P. cordata*, *P. cordata* var. *monostyla*, *Raspalia oblongifolia*, *Raspalia stokoei*, *Raspalia villosa*). Otherwise, the plant-specific primers 18S and 28S designed by Muir and Schlötterer (1999) were applied. Primer combinations were either ITS A/ITS B or 18S/28S. Only in *Staavia phylicoides* did the whole ITS region have to be amplified and sequenced separately in two portions, with ITS A/ITS C and ITS D/ITS B combined, respectively.

DNA extraction, amplification, sequencing, and sequence alignment. Total genomic DNA was extracted from leaves using the plant extraction kit DNeasy (Qiagen, Hilden, Germany). PCRs were performed in a Whatman Biometra TGradient Thermocycler (Biometra GmbH, Göttingen, Germany) following the protocol of Palumbi (1996). The temperature profile was as follows: pretreatment 94°C (1 min); 35 cycles 94°C (3 s), 55°C (5 s), 72°C (1 min); post-treatment 55°C (1.3 min), 72° C (8 min).

PCR products were checked through electrophoresis in agarose and purified using the NucleoSpin Extract Purification Kit (Macherey and Nagel GmbH, Düren, Germany). Sequencing reactions were carried out with the PCR products using the Big-Dye Terminator Cycle Sequencing Kit plus AmpliTaq DNA Polymerase (Applied Biosystems, Norwalk, CT). The following temperature profile was applied: 96°C (1 min); 27 cycles 96°C (1 s), 55°C (2 s), 60°C (4 min); and finally 51.4°C (1 s), 60°C (4 min). Samples were analyzed with automated sequencers (ABI 373 and ABI 377).

Editing and alignment were performed in Sequencher 3.0 (GeneCodes, Ann Arbor, MI) comparing forward and backward strands to create consensus sequences. Alignment of *mat*K data was straightforward. ITS alignments remained restricted to selected clades because of alignment problems across genera. Indels were generally treated as missing data, which would also apply to indel events and thus result in a loss of potential phylogenetic information. Potentially informative indels were therefore coded separately in an additional data matrix (if not specified otherwise), allowing comparisons between reconstructed trees including or excluding indel characters (only maximum parsimony [MP] calculations of *mat*K). Indels were coded as binary characters following the method of Graham et al. (2000) and were added to the respective nucleotide data matrix. Because indel events in ITS generally were of various lengths and unclear homologies, no indels were coded in ITS. Sequences and alignments were simultaneously submitted to the European Molecular Biology Laboratory gene bank using Sequin 5.16 (http://www.ncbi.nlm.nih.gov/Sequin/) (table A1).

Phylogenetic analyses. All sequence data were analyzed in PAUP* (ver. 4b4a-b8; Swofford 2000). Generally, all molecular data sets were analyzed under the MP criterion using Fitch parsimony (Fitch 1971). If more than one equally parsimonious tree was found, strict consensus trees were computed. *Columellia oblonga* and *Desfontainia spinosa* were taken as outgroup taxa in all analyses concerning intergeneric relationships (*mat*K). ITS trees were based on unrooted analyses.

Depending on the number of analyzed taxa, different MP search options were applied. Exhaustive searches were conducted with the ITS data sets. With *mat*K data, only heuristic searches were conducted, using 1000 replicated searches with branch swapping by tree bisection reconnection. Uninformative characters were excluded.

The *mat*K data were also subjected to heuristic maximum likelihood (ML) searches. The determination of the best-fit model of evolution was performed with MODELTEST, version 3.06 (Posada and Crandall 1998), in a hierarchical likelihood ratio test (Felsenstein 1981, 1988; Goldman 1993; Sanderson 1998; Posada and Crandall 2001). The parameters of the best-fit model resulting from the model test procedure serve as likelihood settings for the actual ML calculation, using the same search options as in MP analyses of *mat*K.

Branch support was assessed by bootstrapping (Felsenstein 1985) as implemented in PAUP* (1000 replicates). Further search options were the same as for the original data set. Homoplasy in the data sets was evaluated with the consistency index (Kluge and Farris 1969) and the retention index (Farris 1989). We used the partition homogeneity test (Farris et al. 1995) implemented in PAUP* to test the significance of topological incongruencies between ITS data sets and corresponding taxon subsets of *mat*K data.

Results

The *mat*K data set had 2612 aligned bases, including 25 insertion/deletion gaps. Four indels were found in the coding region (following multiples of three) and 21 in the intron regions. Phylogenetic inference with ML yielded two equally likely trees ($\ln L = -9106.10939$; strict consensus in fig. 1).

Linconia (Linconieae) is sister to Audouinieae and Brunieae. The Audouinieae comprise two major clades: *Audouinia* plus *Tittmannia* and *Thamnea* along with *Pseudobaeckea teres* embedded within it, the latter rendering *Thamnea* polyphyletic. *Tittmannia* also appears polyphyletic, with *Audouinia* emerging as weakly supported sister (68%) to *Tittmannia laevis*.

Staavia (100%) is placed as sister to a weakly supported group (61%) comprising the remaining genera. Among the latter, a strongly supported *Berzelia* clade (100%) comprises three species of *Brunia* (*Brunia* I, II) and all species of *Berzelia*. The *Berzelia* clade appears as sister to a major clade representing the rest of the family. The latter is divided into two strongly supported monophyletic groups (94% each) along with *Raspalia dregeana*, whose phylogenetic relationship to either one of these clades remains unresolved.

The *Brunia*/*Pseudobaeckea* clade includes subclades comprising (1) all species of *Nebelia* plus the remaining *Brunia* species (*Brunia* III) and (2) a weakly supported alliance (65%) of three *Raspalia* species (*Raspalia* I) and *Pseudobaeckea* s.str. (excluding *P. teres*). Because of the inclusion of *Brunia macrocephala* in a moderately supported clade (82%) with *Nebelia*, *Brunia* III cannot be termed monophyletic. *Pseudobaeckea* s.str. is weakly supported (62%) as monophyletic. *Pseudobaeckea africana* and *Pseudobaeckea cordata* are strongly monophyletic (97%), with *P. cordata* var. *monostyla* weakly supported as their sister (62%). Within the three *Raspalia* species, *Raspalia oblongifolia* and *Raspalia villosa* are weakly supported (70%) as sister taxa.

Members of *Raspalia* (*Raspalia* II + III), *Mniothamnea*, and *Lonchostoma* form a monophyletic group (94%), with *Raspalia* II (100%) as sister to the remaining taxa (97%). Within the latter, two *Raspalia* species (*Raspalia* III) form a well-supported monophyletic group (100%) together with two *Mniothamnea* species (*Mniothamnea* clade). This clade is sister to *Lonchostoma* (97%).

