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Phylogenetic relationships in the genus *Aspalathus* L. (Fabaceae; Papilionoideae; Crotalarieae) based on molecular and morphological evidence

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ABSTRACT

The genus *Aspalathus*, comprising approximately 300 species predominantly endemic to South Africa's Core Cape Subregion, is the largest genus within the legume (Leguminosae /Fabaceae) family in the subregion. Despite its ecological and economic significance, phylogenetic reconstructions within *Aspalathus* have been hindered by poor resolution, largely due to limited variation in commonly used molecular markers. This study expands sampling within *Aspalathus* and employs four DNA markers—ETS, ITS, *mat*K, and *trn*S-*trn*G—to improve phylogenetic resolution and clarify relationships within the genus. Our results indicate that *Aspalathus*, as currently circumscribed, may not be monophyletic, with two major clades sharing a common ancestor with other Cape Crotalarieae genera. A polytomy at the backbone of the phylogeny suggests unresolved evolutionary relationships, potentially influenced by limited marker variation. Character inference highlights morphological and ecological differences between the two major clades, particularly in leaf lamina morphology, supporting previous hypotheses of taxonomic divergence within the genus. These findings refine the phylogenetic framework for *Aspalathus*, but further studies, like those using the angiosperm353 target capture sequencing approach, are needed to improve resolution. Additionally, broader sampling of related taxa will be essential for accurately determining the position of *Aspalathus* within the tribe Crotalarieae.

1. Introduction

The genus *Aspalathus* L., commonly known as 'Cape gorse' (iNaturalist, 2025), displays extraordinary diversity within the Core Cape Subregion (CCR). Belonging to the Leguminosae family—a group of pod-bearing shrubs and shrublets (Dahlgren, 1960; Lewis et al., 2005). This genus encompasses approximately 296 accepted species (Du Preez and Stirton, 2024; Stirton et al., 2024; POWO, 2025), whereby *Aspalathus* is the second most species-rich genus in the CCR, with an exceptional 98 % endemism (Goldblatt and Manning, 2002; Cupido, 2007; Manning and Goldblatt, 2012). Species predominantly colonise the western and southern Cape, particularly the mountainous regions between Cape Hangklip and Ceres, as well as the Cape Peninsula (Dahlgren, 1963). While primarily restricted to the CCR, their resilience allows them to extend into the Eastern Cape and parts of KwaZulu-Natal (Stirton and Muasya, 2011, 2016; POWO, 2025), particularly in fire-disturbed shrublands with acidic, nutrient-poor soils (Linder, 2003; Manning and Goldblatt, 2012; Chimphango et al., 2015). This is not surprising, given Linder's (2003) assertion that numerous outliers of Cape flora are likely to be found in mountainous environments where oligotrophic sandstone substrates are present.

Molecular systematics has confirmed that *Aspalathus* belongs to the tribe Crotalarieae (Benth.) Hutch., which is part of the Papilionoideae subfamily (Kajita et al., 2001; Lewis et al., 2005). The Crotalarieae, mostly found in Africa, comprises about 51 % of the genistoid legumes, with approximately 1204 species spread across 16 genera (Boatwright et al. 2011; Cardoso et al., 2013; Yaradua, 2018). Notably, most species within the Crotalarieae fall into two genera: *Crotalaria* L. (Polhill 1982; ca. 714 species, POWO, 2025) and *Aspalathus* (Boatwright et al., 2008; Cardoso et al., 2013; Yaradua, 2018; POWO, 2025). Understanding

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relationships within this tribe has proven challenging due to factors such as reticulation and convergence, contributing to the complexity of their evolutionary history (Dahlgren, 1970a; Polhill, 1976, 1982; Van Wyk, 1991; Boatwright et al., 2008). Studies by Boatwright et al. (2008, 2011) identified three main groups within the tribe Crotalarieae: the 'Cape' group, the 'Lotononis' group, and the 'Crotalaria' group. The genus *Aspalathus* belongs to the 'Cape' group, along with six other related genera: *Calobota* Eckl. & Zeyh., *Ezoloba* B.-E. van Wyk & Boatwr., *Lebeckia* Thunb., *Rafnia* Thunb., *Wiborgia* Thunb., and *Wiborgiella* Boatwr. & B.-E. van Wyk (Boatwright et al., 2008, 2011; Le Roux and Van Wyk, 2012; Yaradua, 2018). Most of these genera are endemic to the Greater Cape Floristic Region, with *Calobota* extending throughout the Cape to parts of Namibia and disjunct into North Africa (Boatwright et al., 2008; Yaradua, 2018; POWO, 2025). Recent revisionary studies by Wojciechowski (2003), Boatwright et al. (2008, 2011), and Cardoso et al. (2013) consistently found the tribe to be monophyletic and sister to the tribe Genisteae (Bronn) Dumort. Although Crotalarieae was confirmed by the above authors to be monophyletic, the position of *Aspalathus* within the tribe remains uncertain. Previous phylogenetic studies by Edwards and Hawkins (2007) using ITS could not determine the exact placement of this genus due to limited sampling and reliance on a single gene region. Boatwright et al. (2008, 2011) conducted an exhaustive phylogenetic study of all genera within Crotalarieae, identifying distinct clades with strong support. This study confirmed that *Aspalathus* is monophyletic with substantial bootstrap support (99 %). However, the relationships between *Aspalathus* and its apparent close relatives, *Wiborgia* and *Wiborgiella*, remain unresolved.

The generic and infrageneric divisions in *Aspalathus* sensu lato are complex and have been adequately reviewed by Dahlgren (1963). In this study, he recognised six subgenera – *Rafnoides, Aspalathus, Ecklonella, Nortieria, Triplobractea*, and *Purpureipetala* –each further subdivided into groups without assigned rank. However, a subsequent revision (Dahlgren, 1988) recognized only five subgenera; *Aspalathus, Ecklonella, Nortieria* (including *Rafnoides*), *Triplobractea*, and *Purpureipetala*; along with 34 informal groups or morphotypes. Both revisions provided detailed descriptions encompassing various aspects such as morphology, anatomy, palynology, cytology, ecology, and phytogeography. Additional studies, including karyological investigations (Dahlgren, 1970b) and detailed phytogeographic analyses (Dahlgren, 1968) resulted in the development of a comprehensive key to the genus. Despite the valuable insights provided by these publications regarding the general similarities among observed groups, none of the taxonomic units at any rank underwent phylogenetic analysis.

