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RESEARCH ARTICLE

A phylogenomic framework for species delimitation in *Kirganelia* (Phyllanthaceae): Tracing a history of reticulate evolution

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DOI https://doi.org/10.1002/tax.70170

Abstract *Kirganelia* (Phyllanthaceae) is a palaeotropical genus recently reinstated as separate from *Phyllanthus*, yet species boundaries remain unclear, especially within the widespread and morphologically variable *K. reticulata* complex. To resolve this, we generated Angiosperms353-based phylogenomic data from 233 samples and applied an integrative framework combining maximum likelihood and coalescent-based trees, ADMIXTURE analysis, phylogenetic networks (PhyloNet, SplitsTree), and diagnostic traits. These approaches consistently supported three main lineages and justified the recognition of 30 clades of individuals, here hypothesized to represent species. One lineage—including *K. zippeliana*, *K. glaucina*, four undescribed taxa, and *Moeroris retinervis*—is sufficiently distinct to be treated as a new section within *Kirganelia*, with nomenclatural changes to follow. Within the *K. reticulata* complex, we recognize five species: two from Africa (*K. polysperma* and a yet unpublished species), and three non-African species (*K. baccata*, *K. microcarpa*, *K. reticulata*). Network analyses revealed hybrid ancestry in multiple taxa, particularly within the "Malagasy" clade, highlighting reticulate evolution as a key driver of morphological overlap and phylogenetic discordance. These findings demonstrate the limitations of relying solely on monophyly for species delimitation in recently diverged lineages and support using multiple lines of evidence to define coherent evolutionary units. This work provides an evolutionary foundation for future studies of *Kirganelia* taxonomy, diversity, and biogeography.

Keywords Angiosperms353; introgression; Phyllanthaceae; phylogenomics; species complex; target capture

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

Phyllanthus L. (Phyllanthaceae) poses a taxonomic challenge because of the large number of species, very small unisexual flowers, and strikingly similar growth forms found among unrelated species (Luo & al., 2011). It has a pantropical distribution and its number of species depends on the circumscription adopted. For example, Bouman & al. (2018) accepted c. 900 species, and Moonlight & al. (2024) 1025, ranking it 27th among the largest Angiosperm genera. The genus exhibits a wide range of growth forms, from aquatic herbs to trees. Morphologically, the unisexual flowers are typically apetalous and characterized by the presence of a disc or disc glands. In this circumscription, genera such as *Breynia* J.R. Forst. & G.Forst., *Glochidion* R.Forst. & G.Forst., *Sauropus* Blume, and *Synostemon* F.Muell. are excluded, as they lack disc structures. After various phylogenetic analyses (Wurdack & al., 2004; Kathriarachchi & al., 2005, 2006; Samuel & al., 2005; Pruesapan & al., 2008, 2012), it became apparent that *Phyllanthus* was paraphyletic and *Breynia* (including *Sauropus*), *Glochidion* and *Synostemon* should be included to make it monophyletic (e.g., Hoffmann & al., 2006; Kathriarachchi & al., 2006). An alternative solution proposed by Van Welzen & al. (2014) was to split *Phyllanthus* into several recognizable monophyletic genera. This approach was implemented by Bouman & al. (2021, 2022), who constructed the largest phylogeny of *Phyllanthus* to date and used it to redefine the genus into monophyletic groups.

Kirganelia Juss. is one of nine genera reinstated from within the formerly broadly defined *Phyllanthus*, based on the comprehensive reclassification of tribe Phyllantheae by Bouman & al. (2022). Originally established as a genus by

Article history: Received: 17 Jul 2025 | returned for (first) revision: 22 Oct 2025 | (last) revision received: 20 Dec 2025 | accepted: 6 Feb 2026
Associate Editor: Seth Musker |

A.L. de Jussieu (1789), *Kirganelia* was initially characterized by its monoecious habit, five-parted calyx, staminate flowers with five stamens completely fused into a column, with three terminal and two lateral adnate anthers, and a small berry with a trilocular fruit structure containing six distinct seeds. The genus has since been treated at various ranks by different authors: as a genus by some (A. de Jussieu, 1824; Rafinesque, 1838; Baillon, 1858; Das, 1940), as a section under *Phyllanthus* by others (Müller, 1863; Bentham, 1873; Boerlage, 1900; Brunel, 1987), as a subgenus under *Cicca* L. (Kurz, 1873), and even as a subsection under *Phyllanthus* sect. *Typophyllanthus* Kuntze (Post & Kuntze, 1904). In more recent treatments, it was widely considered a subgenus of *Phyllanthus* (Webster, 1956; Kathriarachchi & al., 2006; Ralimanana & Hoffmann, 2011), until Bouman & al. (2022) clarified its generic status based on phylogenetic evidence. When treated as a subgenus, the group was associated with a heterogeneous assemblage of sections, including *Kirganelia* sect. *Anisonemopsis* Baill., *Phyllanthus* sect. *Fueggeopsis* Müll.Arg., *P.* sect. *Hemicicca* (Baill.) Müll.Arg., and *P.* sect. *Floribundi* Pax & K.Hoffm., as well as several sections historically associated with *Cicca* (Müller, 1863; Brunel, 1987). Following the phylogenetic reassessment of Bouman & al. (2022), these sectional entities are treated as synonyms of *Kirganelia* sensu stricto, and only *Phyllanthus* sect. *Kirganelia* (A.Juss.) Müll. Arg. and sect. *Pseudomenarda* Müll.Arg. are currently retained within the genus.

Although *Kirganelia* has been treated at various taxonomic ranks, a comprehensive revision is still lacking, and preliminary observations indicate notable morphological variation among specimens currently assigned to the same species. The group centred on *K. reticulata* (Poir.) Baill. has long been problematic: in former *Phyllanthus* classifications, *P. reticulatus* (Poir.) Baill. sensu lato was described as morphologically variable and taxonomically confusing, and studies of the *Phyllanthus–Epicephala* mutualism have referred to a problematic "*P. reticulatus* species complex" with unclear limits (Stevens, 2001; Kawakita & Kato, 2004, 2006). Building on this background, we use the term *K. reticulata* complex to refer to this historically heterogeneous assemblage, which ranges from Africa through Southeast Asia and Malesia to Australia. The ITS data from Luo & al. (2011) provided the first molecular evidence that this broadly defined entity comprises at least three distinct species: *K. polysperma* (Schumach. & Thonn.) R.W.Bouman in Africa, and *K. microcarpa* (Benth.) Hurus. & Yas.Tanaka and *K. reticulata* in Asia and Australia. However, Luo & al.'s sampling of Asian members lacked representation from key regions of Southeast Asia, notably Thailand and Indonesia, and the morphological characters proposed to distinguish these taxa—including differences in habit, floral composition, tepal colour, and style development—were not consistently correlated across their geographic ranges. Consequently, broader geographic sampling, inclusion of additional specimens, and a more comprehensive molecular framework are required to confirm species delimitation within the complex.

A second group of *Kirganelia* species occurs in Madagascar and the nearby islands, indicating that geographic context may also be relevant for interpreting phylogenetic patterns. Herbarium examination also revealed that some Malagasy taxa show morphological variation that can be difficult to interpret using the brief original descriptions available, whereas certain specimens assigned to different names share overlapping character states. These patterns do not necessarily indicate synonymy or misidentification, but they highlight areas where species limits are not entirely clear based on morphology alone. To provide a practical term for addressing this uncertainty, we refer to these Malagasy taxa as the "Malagasy complex", using the term in an operational sense to group morphologically similar species whose relationships and boundaries warrant further phylogenomic investigation.

Regarding phylogenomically grouped specimens as potential species, a reduced-representation genomic study using target capture of genetic data ("Hyb-Seq") with the Angiosperms353 bait set has the potential to genetically identify species within *Kirganelia* when applied at the species level (Johnson & al., 2019). Given *Kirganelia*'s palaeotropical distribution, herbarium specimens, paired with next-generation sequencing (NGS), offer a practical alternative to the logistically challenging task of collecting fresh material, and enables the recovery of low-copy nuclear genes from degraded samples (Kates & al., 2021). The Angiosperms353 kit was designed from over 600 species across the flowering plants and targeting 353 universal low-copy nuclear genes (Johnson & al., 2019). As a bycatch, the recovery of flanking noncoding regions makes Angiosperms353 useful for in-depth phylogenetic analyses below the species level, including population-level genetic structures (Johnson & al., 2019). As summarized by Baker & al. (2021), Angiosperms353 has been successfully applied across a wide range of taxonomic levels, from orders to the species complex and intraspecific scales, with recent studies further demonstrating its broad utility in various plant taxa (e.g., Dahal & al., 2025; Kitur & al., 2025; Liu & al., 2025; Nge & al., 2025; Orel & al., 2025; Vasile & al., 2025). In this context, we also evaluate the performance of the Angiosperms353 marker set for *Kirganelia* using basic recovery metrics, with the expectation of generally high locus capture from herbarium material and sufficient usable sequence data for downstream phylogenomic analyses.

We generated a phylogenomic framework for *Kirganelia*, with a particular focus on assessing lineage cohesion within the *K. reticulata* and Malagasy complexes, including tests of monophyly and other phylogenomic signals relevant to species boundaries, and to outline explicit expectations for species delimitation based solely on the molecular data presented here. We will treat concordant monophyly across tree-based and coalescent methods as initial evidence for cohesive species-level units. Conversely, non-monophyly is interpreted in the context of incomplete lineage sorting, historical gene flow, or broader species limits. Signals of admixture or reticulation detected through population genomic

clustering and network-based approaches are taken to indicate past genetic exchange or recent divergence, and are used only to outline broad patterns of evolutionary relationships consistent with the molecular data.

When molecular phylogenetic analyses fail to yield fully resolved relationships, a step-wise analytical framework that incorporates additional lines of evidence is often required to evaluate species limits. In Phyllanthaceae, floral morphology—particularly of pistillate structures—has repeatedly been shaped by pollination biology, as shown in lineages involved in obligate *Epicephala*–plant mutualisms in which convergent modifications of stigmatic tissues (less surface by either shortening the stigmas or folding them together) are interpreted as adaptations to specific pollination syndromes (Kawakita & Kato, 2004; Van Welzen & al.; 2023). This broader pattern highlights that stigma morphology can carry functionally meaningful variation, and may therefore provide complementary information for assessing evolutionary differentiation in cases where molecular data alone remain ambiguous.

■ MATERIALS AND METHODS

Taxon sampling. — Herbarium specimens of *Kirganelia* were primarily obtained from the Naturalis Biodiversity Center (L, U, WAG), with additional material from other herbaria if necessary. Type specimens were sampled when possible. Herbarium specimens were selected comprising all 24 species according to Bouman & al. (2022), covering their distribution ranges. Visible morphological variations were another selection criterion aside from distribution. A total of 495 specimens (see Appendix 1 for the selection finally used for the phylogeny) were selected and sampled for 10–25 mg of leaf tissue. The sequence of *Flueggea leucopyrus* Willd., which was used as the designated outgroup to root the phylogeny, was obtained from PAFTOL (Baker & al., 2022).

DNA extraction and library preparation. — DNA extraction was performed in two rounds, using different protocols to optimize yield. In the first round, 166 samples were extracted using a modified DNeasy Plant Mini Kit protocol (Qiagen, Hilden, Germany), but samples with DNA concentrations below 4 ng/µl were re-extracted using a modified CTAB protocol, which consistently produced higher yields. The second round, targeting underrepresented species and poorly supported clades, used only the CTAB method with key modifications, including overnight lysis at 42°C with proteinase K and PVPP, and extended precipitation with GlycoBlue for two weeks. DNA was quantified with a Fragment Analyzer DNF-468 kit (Agilent, Santa Clara, California, U.S.A.), and only samples with ≥4 ng/µl were used for library preparation. Fragmentation was performed either by 1-minute sonication (Covaris M220, Woburn, Massachusetts, U.S.A.) or size selection with NucleoMag magnetic beads (Macherey-Nagel, Düren, Germany).

Library preparation used 100–200 ng of DNA in 50 µl input, adjusted to maintain a minimum 3:1 water-to-DNA ratio for viscous samples to ensure bead efficiency. Initial cleanup involved 60 µl of magnetic beads and three washes with 80% ethanol in 96-well plates. The NEBNext Ultra II FS kit (New England Biolabs, Ipswich, Massachusetts, U.S.A.) was used for library construction, with IDT 10 indexing primers and 9–16 PCR cycles. Low-yield libraries (<5 ng) were repeated if sufficient DNA remained and pooled via vacuum centrifugation. Final genomic library pools were assembled based on Fragment Analyzer profiles using the HS Small Fragment DNF-477 kit (Agilent), considering average and peak fragment length, quantity, and trace shape to ensure balanced representation across all samples during hybridization capture and sequencing.

