Systematic Botany (2008), 33(1): pp. 133–147

Systematic Position of the Anomalous Genus *Cadia* and the Phylogeny of the Tribe Podalyrieae (Fabaceae)

James S. Boatwright,[1,4] Vincent Savolainen,[2] Ben-Erik van Wyk,[1] Anne Lise Schutte-Vlok,[3] Félix Forest,[2] and Michelle van der Bank[1]

[1]Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, Johannesburg, South Africa
[2]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, U.K.
[3]CapeNature, Private Bag X658, Oudtshoorn, 6620, South Africa
[4]Author for correspondence (920103313@student.uj.ac.za)

Communicating Editor: James F. Smith

***Abstract*—**Tribe Podalyrieae is a group of papilionoid legumes that are largely endemic to the Cape Floristic Region of South Africa, possessing fire survival strategies with both nonsprouting and sprouting species. A phylogenetic study of the tribe was undertaken using gene sequences obtained from the internal transcribed spacer (ITS) of nuclear ribosomal DNA as well as the plastid *rbcL* gene (107 species). Several clades were identified within the tribe. Subtribe Xiphothecinae consists of the genera *Amphithalea* and *Xiphotheca*. Subtribe Podalyriinae was paraphyletic. Based on the results of this study, *Liparia* (except *L. calycina*) and *Podalyria* are sister genera with *Stirtonanthus* sister to both of these. While *Podalyria* and *Stirtonanthus* are monophyletic, the monophyly of *Liparia* is still uncertain. *Virgilia* and *Calpurnia* are closely related and *Cyclopia* retains an isolated, monophyletic position within the tribe. *Cadia* is monophyletic and sister to the rest of the Podalyrieae. The placement of this genus has, until now, been uncertain due to their actinomorphic flowers that are unusual among papilionoid legumes. The data from this study indicate that actinomorphic flowers may be interpreted as an apomorphy for *Cadia* and it shares many characters with Podalyrieae. We therefore propose that *Cadia* be transferred to Podalyrieae from the paraphyletic tribe Sophoreae. The age of the root node of the tribe Podalyrieae s.s. was estimated at 30.5 ± 2.6 million years (Ma) using nonparametric rate smoothing (NPRS) and 34.7 Ma (confidence intervals: 25.1–44.1 Ma), using a Bayesian relaxed clock, indicating that a major radiation has taken place during the middle to late Miocene and early Pliocene. Finally, we found that nonsprouting species have a higher rate of molecular evolution than sprouting species.

***Keywords*—**Bayesian analysis, independent contrasts, internal transcribed spacer (ITS), maximum parsimony, phylogeny, *rbcL*.

Cadia is an anomalous genus in Papilionoideae that consists of seven species. Six of the species are endemic to Madagascar, while *Cadia purpurea* occurs in East and North-East Africa, extending to southwestern Arabia (Du Puy et al. 2002). The placement of *Cadia* within Fabaceae is uncertain although affinities with tribe Podalyrieae (Schutte and van Wyk 1998) and tribe Sophoreae (Polhill 1981) have been proposed. Recent evidence by Doyle et al. (2000), Kajita et al. (2001), Pennington et al. (2001), Wink (2003), and Wink and Mohamed (2003) confirms the close relationship between *Cadia purpurea* and Podalyrieae based on floral development, embryology, chromosome numbers, quinolizidine alkaloids, and DNA sequence data. *Cadia purpurea* was supported as a member of Podalyrieae and previous authors suggest that the placement of *Cadia* should be reconsidered (Doyle et al. 2000).

The actinomorphic (radially symmetrical) flowers of *Cadia* (similar to those of legume genera such as *Acosmium*, *Baphiopsis* Benth. ex Baker, and *Dicraeopetalum* Harms) are the most interesting and confusing character that creates uncertainty regarding the correct tribal placement of the genus. Several recent studies investigated the radial floral symmetry and unstable petal aestivation of these genera (Tucker 1987, 2002, 2003). Tucker (2002) suggested that the change to radial symmetry in *Cadia purpurea* could be due to the neotenous nature of the flowers; that is, they retain the juvenile state of radial symmetry. The change to the more common papilionoid (zygomorphic) flower occurs late in floral development and flowers that appear radial at anthesis lack the final events that result in a zygomorphic flower. In terms of floral development, *Cadia* conforms to the consistently unidirectional organogenesis found in other Sophoreae and is in agreement with the majority of other papilionoid legumes from various tribes (Tucker 2002). Pennington et al. (2000) used sequence data from the plastid *trnL* intron to evaluate floral evolution in the early diverging Papilionoideae. They interpreted the shift from the typical zygomorphic, papilionoid flower in *Cadia* as a reversal, due to unusual pollination biology and the need to attract different pollinators. They concluded that the floral characters of *Cadia* and other genera that deviate from the typical papilionoid flower are autapomorphies and not necessarily 'primitive' characters for these groups. More recently, Citerne et al. (2006) demonstrated that the radial symmetry of *Cadia* is a homeotic transformation rather than a reversal. They showed, through the study of *CYCLOIDEA* (*CYC*)-like genes, that gene-expression in *Cadia* flowers is similar to that of typical zygomorphic flowers during the early developmental stages. However, the floral organs of *Cadia*, in the later stages, develop equally along the dorsoventral axis instead of unequally as in typical papilionoid flowers. The expression of the *LegCYC1B* gene in all five petals of *Cadia* flowers, instead of only the adaxial petals as in most cases, results in all the petals looking like the dorsal petal and, therefore, five bilaterally symmetrical petals are formed (Citerne et al. 2006).

The tribe Podalyrieae is a group of papilionoid legumes that, with the exception of *Calpurnia* and one species of *Podalyria*, are endemic to the Cape Floristic Region (CFR) of South Africa. It is one of the so-called 'Cape floral clades' together with two other legume groups, Crotalarieae pro parte (*Aspalathus* and *Rafnia*) and Psoraleeae pro parte (*Psoralea* L. and *Otholobium* C.H. Stirton). These clades, according to Linder (2003), can be defined as those that have had most of their evolutionary history in the CFR and have been there since the Pliocene. According to the latest revision by Schutte and van Wyk (1998), Podalyrieae contains eight genera: *Amphithalea*, *Calpurnia*, *Cyclopia*, *Liparia*, *Podalyria*, *Stirtonanthus*, *Virgilia*, and *Xiphotheca*. All species are long-lived perennials with notable variation in growth form and both fire survival strategies, nonsprouting and sprouting, are found in these

genera. The latter resprout from an underground lignotuber after fire, whereas nonsprouting species can only regenerate from seed (Schutte et al. 1995). Growth forms range from tall, upright trees to erect woody shrubs and subshrubs or sprawling shrublets. Almost the full range of leaf diversity of genistoid legumes can be found in this tribe, varying from imparipinnately compound in *Calpurnia* and *Virgilia*, to trifoliate in *Cyclopia* and simple in *Amphithalea*, *Liparia*, *Podalyria*, *Stirtonanthus,* and *Xiphotheca*. The structure of the inflorescence is a useful taxonomic character at both generic and infrageneric levels. Inflorescences are either racemes or panicles in *Calpurnia* and *Virgilia* and variously modified in the rest of the tribe. The flowers are firm-textured in some genera and adapted for pollination by xylocopid bees, but alternative pollination vectors for some species are known (Schutte and van Wyk 1998). Podalyrieae are currently divided (Schutte and van Wyk 1998) into two subtribes: Podalyriinae (consisting of *Calpurnia*, *Cyclopia*, *Liparia*, *Podalyria*, *Stirtonanthus,* and *Virgilia*) and Xiphothecinae (consisting of *Amphithalea* and *Xiphotheca*). Detailed species level phylogenetic trees, based on morphological, chemical, and cytological data exist for most of the genera of the Podalyrieae as currently circumscribed (Schutte and van Wyk 1998, and references cited therein). A phylogenetic tree for 28 species of the tribe, based on combined nuclear ribosomal DNA (internal transcribed spacer, ITS), morphological, and chemical data was published by van der Bank et al. (2002). They confirmed the monophyly of Liparieae and Podalyrieae, but found subtribe Podalyriinae to be paraphyletic, with *Cyclopia* forming a clade sister to the rest of the tribe. These authors suggested that a broader concept of Podalyrieae should include *Cyclopia*, rather than erecting another subtribe to accommodate this genus.

Here, a species-level phylogenetic tree based on ITS and *rbcL* data is presented for 107 species out of a total of about 128 species in the tribe. The purpose of this study was to, first, infer the placement of the Podalyrieae genera within the genistoid alliance as a whole and to estimate the age of the tribe based on ITS sequence data for 69 genera and 197 species of genistoid legumes. Second, this analysis will also allow us to evaluate the affinity of genus *Cadia* in relation to Podalyrieae. Analyses of ITS and *rbcL* are presented for the African *Cadia purpurea* and three of the six rare Madagascan endemic members of the genus. Finally, we aim to reassess generic relationships within the tribe based on an almost complete sampling at species level and test whether nonsprouters and sprouters have different rates of molecular evolution.

Material and Methods

Choice of Genes and Outgroups—The choice of ITS and *rbcL* to infer relationships was based on their extensive use in legume systematics, especially genistoid legumes (e.g. Käss and Wink 1995, 1996, 1997; Ainouche and Bayer 1999; Crisp et al. 2000; Doyle et al. 2000; Schnabel et al. 2003; Wink 2003; Wink and Mohamed 2003; Heenan et al. 2004; Pardo et al. 2004; Lavin et al. 2005; Degtjareva et al. 2006; Wang et al. 2006). Representatives of the 'core' genistoids were chosen as outgroups due to the close relationship known to exist between Podalyrieae and other genistoid tribes, particularly the Crotalarieae and Genisteae (van Wyk and Schutte 1995; Crisp et al. 2000). Voucher specimen information and GenBank accession numbers of the taxa of Podalyrieae and *Cadia* used in the analyses, as well as the outgroup taxa, are listed in Appendix 1.

DNA Extraction, PCR, and Sequencing—Total DNA was extracted from herbarium or silica dried leaf material (0.1–0.3 g) using the 2x hexadecyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (1987) and purified with QIAquick silica columns (Qiagen Inc., Hilden, Germany) according to the manufacturer's protocol. Amplification of ITS and *rbcL* was carried out by polymerase chain reactions (PCR), in 50 μl reactions containing: 25 μl PCR Mastermix [50 units/ml *Taq* DNA Polymerase (pH 8.5), 400 μM each of deoxyribonucleotide triphosphate (dNTP) and 3 mM $MgCl_2$ (Promega Corporation, Madison, Wisconsin)]; 0.5 μl of both forward and reverse primers (0.1 ng/μl); 1 μl 0.004% bovine serum albumin (BSA); 2% dimethyl sulfoxide (DMSO; for ITS only); 20–50 ng DNA template; and sterile distilled water to make up a final volume of 50 μl. The PCR cycle used for ITS consisted of 26 cycles of 1 min denaturation at 94°C, 1 min annealing at 48°C, 3 min extension at 72°C and 7 min final extension at 72°C; and for *rbcL* of 28 cycles of 1 min denaturation at 94°C, 1 min annealing at 48°C, 1:30 min extension at 72°C and 7 min final extension at 72°C. The primer combinations of Olmstead et al. (1992) were used for amplification of *rbcL* and those of White et al. (1990) and Sun et al. (1994) for the ITS region. The PCR products were purified using a QIAquick PCR purification kit following manufacturer's instructions (Qiagen Inc.). Cycle sequencing reactions were performed in 10 μl reactions consisting of: 40 ng cleaned PCR product; 0.5 μl Big Dye Terminator v. 3.1 (Applied Biosystems Inc., Foster City, California, USA); 0.3 μl primer (0.1 ng/μl; PCR primers used as sequencing primers); 2.0 μl sequencing buffer prepared according to manufacturers instructions; 5% DMSO (for ITS reactions); and sterile distilled water to make up a final volume of 10 μl. The cycle sequencing thermal profile consisted of 26 cycles of 10 sec denaturation at 96°C, 5 sec annealing at 50°C and 4 min at 60°C in a thermal cycler (GeneAmp PCR system 9700). The products were purified using ethanol precipitation to remove any excess dye terminator. Cleaned cycle sequencing products were then directly sequenced on a 3130 *xl* Genetic Analyzer (Applied Biosystems Inc.).

Sequence Alignment and Phylogenetic Analyses—Complimentary strands were assembled and edited using Sequencher v. 3.1.2 (Gene Codes Corporation), and aligned manually in PAUP* v. 4.0bl (Swofford 1998). Matrices used in this study are available from TreeBASE (study number S1913). At first, insertions and deletions of nucleotides (indels) were scored as missing data and thus did not contribute to the analysis. No mixture of sequences was detected in the ITS sequences when looking at the electropherograms and DMSO (2%) was added to the amplification reactions to improve PCR specificity (Álvarez and Wendel 2003). Therefore, we did not need to clone the ITS fragment to obtain clean sequences, suggesting that there are no paralogues in these taxa.