The genus *Aspalathus* exhibits wide morphological diversity (Fig. 1, Dahlgren, 1963, 1988). Species within the genus display a wide range of growth habits, with many adopting prostrate or mat-like forms, while others are erect or ascending, typically reaching heights of 0.1 – 1.5 m and occasionally up to 5 m (Dahlgren, 1988). A notable aspect of Aspalathus species is the wide range of leaf types, including simple, subterete leaves in some species (mostly in the Sericea group (Dahlgren, 1963) and trifoliolate leaves in others (Dahlgren, 1988). The trifoliolate leaves are commonly gathered into fascicles that may be laxly separated

Fig. 1. Side view variations in *Aspalathus* flower, illustrating differences in the shape of calyx and petals, along with a spectrum of flower colours ranging from yellow to pink/magenta. Photos: A, D, E, G, H, K, R, by © Charles Stirton, B by © Carina, C and I by © Euston Brown, F, M, O, S by © Brian du Preez, J by © Nicky, L by © Nicola van Berkel (www.inaturalist.org).

or congested on shoots. Several species feature leaf base extensions into median abaxial tubercles, spurs, or spines, collectively referred to as “leaf base processes” (Dahlgren, 1965). This characteristic is particularly prevalent in species such as *A. aculeata* Thunb., *A. desertorum* Bolus, *A. uniflora* L., *A. ternata* (Thunb.) Druce, *A. calcarata* Harv., *A. opaca* Eckl. & Zeyh., and *A. tridentata* L. Variable spines are observed in some members of the genus. Species like *A. spinosa* L., *A. spinifera* C.H. Stirt. & Muasya, *A. acuminata* Lam., and *A. secunda* E. Mey. have spines that are typically long, straight, stiff, and readily distinguished from leaf-apex spines observed in species like A. aculeata (Dahlgren, 1963). Inflorescences in *Aspalathus* are typically borne on main branches, may be terminal or scattered along the branches and form racemes, umbels, spikes, or heads with variations in flower numbers (Dahlgren, 1965). The corolla generally follows the common papilionaceous type, with vexilla usually ovate, obovate, or circular. Petal colour ranges from yellow to violet-purple, with occasional occurrences of white, pink, or red petals. Some species, e.g., the Purpureae group, feature petals in white, violet, or pink, excluding yellow (Dahlgren, 1965, 1988). Pod characters, including shape, hairiness, and surface structure, vary among species, with occasional occurrences of inflated pods (Fig. 2; Dahlgren, 1963, 1988). Seed size, shape, and colour also exhibit considerable variation, with exceptional occurrences of long and compressed seeds in certain groups, e.g., in *A. juniperina* Thunb. and *A. divaricata* Thunb. (Dahlgren, 1965).

Previous molecular studies of Crotalarieae (Boatwright et al., 2008; 2009, 2010, 2011) included *Aspalathus* primarily to provide a generic context within the Cape clade. While these studies clarified tribal-level relationships, the relationships within *Aspalathus* remained unresolved due to limited taxon sampling and low sequence divergence in the markers employed (Edwards and Hawkins, 2007). Consequently, infrageneric relationships and the validity of Dahlgren’s (1988) morphologically defined groups have not been thoroughly tested using molecular data.

The present study focuses on resolving infrageneric relationships within *Aspalathus* through expanded taxon sampling and combined nuclear and plastid DNA markers. Closely related Cape genera, including *Wiborgia, Wiborgiella, Lebeckia*, and *Rafnia*, were included to provide phylogenetic context, while *Crotalaria*, from the distantly related *Crotalaria* clade, was used to root the phylogeny. Rooting with a single, well-supported outgroup was sufficient for this infrageneric focus, as including multiple deep outgroups could introduce long-branch artefacts and obscure internal relationships (Boatwright et al., 2008). The study hypothesises that *Aspalathus*, as currently circumscribed, comprises multiple distinct evolutionary lineages corresponding broadly to Dahlgren’s infrageneric groups and that these lineages are supported by both molecular and morphological synapomorphies. Alternatively,

Fig. 2. Diversity in pod shape, size, and texture. Photos: A, C, D, F by © Charles Stirton, B, E, G by © Brian du Preez, H by © Annekevet, I by © Nick Helme (www.inaturalist.org).

incongruence between molecular and morphological evidence would suggest convergent evolution of certain diagnostic traits, resulting in non-monophyletic assemblages.

The main objectives are: (i) to construct a molecular phylogenetic framework for *Aspalathus* using nuclear and plastid DNA markers, (ii) to evaluate the monophyly and phylogenetic coherence of Dahlgren's morphology-based infrageneric groups, and (iii) to reconstruct the evolutionary history of selected morphological traits within the genus.

2. Materials and methods

2.1. Outgroup selection

In accordance with the methodology established by Edward and Hawkins (2007), this study employed the genus *Crotalaria* as an outgroup, which is represented by only five species within the CCR. This selection was predicated on its inferred phylogenetic position as a sister group to the "Cape clade" (Boatwright et al. 2010). We note that *Ezoloba macrocarpa* (Eckl. & Zeyh.) B.-E.van Wyk & Boatwr. is sister to the 'Cape' group and/or at least early diverging lineages together with *Calobota* (Boatwright et al. 2011), but these taxa could not be included in the current study due to paucity of suitable DNA material.

2.2. Taxon and loci sampling

The study involved sampling nearly 40 % of named *Aspalathus* species, represented by 137 taxa plus 6 unidentified species that exhibit distinctive characteristics that suggest the presence of new, undescribed taxa (see Supplementary materials, Table S1). Additionally, the study included 22 representative species from other genera within the Cape clade, specifically *Lebeckia* (3 of 14 species), *Rafnia* (6 of 29 species), *Wiborgia* (5 of 9 species), and *Wiborgiella* (8 of 10 species) (Boatwright et al. 2010; POWO, 2025). Our sampling strategy utilised well-established and phylogenetically informative nuclear ribosomal DNA (nrDNA) regions, specifically the Internal Transcribed Spacer (ITS) and External Transcribed Spacer (ETS), in addition to the chloroplast DNA *mat*K gene and intergenic spacers *trn*S-*trn*G (Edwards and Hawkins, 2007; Rockinger et al., 2017; Moiloa et al., 2018). The *trn*S-*trn*G region presented sequencing challenges, including long mononucleotide repeats that impeded sequencing success in certain species, as well as sequence inversions that could lead to alignment difficulties. Consequently, only those taxa that were successfully sequenced from this region were included in the analysis, resulting in some instances of missing data. Prior research had indicated that analyses incorporating some missing data did not significantly undermine phylogenetic accuracy (Rockinger et al., 2017; Moiloa et al., 2018).

2.3. DNA extraction, amplification, and sequencing

Plant genomic DNA was extracted from silica-dried or herbarium leaf material using the Cetyltrimethylammonium bromide (CTAB)-2-mercaptoethanol method of Doyle and Doyle (1987). Polymerase Chain Reaction (PCR) amplifications were performed in 25 µL reaction volumes, each comprising 2 µM of each primer (1 µM reverse primer and 1 µM forward primer), 1 mM of DNTP, 2.5 mM MgCl2, 0.25 U/µl Taq polymerase, 2.5 µl 10X buffer, 0.5 µl DMSO, 1 ng/µl DNA template, and 15.25 µl dH2O. All PCR amplifications were conducted on an Applied Biosystems GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Four DNA regions were amplified and sequenced using two primers for each region (ITS: ITS5/ITS 4, White *et al.*, 1990; ETS: 28IF, Cubas et al., 2010, 18S – IGS Baldwin and Markos, 1998; *mat*K: 1RKIM/3FKIM, Kuzmina et al., 2012; *trn*S-*trn*G: trnSGCU (C4)/3′trnGUUG (CI), Hamilton, 1999), resulting in a total of eight primers (thermal reaction profiles indicated in Table 1). The primers utilised in the PCR were also employed during the cycle sequencing stage. For each sample, a portion (3 – 5 µL) of amplicons was loaded into an electrophoresis tank containing 0.5× TBE buffer and run at 100 V for 20–30 min, subsequently visualised under ultraviolet (UV) light. The amplicons from all regions were sent to the Central Analytical Facilities at Stellenbosch University for post-PCR cleaning and sequencing.

