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Phylogenetics of *Ochna* (Ochnaceae) and a new infrageneric classification

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Advances in high-throughput DNA sequencing are allowing faster and more affordable generation of molecular phylogenetic trees for many organisms. However, resolving relationships at species level is still challenging, particularly for taxonomically difficult groups. Until recently, the classification of *Ochna* had been based only on morphological data. Here, we present the first comprehensive phylogenomic study for the genus using targeted sequencing with a custom probe kit. We sampled *c*. 85% of species to evaluate the current infrageneric classification. Our results show that the data generated using the custom probe kit are effective in resolving relationships in the genus, revealing three sections consistent with the current classification and a new section consisting of species from Madagascar and the Mascarene Islands. Our results provide the first insights into the evolutionary relationships of several widespread and morphologically diverse species numerous poorly known and potentially new species to science. We demonstrate that for morphologically challenging groups such as *Ochna*, an integrated approach to classification is essential. Phylogenomic results are only informative when derived from accurately named samples. There is a symbiotic relationship between molecular phylogenomics and morphology-based taxonomy, with taxonomic expertise a requirement to accurately interpret the phylogenomic results.

ADDITIONAL KEYWORDS: anther dehiscence – HybSeq – phylogenomics – style branching – systematics – taxonomic revision.

INTRODUCTION

Ochna L. is the second largest genus of Ochnaceae with *c*. 80 species (Verdcourt, 2005; Amaral & Bittrich, 2014, POWO, 2019). The genus is palaeotropical, distributed throughout continental Africa, Madagascar and the Mascarene Islands, and has six species native to Asia (Kanis, 1968; Verdcourt, 2005). Consisting mainly of shrubs, small trees and geoxylic suffrutices, the genus holds much ecological and cultural importance. For example, *O. pulchra* Hook. is a dominant tree species in the *Burkea* Hook. – *Ochna* open deciduous savanna woodland of southern Africa (Rutherford

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& Panagos, 1982; Rutherford, 1983; Frost, 1996), *O. schweinfurthiana* F.Hoffm. is frequent in miombo woodlands and scrub woodland at the edges of dambo grassland in the Zambezian floristic regions, and *O. afzelii* R.Br. ex Oliv. is a prominent species in the wetter Sudanian savanna woodlands (White, 1983). *Ochna* spp. make up a significant portion of woody vegetation in many parts of their ranges and are an important food source for birds (Kanis, 1968) and other fauna. Other species are widely cultivated as ornamentals due to their characteristic drupaceous fruits and bright-yellow flowers (Christenhusz, Fay & Chase, 2017; Fig. 1), such as *O. serrulata* (Hochst.) Walp., which has become highly invasive in the eastern coastal districts of Australia (Gosper, Vivian-Smith & Hoad, 2006), and naturalized in parts of Hawai'i (Starr, Starr & Loope, 2003). Moreover, there have been numerous reports of medical uses of *Ochna* spp. in herbal remedies throughout Africa and Asia (Kokwaro, 1976; Perry & Metzger, 1980; Bandi *et al.*, 2012; Abdullahi *et al.*, 2014).

So far, as far as we know, there has been no molecular phylogenetic study dedicated to resolving the relationships between *Ochna* spp. Based only on morphology, early accounts of the family suggested that the genus was paraphyletic, with a doubtful separation between *Ochna* and *Brackenridgea* A.Gray (Amaral & Bittrich, 2014). The molecular phylogenetic studies of Ochnaceae of Bissiengou (2014) and Schneider *et al.* (2014) included just eight and nine samples of *Ochna*, respectively. They confirmed the monophyly of the genus, but its sister group remained uncertain. Bissiengou (2014) resolved *Ochna* as sister to *Brackenridgea* based on three plastid markers. Around the same time, Schneider *et al.* (2014) used five plastid and nuclear markers and resolved *Ochna* as sister to *Rhabdophyllum* Tiegh. The relevant nodes in the molecular phylogenetic hypotheses lacked support and were in direct conflict with morphology (Bissiengou, 2014; Schneider *et al.*, 2014), thus requiring further study. Recently, more robust analyses using high-throughput DNA sequencing (HTS) technologies, including hundreds of genes and dense taxon sampling, have provided better insights into the relationships between *Ochna* and closely related genera. A study using targeted sequencing of nuclear loci with a custom probe kit for Ochnaceae (Schneider *et al.*, 2020) resolved *Ochna* as sister to a group of palaeotropical genera comprising *Brackenridgea*, clade B of the polyphyletic genus *Campylospermum* Tiegh. and *Idertia* Farron with strong support. This result was corroborated by Shah *et al.* (2021) who used the same technique but with the universal Angiosperms353 probe kit (Johnson *et al.*, 2019). In contrast, based on plastome data, Schneider *et al.* (2021) inferred *Ochna* as sister to clade A of a polyphyletic *Campylospermum*, although *Rhabdophyllum* was lacking from the sampling. A summary of these findings is shown in Figure 2. These HTS studies provide a reliable framework for further investigation of the understudied and taxonomically difficult, but ecologically important species-rich genera of Ochnaceae: palaeotropical *Campylospermum* and *Ochna* and Neotropical *Ouratea* Aubl. So far, only *Campylospermum* has undergone a comprehensive global taxonomic revision (Bissiengou, 2014), which needs revisiting in light of recent HTS results highlighting the genus to be polyphyletic (Schneider *et al.*, 2020, 2021; Shah *et al.*, 2021).