Target enrichment and sequencing. — Target enrichment was conducted using the Angiosperms353 bait set (Johnson & al., 2019; Arbor Biosciences, Ann Arbor, Michigan, U.S.A.). Hybridization temperature was conducted at 60°C. Library recovery enrichment without polymerase systems followed by library amplification of 8–14 PCR cycles to produce a minimum of 10 nmol without any sign of overamplification based on measurement with a Tape Station HS D5000 (Agilent Technologies). A 0.9 times ratio bead cleanup step was conducted to the amplified libraries and then sequenced using an Illumina NovaSeq 6000 at BaseClear (Leiden, The Netherlands) with a range of requirement 2–5 GB per capture pool. A total of 410 samples were sent to BaseClear for sequencing.

Data processing. — All analyses were conducted using the computer resources from the Academic Leiden Interdisciplinary Cluster Environment (ALICE) provided by Leiden University, the Netherlands. Raw reads were trimmed with Trimmomatic v0.39 (Bolger & al., 2014) using the parameters ILLUMINACLIP:../TruSeq3-PE.fa:2:30:10 LEADING:10 TRAILING:10 SLIDINGWINDOW:4:20 MINLEN:40. Cleaned reads were mapped to target genes using HybPiper v.2.1.5 (Johnson & al., 2016), and sample quality was assessed with HybPiper statistics script (Hybstat). Samples recovering >10% of loci at ≥25% target length were retained (n = 297), with a further filter based on ≥5% nucleotide coverage (of 23,520 bp) yielding 249 samples for downstream analyses. Exon sequences were aligned using MACSE v.2.06 (Ranwez & al., 2018), supercontigs with MAFFT v.7.505 (Katoh & Standley, 2013), and all alignments were trimmed using TrimAl v.1.4 (Capella-Gutiérrez & al., 2009) with the "automated1" setting.

Gene and sample filtering. — From 410 sequencing results, 159 samples were excluded based on HybPiper summary statistics and low nucleotide coverage (suppl. Table S1), leaving 249 specimens for a preliminary analysis. To evaluate the impact of gene filtering, we used a standardized trimming strategy (resoverlap = 0.75, seqoverlap = 80, gt = 0.40) and compared three datasets during Shimodaira-Hasegawa Approximately Unbiased (SH-AU, Shimodaira & Hasegawa, 1999; Shimodaira, 2002) testing (suppl. Table S2): (1) genes with ≤75% missing data and ≥400 parsimony-informative (PI) sites (93 genes), (2) genes with

≥100-taxon coverage and ≥400 PI sites (85 genes), and (3) an unfiltered dataset. All were supported (p-AU ≥ 0.668), but the unfiltered dataset yielded the strongest support (p-AU = 1.0), suggesting that filtering may reduce informative signals relevant to processes like incomplete lineage sorting or introgression. Therefore, we proceeded with all loci retained and trimmed using trimAl's "-automated1" setting, which adapts to each alignment. Lastly, we individually reassessed all specimens that produced unusually long branches by conducting detailed microscopic examinations, applying the available identification keys, and comparing them with type material. These evaluations showed that all such specimens had been misidentified and did not belong to *Kirganelia*. These were excluded, resulting in a final ingroup of 233 specimens (Appendix 1), for which raw sequencing data are available in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1456579.

Phylogenetic reconstruction. — We generated maximum likelihood, using a concatenated supermatrix, and multi-species coalescent-based phylogenies from two datasets: an *exon* dataset containing coding sequences (CDS) only, and a supercontig dataset including exons plus flanking intronic regions. Gene alignments were concatenated using catsequences (Creevey, 2023) and analyzed in IQ-TREE v.2.2.5 (Minh & al., 2020) under the GTR + F + R model with 1000 ultrafast bootstrap replicates (-B) and branch lengths retained (-wbtl). Individual gene trees were inferred using IQ-TREE and concatenated via Unix cat. Gene and site concordance factors (gCF, sCF) were calculated with IQ-TREE to compare dataset consistency. Next, we performed a coalescent-based species phylogeny using ASTRAL-III (C. Zhang & al., 2018) and ASTRAL-Pro v.1.16.1.3 (C. Zhang & al., 2020), with all gene trees as input and with *Flueggea leucopyrus* as the outgroup. Quartet-support pie-chart visualizations were generated using ASTRAL-Pro (C. Zhang & al., 2020) after confirming topological equivalence between ASTRAL-III and ASTRAL-Pro using the "cophylo" function in the Phytools package (Revell, 2012). Trees were visualized in R v.4.3.1 (R Core Team, 2023) using ape v.5.7-1 (Paradis & Schliep, 2019). Final trees were annotated and edited using Inkscape v.1.2 (Inkscape Project, https://inkscape.org).

To evaluate species tree topologies and establish a framework for species delimitation, we compared maximum likelihood (supermatrix) and coalescent-based (ASTRAL) phylogenies. The supermatrix tree, supported by bootstrap (BS) values, was used as the primary backbone when topologies were congruent and species boundaries well-resolved. ASTRAL trees, supported by LPP values, were preferred in cases of topological conflicts or significant gene tree discordance. When overall concordance was observed through cophylogenetic visualization, the supermatrix topology was retained for downstream analyses, including reticulation and introgression.

To explore whether morphologically paraphyletic species may contain genetically monophyletic lineages, we used a custom Python script to assess monophyly across all 353 gene trees, available at https://github.com/EvaKP1990/baitset-performances (Putri, 2025). Genetic population structure was analyzed using ADMIXTURE v.1.3.0 (Alexander & al., 2009), with analyses initiated at $K = 2$ and extended to dataset-specific upper K values informed by a priori expectations of major lineages or genetic groups. Cross-validation was used to guide K selection, but biological interpretation was based on concordance with phylogenetic evidence rather than solely on the lowest cross-validation error. Prior to ADMIXTURE and principal component analysis (PCA), single nucleotide polymorphisms (SNPs) were filtered using PLINK v.1.9 (Purcell & al., 2007; Chang & al., 2015) with LD (linkage disequilibrium) pruning (--indep-pairwise 50 10 0.2) to reduce redundancy and improve interpretability. This ensured a consistent set of approximately independent SNPs across both analyses, enhancing resolution while meeting model assumptions. Where incongruence or signal conflict was observed between clusters and species, additional analyses were conducted to detect potential reticulate evolution. A subset of individuals was selected based on clusters defined by dominant ADMIXTURE ancestry colours and limited to species involved in the detected conflicts.

Reticulate evolution was investigated using PhyloNet v.3.8.2 (Than & al., 2008) under the maximum pseudo-likelihood framework, inferring networks with 1–3 reticulations to detect potential hybridization and introgression events. Model selection was based on the Akaike information criterion (AIC; Akaike, 1974) and the Bayesian information criterion (BIC; Schwarz, 1978), and inheritance probabilities were visualized with Dendroscope v.3 (Huson & Scornavacca, 2012). Complementary consensus networks were generated with NeighborNet in SplitsTree v.6.4.14 (Huson & Bryant, 2006) using trimAl-trimmed alignments. To further explore genetic structure, PCA was performed on pruned SNPs (via PLINK), with eigenvectors visualized in Python. Pairwise patristic distances from a maximum likelihood tree were also calculated to approximate genetic divergence, supporting species delimitation with multiple lines of evidence.

Targeted examination of stigma characters. — Detailed stigma observations were conducted only on a subset of taxa—specifically, those whose relationships remained unresolved based on the molecular analyses. Pistillate flowers from the selected herbarium specimens were rehydrated by briefly boiling the flowers in water, then dissected and examined under a Zeiss Discovery V20 stereo microscope equipped with an Axiocam 305 colour camera, Sycop3 joystick, CL9000 LED illumination, and Zeiss Zen v.3.8 software. Stigma characters, including lobe number, fusion, bifurcation, and orientation, were documented for subsequent character mapping.

■ RESULTS

Gene and sample recovery. — We constructed a dataset of 233 samples to evaluate the performance of the

Angiosperms353 bait set for *Kirganelia*. Sequencing yielded an average of 1.26 ± 0.82 million reads per sample, with a mean on-target mapping rate of 47.32% ± 20.68%, which is expected for a universal bait set like Angiosperms353 designed for broad angiosperm diversity. HybPiper successfully recovered an average of 322 ± 44 genes per sample (range: 104–352), corresponding to a mean recovery rate of 91.2% ± 12.6%. Trimmed alignment lengths across the 353 loci ranged from 89 to 6550 bp (mean ~982 bp), with 161 loci retrieved at ≥75% of their target length.

To assess the phylogenetically informative portion of this dataset, we evaluated bait performance after alignment and TrimAl filtering. Most loci showed strong recovery of samples, with the majority capturing over 200 samples and only seven falling below 100. At the sample level, most accessions recovered over 90% of loci, although 25 samples recovered less than 75%. Detailed patterns of gene- and sample-level recovery are provided in suppl. Fig. S1.

We calculated gCF and sCF from both exon and supercontig alignments (Table 1). The supercontig dataset yielded longer loci and more total sites, resulting in slightly higher concordance values. Based on these metrics, supercontigs were used for downstream analyses.

Species-level patterns in the supermatrix tree. — The specimen phylogeny from the concatenated supermatrix dataset resulted in three major clades within *Kirganelia* (Fig. 1A, full version in suppl. Fig. S2, support values in suppl. Table S3). The first clade, located at the lower part of Fig. 1A, can be defined as a new section in the future containing seven putative species (see below). The second corresponds to *K.* sect. *Pseudomenarda* (Müll.Arg.) R.W. Bouman, comprising two well-defined species: *K. purpurea* (Müll.Arg.) R.W.Bouman and *K. somalensis* (Pax) R.W.Bouman. The third and largest clade, *K.* sect. *Kirganelia*, includes several clearly delimited species with matching geographic and morphological patterns. However, two groups within this clade—the *K. reticulata* complex and the Malagasy complex, which comprise species distributed mainly in Madagascar and nearby western Indian Ocean islands (Mayotte, Seychelles, Mauritius)—exhibit greater phylogenetic complexity and morphological overlap, and are therefore examined in more detail in the following sections.

The earliest-diverging clade within section *Kirganelia* comprises two species, *K. glauca* (Wall. ex Müll.Arg.) R.W. Bouman and *K. flexuosa* (Siebold & Zucc.) R.W.Bouman, forming a strongly supported lineage (BS = 100). Within this clade, sample EP186, *K. oligosperma* (Hayata) R.W.Bouman is nested in the *K. glauca* part of the clade. *Kirganelia oligosperma* is the younger name and has to be considered as a synonym of *K. glauca*.

The next divergence within the clade involves *Kirganelia ovalifolia* (Forssk.) R.W.Bouman as a separate lineage, followed by a clade comprising *K. muelleriana* (Kuntze) R.W. Bouman and *K. dinklagei* (Pax) R.W.Bouman. These species are each strongly supported as distinct lineages (BS = 100) and hereby confirmed as distinct species.

Other well-recognized species include *Kirganelia archboldiana* (Airy Shaw & G.L.Webster) R.W.Bouman, *K. ciccoides* (Müll.Arg.) R.W.Bouman, *K. vieillardii* Baill., and *K. novae-hollandiae* (Müll.Arg.) R.W.Bouman. Notably, the clade containing *K. vieillardii* and *K. novae-hollandiae* also includes a sample (EK36) that appears genetically distinct. However, its placement in the multi-coalescent species tree support assigning this specimen to *K. novae-hollandiae* (Fig. 2).

Although four additional species (*Kirganelia archboldiana*, *K. ciccoides*, *K. novae-hollandiae*, *K. vieillardii*) are nested within the same broader monophyletic lineage as the *K. reticulata* complex, the SplitsTree network including all 125 individuals shows that these taxa form clusters distinct from the five species comprising the complex (Fig. 3A). To evaluate population structure across this expanded clade, we also conducted ADMIXTURE analyses using the 125-individual dataset. However, these runs produced no stable or biologically interpretable clustering patterns across $K = 2, \ldots, 9$, with individuals from all species showing mixed or inconsistent assignment proportions (suppl. Fig. S3). Accordingly, all subsequent clustering inferences focus on the 100 individuals belonging to the five species of the *K. reticulata* complex, for which ADMIXTURE yields coherent and biologically meaningful structure.

***Kirganelia reticulata* species complex. —** Our phylogeny confidently resolved the *Kirganelia reticulata* species complex into two major clades (BS of 97 and 100, respectively; Fig. 1A). The first clade includes three complex groups, with two African clades separated by a non-African lineage. They are identified as *K. polysperma* (Schumach. & Thonn.) R.W. Bouman (African), *K. reticulata* (non-African), and a new African species (sp. 1). The second clade comprises two non-African clades, *K. baccata* (F.Muell. ex Benth.) R.W.Bouman and *K. microcarpa*.

A cophylogenetic comparison indicated that ASTRAL-III and ASTRAL-Pro produced equivalent topologies (suppl. Fig. S4), confirming that the use of ASTRAL-Pro for quartet-support visualization did not affect the interpretation of the species tree. For nodes representing each of the five

Table 1. Comparison of the exon and supercontigs datasets performed by the Angiosperms353 bait kit.