The *mat*K data set was also subjected to the MP optimality criterion. In a first MP analysis, indels were not coded in an additional matrix but were treated as missing data. In a second MP approach, indel information was added that did not alter the tree topology and yielded comparable bootstrap values (not shown). The tree resulting from MP analysis (fig. 2*a*; table 1) differs from the ML topologies in *Berzelia lanuginosa* and *Berzelia abrotanoides*, forming a weakly supported sister pair (53%) and no support for a diverging *Berzelia rubra*. Further differences are a collapse of the root of *Raspalia virgata* and the two *Mniothamnea* species and of the branch leading to *Raspalia* I and the *Pseudobaeckea* species. Generally, ML bootstrap support values correspond favorably to support values of the MP results and vice versa.

Relationships within selected clades (ITS). The partition homogeneity test found ITS data sets for topologies in figures 2*c*, 2*d*, 2*f* significantly incongruent ($P < 0.05$) compared with topologies based on *mat*K sequences for corresponding taxa. Only after eliminating a combination of several taxa did the P value reach $P \geq 0.05$ (table 2). ITS and *mat*K data were therefore not combined for those particular subtrees of the phylogeny.

Linconia contains three species in this analysis. Tree searches are not feasible below four taxa, but ITS alignment of the three species clearly reveals the closer similarity between *Linconia cuspidata* and *Linconia ericoides* compared with *Linconia alopecuroidea* (table 3). Therefore, an inferred ITS cladogram showing the relationships within *Linconia* is presented in figure 2*b*.

ITS sequences were gained of all species present in the Audouinieae clade apart from *Thamnea uniflora* and *Tittmannia laxa* (fig. 2*c*). *Thamnea* forms a well-supported monophyletic group (100%) with *Thamnea diosmoides*/*Thamnea hirtella* and *Thamnea massoniana*/*Thamnea thesioides* as sister pairs. The sister clade to *Thamnea* is a group comprising *Audouinia capitata*, *P. teres*, and two *Tittmannia* species that are in turn sisters to each other.

All species of *Staavia* present in the enlarged *mat*K data set yielded ITS sequences. As in the *mat*K tree, *Staavia phylicoides* and *Staavia verticillata* are the first diverging lineages and are placed in a paraphyletic grade as sister to the group of the remaining species (fig. 2*d*). Within this monophyletic group, *Staavia brownii* and *Staavia comosa* are placed in a polytomy as sisters to a monophyletic group of the five remaining species. Herein, *Staavia zeyheri* is sister to *Staavia radiata*/*Staavia dodii* and *Staavia dregeana*/*Staavia glutinosa*.

In combination with *mat*K data, ITS sequences of *Berzelia* species give a slightly better resolution, with *B. abrotanoides* emerging as sister to *Berzelia arachnoidea* and *B. rubra* (fig. 2*e*).

Within the *Brunia*/*Pseudobaeckea* clade, two members of *Brunia* (*Brunia* III) and *Nebelia* are sister to a well-supported monophyletic group (100%) of two *Pseudobaeckea* taxa and three *Raspalia* species. The two *Brunia* species form a sister group to all *Nebelia* species. *Nebelia sphaerocephala* is sister

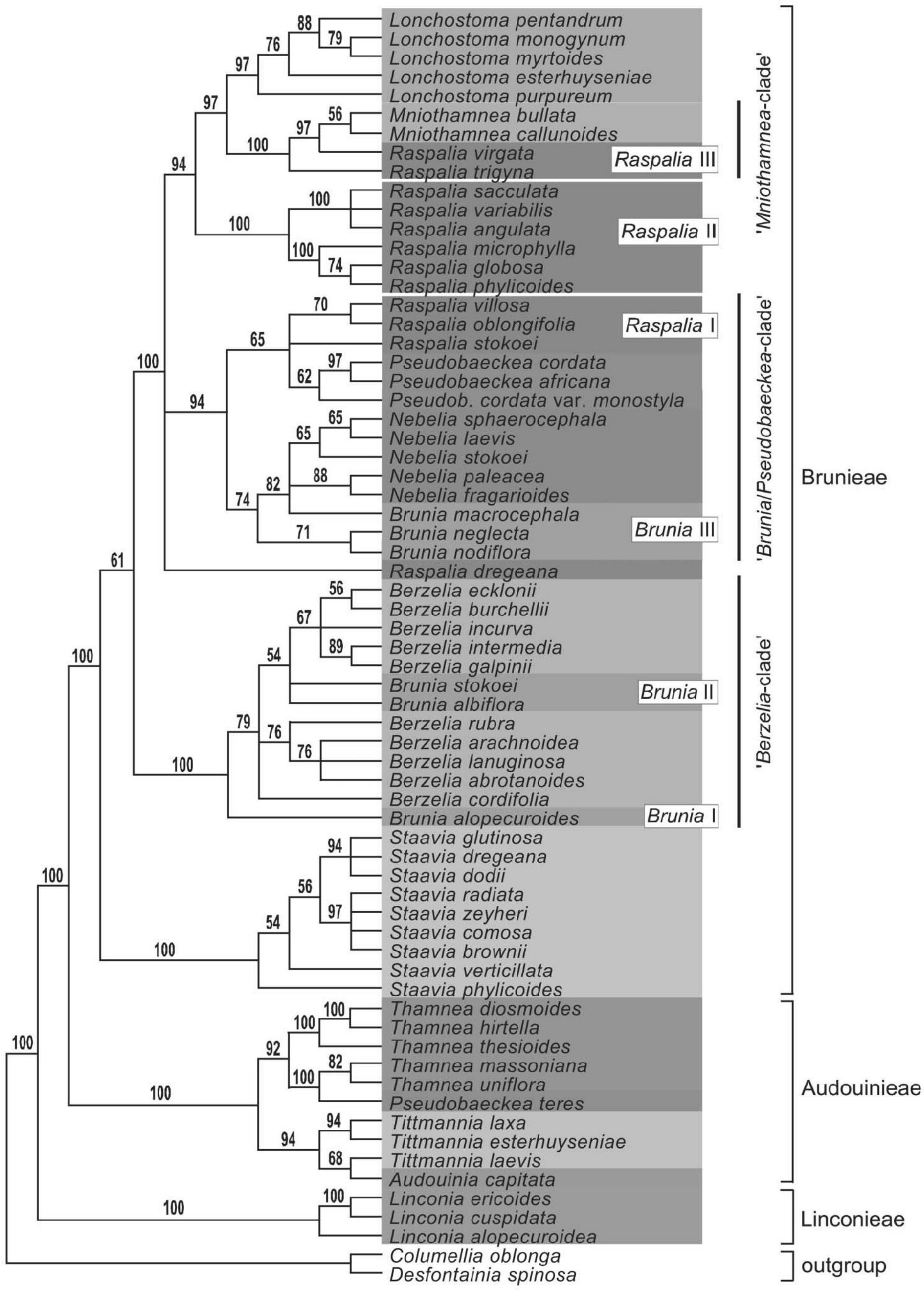

Fig. 1 Strict consensus tree of two equally likely trees ($\ln L = -9106.10939$). The *mat*K sequence data of 65 taxa of Bruniaceae and two Columelliaceae (outgroup) were calculated with maximum likelihood. Bootstrap values $\geq 50\%$ are indicated above branches. The roman numerals following the generic names have only a descriptive meaning and do not indicate monophyletic groups.

to the rest, in which *Nebelia fragarioides*, *Nebelia paleacea*, and *Nebelia stokoei* are monophyletic, with the latter two as a sister pair. *Pseudobaeckea* s.str. is monophyletic and sister to three *Raspalia* species (fig. 2*f*).

