*Annals of Botany* **00**: 1–17, 2025
https://doi.org/10.1093/aob/mcaf109, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Nonprofit since 1887

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

ORIGINAL ARTICLE

# Nuclear phylogenomics reveals strong geographic patterns in the evolutionary history of *Aloe* and related genera (alooids)

**Yannick Woudstra[1,2,3,*], Paul Rees[2], Solofo E. Rakotoarisoa[4], Ronell R. Klopper[5,6], Gideon F. Smith[7], Nina Rønsted[3] and Olwen M. Grace[2,8,*]**

*[1]Department for Ecology, Environment and Plant Sciences, Stockholm University, Stockholm 106 91, Sweden, [2]Accelerated Taxonomy, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AE, UK, [3]Natural History Museum of Denmark, University of Copenhagen, Copenhagen K 1350, Denmark, [4]Conservation Allies, VO 12 Bis A, Manakambahiny, Antananarivo 101, Madagascar, [5]Research and Scientific Services Division, South Africa National Biodiversity Institute, Brummeria, Pretoria 0184, Republic of South Africa, [6]HGWJ Schweickerdt Herbarium, Department of Plant and Soil Sciences, University of Pretoria, Hatfield, Pretoria 0002, Republic of South Africa, [7]Ria Oliver Herbarium, Department of Botany, Nelson Mandela University, University Way, Summerstrand, Gqeberha 6019, South Africa, and [8]The Herbarium, Royal Botanic Garden Edinburgh, Edinburgh EH3 5LR, UK*

*[*]For correspondence. E-mail yannickwoudstra@outlook.com or ograce@rbge.org.uk*

Received: 27 February 2025 Returned for revision: 19 May 2025 Editorial decision: 29 May 2025 Accepted: 29 May 2025

- **Background and Aims** With >700 species, *Aloe* and its generic kin (alooids) are a morphologically diverse group of succulent plants with a wide range across Africa, Madagascar and the Arabian Peninsula. Species such as *Aloe vera* and *A. ferox* are cultivated at scale for natural products, whole foods, and cosmetics. Despite substantial α-taxonomy contributions, infrageneric classification of *Aloe* has remained unresolved. Molecular systematics has been compromised by the lack of informative characters in standard markers and high costs of obtaining informative nuclear loci from large genomes (e.g. >15 Gbp), and the difficulty of obtaining quality DNA extractions from material of known provenance. Here these constraints are overcome with target capture sequencing, which allows cost-effective sequencing of informative low-copy nuclear loci and unlocks genetic resources from preserved specimens in herbaria as well as silica-dried tissues.
- **Methods** Using a custom kit for alooids, 189 nuclear loci were sequenced in 294 species, including 50 herbarium specimens, to build a new phylogenomic framework for the big genus *Aloe* and 11 closely related alooid genera. Genus-level representations of non-alooid members of Asphodelaceae were sequenced with the same tool. The monophyly of Asphodelaceae subfamilies, alooid genera and infrageneric taxonomic groups of *Aloe sensu stricto* was tested with the obtained topologies.
- **Key Results** The new phylogenomic framework demonstrates the monophyly of the alooids and confirms recent classifications in which smaller genera (*Aloidendron*, *Aloiampelos*, *Aristaloe*, *Gonialoe*, *Kumara*) are separated. Strong geographic patterns in the *Aloe* phylogeny are contrasted by less obvious phylogenomic structure in habit (growth form), and vegetative or reproductive morphology, which are mainstays of α-taxonomy.
- **Conclusions** Repeated incidents of adaptive radiation and niche specialization appear to underlie species diversity in *Aloe*. This study illustrates the power of combined (nuclear) phylogenomic and α-taxonomic inference, including the utility of herbarium genomics, in resolving the systematics of big genera.

**Key words:** Adaptive radiation, African plants, *Aloe*, big genera, herbarium genomics, Hyb-Seq, molecular systematics, monocots, museomics, plant taxonomy, succulent plants, target capture sequencing.

## INTRODUCTION

Molecular phylogenies have become the framework for integrated systematics (Grace *et al.*, 2021). Reconstructed evolutionary history can be contrasted with available taxonomic hypotheses to provide additional evidence for retaining or revising classifications. This has led to the presentation of amended systematics for morphologically diverse and taxonomically complex plant families [e.g. Fabaceae (Legume Phylogeny Working Group, 2017), Asteraceae (Mandel *et al.*, 2019), Poaceae (Grass Phylogeny Working Group III, 2024), and, at a higher taxonomic rank, flowering plants in general (The Angiosperm Phylogeny Group, 2016)]. In addition, a

© The Author(s) 2025. Published by Oxford University Press on behalf of the Annals of Botany Company.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

molecular phylogeny provides clues to the evolutionary history that gave rise to the observed morphological variation (e.g. Zuntini *et al*., 2024). In many plant groups, however, the implementation of molecular systematics has unfortunately been hindered by (1) a lack of variation in conventional DNA markers, leading to high levels of uncertainty in molecular phylogeny inference (Hollingsworth *et al*., 2011) and (2) difficulties in obtaining high-quality DNA samples from taxonomically verified reference material. This is particularly the case for highly diverse, 'big' genera of >500 species (Frodin, 2004), which are often characterized by high levels of rapid diversification and comprise many difficult-to-collect species, which may require considerable resources and skills to be collected for study.

The transition from phylogenetics (one to several genes) to nuclear phylogenomics (hundreds of genes), particularly through the use of (solution hybridization) target capture sequencing (Gnirke *et al*., 2009), has helped to overcome these limitations for many plant lineages (Dodsworth *et al*., 2019). With this modern technique, hundreds of informative nuclear loci can be captured with target-specific baits and sequenced with high coverage at a relatively low cost (Woudstra *et al*., 2022). Target capture sequencing also works well on degraded DNA from historical specimens as the small bait size (80–120 nucleotides) allows the efficient capture of even very short fragments (Hart *et al*., 2016; Quatela *et al*., 2023), unlocking a 'treasure vault' of genetic evidence represented by centuries of botanical specimens with near-global coverage. The inclusion of herbarium specimens, particularly type specimens, is considered the gold standard in molecular systematics (Renner *et al*., 2024) because the application of taxonomic names is based on these specimens, thereby providing the best material with which to test taxonomic hypotheses using molecular data. Target capture sequencing tools are available as universal kits (e.g. Angiosperms353; Johnson *et al*., 2019) or clade-specific ranging from family (e.g. Asteraceae1061; Mandel *et al*., 2014) to genus level (e.g. *Inga*; Nicholls *et al*., 2015). Although they are available at a lower cost, universal kits target more conserved loci and may therefore be less suitable for molecular systematics in big genera. In fact, most big-genus phylogenomic studies use a customized approach to target more variable loci [e.g. *Dioscorea* with >600 species (Soto Gomez *et al*., 2019), *Silene* with >1000 species (Quatela *et al*., 2023) and *Begonia* with >2000 species (Michel *et al*., 2022)].

In terms of morphology and ecology, *Aloe sensu stricto* is highly diverse and, in terms of species numbers, qualifies as a big genus, comprising 591 species (Newton, 2020) with a relatively recent evolutionary history (±15–25 million years) (Grace *et al*., 2015; Thompson *et al*., 2023). *Aloe* and its generic kin (alooids) are a predominantly African group of succulent plants, with a natural geographical distribution range covering much of the continent, as well as Madagascar, the Mascarene Islands, Socotra and the Arabian Peninsula. Several *Aloe* species are economically important, for example for the medicinal value derived from various fractions of their leaf extracts, supporting a global health products industry, most notably by *A. vera* and *A. ferox*. The inner leaf mesophyll tissue (clear gel) of these species is used in foods, commodities and cosmetic products, while the leaf exudate of several species, rich in secondary metabolites with antioxidative properties, has been used in traditional medicine to treat, amongst others, indigestion, malaria and cancer (Grace, 2011; Amir *et al*., 2019). Despite its relatively recent diversification, the genus presents a remarkable level of morphological and ecological variation (Fig. 1), reflected by the publication of names for at least 56 infrageneric groups, some recognized at the ranks of section and series and others informally named morphogroups.

Aloes are members of the monocotyledonous asphodel family (Asphodelaceae), which consists of three subfamilies: the predominantly Australasian Hemerocallidoideae (day lilies), the fire-adapted monogeneric Xanthorrhoeoideae (grass trees), and the diverse Asphodeloideae with many leaf succulents, which are mostly alooids. Although the formal nomenclatural and taxonomic history of *Aloe* began in the mid-18th century (Linnaeus, 1753), the first monograph of the genus was published more than 150 years later (Berger, 1908). Berger (1908) recognized 181 species that he arranged in more than 30 infrageneric groups; many of these are still upheld today. Berger (1908) based his work on herbarium collections as well as living material cultivated in the Hanbury Garden at La Mortola, Italy. As far as is known, Berger never studied aloes in their natural habitats. Within 20 years following the publication of Berger's (1908) classification, locally active botanists, such as H. B. Christian (south tropical Africa), H. Perrier de la Bâthie (Madagascar) and G. W. Reynolds (southern Africa), together described more than 140 species that are still accepted today (Klopper and Smith, 2013). Reynolds produced the most extensive monographs on *Aloe* to date, focusing on southern Africa first (Reynolds, 1950) and later complementing this work with treatments of the *Aloe* taxa of Madagascar and tropical Africa (Reynolds, 1966). In the South African monograph, Reynolds (1950) followed Berger's classification (1908) but refined and expanded other formally published infrageneric groups. Many of the new species described from tropical Africa and Madagascar, however, did not comfortably fit within the framework of Berger (1908), leading Reynolds (1966) to group them into newly circumscribed morphogroups. In subsequent decades the number of new species discovered and described increased steadily (Klopper and Smith, 2013), with the novel taxa mainly originating from tropical Africa and Madagascar. By the early 21st century, molecular phylogenies started influencing the classification of the alooids, especially at the rank of genus.

Historically, many genera were segregated from *Aloe*, but these were often eventually reincorporated. Notable alooid segregates reunited with *Aloe* are *Leptaloe* (grass aloes) and *Lomatophyllum* (berried aloes), with *Leptaloe* at present regarded as fitting in *A*. sect. *Leptoaloe*, along with the robust grass aloes, also referred to as slender aloes in the vernacular. The berried aloes are at present treated at the rank of section in the genus *Aloe* (Glen and Hardy, 2000; Grace *et al*., 2013; Newton, 2020). Representatives of *Chortolirion* (bulbous, grass-like aloes), which have a decidedly haworthioid appearance, are now similarly included in an expanded concept of *Aloe* (Manning *et al*., 2014). In contrast, several genera have been reinstated or newly published based on molecular evidence (Grace *et al*., 2013; Manning *et al*., 2014; Grace *et al*., 2015): *Aloestrela* (ancient aloe) (Smith and Molteno, 2019) and *Aristaloe* (awn-leaf aloe) (both monotypic); *Kumara* (fan aloes; two species); *Aloiampelos* (rambling aloes; seven species); *Aloidendron* (tree aloes; six species); and *Gonialoe* (kanniedood aloes; four species). In addition, the genera *Astroloba*

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

(11 species), *Haworthia* (57 species), *Haworthiopsis* (18 species), *Gasteria* (28 species) and *Tulista* (4 species) are all treated as accepted and distinct from *Aloe sensu stricto* (Newton, 2020). As a result, *Aloe sensu stricto* now includes 591 species (excluding natural and artificial hybrids; CITES Secretariat, 2024), many of which have yet to be placed in any of the infrageneric groups available for the genus.

The taxonomy of *Aloe* and some of its generic kin is therefore in a state of flux. A lack of character variation (and thus resolution) in traditional molecular phylogenetics (several organellar genes; Manning *et al*., 2014; Grace *et al*., 2015; Dee *et al*., 2018), however, resulted in authors of recent taxonomic treatments (Carter *et al*., 2011; Newton, 2020) being reluctant to incorporate at least some of the proposed changes at various generic and infrageneric ranks. Expanding the molecular dataset with plastid phylogenomics (e.g. using whole chloroplast genomes from genome skimming data) can improve this situation, particularly when herbarium material, including type specimens, is incorporated, as evidenced by Malakasi *et al.* (2019) in a study of the tree aloes (*Aloidendron*). For the big genus *Aloe*, however, more variable data from the nuclear genome is needed to track species boundaries (Woudstra *et al*., 2021).

This study aimed to test a highly reduced representation approach to resolve long-standing uncertainties in the *Aloe* molecular phylogeny and the classification of alooid genera. A customized target capture sequencing tool (Woudstra *et al*., 2021) was applied to generate an adequate nuclear genomic reference database (Woudstra *et al*., 2024) from which a phylogenomic framework for the alooids (Asphodelaceae subfam. Asphodeloideae) was built. These new phylogenomic data consist of a comprehensively expanded species-level sampling of alooids, and a genus-level sampling of the family Asphodelaceae to test the monophyly of the alooids and their placement among the subfamilies of Asphodelaceae. The resulting phylogeny was then interpreted taxonomically to inform the stability, and remaining uncertainties, of the classification of *Aloe*-related alooid genera (species level), as well as Asphodelaceae subfam. Asphodeloideae and subfam. Hemerocallidoideae (both genus level). The alooid species-level phylogeny was further annotated with taxonomically and ecologically important characters (geographic origin, life form and habitat) to provide further insight into the evolutionary history of the alooids.