	Total loci analyzed	Total sites after trim	Min-max sites per locus	Mean sites per locus	Mean gCF	Median gCF	Mean sCF	Median sCF
Exon	230	95,515	29–3427	415.28	8.12	3.23	42.22	39.89
Supercontig	230	139,603	28–7242	606.97	10.15	4.11	43.51	39.88

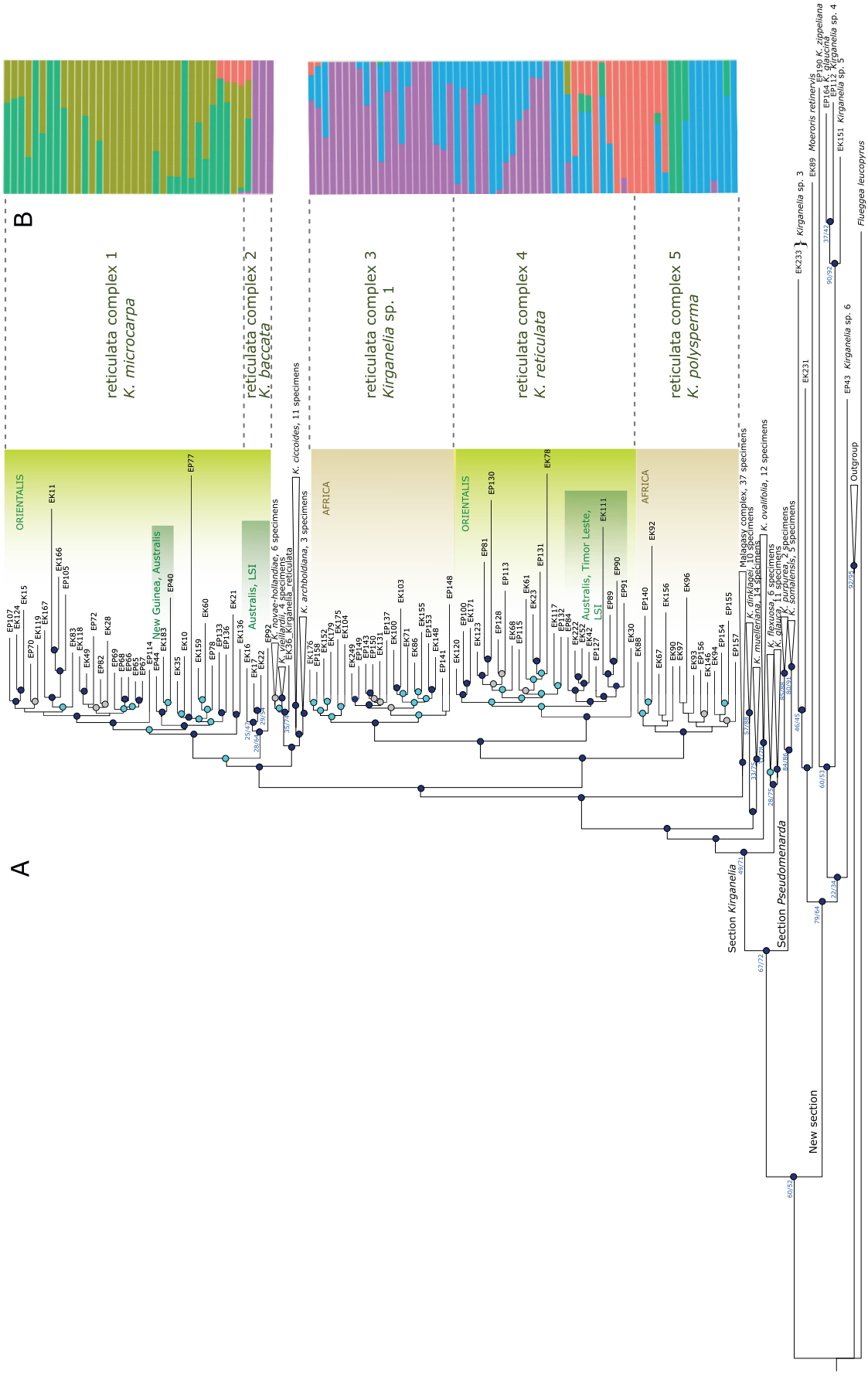

Fig. 1. Phylogenomic relationships and population structure of *Kirganelia*, with emphasis on the *K. reticulata* complex. **A**, Maximum likelihood tree of 233 samples based on a supercontig dataset generated using the Angiosperms353 bait set. Coloured circles indicate node support: dark blue for 100% bootstrap, light blue for 90%–99%, and grey for 80%–89%. Blue text to the left of selected nodes indicates the gCF/sCF values, shown only for branches with gCF ≥ 20%. Collapsed clades represent well-defined species identifications, with numbers indicating the number of specimens per clade. The full tree is provided in suppl. Fig. S2. **B**, Genetic population structure of 100 individuals from the *K. reticulata* complex inferred using ADMIXTURE at $K = 5$.

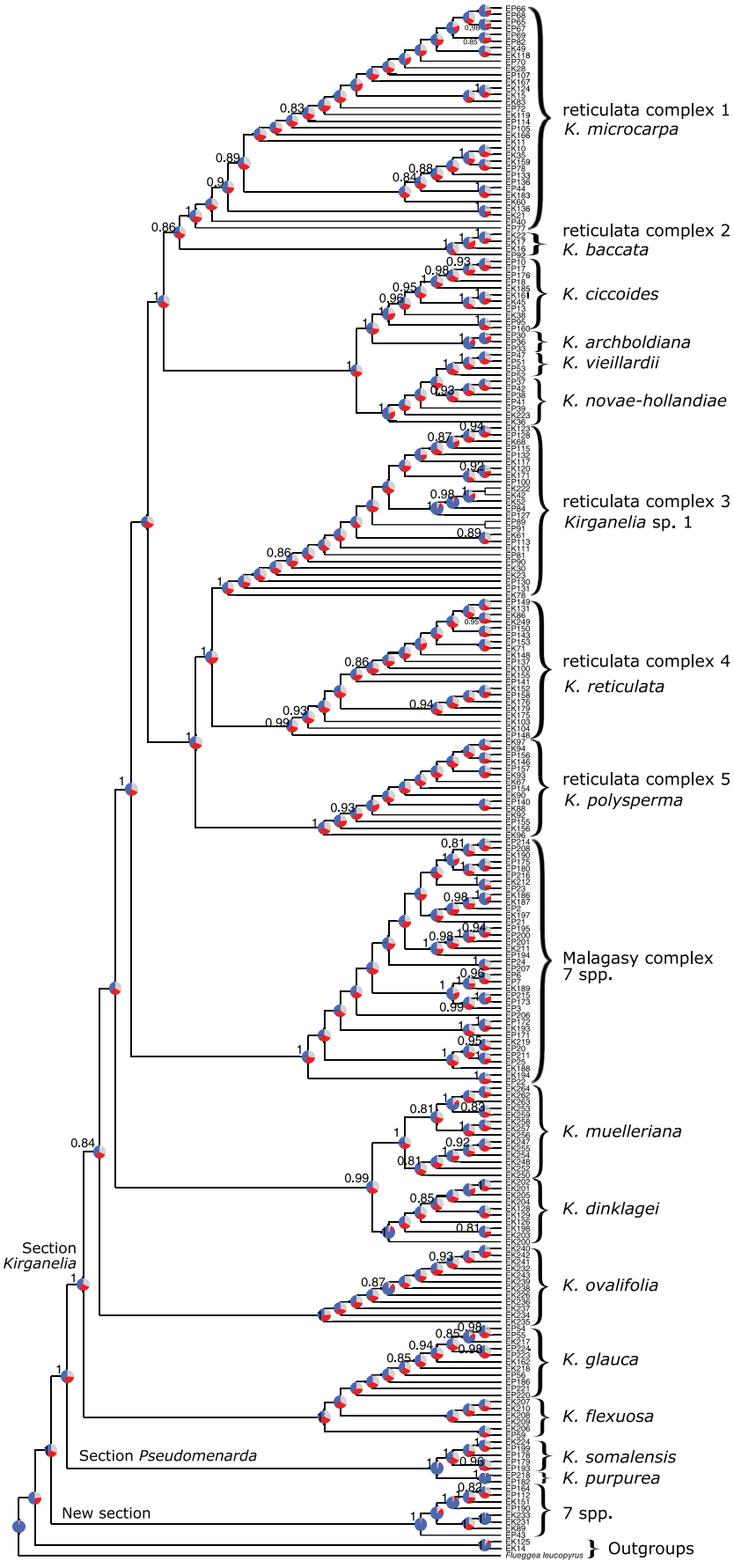

Fig. 2. ASTRAL species tree of *Kirganelia* based on a supercontig dataset (233 samples) mapped using the Angiosperms353 bait set. At each internal node, pie charts indicate quartet support proportions: blue portions (q1) represent the proportion of concordant quartets supporting the main topology, while red (q2) and grey (q3) portions represent the two alternative topologies, respectively. Local posterior probability (LPP) scores ≥0.8 are shown as numeric labels adjacent to the corresponding nodes. Curly braces next to terminal taxa denote cohesive specimen groups that are likely to represent distinct species; the composition of each group corresponds to the same specimen sets shown in the supermatrix tree (Fig. 1A).

species within the *Kirganelia reticulata* complex, the concatenated supermatrix tree showed gCF values below 10% and sCF values of 43%–59%, indicating low gene-tree support but moderate site signal for these splits. In contrast, the ASTRAL species tree recovered the same nodes with high LPP (0.99–1.00), reflecting strong confidence of the model in these nodes despite gene tree discordance, and moderate quartet concordance (q1, 0.46–0.60), indicating partial support for the dominant topology across quartets.

ADMIXTURE analyses ($K = 2, \ldots, 10$) revealed patterns of genetic admixture among lineages (Fig. 1B, cross validations are provided in suppl. Fig. S3B,C). Based on 1976 SNPs retained after LD pruning (from an initial 50,449 variants; 86.13% genotyping rate), the analysis identified distinct ancestral clusters and varying levels of shared ancestry across geographic regions. Although the lowest cross-validation error was observed at $K = 6$, the clustering pattern at $K = 5$ corresponded more closely with the major clades inferred from phylogenetic and network-based analyses (Fig. 1B). At this level, the ADMIXTURE bar plots broadly reflected the primary clade structure. However, the genetic boundaries between species complexes were not sharply defined. Rather than forming clearly distinct clusters, the ancestry proportions inferred from the ADMIXTURE analysis show gradual transitions and overlapping patterns, particularly among individuals located in between species groups.

The NeighborNet network based on 125 individuals revealed the same five major genetic clusters recovered in the phylogenetic analyses, but with clearer separation among species and more pronounced reticulation near the network centre. As in the previous analysis, these clusters correspond to five previously delimited species and fall into two broader clades that reflect higher-level relationships. Within the first major clade, *Kirganelia polysperma* remains well separated with long, coherent branches and virtually no internal reticulation. *Kirganelia reticulata* forms a large and dense cluster with substantial branch length and moderate reticulation at its base. The African lineage "sp. 1" occupies a position closer to the central hub of the network and shows increased reticulation, consistent with a more complex evolutionary signal. In the second major clade, *K. microcarpa* forms an elongated but relatively tidy cluster with little internal reticulation, whereas *K. baccata*, *K. ciccoides*, and the New Caledonian species (*K. novae-hollandiae*, *K. vieillardii*) appear as compact clusters radiating from a more reticulated central region, reflecting their closer relationships and potential historical gene flow.

The Malagasy complex. — Thirty-seven specimens from the Madagascar and neighbouring islands formed a complex group, which we here define as the Malagasy complex. We tested whether the 37 specimens formed a monophyletic group across all 353 gene trees to investigate the causes of its non-monophyly. The group was either paraphyletic or polyphyletic in 334 loci, indicating high topological discordance among gene trees. Quartet support analysis (Fig. 2) revealed substantial phylogenetic conflict, with nearly equal frequencies of alternative resolutions across internal nodes. This pattern suggests weak phylogenetic concordance among the four morphologically defined species: *Kirganelia fuscolurida* (Müll.Arg.) R.W.Bouman, *K. castica* (P.Willemet) R.W.