Table 1

DNA regions sequenced and profiles used for PCR amplification.

Region	Pre-melt	PCR profile Annealing temperature and time	Extension	Cycles
ITS	95 °C (3 min)	95 °C (30 secs) + 54 °C (1 min) + 68 °C (1 min)	68 °C (10 min)	39
ETS	95 °C (3 min)	95 °C (30 s) + 54 °C (1 min) + 68 °C (1 min)	68 °C (10 min)	39
***mat*K**	94 °C (2 min)	94° (1 min) + 55 °C (1 min) + 72 °C (2 min)	72 °C (5 min)	30
***trn*S-*trn*G**	96 °C (5 min)	96 °C (45 secs) + 48 °C (1 min) + 72 °C (30 secs)	72 °C (1 min)	40

2.4. Sequence alignment and phylogenetic analysis

Sequences were assembled and edited using ChromasPro v 2.1.10 (2021). Consensus sequences were aligned using ClustalW in MAFFT (Kuraku et al., 2013; Katoh et al., 2019), and manual adjustments to the alignments were made using BioEdit v. 7.2.5. (Hall, 1999). The final alignments were converted to Nexus format and concatenated using Mesquite v. 3.11 (Maddison and Maddison, 2018). Phylogenetic analyses for all markers were performed using Maximum likelihood (ML), and Bayesian inference (BI) methods. For the initial Maximum Likelihood (ML) analysis, IQ-TREE v2.1.1 (Chernomore et al., 2016) was used. Model tests for all regions, including concatenated nuclear-chloroplast sequences, were selected according to the Bayesian Information Criterion (BIC) (Kalyaanamoorthy *et al.*, 2017). The analysis included ultrafast bootstraps (UFBoot, Hoang et al., 2018) and utilised bootstrapping with 1000 replicates to assess branch support. In the combined analysis, the four gene datasets were partitioned, and the models assigned were same as those applied to the separate unlinked partitions. Taxa absent in any of the five partitions were coded as missing. The congruence of the separate matrices as well as combined datasets was assessed by visually evaluating the topology of the ML/BI trees. The following scale was used for the UFBoot values: weak support (≤70 %); moderate support (71–94 %); and strong support (≥95 %) (Minh et al., 2013). Both separate matrices and a combined matrix underwent Bayesian inference analysis using MrBayes on XSEDE version 3.2.6 (Ronquist et al., 2012), accessed through the Cyber Infrastructure for Phylogenetic Research (CIPRES) site 2.0 (Miller et al., 2011). The best-fitting model was determined for each dataset and multiple partitions using the nst=mixed command. Two independent Metropolis coupled Markov chain Monte Carlo (MCMC) runs, each with one cold and three heated chains, were performed for 50 million generations. Convergence was evaluated using the standard deviation of split frequencies, discarding the initial 25 % as burn-in. The results were summarised as a 50 % majority rule consensus tree and viewed in FigTree 1.4.0 (Rambaut, 2014; available at http://tree.bio.ed.ac.uk/software/figtree/), reflecting branch support as posterior probabilities. Posterior probability (PP) values were scaled according to Shi and Yin (2021), where values between 0.50–0.84 are considered weak support, 0.85–0.94 are moderate support, and >0.95 are strong support.

2.5. Morphological character scoring

To evaluate the morphological synapomorphies of the "Cape Clade," we investigated 19 *a priori* taxonomically significant characters (Table 2). A character is considered taxonomically significant if it shows variation between groups, remains relatively consistent within a group, reflects phylogenetic signal, and provides diagnostic value for distinguishing taxa (Freudenstein, 2005). These characters were chosen for

Table 2
Coded characters and the states used to identify synapomorphies in *Aspalathus* clades and related taxa.

	Characters	States		
1	Growth habit	Sub shrub=0	Shrub=1	
2	Stem type	Herbaceous=0	Woody=1	
3	Shoot indument	Glabrous=0	Hairy=1	
4	Bark surface	Smooth=0	Striated=1	Corky=2
5	Petiole	Present=0	Absent=1	
6	Petiolar spur	Present =0	Absent=1	
7	Leaf range	Unifoliolate=0	Trifoliolate/ fascicles=1	
8	Leaf lamina	Flat=0	Terete/sub-terete=1	
9	Leaf base	Spines=0	tubercle or no spines=1	
10	Leaflet tips	Spine-tipped =0	Not spine-tipped=1	
11	Flower size	Small (<8 mm)= 0	Medium (9–10 mm)=1	Large (>11 mm)=2
12	Flower colour	Yellow=0	Not yellow=1	
13	Flower colour changes with age	Retains colour=0	Changes colour=1	
14	Wing petals length relative to keel	Wing longer than Keel=0	Wing same length as Keel =1	Wing shorter than Keel =2
15	Ovule number	Few ovule 1–6 = 0	Many ovulate (>6) =2	
16	Anther configuration	5 + 5 = 0	4 + 6 = 1	4 + 1 + 5 = 2
17	Pods form	Inflated=0	Laterally compressed=1	
18	Pods type	Dehiscent=0	Indehiscent=1	
19	Pod wings	Wings absent=0	Wings present=1	

their utility in defining or differentiating *Aspalathus* and its infrageneric groups (Dahlgren, 1963), as well as their historical relevance in the classification of the tribe Crotalarieae (Boatwright et al., 2008; Le Roux and van Wyk, 2012). Out of the 19 characters initially chosen, 11 were determined to possess a robust phylogenetic signal. These included traits that are the primary focus of this study such as petiolar spur, presence of petiole, leaf range, leaf lamina, wing petal length relative to keel, anther configuration, pod wing, pod type, and pod form, Each character state was assessed based on previously published research (Dahlgren, 1960, 1984, 1988; Campbell and Van Wyk, 2001; Cupido, 2007; Boatwright et al., 2008, 2011; Le Roux and Van Wyk, 2008; 2009; Stirton and Muasya, 2011; Curtis et al., 2013; Stirton et al., 2024) and through our observations of herbarium material (BOL, NBG, PRE) and fresh specimens. The character set included both binary and multi-state characters, with all character states treated as unordered, polarised in comparison to the outgroup. The characters were optimised onto the single tree obtained from the phylogenetic analysis using Maximum Likelihood method to study their evolution and importance. Maximum Likelihood ancestral character state reconstruction was conducted using the Mk1 model as implemented in Mesquite (Maddison and Maddison, 2018). Additionally, characters were mapped in Reconstruct Ancestral State in Phylogenies (RASP) using the default Maximum Likelihood settings (Yu et al., 2020).

3. Results

The final combined molecular dataset comprised 211 sequences representing all four targeted regions: ITS, ETS, *mat*K and *trn*S–*trn*G, with a total of 2359 bases and 966 variable sites. Among these, 399 sites were parsimony-informative, 370 were singleton sites, and 1590 were constant. Table 3 summarises the characteristics of the individual datasets used in the Maximum Likelihood analysis performed with IQ-TREE2. The combined nuclear regions (ITS + ETS) exhibited a stronger phylogenetic signal than the plastid markers (*mat*K + *trn*S-*trn*G), indicating that the nrDNA data were particularly informative for resolving relationships within *Aspalathus*.