## MATERIALS AND METHODS

Online supporting material referred to in this manuscript is deposited in FigShare at DOI https://doi.org/10.6084/m9.figshare.28435394.

### *Sampling*

The basis of this study was formed by sampling preserved (herbarium) and living botanical collections at the Royal Botanic Gardens, Kew (K); the Natural History Museum of Denmark, Copenhagen (C); the East African Herbarium (EA), the Muséum national d'Histoire naturelle, Paris (P); and the botanic gardens of the Universities of Potsdam, Uppsala, Göteborg and Cambridge. The sampling was designed to build (1) a genus-level backbone phylogeny for the family Asphodelaceae and (2) a comprehensive phylogeny for *Aloe sensu stricto* and related genera (the alooids). Here we apply the term 'alooid' to all currently and formerly accepted species and infraspecific taxa of the genus *Aloe* and related genera. These comprise *Aloe sensu stricto* (591 species and 71 additional infraspecific taxa), the smaller genera *Aloiampelos* (7 species and 3 additional varieties), *Aloidendron* (6 species), *Gonialoe* (4 species) and *Kumara* (2 species), the monotypic genera *Aloestrela* and *Aristaloe*, and the related genera *Astroloba*, *Gasteria*, *Haworthia*, *Haworthiopsis* and *Tulista*. All taxon names referred to in this study follow the checklist provided by the World Flora Online Plant List (WFO Plant List, 2025), unless stated otherwise.

For the Asphodelaceae backbone phylogeny, DNA extracts and ready-to-sequence DNA libraries for one member of each genus in the Asphodelaceae were obtained, except *Kniphofia* and *Haworthia*. These genera were sampled as part of the ongoing Plant and Fungal Trees of Life (PAFTOL) project at the Royal Botanic Gardens, Kew (Baker *et al*., 2022), and included samples from the related alooid genera *Astroloba*, *Gasteria*, *Haworthia*, *Haworthiopsis* and *Tulista*. For *Haworthia*, we sampled *H. coarctata*, which has since been placed in *Haworthiopsis* (Rowley, 2013) and therefore samples representing *Haworthia sensu stricto* are lacking in this study.

For the other alooids, we used samples from two previous studies (Woudstra *et al*., 2021, 2024) covering *Aloe* (401 samples, 378 species, 4 additional subspecies and 8 additional varieties), *Aloestrela* (1 species), *Aloiampelos* (3 species), *Aloidendron* (4 species), *Aristaloe* (1 species), *Gonialoe* (1 species) and *Kumara* (1 species). The sampling for *Aloe* covered the main taxonomic groups (Berger, 1908; Reynolds, 1950, 1966; Glen and Hardy, 2000), clades in molecular phylogenies (Manning *et al*., 2014; Grace *et al*., 2015; Dee *et al*., 2018) and geographic centres of diversity (Carter *et al*., 2011; Grace *et al,* 2015). Detailed information on sample origins can be found in the Accession Information file (in the online supporting material). The procedure for sampling followed the methods outlined by Woudstra *et al.* (2024).

### *DNA isolation and sequencing*

DNA was isolated using either a Qiagen DNEasy kit (for high molecular weight DNA from silica-dried leaf material) using silica columns for purification (Qiagen, Holden, Germany) or using a CTAB protocol (Doyle and Doyle, 1987) combined with a purification protocol optimized for herbarium material (Quatela *et al*., 2023) using AMPure XP beads (Beckman Coulter, Brea, CA, USA). For some herbarium samples, a cleaning step was performed before CTAB extraction to remove high amounts of polysaccharides and polyphenol (Shepherd and McLay, 2011). High molecular weight DNA samples were fragmented by ultrasonication (Covaris M220, Covaris, Woburn, MA, USA). Libraries were prepared using NEBNext Ultra II library preparation kits for Illumina sequencing using Multiplex Dual Index sets 1 and 2 (New England Biolabs, Ipswich, MA, USA). Pools of 8–27 equimolar indexed library samples were then enriched with the alooid target capture bait panel (Woudstra *et al*., 2021), which uses myBaits® v3 chemistry (Daicel Arbor, Ann Arbor, MI, USA).

High-throughput Illumina® paired-end sequence data were obtained for the samples described above. As datasets from different studies were combined (Woudstra *et al*., 2021, 2024), the sequence platform and read length varied from 350 bp paired end (PE) on Illumina MiSeq to 150 bp PE on Illumina HiSeq.

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

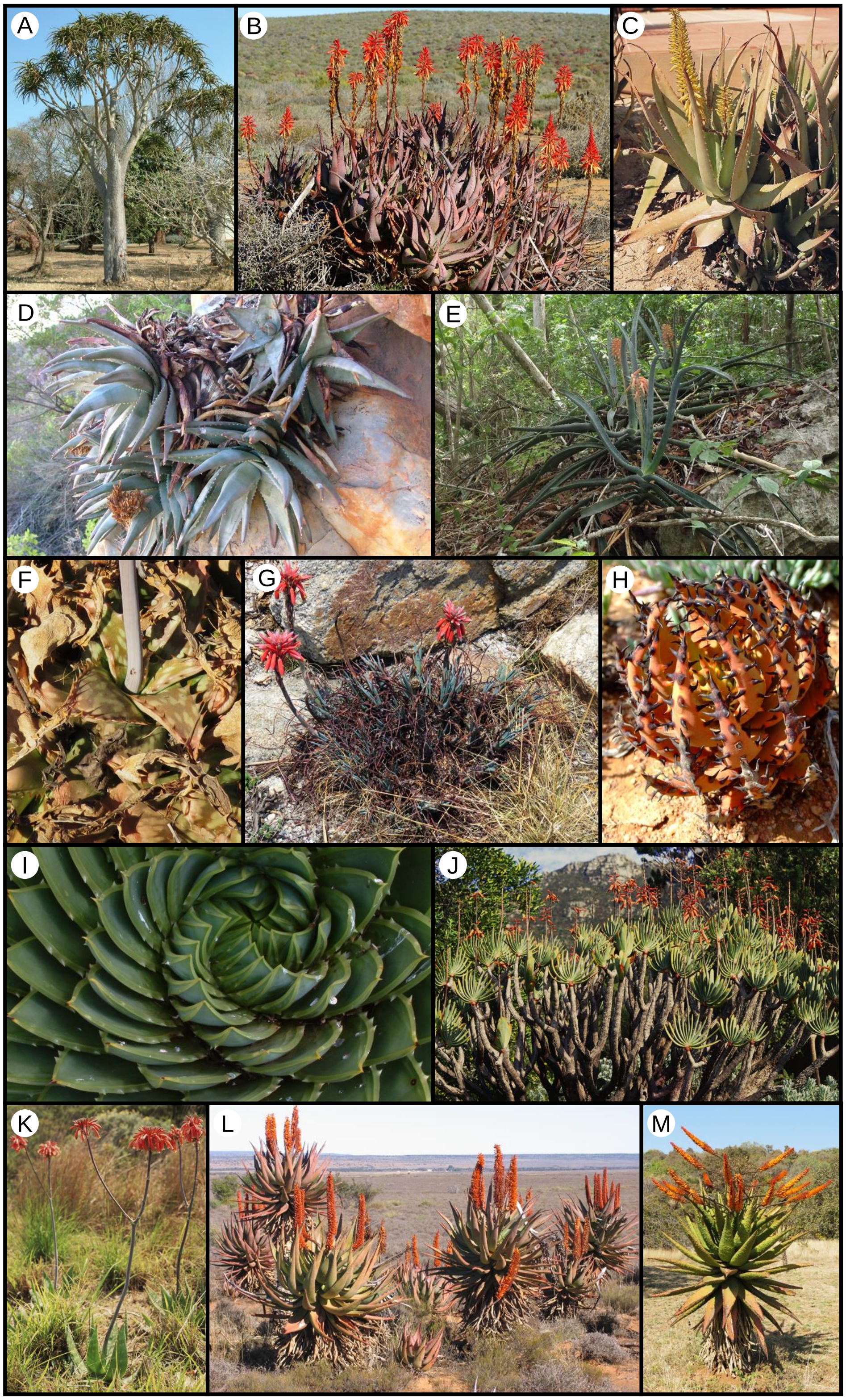

FIG. 1. Morphological and ecological diversity of alooid species. Growth form ranges from tall trees (A; *Aloidendron barberae*) to clump-forming shrubs (B; *Aloe framesii*) and small stemless plants (C; *Aloe vera*). Alooids occupy a wide range of habitats (Supplementary Data Fig. S2) ranging from hyper-arid cliff faces (D; *Aloe meyeri*) to forest understories (E; *Aloe namorokaensis*). Leaf shape is extremely variable, with many species displaying spotted (maculate) patterns (F; *Aloe davyana*), where sometimes the ends of the leaves dry up but stay on. More than 60 species form grass tuft-like leaves (G; *Aloe chortolirioides*) acting as camouflage, whereas others display conspicuous black spines all over the leaves (H; *Aloe melanacantha*). The arrangement of the leaves is usually in a rosette, the most striking example of which is found in the spiral arrangement of *Aloe polyphylla* (I), although distichous (plicate) arrangements are also found (J; *Kumara plicatilis*). Finally, strikingly different inflorescence types are found among the alooids, with ball-shaped terminal (capitate) arrangements (K; *Aloe maculata*) and cylindrical racemose panicles (L; *Aloe ferox*) the most common types. Nearly horizontal (oblique) arrangements of inflorescences are also found (M; *Aloe marlothii*). Photo credits: (A, I, L) N. R. Crouch; (B) A. W. Klopper; (C, F, G, J, K, M) G. F. Smith; (D) E. J. van Jaarsveld; (E) L. Nusbaumer, © Conservatoire & Jardin Botaniques Genève; (H) H. M. Steyn.

Four samples were generated from Illumina RNAseq data assembled into transcripts (Woudstra *et al*., 2021). Details for each included sample can be found in the sequencing information file (online supporting material).

### *Phylogenomics*

Sequence data were analysed to produce an updated phylogenomic framework for (1) genus-level infrafamilial relationships for genera in Asphodelaceae and (2) species-level inter- and infrageneric relationships among species in the alooid clade in the subfamily Asphodeloideae. Uneven taxon sampling, i.e. considerably denser representation within *Aloe* compared with related genera, may influence phylogenomic inference by biasing branch length estimates and affecting resolution at deeper nodes (Zwickl and Hillis, 2002; Heath *et al*., 2008). The genus-level backbone phylogeny for Asphodelaceae and species-level alooid phylogeny were therefore inferred separately with sampling strategies optimized for each phylogeny individually. For the Asphodelaceae backbone phylogeny, a subsampling with one representative of each genus was used, together with four representatives for the big genus *Aloe*: the southern African *A. polyphylla*, the eastern African *A. secundiflora*, the Mascarene *A. tormentorii* and the iconic *A. vera*, which has a strong affinity with Arabian aloes. For the alooid species tree, all suitable alooid samples were used, together with *Bulbine frutescens* as outgroup. Non-alooid members of the Asphodelaceae were excluded from the alooid species phylogeny.

A conservative and curated approach was taken to the phylogenomic analysis to minimize issues with sequence alignment and paralogy affecting the inference of phylogenomic relationships. Firstly, information from (overhanging) off-target reads, which can sometimes be assembled in intronic and intergenic regions, were not used as the recovery and coverage of these regions would be relatively random due to the baits being designed to capture exons (Woudstra *et al*., 2021). Secondly, all loci with high rates of paralogy were removed, as identified by the HybPiper paralogy warning script (Johnson *et al*., 2016) and visual inspection of alignments (Woudstra *et al*., 2021). Thirdly, poorly recovered loci (<200 samples) and samples with <50 % total recovered target length (≤175 kb) were excluded. Finally, the resulting phylogeny was curated by removing all samples with questionable phylogenomic placement due to human error in mislabelling of original DNA isolates or in DNA library preparation (Sequencing Information file in the online supporting material).

Sequence reads were assembled into contigs and processed into clean multiple sequence alignments (MSAs) using the following pipeline: (1) raw reads were trimmed using Trimmomatic v0.39 (Bolger *et al*., 2014) to remove low-quality (terminal parts of) reads (phred-33 score <30 to <99.9 % accuracy); (2) trimmed reads were assembled into 189 low-copy nuclear target loci using HybPiper v1.3.1 (Johnson *et al*., 2016) against reference sequences from the original transcriptomes used for the aloe TCS design (Woudstra *et al*., 2021); (3) assembled target exon sequences were assembled into MSAs using MAFFT v7.429 (Katoh and Standley, 2013) and were subsequently trimmed to remove (4a) insertions (<650 bp) and deletions that are present in less than half of all samples using CIAlign v1.0.14 (Tumescheit *et al*., 2022); (4b) nucleotide sites covered by less than a fifth of all samples using Phyutility v2.2 (Smith and Dunn, 2008); and (4c) previously undiscovered putative paralogous regions by visualization and manual cleaning in Geneious 9.1 Pro. Sequences <100 bp were removed from the MSAs. Woudstra *et al*. (2024) provide more information.