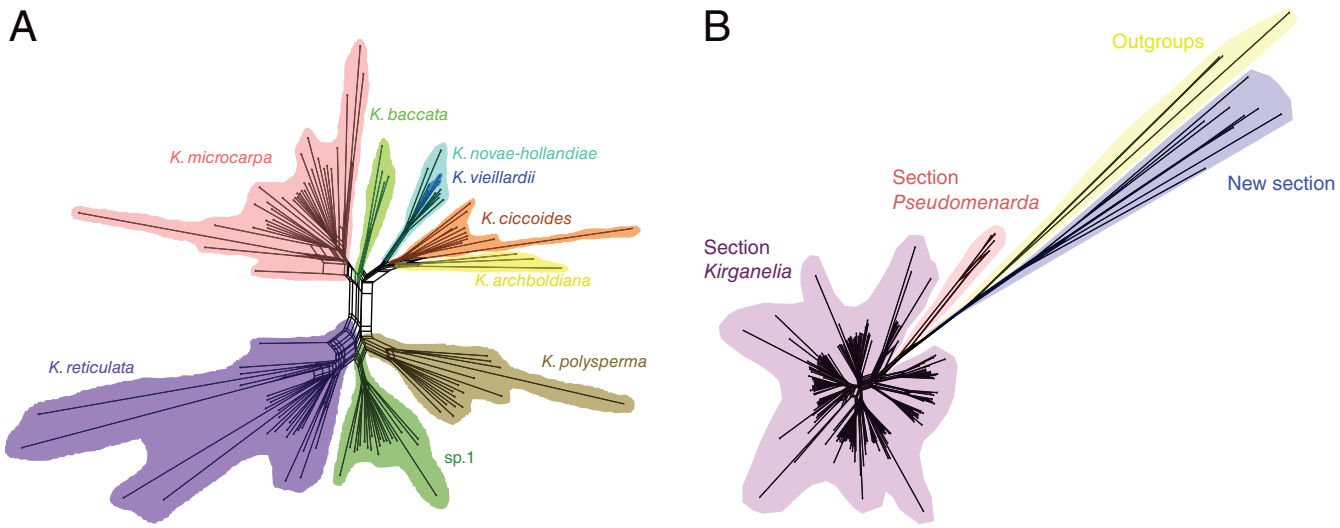

Fig. 3. NeighborNet networks of *Kirganelia* generated using SplitsTree. **A**, Splitstree NeighborNet network of 125 individuals representing nine species of *Kirganelia*. Colours indicate species identity: *K. reticulata* (purple), *Kirganelia* sp. 1 (dark green), *K. polysperma* (brown), *K. baccata* (light green), *K. microcarpa* (pink), *K. ciccoides* (orange), *K. archboldiana* (yellow), *K. vieillardii* (blue), and *K. novae-hollandiae* (cyan). The five species treated here as the *K. reticulata* complex form a compact and clearly delimited cluster, whereas *K. ciccoides*, *K. archboldiana*, *K. vieillardii*, and *K. novae-hollandiae* each form separate groupings within the broader monophyletic lineage. **B**, Network of all samples based on the full concatenated alignment. The core *Kirganelia* clade is visualized as a compact, short-branched radial cluster, while *K. zippeliana* and *K. glaucina*, both part of the newly proposed section, appear as long-branched lineages with reticulate connections, supporting their deep divergence within *Kirganelia* and justifying their recognition as a distinct section.

Bouman, *K. pervilleana* Baill., and *K. matitanensis* (Leandri) R.W.Bouman.

A detailed morphological reassessment, focusing on stigma traits and based on specimens or high-resolution images, was necessary due to widespread misidentifications within the genus; in fact, even some non-*Kirganelia* specimens were originally part of this group. Mapping the revised morphospecies onto the phylogeny revealed several polyphyletic species, indicating discrepancies between morphology and molecular data (Fig. 4A). To explore these mismatches, population structure was assessed using ADMIXTURE analysis of 37 specimens ($K = 2, \ldots, 8$; suppl. Fig. S5). Although cross-validation error was lowest at $K = 2$ and $K = 3$, $K = 4$ was selected for interpretation, as it aligned with morphologically defined species and mirrored the major clades in the supermatrix tree (Fig. 4A), and served as the basis for defining the clusters shown in Fig. 4B. Subdivision at $K = 5$ did not substantially alter the pattern, suggesting that the inferred structure is stable and biologically meaningful.

Cluster A (Fig. 4B) appeared three times and predominantly represented *Kirganelia fuscolurida* (holotype, EP206, included). Interestingly, this cluster was recovered three times across the ADMIXTURE results, roughly corresponding to phylogenetic clades identified morphologically as *K. fuscolurida*. In contrast, two samples (EP23, EK212) identified as *K. fuscolurida* fell into cluster B, which also contained three samples identified as *K. matitanensis* with a longer style. All samples in cluster A, added with samples EP23 and EK212 from cluster B, were used as input for the PhyloNet analysis (Fig. 5A). The PhyloNet results showed that all samples identified as *K. fuscolurida* were grouped within the same clade and that the *K. matitanensis* samples with longer styles formed a separate clade, with gene flow detected from *K. matitanensis* into two *K. fuscolurida* samples (EP24, EP207).

Cluster B (Fig. 4B) predominantly represented *Kirganelia pervilleana*. However, the isolectotype of *K. pervilleana* (designated by Friedmann, 1944; sample EP173), together with another sample of *K. pervilleana* (EP215), was unexpectedly grouped into cluster C along with one of the two *K. castica* morphotypes: *K. castica* "small leaves" (Fig. 4C). Notably, the other *K. castica* morphotype, *K. castica* "big leaves" did not group with the "small leaves", showing a separation between the two morphotypes. To clarify this pattern, the PhyloNet analysis was performed jointly on samples from clusters B and C. The PhyloNet results (Fig. 5B) showed gene flow between *K. pervilleana* and both big- and small-leaved *K. castica*. The former hybridization resulted in sample EK197, while the latter resulted in the clade containing samples EP215 and EP173. In addition, Fig. 5B supported the separation of *K. castica* big- and small-leaves morphotypes into distinct species, as they were placed in different clades.

Cluster D consisted of five samples identified as *Kirganelia matitanensis* and two samples suspected to represent a new species. In addition, three samples identified as *K. matitanensis*, noted for having longer styles, were assigned to cluster A. Therefore, a combined PhyloNet analysis was performed for clusters A and D (Fig. 5C). The

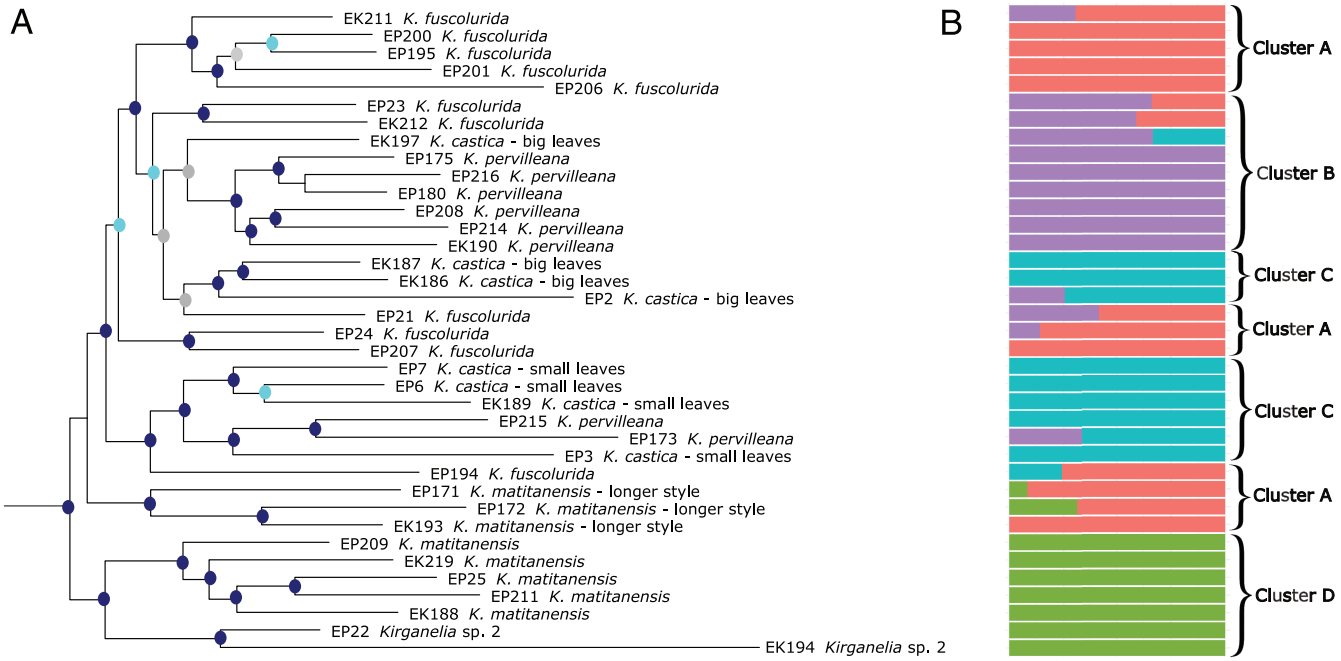

Fig. 4. Phylogenetic and population structure analyses of the Malagasy complex. **A**, Maximum likelihood supermatrix tree. Node support is indicated by coloured circles: dark blue for 100% bootstrap, light blue for 90%–99%, and grey for 80%–89%. Sample names in black represent proposed morphospecies identified in this study. **B**, ADMIXTURE analysis showing the genetic structure of four clusters: cluster A (red), cluster B (purple), cluster C (blue), and cluster D (green).

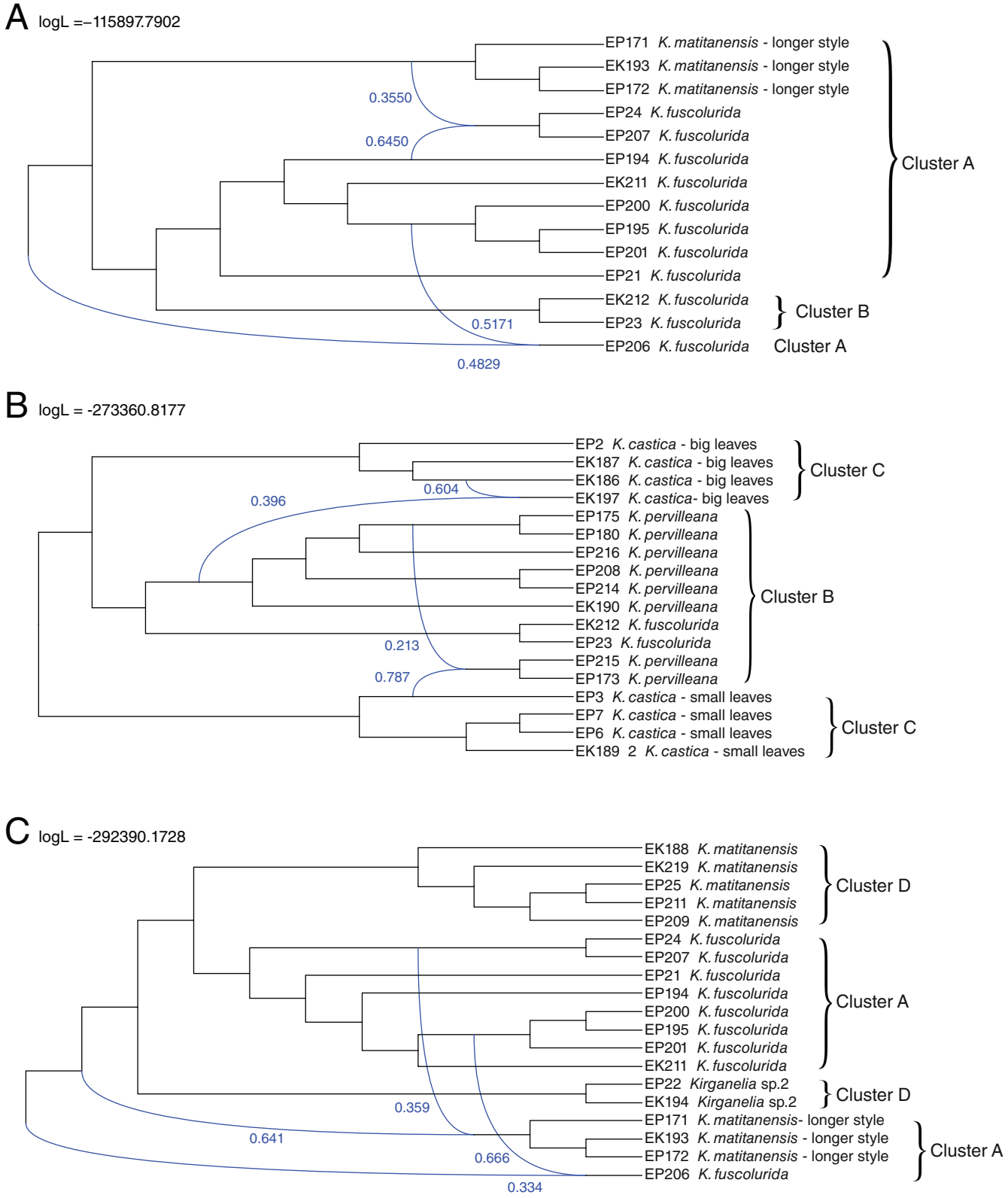

Fig. 5. Reticulation networks inferred by PhyloNet using the best-fit model with two reticulation events, selected based on AIC and BIC comparisons across models with one to three reticulations and their equivalent biological interpretation. Blue branches indicate inferred gene flow, with values denoting inheritance probabilities from parental lineages. Each subfigure represents a subset of samples grouped by dominant Admixture colours: **A**, Cluster A with EK212 & EP23 from cluster B; **B**, Cluster B & C; **C**, Cluster A & D.

results showed a clear separation between *K. matitanensis* in cluster A and cluster D, supporting the observed morphological difference in style length for cluster A. There was also evidence of gene flow from *K. fuscolurida* to *K. matitanensis* with longer styles, explaining their placement together in cluster A. Furthermore, the distinct position of sample EP22 and EK194 relative to the remainder of cluster D (Fig. 5C) represents a phylogenomic pattern consistent with the recognition of a distinct, yet undescribed species.