3.1. Phylogenetic relationships

Phylogenetic analyses based on plastid and nuclear datasets produced largely congruent trees, and tanglegram comparisons revealed no significant conflicts, justifying the combined use of these markers (Fig. 3A–C). The combined ML and BI analyses of these datasets (Fig. 4; see tip details in supplementary materials Fig. S1 and S2) were also consistent, with minor differences restricted to poorly supported branches.

Aspalathus was consistently recovered as non-monophyletic, forming two strongly supported clades. Clade I (UFBoot = 100 %, PP = 1.00) is a heterogeneous assemblage of species with variable morphology, whereas Clade II (UFBoot = 100 %, PP = 1.00) predominantly contains species from Dahlgren’s Group 1 (Sericeae). Exceptions to this pattern were observed, with *A. rugosa* and *A. bidouwensis*, morphologically assigned to Group 1, nested within Clade I. This discordance highlights that Sericeae, as currently defined, does not form a monophyletic lineage and that infrageneric boundaries within *Aspalathus* require reevaluation (Supplementary materials Fig. S1, S2).

The nuclear dataset alone produced a comb-like polytomy within Clade I, although backbone nodes were strongly supported. Similarly, the plastid dataset separated the genus into two main clades but showed limited resolution along deeper nodes, reflecting a pattern previously noted in Cape Crotalarieae, where plastid markers have limited power to resolve rapid radiations. Both ML and BI analyses placed Clade II as strongly supported, whereas Clade I received moderate support in BI (PP = 0.78) and strong support in ML (UFBoot = 95 %).

The relationship of *Aspalathus* to other Cape genera remains unresolved due to weak backbone support and persistent polytomies. In particular, the placement of *Wiborgia* and *Wiborgiella* differs slightly between analyses. In ML, these genera were unsupported as a sister to the remaining Cape genera, whereas BI positioned them as sister to the two *Aspalathus* clades with moderate support (PP = 0.89). These results indicate close but unresolved relationships, and collectively the two *Aspalathus* clades form a poorly resolved polytomy with other Cape genera, reflecting uncertainty in identifying the genus’s closest relatives.

3.2. Ancestral character analysis

Ancestral character reconstruction (Supplementary materials Table S2) revealed substantial morphological variation within

Table 3
Properties of the datasets used for phylogenetic analysis. PIC = parsimony-informative characters.

Data description				Locus			
	ITS	ETS	*mat*K	*trn*S-*trn*G	Combined nuclear	Combined plastid	Combined markers
No. of accessions	125	163	156	81	245	192	211
Best-fit model (BIC)	TN+*F* + R2	TIM2+*F* + *I* + R2	TVM+*F* + R2	K3Pu+*F* + *I* + R2	GTR+*F* + *I* + G4	GTR+*F* + *I* + G4	
Matrix length	558	414	684	703	972	1387	2359
Number (%) PIC	72 (13 %)	135 (33 %)	74 (11 %)	118 (17 %)	207 (21 %)	192 (14 %)	399 (17 %)
Singletons sites	65	75	101	129	140	230	370
Constant sites	421	204	509	456	625	965	1590

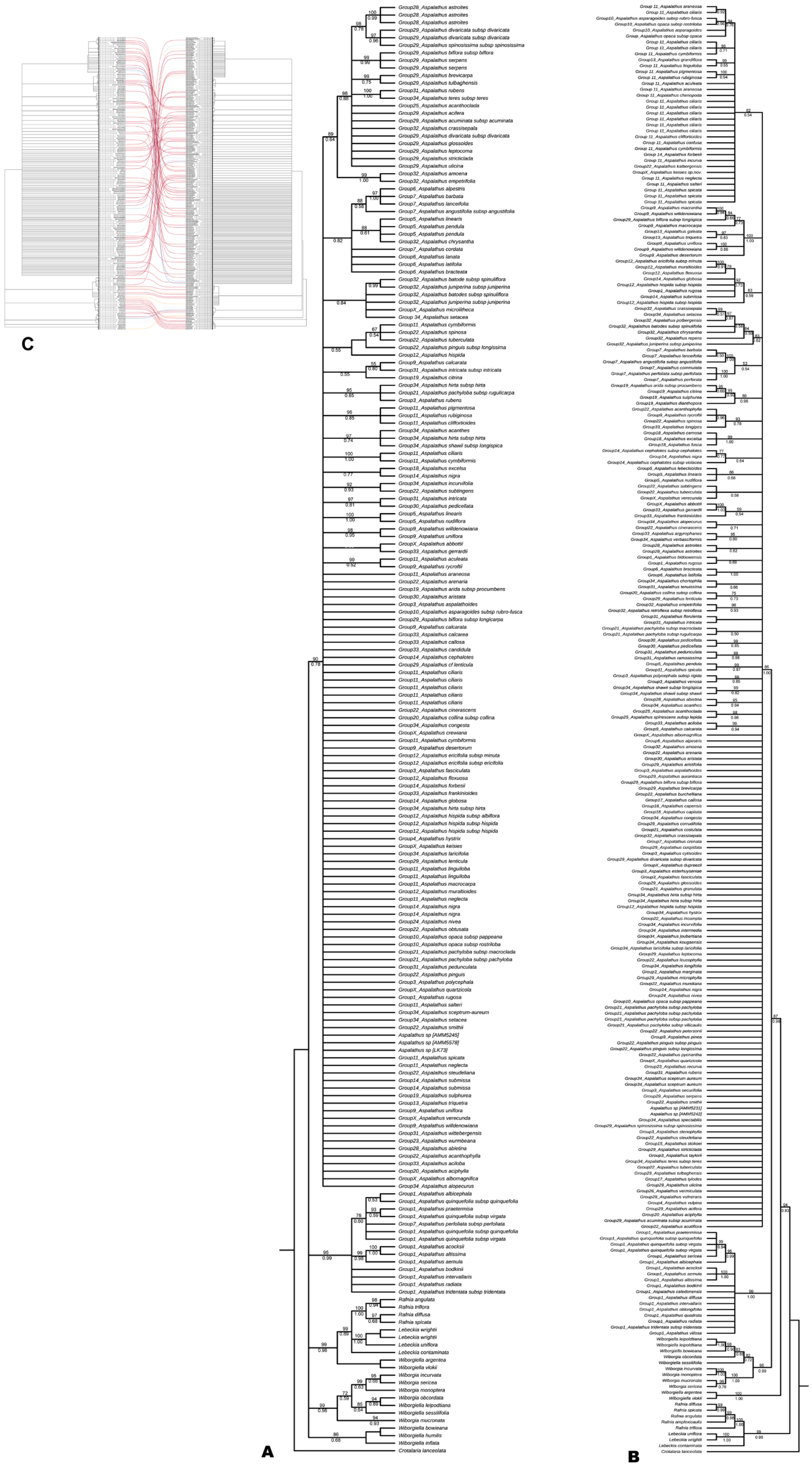

Fig. 3. Phylogenetic relationships in Cape Crotalarieae, showing (A) plastid DNA, (B) nrDNA and (C) tanglegram comparing gene trees. Bootstrap support values (BS) from Maximum Likelihood (≥ 50%) are shown above the branches, while posterior probability values (pp) from Bayesian inference (≥.50) are displayed below the branches.