To estimate phylogenomic relationships, we followed a standard approach for large nuclear phylogenomic datasets (e.g. Soto-Gomez *et al*., 2019; Grass Phylogeny Working Group III, 2024; Zuntini *et al*., 2024), namely maximum likelihood gene tree topologies summarized into a species tree with a coalescent-based approach. Compared with computationally intensive approaches involving Bayesian inference (e.g. *BEAST), this is an efficient protocol to infer a robust phylogenomic species tree for a large nuclear dataset typical of a big genus (e.g. 173 genes, 306 675 nt, 304 samples). Incomplete lineage sorting, which is a common source of low resolution and conflict in molecular phylogenies, particularly in cases of rapid speciation such as aloes (Degnan and Rosenberg, 2009), is accounted for by the coalescent model by estimating this through gene tree discordance (Knowles and Kubatko, 2010), defined as the ratio of quartets shared between all gene trees and the species tree. This coalescent approach outperforms the 'concatenated supermatrix' approach (where all gene sequences are concatenated and analysed as one big sequence) as it incorporates the biologically relevant information on incomplete lineage sorting in the topology and node support of the species tree (Mirarab *et al*., 2014). The superiority of a coalescent approach has been tested and verified for alooid phylogenomics (Woudstra *et al*., 2024).

Maximum likelihood gene trees were generated using IQ-TREE v1.6.12 (Nguyen *et al*., 2015) for each of the loci remaining after the filtering, trimming and manual cleaning steps. A general time-reversible model for substitution rates combined with a γ distribution for rate heterogeneity (option +G) and a correction for invariable sites (option +I) was deployed. This substitution model treats gaps in alignments as missing data and therefore ignores them in the likelihood calculation and subsequent computation of branch lengths and node support. Node support was calculated using 1000 ultra-fast bootstrap replications (option −bb). The 173 resulting gene trees were summarized into a species tree using the coalescent-based model implemented in ASTRAL-III (Zhang *et al*., 2018). Branch support in this summary species tree is expressed as local posterior probability (LPP, ranging from 0 to 1), which is the probability of a correct branch placement based on the quartet topology frequencies across the input gene trees (Sayyari and Mirarab, 2016). The resulting summary trees were visualized and annotated in the interactive Tree of Life programme (Letunic and Bork, 2021). The Asphodelaceae tree was rooted on the branch containing the *Xanthorrhoea preissii* sample, following the Kew Tree of Life (Baker *et al*., 2022) for Asphodelaceae. The alooid species tree was rooted on the branch containing the outgroup sample for *Bulbine frutescens* (subfam. Asphodeloideae).

### *Taxonomic hypothesis testing*

The monophyly and phylogenomic placement of the Asphodelaceae subfamilies (Asphodeloideae, Hemerocallidoideae, Xanthorrhoeoideae), alooid genera and infrageneric taxonomic groups of *Aloe sensu stricto*, according to current consensus

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

(Table 1; Species and Sections file in the online supporting material) were tested with the newly obtained molecular phylogeny. The most comprehensive treatments for *Aloe* were used as taxonomic hypotheses for phylogenomic testing: the monograph of *Aloe* by Berger (1908); the expanded treatments of Reynolds for southern Africa (Reynolds, 1950), tropical Africa (Reynolds, 1966) and Madagascar (Reynolds, 1966); and the floristic treatment of *Aloe* by Glen and Hardy (2000) as part of the *Flora of southern Africa* series. Only groups with two or more representatives in the *Aloe sensu stricto* phylogeny were tested for monophyly. The groups of Malagasy aloes recognized by Castillon and Castillon (2010) were not tested as the sampling in the present study did not have sufficient coverage of all the groups recognized. Using the taxonomic concepts for sections and series described in these publications, validly published species names were allocated to taxonomic groups (online supporting material). Using the interactive Tree of Life annotation software (Letunic and Bork, 2021) the alooid phylogeny was then annotated with the main infrageneric groups (sections and series) in *Aloe* according to Berger (1908) before adding additional detail from other treatments by Reynolds (1950, 1966) and Glen and Hardy (2000).

### *Phylogenomic patterns of relevant traits*

Finally, phylogenomic patterns for several taxonomically and ecologically important characters were visualized by annotating the molecular phylogeny (for details, see section Taxonomic hypothesis testing). Geographic distribution was categorized in eight areas of diversity, based on Carter *et al.* (2011) and Grace *et al.* (2015): southern Africa, including all of South Africa, Lesotho and Eswatini; southwestern Africa, including all of Namibia and the dry, southwestern parts of Angola; south tropical Africa, corresponding roughly to Zambezia including Botswana, Mozambique, Zambia, Zimbabwe and the eastern parts of Angola; tropical East Africa, including all of Kenya, Uganda, Tanzania, Rwanda and Burundi; tropical West Africa, including the tropical wetter parts of the Congo and the Gold Coast; the Horn of Africa, including all of Ethiopia, Somalia, Eritrea, Djibouti, Sudan and South Sudan; the Arabian Peninsula, including all of Saudi Arabia, Oman and Yemen, including Socotra; Madagascar and the Mascarene islands, including La Réunion, Mauritius, Mayotte, the Comoros and the southern Seychelles.

Information on life form (habit) and habitat was obtained from species descriptions compiled by Newton (2020). Four life form categories were used: Stemless – plants without stems; Short-stemmed – plants with singular unbranched stems up to 1.5 m above the ground; Shrub – plants with branched stems up to 200 m above the ground; and Tree – plants with erect stems (branched or unbranched) at least 2 m above the ground. Habitat was categorized in seven categories: Grassland; Rocky hills; Forest; Sandy plains; Bush; Cliffs; and Crevices.

## RESULTS

This study presents a considerable increase in sequence information for the alooids (Asphodelaceae subfam. Asphodeloideae; *Aloe* and related genera) covering all 12 genera (with the exception of *Haworthia sensu stricto*) and a total of 303 taxa (294 species, of which 278 belong to *Aloe*). In fact, 142 of these taxa (133 species) have never been sequenced before (Species Information file in the online supporting material). The amount of sequence data underlying the phylogeny was considerably increased for all species, from up to 7 plastid and nuclear ribosomal markers (previous studies) to 173 low-copy nuclear genes (this study), discounting 16 genes removed due to paralogy or low recovery. The total target dataset (all 189 genes) comprised 350 347 bp compared with 4693 bp using traditional markers (Woudstra *et al.*, 2021). The sequence data for alooids come from previously published studies on alooid phylogenomics (Woudstra *et al.*, 2021, 2024), while the samples for the Asphodelaceae backbone phylogeny were obtained from an ongoing Asphodelaceae phylogenomics project as part of the PAFTOL project (Baker *et al.*, 2022) and were newly sequenced for this study with the alooid target capture sequencing tool (Woudstra *et al.*, 2021). An overview of sample and sequence data origin is presented in Table 2.

### *The genera of the alooid clade*

The alooids (12 genera) comprise a monophyletic clade (Fig. 2) embedded within subfamily Asphodeloideae. The species-level topology for alooids confirms the separation of *Aloestrela*, *Aloidendron*, *Aristaloe*, *Gonialoe* and *Kumara* from *Aloe sensu stricto* with full support (Fig. 2B). *Aloestrela* is nested within *Aloidendron* and together they form a fully supported monophyletic clade of five species in this topology. *Aloiampelos ciliaris* and *A. striatula* are monophyletic and form a larger monophyletic clade together with *Aloidendron*. *Kumara* is sister to the remaining members of the alooid clade, with the 279 species of *Aloe sensu stricto* forming a large, fully supported monophyletic clade. Although two out of the three sequenced species of *Aloiampelos* are phylogenomically distinct from *Aloe sensu stricto*, *Aloiampelos* is not resolved as a monophyletic clade because *Aloiampelos commixta* is sister to *Aloe sensu stricto* (Fig. 2B).

*Gonialoe* and *Aristaloe* form a fully supported clade with the larger genera *Haworthiopsis* and *Gasteria*, as well as *Astroloba* and *Tulista*. The genus-level phylogeny of Asphodelaceae (clade C, Fig. 2A) and the species-level phylogeny of the alooids (Fig. 2B) disagree about the relationship between *Gonialoe*, *Astroloba* and the other genera: in the former, *Astroloba* is sister to the rest, while in the latter *Gonialoe* is in this position. In both cases these nodes are not supported ($\text{LPP} = 0.56$ and $0.36$, respectively) and the sister genus placement therefore remains ambiguous. Both topologies do, however, agree on the placement of *Aristaloe* as sister to *Gasteria*, *Haworthiopsis* and *Tulista*, with the latter three forming a monophyletic clade.

### *Infrageneric relationships in* Aloe sensu stricto

The final phylogeny of *Aloe sensu stricto* presented here comprises 279 species with five additional subspecies and three additional varieties. Support was generally high ($\text{LPP} \geq 0.80$), particularly for the deeper nodes. Gene tree discordance was detected with a normalized quartet score of 0.625, causing low support in some of the shallower nodes of diverse clades (e.g. maculate aloes). The new topology generally favours the classification of Berger (1908) for the southern African clades (Fig. 3A), whereas the tropical African clades, which are younger, are better characterized by geographic distribution patterns (Fig. 4) than by morphology (Fig. 3B). Based on these phylogenomic patterns in taxonomy

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

TABLE 1. Overview of alooid genera and infrageneric groups recognized in *Aloe sensu stricto* treated in this study. The number of accepted and included species are indicated, with the numbers of infraspecific taxa (number of subspecies/number of varieties) indicated in brackets, following the currently accepted taxonomy for alooids in the World Flora Online (https://www.worldfloraonline.org/; see also 'Species and Section' file in the online supporting material). The results of the monophyly tests, presented in Fig. 2B for the alooid genera and Fig. 3 for the infrageneric groups for *Aloe sensu stricto*, are summarized in the final column, by the number of taxa forming a monophyletic group, 'pol.' indicates polyphyletic and 'par.' indicates paraphyletic

| Genus/Section | Number of accepted species | Number of species included | Monophyly |
|---|---|---|---|
| *Aloestrela* | 1 | 1 | – |
| *Aloiampelos* | 7 (0/9) | 3 (0) | 2/3 (pol.) |
| *Aloidendron* | 6 | 4 | 4/4 |
| *Aristaloe* | 1 | 1 | – |
| *Astroloba* | 11 (0/1) | 1 (0) | – |
| *Gasteria* | 28 (2/10) | 1 (0) | – |
| *Gonialoe* | 4 | 1 | – |
| *Haworthia* | 57 (0/97) | 0 | – |
| *Haworthiopsis* | 18 (0/21) | 2 | par. |
| *Kumara* | 2 | 1 | – |
| *Tulista* | 4 | 1 | – |
| *Aloe sensu stricto* | 595 (31/43) | 278 (5/4) | 595/595 |
| sect. *Aloe* L. | 9 | 7 | 6/7 (pol.) |
| sect. *Anguialoe* Reynolds | 9 | 2 | par. |
| sect. *Arborescentes* Salm-Dyck | 5 | 3 | 2/3 (pol.) |
| sect. *Asperifoliae* (A.Berger) Glen & D.S.Hardy | 11 | 7 | 7/7 |
| sect. *Chabaudia* Glen & D.S.Hardy | 10 (0/2) | 7 (0/0) | 3/7 (pol.) |
| sect. *Echinatae* Salm-Dyck | 5 | 5 | 2/5 (pol.) |
| sect. *Latebracteatae* (A.Berger) Glen & D.S.Hardy | 7 (0/1) | 5 (0/0) | 3/5 and 2/5 (pol.) |
| sect. *Leptoaloe* Reynolds | 49 (1/6) | 14 (0/0) | 13/14 (pol.) |
| sect. *Lomatophyllum* G.D.Rowley | 33 (1/0) | 8 (0/0) | 8/8 |
| sect. *Longistylae* (A.Berger) Glen & D.S.Hardy | 4 (0/1) | 3 (0/0) | 2/3 (par.) |
| sect. *Ortholophae* (Christian) Glen & D.S.Hardy | 20 (1/1) | 11 (0/1) | 3 (0/1)/11 (pol.) |
| sect. *Pachydendron* (Haw.) Salm-Dyck | 19 (1/1) | 11 (0/0) | 4/11 and 3/11 (pol.) |
| sect. *Pachythamnos* Glen & D.S.Hardy | 4 (0/1) | 3 (0/0) | 3/3 |
| ser. *Paniculatae* Salm-Dyck ex Kunth | 6 (1/0) | 5 (0/0) | 4/5 (par.) |
| sect. *Pictae* Salm-Dyck | 61 (2/3) | 32 (1/1) | 28 (1/1)/32 (pol.) |
| sect. *Purpurascentes* Salm-Dyck | 7 (2/0) | 5 (0/0) | 2/5 (pol.) |
| sect. *Rhodocanthae* Salm-Dyck | 6 (0/1) | 4 (0/0) | 2/4 (pol.) |
| Reynolds' tropical group 4 (striped perianth) | 16 | 13 | 5/13 and 2/13 (pol.) |
| Reynolds' tropical group 5 (*A. dorothaea* + allies) | 4 | 4 | 3/4 (pol.) |
| Reynolds' tropical group 9 (sect. *Verae* A.Berger) | 34 (1/2) | 16 (0/1) | 14 (0/1)/16 (par.) |
| Reynolds' tropical group 10 (pendent habit) | 13 | 7 | 2/7 (pol.) |
| Reynolds' tropical group 13 (clavate perianth) | 15 | 7 | 4/7 (pol.) |
| Reynolds' tropical group 15 (bottle-brush raceme) | 5 | 3 | 2/3 (pol.) |
| Reynolds' tropical group 16 (large compact rosette) | 20 | 12 | 4/12 (pol.) |
| Reynolds' tropical group 17 (canaliculate leaves) | 11 (2/0) | 10 (2/0) | 0/10 (pol.) |
| Reynolds' tropical group 19 (shrubs) | 34 (0/2) | 23 (0/1) | 6/23 (pol.) |
| Reynolds' Madagascar group 1 (very small plants) | 21 (1/1) | 9 (1/0) | 2/9 and 2/9 (pol.) |
| Reynolds' Madagascar group 4 (lineate leaves) | 14 (1/6) | 6 (0/0) | 0/6 (pol.) |
| Reynolds' Madagascar group 6 (capitate racemes) | 13 (1/6) | 3 (0/0) | 3/3 (par.) |
| Reynolds' Madagascar group 7 (cylindrical racemes) | 9 | 3 | 0/3 (pol.) |
| Reynolds' Madagascar group 8 (shrubs) | 13 (4/4) | 8 (0/0) | 2/8 and 2/8 (pol.) |

