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Ancient or recent? Insights into the temporal evolution of the Bruniaceae

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Abstract

The Bruniaceae are a South African plant family endemic to the Cape Floristic Region with one geographic outlier (*Raspalia trigyna*) in the Natal Province. Recent molecular phylogenetic analyses have cast new light upon inter- and intra-generic relationships within the family. The present work uses those data to gain insights into the temporal evolution of Bruniaceae by inferring a molecular clock. For calibration, the inferred age of *Berzelia cordifolia* (3–5 My) was used, based on its distribution restricted to the geologically young limestone area around Bredasdorp. The results are consistent with the purported Cretaceous age of the family ('palaeoendemics'), but also suggest that most extant species are relatively young. The major diversification of the family may have happened within relatively recent times (between 18 and 3 Mya), simultaneously with the establishment of the present Mediterranean climate in their environment. The disjunct distribution of *Raspalia trigyna* may be attributable to migration over sandstone exposed during a brief marine regression of the Indian Ocean at the Miocene–Pliocene boundary.

Keywords: Bruniaceae; Cape floral clade; Molecular clock; Palaeoendemics; Late Tertiary radiation

Introduction

The Cape Floristic Region (CFR) not only harbours one of the most species-rich floras of the world, but is also unique in its floristic composition with a high species-to-genus ratio and almost 70% endemics (Linder 2003; Linder and Hardy 2005; Mucina and Rutherford 2006).

In the past a Gondwanan origin was assumed for part of the Cape floral elements, but recent climatic and phylogenetic reconstructions point to much younger radiation events (Linder 2005).

The Bruniaceae (12 genera, 64–78 species) are a characteristic element of the fynbos biome. With only one species in Pondoland, it is near-endemic to the CFR and represents one of the 33 'Cape floral clades' that together include 50% of the angiosperm species of the CFR (Linder 2003). Reconstruction of their radiation by dating phylogenies and identifying the triggers for speciation will help to understand the evolution of the unique Cape flora.

Raspalia trigyna is the only geographic outlier. It is known from two populations growing on sandstone soils in Natal/Pondoland (Natal Group), at a distance of c. 600 km the nearest sandstone outcrop of significant size to the Cape Supergroup (van Wyk 1990a). The strong floristic links between the Cape flora and the Pondoland Centre (Sim 1907; Acocks 1953) are also known from other genera (e.g. *Leucadendron, Leucospermum, Erica*, *Aspalathus*, *Lotononis*, *Phylica*, *Watsonia*, *Felicia*, *Athanasia*, *Psoralea* and *Relhania*; van Wyk 1990a).

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Generally, Bruniaceae grow on acidic, low-nutrient soils derived from quartzitic sandstone bedrock. The only exception to the sandstone habitat is *Berzelia cordifolia*. This species occurs in the Bredasdorp area (Agulhas Plain) as a limestone endemic (A. Hall, pers. comm.). It is not found on other limestone soils (i.e. not in the Riversdale area). The habitat of *Berzelia cordifolia* was apparently flooded by the ocean until at least 5 Mya or up to 3 Mya (Siesser and Dingle 1980; Rogers 1987; Maud and Botha 2000; Mucina and Rutherford 2006).

Many species occur on the predominantly moist, cloud-covered southeastern mountain slopes frequently characterised as a relict habitat reflecting ancient climatic conditions (Hall 1988; Goldblatt and Manning 2002). Representatives of Bruniaceae are considered palaeoendemics (Hall 1987, 1988; Carlquist 1991; S.E. de Villiers, pers. comm.), i.e. as taxonomically isolated descendants from an ancient stock with no close relatives in proximity. As Cape floral lineages (e.g. Proteaceae, Restionaceae, Ericaceae) dating back to the Tertiary (71–64 Mya; Scholtz 1985) radiated only between 18 and 8 Mya (Linder 2003, 2005), the question arises whether the Bruniaceae would also fit this pattern of ancient origin and recent speciation. This hypothesis is tested in the present study by reconstructing the temporal evolution of the family.

Material and methods

Molecular data sets

Temporal aspects of the evolution of Bruniaceae are assessed by reanalyzing published *mat*K, *rbc*L and ITS data (for details see Quint 2004; Quint and Claßen-Bockhoff 2006a) with molecular clock constraints. The *mat*K data set covering 82% of the species is the largest one and serves as the basis for estimating temporal evolution in the family. It identifies the Linconieae (with *Linconia* only) as sister to the remaining Audouinieae (*Audouinia, Pseudobaeckea teres*, *Thamnea*, *Tittmannia*) and Brunieae (*Staavia*, *Berzelia*, *Brunia*, *Lonchostoma*, *Mniothamnea*, *Nebelia*, *Pseudobaeckea* p.p., *Raspalia*) each representing monophyletic groups. Bootstrap values are generally high (Quint and Claßen-Bockhoff 2006a).

For *rbc*L, 12 species were selected representing all recognised genera in Bruniaceae. ITS sequences could not be aligned across major genera or clades, thus were analysed separately.

Checking for constancy of evolutionary rates

To check molecular data sets for constancy of evolutionary rates, maximum likelihood (ML) calculations were conducted in PAUP, both with a molecular clock enforced and not enforced. Scores of ML trees (clock vs. non-clock) were subjected to a modified χ^2-test, to test for significant rejection of the null-hypothesis (i.e. constant substitution rates). It is based on the formula $\delta = 2(\ln L_{\text{nonclock}} - \ln L_{\text{clock}})$, with δ following a χ^2-distribution (Yang et al. 1995), L_{nonclock} being the model with molecular clock not enforced, L_{clock} being the model with molecular clock enforced (null-hypothesis), and $n-2$ degrees of freedom (n = number of sequences in the data set). The presence of a global molecular clock can be postulated if the difference between the two ML values is not significant. The global likelihood ratio test (LRT) can be considered as superior to the frequently used relative rate test, which is restricted to local testing as it averages only over sublineages of a given phylogeny and does not test rate constancy from a branch earlier in time to one later in time (Sanderson 1998).