The *Mniothamnea* clade comprises two species of *Raspalia* (*Raspalia trigyna*, *R. virgata*) and the only two *Mniothamnea* species (*Mniothamnea bullata*, *Mniothamnea callunoides*). In the tree topology of the ITS subtree, *M. bullata* and *M. callunoides* are well-supported sisters (96%, not shown). Combination with *mat*K data does not alter the tree topology but enhances the latter bootstrap value to 99% (fig. 2*g*).

Discussion

Subdivision of the Bruniaceae

The molecular data of this study, provided for Bruniaceae for the first time, offer reliable new arguments for a systematic

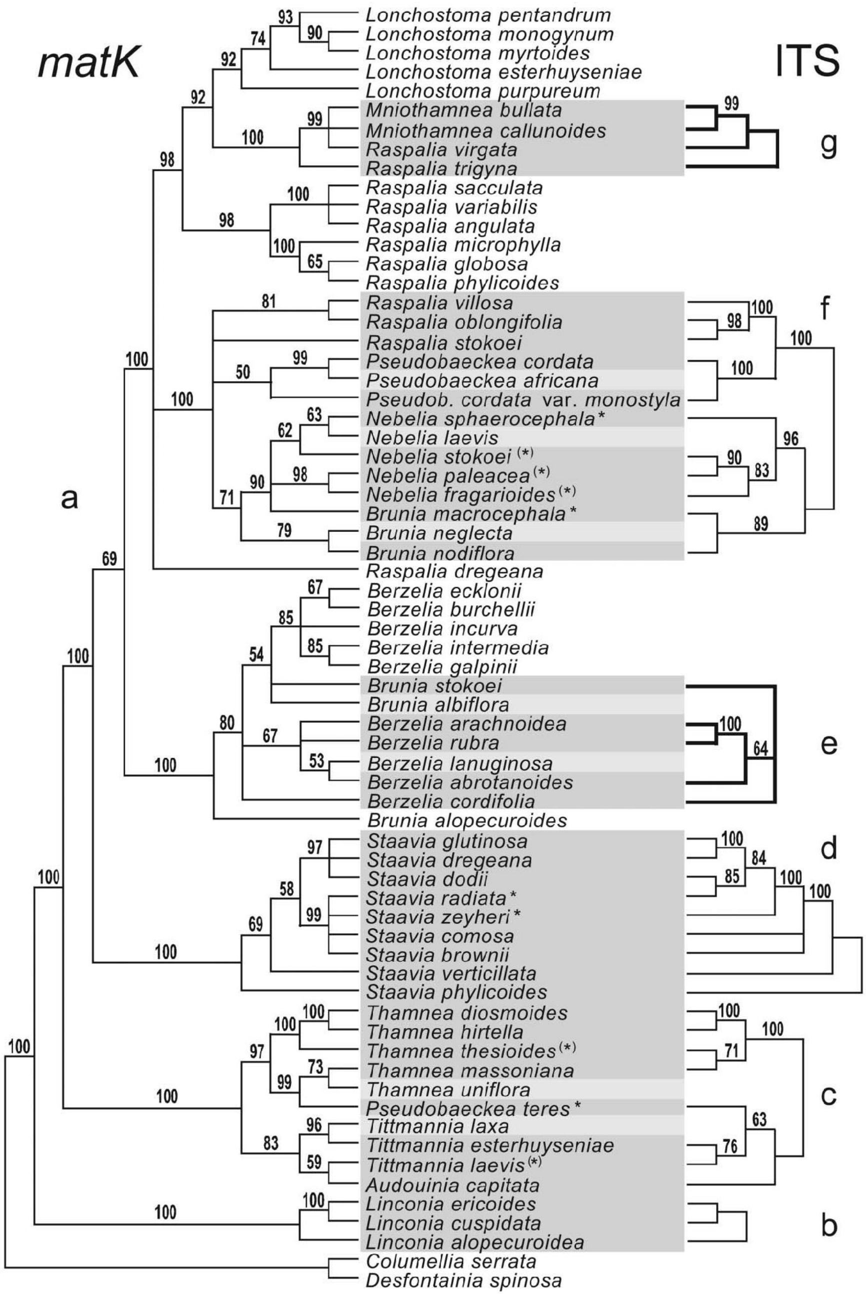

Fig. 2 Opposed tree topologies of *mat*K maximum parsimony (MP) analysis (*a*) and internal transcribed spacer (ITS) MP analyses of selected clades (*b–g*; in *b*, the ITS cladogram is inferred from alignment). Combined ITS and *mat*K data in the case of congruence led to the tress marked with bold lines (bootstrap values resulting from combined analysis). Taxa of selected clades, for which ITS sequences are not available, are highlighted with light gray. Taxa causing incongruence according to the partition homogeneity test are marked with asterisks (asterisk = taxon has to be eliminated to reach congruence, asterisk in parentheses = one of these taxa has to be eliminated to reach congruence). Bootstrap values ≥50% are indicated above branches.

revision of the family that to a large extent questions the hitherto proposed classification. Takhtajan (1987) recognized Audouinioideae (*Audouinia*, *Thamnea*, *Tittmannia*), Brunioideae (*Brunia*, *Linconia*, *Nebelia*, *Pseudobaeckea*, *Raspalia*, *Staavia*), Lonchostomoideae (*Lonchostoma*), and Berzelioideae (*Berzelia*, *Mniothamnea*), whereas our molecular studies recovered only monophyletic Audouinioideae and Lonchostomoideae. Takhtajan's other subfamilies are clearly polyphyletic (see fig. 1). A more convincing systematic pattern in respect to our molecular findings are the traditional tribes Audouinieae (*Audouinia*, *Thamnea*, *Tittmannia*) and Brunieae (remaining genera) suggested by Niedenzu and Harms

Table 1

Tree Statistics of Figure 2

Figure	Marker	Ingroup	Outgroup	Alignment length (bp)	Parsimony-informative characters	No. MP trees	Tree length	CI	RI
2*a*	*mat*K	65	2	2612	392 (15%)	382	862	0.816	0.938
2*c*	ITS	Unrooted	Unrooted	660	48 (7.2%)	1	198	0.909	0.780
2*d*	ITS	Unrooted	Unrooted	644	49 (7.6%)	2	177	0.921	0,811
2*e*	ITS + *mat*K	Unrooted	Unrooted	3249	48 (1.5%)	1	213	0.930	0.688
2*f*	ITS	Unrooted	Unrooted	630	41 (6.5%)	1	82	0.939	0.943
2*g*	ITS + *mat*K	Unrooted	Unrooted	3249	4 (0.1%)	1	101	1	1

Note. CI = consistency index, RI = retention index. For figure 2*b*, see table 3.