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

Table 2. Overview of samples and published sources used in the phylogenomic inference of alooids and other members of the Asphodelaceae. 'Herb.' refers to herbarium material, 'Fresh' to silica-dried leaf material sampled from living plants, and 'DNA' to DNA samples

| Clade | Total | Woudstra *et al.*, 2021 | | | Woudstra *et al.*, 2024 | | | PAFTOL (Baker *et al.*, 2022) |
|---|---|---|---|---|---|---|---|---|
| | | Herb. | Fresh | DNA | Herb. | Fresh | DNA | DNA |
| *Aloe sensu stricto* | **287** | **1**/1 | **16*** | **7**/1 | **48**/76 | **169**/10 | **46**/4 | – |
| Other alooids | **17** | – | **2*** | – | – | **3**/1 | **4** | **8** |
| Asphodeloideae (non-alooids) | **7** | – | – | – | – | – | – | **7** |
| Xanthorrhoeoideae | **1** | – | – | – | – | – | – | **1** |
| Hemerocallidoideae | **20** | – | – | – | – | – | – | **20** |

Numbers in bold indicate the number of samples that passed the quality-filtering criteria and were included in the final phylogenomic framework; numbers in non-bold font indicate samples that failed to pass quality-filtering criteria.

*Includes the nuclear gene sequences obtained from transcriptomes of four alooid species (3 *Aloe*, 1 *Aloidendron*) that were used to design the target capture sequencing tool (Woudstra *et al.*, 2021) used in the current study.

and geography (detailed below), an annotated species-level phylogeny of *Aloe sensu stricto* was determined (Fig. 5).

The older clades predominantly comprise southern African taxa (Fig. 4). Among these, many of the sections, especially those first included in the monograph of Berger (1908), form clear phylogenomic patterns (Fig. 3A). *Aloe* sect. *Aloe* is sister to the rest of the genus, with two species from *A.* sect. *Echinatae* (i.e. *A. erinacea* and *A. melanacantha*) embedded within it. *Aloe* sect. *Leptoaloe* is a monophyletic clade (LPP = 0.73), the only exception being the bulb-forming *A. buettneri*, which is sister to *A.* sect. *Pictae*. In *A.* sect. *Pictae*, *A.* ser. *Paniculatae* is embedded as a paraphyletic clade. *Aloe* sect. *Latebracteatae* is monophyletic and forms a clade with two paraphyletic members of *A.* sect. *Anguialoe* (i.e. *A. alooides* and *A. vryheidensis*) and *A. globuligemma* (*A.* sect. *Ortholophae*).

With the exception of one species (*A. rupicola*), *A.* sect. *Pachydendron* (single-stemmed, tree-like aloes) is monophyletic and comprises only southern African species with *A.* sect. *Pachythamnos* embedded within it, along with *A. marlothii* [which Berger (1908) also placed in *A.* sect. *Pachydendron*; this species is placed in *A.* sect. *Ortholophae* by Glen and Hardy, 2000]. *Aloe rupicola* (*A.* sect. *Pachydendron*) is instead embedded within the monophyletic *A.* sect. *Asperifoliae*, which comprises species predominantly from Southwestern Africa. Although paraphyletic, the three members of *A.* sect. *Longistylae* (i.e. *A. broomii*, *A. chlorantha* and *A. longistyla*) are all in the same clade together with three of the five members of *A.* sect. *Purpurascentes* (i.e. *A. framesii*, *A. khamiesensis* and *A. microstigma*) and two monophyletic members of *A.* sect. *Rhodocanthae* (i.e. *A. glauca* and *A. lineata*). Also included in this clade are *A. humilis* and *A. pictifolia* (from *A.* sect. *Echinatae*), but *A. humilis* resolves as sister to the clade containing the two species of *A.* sect. *Rhodocanthae*, while *A. pictifolia* is sister to *A. microstigma*. *Aloe arborescens* and *A. mutabilis* from *A.* sect. *Arborescentes* are monophyletic, while *A. pluridens* is sister to *A. succotrina* (from *A.* sect. *Purpurascentes*). Another southern African clade comprises *A. comosa* (from *A.* sect. *Rhodacanthae*), *A. gariepensis* (from *A.* sect. *Purpurascentes*) and *A. krapohliana* (from *A.* sect. *Echinatae*).

While southern African taxa of *A.* sect. *Aloe*, *A.* sect. *Latebracteatae*, *A.* sect. *Leptoaloe*, *A.* sect. *Pachydendron* and *A.* sect. *Pictae* form strongly supported coherent clades, several sections are polyphyletic: *A.* sect. *Arborescentes*, *A.* sect. *Echinatae*, *A.* sect. *Purpurascentes* and *A.* sect. *Rhodacanthae*.

The taxonomic sections hitherto recognized in *Aloe* are not recovered intact in the phylogenomic clades of tropical Africa (Fig. 3A). *Aloe* sect. *Chabaudia* (7 species), *A.* sect. *Ortholophae* (10 species) and *A.* sect. *Pachydendron* (6 species) are all represented by species spread across three clades covering substantial phylogenomic distances. A similar pattern is found for the three tropical African members of *A.* sect. *Pictae*, which are spread across two clades. *Aloe* sect. *Latebracteatae* is the only monophyletic section among the tropical African aloes, though separated from the southern African taxa.

All of the tropical African morphogroups recognized by Reynolds (1966) are polyphyletic (Fig. 3B) with only a few groups forming monophyletic clades, but never with all members sampled. Of these, the strongest coherence is found in group 9 of Reynolds (1966), which is an expanded version of *A.* sect. *Verae*, where only two members are recovered in a separate clade. *Aloe dorotheae* (Reynolds group 5) forms a monophyletic clade with two of its close relatives but with a third species separate (*A. canarina*). Based on perianth structure, two groups can be formed: those with striped perianths (Reynolds group 4; clade I, Fig. 3B; five species), which comprise the *A. somaliensis* species complex (Fig. 5); and those with clavate perianths (Reynolds group 13; clade II, Fig. 3B; four species).

Geography (Fig. 4) is a better classifier than morphology in *Aloe sensu stricto*, with the tropical African aloes roughly falling into three geographic clades: southwestern Africa, tropical East Africa, and aloes from the Horn of African and Arabian Peninsula. A shrubby growth form is the predominant type of habit among tropical East African species of *Aloe* (24 species, Reynolds group 19, Fig. 3B; Supplementary Data Fig. S1). The *A. somaliensis* cluster of species mentioned above is nested within the tropical East African clade and *A.* sect. *Verae* comprises predominantly Arabian aloes. Within *A.* sect. *Pictae*, a monophyletic group is formed by aloes from tropical East Africa and the Horn of Africa.

The Malagasy taxa in *Aloe sensu stricto* form a fully supported monophyletic clade with the monophyletic clade with *A.* sect. *Lomatophyllum* embedded in it (Fig. 3C). As is the case for the tropical African aloes, the Malagasy morphogroups recognized by Reynolds (1966) are not recovered as phylogenomic units. The only coherent group of species from Madagascar comprises three paraphyletic species with capitate inflorescences.

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

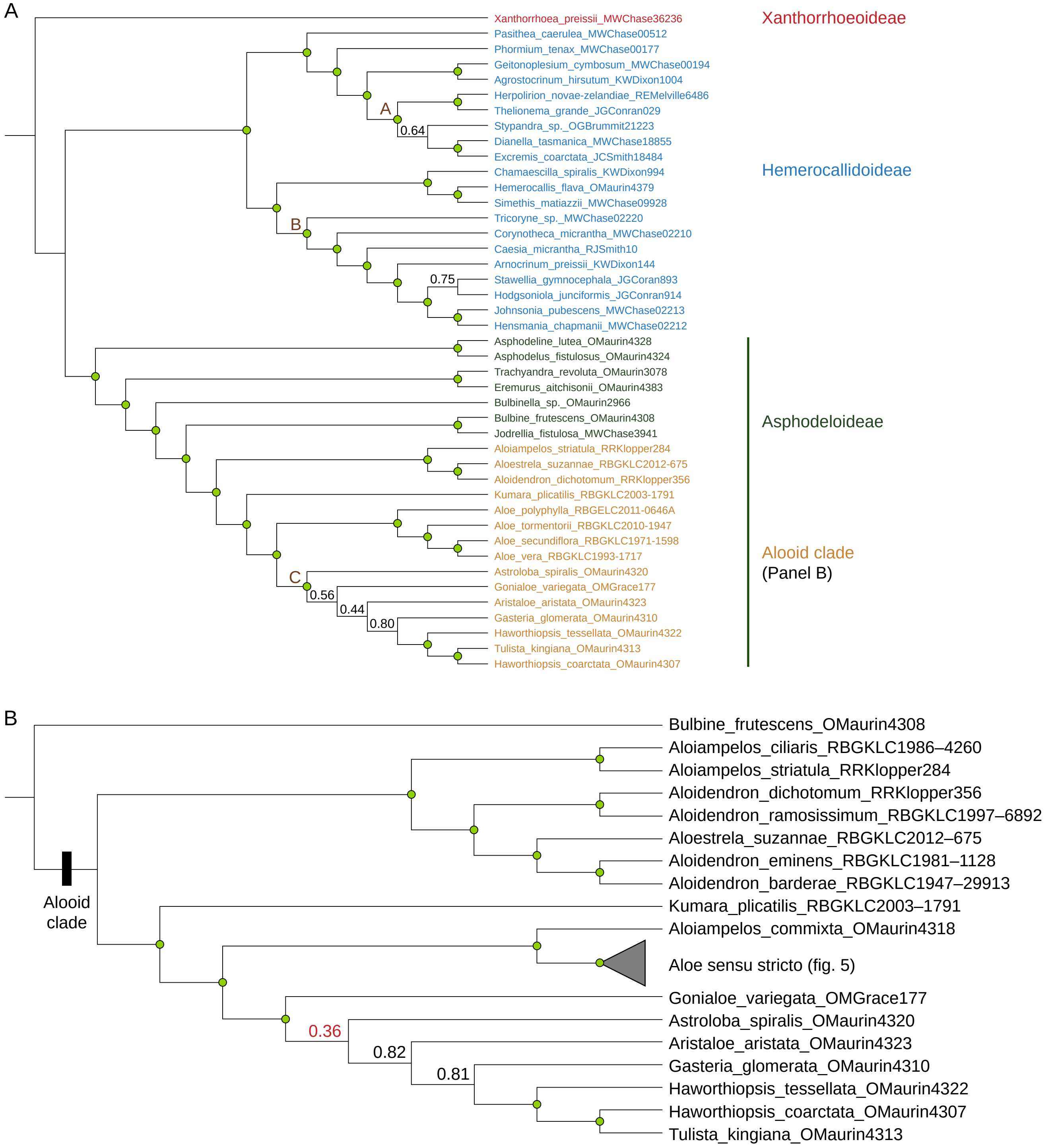

FIG. 2. Coalescent summary trees (generated with ASTRAL-III) of the genus-level backbone phylogeny for Asphodelaceae (A) and a species-level phylogeny for the alooid clade (B). The Asphodelaceae backbone phylogeny was manually rooted at the branch for *Xanthorrhoea preissii*, and the alooid phylogeny was manually rooted at the branch for *Bulbine frutescens*, causing the absence of node support for nodes between subfamilies. Nodes that are fully supported (LPP = 1) are indicated with a green circle and are specified in full if lower (LPP < 1). All three defined subfamilies in Asphodelaceae are monophyletic, with the alooids forming a monophyletic clade in Asphodelaceae subfam. Asphodeloideae. Three clades are highlighted in (A) with letters at the relevant node: A – core phormioids; B – former Johnsoniaceae; C – haworthioids. *Aloe sensu stricto* is collapsed in (B) to focus on the intergeneric classification of alooids. A detailed annotated phylogeny for *Aloe sensu stricto* is presented in Fig. 5. The trees can be explored using the following link: https://itol.embl.de/shared/YWoudstra.