Phylogenetic analyses were performed using PAUP*. Tree searches were carried out using a heuristic search with 1,000 random sequence additions, tree bisection-reconnection (TBR) branch swapping and the MULTREES option in effect, but keeping only 10 trees per replicate. All character transformations were treated as equally likely (Fitch parsimony; Fitch 1971). Trees collected in the 1,000 replicates were used as starting trees for another similar search, but without a tree limit. Delayed transformation character optimisation (DELTRAN) was used to calculate branch lengths, due to reported errors with accelerated transformation optimisation (ACCTRAN) in PAUP v. 4.0bl. In addition, successive approximations weighting (SW; Farris 1969) was used in the combined analysis to down-weight base positions that changed excessively and to determine the effects of such characters on the tree topology. This reduces the effect of unstable taxa and for this reason was implemented in this and previous studies such as Chase et al. (2000) and Goldblatt et al. (2002). We used the Shimodaira-Hasegawa test (SH; Shimodaira and Hasegawa 1999) to evaluate whether there is a significance difference between the topology of the combined analysis and a topology where *Calpurnia, Liparia,* and *Xiphotheca* were forced into monophyly. The SH test was implemented in PAUP*, using 1,000 bootstrap replicates and applying the RELL resampling method. An additional search with gaps from the ITS dataset coded as binary characters (no gaps present in the *rbcL* dataset) was performed. This time, gaps were coded in SeqState v. 1.32 (Müller 2005) using simple indel coding as described by Simmons and Ochoterena (2000) and resulted in identical clade resolution.

Internal support was estimated with 1,000 bootstrap replicates (Felsenstein 1985) using TBR and holding 10 trees per replicate. The following scale for bootstrap support percentages (BP) was used: 50–74%, low; 75–84%, moderate; 85–100%, strong. Congruence of the separate datasets was assessed by examining the individual bootstrap consensus trees. The bootstrap trees were considered incongruent only if they displayed 'hard' (i.e. high bootstrap support) rather than 'soft' (i.e. low bootstrap support) incongruence (Seelanan et al. 1997; Wiens 1998). 'Incongruence tests' such as the incongruence length difference test (ILD) can be unreliable (Reeves et al. 2001; Yoder et al. 2001) and therefore were not used in this study.

A few taxa could not be amplified for ITS and *rbcL* due to low DNA

yield during extraction: we failed to amplify *Podalyria velutina* for ITS and *Cadia pubescens*, *Calpurnia aurea*, *Cyclopia burtonii,* and *Podalyria intermedia* for *rbcL*. Missing data represented 10.5% of the entire combined matrix because only ITS sequences (not *rbcL*) could be obtained for many of the outgroup taxa. For some taxa, despite repeated attempts, we were only able to amplify about half of the ITS region: *Amphithalea rostrata*, *Cadia commersoniana*, *C. pedicellata*, *C. pubescens*, *C. purpurea*, *Calpurnia woodii*, *Cyclopia alopecuroides*, *C. falcata*, *C. glabra*, *C. plicata*, *C. sessiliflora*, *Podalyria calyptrata*, and *P. canescens*. We could also amplify only about half of *rbcL* for *Amphithalea dahlgrenii*, *Cyclopia pubescens*, *Podalyria canescens*, *P. pearsonii*, *P. velutina*, *Stirtonanthus chrysanthus*, *Xiphotheca canescens*, and *X. cordifolia*.

Bayesian analysis (BI; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was performed, using MrBayes v. 2.01. Settings for the TIM + I + G model [indicated by Modeltest v. 3.06 (Posada and Crandall 1998) as the best model] were: number of rate parameters = 6, rates = invgamma, base frequency = empirical, clock = unconstrained and number of generations = 1,000,000. The resulting trees were plotted against their likelihoods in order to determine where the likelihoods converge on a maximum value. All the trees before this convergence were discarded as the 'burn-in' phase. The remaining trees were imported into PAUP* and a majority rule consensus tree was produced in order to show the posterior probabilities (PP) of all observed bipartitions. The following scale was used to evaluate the PPs: 0.50–0.74, low; 0.75–0.84, moderate; 0.85–1.0, strong.

Age Estimation of the 'Core' Genistoids—Additional ITS sequences were obtained from GenBank and combined with our data for Podalyrieae to compile a high-level ITS matrix that was used to evaluate the position of *Cadia* and to date the 'core' genistoids and Podalyrieae (see Appendix 1). It is not possible to align reliably the ITS region across all Fabaceae (Lavin et al. 2005) and therefore only the genistoid tribes were included in this analysis. Because a likelihood ratio test indicated rate heterogeneity across lineages (likelihood score with clock = 16,935.40457; likelihood score without clock = 16,435.28640; Λ = 1,000.23634; Df = 196; $p < 0.0001$), we used two dating methods to estimate time, the nonparametric rate smoothing method (NPRS; Sanderson 1997) and a Bayesian relaxed clock (Thorne et al. 1998; Kishino et al. 2001; Thorne and Kishino 2002). The Fitch tree used for the NPRS analysis was generated using a heuristic search (of the high-level ITS dataset) identical to that described earlier for the combined ITS and *rbcL* analysis and branch lengths calculated using DELTRAN. The first of the trees obtained was imported into TreeEdit v. 1.0a 4.61 (Rambaut and Charleston 2000) where an ultrametric NPRS tree was produced. To compute an error estimate for the root node of Podalyrieae, the NPRS procedure was applied to 100 bootstrapped matrices (Sanderson 1997).

We also used a Bayesian dating method (Thorne et al. 1998; Kishino et al. 2001; Thorne and Kishino 2002; see also Rutschmann 2004). The model parameters (F84 + G; Felsenstein 1993) were estimated from the data using the software *baseml* (PAML; Yang 1997). Second, maximum likelihood branch lengths and their variance-covariance matrix were estimated using the program *estbranches* (Thorne et al. 1998). Finally, the program *multidivtime* (Kishino et al. 2001; Thorne and Kishino 2002) was used to determine the posterior distributions of substitution rates and divergence times using the following settings: *rttm* set at 60 million years ago (Ma) and *rttmsd* set at 30 Ma; *rtrate* and *rtratesd* set at 0.0309 (based on average root-to-tip branch lengths of the tree obtained by *estbranches*), *brownmean* and *brownsd* set at 0.167 (set at 1/*rttm* as suggested by J. Thorne), *bigtime* set at 121 Ma, the age of the eudicots inferred from pollen fossil records (see documentation on www.statgen.ncsu.edu/thorne/multidivtime.html for more details). Following an initial burn-in of 10^5 generations, the Markov chain was run for 8×10^5 generations and sampled every 100 generations.

Diplotropis, a sophoroid fossil from the Eocene (Herendeen and Dilcher 1990) with an estimated date of 56 Ma, was used to calibrate the tree (Lavin et al. 2005). *Diplotropis* is an early diverging lineage in the genistoid legumes and thus placed close to the Brongniartieae and *Acosmium* and essentially represents the root node of the genistoid legumes.

Rates of Evolution—Because nonsprouters are thought to go through more life cycles than sprouters, it has been hypothesized that they might evolve faster. We tested this hypothesis with independent contrasts using the software package CAIC v. 2.6.9 (Comparative Analysis by Independent Contrasts, Purvis and Rambaut 1995). Molecular branch lengths from the BI tree were used as surrogates for molecular evolution, and we ask whether nonsprouters have, on average, longer branch lengths than sprouters.

The resulting tree from BI (results.t.con files from MrBayes) for the combined ITS and *rbcL* matrix was imported into TreeEdit v. 1.0a 4.61 (Rambaut and Charleston 2000) and exported in CAIC format, which resulted in plain text, coded phylogeny and branch length files. A table (tab delimited) was compiled containing branch lengths (continuous variable) from BI and sprouting/nonsprouting information (categorical variable), scored as 0 for sprouting and 1 for nonsprouting, for each species in the dataset. All data were read into CAIC following the procedure set out in the user's guide. The 'brunch' function of the CAIC program was used because of the categorical character sprouter/nonsprouter. A sign test was performed (www.fon.hum.uva.nl) to determine whether the contrasts were significantly different.

Optimisation of sprouter/nonsprouter characters was performed using maximum parsimony (MP) and ACCTRAN in MacClade v. 4.03 (Maddison and Maddison 2001) and the combined ITS and *rbcL* tree.

Results

Phylogenetic Analyses—The length of the *rbcL* gene included in the analysis was 1,415 positions of which 232 were variable and 154 potentially informative. Analysis resulted in 173 equally parsimonious trees of 447 steps with a consistency index (CI) of 0.62 and a retention index (RI) of 0.83. The bootstrap consensus tree based on *rbcL* alone is poorly resolved (tree not shown). *Cadia* is weakly supported as part of Podalyrieae (53 BP), but there is no support for the monophyly of the genus. The monophyly of *Calpurnia* (excluding *C. intrusa*) as well as *Virgilia*, received high support (99 BP and 97 BP, respectively).

Analysis of the ITS region consisted of 734 positions of which 375 were variable and 234 potentially informative. A total of 6,650 equally parsimonious trees of 891 steps with a CI of 0.61 and a RI of 0.83 were obtained. The bootstrap consensus tree for ITS alone is better resolved than *rbcL* (Fig. 1). *Cadia* is strongly supported as a monophyletic (100 BP) part of Podalyrieae (95 BP). Support for the monophyly of *Calpurnia* (excluding *C. intrusa*), *Cyclopia,* and *Virgilia* was high (99 BP, 100 BP, and 100 BP respectively), while the monophyly of the other genera was not supported in this analysis. Gap coding did not improve the resolution within Podalyrieae, but bootstrap percentages were higher for some groups (Fig. 1).

Visual inspection of the separate ITS and *rbcL* bootstrap consensus trees displayed no strongly supported incongruent patterns and thus were directly combined. The parsimony analysis of ITS and *rbcL* combined produced 140 equally most parsimonious trees (Fig. 2 ; tree length (TL) = 1,176; CI = 0.61; RI = 0.83). Successive weighting resulted in 950 trees (TL = 882.257; CI = 0.61; RI = 0.83). The matrix included 2,148 characters of which 530 were variable and 323 potentially parsimony informative.

The topology of the majority rule consensus tree from BI (tree not shown), although largely the same, differed slightly from the Fitch tree. *Amphithalea* and *Xiphotheca* are strongly supported to be closely related (PP 1.0), but based on these results *Xiphotheca* is not monophyletic. Some of the *Liparia* species and *Podalyria* form a strongly supported clade (PP 1.0). *Liparia* is paraphyletic, with *L. calycina* and *L. umbellifera* not included in the *Liparia* clade. *Virgilia* and *Calpurnia* form separate low to strongly supported clades (PP 1.0 for *Virgilia*; PP 0.73 for *Calpurnia*). This differs from the Fitch tree where they form a clade, albeit without bootstrap support. In this analysis *Calpurnia* is supported to be monophyletic, with *C. intrusa* included in the *Calpurnia* clade (PP 0.73). *Cyclopia* forms the next clade and its monophyly receives high support (PP 1.0). *Cadia* is well supported to be monophyletic (PP 1.0) and sister to Podalyrieae (PP 1.0).