Molecular clock analysis

Search options for maximum likelihood calculations were generally restricted to heuristic searches (random taxon addition, tree bisection reconnection (TBR)) analysing each locus separately. For molecular clock calculations, indel events were excluded from the original *matK* alignment (Quint and Claßen-Bockhoff 2006a) to reduce the computational effort for missing-data entries in the data set. Considering the uncertainty about the sister taxon of Bruniaceae (APG 1998; Savolainen et al. 2000; Soltis et al. 2000; Albach et al. 2001; Bremer et al. 2001, 2002), we calculated the molecular clock within Bruniaceae only, thus excluding bias caused by rate heterogeneity due to distantly related taxa. Using *Linconia* as the outgroup seems justified, since this genus appears as sister to all other Bruniaceae in all analyses of different genes and different potential outgroups from Euasterid II (Quint 2004). The ML analysis was carried out using the model TVM + Γ selected by MODELTEST 3.06 (Posada and Crandall 1998).

In functionally highly constrained protein coding genes such as *rbc*L, non-synonymous substitutions are likely to be non-clocklike in closely related lineages, thus may not be suitable for estimating divergence times (Albert et al. 1994; Davis et al. 1998; Xiang et al. 2000). Non-synonymous substitutions were therefore excluded from the *rbc*L molecular clock analysis (Albert et al. 1994; Davis et al. 1998; Xiang et al. 2000). For the *rbc*L data set, MODELTEST suggested the HKY + Γ model. For the much less constrained *mat*K gene, Hilu and Liang (1997) have suggested that amino acid and nucleotide variation are indicative of neutral selection. Even lower functional constraints and quasi-neutral selection have also been inferred for the non-coding ITS

region (Baldwin et al. 1995; Soltis and Soltis 1998). Accordingly, molecular clock calculations of *mat*K and ITS data were performed over all substitutions.

ITS data could be provided for separate clades only (Quint and Claßen-Bockhoff 2006a). Molecular clock calculations in ITS involve the *Brunia*/*Pseudobaeckea*-clade and *Staavia*. For the *Brunia*/*Pseudobaeckea*-clade, MODELTEST determined HKY + Γ as the best-fitting model; for *Staavia* that choice was K81uf + Γ.

Calibration

As direct (fossils) and indirect evidence (relative split between Bruniaceae and its sister family) is lacking, a calibration point based on the endemism of *Berzelia cordifolia* is used in the present study. Though calibration at internal nodes is not recommended, because the sources of error are accumulated along basal nodes (Magallon 2004), there is no alternative to that procedure at this time.

Since the limestone habitat of *Berzelia cordifolia* was inundated until at least 5 Mya, a maximum age for this particular species is provided. The maximum age of 5 My must be regarded as conservative, because it is highly probable that the species emerged at a somewhat later point in time. Exposure of the highest limestone hills in the Bredasdorp area should not have happened later than c. 3 Mya (Siesser and Dingle 1980; Rogers 1987; Linder 2003). Therefore, a second calibration point at 3 Mya was used. It should be noted that both calibration points provide maximum ages, reflecting the time-span uncertainty of the geological findings.

Lineages through time

The lineages-through-time approach provides a means to illustrate the development of species numbers in a given period of time. It may form a base for estimations of speciation and extinction rates in a phylogeny of species, and potentially for quantifying how these rates have changed over time (Hey 1992; Harvey et al. 1994; Nee et al. 1994; Avise 2000; Nee 2001; Barraclough and Vogler 2002). A detailed statistical survey of speciation and extinction rates is not provided here. Lineages-through-time plots are used, however, to give basic information on the possible timing of diversifications which can be extracted from a dated phylogeny of Bruniaceae.

The phylogeny of the relatively comprehensive *mat*K data set (64 species and 1 variety) was extended by adding data for the 14 missing species (78 species have been recognised to date), since leaving out the missing species would have caused a bias towards extinction (Losos 1990; Barraclough and Vogler 2002). The 14 species not sampled for molecular analysis (*Berzelia commutata*, *Berzelia dregeana*, *Brunia laevis*, *Linconia deusta*, *Nebelia tulbaghensis*, *Pseudobaeckea stokoei*, *Raspalia barnardii*, *Raspalia palustris*, *Raspalia schlechteri*, *Raspalia staavioides*, *Staavia trichotoma*, *Thamnea depressa*, *Thamnea gracilis*, *Tittmannia hispida*) were inserted in their respective most likely places in the tree on the basis of taxonomic accounts (Pillans 1947) and our own morphological observations (Quint 2004). Note that it is not possible to determine branch lengths for those added species. Instead, each node for such a species was set at the midpoint along the branch it most likely descended from (Losos 1990; Barraclough and Vogler 2002). Since it appears more appropriate to rank *Pseudobaeckea cordata* var. *monostyla* as a species (Quint and Claßen-Bockhoff 2006a), this taxon was also included in the lineages-through-time plots, resulting in a set of 79 species. Lineages-through-time plots were calculated for the whole family as well as for the genera *Berzelia* (including *Brunia alopecuroides*, *Brunia albiflora*, *Brunia stokoei*) and *Staavia* (including *Raspalia staavioides*).

Results

The global LRT results support rate constancy ($p > 0.01$) as a precondition for a molecular clock (except for the ITS analysis of Audouinieae, which was thus not pursued any further; Table 1).