The phylogenetic network inferred using SplitsTree reflects the reticulation events estimated from PhyloNet (Fig. 6). The network preserves the major groupings observed in both the phylogenetic tree and ADMIXTURE analyses. Samples EP22 and EK194 are recovered as a distinct group, supporting our conclusion that these specimens represent a distinct, undescribed species. The two subclades of *Kirganelia castica* remain visually distinct, with EP173 and EP215 positioned closer to other *K. castica* samples than to *K. pervilleana*, retaining the reticulation observed from PhyloNet (Fig. 5B). This placement is consistent with their assignment to the cluster C, despite their original morphological identification as *K. pervilleana*. The network structure reveals ambiguous affiliations among several lineages (e.g., *K. fuscolurida* and *K. pervilleana*), but does not show clear reticulate connections, such as between EP173/EP215 and the rest of *K. pervilleana*. The broader separation of *K. matitanensis* and *K. fuscolurida* clades is also retained.

To further evaluate whether a single PhyloNet analysis could recover a reticulated history uniting all major Malagasy lineages, we conducted four additional maximum pseudo-likelihood network inferences using alternative taxon subsets defined by ADMIXTURE clusters, SplitsTree structure, and placements in the supermatrix phylogeny. Each subset consistently recovered several cross-cluster introgression edges and reproduced key reticulation signals observed in the cluster-based analyses. However, no individual subset yielded a network that simultaneously accounted for the full range of relationships among the focal lineages. In particular, some subsets recovered clear gene-flow patterns for *Kirganelia fuscolurida* but not for *K. pervilleana*, whereas others resolved the latter but left portions of *K. fuscolurida* unexplained (suppl. Fig. S6). These outcomes indicate that the heterogeneous and taxon-specific signals underlying the Malagasy complex are not fully recoverable under a single-run configuration of the maximum pseudo-likelihood framework and that reticulation patterns remain sensitive to taxon sampling. Detailed networks for all four subset analyses are provided in suppl. Fig. S6.

■ DISCUSSION

Angiosperms353 performance for *Kirganelia*. — The Angiosperms353 bait set recovered loci efficiently across *Kirganelia* samples. Only seven loci consistently showed poor recovery (<100 of 233 samples), while at the sample level, 88% of accessions recovered more than 90% of loci and 25 samples recovered fewer than 75% (suppl. Fig. S1), likely due to

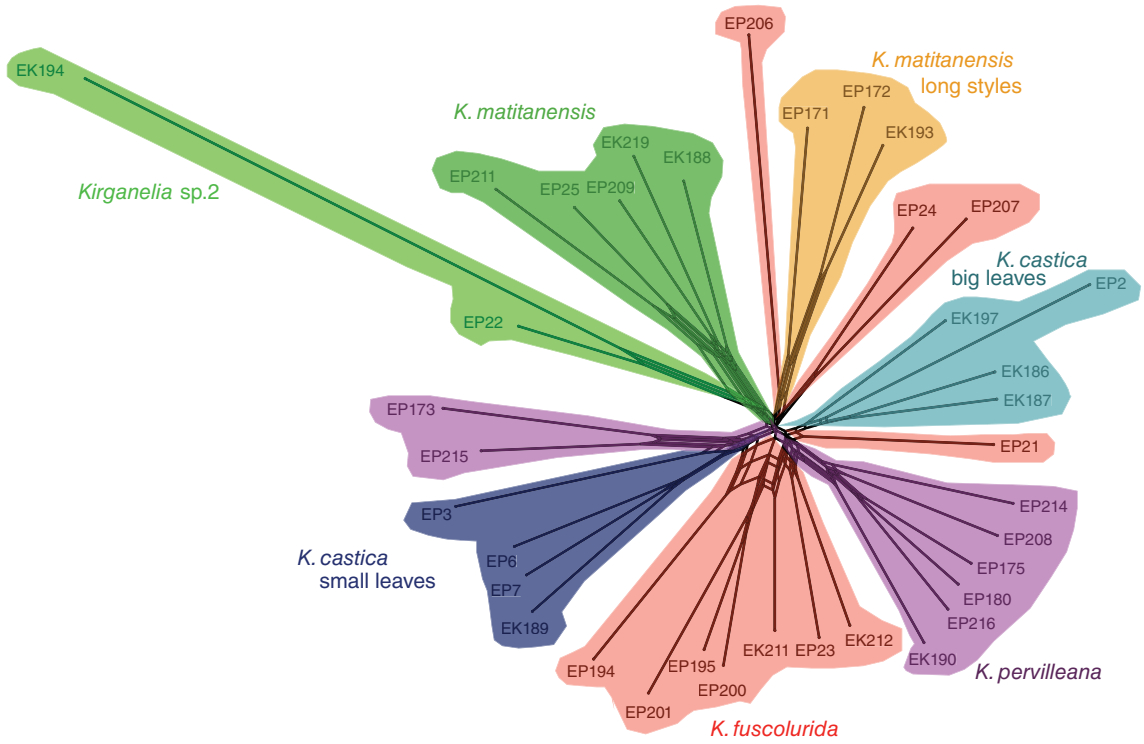

Fig. 6. NeighborNet network of 37 individuals of the Malagasy complex, based on a concatenated alignment of 353 loci. Reticulations indicate potential gene flow or incomplete lineage sorting among subclades.

degraded DNA from historical herbarium specimens. These results confirm the bait set's suitability for *Kirganelia*, but also emphasize that DNA quality remains critical. Additionally, including non-coding regions in the supercontigs increased alignment lengths and slightly improved gene and site concordance factors (Table 1), reflecting the additional informative variation contributed by captured introns and flanking regions.

Choosing the backbone tree: supermatrix versus coalescent approaches. — Our dataset showed a strong gene tree discordance, with quartet support (QS) values at key nodes indicating nearly equal support for alternative topologies (Fig. 2). This likely reflects complexities in the evolutionary history of *Kirganelia*, including ancestral polymorphism, introgression, or hybridization (Figs. 3, 5). Similar patterns of discordance have been observed in other plant groups, such as *Loricaria* (Kandziora & al., 2021) and *Tulipa* (Z. Zhang & al., 2025), where extensive incomplete lineage sorting and reticulate evolution contributed to pervasive phylogenomic conflict. Comparison of the supermatrix and coalescent-based trees showed that they were broadly similar, differing only slightly in specimen placement within species and supporting comparable conclusions (suppl. Fig. S7). Notably, the coalescent tree showed *K. novae-hollandiae* to be paraphyletic and split into multiple subclades, while *K. vieillardii* remained monophyletic; similar within-species splitting appeared in *K. glauca* and *K. flexuosa*. When samples were ordered according to the supermatrix topology, ADMIXTURE cluster patterns aligned more clearly with major clades, confirming interpretation (Fig. 4). For these reasons, we, prefer the supermatrix tree as the primary framework for interpreting interspecific relationships, providing a clear framework for comparative and integrative analyses.

A new section for *Kirganelia*. — Although *Kirganelia zippeliana* and *K. glaucina* were previously classified in *K.* sect. *Kirganelia* (Bouman & al., 2022), our analyses place them outside this group, forming a distinct clade together with several related taxa (Fig. 1). Genetic distance and gene tree placement analyses across 350 loci showed that this clade is closer to the core *Kirganelia* sections than to any outgroup, yet remains relatively divergent (interspecific distance 0.1696; suppl. Table S4) and was recovered within *Kirganelia* in ~79% of gene trees. To further examine this clade, we conducted a preliminary morphological assessment focusing on fruit and stigma traits, which identified seven coherent groups, including *K. zippeliana*, *K. glaucina*, and five additional taxa—four likely undescribed species and *Moeroris retinervis* (Hutch.) R.W.Bouman. These combined molecular and morphological observations suggest that this clade may merit sectional recognition and further taxonomic study.

Species delimitation in *Kirganelia* sect. *Kirganelia*. — While *Kirganelia* sect. *Pseudomenarda* follows Bouman & al. (2022), *K.* sect. *Kirganelia* is redefined with notable changes and now comprises 21 species: nine well-defined species, five from the *K. reticulata* complex, and seven from the Malagasy complex (Fig. 1). The nine well-defined species, detailed here in alphabetical order, were recovered as monophyletic in the supermatrix analysis: *K. archboldiana*, *K. ciccoides*, *K. dinklagei*, *K. flexuosa*, *K. glauca*, *K. muelleriana*, *K. novae-hollandiae*, *K. ovalifolia*, and *K. vieillardii*. The monophyly of these nine species was further supported by morphological examination, comparison with the original protologues, and verification of type specimens, either by physical inspection or, when access was not possible, by consulting high-resolution digital images from online herbarium databases. Among the available morphological traits, stigma structure was the feature most consistent with the molecularly inferred species boundaries, particularly in lobe number, fusion, bifurcation, and orientation. This aligns in part with historical classifications, as Brunel (1987) treated *K.* sect. *Kirganelia* and sect. *Anisonema* together and placed *K. muelleriana* within that broader group. *Kirganelia* sect. *Pseudomenarda* was originally proposed by Müller (1863) and later adopted by Brunel (1987). The two species complexes are discussed separately in the following sections, and a full taxonomic treatment for the entire section will be presented in a forthcoming publication.

Molecular confirmation could not be obtained for *Kirganelia angavensis* (Leandri) R.W.Bouman and *K. keyensis*. Of *K. keyensis*, only the type is known, and sampling was not permitted due to limited leaf material. However, based on stigma characters of the type and its documented distribution, we propose to synonymize it with *K. microcarpa* (Benth.) Hurus. & Yas.Tanaka. For *K. angavensis*, four specimens were sampled but failed during processing or did not meet quality standards for analysis. The Plants of the World Online (POWO; https://powo.science.kew.org/) database recognizes *Phyllanthus angavensis* Leandri as a synonym of *P. casticum* P.Willemet. To clarify this, we compared the types, which showed identical stigma characters in number, bifurcation, and orientation. Based on this morphological congruence and lacking molecular support, we treat *K. angavensis* as a heterotypic synonym of the *K. castica* "big leaves" group.

Five lineages within the *Kirganelia reticulata* complex. — Phylogenetic and genomic analyses consistently support dividing the *Kirganelia reticulata* complex into five distinct lineages, each broadly matching geographic regions, as resolved by the supermatrix phylogeny (Fig. 1A) and the ASTRAL tree (Fig. 2). Four of these lineages match existing species delimitations, leaving the fifth as a new species to be described. Despite this, the balanced QS proportions, low gCF, and high LPP scores at key nodes indicate well-supported but also conflicting signals, likely due to incomplete lineage sorting and gene flow. The SplitsTree Neighbor-Net (Fig. 3A) supports the five-group structure while showing varying degrees of reticulation: *Kirganelia microcarpa* and *K. baccata* diverge clearly, whereas the other groups cluster centrally with more complex connections, suggesting hybridization or other non-bifurcating processes. ADMIXTURE results show gradual ancestry transitions within the complex (Fig. 1B), rather than sharp boundaries, consistent with recent divergence and gene flow. Together, these patterns underscore

the complexity of lineage formation in *K. reticulata*, likely shaped by ecological factors such as moth pollination and fruit traits influencing parasitism and reproduction.

In this study, *Kirganelia baccata* is treated as a distinct species, despite its listing as a synonym of *Phyllanthus ciccoides* Müll.Arg. var. *ciccoides* in major taxonomic databases, including the Australian Plant Name Index (APNI; https://biodiversity.org.au/nsl/services/apni), the Atlas of Living Australia (ALA; https://www.ala.org.au/), and POWO, and as unchecked in WFO (World Flora Online). Notably, none of these sources cite a specific taxonomic revision supporting the synonymy. Our phylogenetic analyses place *K. baccata* in a clade clearly separated from *P. ciccoides* and instead closely allied with *K. microcarpa*. Morphologically, *K. baccata* is distinguished from *K. microcarpa* by its larger, globose fruits and broader leaves, and by its geographical distribution, which is restricted to northeastern Australia and the Lesser Sunda Islands. These consistent molecular and morphological differences support its recognition as a distinct species, in line with the treatment by Bouman & al. (2022).

The previous work by Luo & al. (2011), based on only 13 specimens, recognized *Kirganelia reticulata* and *K. microcarpa* for Asian entities and *K. polysperma* for African ones. By incorporating over 100 specimens from a wider geographic range, covering Asia (tropical and subtropical regions), Africa, Malesia, Oceania, and Australia, our study resolves five distinct species: two in Africa (*K. polysperma*, *Kirganelia* sp. 2), two spanning Asia and Oceania (*K. microcarpa*, *K. reticulata*), and one restricted to Australia and the Lesser Sunda Islands (*K. baccata*). Although each lineage shows varying degrees of genetic cohesion and some gene tree discordance remains, the combined phylogenetic, morphological, geographic, and population-level evidence supports recognizing these as evolutionarily distinct species. A comprehensive taxonomic revision, including formal species descriptions and nomenclatural treatment, will be provided in a subsequent publication.