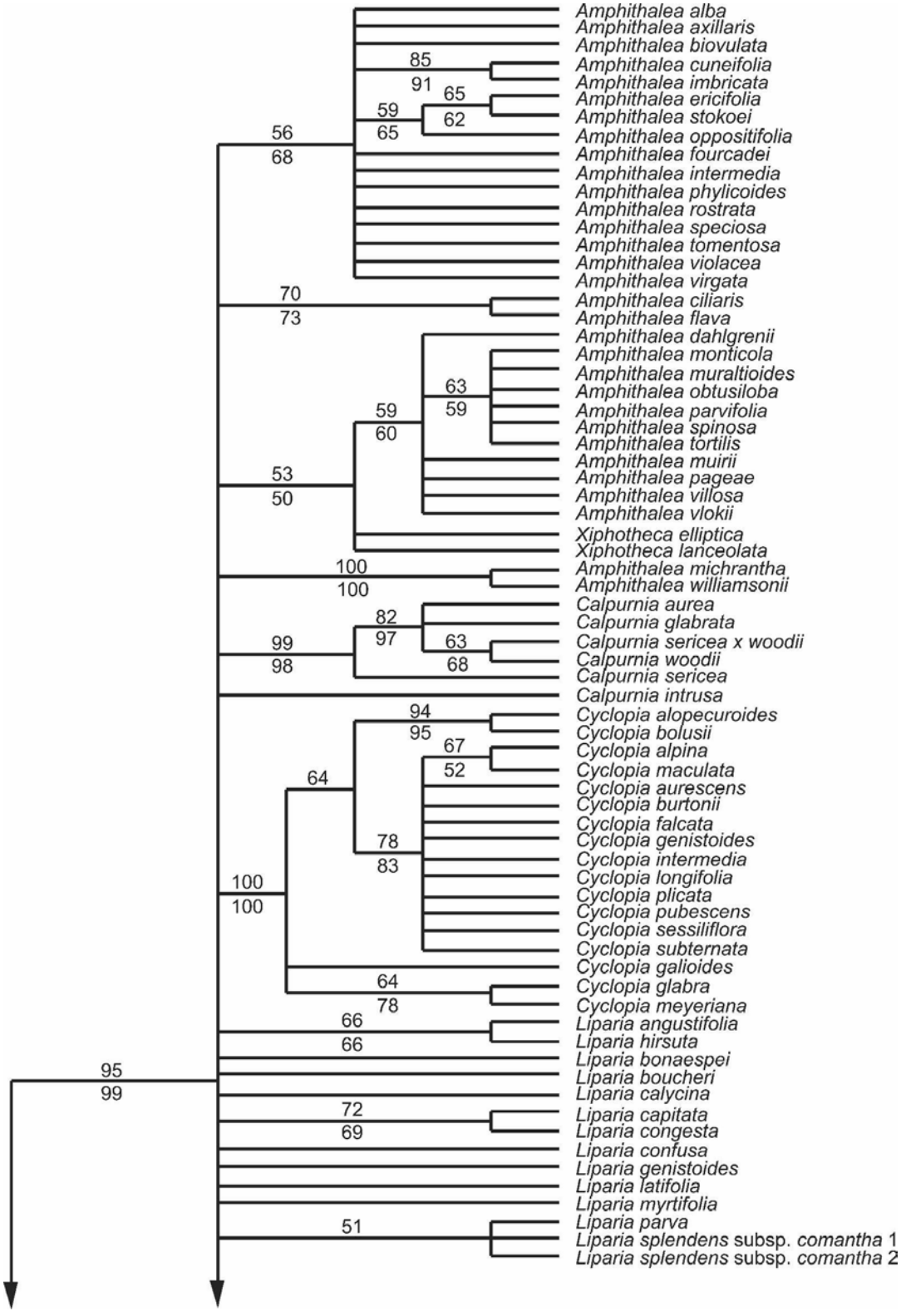

FIG. 1. Bootstrap consensus tree of the ITS analysis of Podalyrieae. Bootstrap percentages above 50% for the ITS analysis without gap coding is shown above the branches, and with gap coding below the branches.

Several major clades were identified within the tribe based on the BI and MP analyses. The first major clade contains the genera *Amphithalea* and *Xiphotheca*. The grouping of these genera received low bootstrap support (53 BP, 54 SW), but is strongly supported with BI (PP 1.0). There is no support for the monophyly of either of these genera, as some species of *Xiphotheca* are embedded within *Amphithalea*. Sister to these genera is a clade containing *Liparia*, *Podalyria* and *Stirtonanthus*. *Podalyria* is moderately to strongly supported as monophyletic (63 BP, 65 SW, PP 1.0) and is sister to all species of *Liparia* except *L. calycina* (56 BP, 59 SW, PP 1.0). *Liparia* is paraphyletic, with *L. calycina* not included in the *Liparia* clade

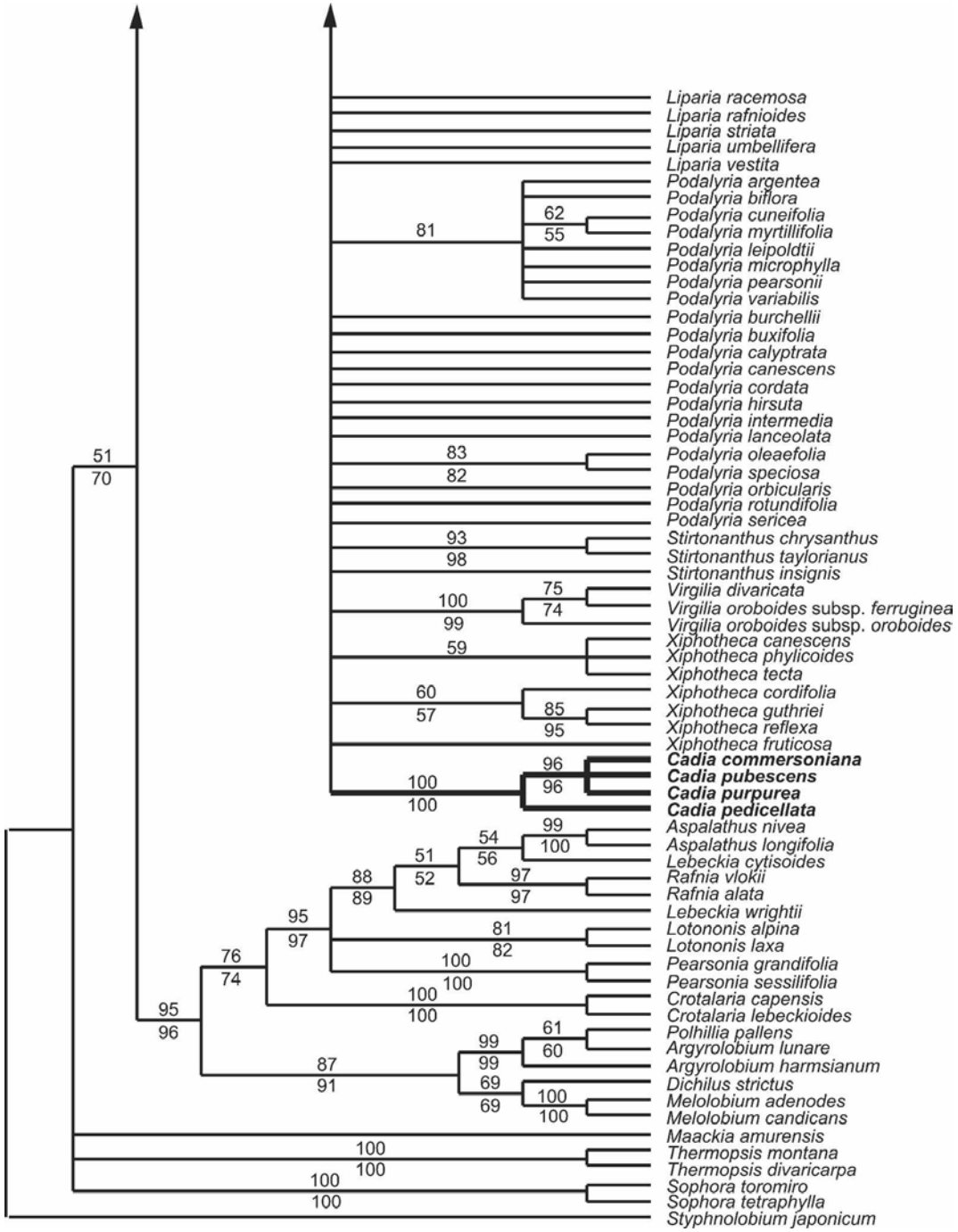

FIG. 1. (Continued)

(both *L. calycina* and *L. umbellifera* in BI). *Stirtonanthus* is sister to the *Liparia/Podalyria* clade (PP 0.73) and supported as monophyletic (69 BP, 71 SW, PP 0.92). *Stirtonanthus chrysanthus* and *S. taylorianus* are strongly supported as sister taxa (95 BP, 94 SW, PP 1.0). The next clade sister to the *Amphithalea/Xiphotheca* and the *Liparia/Podalyria/Stirtonanthus* clades contains the genera *Calpurnia* and *Virgilia*. This clade is not represented in BI and lacks support with MP. *Calpurnia* is paraphyletic with MP, while *Virgilia* is strongly supported to be monophyletic (100 BP, 100 SW, PP 1.0). *Virgilia divaricata* and *V. oroboides* subsp. *ferruginea* are supported as sister taxa (75 BP, 77 SW, PP 0.96). The members of *Cyclopia* form the subsequent clade sister to the rest of Podalyrieae and the monophyly of the genus received high support (99 BP, 98 SW, PP 1.0). The support for the monophyly of tribe Podalyrieae excluding *Cadia* is low (56 BP, 54 SW, PP 0.68), and *Cadia* is well supported as sister to Podalyrieae (92 BP, 94 SW, PP 1.0). The monophyly of *Cadia* received high support (100 BP, 100 SW, PP 1.0) and a sister relationship between *C. commersoniana* and *C. pubescens/C. purpurea* is strongly supported (95 BP, 96 SW, PP 1.0). The SH test rejected the null hypothesis that the constrained trees were significantly different from the unconstrained ($p < 0.05$) trees. This indicates that although the genera tested are paraphyletic in the phylogeny, they could equally be monophyletic and that this result might be due to a lack of signal or resolution in these clades.

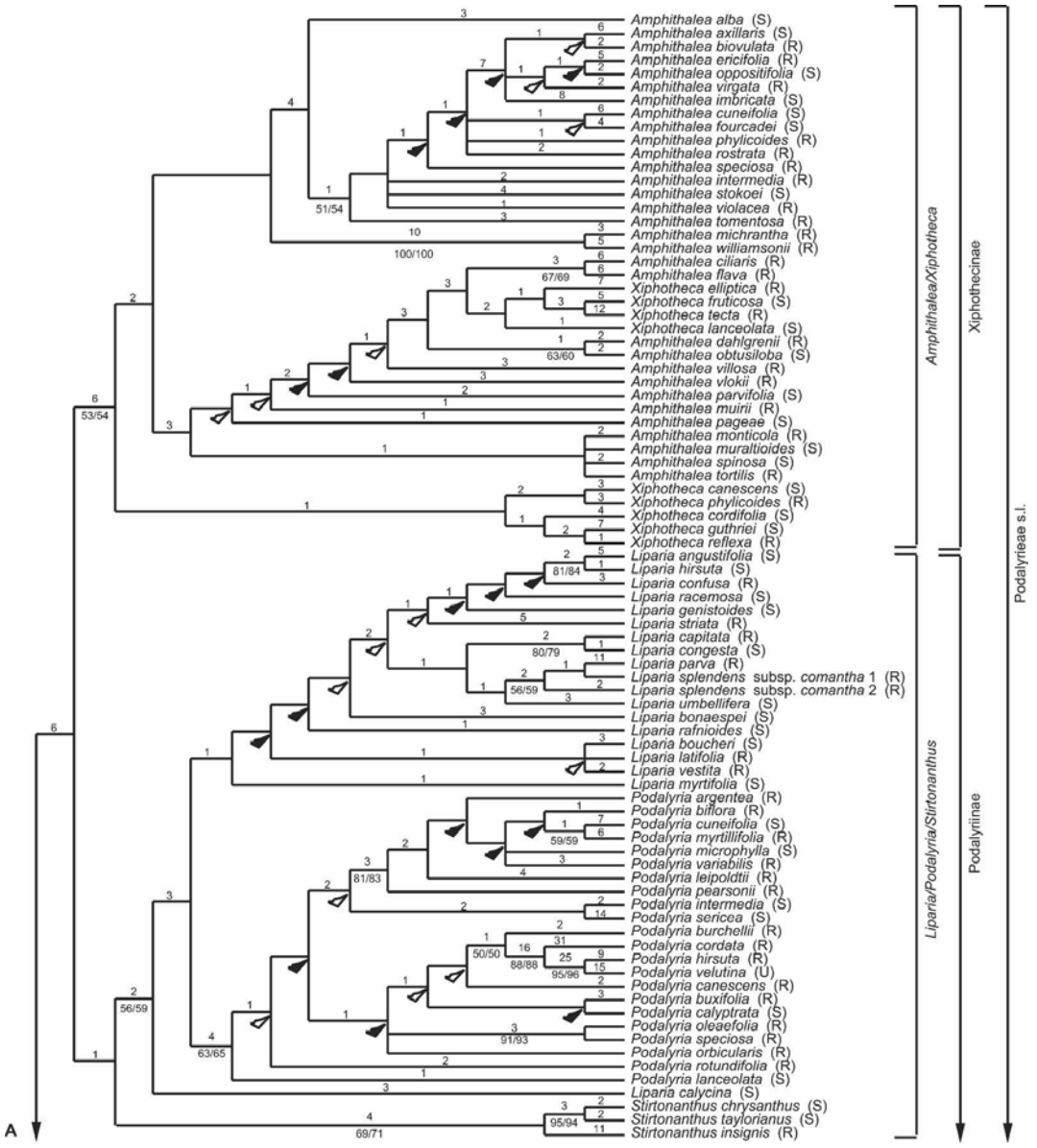

FIG. 2. One of the 140 equally most parsimonious trees from the combined molecular analysis of ITS and *rbcL* data for Podalyrieae. Numbers above the branches are Fitch lengths (DELTRAN optimisation) and the values below branches are bootstrap percentages above 50%, equal weights (EW) before and successive weights (SW) after the slash. Solid arrows indicate groups that collapse in the EW strict consensus tree whereas open arrows indicate groups that collapse in the SW strict consensus tree. Fire survival strategy is indicated for Podalyrieae: nonsprouters or seeders (S), sprouters (R) and unknown strategy (U).