The ML analysis of the *mat*K data set resulted in 36 most likely trees ($\ln L_{\text{nonclock}} = -7741.89805$). Conducting a search under the same parameters, but with a molecular clock enforced, also led to 36 ML trees of identical tree topology ($\ln L_{\text{clock}} = -7786.0666$). The ultrametric *mat*$K_{\text{clock}}$tree is shown in Fig. 1. Considering two calibration points for the age of *Berzelia cordifolia*, resulting evolutionary rates for the

Table 1. Results of the global LRT (rate constancy)

Data set	$\Delta = 2\ (\ln L_{\text{nonclock}} - \ln L_{\text{clock}})$	df = $n-2$	$p > 0.01$	Rate constancy
*mat*K (Bruniaceae)	88.3372	63	0.019320	Yes
*rbc*L (Bruniaceae)	8.7658	10	0.554435	Yes
ITS (Audouinieae)	56.9880	6	0.00	No
ITS (*Staavia*)	15.9026	7	0.026021	Yes
ITS ('*Brunia*/*Pseudobaeckea* clade')	16.8178	9	0.051646	Yes

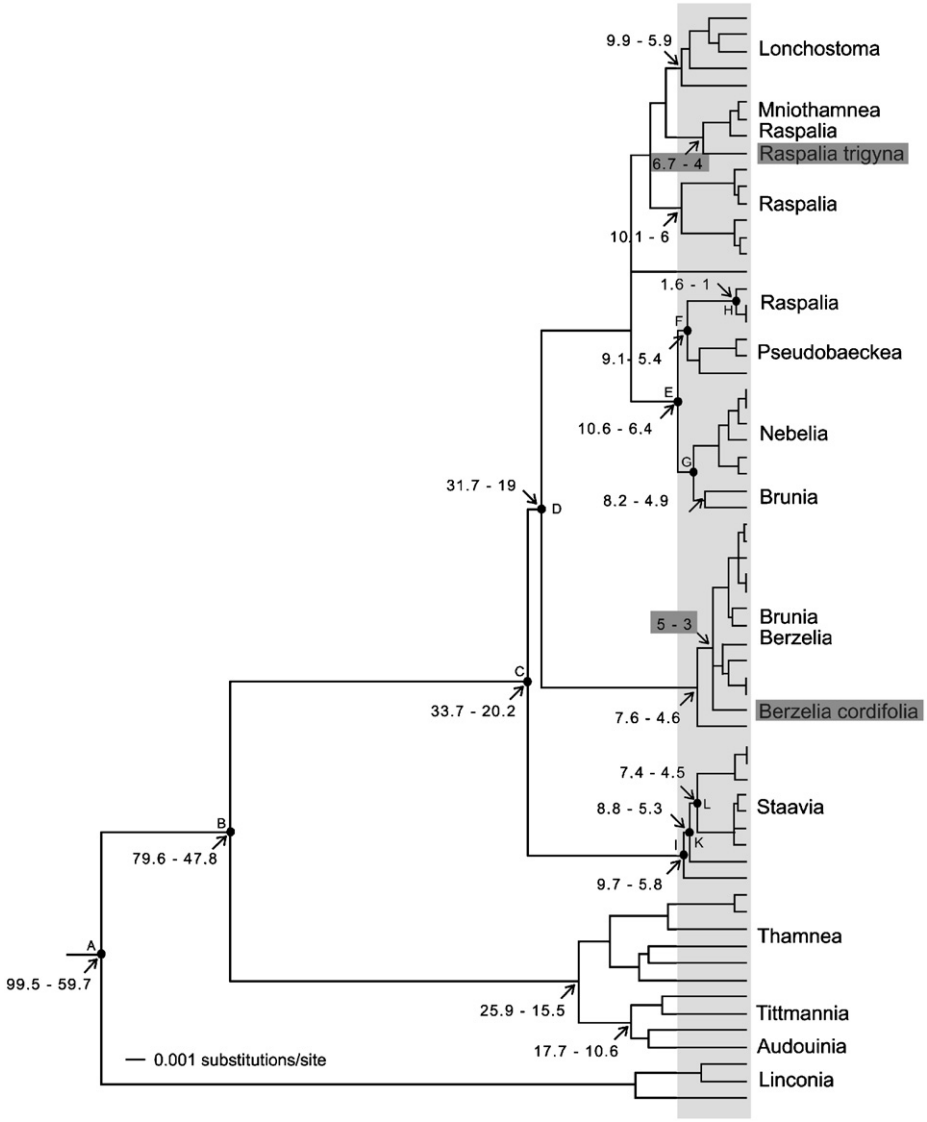

Fig. 1. Ultrametric *mat*K$_{clock}$tree (ln $L_{clock} = -7786.0666$) of 65 Bruniaceae taxa. Emergence of *Berzelia cordifolia* (5–3 Mya) taken as calibration point (grey shading). Emergence of the geographically disjunct *Raspalia trigyna* (6.7–4 Mya) likewise shaded in grey. Nodes marked with black dots and labelled with letters correspond to nodes in Figs. 2–4. For selected nodes, age estimates (in Mya) are assigned via arrows.

*mat*K gene range from 3.78×10^{-10} to 6.3×10^{-10} substitutions/site/year.

ML analysis of the *rbc*L data set resulted in one tree (ln $L_{nonclock} = -2166.1248$). Invoking a molecular clock yielded the same tree topology (ln $L_{clock} = -2170.50775$; Fig. 2).

Molecular-clock calculations in ITS involved the '*Brunia*/*Pseudobaeckea*-clade' and *Staavia* (Figs. 3 and 4). The ML analysis of the '*Brunia*/*Pseudobaeckea*-clade' resulted in one most likely tree (ln $L_{nonclock} = -1338.06096$). Enforcement of a molecular clock led to identical tree topology (ln $L_{clock} = -1346.46982$). In *Staavia*, likelihood scores were ln $L_{nonclock} = -1725.1636$ and ln $L_{clock} = -1733.1149$, also resulting in identical tree topology.

Node ages from the ultrametric *mat*K$_{clock}$tree (Fig. 1) were assigned to nodes in the *rbc*L and ITS data sets, respectively, which were readily comparable concerning congruence, resolution and species composition. The different evolutionary rates inferred from multiple nodes were averaged for the two calibration points, respectively (Table 2).