Taxonomic history and classification of *Ochna*

Early concepts of *Ochna* were unclear, leading to confusion in subsequent taxonomic accounts. This was largely due to Linnaeus changing his definition during the course of his work (Robson, 1962a). The confusion arose from description of *Ochna* from a specimen collected from the West Indies, contradictory to our current understanding of the palaeotropical distribution of *Ochna*. In 1744, Linnaeus received specimens from Sri Lanka (as Ceylon), leading him to amend his generic concept of *Ochna*, describing a plant with persistent sepals and numerous stamens. This description was included in subsequent literature (Linnaeus, 1737, 1747, 1752, 1754). Despite the confusion, the type species for the genus was designated by Robson (1962a) as *O. jabotapita* L. with a lectotype selected from the collection from Sri Lanka seen by Linnaeus. So far, there has been no global revision of the genus, mainly due to the difficulty of species delimitation. This is largely due to the lack of fertile herbarium specimens which has led to uncertainty in species boundaries particularly resulting from the absence of important identification characters in the flowers, such as the anthers.

The earliest informal infrageneric treatment of *Ochna* by Sprengel (1826) was based on stigmatic division. His treatment listed ten species in two groups: (1) species with a capitate stigma; and (2) species with a branched stigma. His infrageneric groups based on stigma division were not carried forward in subsequent treatments. In the *Flora of Tropical Africa* (Oliver, 1868), five *Ochna* spp. were described and separated in the key by inflorescence arrangement, again not followed by later authors. Later, in his account of African Ochnaceae, Engler (1893) mentioned eight *Ochna* spp., which he placed in two sections: section *Schizanthera* Engl. based on the presence of longitudinal anther dehiscence; and section *Diporidium* (Wendl.) Engl. based on poricidal anther dehiscence.