Dating—The analysis of the ITS region alone, for the representatives of genistoid legumes, consisted of 764 characters of which 509 were variable and 408 potentially informative. This high taxonomic level analysis resulted in 930 MP trees of 2,938 steps, with a CI of 0.34 and a RI of 0.75. The age of the root node of Podalyrieae (excluding *Cadia*) was estimated at 30.5 Ma (Fig. 3A), with the bootstrap distribution of ages giving an error estimate of 2.6 Ma (Bayesian: 34.7 Ma [confidence interval, ci: 25.1–44.1 Ma]; not shown). In this analysis *Cadia* is again strongly supported to be monophyletic (100 BP) and sister to Podalyrieae (99 BP), although the support for the monophyly of Podalyrieae excluding *Cadia* is low (61 BP). Due to low resolution in these trees, *Calpurnia* and *Virgilia* are present as separate clades as opposed to a single clade in the combined ITS and *rbcL* analysis. Also *Cyclopia* is sister to the *Virgilia*/*Liparia*/*Podalyria*/*Stirtonanthus* clade, instead of the whole of Podalyrieae as in the combined MP analysis. The rest of the ITS-based topology for Podalyrieae is the same as the combined analysis. Sister to the Podalyrieae/*Cadia* clade are the tribes Crotalarieae and Genisteae. The estimated ages for these tribes are 31.2 ± 3.4 and 32.3 ± 2.9 Ma, respectively (Fig. 3B; Bayesian: 35.2 Ma [ci: 23.3–45.6 Ma] and 37.5 Ma [ci: 27.6–46.8 Ma], respectively; not shown). These are moderately supported as sister tribes (69 BP). Sophoreae (in part) and Thermopsideae form a clade with low support (61 BP) and are sister to the above-mentioned tribes (51 BP). All these tribes constitute the 'core' genistoids and retain an isolated, monophyletic position within the genistoid legumes. The root node of the 'core' genistoids was dated at 45.2 ± 2.3 Ma (Fig. 3B; Bayesian: 51.2 Ma [ci: 43.9–55.3 Ma]). Age estimates obtained using NPRS are in general slightly younger than those obtained using the Bayesian

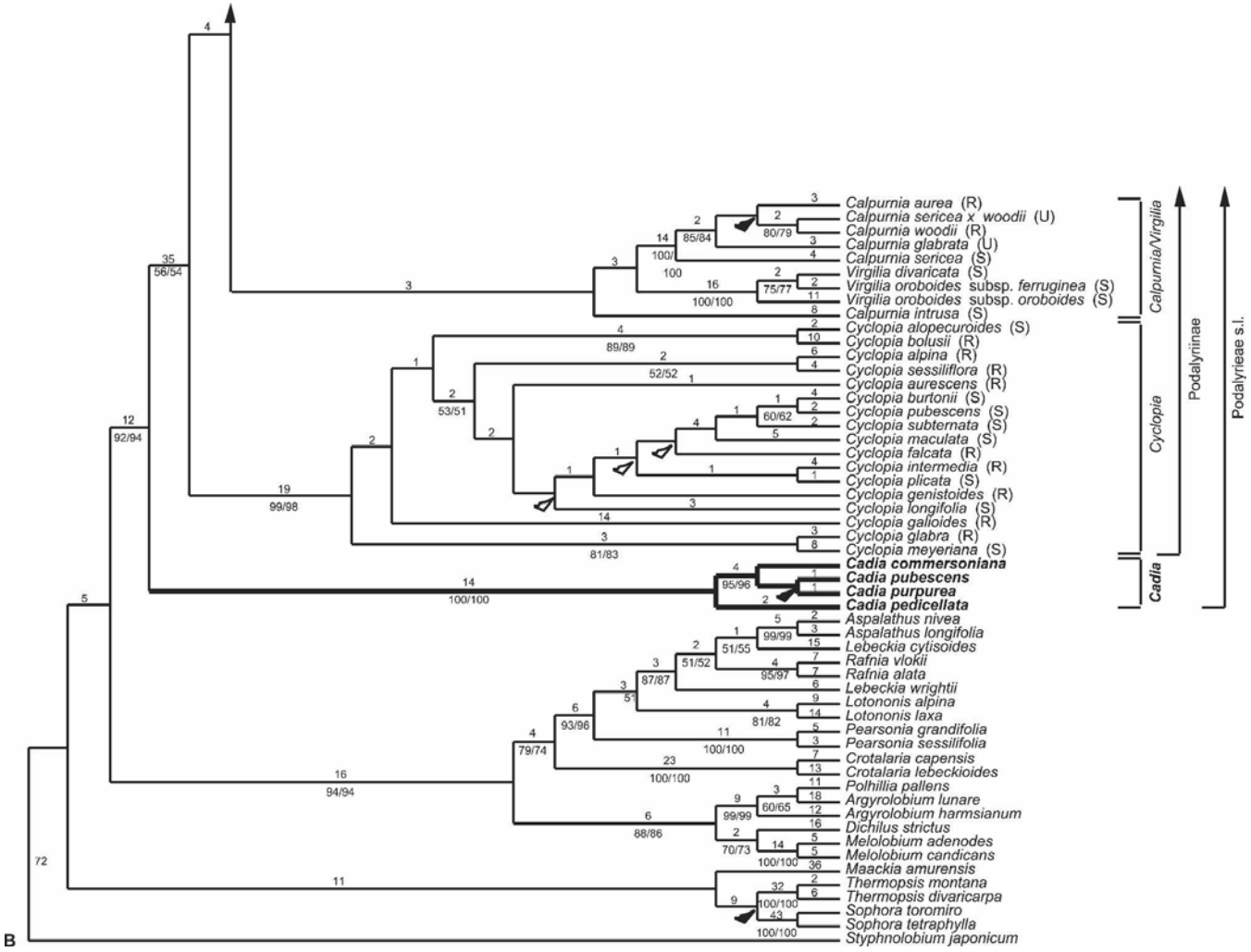

FIG. 2. (Continued)

method, but standard errors (NPRS) and confidence intervals (Bayesian) always overlapped.

Rates of Evolution—The CAIC analysis produced 33 contrasts for Podalyrieae, 21 positive contrasts and 12 negative contrasts (raw data from the analysis is available on request). For a given pair of taxa, a positive contrast indicates that the branch starting at their common ancestor and leading to a nonsprouter is longer than the one leading to the sprouter. A negative contrast indicates the opposite situation. Given that we have found more positive contrasts, our results are in agreement with the hypothesis that nonsprouters have higher rates of molecular evolution due to their higher number of life cycles. However, the sign test was not significant ($p = 0.163$). Optimisation of sprouters/nonsprouters showed an equivocal ancestral state for the first two nodes within Podalyrieae, although the next nodes upwards were all optimised with a nonsprouter ancestral state (data not shown).

DISCUSSION

Evolutionary Relationships within Podalyrieae—The relationship between *Amphithalea* and *Xiphotheca*, although weakly supported with MP, agrees with the results of Schutte and van Wyk (1998) and van der Bank et al. (2002). These two genera constitute the subtribe Xiphothecinae and share several morphological characters: a nonintrusive calyx base, obtuse keel petal, reduced number of ovules, and wing petals with a thickened lobe on the abaxial surface. No clear explanation is apparent for the species of *Xiphotheca* that are embedded within *Amphithalea*. These genera differ in the shape of the seed aril, the number of ovules and the presence or absence of ammodendrine as a major alkaloid (Schutte 1997a). Molecular systematic studies of these genera, involving more variable genes, will provide valuable insight into the relationship between them and whether they are monophyletic.

The subtribe Podalyriinae is clearly not monophyletic. This confirms the results obtained by van der Bank et al. (2002) where *Cyclopia* is sister to the rest of Podalyrieae. The six genera that constitute the subtribe all have an intrusive calyx base, rostrate (beaked) keel petals and robust, strongly textured flowers. In this study, three clades are found within Podalyriinae: *Liparia/Podalyria/Stirtonanthus*, *Calpurnia/Virgilia*, and *Cyclopia*. The clade containing *Liparia*, *Podalyria*, and *Stirtonanthus* is sister to *Amphithalea* and *Xiphotheca* in the combined analyses with high support with BI. A close relationship between *Liparia* and *Podalyria* is also strongly supported with BI. These genera share the intrusive calyx base and rostrate keel petals that are characteristic of Podalyriinae

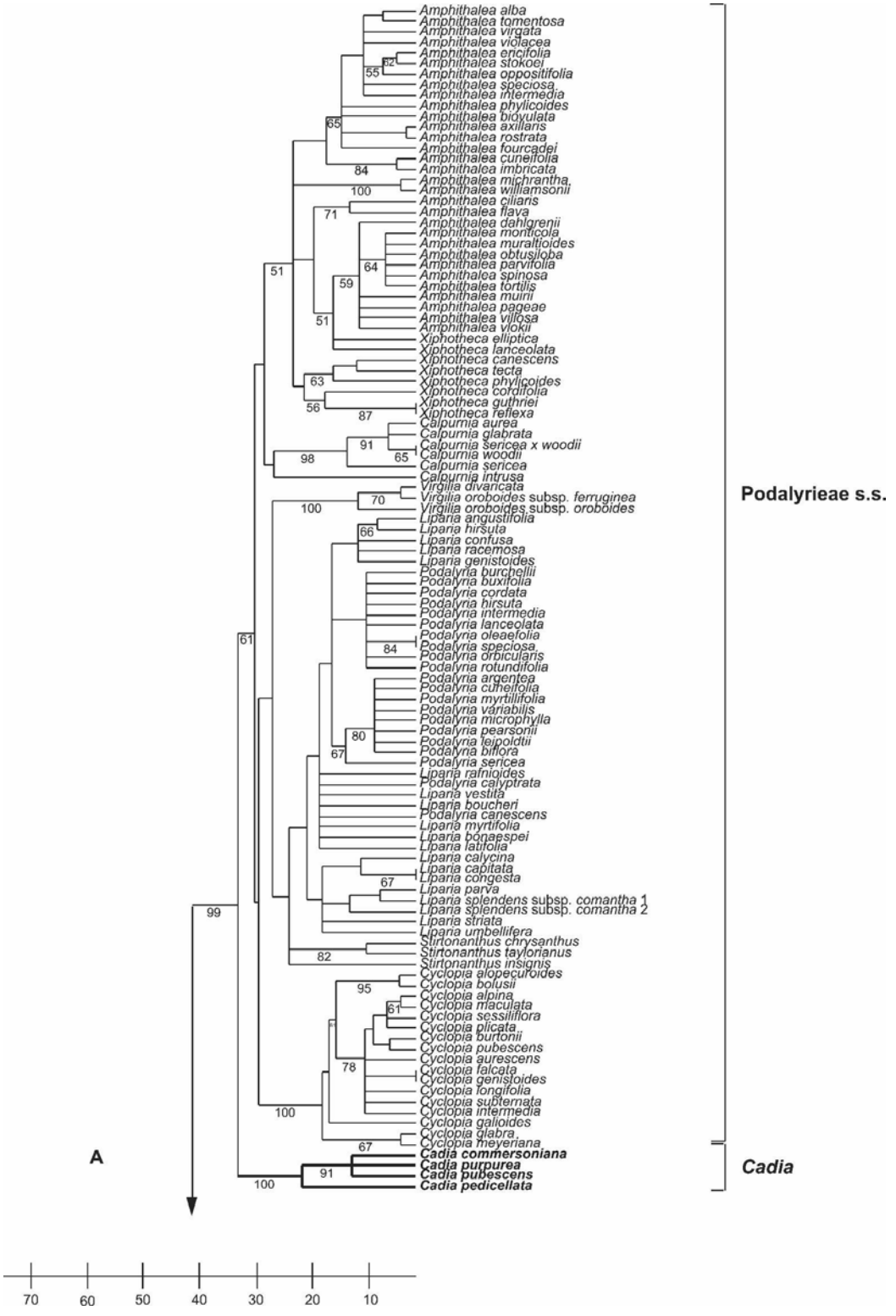

FIG. 3. Ultrametric tree based on ITS data and produced by nonparametric rate smoothing, showing the age estimates of Podalyrieae s.s. and the 'core' genistoids. Only PPs above 0.5 are reported. The tree was calibrated using the age of the fossil *Diplotropis* (indicated by the arrow). The time scale is in million years. Note that the age of Podalyrieae s.s. is 30.5 ± 2.6 million years; the ages of Crotalarieae and Genisteae are 31.2 ± 3.4 and 32.3 ± 2.9 million years respectively and the split between these two tribes is dated at 36.9 ± 2.5 million years. The age of the 'core' genistoids was estimated at 45.2 ± 2.3 million years.