A family-wide lineages-through-time plot for Bruniaceae and lineages-through-time plots for the '*Berzelia*-clade' and for *Staavia* with 5 Mya as the calibration point shows a roughly linear increase in diversification with two shifts

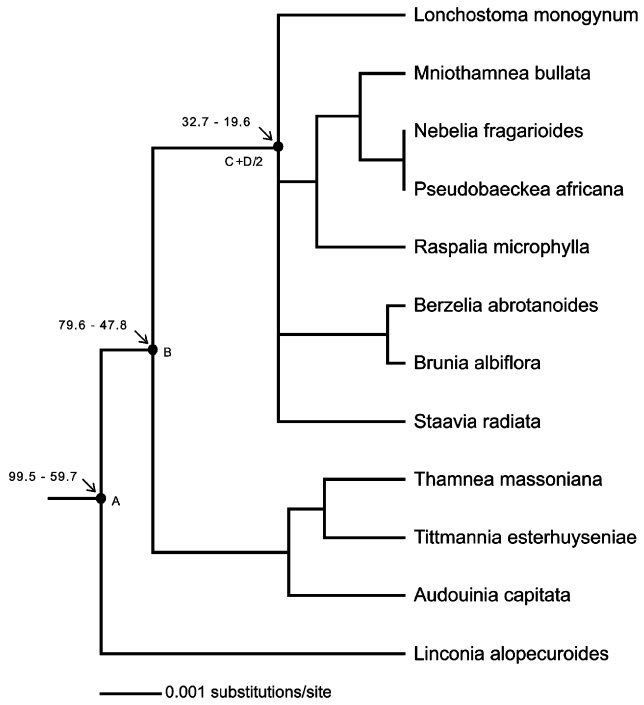

Fig. 2. Ultrametric *rbc*L_{clock}tree ($\ln L_{clock} = -2170.50775$) of representatives of Bruniaceae from 12 different genera. Age estimates (in Mya; assigned via arrows) and nodes (black dots) are the respective specifications of the ultrametric *mat*K_{clock}tree (Fig. 1). C + D/2 = average age of nodes C and D combined (see Table 2).

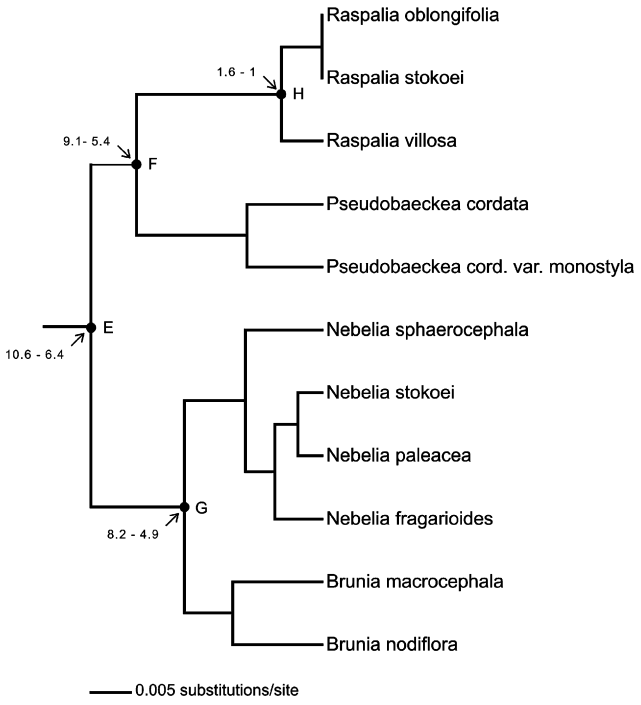

Fig. 3. Ultrametric ITS$_{clock}$tree ($\ln L_{clock} = -1346.46982$) of the '*Brunia*/*Pseudobaeckea*-clade'. Age estimates (in Mya; assigned via arrows) and nodes (black dots) are the respective specifications of the ultrametric *mat*K_{clock}tree (Fig. 1).

(Fig. 5): a minor one within the last 33 My and a marked, consistent diversification shift within 18 My. *Staavia* shows a tremendous shift in diversification within 9 My and the *Berzelia*-clade within 6 My. If the molecular tree was calibrated to a point 3 Mya, the general pattern of the lineages-through-time plots remained identical, but major shifts of the diversification appeared within the last 11 My for the whole family, within 5 My for *Staavia*, and within 3 My for the *Berzelia*-clade.

Discussion

The age of *Berzelia cordifolia* as an internal calibration point

The molecular-clock calculations assessing the temporal evolution of Bruniaceae relied on the origin of *Berzelia cordifolia* as the calibration point. *Berzelia cordifolia* has been recorded only from a few limestone sites in the Potberg region near Bredasdorp, not from other sites providing alkaline substrates (Pillans 1947; A. Hall, pers. comm.; Fig. 6). This region was apparently flooded by the ocean until at least 5 Mya or up to 2–3 Mya (Siesser and Dingle 1980; Midgley 1987; Rogers 1987; Partridge and Maud 2000). The limestone