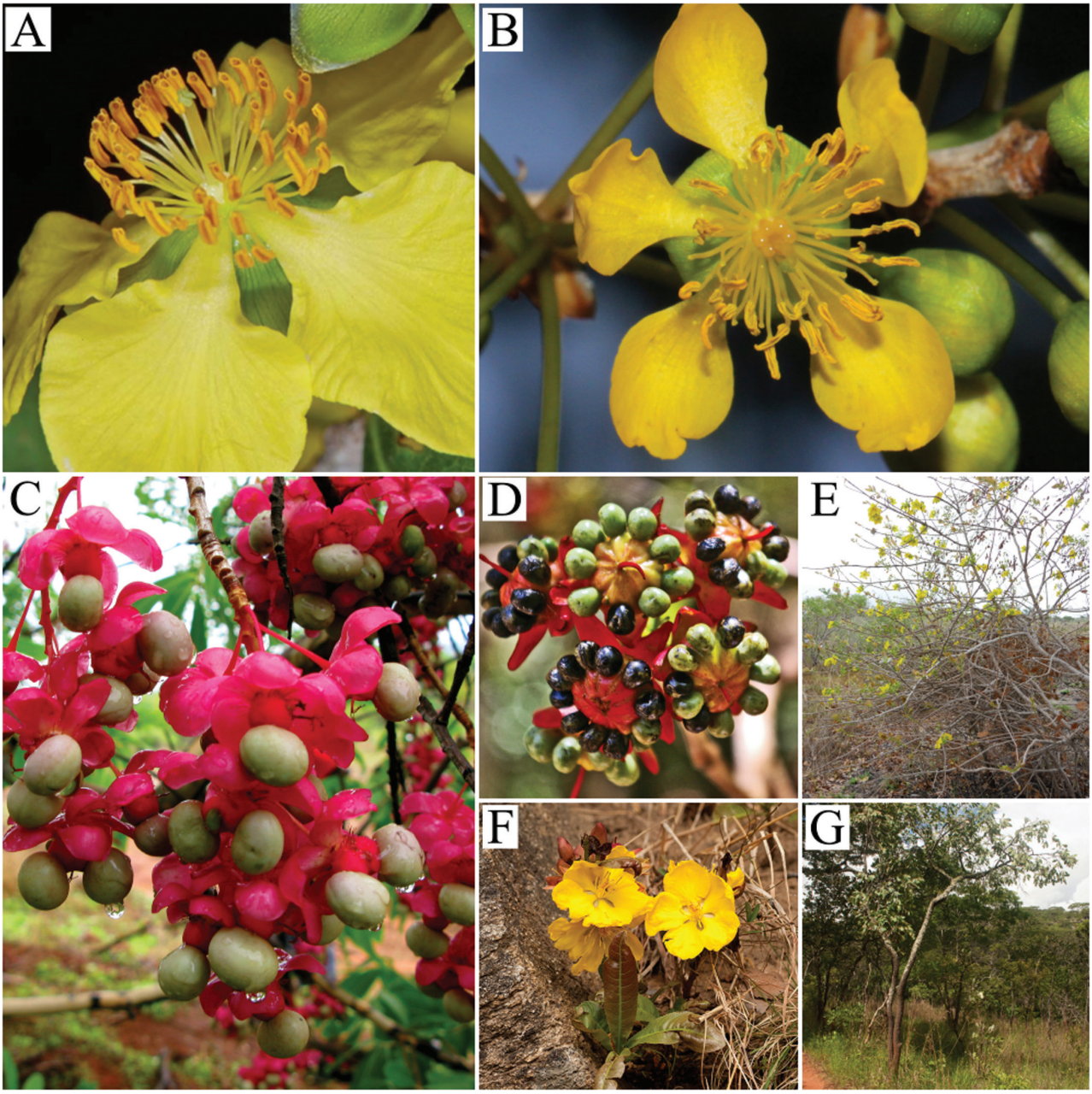

Figure 1. Overview of the morphological diversity of *Ochna*. A, B, *Ochna* flowers and anther dehiscence. A, *O. barbosae* showing distinct poricidal anther dehiscence and a style free at apex. B, *O. schweinfurthiana* showing anthers with longitudinal anther dehiscence and gynobasic style. C, D, Drupe shape and attachment. C, *O. pulchra* with reniform drupes attached in the middle. D, *O. atropurpurea* with sub-globose drupes attached at base. E–G, Variation in habit. E, *O. kirkii* as much branched shrub. F, *O. macrocalyx* as geoxylic suffrutex. G, *O. gambleoides* as small tree. Photographs: A, Sune Holt; B, F, G, Bart Wursten; C, Helen Pickering; D, Meg Coates Palgrave and E, Joanna Osborne.

The global revision by van Tieghem (1902) split *Ochna* from a single genus into 15 segregate genera. His classification was based on anther dehiscence, embryo morphology and number of carpels (van Tieghem, 1902). This extensive splitting of *Ochna* was not widely accepted, and subsequent authors criticized his treatment for having too narrow generic limits (Callmander & Phillipson, 2012). Only five of the segregate genera were retained in a subsequent revision of Ochnaceae for Madagascar by Perrier de la Bâthie (1941, 1951), three of which are now considered synonyms of *Ochna*. More recent revisions and flora