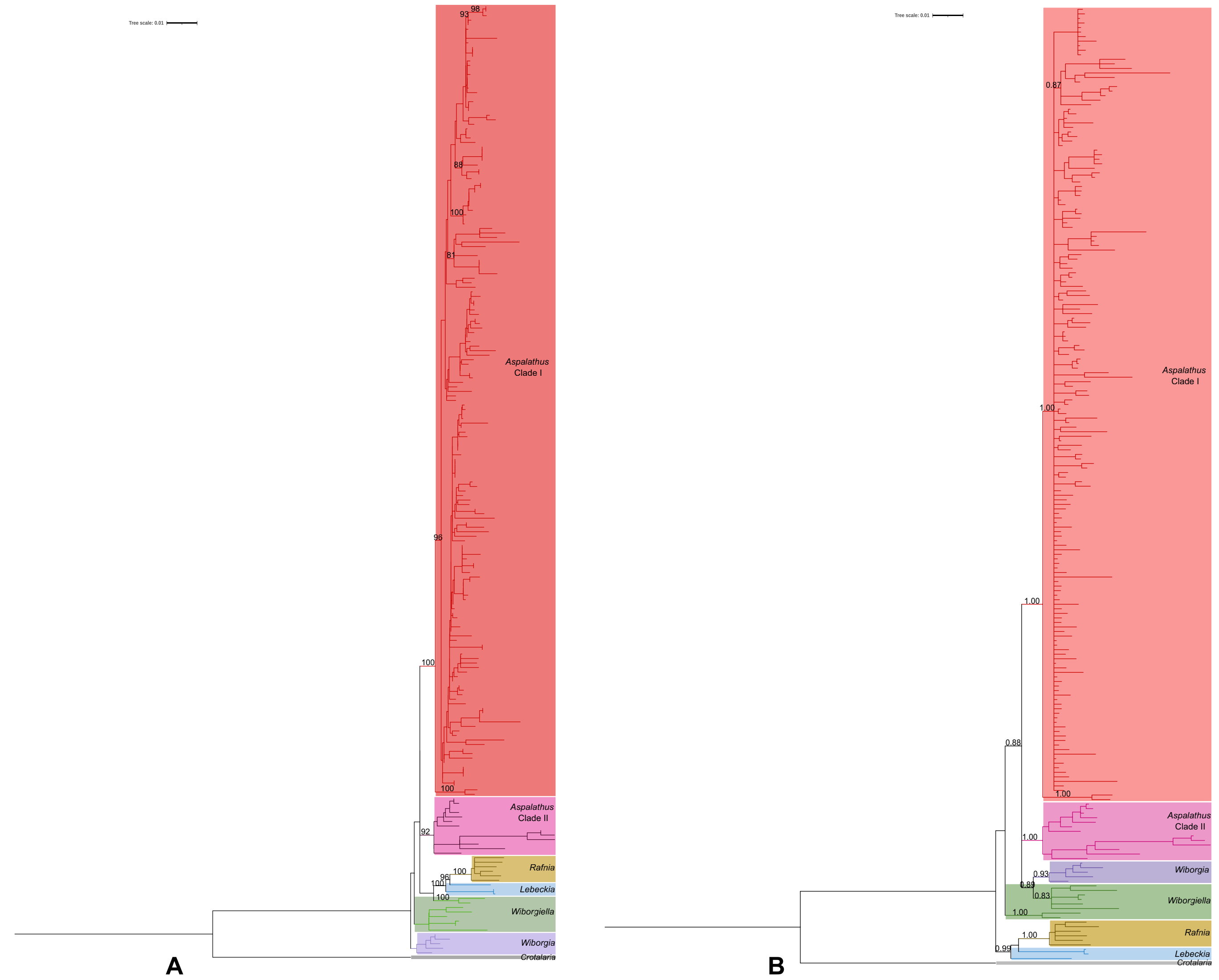

Fig. 4. Phylogenetic trees of the tribe Crotalarieae generated using (A) Bayesian Inference and (B) Maximum Likelihood methods. Bootstrap support (A, ≥80 %) and posterior probabilities (B, ≥0.80) are indicated above the branches.

Aspalathus, providing phylogenetically informative patterns that correspond to the two major clades identified in our molecular analyses. Leaf lamina shape distinguishes the clades, with Clade I predominantly exhibiting terete to subterete leaflets, which are nearly cylindrical and occasionally appear succulent, whereas Clade II species generally have flat leaflets (Fig. 5A). Leaflet tips also differ between the clades. Clade I contains lineages with sharp leaflet tips, including the Laterales (Group 34), Calcicolae (Group 33), Teretilobae (Group 32), Niveae (Group 24), and Pingues (Group 22) groups, whereas Clade II species mostly have rounded leaflet tips (Fig. 5B). Leaf arrangement varies as well, with Clade I lineages often producing multiple leaflets per node, whereas Clade II species typically bear trifoliolate leaflets at each node (Fig. 5C). These patterns of leaf morphology provide useful diagnostic traits for distinguishing the two major clades, although exceptions occur within each group.

Leaflets in *Aspalathus* are generally sessile and attach directly to the stem. In many species, a small basal swelling or spur occurs at the leaflet base (Fig. 5D–E). This structure is present across both clades, although its size and prominence vary among species. In *Wiborgia* and *Wiborgiella*, leaflets are petiolate, and the petiole base becomes swollen and persistent after leaflet abscission, forming a distinct tubercle-like structure. The position and form of these structures differ between *Aspalathus* and its sister genera, with the spur in *Aspalathus* occurring at the leaflet base and the persistent swelling in *Wiborgia* and *Wiborgiella* occurring at the petiole base.

Floral morphology shows additional synapomorphic patterns that differentiate the clades. Flower colour is predominantly yellow across both Clade I and Clade II, but several lineages in Clade I, including the Purpureae (Group 14) and Rostratae (Group 9) groups, display white or pink flowers. Clade II also contains species with non-yellow flowers, such as *Aspalathus albicephala* and *A. argyrella*, although yellow remains the dominant colour (Fig. 5F). Petal proportions differ consistently between clades. In Clade I, the keel petal is larger than the wing petals. In Clade II, the wing petals are equal to or larger than the keel petals, mirroring the floral morphology observed in the sister genera *Wiborgia* and *Wiborgiella* (Fig. 5G). Anther configuration is largely conserved across *Aspalathus, Rafnia*, and *Wiborgia*, with a pattern of five short dorsifixed anthers, four long basifixed anthers, and one intermediate carinal anther (Fig. 5H).