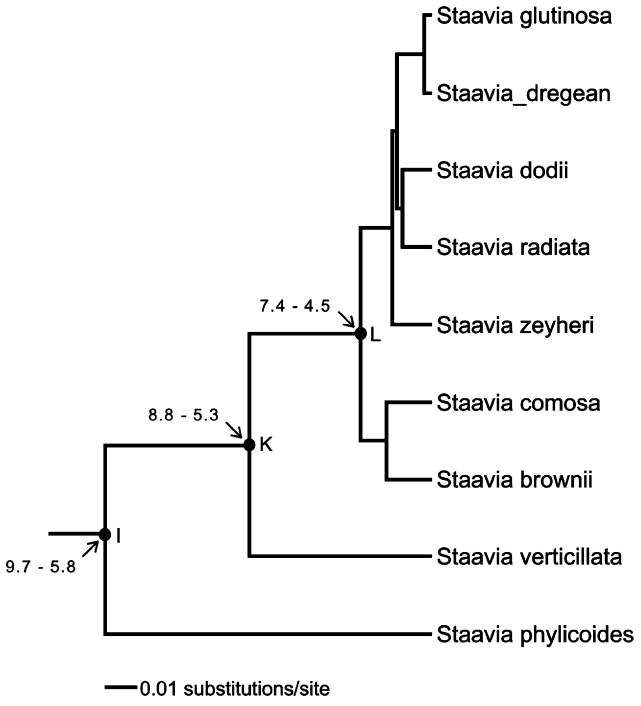

Fig. 4. Ultrametric ITS$_{clock}$tree ($\ln L_{clock} = -1733.1149$) of *Staavia* species. Age estimates (in Mya; assigned via arrows) and nodes (black dots) are the respective specifications of the ultrametric *mat*K_{clock}tree (Fig. 1).

Table 2. Evolutionary rates of *rbc*L and ITS inferred from *mat*K node ages

Data set		Evolutionary rate s/s/y	
		Calibr. point 5 Mya	Calibr. point 3 Mya
*mat*K, analysis 3 (mod.)		3.78×10^{-10}	6.3×10^{-10}
*rbc*L, analysis 2a (mod.)	Node A:	0.51×10^{-10}	0.86×10^{-10}
	Node B:	0.53×10^{-10}	0.89×10^{-10}
	Node (C + D)/2[a]:	0.65×10^{-10}	1.09×10^{-10}
	Average:	0.56×10^{-10}	0.95×10^{-10}
ITS, '*Brunia*/*Pseudobaeckea*	Node E:	2.77×10^{-9}	3.54×10^{-9}
clade', analysis 4e	Node F:	2.61×10^{-9}	4.62×10^{-9}
	Node G:	2.13×10^{-9}	4.35×10^{-9}
	Node H:	3.15×10^{-9}	5.27×10^{-9}
	Average:	2.67×10^{-9}	4.45×10^{-9}
ITS, *Staavia*, analysis 4c	Node I:	11.71×10^{-9}	19.54×10^{-9}
	Node K:	7.15×10^{-9}	11.90×10^{-9}
	Node L:	3.28×10^{-9}	5.47×10^{-9}
	Average:	7.38×10^{-9}	12.30×10^{-9}

[a]Nodes C and D collapse in a polytomy in the *rbc*L data set if calculated with nonsynonymous sites only. However, the timings of nodes C and D are very close in the *mat*K data set (see Fig. 1). It was therefore decided to apply the average time C + D/2 to the *rbc*L data for the node leading to the unresolved Brunieae (see Fig. 2).

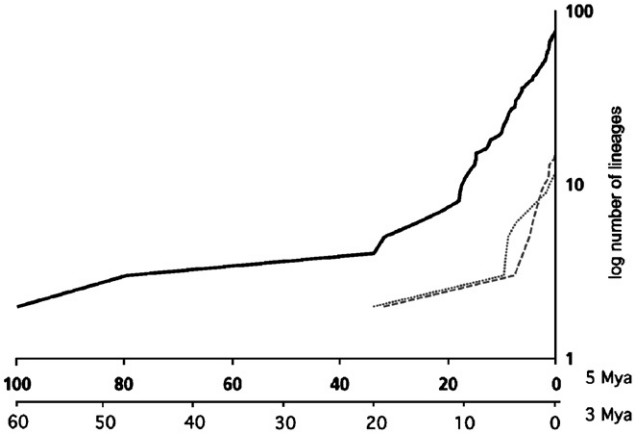

Fig. 5. Lineages-through-time plots with 5 and 3 Mya as respective calibration points for the ultrametric *mat*K_{clock}tree. Solid line: all recognised species of Bruniaceae; dotted line: all species of *Staavia*; dashed line: all species of the '*Berzelia*-clade'.

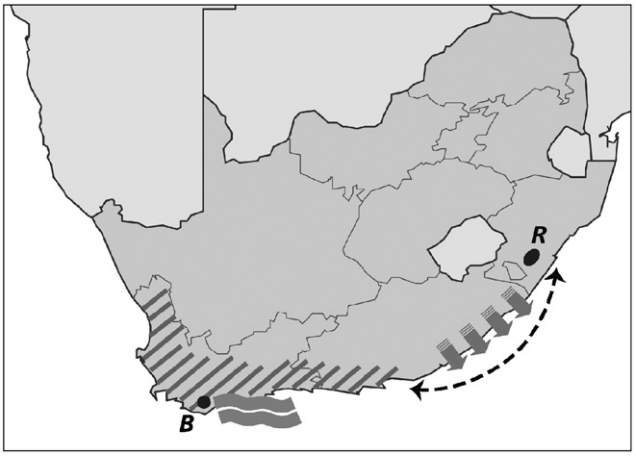

Fig. 6. Geological events used for calibration. *Berzelia cordata* (B) occurs only on the limestone in the Bredasdorp area. This area was flooded by the ocean until 5 Mya, providing the species with an approximate age of 5–3 My. *Raspalia trigyna* (R) is the only outlier of the Bruniaceae occurring on sandstone in Natal/Pondoland. A sandstone bridge might have existed 7–6 Mya, which would explain the inferred divergence time between the species and its Cape relatives.

hills have long been interpreted as deposits laid down by a regressive sea (Haughton 1925; Siesser 1972), although the extent of the last Neogene transgression remains controversial (Siesser and Dingle 1980; Maud and Partridge 1987; Rogers 1987). The maximum height of the limestone hills in the Bredasdorp area is 200 m, which coincides with the highest Neogene sea-level (Linder 2003). This recently exposed limestone habitat has been associated with evolutionary novelties: a minor species radiation of the Restionaceae genus *Thamnochortus* (Linder and Mann 1998), evolution of new 'lowland' species in the section *Herschelia* of the orchid genus *Disa* from montane 'ancestral' species (Linder 1995), and adaptation of *Leucodendron* (Proteaceae) species to the alkaline substrate (Midgley 1987; van Wyk 1990b).