and both have few-flowered, racemose inflorescences (Schutte and van Wyk 1998). Schutte (1997b) described a close relationship between *Cyclopia* and *Liparia*, due to the presence of prominent, decurrent leaf bases and sterile bracts at the base of the inflorescences in both genera. Here *Cyclopia* retains an isolated position within Podalyrieae sister to the rest of the tribe in the combined analyses and is not closely allied to *Liparia*. Crisp et al. (2000) mention a similar close relationship

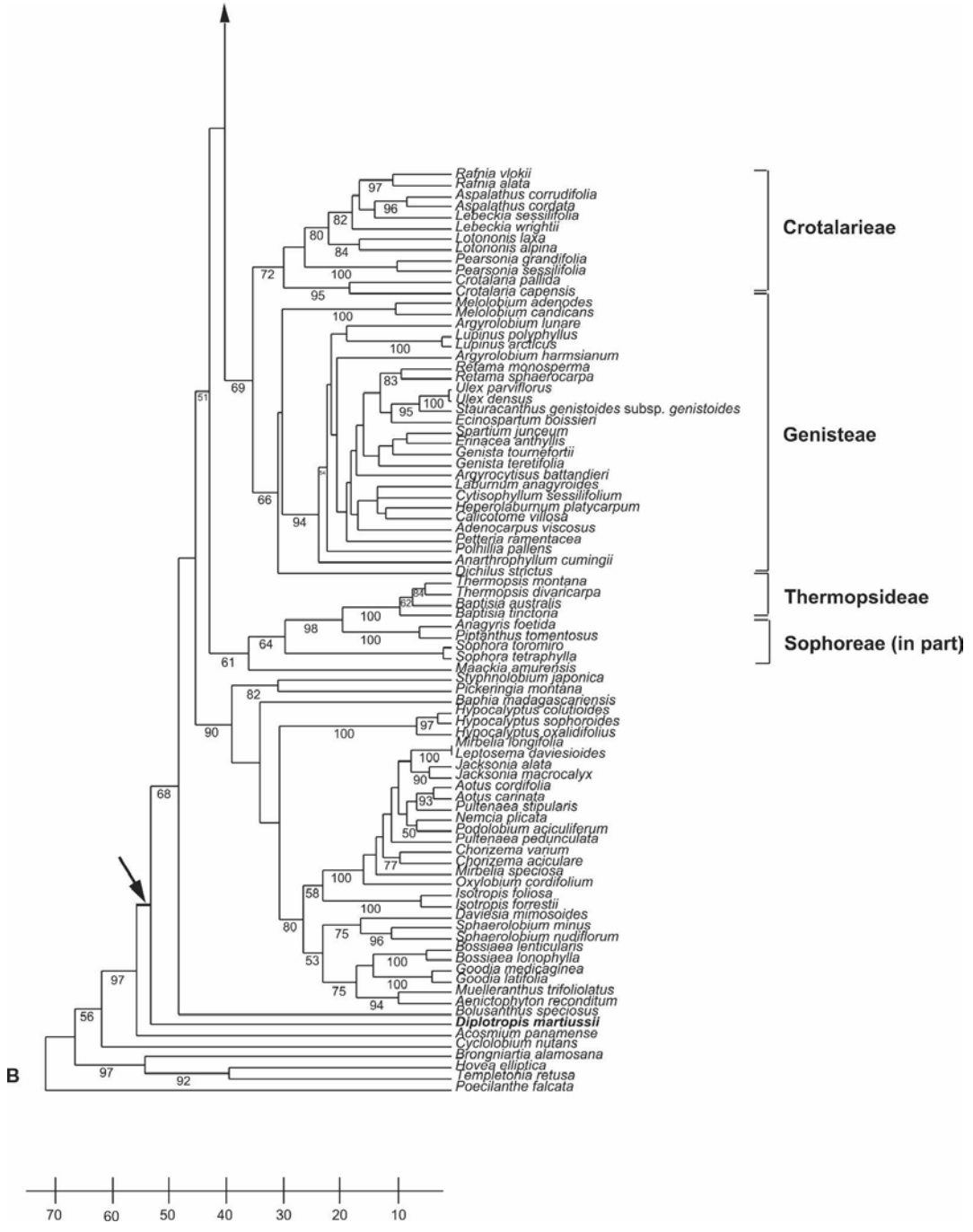

FIG. 3. (Continued)

between *Liparia* and *Podalyria*. In their molecular phylogenetic analysis, the genera form a clade with moderate bootstrap support. *Liparia* is easily distinguished from other genera in the tribe by the sessile leaves, unusual leaf venation pattern, the presence of a terminal rachis extension on the inflorescences and the unique combination of alkaloids found in the genus (Schutte 1997c). All analyses indicate that *Liparia* is not monophyletic, with *L. calycina* (and *L. umbellifera* with BI) not included in the *Liparia* clade. Schutte (1995, 1997c) described a close relationship between *L. calycina* and *L. vestita*, and these two species share a sympatric distribution. *Liparia vestita* in this case is embedded in the *Liparia* clade closer to *L. boucheri* and *L. latifolia*, although this is not well resolved. Crisp et al. (2000) also commented on the possible paraphyly of *Liparia*, with some species of *Liparia* in their analysis being sister to *Cyclopia* and others to *Podalyria*. They ascribed this result to the amalgamation of *Liparia* and *Priestleya* DC. and recommended that this should be evaluated with a larger sampling from both genera. In our study almost all species of *Liparia* were included. Only *L. graminifolia*, which is presumably extinct, and *L. laevigata* were not included. The combination of the two genera does not seem to be the problem, seeing that the rest of *Liparia* group together. Low resolution across the tree may account for the position of *L. calycina* as indicated by the result of the SH-test, but recollecting material from other populations of these species may also be necessary before making a definite conclusion regarding the monophyly of the genus. The presence of

several morphological and chemical apomorphies support the monophyly of this genus (Schutte 1997c).

The support for *Podalyria* to be monophyletic, although low with MP, differs from the result obtained by van der Bank et al. (2002) where *Podalyria* was paraphyletic. According to Schutte (1995) there is no single autapomorphy for the genus, but it has a unique combination of characters, namely simple, distinctly petiolate leaves (shared with *Stirtonanthus* and *Xiphotheca*), pink, purple or white flowers (shared with *Virgilia* and *Amphithalea*), few-flowered racemose inflorescences (shared with some species of *Liparia*) and a characteristic combination of alkaloids. *Stirtonanthus* (then part of *Podalyria*) differs in accumulating virgiline, 13α-hydroxylupanine and different esters (van Wyk et al. 1992). Our study indicates that *Podalyria* is monophyletic.

Stirtonanthus is supported with BI to be sister to the *Liparia*/*Podalyria* clade. A sister relationship between *S. chrysanthus* and *S. taylorianus* is indicated in both the BI and MP. These have inflated pods, as opposed to the compressed pods of *S. insignis*, and are both nonsprouters (van Wyk and Schutte 1994). The placement of *Podalyria* in closer relation to *Liparia* than *Stirtonanthus* is unexpected, seeing that the species of *Stirtonanthus* were originally included within *Podalyria*. A sister relationship between *Podalyria* and *Stirtonanthus* with *Liparia* sister to them, based on a cladistic analysis of morphological data, was shown by Schutte (1995) and Schutte and van Wyk (1998).

The second clade in Podalyriinae consists of *Calpurnia* and *Virgilia*. These genera were both originally placed in Sophoreae (van Wyk 1986; Beaumont et al. 1999). Although there is no support for this clade, the genera share several characters, including imparipinnately compound leaves and carboxylic acid esters of quinolizidine alkaloids (van Wyk and Schutte 1995; Schutte and van Wyk 1998). *Calpurnia intrusa* is not included in the *Calpurnia* clade with MP. It is interesting to note, however, that *C. intrusa* is the only Cape species in *Calpurnia*. A hybrid between *C. sericea* and *C. woodii* was described by Beaumont et al. (1999). Both putative parent species of this hybrid were also included in the study and a possible relationship of the hybrid with only *C. woodii* was found. A clear explanation for this is not apparent, but sampling material from the parent species at the hybrid locality might prove valuable. In *Virgilia*, *V. divaricata* and *V. oroboides* subsp. *ferruginea* form a strongly supported clade. van Wyk (1986) suggested that *V. oroboides* subsp. *ferruginea* probably originated as a hybrid between *V. divaricata* and V. *oroboides* subsp. *oroboides*. This and the fact that it is more or less geographically isolated from *V. oroboides* subsp. *oroboides* could explain the close relationship with *V. divaricata*. Based on enzyme electrophoretic data, van der Bank et al. (1996) also suggest that divergence followed by introgression could account for the similarity of the taxa. They speculate that there could have been an initial divergence in two species, *V. divaricata* and *V. oroboides*, with introgression resulting in a morphologically intermediate *V. oroboides* subsp. *ferruginea*.

Cyclopia is strongly supported as monophyletic. The genus is unique in Podalyrieae, as it is the only member that has trifoliate leaves, single-flowered inflorescences and a total absence of alkaloids (Schutte 1997b). It has been suggested that *Cyclopia* shares a close relationship with *Liparia* and *Podalyria* (Schutte and van Wyk 1998), but our results do not support this.

***Position of Cadia*—**While the monophyly of Podalyrieae excluding *Cadia* is only weakly supported, the support for a sister relationship between *Cadia* and Podalyrieae is high. Although Schutte and van Wyk (1998) excluded *Cadia* from Podalyrieae, they mention that subsequent studies involving chemistry or DNA might place *Cadia* as sister to the tribe.

Cadia species have tufted, imparipinnate leaves and axillary, racemose inflorescences with pendulous actinomorphic, pink to purple flowers (Du Puy et al. 2002). It is clear that the genus is monophyletic and that the widely distributed *Cadia purpurea* is closely related to the Madagascan species (Fig. 2B). A sister relationship exists between *C. commersoniana*, *C. pubescens* and *C. purpurea*. *Cadia commersoniana* and *C. pubescens* both have broad, leafy bracts on the inflorescence and can be distinguished from each other by the pubescent leaves and stems of *C. pubescens*, together with the smaller number of leaflets found in this species. *Cadia pedicellata* has a similar distribution to *C. pubescens*, but is much less pubescent and has narrow, nonleafy bracts on the inflorescence (Du Puy et al. 2002).

Floral symmetry is a distinguishing character between the three subfamilies of Fabaceae. Most members of the Caesalpinioideae and Mimosoideae have radially symmetrical flowers, although some flowers in Caesalpinioideae may be strongly zygomophic (Tucker 2003). In Papilionoideae the flowers are usually highly specialised and differentiated into standard, wing, and keel petals. They are strongly zygomorphic with uniform ontogenies, but *Cadia* is a rare example with radially symmetrical flowers. The floral ontogeny of most legume flowers is similar, especially the early developmental stages, with modifications to floral symmetry occurring later in development. In the case of *Cadia*, the lack of petal differentiation leads to a radially symmetrical flower (Tucker 1987, 2002). Although the flower of *Cadia* is plesiomorphic and relatively unspecialised, Tucker (2002) suggests that it may not necessarily indicate early divergence, but rather the retention of the symmetry of early developmental stages (neoteny). It is clear now however, that the occurrence of actinomorphic flowers is in fact a homeotic transformation due to the dorsalized petals in *Cadia* and not an evolutionary reversal (Citerne et al. 2006). This type of radial floral symmetry is therefore unique to the genus, i.e. an autapomorphy, as interpreted by Pennington et al. (2000). In Podalyrieae the flowers are normally adapted to pollination by xylocopid bees, but some species of *Liparia*, e.g. *L. splendens* and *L. parva*, display structural modifications in their flowers and inflorescences as an adaptation to sunbird and rodent pollination. Some of these changes include forwardly directed petals for bird pollination, yeast odours and inflorescences borne at ground level for rodent pollination (Schutte and van Wyk 1994; Schutte 1997c). Pennington et al. (2000) mention that little is known about the pollination biology of the basal papilionoids, but that it is possible that deviations from the typical, zygomorphic flowers may be due to pollination pressure, as may be the case in *Cadia*. Observations of the flowers of *Cadia* suggest possible pollination by birds, seeing that they produce abundant nectar and have no scent (Pennington et al. 2000).