(1930). The only exception to their taxonomy is the genus *Linconia*, which in our studies clearly constitutes a well-defined, isolated group positioned as sister to the remainder of the family. We therefore exclude a monogeneric tribe Linconieae from the Brunieae *sensu* Niedenzu and Harms (1930). The Audouinieae *sensu* Niedenzu and Harms (1930) are maintained.

It is very difficult to find synapomorphic characters that distinguish the tribal groupings exclusively and that hold true for all members of the respective tribe. One approach would be to extend the delimitation by Niedenzu and Harms (1930), who based their distinction of the tribes on anther morphology.

Synapomorphies for the Linconieae are anthers that are distinctly sagittate (distal ends of thecae clearly diverging and apical ends never spreading) and pollen sacs ending apically in a conspicuous fused, sterile tip (Quint 2004) (fig. 3*a*). Further synapomorphies are a hard, inflexible petal texture and sepals reduced to inconspicuous lobes without apicula (sepals of *Pseudobaeckea* taxa within the Brunieae lack the apicula as well, but they are larger and petaloid). The Audouinieae are characterized by linear anthers, the pollen-sacs fused with the connective on the entire length (fig. 3*b*) (although these characters have to be confirmed in the species of *Thamnea*). The Brunieae have versatile anthers of sagittate, oval, or lineal form, with thecae that can diverge apically. In the case of exserted stamens, adult anthers generally tip over, with the apex pointing toward the flower base (fig. 3*c*).

Intergeneric Relationships in the Bruniaceae

In the following, the phylogeny of Bruniaceae will be reconsidered on the basis of all data available. The different molecular data sets will be compared and discussed with respect to morphological data.

Linconieae. *Linconia* is positioned as the sister of all other Bruniaceae with high bootstrap support values (figs. 1, 2*a*), which has previously not been suggested. The monotypic genus *Audouinia* has consistently been the most likely candidate (Pillans 1947; Goldblatt 1981; Takhtajan 1987; Hall 1988; Carlquist 1991; Classen-Bockhoff 2000). One of the initial notions is the primitive, trimerous ovary of *Audouinia* and interpretation of the dimerous or monomerous ovaries of the other genera as a reduction of ovary carpels. But the number of ovary carpels seems to be less consistent in Bruniaceae than assumed because species of *Linconia* and *Tittmannia* rarely form tricarpellate (and trilocular) ovaries as well (M. Quint, personal observation). Furthermore, *Audouinia* also has been reported to occasionally show bilocular (and tetra- and pentalocular) ovaries (Pillans 1947; de Lange et al. 1993). Apparently, this character is less fixed and therefore less diagnostic for phylogenetic interpretations in Bruniaceae than previously inferred.

Table 2

Results of the Partition Homogeneity Test (cf. Fig. 2)

Taxa analyzed: data set 1 (ITS), data set 2 (*mat*K), respectively	*P* value	Incongruence	Taxa causing incongruence
Brunia/Pseudobaeckea[a]	0.01	Yes	*Brunia macrocephala*, *Nebelia sphaerocephala*, *Nebelia stokoei/ Nebelia fragaroides/Nebelia paleacea*[b]
Staavia[c]	0.01	Yes	*Staavia radiata*, *Staavia zeyheri*
Audouinieae[d]	0.01	Yes	*Pseudobaeckea teres*, *Thamnea thesioides/Tittmannia laevis*[b]

[a] *Brunia macrocephala*, *Brunia nodiflora*, *Nebelia fragarioides*, *Nebelia paleacea*, *Nebelia sphaerocephala*, *Nebelia stokoei*, *Pseudobaeckea cordata*, *Pseudobaeckea cordata* var. *monostyla*, *Raspalia oblongifolia*, *Raspalia stokoei*, *Raspalia villosa*.

[b] One of the species connected with a slash has to be eliminated to reach congruence.

[c] *Staavia brownii*, *Staavia comosa*, *Staavia dodii*, *Staavia dregeana*, *Staavia glutinosa*, *Staavia radiata*, *Staavia phylicoides*, *Staavia verticillata*, *Staavia zeyheri*.

[d] *Audouinia capitata*, *Pseudobaeckea teres*, *Thamnea diosmoides*, *Thamnea hirtella*, *Thamnea massoniana*, *Thamnea thesioides*, *Tittmannia esterhuyseniae*, *Tittmannia laevis*.

Table 3

Number and Percentage of Character States Shared between Two *Linconia* Species

	L. alopecuroidea	*L. cuspidata*	*L. ericoides*
L. alopecuroidea	...		
L. cuspidata	1 (0.9%)	...	
L. ericoides	4 (3.6%)	105 (95.5%)	...

Note. Table shows 110 parsimony-informative sites.

Scott (1999) proposed *Lonchostoma* as the most primitive genus of the family, partly on the basis of the assumption that the largely sympetalous Epacridaceae are the closest relatives of the family. The sympetaly of *Lonchostoma* would thus present a symplesiomorphic, ancestral character state. Flower morphological studies, however, reveal that *Lonchostoma* flowers are not characterized by true sympetaly but by a fused petal-stamen tube (Leinfellner 1964).