Differences in soil types strongly influence vegetation community boundaries in the fynbos (Mucina and Rutherford 2006), and many fynbos taxa (i.e. Restionaceae, Proteaceae, Ericaceae) are mostly restricted

to certain soils (Marloth 1908; Cowling and Campbell 1980, 1983; Linder 2003). The limitation of taxa to specific soils is also found in Bruniaceae, whose affinity to table mountain sandstone is consistent, with the exception of *Berzelia cordifolia*, which is apparently restricted to the limestones and coastal sands of the Bredasdorp area, although this soil affinity needs to be confirmed by transplantation experiments. It cannot be ruled out that *Berzelia cordifolia* existed prior to the availability of its present habitat, but then one would expect to find its actual distribution range to extend beyond the once inundated Bredasdorp area into the table mountain sandstone of the Cape Mountains. In the CFR, soil chemistry appears to be important in preventing the invasion of limestone by calcifuge species (Newton et al. 1991; Cowling et al. 1992), resulting in the establishment of species boundaries (Rourke 1972; Williams 1972; Goldblatt 1979, 1982; Linder and Ellis 1990; Kurzweil et al. 1991). Thus, the exclusive occurrence of *Berzelia cordifolia* near Bredasdorp may reflect adaptation and speciation on a new, edaphically distinctive habitat.

Testing the calculation by inferring evolutionary rates

Fossils are needed to enhance the accuracy of the dating procedure and to test this geology-based calibration of the molecular clock. Judging from the present diversity of bruniaceous pollen (Hall 1988), palynological fossil studies should be able to discriminate between at least some genera, thus possibly providing new internal calibration points. Indeed, fossil pollen grains similar to those in Bruniaceae have been recorded from Hondeklip Bay in Namaqualand (de Villiers and Cadman 1997; S.E. de Villiers, pers. comm.). But as the dating of these deposits is not clear (Linder 2003), they are not considered in the present study.

Lacking such a minimal-age calibration point, we tested the above molecular-clock calculations indirectly by imposing *mat*K-derived time estimates on *rbc*L and ITS subsets of Bruniaceae and inferring evolutionary rates of the latter markers (Table 2). For *mat*K, an evolutionary rate of 4×10^{-10} substitutions/site/year has been reported in *Paeonia* (Sang et al. 1997) and Rhizophoraceae (Zhong et al. 2000). This compares well to the lower range of *mat*K evolutionary rates observed in Bruniaceae (5 Mya: 3.78×10^{-10}; 3 Mya: 6.3×10^{-10}) and would favour 5 Mya as the date of emergence of the limestone habitat and *Berzelia cordifolia*, respectively. A calibration point of 5 Mya, however, results in a relatively low evolutionary rate for *rbc*L (0.56×10^{-10}; versus for 3 Mya: 0.95×10^{-10}), which would lie below the lowest evolutionary rate quoted for land plants (0.84×10^{-10}; Albert et al. 1994). On the other hand, the calculation would result in higher values if non-synonymous nucleotide substitutions were not strictly excluded (as advocated by Albert et al. 1994; Davis et al. 1998; Xiang et al. 2000). For ITS, Richardson et al. (2001a) reported evolutionary rates ranging from 3.2×10^{-10} to 9.0×10^{-9} (average: 4.66×10^{-9}). ITS rates obtained for the '*Brunia*/*Pseudobaeckea*-clade' (5 Mya: 2.67×10^{-9}; 3 Mya: 4.45×10^{-9}) fall within the range of known ITS rates for 3 Mya as the calibration point, and slightly below the range for 5 Mya. The ITS rates inferred for *Staavia* (5 Mya: 7.38×10^{-9}; 3 Mya: 12.3×10^{-9}) lie in the upper range or exceed the ITS rates given by Richardson et al. (2001a), but rates for the three nodes used in this particular data set differ markedly and may be the result of branch-length differences between *mat*K and ITS topologies (compare Figs. 1 and 4). These differences cause an acceleration bias, when – in the absence of independent calibration points – only node ages from *mat*K are superimposed. Referring to node L (Fig. 4) exclusively, the inferred rates (5 Mya: 3.28×10^{-9}; 3 Mya: 5.47×10^{-9}) are readily comparable to those obtained for the *Brunia*/*Pseudobaeckea* clade (see above). Due to pronounced branch-length differences among the three markers, it remains problematic to impose node ages obtained from one gene on topologies based on other genes. Independent external calibration points for the *rbc*L or ITS data sets would yield a more robust test of *mat*K-derived time estimates. Likewise, comparison to average values taken from the literature remains equivocal, since evolutionary rates may differ markedly from lineage to lineage (Britten 1986; Avise 1994; Warren and Hawkins 2006) and may not have much explanatory power. The comparisons outlined above, however, may not be able to tip the scales in favour of either 5 or 3 Mya as the calibration point, but the general picture remains that evolutionary rates of Bruniaceae are in general agreement with those obtained by other authors for various plant lineages.