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

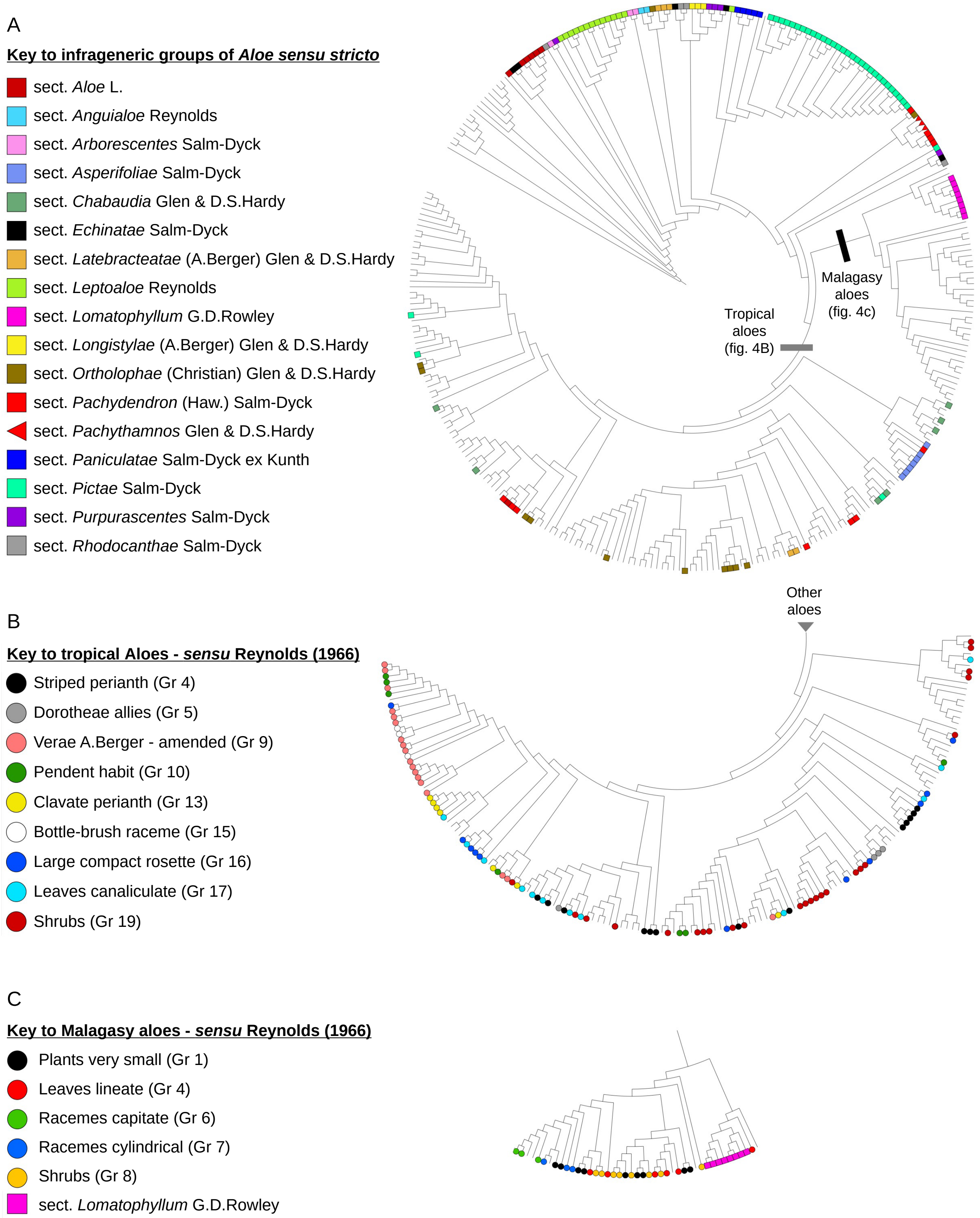

Fig. 3. Phylogenomic testing of standing taxonomic hypotheses for the genus *Aloe sensu stricto*. (A) Main sections according to the current consensus (Table 1), following the classification by Berger (1908) with amendments by Reynolds (1950), Rowley (1996) and Glen and Hardy (2000). (B) Morphogroups for the aloes of tropical Africa and the Arabian Peninsula following the classification by Reynolds (1966). (C) Morphogroups for the aloes of Madagascar and the Mascarene islands, including *Aloe* sect. *Lomatophyllum* as circumscribed by Rowley (1996), and following the classification by Reynolds (1966). Topologies correspond to the coalescent-based summary tree for the alooids (Fig. 5) and can be explored using the following link: https://itol.embl.de/shared/YWoudstra.

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

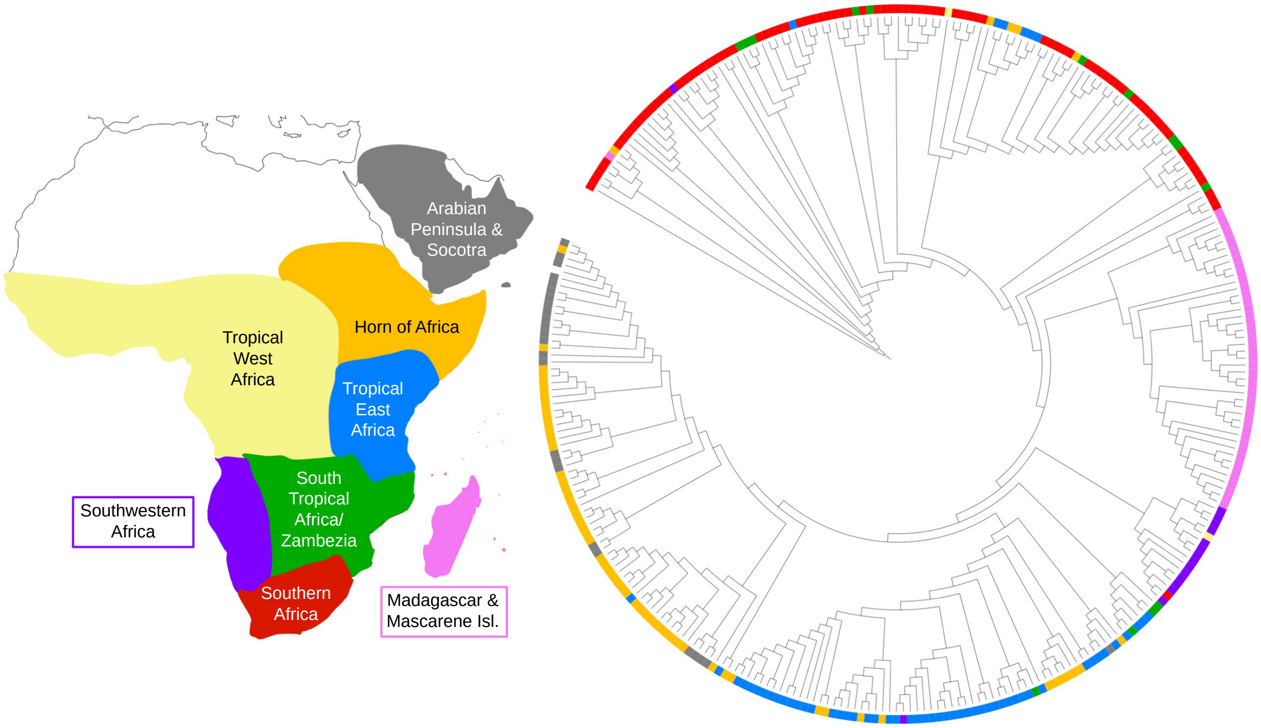

Fig. 4. Phylogenomic patterns of geographic distribution in the alooids. Geographic regions are based on the classifications by Carter *et al*. (2011) and Grace *et al*. (2015). Topology corresponds to the coalescent-based summary tree for alooids (Fig. 5) and can be explored using the following link: https://itol.embl.de/shared/YWoudstra.

### *A fully resolved backbone phylogeny for Asphodelaceae*

The final backbone phylogeny of Asphodelaceae (Fig. 2A) comprises all but two (*Kniphofia* and *Haworthia*) of the currently accepted genera. Both Hemerocallidoideae (20 genera) and Asphodeloideae (19 genera) are monophyletic, with the monogeneric Xanthorrhoeoideae sister to Asphodeloideae. All except five nodes were fully supported (LPP > 1), with relationships between *Gonialoe*, *Aristaloe* and *Gasteria* receiving the lowest support.

In Asphodeloideae, *Asphodelus* is monophyletic with *Asphodeline* in a clade that is sister to the rest of the genera. *Trachyandra* and *Eremurus* are also monophyletic, while *Bulbinella* is recovered on a separate branch. *Bulbine* and *Jodrellia* are sister to the monophyletic alooid clade. Within this, *Aloidendron* and *Aloestrela* form a monophyletic clade and, together with *Aloiampelos*, are sister to the rest of the alooid genera. *Kumara* is recovered on a distinct branch, sister to the 'core' alooids. *Aloe sensu stricto* is monophyletic and distinct from a clade comprising *Gonialoe* and *Aristaloe*, which were previously included in *Aloe* (clade C, Fig. 2A). In clade C, *Astroloba* is sister genus to the rest, but the nodes separating *Gonialoe* and *Aristaloe* are poorly supported (LPP = 0.56 and 0.44, respectively). *Haworthiopsis tessellata* is separate from *Haworthia coarctata* (i.e. *Haworthiopsis coarctata*), which is monophyletic together with *Tulista*. *Haworthiopsis* is therefore paraphyletic in this topology.

Two monophyletic clades are recovered in Asphodelaceae subfam. Hemerocallidoideae. In the smaller clade, the genus *Pasithea* is sister to eight other genera and *Phormium* is sister to the remaining seven. *Geitonoplesium* and *Agrostocrinum* are monophyletic, as are *Herpolirion* and *Thelionema*. The monophyly of *Stypandra* with *Dianella* and *Excremis* is poorly supported (LPP = 0.64), while there is full support for monophyly between the latter two. The larger clade involves *Hemerocallis*, which is monophyletic together with *Simethis* and *Chamaescilla*. This group is sister to the former Johnsoniaceae (clade B, Fig. 2A), comprising *Tricoryne*, *Corynotheca*, *Caesia*, *Arnocrinum*, *Stawellia*, *Hodgsoniola*, *Johnsonia* and *Hensmania*. *Hodgsoniola* is monophyletic with *Stawellia* with moderate support (LPP = 0.75), while the monophyly of *Johnsonia* and *Hensmania* is fully supported.

## DISCUSSION

In an effort to stabilize the classification of the alooids, one of the most diverse succulent plant lineages, nuclear phylogenomic evidence was generated to determine the species-level phylogeny of *Aloe* and related genera, as well as the genus-level phylogeny of the family Asphodelaceae. The monophyly of the alooid clade was confirmed within Asphodelaceae subfam. Asphodeloideae, wherein *Aloe sensu stricto* was confirmed as a monophyletic genus. The phylogeny of the big genus *Aloe sensu stricto* (278 out of 595 accepted species) is best characterized by geography, rather than vegetative and reproductive characters, which are mainstays in the taxonomic classification of aloes. The genus-level phylogenomic inference of subfam. Asphodeloideae and subfam. Hemerocallidoideae bring further stabilization to the classification of Asphodelaceae as a family.