Remarkably, *Linconia* as sister to all other Bruniaceae shares many morphological characters that by broad agreement characterize the less derived members of the family: consistently tricolporate pollen grains (*Audouinia*, *Tittmannia*, *Pseudobaeckea teres*, *Berzelia*, *Brunia* pp.) (Hall 1988), solitary flowers at the top of bracteate short shoots (*Audouinia*, *Tittmannia*, *P. teres*) (Classen-Bockhoff 2000), ovary glabrous (*Audouinia*, *Thamnea*, *P. teres*, *Tittmannia*, the latter two with small papillae), presence of a flower pedicel (*Audouinia*, *Tittmannia*), and presence of a cuticular rim around the stomata (*Audouinia*, *Tittmannia*) (Carlquist 1991). However, the unusual leaf morphological combination of a character involving fiber strands with a character involving occurrence of crystalline deposits found by Carlquist (1991) allies *Linconia* with the more derived species *Lonchostoma*, *Mniothamnea*, *Pseudobaeckea* (without *P. teres*), *Raspalia*, and *Staavia*, while the more basal taxa *Audouinia*, *Tittmannia*, *Thamnea*, and *P. teres* are allied with *Berzelia*, *Brunia*, and *Nebelia*. According to Goldblatt (1981), the basal chromosome number in the family is $n = 11$. Goldblatt (1981) strongly supports the view of *Audouinia* as the primitive member of the family because *Audouinia* reveals a palaeodiploid state of $2n = 22$, whereas the other species under study have higher ploidy levels. Unfortunately, *Linconia* (as well as the other supposedly basal taxa *Tittmannia*, *Thamnea*, and *P. teres*) are missing in his study. Presupposing a progression from lower to higher ploidy levels, a low ploidy level in *Linconia* could give further evidence to its sister position to all other Bruniaceae.

Within the genus *Linconia*, *Linconia ericoides* and *Linconia cuspidata* clearly form a sister pair compared with *Linconia alopecuroidea*. All three *Linconia* species are very rare and occur only in isolated populations. Florally, *L. ericoides* and *L. alopecuroidea* are similar to each other, whereas vegetatively, *L. cuspidata* and *L. ericoides* resemble each other more closely (Oliver 1999). The close relationship between *L. cuspidata* and *L. ericoides* is also indicated by their particular microhabitat: both thrive in dry rock crevices in mountainous areas, whereas *L. alopecuroides* occurs on moist, swampy meadows with peaty soil. The only known locality of *L. ericoides* (Stormsvlei, Riviersonderend Mountains) is closest to an unspecific locality in the Riviersonderend Mountains of *L. cuspidata*, whereas *L. alopecuroidea* occurs in only a few scattered populations in the Langeberg range (Oliver 1999).

Audouinieae. Agreement prevails that the Audouinieae reflect a natural group comprising the monotypic *Audouinia capitata* and the genera *Tittmannia* and *Thamnea* (Pillans 1947; Goldblatt 1981; Hall 1988; Carlquist 1991; Classen-Bockhoff 2000). Carlquist (1991) and Classen-Bockhoff (2000) have additionally emphasized that *P. teres* is misplaced in the genus *Pseudobaeckea sensu* Pillans and pinpoint an affinity to the Audouinieae. Pollen data offered by Hall (1988) would also confirm an alliance of *P. teres* with the genera with likewise three pollen colpi (e.g., *Audouinia*, *Thamnea* pp., or *Tittmannia*). The distinctive densely granular tectal pollen surface finds no match in the family and must be interpreted as autapomorphic.

While the position of *P. teres* in the Audouinieae is undisputed, its relationship to the genera in question is less clear. Classen-Bockhoff (2000) favors an alliance of *P. teres* with the genus *Tittmannia* on the basis of inflorescence studies. This relationship is also weakly (63%) supported in our ITS studies (fig. 2*c*) but is strongly objected in our *mat*K analyses

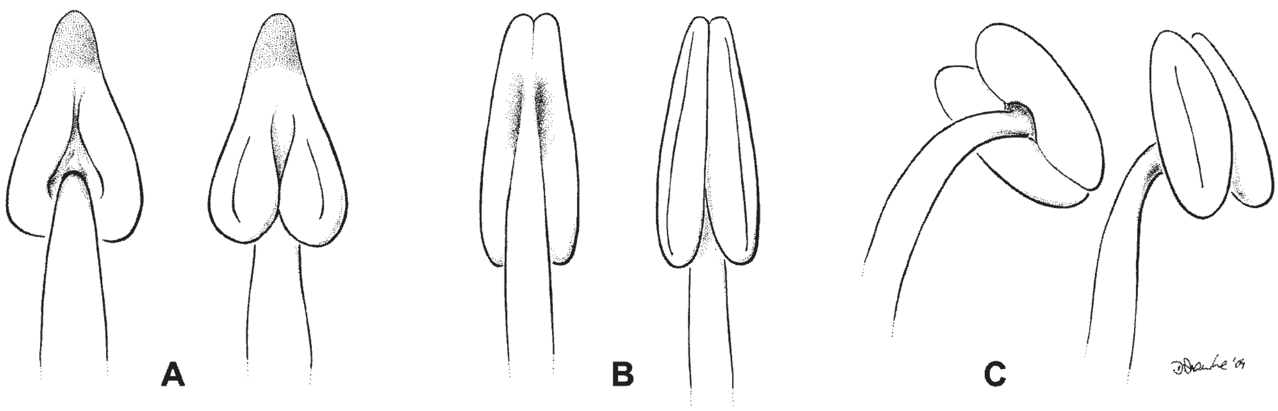

Fig. 3 Anther morphology in Bruniaceae showing the typical features of the tribes Linconieae (*A*), Audouinieae (*B*), and Brunieae (*C*).

(≥92%), where *P. teres* is embedded within the genus *Thamnea*, forming a strongly supported monophyletic group (≥97%) with the species *Thamnea massoniana* and *Thamnea uniflora* (fig. 1, 2*a*).

The latter two species and *Thamnea thesioides* are notably the only *Thamnea* species in our molecular study that likewise possess tricolporate pollen. When we take Linconieae as outgroup, tricolporate pollen must at present be considered symplesiomorphic for *Thamnea* and the other consistently tricolporate Audouinieae and does thus not provide an indication for an alliance with *P. teres*. Similarly, a cuticular rim around the stomata, observed in *Audouinia*, *Tittmannia*, *Linconia*, and *P. teres*, is apparently a symplesiomorphic feature for all basal Bruniaceae, the loss being a synapomorphy for *Thamnea*. A morphological feature indicating an affinity of *P. teres* to *Thamnea* is the absence of a flower pedicel ("flowers sessile"; Pillans 1947), which is in turn present in *Audouinia*, *Tittmannia*, and *Linconia*. *Thamnea* species and *P. teres* furthermore share the following features: singular flowers dispersed (Classen-Bockhoff 2000), scalelike small leaves, and prostrate growth. Studies on petal morphology also confirm an affinity between *P. teres* and *Thamnea* because both form petal bulges of the *Thamnea* type, with two separate, rather thin parallel ridges that are not fused at the base of the petal (Quint and Classen-Bockhoff, forthcoming).