The Malagasy complex: from complexity to clarity. — Phylogenomic evidence supports redefining the Malagasy complex from four to seven species-level lineages (Figs. 4, 5), expanding upon the four morphologically recognized species described by Ralimanana & Hoffmann (2011): *Kirganelia castica*, *K. fuscolurida*, *K. matitanensis*, and *K. pervilleana*. While their study provided a well-resolved treatment based on morphology, our results reveal previously undetected genetic structure, reticulation, and polyphyly, justifying the use of the term "complex". We recover seven lineages—*K. castica* (with big- and small-leaved forms), *K. fuscolurida*, *K. matitanensis*, *K. matitanensis* with longer styles, *K. pervilleana*, and a novel lineage (EP22, EK194) provisionally labelled *Kirganelia* sp. 2. This is supported by the supermatrix phylogeny, ADMIXTURE clustering (Fig. 4), and phylogenetic networks (Figs. 5, 6), which indicate shifting ancestry and historical gene flow (~0.2–0.7), especially between *K. fuscolurida* and *K. matitanensis*. The following sections detail the evidence for each lineage.

Our analyses identify two distinct species within what was previously treated as *Kirganelia castica*, distinguishable by leaf size and stigma characteristics (Fig. 5B): the small-leaf lineage has three free stigmas, while the large-leaf lineage has partially fused styles with three lobes. Although stigma traits may become ambiguous in mature fruits, they remain taxonomically informative and the morphological patterns found are congruent with phylogenetic, ADMIXTURE, network, and PCA analyses (Euclidean distance between lineages: 0.33; within-group: 0.13; Fig. 7). Patristic distances mirror these findings (0.08 between vs ~0.02 within). One ambiguous sample, EK197, shares stigma traits with the large-leaf lineage but shows conflicting signals: it clusters with *K. pervilleana* in phylogeny and ADMIXTURE (Figs. 4A,B), and patristic distance places it closer to *K. pervilleana*; yet PhyloNet identifies it as a hybrid with *K. castica* as the major parent, and SplitsTree groups it clearly with *K. castica* "big leaves" (Figs. 5B, 6). We therefore treat EK197 as part of *K. castica* "big leaves", likely reflecting historical introgression.

Sample EP173, the isolectotype of *Kirganelia pervilleana*, and EP215, a morphologically matching specimen, are both distinct morphologically from *K. castica*, particularly in stigma structure, yet phylogenetically fall within cluster C alongside small-leaf *K. castica* specimens (Fig. 4B). Patristic distances reflect this conflict: EP173 is genetically closer to small-leaf *K. castica* (0.023) than to other *K. pervilleana* specimens (0.027), and EP215 shows a similar trend (0.019 vs 0.023). Interestingly, EK197, a representative of big-leaf *K. castica*, also shows evidence of genetic proximity to this cluster, suggesting that gene flow may have occurred across all three entities. Despite these affinities, EP173 and EP215 retain diagnostic morphological traits consistent with *K. pervilleana*. Their intermediate genetic positions, supported by PhyloNet analyses indicating introgression (Fig. 5B), suggest historical gene flow among small-leaf *K. castica*, big-leaf *K. castica*, and *K. pervilleana*. In the absence of a clear molecular separation, we rely on consistent morphological differences—especially in stigma structure and leaf architecture—to maintain the recognition of *K. pervilleana* as a distinct species with a complex evolutionary history.

The morphospecies *Kirganelia matitanensis* comprises two genetically distinct lineages: individuals with longer styles in ADMIXTURE cluster A, and typical *K. matitanensis* in cluster D. This pattern is evident in ADMIXTURE results (Fig. 4B) and is supported by the phylogeny (Fig. 4A), network analyses (Fig. 6), and PhyloNet-inferred parentage (Fig. 5C). The three long-style individuals originate from different historical names that were synonymized under *Phyllanthus matitanensis* by Ralimanana & Hoffmann (2011). Specifically, EP171 (P00535922) is the holotype of *Phyllanthus albolapidosi* Leandri, EP172 (P00535918) is the holotype of *P. mantsakariva* Leandri, and EK193 closely resembles both. PCA further separates the two lineages (Euclidean distance 0.44 vs 0.08; Fig. 7), and patristic distances reflect the same pattern (0.0235

between clusters vs 0.0157 within cluster A and 0.0134 within cluster D; suppl. Table S4). These results support recognizing both lineages as distinct species and challenge the current synonymization of *P. albolapidosi* and *P. mantsakariva* under *K. matitanensis*, as the correct name depends on nomenclatural priority.

Although cluster A in Fig. 4B predominantly includes *Kirganelia fuscolurida* and appears scattered across multiple subclades in the phylogenetic tree, both genetic cohesion and morphological consistency support treating it as a single species. PCA results (Fig. 7) show that core *K. fuscolurida* samples form a distinct cluster (mean distance = 0.198), substantially lower than intergroup distances (0.382–0.761); the closest genetic neighbour, *K. matitanensis*, is clearly separated despite some phylogenetic substructuring. Patristic distances are similarly low within *K. fuscolurida* (0.0165) compared to values between groups, such as 0.0235 between clades A and D of *K. matitanensis* (suppl. Table S4). Although the holotype EP206 is slightly peripheral in some analyses, it shares the same ADMIXTURE cluster and shows gene flow with the main group, supporting its inclusion. Two specimens (EK212, EP23) fall into cluster B (*K. pervilleana*) in ADMIXTURE, but exhibit key *K. fuscolurida* traits such as short peduncles, and are placed in reticulate positions by both PhyloNet (Fig. 5A) and SplitsTree (Fig. 6), consistent with intermediate ancestry. A comparable situation is reported in *Primula elatior* (Stubbs & al., 2022), which also exhibits phylogenetic non-monophyly due to historical admixture,

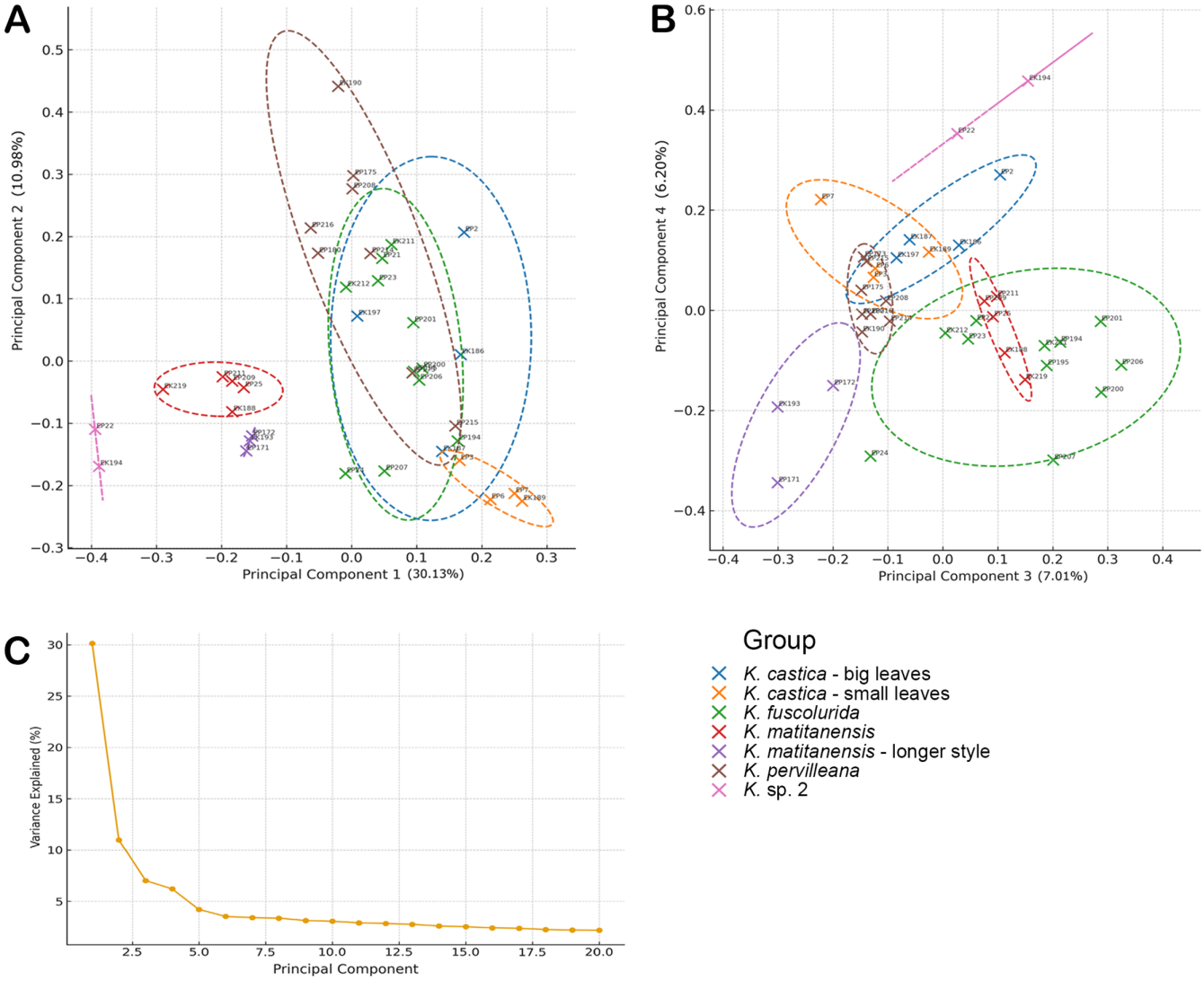

Fig. 7. Principal component analysis (PCA) of *Kirganelia* samples based on genetic variation. **A**, PC1 vs PC2; **B**, PC3 vs PC4; **C**, Scree plot showing the proportion of variance explained by each principal component. — Samples are coloured and grouped according to final species delimitations. Ellipses represent 95% confidence intervals for each inferred species cluster. The PCA reveals clear separation among several lineages, including *K. fuscolurida*, *K. castica* (big- and small-leaved forms), *K. pervilleana*, *K. matitanensis*, *K. matitanensis* with longer-style form, and *Kirganelia* sp. 2.

yet is retained as a single species based on morphological uniformity and overall genetic continuity. In light of this, we interpret *K. fuscolurida* as a single evolutionary unit shaped by reticulate history, emphasizing the value of integrating phylogenetic, population, and morphological data in species delimitation.

Our results indicate that samples EP22 and EK194 represent a previously unrecognized species within *Kirganelia*. Sample EP22 was initially identified as *K. fuscolurida* due to its short peduncles, but unlike *K. fuscolurida*, it has 5–7 free stigmas rather than partially fused ones. Sample EK194, by contrast, was first assigned to *K. castica* (big leaves) owing to the absence of peduncles; however, this likely reflects a fruiting-stage condition rather than true morphological affinity. Both samples fall within the cluster D associated with *K. matitanensis* (Fig. 4A) but differ from it in key traits. PhyloNet places them in a separate clade with no gene flow with *K. matitanensis* (Fig. 5C), and PCA confirms strong cohesion between the two (Euclidean distance 0.18; Fig. 7) and clear separation from *K. fuscolurida*, *K. castica* (big leaves), and *K. matitanensis* (distances all >0.74). SplitsTree shows limited reticulation with *K. matitanensis* (Fig. 6), indicating historical contact but genetic independence. Patristic distances further support this distinction: EP22 and EK194 are closest to each other (0.0242), with EK194 showing distances >0.035 to all other species groups, and EP22 consistently closer to EK194 than to any existing lineage (suppl. Table S4).

Ralimanana & Hoffmann (2011) recognized two varieties of *Phyllanthus fuscoluridus*: var. *fuscoluridus* (3–7 stigmas) and var. *villosus* (Leandri) Ralim. & Petra Hoffm. (3–4 stigmas). However, our phylogenetic results indicate a much higher correlation between stigma characters than indument characters. This likely has to do with the very specific moth pollination between Phyllantheae and the moth genus *Epicephala* (Kato & Kawakita, 2017). Therefore, we treat *Kirganelia fuscolurida* as a single taxon characterized by 3–5 stigmas partly fused into a column, without recognizing varieties. Specimens previously identified as *P. fuscoluridus* var. *fuscoluridus*, typically glabrous and bearing 3–7 stigmas, may fall into two groups: those with 5–7 free stigmas correspond to the clade represented by EP22 and EK194 and are here considered as a new species, while those with 3–5 partly fused stigmas are considered to be *K. fuscolurida*.

While this study focuses on resolving seven core lineages within the Malagasy complex, several peripheral samples warrant brief mention due to unexpected placements. EP3 from Mauritius shows a full cluster C ADMIXTURE profile consistent with *Kirganelia castica* (small-leaf form), but falls outside the main *K. castica* clade, suggesting a distinct insular lineage. EP173 from the Seychelles, the type of *K. pervilleana*, shows mixed cluster B–C ancestry and clusters with EP215 (Madagascar) and EP3, rather than with the core *K. pervilleana* clade. In contrast, EP175 and EP180 from Mayotte have uniform cluster B profiles and group with the core *K. pervilleana*, indicating a stable island extension. These peripheral cases highlight additional diversity and biogeographic structure in the Mascarene region, meriting further study.