Pod morphology within *Aspalathus* shows notable variation, although certain features are broadly conserved across the genus. Nearly all species examined possess wingless, dehiscent pods, which represent a stable condition within the genus (Fig. 5I–J). Pod form ranges from laterally compressed to inflated, with this variation occurring across

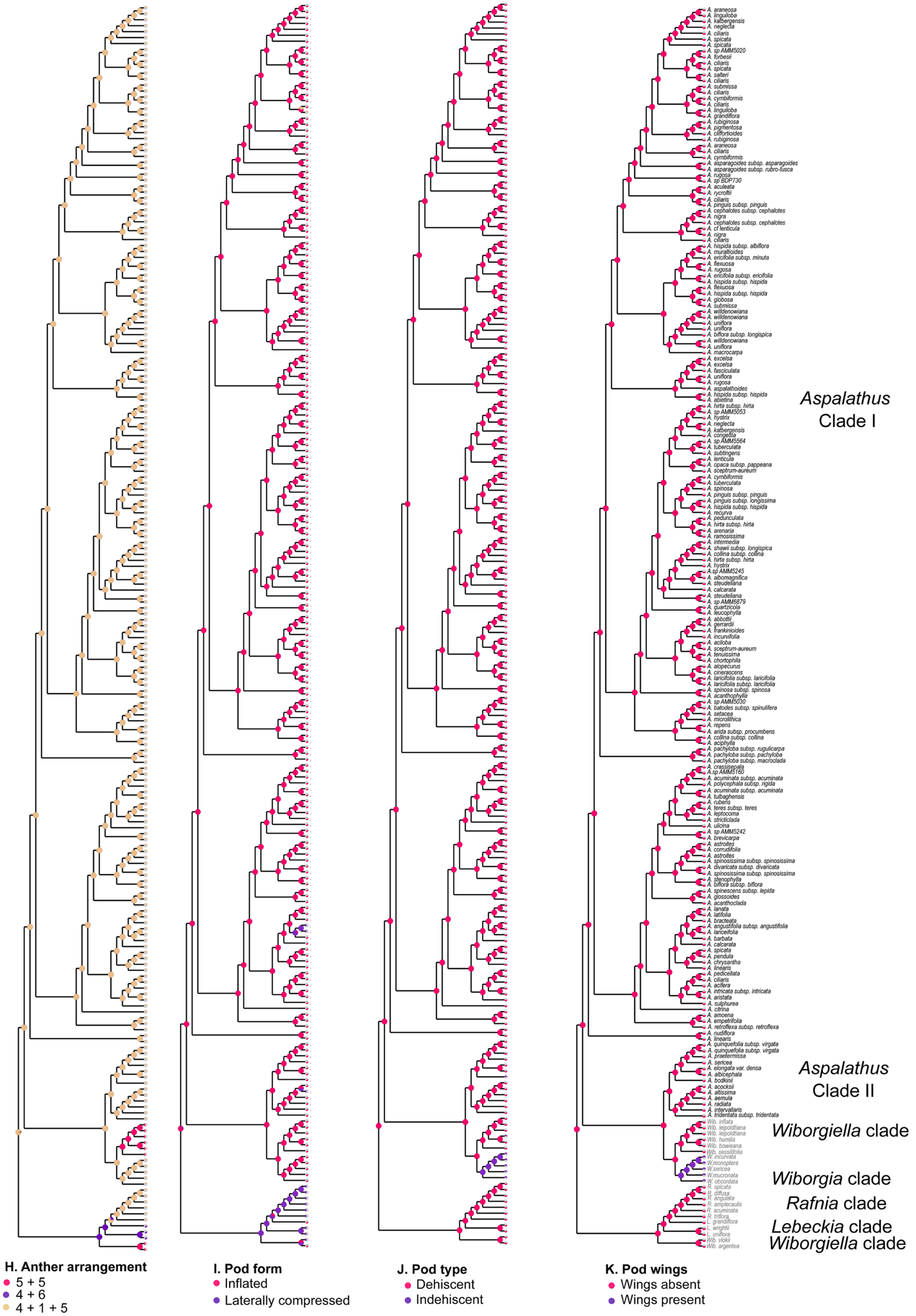

Fig. 5. Morphological character states for (A) leaf lamina, (B) leaf tips, (C) leaf range, (D) petiole, (E) petiolar spur, (F) flower colour, (G) wing petal relative to keel, (H) anther arrangement, (I) pod form, (J) pod type, (K) pod wings.

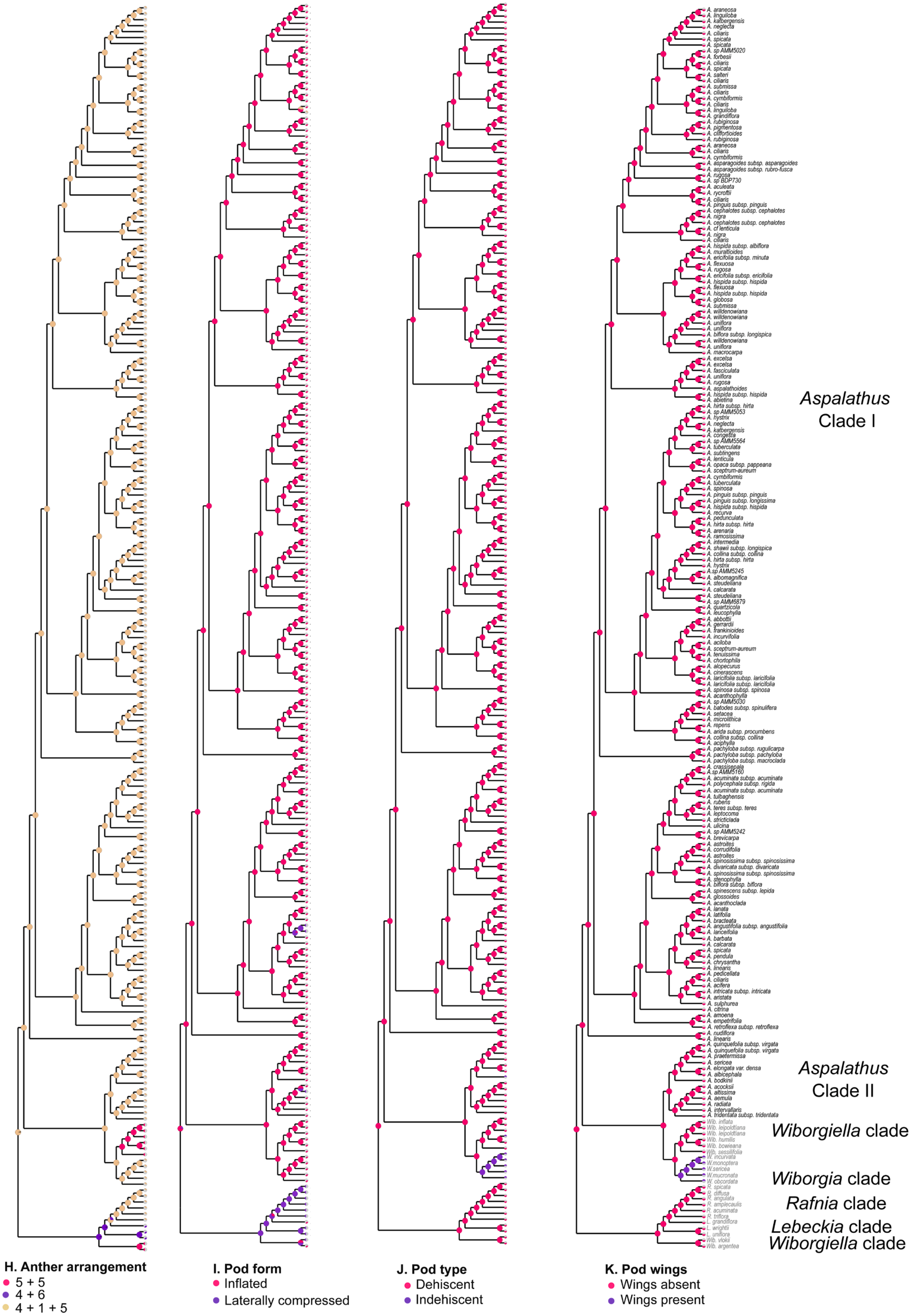

Fig. 5. (*continued*).