Cadia shares several characters with members of Podalyrieae: a chromosome base number of $x = 9$ (Goldblatt 1981), imparipinnately compound leaves as in *Calpurnia* and *Virgilia*, axillary racemose inflorescences as in most Podalyrieae, carboxylic acid esters of quinolizidine alkaloids as

found in *Calpurnia*, *Stirtonanthus*, *Virgilia* and some species of *Liparia* (van Wyk 2003; Wink 2003; Wink and Mohamed 2003), and the isoflavone 3′-hydroxydaidzein as a major seed flavonoid (De Nysschen et al. 1998). The formal transfer of *Cadia* to the tribe Podalyrieae therefore seems long overdue and is proposed here. Molecular data from this and previous studies (van der Bank et al. 2002) do not support the monophyly of the subtribe Podalyriinae of Schutte and van Wyk (1998). Pending clarification of the possible inclusion of *Sophora inhambanensis* Klotzsch in the tribe Podalyrieae (van Wyk 2003), a revision of the subtribal classification system is necessary.

***Age of Podalyrieae*—**The use of molecular sequence data to infer ages of lineages and clade diversification has become more frequent in recent years. Several studies have determined the ages of well-known Cape plant groups. Richardson et al. (2001) dated the major proliferation of *Phylica* L. at 7–8 Ma. Reeves (2001) dated the radiation of *Protea* L. at 25 Ma. The divergence of the sister pair *Ferraria* Burm. ex Mill. and *Moraea* Mill. was dated at 25 Ma (Goldblatt et al. 2002), and Klak et al. (2004) dated the radiation of the 'core' Ruschioideae between 3.8–8.7 Ma. Linder (2005) discusses the evolution of diversity in the Cape flora and mentions that the greatest diversity and most recent radiations in southern Africa are found in the more arid western parts of the subcontinent. The largely gradual transformation in climate that has taken place throughout the evolutionary history of South Africa means that there was no single, obvious trigger for the radiation of the Cape flora and this subsequently accounts for the great spread in the dates of initiation of the radiation of various lineages.

The relationships inferred in the high taxonomic level analysis of the genistoid legumes support those of van Wyk and Schutte (1995) and Crisp et al. (2000) and represent the current hypotheses of relationships within these groups. Lavin et al. (2005) studied the rates of evolution of legumes and found that a rapid diversification took place in the Tertiary, soon after the origin of the family about 60 Ma ago. In their study, *Diplotropis* was also used to fix the age of the genistoid crown clade at 56 Ma. In our study, the root node of Podalyrieae s.s. was dated at 30.5 ± 2.6 Ma using NPRS and 34.7 Ma (ci: 25.1–44.1 Ma) based on the Bayesian method (not shown). This date indicates that Podalyrieae started to diversify in the late Oligocene. The date estimated for the 'core' genistoids (45.2 ± 2.3 and 51.2 Ma [ci: 43.9–55.3 Ma] NPRS and Bayesian [not shown] respectively) also corresponds well with that of Lavin et al. (2005; i.e. 45.5 Ma). Their date for the origin of Podalyrieae (44 Ma) is older than the age estimated in our study, and this could be due to undersampling of Podalyrieae in Lavin et al.'s study or the fact that a different dating method was used.

Linder (2003) suggests that two environmental changes in the Tertiary could have triggered the radiations that took place, namely fluctuations in sea level and climatic changes. At the end of the Oligocene there was a stabilisation of the climate in the Cape. The Miocene was characterised by high sea levels, with only ephemeral ice sheets on Antarctica. Climatic gradients from the equator to the poles became steeper in the Middle Miocene and seasonal aridity became more pronounced in the late Miocene after the glaciation of the Northern Hemisphere that led to a symmetrical zonal climate. The South Atlantic high pressure caused the fynbos region to become dryer (Hendey 1983; Deacon et al. 1992; Hallam 1994; Linder 2003) and with the inception of the Mediterranean type climate during the Pliocene, the climate in South Africa finally stabilised. It is during the middle and late Miocene that most clades within Podalyrieae diverged and the diversification continued into the Pliocene and corresponds to the inception of the Mediterranean type climate in the CFR.

***Rates of Evolution*—**There are two explanations why nonsprouters would have higher rates of molecular evolution: (i) shorter generation time and (ii) smaller population size. Schutte et al. (1995) suggested that speciation rates are likely higher in nonsprouting taxa due to the temporal isolation of these individuals and the limited interbreeding that occurs between parents and seedlings. These authors also indicated that a higher number of species in Podalyrieae are habitat specialists and that very few nonsprouters have wide distribution ranges. Such small populations could be prone to genetic drift and this could lead to an increase in the rate of molecular evolution. Wells (1969) argues that nonsprouters have shorter generation times than sprouters and that they are subject to selection pressures acting on each discrete generation of seedlings. Therefore, nonsprouters might fix more mutations than sprouters. In our case, although not significant, we have shown that the rate of molecular evolution seems to be higher in nonsprouters compared to sprouters, which is in agreement with both hypotheses. More studies in Podalyrieae and other groups (e.g. *Protea*) will help to resolve this hypothesis.

The higher rates of molecular evolution found in this study might affect the rate of speciation as well, given that rates of molecular and morphological evolution and diversification are marginally correlated in plants (Davies and Savolainen 2006). Further studies need to be conducted within Podalyrieae to better understand the causes of this radiation. Whether sprouting or nonsprouting is a derived trait may also differ between taxa (Le Maitre and Midgley 1992). In the genus *Erica* L., Verdaguer and Ojeda (2005) suggest that sprouting is ancestral to the nonsprouting life strategy and they propose that the marked species diversity and narrow endemism in this genus could be associated with the nonsprouting habit. In *Aspalathus,* however, van der Bank et al. (1999) demonstrated through morphological and genetic analyses that nonsprouting could be a pleisiomorphic character state with sprouting developing as a fire-survival strategy. They suggest that switches between the two strategies are possible, e.g. in *Cyclopia*, *Podalyria,* and *Hypocalyptus*, and that it must still be demonstrated whether the change from nonsprouting to sprouting was a single evolutionary event or convergence in different populations of *Aspalathus*. In our case, optimisations of sprouters/nonsprouters show that it is likely that the ancestral state in the early nodes within Podalyrieae are nonsprouters with perhaps at least 16 changes from nonsprouters to sprouters (data not shown but available on request).

ACKNOWLEDGMENTS. The authors thank Dr. B. Schrire (Kew Herbarium), Mr. M. Johns (CapeNature) and Mr. N. Helme (Nick Helme Botanical Surveys) for plant samples, the Darwin Initiative and the Kirstenbosch Botanical Gardens for DNA aliquots supplied, J. Thorne for providing sources codes from the Multidistribute package, and T. Fulcher for help with UNIX. The Thuthuka Program (National Research Foundation) and University of Johannesburg are gratefully acknowledged for financial support.

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APPENDIX 1. Voucher information and GenBank accession numbers for the species sampled for the separate and combined analyses of ITS and *rbcL*, and in the high taxonomic level ITS analysis. Information is provided as follows: species, voucher specimen, GenBank accession number *rbcL*, GenBank accession number ITS, literature citation ([1]Unpublished).