### *The alooid clade: nuclear phylogenomics brings stabilization*

The findings of this study bring hitherto elusive nuclear phylogenomic evidence to stabilize the current classification of the

FIG. 5. Phylogenomic relationships within *Aloe sensu stricto* as determined with a coalescent-based summary tree from 173 low-copy nuclear gene trees. Tip labels are coloured according to fully supported taxonomic sections (Fig. 3) or geographic distribution (Fig. 4). Node support is indicated with coloured circles, green indicating high support (LPP ≥ 0.8) and yellow indicating moderate support (LPP = 0.60–0.79). Nodes without coloured dots are poorly supported in this topology (LPP < 0.60). The clades for the haworthioids (*Aristaloe*, *Astroloba*, *Gasteria*, *Gonialoe*, *Haworthiopsis* and *Tulista*) and for *Aloiampelos* and *Aloidendron* have been collapsed and are presented in detail in Fig. 2B. The tree can be explored using the following link: https://itol.embl.de/shared/YWoudstra.

alooids. The taxonomy of the alooids has been revised significantly in the last three decades, with several genera being split from the core *Aloe* genus (Grace *et al.*, 2013; Manning *et al.*, 2014; Smith and Molteno, 2019) and older genera being reincorporated (Rowley, 1996; Glen and Hardy, 2000; Grace *et al.*, 2013). With a few minor changes, which are detailed below, an intergeneric classification of 11 alooid genera (comprising 730 species) can be stabilized. The present study furthermore provides a framework and genomic tool with which to add previously uninvestigated species as sequences become available to complete the *Aloe* tree of life.

One of the more easily distinguishable groups of alooids is the tree aloes, i.e. the genus *Aloidendron*, based on their distinctive arborescent, often multi-branched growth form (Malakasi *et al.*, 2019). Nevertheless, the systematics surrounding this group has become rather complicated in recent years. Although the arborescent growth form has likely evolved multiple times in the alooids (Supplementary Data Fig. S1), species included in *Aloidendron* are distinct from the often single-stemmed trees included in *Aloe sensu stricto* by their height (>3 m), dichotomous branching and non-retention of dead leaves on the stem and branches (Grace *et al.*, 2013). For these reasons, the (usually) unbranched

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

Malagasy tree aloe *Aloe suzannae*, which showed phylogenomic affinity with *Aloidendron*, was raised to its own monotypic genus, *Aloestrela* (Smith and Molteno, 2019). *Aloe sabaea*, on the other hand, was placed in *Aloidendron* (Manning *et al*., 2014) based on an erroneous record of a 9-m-tall specimen by Reynolds (1966) (Malakasi *et al*., 2019). *Kumara*, the other genus of which one representative attains tree-like dimensions, has distinctly distichous (plicate) leaves. Following the results of Malakasi *et al*. (2019), the present detailed nuclear phylogenomic study of the alooids now reinforces the acceptance of *Aloidendron* and *Kumara* at the rank of genus. *Aloestrela suzannae* is nested well within *Aloidendron sensu stricto* and could be transferred to *Aloidendron*. *Aloe sabaea* is clearly distinct from *Aloidendron* and is related to tropical African tree-like species, such as *Aloe ballyi*, included in *Aloe sensu stricto*, and has already been returned to the genus *Aloe* (Smith *et al*., 2019). *Kumara plicatilis* is also clearly distinct, occurring on a separate branch as sister to the remaining genera in the alooid clade, and the reinstatement of this genus is therefore warranted. Although *Aloidendron pillansii* was not sequenced in this study, its placement in *Aloidendron* was confirmed by Malakasi *et al*. (2019). This species was, however, paraphyletic in that study and thus requires further investigation for its taxonomic status. *Aloidendron tongaense* has yet to be sequenced and investigated in a molecular phylogeny and therefore requires further attention to complete systematic studies on *Aloidendron.* At present, the genus *Aloidendron* should therefore comprise seven species, with the inclusion of *Aloestrela suzannae*.

The genus *Aloiampelos* also comprises seven species and is distinguishable by its variously rambling, leaning or creeping habit, characteristic leaf sheaths and comparatively long internodes (Grace *et al*., 2013). Contrary to *Aloidendron*, this genus is polyphyletic in the present topology (Fig. 2B), with two species showing strong affinity with *Aloidendron* and one, i.e. *Aloiampelos commixta*, being sister to *Aloe sensu stricto*. The placement of this *Aloiampelos commixta* sample was also recovered using the Angiosperms353 universal target capture kit (Baker *et al*., 2022). In a taxonomic study of *Aloiampelos* by Ellis (2013), *A. commixta* consistently resolved within *Aloiampelos*, often sister to *A. juddii*. However, the various gene trees (based on ITS sequences) presented in that study generally did not show good node support (Ellis, 2013). Further phylogenomic investigation using other accessions, preferably including the type specimen of *A. commixta* and material from *A. juddii*, should confirm whether this taxon does indeed not belong to *Aloiampelos.* Returning this taxon to *Aloe sensu stricto* would be one possible solution, but further investigation is required before any changes to the circumscription of the genus *Aloiampelos* is made.

The kanniedood aloes (genus *Gonialoe*) and the awn-leaf aloe (monotypic genus *Aristaloe*) were separated from *Aloe* based on their phylogenetic affinity with *Haworthia* and *Gasteria* (Manning *et al*., 2014). The results presented here support this separation showing *Gonialoe variegata* and *Aristaloe aristata* as phylogenomically distinct from *Aloe sensu stricto*. *Gonialoe* is based on *A.* sect. *Serrulatae* and now includes four species. To test the monophyly of this small genus of alooids, phylogenomic analysis of the remaining members should be prioritized.

In summary, our data currently support an alooid classification of 11 genera, recognizing *Aloe sensu stricto*, *Aloiampelos*, *Aloidendron*, *Aristaloe*, *Astroloba*, *Gasteria*, *Gonialoe*, *Haworthia*, *Haworthiopsis*, *Kumara* and *Tulista* at this rank, with *Aloestrela* incorporated into *Aloidendron*.

### Aloe sensu stricto*: clarifying infrageneric sections*

The 278 species of *Aloe sensu stricto* included in the present study are monophyletic. The use of nuclear phylogenomics through target capture sequencing has afforded a significant improvement in the molecular systematics of this big genus. Herbarium genomics furthermore allowed the inclusion of many difficult-to-collect and difficult-to-cultivate (e.g. grass aloes) species in the present study, expanding the molecular phylogeny framework from 197 species of *Aloe* (Grace *et al.*, 2015) to 279 out of the 591 currently accepted species. With this expanded phylogenomic framework, it was possible to test existing taxonomic hypotheses based mostly on vegetative and reproductive morphology using molecular data. The results confirm strong geographic patterns in the evolutionary history of *Aloe*, and the monophyly of five large sections for predominantly southern African taxa and one section for Arabian taxa.

Historically, southern African alooid genera have received more taxonomic attention because of a considerable number of botanists working on the group in the region. Dedicated collecting trips by botanists working with institutes such as the Royal Botanic Gardens, Kew, the South African National Biodiversity Institute and some South African universities have expanded the available living and preserved collections. As this present study was, for the main part, based on the collections at Kew, good coverage is available for this region. The general emerging pattern is that the infrageneric groupings recognized by Berger (1908), often as they were expanded by Reynolds (1950), are coherent, whereas the groupings published later, e.g. by Reynolds (1966) and Glen and Hardy (2000), are often polyphyletic. Several well-defined groups can be distinguished with, necessarily, their circumscription refined as new taxa are described.

Aptly, the clade that is sister to all other groups recognized in *Aloe sensu stricto* is *A.* sect. *Aloe*, currently comprising the mitre-forming aloes (following the concept of Glen and Hardy, 2000, but see Klopper *et al*., 2025 and Smith *et al*., 2025), e.g. *A. arenicola*, *A. mitriformis* and *A. pearsonii*. These species with their capitate inflorescences are predominantly distributed along the west coast of the Western and Northern Cape provinces of South Africa, as well as in southern Namibia. In the phylogenomic inference presented here, this clade also includes two species, i.e. *A. erinacea* and *A. melanacantha*, from *A.* sect. *Echinatae*, with sharper, more pronounced teeth on the leaf margins and scattered on one or both leaf surfaces. Like representatives of *A.* sect. *Aloe*, these two species also sucker basally to form clumps of variable density, but their racemes are conical–cylindrical and not capitate.

With their grass tuft-like habit and capitate to near-capitate inflorescences, the grass aloes, i.e. *A.* sect. *Leptoaloe*, form one of the most distinctive and visually recognizable clades of aloes. Their association with grasslands and grassy patches on cliffs provides effective camouflage to limit herbivory. These species generally do not succumb to veld fires and are able to resprout from their basally thickened stems and often fusiform roots (Cousins and Witkowski, 2012). These ecological innovations have allowed this group to diversify significantly and it currently includes 49 accepted species (Species and Sections file in the online supporting material), >8 % of the *Aloe* taxa recognized at present. One grass aloe, *A. myriacantha*, has the widest natural geographic distribution of all the aloes, ranging from southern South Africa in a northerly direction to well beyond the equator (Newton, 2020). The grass aloes are clearly monophyletic in the present study with a fully supported node, the only exception being the bulb-forming *A. buettneri*, which resolves here as a member of the maculate aloes clade.

The maculate aloes, i.e. *A.* sect. *Pictae*, are the most diverse section in *Aloe*, represented in the present study by 32 out of 61 currently accepted species (Table 1, Fig. 3A, 5). Although typical characters of this group, such as (generally) transversely arranged leaf maculations (H-shaped spots) and flower tubes that are constricted above the ovaries, are not unique to *A.* sect. *Pictae*, they are well defined on a combination of these characters and their leaf surface sculpturing (Grace *et al.*, 2009). The nuclear phylogenomic results presented here confirm the monophyly of this group for all southern African species and most of the tropical African species. *Aloe* ser. *Paniculatae*, the coral aloes, is embedded within *A.* sect. *Pictae*. The floral morphology of *A. buettneri* and the coral aloes is similar to that of the maculate aloes, supporting their possible inclusion in *A.* sect. *Pictae*. Although *A. swynnertonii* appears on a separate branch in the final phylogeny, the node is not supported (LPP < 0.60) and in many of the gene trees this species is part of *A.* sect. *Pictae*, being sister to the other members. Low node support is relatively frequent towards the tips of the maculate aloes clade (Fig. 5), which indicates high levels of incomplete lineage sorting. This, in turn, would indicate that the maculate aloes have rapidly diversified in recent evolutionary history (i.e. <5 million years; Grace *et al.*, 2015) and that genetic differentiation does not (yet) reflect the morphological diversity observed in this clade. The tropical aloes *A. citrina*, *A. congdonii* and *A. weloensis* are clearly not part of *A.* sect. *Pictae* because they are placed at some distance from the rest of this section (Fig. 3A). Further investigation of the tropical members of *A.* sect. *Pictae* would therefore be warranted to determine their placement as part of the section.

The only section that is monophyletic for both southern African and tropical African aloes, albeit in two separate clades, is *A.* sect. *Latebracteatae*, indicating the likely loss and subsequent gain of shared features used to define the section (large floral bracts, flowers on long pedicels (>10 mm), anthers and styles not (or very shortly) exserted from the flower tube; Glen and Hardy, 2000). This is a general pattern across the *Aloe* phylogeny, perhaps best exemplified by the multiple separate occurrences of the (generally single-stemmed) tree habit (>3 m, Supplementary Data Fig. S1). Unbranched trees occur in the southern African (e.g. *Aloe ferox*), Malagasy (e.g. *A. helenae*), tropical East African (e.g. *A. volkensii*) and Horn of Africa (e.g. *A. gracilicaulis*) clades. It is therefore not surprising that *A.* sect. *Pachydendron* is polyphyletic.

Other monophyletic groups among the tropical African aloes are *A.* sect. *Verae* (Reynolds' group 9), which includes the popular *Aloe vera*, and a group of small montane aloes from Somalia (Somaliensis group, Fig. 5). This last group has been treated previously by Carter *et al.* (1984) and includes several members of Reynolds' group 4 (striped perianth): *A. somaliensis*, *A. hemmingii* (synonymized by Carter *et al.* under *A. somaliensis*), *A. jucunda* and *A. peckii*; with *A. mcloughlinii* and *A. erensii* considered to be close relatives. The nuclear phylogenomic topology in this study clarifies the monophyly of the four species in this group together with *A. mcloughlinii* and the Northern Ethiopian *A. monticola*. The Kenyan *A. erensii*, however, is more distantly related and is monophyletic with *A. diolii* from South Sudan. The monophyly of *A. hemmingii* with *A. somaliensis* would support its synonymy.

### *The non-alooid members of Asphodelaceae subfam. Asphodeloideae*

The arrangement of non-alooid genera in the subfamily Asphodeloideae agrees with previous traditional phylogenetic studies (Seberg *et al.*, 2012; Grace *et al.*, 2015; McLay and Bayly, 2016) regarding the monophyly of *Asphodeline*–*Asphodelus* and the sister relationship of *Bulbine* to the alooid clade. Disagreement is, however, found in the relationships between *Bulbinella*, *Eremurus* and *Trachyandra*. The present topology agrees with Seberg *et al.* (2012) in placing *Eremurus* and *Trachyandra* together and recovering *Bulbinella* on a separate branch. *Kniphofia* is expected to occupy a separate branch nested among the non-alooid members of the subfamily, according to nuclear data (Baker *et al.*, 2022). The monotypic *Jodrellia* is currently synonymized with *Bulbine* (Boatwright and Manning, 2010), a decision that is supported by the data from the present study, although additional species of *Bulbine* need to be investigated to confirm the monophyly of this genus.