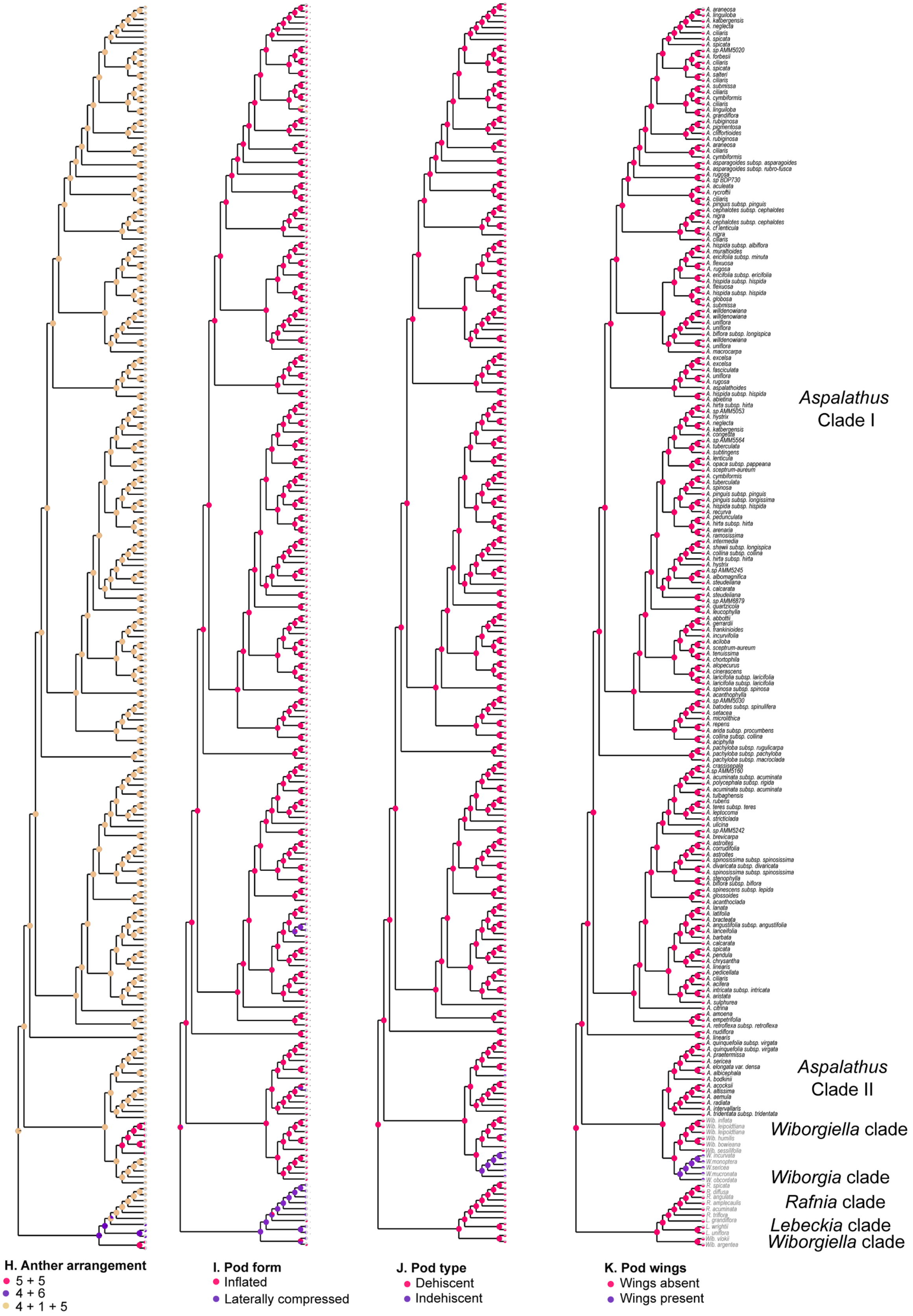

Fig. 5. (*continued*).

both major clades. In a few species, such as *A. bodkinii*, pods remain closed for extended periods and behave functionally as small nut-like structures (Fig. 5K), highlighting a rare deviation from the otherwise consistent dehiscent condition. Although inflated pods also occur in the sister genus *Wiborgiella*, this feature arises sporadically within *Aspalathus* (Fig. 5L) and therefore does not correspond to deeper phylogenetic divisions within the group.

4. Discussion

4.1. Phylogenetic relationships in Aspalathus

The genus *Aspalathus* has long presented taxonomic challenges due to its remarkable morphological diversity and the difficulty of delineating natural groups within it (Thunberg, 1802; Presl, 1845; Bentham, 1848; Harvey, 1868; Dahlgren, 1960, 1963). Dahlgren's (1988) monograph represented the most comprehensive attempt to resolve this complexity, dividing the genus into morphological groups based on vegetative and floral characters. However, our molecular analyses demonstrate that these groups may not correspond to monophyletic lineages, confirming that morphology alone provides an unreliable basis for intrageneric classification.

Across all analyses, two well-supported molecular clades were consistently recovered within *Aspalathus*. Clade I comprises a heterogeneous assemblage of species with variable morphology, whereas Clade II largely corresponds to Dahlgren's Group 1 (Sericeae). Despite this partial correspondence, the placement of *A. rugosa*—morphologically aligned with Sericeae—within Clade I highlights potential homoplasy. This discordance between morphological and molecular evidence indicates that many traits traditionally used to classify *Aspalathus*, including leaf texture, growth habit, and floral form, are homoplastic, having arisen independently in separate lineages. Furthermore, the polytomous structure observed within Clade I suggests a recent or rapid diversification, possibly driven by ecological specialisation within the Cape Floristic Region (Linder, 2003).

In a broader phylogenetic context, *Aspalathus* is consistently recovered as potentially non-monophyletic, forming two major clades that associate variably with the Cape genera *Wiborgia* and *Wiborgiella*. However, these relationships remain unresolved due to weak backbone support and persistent polytomies, making it difficult to determine the closest relatives of *Aspalathus* within the Cape clade. This pattern mirrors previous studies of the tribe Crotalarieae (Boatwright et al., 2008), which similarly reported uncertain relationships among the Cape genera.

The combination of non-monophyly and extensive morphological convergence indicates that current generic and infrageneric delimitations in *Aspalathus* do not accurately reflect evolutionary history. The polytomy in Clade I further supports the occurrence of a recent and rapid diversification, consistent with other lineages in the Cape flora where bursts of speciation often followed environmental shifts (Bouchenak-Khelladi and Linder, 2017).

4.2. Character evolution

Morphological variation within *Aspalathus* is extensive, complicating the interpretation of evolutionary relationships within the genus and its position among Cape Crotalarieae (Dahlgren, 1988; Boatwright et al., 2008; Edward et al., 2008; Le Roux and van Wyk, 2012). To provide a robust framework for understanding character evolution, this study focused on traits that were consistently observable across taxa and phylogenetically informative. These included leaf lamina shape, petiole presence or reduction, floral morphology, and pod structure. Characters previously highlighted in taxonomic studies, such as calyx lobe arrangement, branch apex colour, and growth form, were excluded due to limited variation, inconsistent scoring across herbarium specimens, or high homoplasy, which could obscure phylogenetic signal (Dahlgren, 1963, 1988; Moiloa et al., 2018; Campbell and van Wyk, 2001).