***Acosmium* Schott:** *A. panamense* (Benth.) Yakovlev, *Hughes 1308* (FHO), -, AF187084, Lavin et al. (2001). ***Adenocarpus* DC.:** *A. viscosus* (Willd.) Webb and Berthel, *Käss 343*, -, Z72300 and Z72301, Käss (1995). ***Aenictophyton* A.T. Lee:** *A. reconditum* A.T. Lee, *Fryxell 4500* (CANB), -, AF287654, Crisp et al. (2000). ***Amphithalea* Eckl. and Zeyh.:** *A. alba* Granby, *van Wyk 2125* (SCHG), AM180171, AM261217, present study; *A. axillaris* Granby, *Vlok and Schutte 17* (SCHG), AM180172, AM261218, present study; *A. biovulata* (Bolus) Granby, *M. Johns s.n.* (JRAU), AM180173, AM261219, present study; *A. ciliaris* Eckl. and Zeyh., *Vlok and Schutte 193* (SCHG), AM180174, AM261220, present study; *A. cuneifolia* Eckl. and Zeyh., *Vlok and Schutte 381* (SCHG), AM180176, AM261221, present study; *A. dahlgrenii* (Granby) A.L. Schutte, *Vlok and Schutte 221* (SCHG), AM180175, AM261222, present study; *A. ericifolia* L., *Vlok and Schutte 368* (SCHG), AM180177, AM261223, present study; *A. flava* (Granby) A.L. Schutte, *Schutte 652* (SCHG), AM180178, AM261224, present study; *A. fourcadei* Compton, *Vlok and Schutte 394* (SCHG), AM180179, AM261225, present study; *A. imbricata* (L.) Druce, *N.A. Helme 3426* (NBG), AM180180, AM268390 and AM268391, present study; *A. intermedia* Eckl. and Zeyh., *Schutte 828* (SCHG), AM180181, AM261226, present study; *A. micrantha* (E. Mey.) Walp., *Schutte 751* (SCHG), AM180182, AM261227, present study; *A. monticola* A.L. Schutte, Matroosberg, *Schutte 562* (SCHG), AM180183, AM261228, present study; *A. muirii* (Granby) A.L. Schutte, *Vlok and Schutte 157* (SCHG), AM180184, AM261229, present study; *A. muraltioides* (Benth.) A.L. Schutte, *Vlok and Schutte 354* (SCHG), AM180185, AM261230, present study; *A. obtusiloba* (Granby) A.L. Schutte, *N.A. Helme 3449* (NBG), AM180186, AM268392 and AM268393, present study; *A. oppositifolia* L. Bolus, *M. Johns s.n.* (JRAU), AM180187, AM261231, present study; *A. pageae* (L. Bolus) A.L. Schutte, *Vlok and Schutte 215* (SCHG), AM180188, AM261232, present study; *A. parvifolia* (Thunb.) A.L. Schutte, *Vlok and Schutte 190* (SCHG), AM180189, AM261233, present study; *A. phylicoides* Eckl. and Zeyh., *Vlok and Schutte 18* (SCHG), AM180190, AM261234, present study; *A. rostrata* A.L. Schutte and B.-E. van Wyk, *Vlok and Schutte 69* (SCHG), AM177361, AM261730, present study; *A. speciosa* Schltr., *Vlok and Schutte 367* (SCHG), AM177362, AM261235, present study; *A. spinosa* (Harv.) A.L. Schutte, *van Wyk 2195* (SCHG), AM177363, AM261236, present study; *A. stokoei* L. Bolus, *Vlok and Schutte 297* (SCHG), AM177364, AM261429, present study; *A. tomentosa* (Thunb.) Granby, *Vlok, van Wyk and Schutte 92* (SCHG), AM177365, AM261430, present study; *A. tortilis* (E. Mey.) Steud., *Schutte 599* (SCHG), AM177366, AM261431, present study; *A. villosa* Schltr., *Vlok and Schutte 117* (SCHG), AM177367, AM261432, present study; *A. violacea* (E. Mey.) Benth., *Vlok and Schutte 407* (SCHG), AM177368, AM261433, present study; *A. virgata* Eckl. and Zeyh., *Boatwright and Magee 65* (JRAU), AM177369, AM261434, present study; *A. vlokii* (A.L. Schutte and B.-E. van Wyk) A.L. Schutte, *Schutte 744* (SCHG), AM177370, AM261435, present study; *A. williamsonii* Harv., *Euston-Brown s.n.* (SCHG), AM177372, AM261436, present study. ***Anagyris* L.:** *A. foetida* L., *Wang H.C., KBG-127* (KUN), -, AY091571, Wang et al. (2006). ***Anarthrophyllum* Benth.:** *A. cumingii* (Hook. and Arn.) F. Phil., *AC 23756* (G), -, AY609186 and AY609196, Ainouche and Misset[1]. ***Aotus* Sm.:** *A. carinata* Meisn., *Chappill 6581*, -, AY883352, Orthia et al. (2005); *A. cordifolia* Benth., *Chappill 6587*, -, AY883353, Orthia et al. (2005). ***Argyrocytisus* (Maire) Raynaud:** *A. battandieri* (Maire) Raynaud, *Wink 397*, -, Z95580 and Z95581, Käss and Wink (1997). ***Argyrolobium* Eckl. and Zeyh.:** *A. harmsianum* Schltr. ex Harms, *Crisp 9042* (CANB), -, AF287685, Crisp et al. (2000); *A. lunare* Druce, *Crisp 9039* (CANB), -, AF287686, Crisp et al. (2000); *A. marginatum* Bolus, *T. Edwards 471*, Z95547, -, Käss and Wink (1997); *A. uniflorum* (Decne.) Jaub and Spach, *El-Shazly 477*, Z95548, -, Käss and Wink (1997). ***Aspalathus* L.:** *A. cephalotes* Thunb., *Heidrich 373*, Z70132, -, Käss (1995); *A. cordata* (L.) R. Dahlgr., *Crisp 9067* (CANB), -, AF287681, Crisp et al. (2000); *A. corrudifolia* P.J. Bergius, *Crisp 9037* (CANB), -, AF287682, Crisp et al. (2000); *A. longifolia* Benth., *B.-E. van Wyk 2799* (JRAU), -, AM262449, Motsi (2004); *A. nivea* Thunb., *B.-E. van Wyk 2938* (JRAU), -, AM262447, Motsi (2004). ***Baphia* Afzel. ex Lodd.:** *B. madagascariensis* C.H. Stirton and Du Puy, *D.J. Du Puy M554* (K), -, U59888, Hu et al. (2002). ***Baptisia* Vent.:** *B. australis* (L.) R. Br., *Jones P.D., NCBG-05* (KUN), -, AY091572, Wang et al. (2006); *B. tinctoria* (L.) R. Br., Botanical Gardens Heidelberg, Germany, -, Z72314 and Z72315, Käss (1995). ***Bolusanthus* Harms:** *B. speciosus* (Bolus) Harms, *J.P. 37 et* (H.G.), -, AM262451, Motsi (2004). ***Bossiaea* Vent.:** *B. lenticularis* DC., *MDC 9289*, -, AF518104, Crisp and Cook (2003); *B. linophylla* R. Br., *Crisp 8927* (CANB), -, AF287657, Crisp et al. (2000). ***Brongniartia* Kunth:** *B. alamosana* Rydb., *Hu 1120* (DAV), -, AF467022, Hu et al. (2002). ***Cadia* Forssk.:** *C. commersoniana* Baill., *Ambri and Arifin W584* (K), AM260749, AM261737, present study; *C. pedicellata* Baker, *J.-N. Labat 2423* (K), AM260750, AM261738, present study; *C. pubescens* Bojer ex Baker, *L.J. Dorr, L.C. Barnett and R. Brooks 3279* (K), -, AM261739, present study; *C. purpurea* (Ait.) Forssk., *J.J. Beckett 1702* (K), AM260751, AM261740, present study. ***Calicotome* Link:** *C. villosa* (Poir.) Link, *Käss 175*, -, Z72252 and Z72253, Käss (1995). ***Calpurnia* E. Mey.:** *C. aurea* (Aiton) Benth., *RBG, Kew 1991–1626* (K), -, AJ409913, van der Bank et al. (2002); *C. glabrata* Brummit, *K. Baldwin and M.-J. Baldwin 8502* (J), AM177372, AM261437, present study; *C. intrusa* (R. Br. in W.T. Aiton) E. Mey., *Schutte s.n.* (SCHG), AM177373, AM261438, present study; *C. sericea* Harv., *Boatwright 86* (JRAU), AM177374, AM268374 and AM268375, present study; *C. sericea x C. woodii*, *Beaumont s.n.* (NU), AM261726, AM261477, present study; *C. woodii* Schinz., *Beaumont s.n.* (NU), AM177375, AM261731, present study. ***Chorizema* Labill.:** *C. aciculare* (DC.) C.A. Gardner, *MDC 9202*, -, AF518108, Crisp and Cook (2003); *C. varium* Benth. ex Lindl., *MDC 8528*, -, AF518112, Crisp and Cook (2003). ***Crotalaria* L.:** *C. capensis* Jacq., *Heidrich 366*, Z70133, Z72310 and Z72311, Käss (1995); *C. lebeckioides* Bond, *B.-E. van Wyk 3315* (JRAU), -, AM262454, Motsi (2004); *C. incana* L., Botanical Gardens Coimbra, Portugal, Z70134, -, Käss and Wink (1995); *C. pallida* W.T. Aiton, Botanical Gardens Lome, Togo, -, Z72312 and Z72313, Käss (1995). ***Cyclolobium* Benth.:** *C. nutans* C.T. Rizzini and E.P. Heringer, *Ratter et al. 7431* (E), -, AF467041, Hu et al. (2002). ***Cyclopia* Vent.:** *C. alopecuroides* A.L. Schutte, *Vlok and Schutte 129* (SCHG), AM261711, AM050828, present study; *C. alpina* A.L. Schutte, *Vlok and Schutte 250* (SCHG), AM261712, AM050830, present study; *C. aurescens* Kies, *Schutte and van Wyk 771b* (JRAU), AM261713, AM050826, present study; *C. bolusii* Hofmeyr and E. Phillips, *Schutte 826* (SCHG), AM263058, AM268376 and AM268377, present study; *C. burtonii* Hofmeyr and E. Phillips, *Vlok and van Wyk 189* (JRAU), -, AJ310733, van der Bank et al. (2002); *C. falcata* (Harv.) Kies, *Schutte 598* (JRAU), AM261714, AM261732, present study; *C. galioides* (P.J. Bergius) DC., *De Lange 13* (SCHG), AM261715, AM050825, present study; *C. genistoides* (L.) R. Br., *Boatwright and Magee 53* (JRAU), AM261716, AM050819, present study; *C. glabra* (Hofmeyr and E. Phillips) A.L. Schutte, *Schutte 558* (SCHG), AM261717, AM050830, present study; *C. intermedia* E. Mey., *Schutte 658* (JRAU), AM261718, AM261733, present study; *C. longifolia* Vogel, *Vlok and Schutte 422* (SCHG), AM261719, AM050820, present study; *C. maculata* (Andrews) Kies, *Schutte 609–611* (JRAU), AM261720, AJ409896, van der Bank et al. (2002) and present study; *C. meyeriana* Walp., *Vlok and Schutte 251* (SCHG), AM261721, AM050818, present study; *C. plicata* Kies, *Schutte 670b* (JRAU), AM261722, AM268394, present study; *C. pubescens* Eckl. and Zeyh., *Schutte 685–689* (JRAU), AM261723, AJ409897, van der Bank et al. (2002) and present study; *C. sessiliflora* Eckl. and Zeyh., *Vlok and Schutte 213* (SCHG), AM261724, AM050831, present study; *C. subternata* Vogel, *Boatwright and Magee 35* (JRAU), AM261725, AM050821, present study. ***Cytisophyllum* O. Lang:** *C. sessilifolium* (L.) O. Lang, Botanical Gardens Hohenheim, Germany, -, Z72254 and Z72255, Käss (1995). ***Daviesia* Sm.:** *D. mimosoides* R. Br., *Crisp 9151*, -, AY883356, Orthia et al. (2005). ***Dichilus* DC.:** *D. lebeckioides* DC., *McMurtry 6367* (K), U74223, -, Doyle et al. (1997); *D. strictus* E. Mey., *Crisp 9073* (CANB), -, AF287684, Crisp et al. (2000). ***Diplotropis* Benth.:** *D. martiusii* Benth., *Beck, Henner and Jo Cardosa 166* (US), -, AY553711, Wojciechowski[1]. ***Echinospartum* (Spach) Rothm.:** *E. boissieri* (Spach) Rothm., *MAF 148150*, -, AY609188 and AY609193, Ainouche and Misset[1]. ***Erinacea* Adans.:** *E. anthyllis* Link, Botanical Gardens Tübingen, Germany, -, Z72256 and Z72257, Käss (1995). ***Euchresta* Benn.:** *E. japonica* Hook. f. ex Regel, *Kato and Kuribayashi 930674* (KYO), AB127040, -, Lee et al. (2004). ***Genista* L.:** *G. teretifolia* Willk., *MAF 162924*, -, AY263668, Pardo et al. (2004); *G. tournefortii* Spach, *MAF 160762*, -, AY263669, Pardo et al. (2004). ***Goodia* Salisb.:** *G. lotifolia* Salisb., *ANBG 702052*, -, AF287655, Crisp et al. (2000); *G. medicaginea* F. Muell., *MDC 9274*, -, AF518103, Crisp and Cook (2003). ***Hesperolaburnum* Maire:** *H. platycarpum* (Maire) Maire, *MA 586956*, -, AY263678, Pardo et al. (2004). ***Hovea* R. Br. ex W.T. Aiton:** *H. elliptica* (Sm.) DC., *Crisp 8924* (CANB), -, AF287640, Crisp et al. (2000). ***Hypocalyptus* Thunb.:** *H. coluteoides* (Lam.) R. Dahlgr., *Schutte 730* (JRAU), -, AJ409917, van der Bank et al. (2002); *H. oxalidifolius* (Sims) Baillon, *Schutte 468* (JRAU), -, AJ409918, van der Bank et al. (2002); *H. sophoroides* (P.J. Bergius) Baillon, *B.-E. van Wyk 3012*, *3319* (JRAU), -, AJ409919, van der Bank et al. (2002). ***Isotropis* Benth.:** *I. foliosa* Crisp, *MDC 9121*, -, AF518105, Crisp and Cook (2003); *I. forrestii* F. Muell., *Crisp 9261*, -, AY883357, Orthia et al. (2005). ***Jacksonia* R. Br. ex Sm.:** *J. alata* Benth., *MDC 8956*, -, AF518106, Crisp and Cook (2003); *J. macrocalyx* Meisn., *MDC 9272*, -, AF519107, Crisp and Cook (2003). ***Laburnum* Fabr.:** *L. anagyroides* Medik., *MAF 162279*, -, AY263679, Pardo et al. (2004). ***Lebeckia* Thunb.:** *L. cytisoides* Thunb., *Schutte 286* (JRAU), -, AM262452,