### *Asphodelaceae subfam. Hemerocallidoideae*

The present study presents a fully resolved and, with the exception of two nodes, fully supported phylogeny of the monophyletic subfamily Hemerocallidoideae. Several uncertainties in the taxonomy and molecular phylogeny are resolved with our phylogenomic data. *Geitonoplesium* is confirmed as a member of this subfamily, sister to *Agrostocrinum*, and *Chamaescilla* is confirmed as sister to a monophyletic clade of *Hemerocallis* and *Simethis* (McLay and Bayly, 2016). The historical polytomy at the base of the clade with *Dianella*, *Excremis*, *Stypandra*, *Thelionema* and *Herpolirion* (core phormioids, clade A in Fig. 2A) is resolved and broadly agrees with the topology presented by McLay and Bayly (2016), with *Thelionema* recovered together with *Herpolirion* and *Dianella* with *Excremis*.

The previously recognized family Johnsoniaceae forms a monophyletic clade (clade B in Fig. 2A). In line with previous (traditional) phylogenetic efforts (Wurdack and Dorr, 2009; Seberg *et al.*, 2012; McLay and Bayly, 2016), *Tricoryne* is sister to the rest of the group in the present phylogenomic topology, followed by *Corynotheca*, *Caesia*, *Arnocrinum* and the core group formed by *Hodgsoniola*, *Hensmania*, *Johnsonia* and *Stawellia*. Only one of the aforementioned studies included *Hodgsoniola* (McLay and Bayly, 2016), placing it with *Hensmania*. The phylogenomic inference presented here disagrees with this and recovers *Hodgsoniola* with *Johnsonia* instead. *Hensmania* is monophyletic with *Stawellia* with full support. The monotypic *Hodgsoniola* and *Stawellia* (five species) can be united on a morphological basis as well, with both genera having basally united tepals (Clifford and Conran, 1998). A possible synapomorphy between *Johnsonia* and *Hensmania* could be the presence of bracts around the flowers.

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

*Future work*

One of the most taxonomically problematic groups in *Aloe*, *A*. sect. *Purpurascentes*, remains polyphyletic in the present phylogenomic treatment. Despite recent work to stabilize the taxonomy of this group (Klopper *et al*., 2023), further adjustment may be necessary. Three of the five representatives of this section included in the present study form a coherent but paraphyletic group: *A. framesii*, *A. khamiesensis* and *A. microstigma*. The phylogenomic distance between the three groupings of species from *A*. sect. *Purpurascentes* supports Klopper *et al*.'s (2023) findings that *A. gariepensis* and especially *A. succotrina* comprise monophyletic clades separate from the rest of the core Purpurascentes group (i.e. *A. framesii*, *A. khamiesensis* and *A. microstigma*). Based on their morphological analyses, Klopper *et al*. (2023) concluded that *A. pictifolia*, a species regarded by some as a relative of *A. microstigma*, probably does not belong to the *A*. sect. *Purpurascentes*, despite some morphological similarities that might support such a relationship. However, *A. pictifolia* was not included in the molecular analysis of Klopper *et al*. (2023) and in results from the present study it resolves as sister to *A. microstigma*. Its sectional placement could therefore be reconsidered. A detailed study of all ten species and infraspecific taxa currently included in this section, as well as *A. pictifolia*, using our target capture tool may suggest a refined classification for *A*. sect. *Purpurascentes*.

Another predominantly southern African section that requires further investigation is *A*. sect *Echinatae*, which clearly does not consist of a natural grouping of species based on molecular data. Members of this section resolve in four different phylogenomically distant clades in the phylogenomic results presented here. As mentioned above, the placement of *A. erinacea* and *A. melanacantha* within the clade comprising *A*. sect. *Aloe* and the sister relationship of *A. pictifolia* and *A. microstigma* (from *A*. sect. *Purpurascentes*) could be morphologically justified. More difficult to explain from a morphological perspective is the placement of *A. humilis* as sister to *A. glauca* and *A. lineata* (from *A*. sect. *Rhodacanthae*), as well as the placement of *A. krapohliana* in a clade with *A. comosa* (from *A*. sect. *Rhodacanthae*) and *A. gariepensis* (from *A*. sect. *Purpurascentes*).

Beyond southern Africa, there are three coherent monophyletic groups: *A*. sect. *Asperifoliae*, comprising arid-adapted aloes distributed in Southwestern Africa; the *A. somaliensis* species complex, comprising small aloes from the Horn of Africa; and *A*. sect. *Verae* comprising mainly Arabian aloes, including the iconic *Aloe vera*. The origins of *A. vera* have long been enigmatic (Grace *et al*., 2015) as it is only known from cultivation and individuals that have likely escaped from cultivation. The prolific plantations and wild growth of *A. vera* in the Caribbean have led to the popularization of the now synonymous name *A. barbadensis* (Grace, 2011). Taxonomically, however, it has often been recognized as a close relative of extant wild species on the Arabian Peninsula and the placement of *A. vera* in *A*. sect. *Verae* along with other Arabian aloes in the present study supports this hypothesis.

To further investigate the *Dianella*, *Excremis*, *Stypandra*, *Thelionema* and *Herpolirion* clade in Asphodelaceae subfam. Hemerocallidoideae, it would be useful to sequence the monotypic genus *Rhuacophila*, which is currently synonymized with *Dianella* but shows strong affinity with *Stypandra* (Wurdack and Dorr, 2009; McLay and Bayly, 2016).

Finally, the phylogenomic framework presented here, with improved resolution and a considerable increase in taxa represented, could generate new insights into the ecological adaptation and diversification of this geographically widespread succulent lineage. Succulent plant lineages are generally associated with increased net diversification rates (Thompson *et al*., 2023) and the strong geographic patterns in the alooid phylogeny suggest there have been multiple adaptive radiation events in the evolutionary history of alooids.

*Conclusions*

The customized target capture sequencing method has proved highly suitable as a pragmatic solution to expand sequencing efforts in the alooids, despite the large numbers of species and large genome sizes, partly through the inclusion of degraded DNA samples from herbarium voucher specimens. Phylogenomic inference with 173 low-copy nuclear genes has resolved basal polytomies, both in the Asphodelaceae backbone phylogeny and in the alooid species tree. This approach has further clarified the inter- and infrageneric framework of the alooids by testing the monophyly of alooid genera and infrageneric taxonomic groups in *Aloe*. Strong geographic patterns in the present phylogeny provide a key to further resolve the infrageneric classification of *Aloe*. Reclassification of *Aloe sensu stricto* will therefore have to build on the approach of Reynolds (1950, 1966), with separate treatments for southern Africa, Madagascar and tropical Africa. Much work is still needed for Madagascar and tropical Africa, where the existing morphogroup classifications might not hold up to scrutiny (Fig. 3B, C) and where many recently described species have yet to be sampled and sequenced. A collaborative approach is therefore advocated, involving local botanists and large regional herbaria, such as those of the South African National Biodiversity Institute (SANBI) in Pretoria (PRE) and Cape Town (NBG) for southern Africa and Madagascar, the East African Herbarium (EA) for tropical East Africa and Tsimbazaza Herbarium (TAN) for Madagascar, to achieve a comprehensive revision to the infrageneric classification of *Aloe*.

## SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following. Figures S1 and S2 are available in the PDF file named 'Supporting Information' and contain two graphs displaying alooid topologies annotated with life form (habit; Fig. S1) and habitat (Fig. S2). Figure S1: phylogenomic patterns in life form across Asphodelaceae subfam. Alooideae. Trait data are based on species descriptions listed in the overview by Newton (2020). Different life forms are defined as follows: Stemless – plants without stems; Short-stemmed – plants with singular unbranched stems up to 150 cm above the ground; Shrub – plants with branched stems up to 200 cm above the ground; Tree – plants with erect stems (branched or unbranched) at least 200 cm above the ground. Tree topology is based on the ASTRAL-III summary species tree for Asphodelaceae subfam. Alooideae (Fig. 5 of the main manuscript), which can be explored using the following link: https://itol.embl.de/shared/YWoudstra.

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

Figure S2: phylogenomic patterns in habitat across Asphodelaceae subfam. Alooideae. Habitat data are based on species descriptions listed in the overview by Newton (2020). Tree topology is based on the ASTRAL-III summary species tree for Asphodelaceae subfam. Alooideae (Fig. 3B in the main manuscript), which can be explored using the following link: https://itol.embl.de/shared/YWoudstra. Other data related to this manuscript are available through the online supporting material deposited in FigShare at DOI https://doi.org/10.6084/m9.figshare.28435394. These include (1) the reference sequences for the four species used in the design of the alooid target capture tool, (2) Accession Information file, (3) Sequence Information file, (4) Species Information file containing info on the taxonomy, geographic distribution and growth form of species investigated in this study, (5) Species-and-Sections file containing taxonomic information on all *Aloe* names published to date, and (6) a directory containing the assembled sequences, cleaned alignments, gene trees, species trees and the code to produce these.

## FUNDING

This work was supported by funding from the European Union's Horizon 2020 Framework Programme, as part of the Marie Skłodowska-Curie Actions ITN-ETN 'Plant.ID' (grant number 765000) and a personal stipend from the Sven och Lilly Lawskis Fond för Naturvetenskaplig Forskning (Sweden) [N2023-0017] to Y.W.

## ACKNOWLEDGEMENTS

The authors thank collectors, horticulturalists and herbarium curators at the different botanical institutes that provided provenanced reference material to the study: Royal Botanic Gardens, Kew, and Kew Herbarium (K); Royal Botanic Garden Edinburgh (E); East Africa Herbarium (EA); Herbarium of the Musée National d'Histoire Naturelle, Paris (P); University of Uppsala Herbarium (UPS) and Uppsala Botanic Garden, Gothenburg Botanic Garden; Potsdam Botanic Garden; and Cambridge University Botanic Garden. We thank Anne-Sophie Quatela for advice on DNA extractions from herbarium material, and Kennedy Matheka for providing material of *Aloe ngutwaensis* before the official publication of the species description. Computational resources were provided by UNINETT Sigma2 – the National Infrastructure for High Performance Computing and Data Storage in Norway.

## AUTHOR CONTRIBUTIONS

Conceptualization: O.M.G., Y.W. and N.R.; specimen curation: P.R., O.M.G., R.R.K., G.F.S. and S.E.R.; data generation (DNA sequences): Y.W.; data curation (taxonomic): R.R.K. and G.F.S.; data curation (traits): Y.W.; analysis (phylogenomic inference): Y.W.; manuscript writing and revision: Y.W. with help from all co-authors.

## CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest applicable to this study.

## DATA AVAILABILITY

Raw sequencing data used in this study are deposited in the Sequence Read Archive (SRA) of the US National Center for Biotechnology Information (NCBI) under Bioprojects PRJNA1120847 (for alooids), PRJNA1122593 (for other members of the Asphodelaceae), and PRJNA1120785 [for data from the alooid target capture tool design study (Woudstra *et al.*, 2021)].

## REFERENCES

**Amir HM**, **Grace OM**, **Wabuyele E**, **Manoko MLK. 2019**. Ethnobotany of *Aloe* L. (Asphodelaceae) in Tanzania. *South African Journal of Botany* **122**: 330–335.

**Baker WJ**, **Bailey P**, **Barber V**, ***et al.* 2022**. A comprehensive phylogenomic platform for exploring the angiosperm tree of life. *Systematic Biology* **71**: 301–319.

**Berger A. 1908**. Liliaceae-Asphodeloideae-Aloineae. In: **Engler A.** ed. *Das Pflanzenreich: regni vegetabilis conspectus*. Leipzig: W. Engelmann, 1–360.

**Boatwright JS**, **Manning JC. 2010**. Asphodelaceae. Inclusion of the genus *Jodrellia* in *Bulbine* (Asphodelaceae). *Bothalia* **40**: 59.

**Bolger AM**, **Lohse M**, **Usadel B. 2014**. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114–2120.

**Carter S**, **Cutler DF**, **Reynolds T**, **Brandham PE. 1984**. A multidisciplinary approach to a revision of the *Aloe somaliensis* complex (Liliaceae). *Kew Bulletin* **39**: 611–633.

**Carter S**, **Lavranos JJ**, **Newton LE**, **Walker CC. 2011**. *Aloes: the definitive guide*. London: Royal Botanic Gardens, Kew & British Cactus and Succulent Society.

**Castillon JB**, **Castillon JP. 2010**. *Les aloe de Madagascar*. Antananarivo: J.-P. Castillon.

CITES Secretariat**. 2024**. *CITES checklist for aloe species: an updated checklist to the aloes of the world*. Compiled by: Klopper RR, Carter-Holmes S, Demissew S, et al. (eds). SANBI Biodiversity Series. Pretoria: South African National Biodiversity Institute.