■ CONCLUSION

Species delimitation in *Kirganelia* reveals a spectrum of evolutionary complexity, ranging from well-resolved clades to deeply reticulate lineages. Phylogenetic inference alone was sufficient for some groups, such as the *K. reticulata* complex, whereas the Malagasy lineages required an integrative approach combining morphology, population structure, phylogenetic networks, multivariate genetic analysis, and patristic distances due to their topological discordance and morphological ambiguity. By synthesizing genomic and morphological data, we clarified historically conflated taxa, recognized morphologically subtle groups, and uncovered patterns of genetic differentiation, particularly within the Malagasy complex. These findings demonstrate that phylogeny alone is insufficient for species delimitation in groups shaped by hybridization, introgression, and incomplete lineage sorting, especially under scenarios of recent speciation and overlapping geographic ranges. Instead, species boundaries are supported where phylogenomic, morphological, and geographic evidence are concordant. While some lineages show signals of hybrid ancestry (e.g., in the Malagasy clade), they were recognized as distinct species only when genetically cohesive and morphologically consistent. Our forthcoming revised taxonomy establishes a stable basis for formal nomenclatural treatment, to be published separately, and provides a clarified framework for future biogeographic and evolutionary studies.

■ AUTHOR CONTRIBUTIONS

EKP conceived and designed the study; conducted sampling and molecular laboratory work; performed bioinformatic and formal analyses; curated the data; and led the writing of the original draft. BW contributed to data analysis and interpretation. KPH contributed to data analysis and interpretation. PCvW initiated the research, contributed to its conceptualization, and contributed to data analysis. RWB contributed to the conceptualization of the study through the development of a phylogenetic framework informing sampling design and initial species concepts. RFAB contributed to bioinformatic analyses. SMdO contributed to data analysis and interpretation. All authors contributed to interpreting the results and approved the final version of the manuscript.

■ ACKNOWLEDGEMENTS

This article forms part of the Ph.D. thesis of EKP, funded by the Indonesia Endowment Fund for Education (LPDP, Scholarship No. 202203221508169). We acknowledge the following herbaria for granting sampling, herbarium loans, and/or providing high-quality images: A, BM, BR, E, G, K, L, MO, P, TI, U, WAG. We also acknowledge the PAFTOL project for providing comparative phylogenomic data that informed outgroup designation. Special thanks to Luthfi Nurhidayat for his generous assistance throughout the bioinformatics workflow. This work was performed using the computing resources of the

Academic Leiden Interdisciplinary Cluster Environment (ALICE) provided by Leiden University.

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Appendix 1. Voucher information.

Taxon, specimen ID, origin, collector and collection number (herbarium), NCBI Sequence Read Archive (SRA) accession number.

Kirganelia archboldiana (Airy Shaw & G.L.Webster) R.W.Bouman, EP36, Indonesia, New Guinea, *Brass 11660* (L 0016410), SRR38243939; EP30, Indonesia, New Guinea, *Brass 11661* (L.2252338), SRR38243941; EP33, Indonesia, New Guinea, *Schiefenhövel 416* (L.3957162), SRR38243940; ***Kirganelia baccata*** (F.Muell. ex Benth.) R.W.Bouman, EP92, Indonesia, Sumba, *Verheijen 3940* (L.2247348), SRR38243764; EK17, Australia, Western Australia, *Foulkes 255* (L.2247369), SRR38243753; EK16, Australia, Northern Territory, *Rankin 1269* (L.2247365), SRR38243759; EK22, Australia, Western Australia, *Kenneally 9166* (L.2247291), SRR38243813; ***Kirganelia castica* - big leaves**, EK187, Madagascar, *Eboroke 963* (WAG.1354771), SRR38243872; EK186, Madagascar, *Philipson 2338* (WAG.1354763), SRR38243874; EP2, Madagascar, Herb. Museum Paris 8405 (WAG.1354764), SRR38243829; EK197, Madagascar, *Humbert 19.654* (WAG.1354766), SRR38243866; ***Kirganelia castica* - small leaves**, EP7, Madagascar, *Jongkind & al. 3344* (WAG.1354774), SRR38243777; EP6, Madagascar, *Ramamonjisoa 2030* (WAG.1354769), SRR38243783; EP3, Mauritius, *Splitgerber s.n.* (L.2252768), SRR38243942; EK189, Madagascar, *Unknown 8150* (WAG.1354765), SRR38243870; ***Kirganelia ciccoides*** (Müll.Arg.) R.W.Bouman, EP10, Papua New Guinea, *Hollrung 332* (L.2059158), SRR38243954; EP17, Vanuatu, Uliveo, *Curry 874* (L.2252686), SRR38243880; EK185, Papua New Guinea, *Craven & Schodde 41* (L.2252677), SRR38243875; EK45, Australia, Queensland, *Stoddart 4431* (L.2247370), SRR38243975; EP13, Papua New Guinea, *Brass 28087* (L.3958075), SRR38243903; EP95, Philippines, Luzon, *Vidal y Soler 1768* (L.2247432), SRR38243763; EP160, Papua New Guinea, *Manner & Street 291* (L.2059548), SRR38243882; EP176, Vanuatu, *Cabalion 2196* (K004519410), SRR38243842; EK38, Solomon Islands, Guadalcanal, *Sirute'e & al. 10134* (L.2247378), SRR38243977; EP18, Vanuatu, Espiritu Santo, *Berry 18* (L.2252687), SRR38243839; EK161, Papua New Guinea, *Darbyshire & Hoogland 8074* (L.2059446), SRR38243758; ***Kirganelia dinklagei*** (Pax) R.W.Bouman, EK129, Cameroon, *Leeuwenberg 9998* (WAG.1340288), SRR38243886; EK201, Gabon, *Bissiengou & al. 780* (WAG.1354826), SRR38243863; EK205, Gabon, *Lachenaud 1559* (WAG.1963340), SRR38243858; EK200, Congo, *Maudoux 190* (WAG.1354813), SRR38243864; EK198, Central Cameroon, *Wilde 7626* (WAG.1354814), SRR38243865; EK126, Gabon, *Breteler 6878* (WAG.1340294), SRR38243908; EK202, Gabon, *Wieringa & al. 7830* (WAG.1571761), SRR38243861; EK204, Gabon, *Bissiengou & al. 560* (WAG.1354822), SRR38243859; EK128, Cameroon, *de Wit 7579* (WAG.1340290), SRR38243897; EK203, Cameroon, *Ganbary s.n.* (U.147218), SRR38243860; ***Kirganelia flexuosa*** (Siebold & Zucc.) R.W.Bouman, EK209, Japan, Honshu, *Fukuoka 6497* (U.1472291), SRR38243854; EP59, Japan, Honshu, *Mitsuta &Takamiya s.n.* (L.2246125), SRR38243920; EK206, Japan, Shikoku, *Murata & Shimizu 722* (U.1472292), SRR38243857; EK210, Japan, Honshu, *Taoda 3272* (L.2246129), SRR38243852; EK207, Japan, Honshu, *Yahara 1324* (U.1472288), SRR38243856; EK208, Japan, Honshu, *Murata 11560* (U.1472289), SRR38243855; ***Kirganelia fuscolurida*** (Müll.Arg.) R.W.Bouman, EP201, Madagascar, *Ralimanana & Rajaonarison 345* (BR0000009752054), SRR38243827; EP206, Madagascar, *Bojer s.n.* (P00482899), SRR38243826; EP23, Madagascar, *Antilahimena & al. 4390* (WAG.1354887), SRR38243945; EP195, Madagascar, *Bernardi 11088* (BR0000016659261), SRR38243831; EP21, Madagascar, *Outer & Veenendaal 991* (WAG.1917739), SRR38243822; EP194, Madagascar, *Ralimanana & Rajaonarison 346* (BR0000009751958), SRR38243833; EP207, Madagascar, *Rakotoarivelo & al. 972* (P01155600), SRR38243825; EP200, Madagascar, *Ranaivojaona 355* (BR0000009264939), SRR38243828; EK212, Madagascar, *Humbert & Cours 17510* (WAG.1354886), SRR38243849; EP24, Madagascar, *Dorr & al. 3650* (WAG.1354884), SRR38243944; EK211, Madagascar, *Service Forestier de Madagascar SF25511* (WAG.1354885), SRR38243850; ***Kirganelia glauca*** (Wall. ex Müll.Arg.) R.W.Bouman, EP224, Bhutan, *Grierson & Long 4678* (E01105919), SRR38243947; EK162, Myanmar, *Aung & al. 92433* (L.3981482), SRR38243757; EP56, China, Anhui, *Chow 132* (L.2246194), SRR38243921; EP221, China, Jiangxi, *Min-Xiang 92165* (E00021354), SRR38243949; EP223, Nepal, *Ohba & al. 8340192* (E00113403), SRR38243948; EK217, India, Meghalaya, *Koelz 30317* (L.2246195), SRR38243848; EP54, India, Meghalaya, *Hooker & Thomson s.n.* (L.2246198), SRR38243924; EP55, India, Meghalaya, *Hooker & Thomson s.n.* (L.2246197), SRR38243922; EP220, China, Hunan, *Lin-bo 619* (E00071294), SRR38243950; EP186, Taiwan, *Namba & al. 32* (TI00278456), SRR38243836; EK218, China, Jiangxi, *Min-Xiang 92084* (L.2246193), SRR38243815; ***Kirganelia glaucina*** (Miquel) R.W. Bouman, EP164, Sumatra, *Diepenhorst HB 2105* (U 0001869), SRR38243881; ***Kirganelia macrocarpa*** (Benth.) Hurus. & Yas.Tanaka, EP107, Laos, *Kerr 20874* (L.2246873), SRR38243911; EK21, Philippines, Luzon, *Allen PNH 150066* (L.2247429), SRR38243853; EP40, Australia, Queensland, Cape York, *Hyland 21105 V* (L.2246711), SRR38243933; EK183, Papua New Guinea, *Takeumi & al. 17652* (L.3978286), SRR38243876; EP44, Papua New Guinea, *Schodde 2425* (L.2059453), SRR38243929; EP114, Taiwan, *Gressitt 99* (L.2247027), SRR38243907; EP67, China, Guangxi, *Bouman & al. 41* (L.3981478), SRR38243780; EP77, Malaysia, Borneo, *Puasa & Angian 3958* (L.2059282), SRR38243773; EK159, Indonesia, Borneo, *Kessler & al. PK 1412* (L.2059440), SRR38243760; EK60, Philippines, Luzon, *Layoza 66* (L.2247428), SRR38243972; EP78, Indonesia, Borneo, *Kessler & al. 1049* (L.3957998), SRR38243772; EP133, Indonesia, Sulawesi, *Vogel 5870* (L.2059542), SRR38243899; EP136, Indonesia, Sulawesi, *Elbert 3350* (L.2059567), SRR38243898; EK35, Brunei, Borneo, *Niel 4296* (L.2246987), SRR38243979; EK136, Indonesia, Flores, *Verheyen 741* (L.2247347), SRR38243832; EP65, China, Guangxi, *Bouman & al. 43* (L.3981476), SRR38243782; EP68, China, Guangdong, *Bouman & al. 38* (L.3981479), SRR38243779; EK10, Malaysia, Borneo, *Lugas 2586* (L.3976143), SRR38243983; EP69, Thailand, Chiang Mai, *Maxwell 89-7* (L.2246407), SRR38243778; EK11, China, Hongkong, *Hu & But PP-H 21902* (L.2246827), SRR38243873; EP66, China, Guangdong, *Bouman & al. 39* (L.3981474), SRR38243781; EK166, Laos, *Munzinger & Engelmann 181* (L.2247816), SRR38243755; EP105, Vietnam, *Alleizette s.n.* (L.2247018), SRR38243952; EK83, India, *Kramer & Nair 6605* (U.1472320), SRR38243965; EK49, Indonesia, Bali, *Balgooy 7535* (L.0583238), SRR38243974; EP72, Thailand, Nakhon Ratchasima, *Beusekom & Charoenphol 1961* (L.2246410), SRR38243774; EK118, Laos, *Sengsomphou 28* (L.2246787), SRR38243808; EK167, Northern Thailand, *Maxwell 90.668* (L.2246413), SRR38243754; EK119, Thailand, Prachuap Khiri Khan, *Middleton & al. 2535* (L.2246820), SRR38243797; EP70, Thailand, Teen Tok, *Larsen & al. 3095* (L.2246412), SRR38243775; EK15, Vietnam, *Soejarto & al. 10275* (L.2247015), SRR38243923; EK124, Thailand, Chiang Mai, *Beusekom & Phengklai 2463* (L.2246874), SRR38243970; EK28, China, Guangdong, *K'tung 78 5772* (L.2246834), SRR38243912; EP82, Vietnam, *Toyokuni & al. V 1207* (L.3976157), SRR38243770; ***Kirganelia matitanensis*** (Leandri) R.W.Bouman, EP25, Madagascar, *Antilahimena & al. 3016* (WAG.1801730), SRR38243943; EP209, Madagascar, *Antilahimena & Marcelin 5942* (P05480574), SRR38243823; EK219, Madagascar, *McPherson 17533* (L.4317892), SRR38243814; EP211, Madagascar, *Andriamahefarivo & al. 77* (P05480566), SRR38243820; EK188, Madagascar, *Lam & Meeuse 5291* (WAG.1354775), SRR38243871; ***Kirganelia matitanensis* - longer style**, EP172, Madagascar, *Service Forestier Madagascar 5820 SF* (P00535918), SRR38243846; EK193, Madagascar, *Bernardi 11908* (L.2252774), SRR38243868; EP171, Madagascar, *Humbert 18876* (P00535922), SRR38243847; ***Kirganelia muelleriana*** (Kuntze) R.W.Bouman, EK253, Cameroon, *Wilde & Wilde-Duyfjes 3269* (WAG.1801689), SRR38243787; EK263, Benin, *Adjakdjè 1899* (WAG.1810200), SRR38243914; EK264, Benin, *Sokpon & al. 233* (WAG.1810203), SRR38243913; EK262, Benin, *Akoegninou & al. 1603* (WAG.1801518), SRR38243915; EK259, Cameroon, *Fotius 2078* (WAG.1801728), SRR38243916; EK252, Cameroon, *Leeuwenberg 7715* (WAG.1801722), SRR38243788;

Appendix 1. Continued.