Reconstruction of the Cape-Pondoland disjunction

According to the ultrametric tree the only geographic outlier of the family, *Raspalia trigyna*, could have originated in a time span from 6.7 to 4 Mya. Dispersal abilities of the Cape flora are generally very poor (Goldblatt 1997; but see the discussion on post-Gondwanan intercontinental relationships in Linder 2005). Although impossible to rule out, long-distance dispersal is unlikely in the Bruniaceae considering their indehiscent nuts or dehiscent capsules (Pillans 1947), which lack morphological adaptations to enhance dispersal abilities. Thus, a vicariance event becomes more plausible to explain the characteristic disjunction between the two sandstone regions. A once direct sandstone link between Natal/Pondoland and the Cape

was probably established by the Falkland Plateau, which abutted on the African plate, but broke up in Cretaceous times (van Wyk 1990a). However, this sandstone bridge vanished too long ago (van Wyk 1990a) to explain the relatively short divergence time (6.7–4 My) between *Raspalia trigyna* and its Cape allies. Divergence estimates based on morphological analysis of similarly disjunct *Leucadendron* species make it unlikely that the disjunction of the latter genus is Cretaceous in origin (Midgley 1987; van Wyk 1990b). Midgley (1987) hypothesised that climatic fluctuations during the Pleistocene fragmented populations of *Leucadendron*, but this is difficult to reconcile with the strict edaphic preferences of extant *Leucadendron* species and the Cape flora in general (van Wyk 1990b). However, referring these data to the suggested age of *Raspalia trigyna* a Pleistocene population fragmentation again would be too recent.

A more likely explanation consists in a possible brief and dramatic retreat of the seas on the Miocene–Pliocene boundary (Siesser 1980; Siesser and Dingle 1980; Hallam 1992; Linder 2003) approximately 6–7 Mya, prior to the major transgression c. 5–3 Mya. This retreat could have exposed more or less continuous low-nutrient sandstone outcrops, which are inundated at present (Maud and Botha 2000; Fig. 6). A possible remnant of these now submerged habitats – also occupied by Cape floristic elements – is the Dwesa Nature Reserve, 100 km to the south of the Pondoland Centre (van Wyk 1990b). The opening of this migration route along the coast 6–7 Mya and its subsequent closure by transgression of the sea c. 5 Mya could give the most likely explanation for the inferred divergence time between *Raspalia trigyna* and its Cape relatives (6.7–4 Mya).

Age estimation of the Bruniaceae

There is broad agreement that Bruniaceae should be regarded as an ancient relict family (Hall 1987, 1988; Linder et al. 1992; Cowling and Richardson 1995; Goldblatt and Manning 2002). Hall (1987, 1988) postulated an ancestry of Bruniaceae going back to at least 30–40 Mya. Carlquist (1978) pointed at the highly primitive wood anatomy of Bruniaceae. Considering the size of the family, several morphological features are remarkably diverse (Carlquist 1978, 1991; Hall 1988; Claßen-Bockhoff 2000; Quint and Claßen-Bockhoff 2006b), which can be explained best by considerable age providing enough time to accumulate morphological diversity, and which likewise explains their apparent distinctness from other angiosperm plant groups (Quint 2004). Bruniaceae occur predominantly in mountainous habitats (Carlquist 1978, 1991; Hall 1988; Claßen-Bockhoff 2000), obviously displaying a preference for mesic microclimates that may resemble their original climatic environment prior to the development of more arid and seasonal conditions at the Cape (Hall 1988; Goldblatt and Manning 2002).

In any case, Bruniaceae appear not to be older than 99–107 My, the age estimate given by Wikström et al. (2001) for the Aquifoliales, which stand in a basal position in Euasterids II, to which Bruniaceae belong (Savolainen et al. 2000; Soltis et al. 2000; Albach et al. 2001; Bremer et al. 2001, 2002; Quint 2004), and also the age estimate given by Crepet et al. (2004) for the root node of Asterids I–IV. More precise constraints are precluded by the fact that assignment of Bruniaceae (and Columelliaceae) to a particular order in Euasterids II remains unresolved. It should be taken into consideration, however, that the age estimates for Apiales (85–90 My), Dipsacales (85–90 My) and Asterales (94–101) (Wikström et al. 2001) are comparable to our estimate for Bruniaceae (59.7–99.5 My), which may be sister to one of those two orders in Euasterids II. With only *Berzelia* included, Wikström et al. (2001, electronic appendix) proposed an age of 85–90 My for Bruniaceae.

Recent speciation events in Bruniaceae

The considerable age of the family contrasts with the apparently young age of some of its members. Frequently, closely related species are not older than 3 My or even younger (Fig. 1). Their young age (and, thus, little differentiation) may also allow the occurrence of hybridisation, which has been reported within *Staavia* (Powrie 1969) and *Brunia* (A. Hall, pers. comm.). The term ‘palaeoendemics’, often used in connection with Bruniaceae (Hall 1987, 1988; Linder et al. 1992; Cowling and Richardson 1995; Goldblatt and Manning 2002), seems rather misleading in this context, since the entire family Bruniaceae likely descended from an ancient stock and is now taxonomically isolated (i.e. ‘palaeoendemic’), but most present members actually are ‘neoendemics’, namely relatively recently evolved with close relatives in proximity (for both those terms, see Favarger and Constandriopoulos 1961; Stebbins and Major 1965).

Lineages-through-time plots reveal a distinct acceleration in the increase in species number at c. 18 Mya (Bruniaceae), c. 9 Mya (*Staavia*), and c. 6 Mya (*Berzelia*), respectively, if 5 Mya is used as the calibration point. Consequently, the more recent calibration point of 3 Mya results in more recent time estimates for these ‘radiations’: c. 11 Mya (Bruniaceae), c. 5 Mya (*Staavia*), and c. 3 Mya (*Berzelia*) (Figs. 5 and 7). Although the term ‘radiation’ should be founded on statistically tested changes in speciation and extinction rates (Barraclough and Vogler 2002), and the need for future work on this matter is appreciated,