The evolution of four to five pollen colpi in *Thamnea diosmoides* and *Thamnea hirtella* can be viewed as a synapomorphy for these species. Molecular data from both genomes assert their sister relationship strongly. However, *mat*K and ITS trees are incongruent as to whether *T. thesioides* is sister to the latter two species (100%; figs. 1, 2*a*) or to *T. massoniana* (71%; fig. 2*c*; *T. uniflora* is not present in the ITS data set). No morphological synapomophies could be found for either relationship. Evolution of relatively large flowers of the salver-shaped type (*T. diosmoides*, *T. massoniana*) must be interpreted as convergent evolution in both alternatives. Gain and loss or convergent evolution of the ring-shaped nectary structure (the "elevated ovary-margin" of Pillans 1947; all species of *Thamnea* except *T. thesioides*) is, however, equally likely.

Our molecular studies also remain uncertain concerning the monophyly of *Tittmannia*. While ITS data argue for a sister relationship of *Tittmannia esterhuyseniae* and *Tittmannia laevis* (76%; fig. 2*c*; *Tittmannia laxa* missing in the ITS data set), *mat*K data weakly advocate a closer relationship between *T. laevis* and *A. capitata* (≤68%; figs. 1, 2*a*). From a morphological point of view, *Tittmannia* species differ from *Audouinia* regarding their much smaller, dull white flowers, petals belonging to the *Linconia* type (Quint and Classen-Bockhoff, forthcoming), and dimerous ovaries, so *Tittmannia* may well constitute a monophyletic group.

Brunieae: *Staavia*. The monophyly of the Brunieae has been asserted in all analyses with high bootstrap values. The results provide satisfying resolution among major clades within the Brunieae, but the relationship of *Staavia* within the subfamily remains uncertain. Bootstrap support for this pattern is ≤69% (figs. 1, 2*a*), and both the *Berzelia* clade and its sister clade may be equally apt candidates for this position. While characters like pollen morphology (Hall 1988) and inflorescence position (Classen-Bockhoff 2000) do indeed argue for an affinity of the *Berzelia* clade to the Audouinieae and Linconieae (both having tricolporate pollen grains and an ananthic branching pattern), the position of *Staavia* is upheld again when the ML optimality criterion is applied (fig. 2). ML calculations are less subjected to long-branch attraction (Kuhner and Felsenstein 1994; Gaut and Lewis 1995; Swofford et al. 1996; Lewis 1998), which makes a misplacement of *Staavia* from these effects less probable. We would therefore advocate for *Staavia* as the sister to other Brunieae, although this position needs to be confirmed with new data possibly from nuclear genes with slower evolutionary rates than ITS.

Monophyly of *Staavia* is strongly supported in the enlarged *mat*K analysis (figs. 1, 2*a*). Except *Nebelia paleacea*, *Staavia* is the only genus of Bruniaceae that evolved showy involucres around a bowl-shaped inflorescence. All species of *Staavia* further agree in having a homogeneous petal bulge without any detectable vertical subdivision (Quint and Classen-Bockhoff, forthcoming) and in having fused styles.

The *mat*K and ITS sequences agree in the position for *Staavia phylicoides* and *Staavia verticillata* as a paraphyletic grade, with the remaining species as their terminal group. These species differ from the remaining ones in inserting monopodial shoots in an otherwise regular sympodial branching pattern (Classen-Bockhoff 2000, for *S. phylicoides*; M. Quint, personal observation) and in having involucral bracts that scarcely differ in length and color from the uppermost green leaves. All remaining *Staavia* species (except *Staavia dregeana*) have conspicuous or even showy involucra. The *mat*K and ITS data are partially incongruent for the latter species, which is attributed to the different placement of *Staavia radiata* and *Staavia zeyheri* in the respective analysis (fig. 2). Morphology does not provide convincing arguments for either alternative. While *S. radiata* is a very wide-spread species, all other *Staavia* species are rare and restricted to a few localities. *Staavia glutinosa*, *S. dregeana*, and *Staavia dodii* occur exclusively on the geographically isolated Cape Peninsula, and it is therefore plausible to assume an alliance of these species. Because *Staavia* species (*S. radiata* with *S. dodii* and *Staavia comosa*) have been reported to hybridize (Powrie 1969*b*), evidence of *mat*K data may be flawed because of chloroplast capture (Rieseberg et al. 1996; Wendel and Doyle 1998). Therefore, ITS data may be more accurate, resulting in a sister relationship between *S. radiata* and *S. dodii*.

Brunieae: Berzelia clade. All species of *Berzelia* and three species of *Brunia* form the well supported *Berzelia* clade (fig. 1). Evidence for an exclusion of these *Brunia* species (*Brunia* I and II) from the genus *Brunia* is given by various morphological features (table 4). Among the members of Brunieae, which have inflorescences of the pincushion style with exserted stamens (*Berzelia*, *Brunia*, *Nebelia*, and *Raspalia dregeana*), the *Berzelia* clade is characterized by tricolporate pollen grains, developed stipules, and petiolate leaves.

The distinction between *Brunia* and *Berzelia* suggested by Pillans (1947) is gynoecial: unilocular ovaries and one style in *Berzelia* and imperfectly bilocular ovaries with two styles in *Brunia*. Collapse of the weakly supported branch clustering *Brunia stokoei* and *Brunia albiflora* with five *Berzelia* species would permit one to regard a dimerous ovary as a symplesiomorphic character state for the *Berzelia* clade

Table 4

Features Justifying Two Subgroupings within *Brunia*

Brunia I, II	*Brunia* III
Adult leaves with stipules	Adult leaves without stipules
Stamens equal in length	Stamens unequal in length
Leaves petiolate	Leaves sessile
Uniovulate loculi	Biovulate loculi
Pollen tricolporate	Pollen polycolporate
Pollen tectum foveolate	Pollen tectum psilate or reticulate
Flowering time: spring	Flowering time: summer

Note. Subgroupings are based on work by Pillans (1947), Hall (1988), Classen-Bockhoff (2000), and new observations.

with the possible inference of one reduction event for all *Berzelia* species. Unfortunately, ITS sequence data neither support nor contradict this view because only one of the *Brunia* species in this clade could be successfully sequenced (fig. 2*e*). In the enlarged *mat*K analysis (figs. 1, 2*a*), *Brunia alopecuroides* comes out as sister to the rest of the *Berzelia* clade. Pillans (1947) and Classen-Bockhoff (2000) comment on the distinctness of this species, e.g., on the arrangement and size of the flower heads. Stamens of *B. alopecuroides* are the shortest ones in all species of the pincushion flower type, and it may thus represent an early state in the evolution of flowers with exserted stamens.

Brunia III, *Nebelia*, *Pseudobaeckea* (without *P. teres*), *Raspalia*, *Mniothamnea*, and *Lonchostoma* form a well-supported clade (figs. 1, 2*a*) characterized by sessile leaves and missing stipules at least in the adult stage. For the group consisting of *Pseudobaeckea* pp., *Raspalia oblongifolia*, and *Raspalia stokoei*, one reversal to petiolate leaves is most likely (the leaf feature remaining equivocal for *Raspalia villosa*). Free styles are best interpreted as a symplesiomorphic feature for the group because the closely related three *Brunia* species (*Brunia* I) of the *Berzelia* clade likewise possess free styles.