**Clifford HT**, **Conran JG. 1998**. Johnsoniaceae. In: **Kubitzki K.** ed. *The families and genera of vascular plants, Vol. III: flowering plants. Monocotyledons: Lilianae (except Orchidaceae)*. Berlin: Springer, 336–340.

**Cousins SR**, **Witkowski ETF. 2012**. African aloe ecology: a review. *Journal of Arid Environments* **85**: 1–17.

**Dee R**, **Malakasi P**, **Rakotoarisoa SE**, **Grace OM. 2018**. A phylogenetic analysis of the genus *Aloe* (Asphodelaceae) in Madagascar and the Mascarene Islands. *Botanical Journal of the Linnean Society* **187**: 428–440.

**Degnan JH**, **Rosenberg NA. 2009**. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* **24**: 332–340.

**Dodsworth S**, **Pokorny L**, **Johnson MG**, ***et al.* 2019**. Hyb-Seq for flowering plant systematics. *Trends in Plant Science* **24**: 887–891.

**Doyle JJ**, **Doyle JL. 1987**. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11–15.

**Ellis K. 2013.** *Revision of Aloiampelos Klopper & Gideon F. Sm. (Xanthorrhoeaceae subfam. Asphodeloideae).* MSc Thesis, Nelson Mandela Metropolitan University, Gqeberha, South Africa.

**Frodin DG. 2004**. History and concepts of big plant genera. *Taxon* **53**: 753–776.

**Glen HF**, **Hardy DS. 2000**. Aloaceae (first part): *Aloe*. In: **Germishuizen G.** ed. *Flora of southern Africa 5, part 1, fascicle 1*. Pretoria: National Botanical Institute, 1–159.

**Gnirke A**, **Melnikov A**, **Maguire J**, ***et al.* 2009**. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nature Biotechnology* **27**: 182–189.

**Grace OM. 2011**. Current perspectives on the economic botany of the genus *Aloe* L. (Xanthorrhoeaceae). *South African Journal of Botany* **77**: 980–987.

**Grace OM**, **Buerki S**, **Symonds MR**, ***et al.* 2015**. Evolutionary history and leaf succulence as explanations for medicinal use in aloes and the global popularity of *Aloe vera*. *BMC Evolutionary Biology* **15**: e29.

**Grace OM**, **Klopper RR**, **Smith GF**, ***et al.* 2013**. A revised generic classification for *Aloe* (Xanthorrhoeaceae subfam. Asphodeloideae). *Phytotaxa* **76**: 7–14.

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026

**Grace OM**, **Pérez-Escobar OA**, **Lucas EJ**, ***et al.* 2021**. Botanical monography in the Anthropocene. *Trends in Plant Science* **26**: 433–441.

**Grace OM**, **Simmonds MSJ**, **Smith GF**, **Van Wyk AE. 2009**. Taxonomic significance of leaf surface morphology in *Aloe* section *Pictae* (Xanthorrhoeaceae). *Botanical Journal of the Linnean Society* **160**: 418–428.

**Grass Phylogeny Working Group III. 2024**. A nuclear phylogenomic tree of grasses (Poaceae) recovers current classification despite gene tree incongruence. *New Phytologist* **245**: 818–834.

**Hart ML**, **Forrest LL**, **Nicholls JA**, **Kidner CA. 2016**. Retrieval of hundreds of nuclear loci from herbarium specimens. *Taxon* **65**: 1081–1092.

**Heath TA**, **Hedtke SM**, **Hillis DM. 2008**. Taxon sampling and the accuracy of phylogenetic analyses. *Journal of Systematics and Evolution* **46**: 239–257.

**Hollingsworth PM**, **Graham SW**, **Little DP. 2011**. Choosing and using a plant DNA barcode. *PLoS One* **6**: e19254.

**Johnson MG**, **Gardner EM**, **Liu Y**, ***et al.* 2016**. HybPiper: extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. *Applications in Plant Sciences* **4**: e1600016.

**Johnson MG**, **Pokorny L**, **Dodsworth S**, ***et al.* 2019**. A universal probe set for targeted sequencing of 353 nuclear genes from any flowering plant designed using k-medoids clustering. *Systematic Biology* **68**: 594–606.

**Katoh K**, **Standley DM. 2013**. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

**Klopper RR**, **Grace OM**, **Klopper AW**, **Smith GF**, **Van Wyk AE. 2023**. A taxonomic revision of *Aloe* sect. *Purpurascentes* (Asphodelaceae subfam. Alooideae). *Phytotaxa* **628**: 1–64.

**Klopper RR**, **Smith GF. 2013**. Aloes of the world: when, where and who? *Aloe* **50**: 44–52.

**Klopper RR**, **Smith GF**, **Van Wyk AE. 2025**. (3084–3085) Proposals to conserve the name *Aloe mitriformis* with a conserved type and the name *A. microstigma* against *A. perfoliata* (Asphodelaceae: Alooideae). *Taxon* **74**: 727–729.

**Knowles LL**, **Kubatko LS. 2010**. *Estimating species trees: practical and theoretical aspects*. Hoboken: Wiley-Blackwell.

**Legume Phylogeny Working Group. 2017**. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny: the Legume Phylogeny Working Group (LPWG). *Taxon* **66**: 44–77.

**Letunic I**, **Bork P. 2021**. Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research* **49**: W293–W296.

**Linnaeus C. 1753**. *Species plantarum 1*. Stockholm: Impensis Laurentii Salvii.

**Malakasi P**, **Bellot S**, **Dee R**, **Grace OM. 2019**. Museomics clarifies the classification of *Aloidendron* (Asphodelaceae), the iconic African tree aloes. *Frontiers in Plant Science* **10**: e1227.

**Mandel JR**, **Dikow RB**, **Funk VA**, ***et al.* 2014**. A target enrichment method for gathering phylogenetic information from hundreds of loci: an example from the Compositae. *Applications in Plant Sciences* **2**: e1300085.

**Mandel JR**, **Dikow RB**, **Siniscalchi CM**, ***et al.* 2019**. A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae. *Proceedings of the National Academy of Sciences of the United States of America* **116**: 14083–14088.

**Manning JC**, **Boatwright JS**, **Daru BH**, **Maurin O**, **Van der Bank M. 2014**. A molecular phylogeny and generic classification of Asphodelaceae subfamily Alooideae: a final resolution of the prickly issue of polyphyly in the alooids? *Systematic Botany* **39**: 55–74.

**McLay TGB**, **Bayly MJ. 2016**. A new family placement for Australian blue squill, *Chamaescilla*: Xanthorrhoeaceae (Hemerocallidoideae), not Asparagaceae. *Phytotaxa* **275**: 97–111.

**Michel T**, **Tseng Y-H**, **Wilson H**, **Chung K-F**, **Thomas DC**, **Kidner C. 2022**. A hybrid capture bait set for *Begonia*. *Edinburgh Journal of Botany* **79**: 1–33.

**Mirarab S**, **Reaz R**, **Bayzid MS**, **Zimmermann T**, **Swenson MS**, **Warnow T. 2014**. ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics* **30**: i541–i548.

**Newton LE. 2020**. *Aloe* (Asphodelaceae). In: **Eggli U**, **Nyffeler R.** eds. *Illustrated handbook of succulent plants. Monocotyledons*. Berlin: Springer, 485–696.

**Nguyen L-T**, **Schmidt HA**, **Von Haeseler A**, **Minh BQ. 2015**. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32**: 268–274.

**Nicholls JA**, **Pennington RT**, **Koenen EJ**, ***et al.* 2015**. Using targeted enrichment of nuclear genes to increase phylogenetic resolution in the neotropical rain forest genus *Inga* (Leguminosae: Mimosoideae). *Frontiers in Plant Science* **6**: e710.

**Quatela A-S**, **Cangren P**, **Jafari F**, **Michel T**, **De Boer HJ**, **Oxelman B. 2023**. Retrieval of long DNA reads from herbarium specimens. *AoB Plants* **15**: plad074.

**Renner SS**, **Scherz MD**, **Schoch CL**, **Gottschling M**, **Vences M. 2024**. Improving the gold standard in NCBI GenBank and related databases: DNA sequences from type specimens and type strains. *Systematic Biology* **73**: 486–494.

**Reynolds GW. 1950**. *The aloes of South Africa*. Johannesburg: The Trustees of the Aloes of South Africa Book Fund.

**Reynolds GW. 1966**. *The aloes of tropical Africa and Madagascar*. Mbabane: The Trustees of the Aloes Book Fund.

**Rowley GD. 1996**. The berried aloes: *Aloe* section *Lomatophyllum*. *Excelsa* **17**: 59–62.

**Rowley GD. 2013**. Generic concepts in the Alooideae – part 3. The phylogenetic story. *Alsterworthia International Special Issue* **10**: 3–6.

**Sayyari E**, **Mirarab S. 2016**. Fast coalescent-based computation of local branch support from quartet frequencies. *Molecular Biology and Evolution* **33**: 1654–1668.

**Seberg O**, **Petersen G**, **Davis JI**, ***et al.* 2012**. Phylogeny of the Asparagales based on three plastid and two mitochondrial genes. *American Journal of Botany* **99**: 875–889.

**Shepherd LD**, **McLay TGB. 2011**. Two micro-scale protocols for the isolation of DNA from polysaccharide-rich plant tissue. *Journal of Plant Research* **124**: 311–314.

**Smith SA**, **Dunn CW. 2008**. Phyutility: a phyloinformatics tool for trees, alignments and molecular data. *Bioinformatics* **24**: 715–716.

**Smith GF**, **Klopper RR**, **Grace OM. 2019**. *Aloidendron* (Asphodelaceae subfam. Alooideae) consists of six species, not seven: *Aloe sabaea* is a true aloe. *Phytotaxa* **416**: 88–90.

**Smith GF**, **Klopper RR**, **Woudstra Y**, **Grace OM. 2025**. A further step towards stabilising the nomenclature associated with the genus name *Aloe* (Asphodelaceae subfam. Alooideae): the legitimate name *A. perfoliata* and the illegitimate name *A. mitriformis* are based on the same type, with notes on the identity of *A. mitriformis*. *Phytotaxa* **700**: 223–232.

**Smith GF**, **Molteno S. 2019**. *Aloestrela* Molteno & Gideon F.Sm. (Asphodelaceae: Alooideae), a new alooid genus with *A. suzannae* (Decary) Molteno & Gideon F.Sm. as the only species. *Bradleya* **2019**: 3–7.

**Soto Gomez M**, **Pokorny L**, **Kantar MB**, ***et al.* 2019**. A customized nuclear target enrichment approach for developing a phylogenomic baseline for *Dioscorea* yams (Dioscoreaceae). *Applications in Plant Sciences* **7**: e11254.

**The Angiosperm Phylogeny Group. 2016**. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181**: 1–20.

**Thompson J**, **Ramírez-Barahona S**, **Priest N**, **Herández-Hernández T. 2023**. Did succulents diversify in response to aridity? Evolutionary analyses of major succulent lineages around the world. *bioRxiv* 541957. doi:10.1101/2023.05.23.541957.

**Tumescheit C**, **Firth AE**, **Brown K. 2022**. CIAlign: a highly customisable command line tool to clean, interpret and visualise multiple sequence alignments. *PeerJ* **10**: e12983.

**WFO Plant List. 2025.** *The World Flora Online Plant List*. http://www.worldfloraonline.org (27 Feb 2025, date last accessed).

**Woudstra Y**, **Quatela A-S**, **Kidner C**, ***et al.* 2022**. Target capture. In: **De Boer H**, **Rydmark MO**, **Verstraete B**, **Gravendeel B.** eds. *Molecular identification of plants: from sequence to species*. Sofia: Pensoft Publishers, Advanced Books, 190–212.

**Woudstra Y**, **Rees P**, **Rakotoarisoa SE**, **Rønsted N**, **Howard C**, **Grace OM. 2024.** An updated DNA barcoding tool for Aloe vera and related CITES-regulated species. bioRxiv 602761. doi:10.1101/2024.07.09.602761.

**Woudstra Y**, **Viruel J**, **Fritzsche M**, ***et al.* 2021**. A customised target capture sequencing tool for molecular identification of *Aloe vera* and relatives. *Scientific Reports* **11**: e24347.

**Wurdack KJ**, **Dorr LJ. 2009**. The South American genera of Hemerocallidaceae (*Eccremis* and *Pasithea*): two introductions to the New World. *Taxon* **58**: 1122–1132.

**Zhang C**, **Rabiee M**, **Sayyari E**, **Mirarab S. 2018**. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* **19**: e153.

**Zuntini AR**, **Carruthers T**, **Maurin O**, ***et al.* 2024**. Phylogenomics and the rise of the angiosperms. *Nature* **629**: 843–850.

**Zwickl DJ**, **Hillis DM. 2002**. Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology* **51**: 588–598.

Downloaded from https://academic.oup.com/aob/advance-article/doi/10.1093/aob/mcaf109/8154207 by University of Pretoria, Academic Information Service user on 29 January 2026