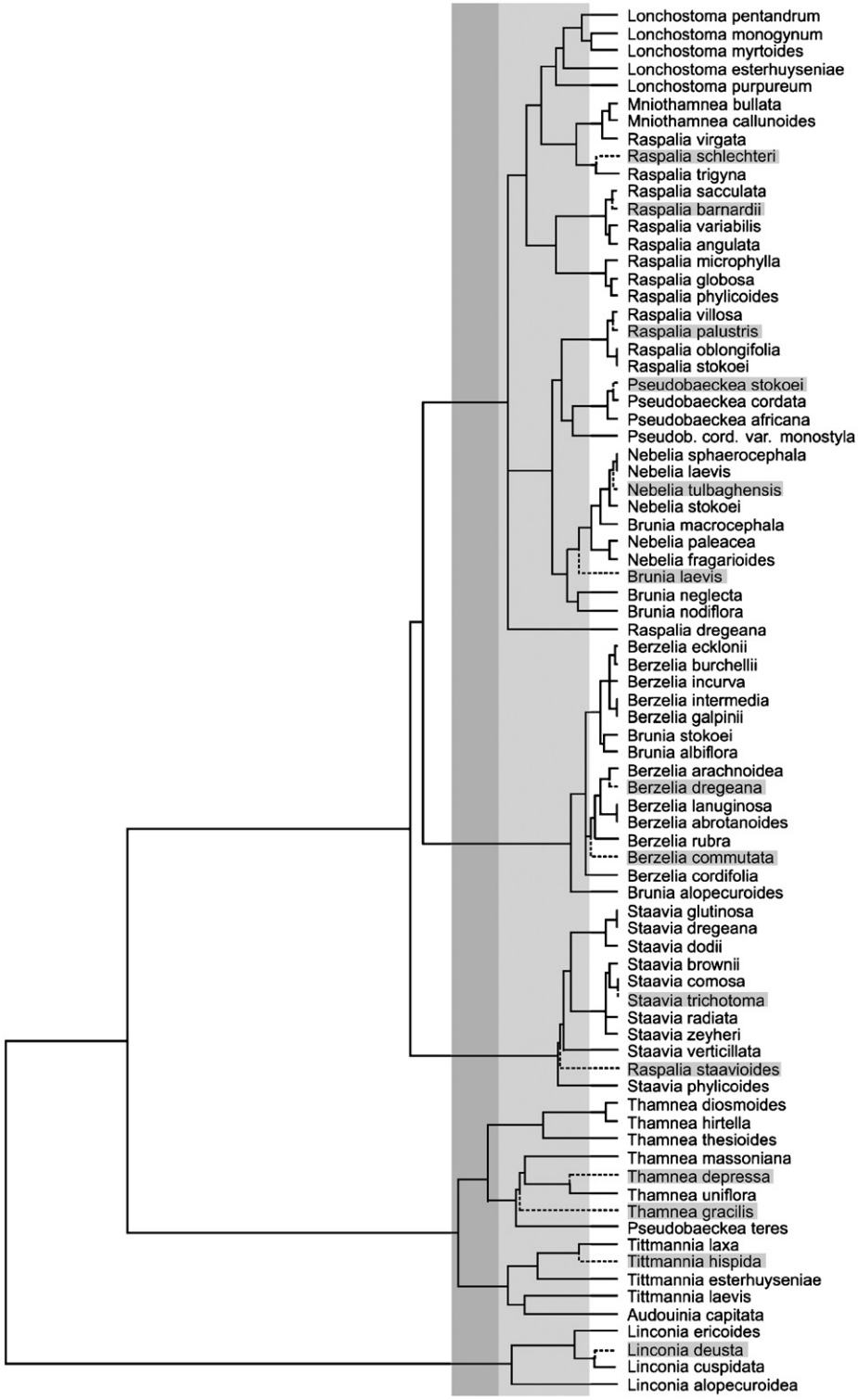

Fig. 7. Recent speciation in Bruniaceae. Taxa missing in the molecular analysis (highlighted in grey) were placed in the ultrametric *mat*K_{clock}tree in their respective most likely positions on the basis of morphological accounts (Pillans 1947; Quint 2004). Note that lengths of dotted lines leading to those taxa are not equivalent to branch lengths for taxa included in the molecular analysis. Dark grey vertical bar: Pliocene; light grey bar: Miocene.

we want to acknowledge the outlined increase in species number by comparing it to already dated molecular phylogenies.

Long branches leading to radiating crown groups (with short branch lengths) have been hypothesized several times in Cape plant phylogenies, and indicate the

survival of only one lineage of a group being present in the area for a long time (Linder 2003, 2005). The emergence of new habitats by climatic change near the Miocene–Pliocene boundary (8–6 Mya) has frequently been named as a radiation trigger (Levyns 1964; Linder et al. 1992; Goldblatt and Manning 2002; Goldblatt et al. 2002; Linder 2005; Warren and Hawkins 2006). Several phylogenies of Cape floral lineages covering a wide phylogenetic spectrum have provided estimates for the initiation of radiations (Bakker et al. 1999, 2004; Goldblatt et al. 2002; Reeves 2001; Richardson et al. 2001b; Verboom et al. 2003; Linder et al. 2005). The suggested dates in these estimates vary substantially (from 20 to 8 Mya) and may indicate that not all radiations started at the same time, which is difficult to reconcile with a general explanation founded on climatic change (Linder 2003). Interestingly, the estimates for the beginnings of radiations lie at the upper boundary for Bruniaceae as a whole and at the lower boundary for two exemplary genera, when compared to the estimates given by other authors.

Conclusions

The present data illustrate that the dating of diversifications in Bruniaceae matches those for other Cape plant lineages reasonably well. Accepting the calibration point used, the Bruniaceae emerge as a plant family with ancient origins and recent speciation events. Dates for the temporal evolution of the family also provide reasonable explanations for a vicariance event resulting in the present disjunction between *Raspalia trigyna* and the remainder of Bruniaceae. Though the triggers for the speciation burst at the Cape summarized by Linder (2003) may predominantly have affected the evolution of the Bruniaceae, further studies on the potential role of pollinators and breeding systems are needed. As one of the exclusively Cape lineages, the Bruniaceae merit further attention since they provide a promising model for the evolution of relict plants that have survived in southern Africa and undergone evolutionary pathways possibly triggered by climatic changes near the end of the Miocene.

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