Affinities of *R. dregeana* cannot be addressed sufficiently from molecular data. The species reflects a mosaic of morphological characters that notably complicates phylogenetic interpretations (Quint 2004). *Raspalia dregeana* may be viewed as a missing link between the major clades of the Brunieae (excluding *Staavia*) and should be focused on in further studies regarding molecular systematics as well as pollination biology. Irrespective of the position of *R. dregeana*, the present pattern would indicate a polyphyletic genus *Raspalia* (figs. 1, 2*a*). The apparent subdivision in *Raspalia* I, II, and III can only tentatively be justified with morphological features. Inflorescence studies imply that truncation of the terminal flower of a flower head has happened in *Nebelia* and *Pseudobaeckea* pp. and is also a consistent feature for the closely related *Raspalia* I, whereas *Raspalia* II and III (except *Raspalia virgata* and *Raspalia sacculata*) have determinate flower heads (Classen-Bockhoff 2000). Leaves of *Raspalia* I are generally ascending to erect-spreading, while leaves in *Raspalia* II and III are closely appressed to the stem (although in some species, the situation is less clear). Tannins are present in *Raspalia* I (and in the related *Nebelia* and *Pseudobaeckea* species) but generally absent in *Raspalia* II (except *Raspalia variabilis*) and III (Carlquist 1978).

Likewise, there are no clear morphological synapomorphies that would allow one to circumscribe the clade comprising *Brunia* III, *Nebelia*, *Pseudobaeckea* pp., and *Raspalia* I (fig. 1). A promising study may be a survey of fruit and seed morphology, which is particularly scanty in *Raspalia* (Pillans 1947). It should be noted that certain species of *Raspalia* are difficult to distinguish, and homoplasies in morphological characters obtained from the literature may also result from misidentifications.

Brunieae: Brunia-Pseudobaeckea clade. The strongly supported clade comprising *Brunia* III, *Nebelia*, *Pseudobaeckea* pp., and *Raspalia* I generally splits into two major subclades allying *Brunia* III and *Nebelia* as well as *Pseudobaeckea* pp. and *Raspalia* I. This split is strongly supported by ITS data (fig. 2*f*) and is also convincing from a morphological point of view. *Brunia* III and *Nebelia* have much larger flowers and inflorescences of the pincushion flower style clustered on stout, erect stems, sessile leaves, fibers on the leaf midvein, as well as rhomboidal crystals in bundle sheath cells (the latter after Carlquist 1991), while *Pseudobaeckea* pp. and *Raspalia* I have smaller flowers and inflorescences with included stamens dispersed on more intricately branched shoot systems, petiolate leaves, and show druses in mesophyll cells together with few or no fibers on leaf midveins (the latter after Carlquist 1991). While ITS data favor the monophyly of *Brunia* II and *Nebelia* (fig. 2*f*, *Brunia neglecta* missing in the ITS data set), *mat*K data ally *Brunia macrocephala* with the *Nebelia* species (figs. 1, 2*a*). Morphological synapomorphies support the monophyly of each, *Brunia* III and *Nebelia*. *Brunia* III is characterized by unequal filament lengths (Classen-Bockhoff 2000) and filaments exceeding petals in length (Pillans 1947), while *Nebelia* has stomata on the abaxial surface restricted to the lower half (R. Classen-Bockhoff, personal observation). Tannins characterize *Nebelia* as well as the related *Pseudobaeckea* taxa (except *Pseudobaeckea africana*) and *Raspalia* I (Carlquist 1978) but are absent in *Brunia* II, while consistently biovulate chambers are present in *Brunia* II but absent in the related taxa. Morphology thus clearly advocates a separation of *Nebelia* and *Brunia* II as reflected by ITS data (fig. 2*f*).

Further incongruencies concern the relationships within *Nebelia*. Again, ITS data are more convincing because *N. paleacea*, *Nebelia stokoei*, and *Nebelia fragarioides* have much smaller (individual) inflorescences than *Nebelia sphaerocephala* and *Nebelia laevis*, which clearly resemble the related *Brunia* species. In *N. stokoei* and *N. fragarioides*, 30–50 individual inflorescences are arranged in spherical aggregates (Classen-Bockhoff 2000). Regarding the molecular markers, either these aggregates evolved parallel, reflecting the general tendency of inflorescences toward compound clusters (Maresquelle 1970; Sell 1976) or they were gained once with a subsequent loss by disintegration to small and often tightly clustered solitary inflorescences in *N. paleacea*.

The subclade comprising *Pseudobaeckea* pp. and *Raspalia* I contains a weakly supported monophyletic group of *Pseudobaeckea* pp. and an unresolved *Raspalia* I (figs. 1, 2*a*). ITS data provide more and stronger resolution for a monophyletic *Pseudobaeckea* pp. and *Raspalia* I, respectively (fig. 2*f*). Both groups also differ in morphological features: *Pseudobaeckea* pp. with a calyx constricted at and articulated with the top of the ovary (Pillans 1947), apiculae lacking on the petaloid calyx lobes, and stomata present on both leaf surfaces (Carlquist 1991), and *Raspalia* I with a different calyx, apiculae present, and stomata present only on adaxial side of the leaves. In this context, a monophyletic origin of *Pseudobaeckea* pp. seems very likely.

Brunieae: Mniothamnea clade. The difficulty in justifying the segregation of *Raspalia* species morphologically becomes even more problematic concerning the apparent molecular differences between a clade comprising *Raspalia* II and an alliance of *Raspalia trigyna* and *R. virgata* (*Raspalia* III) with *Mniothamnea* (figs. 1, 2*a*). At present, there are no convincing morphological characters that would advocate this difference.

The monophyly of *Mniothamnea* is not clear from *mat*K data but becomes evident from ITS data (fig. 2). As expected from the original classification, the two species of *Mniothamnea* are also characterized by morphological novelties: they have solitary flowers at the top of leafy shoots (Classen-Bockhoff 2000) and monomerous ovaries (Pillans 1947), while the related *Raspalia* species (*Raspalia* III) and *Lonchostoma* have flowers aggregated in flower heads and dimerous ovaries.

Lonchostoma, clearly monophyletic by molecular analysis, is distinguished from the remaining Brunieae by having salver-shaped flowers pointing to adaptation to long-tongued insect pollinators. A particular petal type (Quint and Classen-Bockhoff, forthcoming) and fusion of filaments and petals to a tube are synapomorphies probably related to the pollination syndrome. It should be noted that the fusion is imperfect or almost invisible in *Lonchostoma purpureum*, which comes out as sister to the rest of the genus, probably marking the beginning of an evolutionary trend toward fully fused petal-stamen tubes.