EK255, Cameroon, *Elad 413* (WAG.1801708), SRR38243784; EK258, Liberia, *Jongkind & al. 10084* (WAG.1801547), SRR38243917; EK247, Gabon, *Maas & al. 9992* (WAG.1801638), SRR38243792; EK254, Gabon, *Bissiengou & al. 1191* (WAG.1801599), SRR38243785; EK248, Congo, *Lisowski 85850* (WAG.1810152), SRR38243791; EK250, Malawi, *Philips 3117* (WAG.1810164), SRR38243789; EK257, Ivory, *Hepper & Maley 7992* (WAG.1801567), SRR38243918; EK256, Ghana, *Merello & al. 1247* (WAG.1810192), SRR38243919; ***Kirganelia novae-hollandiae*** (Müll.Arg.) R.W.Bouman, EP38, Australia, Queensland, *Forster & Bean 9419* (L.2246721), SRR38243937; EK36, Papua New Guinea, *Pullen 6885* (L.2059549), SRR38243978; EP37, Australia, Queensland, *Forster & Brushe 34964* (L.3800067), SRR38243938; EP41, Australia, Queensland, *Hyland 8134* (L.2246715), SRR38243932; EK223, Australia, Queensland, *Telford & Bruhl 13024* (L.2246712), SRR38243811; EP42, Australia, Queensland, *Batianoff 900449* (L.2246720), SRR38243931; EP39, Australia, Queensland, *Telford & Bruhl 13023* (L.2246713), SRR38243936; ***Kirganelia ovalifolia*** (Forssk.) R.W.Bouman, EK240, Burundi, *Becquet 105* (WAG.1799920), SRR38243796; EK232, Ethiopia, *Seegeler 2960* (WAG.1799930), SRR38243805; EK242, Tanzania, *Tanner 5177* (L.2246136), SRR38243794; EK235, Ethiopia, *Wilde & Wilde-Duyfjes 8826* (WAG.1799935), SRR38243802; EK241, Burundi, *Reekmans 7280* (WAG.1799927), SRR38243795; EK239, Congo, *Malaisse 9711* (WAG.1799921), SRR38243798; EK226, Zambia, *Bingham 9639* (WAG.1799909), SRR38243809; EK237, Congo, *Thiebaud 627* (WAG.1354912), SRR38243800; EK238, Congo, *Pierlot 2954* (WAG.1799923), SRR38243799; EK243, Uganda, *Rwaburindore 4239* (L.2246672), SRR38243793; EK234, Ethiopia, *Wilde 6304* (WAG.1799932), SRR38243803; EK236, Congo, *Witte 8121* (WAG.1354904), SRR38243801; ***Kirganelia pervilleana*** Baill., EP173, Seychelles, *Boivin s.n*. (P00482880), SRR38243845; EK190, Madagascar, *Wohlhauser & al. SW60172* (WAG.1354787), SRR38243869; EP208, Madagascar, *Bardot-Vaucoulon & Andrianantoanina 851* (P00309533), SRR38243824; EP180, Mayotte, *Barthelat 337* (K004864130), SRR38243838; EP216, Madagascar, *Pascal 871* (P00144621), SRR38243817; EP175, Mayotte, *Hoffman & al. 392* (K004864147), SRR38243844; EP214, Madagascar, *Rabenantoandro & al. 1125* (P04796539), SRR38243819; EP215, Madagascar, *Friedmann 4518* (P04796932), SRR38243818; ***Kirganelia polysperma*** (Schumach. & Thonn.) R.W.Bouman, EK97, Mozambique, *Groenendijk 313* (WAG.1340202), SRR38243955; EK156, Mozambique, *Macitela 40* (WAG.1354871), SRR38243761; EP154, South Africa, *Smook 1333* (WAG.1340271), SRR38243888; EK94, Mozambique, *Groenendijk 1837* (WAG.1340215), SRR38243957; EK146, Mozambique, *Koning 7116* (WAG.1340310), SRR38243946; EP156, Malawi, *Pawek 8694* (WAG.1340276), SRR38243885; EK93, Mozambique, *Groenendijk 1781* (WAG.1340211), SRR38243958; EK96, Mozambique, *Silva & al. 133* (WAG.1340203), SRR38243956; EP155, South Africa, *Moll 4389* (WAG.1340273), SRR38243887; EP157, South Africa, *Pienaar 48* (WAG.1340277), SRR38243884; EK90, Tanzania, *Haris 4970* (WAG.1340236), SRR38243961; EK67, Madagascar, *Lam & Meeuse 4941* (L.2247362), SRR38243969; EK92, Mozambique, *Groenendijk & Dungo 497* (WAG.1340208), SRR38243960; EP140, Kenya, *Magogo & Glover 193* (WAG.1340274), SRR38243895; EK88, Tanzania, *Fakih & Abdulla 442* (WAG.1340268), SRR38243963; ***Kirganelia purpurea*** (Müll.Arg.) R.W.Bouman, EP218, Angola, *Welwitsch Iter Angolense 329* (P04832183), SRR38243816; EP182, Namibia, *Leistner & al. 91* (K001519639), SRR38243837; ***Kirganelia reticulata*** (Poir.) Baill., EK42, Australia, Queensland, *Blake 23229* (L.2247372), SRR38243976; EP127, Timor Leste, Timor, *Cowie 11385* (L.3795193), SRR38243905; EK52, New Guinea, *Hoogland & Craven 10*.309 (L.2059555), SRR38243973; EK222, Australia, Queensland, *Hyland 9261* (L.2246710), SRR38243812; EK171, Indonesia, Java, *Popta 189/21* (L.3957978), SRR38243752; EP132, Vietnam, *Poilane 28912* (L.3784877), SRR38243900; EK117, Laos, *Vannachak & al. 1096* (L.2246826), SRR38243851; EK78, Vietnam, *Clemens 3256* (U.1472211), SRR38243966; EP131, Thailand, Phattalung, *Pooma & al. 6645* (L.3799095), SRR38243901; EK23, Srilanka, *Balakrishnan NEK 502* (L.2246800), SRR38243807; EK61, Philippines, Luzon, *Layoza 80* (L.2247417), SRR38243971; EP115, Myanmar, *Armstrong & al. 2586* (L.4432956), SRR38243906; EK68, India, Bihar, *Koelz 18703* (L.2246797), SRR38243968; EP113, Taiwan, *Hsieh & al. 943* (L.2246357), SRR38243909; EP128, Laos, *Elkington & al. 203* (L.3784639), SRR38243904; EP130, Thailand, Saraburi, *Pooma & Phattarahirankanok 6301* (L.3795211), SRR38243902; EK111, Indonesia, Sumbawa, *Elbert 3837* (L.2059565), SRR38243862; EP89, Indonesia, Timor, *Sauveur 106* (L.2247413), SRR38243768; EP84, Australia, Northern Territory, *Craven 6471* (L.2247373), SRR38243769; EP91, Indonesia, Flores, *Verheijen 5462* (L.2247411), SRR38243766; EP90, Indonesia, Timor, *Schmutz 2348* (L.2059544), SRR38243767; EK120, Indonesia, Bali, *Balgooy 7474* (L.2246822), SRR38243786; EK123, Laos, *Bouamanivong & al. 136* (L.2246997), SRR38243981; EP81, Cambodia, *Monyrak & Meng 230* (L.2247819), SRR38243771; EK30, Taiwan, *Tang 1049* (L.2246802), SRR38243980; EP100, Indonesia, Borneo, *Giesen 19 A* (L.2059537), SRR38243953; ***Kirganelia somalensis*** (Pax) R.W.Bouman, EP193, Kenya, *Bally 16865* (BR0000016681613), SRR38243834; EP178, Kenya, *Luke & al. TRP 91* (K004862036), SRR38243841; EK224, Somalia, *Killian 1718 & Lobin 6572* (WAG.1340360), SRR38243810; EP199, Somalia, *Friis I. & al. 4590* (BR0000016681590), SRR38243830; EP179, Kenya, *Lucas 60* (K004862033), SRR38243840; ***Kirganelia* sp. 1**, EP148, Bafatá, *Unknown s.n*. (WAG.1340257), SRR38243892; EP141, Kenya, *Mwangangi & Gwynne 1238* (WAG.1340275), SRR38243894; EK148, West, *Cissé 251 C* (WAG.1340303), SRR38243934; EP153, Benin, *Adjakidjè & al. 2581* (WAG.1340250), SRR38243889; EK86, Guinea, *Yonon Botanic Team 78* (WAG.1340247), SRR38243964; EK71, Cameroon, *Letouzey 9804* (WAG.1340189), SRR38243967; EK103, Congo, *Lisowski 16998* (WAG.1340226), SRR38243935; EK155, Benin, *Schäfer 8600* (WAG.1340149), SRR38243762; EK100, Ethiopia, *de Wilde 4889* (WAG.1340193), SRR38243982; EK176, Gabon, *Valkenburg 2908* (WAG.1340145), SRR38243878; EP137, Chad, *Wilde & al. 5173* (WAG.1340272), SRR38243896; EP158, Namibia, *Giess 8876* (WAG.1340279), SRR38243883; EK152, Gabon, *Wieringa 1235* (WAG.1340138), SRR38243765; EK179, Gabon, *Bergen 487* (WAG.1340139), SRR38243877; EK104, Congo, *Compére 1021* (WAG.1340224), SRR38243756; EK175, Gabon, *Bissiengou & al. 310* (WAG.1340140), SRR38243879; EK249, Senegal, *Wieringa & al. 9305* (WAG.1972408), SRR38243790; EP149, Ghana, *Jongkind & Nieuwenhuis 2491* (WAG.1340264), SRR38243891; EP143, Mali, *Cissé 74* (WAG.1340321), SRR38243893; EP150, Ghana, *Jongkind 4023* (WAG.1340266), SRR38243890; EK131, Liberia, *Jongkind 4993* (WAG.1340262), SRR38243843; ***Kirganelia* sp. 2**, EP22, Madagascar, *Schatz & al. 1737* (WAG.1354883), SRR38243951; EK194, Madagascar, *Rakoto 2* (L.4317891), SRR38243867; ***Kirganelia* sp. 3**, EK233, Ethiopia, *Friis & al. 13789* (WAG.1950464), SRR38243804; EK231, Ethiopia, *Friis & al. 14939* (WAG.1953836), SRR38243806; ***Kirganelia* sp. 4**, EP112, Taiwan, *Chiou & Chiang 11188* (L.2246356), SRR38243910; ***Kirganelia* sp. 5**, EK151, Ivory, *Versteegh & Outer 171* (WAG.1917742), SRR38243776; ***Kirganelia* sp. 6**, EP43, Australia, *Lam 7668* (L.2246718), SRR38243930; ***Kirganelia vieillardii*** Baill., EP52, New Caledonia, *Pancher s.n.* (L.2253038), SRR38243926; EP51, New Caledonia, *MacKee 42395* (L.2253035), SRR38243927; EP53, New Caledonia, *MacKee 2249* (L.2253036), SRR38243925; EP47, New Caledonia, *MacKee 29749* (L.2253032), SRR38243928; ***Kirganelia zippeliana*** (Müll.Arg) R.W.Bouman, EP190, Unknown, *Martens s.n*. (BR0000030504486), SRR38243835; ***Moeroris retinervis*** (Hutchinson) R.W.Bouman, EK89, Tanzania, *Harder & al. 1193* (WAG.1340233), SRR38243962; **Outgroups:** ***Dendrophyllanthus* sp.,** EK14, Vietnam, *Soejarto & Ninh 13619* (L.2246823), SRR38243821; ***Flueggea leucopyrus*** Willd., Flueggea, Ethiopia, *Friis I & al. 6249* (K001382206), ERR10978038; ***Nymphanthus* sp.,** EK125, India, *Sastry 40722* (L.2246807), SRR38243959.