Leaf lamina shape provides one of the clearest signals for differentiating the major clades within *Aspalathus* (Fig. 5A). Clade I is largely characterised by terete to subterete leaf lamina, whereas Clade II predominantly exhibits flat leaflets. This pattern aligns partially with Dahlgren's (1988) recognition of Group 1 species as distinct, yet our results indicate multiple independent transitions and reversals, reflecting the homoplastic nature of this character (Goldblatt and Manning, 2002; Schnitzler et al., 2011). Leaflet tip morphology further distinguishes these clades (Fig. 5B), with Clade I lineages such as Laterals (Group 34), Calcicolae (Group 33), Teretilobae (Group 32), Niveae (Group 24), and Pingues (Group 22) showing predominantly sharp apices, whereas Clade II species mostly have rounded tips. Exceptions within Clade I indicate repeated independent evolution of leaflet tip shapes across the genus, likely in response to ecological pressures such as water stress or herbivory.

Leaflet arrangement is also informative (Fig. 5C). Clade I species typically display nodes bearing multiple fascicles of leaflets (more than three per node), whereas Clade II taxa are largely trifoliolate throughout. Some lineages, including Lebeckiiformes (Group 5), Pedunculares (Group 6), and Borborniae (Group 7), retain simple leaves across nodes, demonstrating repeated simplification or loss in leaf complexity (Linder, 2003). These shifts highlight the dynamic nature of leaflet arrangement in *Aspalathus* and suggest adaptation to specific ecological or functional demands, including maximising photosynthetic efficiency in heterogeneous CFR habitats (Goldblatt and Manning, 2002).

Petiole structure and the development of spur- or tubercle-like swellings offer insight into leaf evolution in the Cape clade (Fig. 5D–E). In most species of *Aspalathus*, leaves are sessile and borne directly on the stem, with a persistent spur derived from the leaf base (Dahlgren, 1963). In contrast, species of *Wiborgiella* bear petiolate leaves with a swollen, persistent petiole base that remains after leaflet abscission (Boatwright et al., 2010). Because the *Aspalathus* spur arises from the leaf base while the swelling in *Wiborgiella* is petiolar, these features are unlikely to be homologous. Instead, they may represent independent structural adaptations to similar ecological pressures in the CFR, potentially enhancing leaf support and persistence under high light, wind exposure, and seasonal drought (Linder, 2003; Schnitzler et al., 2011). Further comparative anatomical work is required to test homology formally.

Flower colour in *Aspalathus* is predominantly yellow, fading to orange or reddish-brown with age (Fig. 5F). While yellow flowers are dominant across most lineages, variation exists within both major clades. In Clade I, some lineages such as Rostratae (Group 9) and Purpureae (Group 14) include species with white or pink flowers. Similarly, Clade II contains species with white (*A. albicephala*) or magenta flowers (*A. argyrella*). This variation suggests that flower colour is labile within the genus and may reflect local adaptation to different pollinator assemblages or microhabitats, rather than representing a fixed phylogenetic signal.

The relative size of the wing and keel petals further distinguishes the two major clades (Fig. 5G). In Clade I, species predominantly exhibit a keel petal larger than the wing petals, which may enhance mechanical precision during explosive pollination (Le Roux and van Wyk, 2012). During this pollination mechanism, reproductive organs are under pressure within the keel, which is released upon pollinator interaction, facilitating effective pollen dispersal (Figueroa Fleming and Etcheverry, 2017). In contrast, Clade II species have wing petals equal to or larger than the keel petals, a trait shared with the sister genera *Wiborgia* and *Wiborgiella*. This morphological distinction not only differentiates the two clades but also suggests shifts in pollination strategies or selective pressures that have driven floral diversification within the genus.

Anther arrangement in *Aspalathus* is highly conserved, with a consistent 4 + 1 + 5 configuration: five short dorsifixed anthers, four long basifixed anthers, and one intermediate carinal anther (Fig. 5H).

This arrangement, shared with *Wiborgia* and *Rafnia*, is considered a generic trait with phylogenetic significance within the Cape genera (van Wyk, 1991; Le Roux and van Wyk, 2012). The dimorphic configuration likely optimises pollen transfer efficiency, particularly in species with explosive pollination, and may have contributed to the diversification of these genera by facilitating specialised interactions with pollinators (Sapir et al., 2017).

Pod morphology in *Aspalathus* is species-specific and generally uninformative at the level of major clades. However, certain features are shared with related genera and have phylogenetic relevance. Members of *Aspalathus* typically produce laterally compressed, dehiscent, and wingless pods (Fig. 5I–J), a character also observed in *Wiborgiella*. Dehiscence is largely consistent across the genus, with the exception of *A. bodkinii*, which produces indehiscent pods similar to those in *Wiborgia*. These shared pod characters provide evidence of a closer evolutionary relationship between *Aspalathus* and *Wiborgiella* and highlight how reproductive traits, even when variable, can retain phylogenetic signal at the genus level.

4.3. Conclusion

This study substantially improves phylogenetic resolution within *Aspalathus* by expanding taxon sampling and incorporating both plastid and nuclear markers. Although backbone relationships remain weakly resolved, the results consistently recover two strongly supported clades within *Aspalathus*, with both clades forming a polytomy alongside the Cape genera *Wiborgia, Wiborgiella, Rafnia*, and *Lebeckia*. The unresolved backbone suggests rapid radiation or incomplete lineage sorting among these lineages, highlighting the need for higher-resolution genomic approaches. Morphological trait evolution analyses corroborate molecular findings and reinforce Dahlgren's early infrageneric observations. The consistent association of terete leaf laminae, sharp leaflet apices, and Keel > Wing petal proportions in Clade I, contrasted with flat leaf laminae, rounded leaflet tips, and Wing ≥ Keel petals in Clade II (largely corresponding to the Sericeae), indicates deep evolutionary divergence within the genus. Ecological patterns, including the predominance of sandstone substrates in the Sericeae and greater edaphic diversity in Clade I lineages, further support this separation. Taken together, the genetic, morphological, and ecological evidence strongly suggests that Clade II represents a cohesive evolutionary lineage that may warrant taxonomic recognition separate from Clade I following further assessment. Despite these advances, definitive conclusions on the placement of *Aspalathus* relative to *Wiborgia* and *Wiborgiella* remain limited by low support at deep nodes. Adoption of high-resolution genomic methods such as Angiosperms353 target-capture sequencing, coupled with expanded sampling of sister genera and unsampled *Aspalathus* lineages (e.g., Group 3 Cephalanthes), will be essential for resolving this outstanding phylogenetic uncertainty and revising the taxonomy of this ecologically significant Cape lineage.

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CRediT authorship contribution statement

L.K. Madika: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **R.J. Sebola:** Writing – review & editing, Supervision. **D.A. Zhigila:** Writing – review & editing, Supervision, Formal analysis. **C.H. Stirton:** Writing – review & editing, Supervision, Methodology, Conceptualization. **A.M. Muasya:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

We have no conflicts of interest to disclose

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2025.11.037.

Data availability

Aligned DNA matrices used in this work are available at the following link, doi:10.25375/uct.30710603.

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