Motsi (2004); *L. sessilifolia* (Eckl. and Zeyh.) Benth., *Crisp 9041* (CANB), -, AF287678, Crisp et al. (2000); *L. wrightii* (Harv.) Bolus, *B.-E. van Wyk 3354* (JRAU), -, AM262447, Motsi (2004). ***Leptosema* Benth.:** *L. daviesioides* (Turcz.) Crisp, *Crisp 9193*, -, AY883360, Orthia et al. (2005). ***Liparia* L.:** *L. angustifolia* (Eckl. and Zeyh.) A.L. Schutte, *Boatwright and Magee 66* (JRAU), AM177376, AM261478, present study; *L. bonaespei* A.L. Schutte, *N.A. Helme and D. Raimondo 3430* (NBG), AM177377, AM261479, present study; *L. boucheri* (E.G.H. Oliv. and Fellingham) A.L. Schutte, *M. Johns s.n.* (JRAU), AM177378, AM261480, present study; *L. calycina* (L. Bolus) A.L. Schutte, *Vlok and Schutte 129* (SCHG), AM177379, AM261481, present study; *L. capitata* Thunb., *Schutte and van Wyk 776* (JRAU), AM177380, AM261482, present study; *L. confusa* A.L. Schutte, *Vlok and Schutte 502* (SCHG), AM259355, AM261483, present study; *L. congesta* A.L. Schutte, *Bean 2619* (SCHG), AM259356, AM261484, present study; *L. genistoides* (Lam.) A.L. Schutte, *Schutte 752* (SCHG), AM261727, AM261485, present study; *L. hirsuta* Thunb., *Boatwright and Magee 33* (JRAU), AM259357, AM261486, present study; *L. latifolia* (Benth.) A.L. Schutte, *N.A. Helme 3455* (NBG), AM259358, AM268378 and AM268379, present study; *L. myrtifolia* Thunb., *van Wyk 2639* (SCHG), AM259359, AM261487, present study; *L. parva* Vogel ex Walp., *van Wyk 3149, 3243* (JRAU), AM259360, AJ409909, van der Bank et al. (2002) and present study; *L. racemosa* A.L. Schutte, *Vlok and Schutte 501* (SCHG), AM259361, AM261488, present study; *L. rafnioides* A.L. Schutte, *M. Johns s.n.* (JRAU), AM259362, AM261489, present study; *L. splendens* (Burm. f.) Bos and De Wit subsp. *comantha* (Eckl. and Zeyh.) Bos and De Wit 1, *Vlok and Schutte 211* (SCHG), AM259363, AM268380 and AM268381, present study; *L. splendens* (Burm. f.) Bos and De Wit subsp. *comantha* 2, *Boatwright and Magee 8* (JRAU), AM261728, AM261490, present study; *L. striata* A.L. Schutte, *Schutte 759* (SCHG), AM259364, AM261491, present study; *L. umbellifera* Thunb., *Schutte 561* (SCHG), AM259365, AM261734, present study; *L. vestita* Thunb., *Boatwright and Magee 62* (JRAU), AM259366, AM261492, present study. ***Lotononis* (DC.) Eckl. and Zeyh.:** *L. alpina* (Eckl. and Zeyh.) B.-E. van Wyk, *B.-E. and M. van Wyk 1478* (JRAU), -, AM262446, Motsi (2004); *L. galpinii* Dümmer, *T. Edwards 480,* Z95538, -, Käss and Wink (1997); *L. laxa* Eckl. and Zeyh., *Crisp 9075* (CANB), -, AF287677, Crisp et al. (2000). ***Lupinus* L.:** *L. arcticus* S. Watson, *Hb, ALTA/95826*, -, AF007495, Ainouche and Bayer (1999); *L. polyphyllus* Lindl., *USDA/ 504404*, -, AF007496, Ainouche and Bayer (1999). ***Muelleranthus* Hutch.:** *M. trifoliolatus* (F. Muell.) A.T. Lee, *Lally 743* (CANB), -, AF287653, Crisp et al. (2000). ***Melolobium* Eckl. and Zeyh.:** *M. adenodes* Eckl. and Zeyh., *B.-E. van Wyk 4036* (JRAU), -, AM050832, Moteetee (2003); *M. candicans* Eckl. and Zeyh., *B.-E van Wyk 4016* (JRAU), -, AM050833, Moteetee (2003); *M. microphyllum* (L.f.) Eckl. and Zeyh., *T. Edwards 470*, Z95539, -, Käss and Wink (1997); *M. obcordatum* Harv., *T. Edwards 469,* Z95540, -, Käss and Wink (1997). ***Mirbelia* Sm.:** *M. longifolia* C.A. Gardner, *Crisp 9263*, -, AY883361, Orthia et al. (2005); *M. speciosa* DC., *ANBG 8100876*, -, AF518116, Crisp and Cook (2003). ***Maackia* Rupr. and Maxim.:** *M. amurensis* Rupr. and Maxim., Botanical Gardens Göttingen, Germany, -, Z72336 and Z72352, Käss (1995); *M. floribunda* (Miq.) Takeda, *Kurosaki and Nagamasu 2324 (*KYO), AB127042, -, Lee et al. (2004); *M. tashiroi* (Yatabe) Makino, *Deguchi et al. 46910* (KYO), AB127043, -, Lee et al. (2004). ***Nemcia* Domin (=***Gastrolobium* R. Br. ex W.T. Aiton): *N. plicata* (Turcz.) Crisp (=*G. plicatum* Turcz.), *MDC 9014*, -, AF518119, Crisp and Cook (2003). ***Oxylobium* Andrews:** *O. cordifolium* Andrews, *MDC 9133*, -, AF518117, Crisp and Cook (2003). ***Pearsonia* Dümmer:** *P. grandifolia* (Bolus) subsp. *latibracteolata* (Dümmer) Polhill, *B.-E. van Wyk 3047* (JRAU), -, AM262450, Motsi (2004); *P. sessilifolia* (Harv.) Dümmer, *Crisp 9078* (CANB), -, AJ287675, Crisp et al. (2000). ***Petteria* C. Presl:** *P. ramentacea* (Sieber) C. Presl, Botanical Gardens Gießen, Germany, -, Z72232 and Z72233, Käss (1995). ***Pickeringia* Nutt. ex Torr. and A. Gray:** *P. montana* Nutt. ex Torr. and A. Gray, Unknown, -, AY091568, Wang et al.[1]. ***Piptanthus* Sweet:** *P. tomentosus* Franchet, *Wang H.C., 0132* (KUN), -, AY091570, Wang et al. (2006). ***Podalyria* Willd.:** *P. argentea* (Salisb.) Salisb., *Vlok, van Wyk and Schutte 4* (SCHG), AM261690, AM261493, present study; *P. biflora* (L.) Lam., *Vlok s.n.* (SCHG), AM261691, AM261494, present study; *P. burchellii* DC., *B.-E. and M. van Wyk 7* (SCHG), AM261692, AM261495, present study; *P. buxifolia* (Retz.) Lam., *Boatwright and Magee 34* (JRAU), AM261693, AM261496, present study; *P. calyptrata* (Retz.) Willd., *Chase 16091* (K), AM261694, AM261735, present study; *P. canescens* E. Mey, *van Wyk 3237* (JRAU), AM261695, AM261736, present study; *P. cordata* (Thunb.) R. Br., *Vlok and Schutte 311* (SCHG), AM261696, AM268382 and AM268383, present study; *P. cuneifolia* Vent., *van Wyk 2888, 3177* (JRAU), AM261697, AJ409904, van der Bank et al. (2002) and present study; *P. hirsuta* (W.T. Aiton) Willd., *Vlok and Schutte 437* (SCHG), AM261698, AM261671, present study; *P. intermedia* Eckl. and Zeyh., *van Wyk 3003* (JRAU), -, AJ409899, van der Bank et al. (2002); *P. lanceolata* (E. Mey) Benth., *Vlok and Schutte 76* (SCHG), AM261699, AM261672, present study; *P. leipoldtii* L. Bolus ex A.L. Schutte, *van Wyk 3128* (JRAU), AM261700, AJ409902, van der Bank et al. (2002) and present study; *P. microphylla* E. Mey., *Vlok and Schutte 423* (SCHG), AM261701, AM261673, present study; *P. myrtillifolia* (Retz.) Willd., *van Wyk 2995, 3004* (JRAU), AM261702, AJ409901, van der Bank et al. (2002) and present study; *P. oleaefolia* Salisb., *Vlok, van Wyk and Schutte 76* (SCHG), AM261703, AM261674, present study; *P. orbicularis* (E. Mey.) Eckl. and Zeyh., *Vlok and Schutte 428* (SCHG), AM261704, AM261675, present study; *P. pearsonii* E. Phillips, *Vlok and Schutte 47* (SCHG), AM261705, AM268384 and AM268385, present study; *P. rotundifolia* (P.J. Bergius) A.L. Schutte, *Vlok and Schutte 441* (SCHG), AM261706, AM261676, present study; *P. sericea* (Andrews) R. Br., *Vlok and Schutte 63b* (JRAU), AM261707, AJ409903, van der Bank et al. (2002) and present study; *P. speciosa* Eckl. and Zeyh., *Boatwright and Magee 79* (JRAU), AM261708, AM261677, present study; *P. variabilis* A.L. Schutte (ined.), *Vlok and Schutte 230* (SCHG), AM261709, AM261678, present study; *P. velutina* Burch. ex Benth., *A.E. van Wyk 337* (PRU), AM261710, -, present study. ***Podolobium* R. Br. ex W.T. Aiton:** *P. aciculiferum* F. Muell., *GTC 606*, -, AF518118, Crisp and Cook (2003). ***Poecilanthe* Benth.:** *P. falcata* (Vell.) E.P. Heringer, *De Lima 2* (RJ), -, AF467492, Hu et al. (2002). ***Polhillia* C.H. Stirton:** *P. pallens* C.H. Stirton, *B.-E. van Wyk 2128* (JRAU), -, AM262453, Motsi (2004). ***Pultenaea* Sm.:** *P. pedunculata* Hook., *De Kok 756*, -, AY883374, Orthia et al. (2005); *P. stipularis* Sm., *De Kok 701*, -, AY883378, Orthia et al. (2005). Rafnia Thunb.: *R. alata* G.J. Campbell and B.-E*. van Wyk,* *Campbell and van Wyk 8* (JRAU), -, AJ744938, Motsi (2004); *R. vlokii* G.J. Campbell and B.-E. van Wyk, *B.-E. van Wyk 3172* (JRAU), -, AJ744937, Motsi (2004). ***Retama*** Raf.: *R. monosperma* (L.) Boiss., *MAF 162126*, -, AY263681, Pardo et al. (2004); *R. sphaerocarpa* (L.) Boiss., *MAF 160442*, -, AY263682, Pardo et al. (2004). ***Spartium* L.:** *S. junceum* L., *MAF 159908*, -, AF351088, Cubas et al. (2002). ***Sphaerolobium* Sm.:** *S. minus* Labill., *MDC 9154*, -, AF518101, Crisp and Cook (2003); *S. nudiflorum* (Meisn.) Benth., *RB 891*, -, AF518102, Crisp and Cook (2003). ***Sophora* L.:** *S. microphylla* Aiton, *CHR 529930,* AY725480, -, Heenan et al. (2004); *S. tetraphylla* J. S. Muell., *RBG, Kew 1977–1212* (K), -, AJ310734, van der Bank et al. (2002); *S. tomentosa* L., *CHR 569752*, AY725481, -, Heenan et al. (2004); *S. toromiro* Skottsb., *RBG, Kew 1994–2331* (K), -, AJ409921, van der Bank et al. (2002). ***Stauracanthus* Link:** *S. genistoides* (Brot.) Samp. subsp. *genistoides*, *MAF 7908*, -, AF384340 and AF384341, Ainouche et al. (2003). ***Stirtonanthus* B.-E. van Wyk and A.L. Schutte:** *S. chrysanthus* (Adamson) B.-E. van Wyk and A.L. Schutte, *van Wyk and Schutte 3297* (JRAU), AM259367, AM268386 and AM268387, present study; *S. insignis* (Compton) B.-E. van Wyk and A.L. Schutte, *Schutte and van Wyk 721* (JRAU), AM259368, AJ 409906, van der Bank et al. (2002) and present study; *S. taylorianus* (L. Bolus) B.-E. van Wyk and A.L. Schutte, *van Wyk and Schutte 3248* (JRAU), AM259369, AJ409907, van der Bank et al. (2002) and present study. ***Styphnolobium* Schott:** *S. japonicum* (L.) Schott, *RBG, Kew 1972–10834* (K), -, AJ409920, van der Bank et al. (2002). ***Templetonia* R. Br. ex W.T. Aiton:** *T. retusa* (Vent.) R. Br., *Crisp 8996* (CANB), -, AF287636, Crisp et al. (2000). ***Thermopsis* R. Br. ex W.T. Aiton:** *T. divaricarpa* Nelson, *Jones P.D., NCBG-01* (KUN), -, AY091575, Wang et al. (2006); *T. montana* Torr. and A. Gray, *HbUR/Ktm 101*, -, AF384336 and AF384337, Ainouche et al. (2003). ***Ulex* L.:** *U. densus* Welw. ex Webb, *HbUR/UD 7*, -, AF384356 and AF384357, Ainouche et al. (2003); *U. parviflorus* Pourr., *LB-UR-Fr/G53*, -, AF007470, Ainouche and Bayer (1999). ***Virgilia* Poir.:** *V. divaricata* Adamson, *van Wyk 879–888* (JRAU), AM260737, AJ409910, van der Bank et al. (2002) and present study; *V. oroboides* (P.J. Bergius) T.M. Salter subsp. *ferruginea* B.-E. van Wyk, *van Wyk 956, 957* (JRAU), AM260738, AJ409911, van der Bank et al. (2002) and present study; *V. oroboides* (P.J. Bergius) T.M. Salter subsp. *oroboides*, *van Wyk 802–806* (JRAU), AM260739, AJ409912, van der Bank et al. (2002) and present study. ***Xiphotheca* Eckl. and Zeyh.:** *X. canescens* (Thunb.) A.L. Schutte and B.-E. van Wyk, *Schutte 595* (JRAU), AM260740, AM268388 and AM268389, present study; *X. cordifolia* A.L. Schutte and B.-E. van Wyk, *N.A. Helme 2852* (NBG), AM260741, AM261679, present study; *X. elliptica* (DC.) A.L. Schutte and B.-E. van Wyk, *M. Johns s.n.* (JRAU), AM260742, AM261680, present study; *X. fruticosa* (L.) A.L. Schutte and B.-E. van Wyk, *Schutte 673–675* (JRAU), AM260743, AJ310726, van der Bank et al. (2002) and present study; *X. guthriei* (L. Bolus) A.L. Schutte and B.-E. van Wyk, *Vlok and Schutte 4* (SCHG), AM260744, AM261741, present study; *X. lanceolata* (E. Mey.) Eckl. and Zeyh., *Vlok and Schutte 424* (SCHG), AM260745, AM261742, present study; *X. phylicoides* A.L. Schutte and B.-E. van Wyk, *Vlok 2500* (SCHG), AM260746, AM261743, present study; *X. reflexa* (Thunb.) A.L. Schutte and B.-E. van Wyk, *Schutte 760* (JRAU), AM260747, AM261744, present study; *X. tecta* (Thunb.) A.L. Schutte and B.-E. van Wyk, *Schutte 714, 738* (JRAU), AM260748, AJ310727, van der Bank et al. (2002) and present study.