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Appendix

Table A1

Plant Material of Bruniaceae and Accession Numbers of Sequences Deposited in the European Molecular Biology Laboratory Gene Bank

Taxon	*mat*K	ITS	Collection and deposition
Audouinia capitata (L.) Brongn.	AY490978	AY494050	CB 4000, MJG 040549
Berzelia abrotanoides (L.) Brongn.	AY490954	AY494009	CB 4025, MJG 040550
B. arachnoidea (Wendl.) Eckl. & Zeyh.	AY490956	AY494010	CB 4006, MJG 040565
B. burchellii Duemmer	AY490948		CB 4005, MJG 040551
B. cordifolia Schldl.	AY490958	AY494012	Quint Q48, MJG 040508
B. ecklonii Pillans	AY490947		CB 4027, MJG 040552
B. galpinii Pillans	AY490951		CB 4029, MJG 040566
B. incurva Pillans	AY490949		Quint Q45, MJG 040505
B. intermedia (Dietr.) Schldl.	AY490950		CB 4030, MJG 040553
B. lanuginosa (L.) Brongn.	AY490955		CB 4032, MJG 040567
B. rubra Schldl.	AY490957	AY494011	Quint Q12, MJG 040296
Brunia albiflora Phill.	AY490953		CB 4033, MJG 040554
B. alopecuroides Thunb.	AY490959		CB 4034, MJG 040555
B. macrocephala Willd.	AY490944		Quint Q17, MJG 040299
B. neglecta Schltr.	AY490945		Quint Q25, MJG 040298
B. nodiflora L.	AY490946		CB 4036, MJG 040556
B. stokoei Phill.	AY490952	AY494008	CB 4014, MJG 040568
Linconia alopecuroidea L.	AY490981	AY494029	Quint Q15, MJG 040287
L. cuspidata (Thunb.) Schwartz	AY490980	AY494028	Quint Q51, MJG 040511
L. ericoides Oliv.	AY490979	AY494027	O 11200, NBG 193190
Lonchostoma esterhuyseniae Strid	AY490920		O 11231, NBG 193163
L. monogynum (Vahl) Pillans	AY490917		CB 4007, MJG 040569
L. myrtoides (Vahl) Pillans	AY490918		Quint Q38, MJG 040494
L. pentandrum (Thunb.) Pillans	AY490919		CB 4008, MJG 040557
L. purpureum Pillans	AY490921	AY494014	Quint Q9b, MJG 040302
Mniothamnea bullata Schltr.	AY490922	AY494030	CB 4017, MJG 040558
M. callunoides (Oliv.) Niedenzu	AY490923	AY494031	Quint Q47, MJG 040507

Table A1

(*Continued*)

Taxon	*mat*K	ITS	Collection and deposition
Nebelia fragarioides (Willd.) Kuntze	AY490942	AY494024	CB 4012, MJG 040559
N. laevis (E. Mey.) Kuntze	AY490939		Quint Q49, MJG 040509
N. paleacea (Berg.) Sweet	AY490941	AY494023	CB 4037, MJG 040560
N. sphaerocephala (Sond.) Kuntze	AY490940	AY494021	Quint Q4, MJG 040291
N. stokoei Pillans	AY490943	AY494022	Quint Q53, MJG 040514
Pseudobaeckea africana (Burm.F.) Pillans	AY490937		CB 4004, MJG 040570
P. cordata (Burm.F.) Pillans	AY490936	AY494019	Quint Q9, MJG 040285
P. cordata var. *monostyla* Pillans	AY490938	AY494020	Quint Q36b, MJG 040492
P. teres (Oliv.) Duemmer	AY490974	AY494044	CB 4020, MJG 040561
Raspalia angulata (Sond.) Niedenzu	AY490928		Quint Q6, MJG 040280
R. dregeana (Sond.) Niedenzu	AY490932	AY494013	Quint Q40, MJG 040499
R. globosa (Lam.) Pillans	AY490930		Quint Q11, MJG 040479
R. microphylla (Thunb.) Brongn.	AY490929		CB 4011, MJG 040571
R. oblongifolia Pillans	AY490933	AY494016	Quint Q41, MJG 040500
R. phylicoides (Thunb.) Arn.	AY490931		Quint Q32, MJG 040486
R. sacculata (Bolus ex Kirchner) Pillans	AY490926		Quint Q24b, MJG 040282
R. stokoei Pillans	AY490934	AY494017	Taylor 8659, NBG[a]
R. trigyna (Schltr.) Duemmer	AY490925	AY494033	de Lange 6, NBG 755709
R. variabilis Pillans	AY490927		Quint Q50, MJG 040510
R. villosa Presl.	AY490935	AY494018	Quint Q39a, MJG 040497
R. virgata (Brongn.) Pillans	AY490924	AY494032	CB 4016, MJG 040562
Staavia brownii Duemmer	AY490966	AY494040	Quint Q26b, MJG 040289
S. comosa Colozza	AY490965	AY494039	Quint Q33a, MJG 040487
S. dodii H. Bol.	AY490962	AY494036	CB 4039, MJG 040563
S. dregeana Presl.	AY490961	AY494035	CB 4040, MJG 040573
S. glutinosa (Berg.) Dahl	AY490960	AY494034	CB 4023, MJG 040574
S. phylicoides Pillans	AY490968	AY494042	P WAP.579, MJG 040529
S. radiata (L.) Dahl	AY490963	AY494037	CB 4042, MJG 040575
S. verticillata (L.f.) Pillans	AY490967	AY494041	Quint Q36a, MJG 040491
S. zeyheri Sond.	AY490964	AY494038	Quint Q16, MJG 040288
Thamnea diosmoides Oliv.	AY490972	AY494046	O 10769, NBG 755709
T. hirtella Oliv.	AY490973	AY494047	Quint Q44, MJG 040504
T. massoniana Duemmer	AY490969	AY494043	CB 4010, MJG 040576
T. thesioides Duemmer	AY490971	AY494045	Quint Q42a, MJG 040501
T. uniflora Sol. ex Brongn.	AY490970		Quint Q24, MJG 040290
Tittmannia esterhuyseniae Powrie	AY490976	AY494048	CB, E 4021, MJG 040564
T. laevis Pillans	AY490977	AY494049	Quint Q35, MJG 040490
T. laxa (Thunb.) Presl	AY490975		Quint Q30, MJG 040481

Note. Nomenclature after Pillans (1947), Strid (1968), Powrie (1969*b*), and Oliver (1999). CB = Claßen-Bockhoff, E = Esterhuysen, O = Oliver, P = Pretorius.

[a] In revision.

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