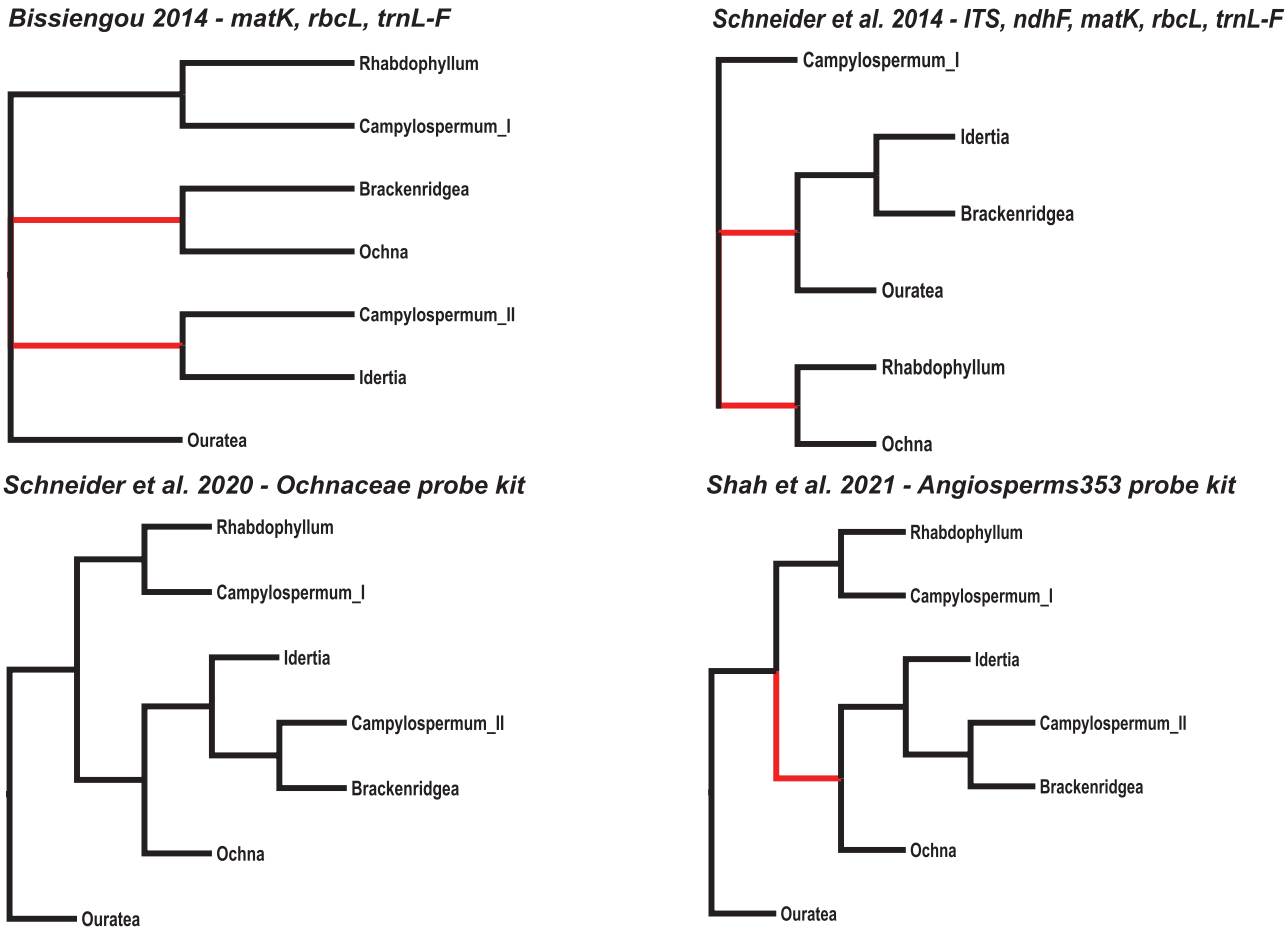

Figure 2. Summary of generic relationships in subtribe Ochninae from recent studies. Comparison of relationships of genera within the subtribe Ochninae (Ochnaceae) in four studies. Showing the variation in gene or genomic data and topology. Poorly supported branches (< 70% BS) indicated in red.

accounts (Robson, 1963; Kanis, 1968; Du Toit & Obermeyer, 1976; Verdcourt, 2005) favoured a broad concept for *Ochna*, with the segregate genera reduced to synonymy.

The genus has been treated in various regional floras in tropical Africa (notably Robson, 1963; Bamps & Farron, 1967; Du Toit & Obermeyer, 1976; Verdcourt, 2005). The currently accepted infrageneric classification of *Ochna* was published by Robson (1962b) based on anther and fruit morphology. He formally recognized three sections: (1) section *Renicarpus* N.Robson, with reniform drupelets attached in the centre and anthers with both longitudinal and poricidal dehiscence; (2) section *Ochna*, with anthers with poricidal dehiscence and (3) section *Schizanthera*, with anthers with longitudinal dehiscence. The latter two sections have ellipsoid to sub-globose drupelets attached at or close to the base. As indicated by the infrageneric classification, a key character for the identification of *Ochna* spp. lies in the flowers, specifically the anthers. However, features of the anthers, essential for identification, are not easily observed in herbarium specimens. This is because most *Ochna* specimens have been collected in fruit, primarily due to their conspicuous brightly coloured persistent sepals and enlarged torus during fruiting, but also due to the longer fruiting times compared to the comparatively short-lived flowers that reduce the likelihood of collecting trips coinciding with the brief flowering time.

The comprehensive family-wide phylogenomic study by Schneider *et al.* (2020) included a wider sampling than previous studies including 41 *Ochna* spp. Their results showed two large clades largely congruent with section *Ochna* and section *Schizanthera* of Robson (1962b). Two further smaller clades were retrieved, one with two species from Madagascar and the second including species from section *Renicarpus* with taxa such as *O. pulchra* and *Ochna latisepala* (Tiegh.) Bamps characteristic of the group with large reniform drupelets. *Ochna arborea* Burch. ex DC., previously placed in section *Renicarpus* because of its reniform, centrally attached drupelets, was

retrieved in the section *Schizanthera* clade, suggesting that section *Renicarpus* may be polyphyletic. These findings highlighted the need for a more in-depth study, with dense taxon sampling to fully understand the infrageneric groups in the genus (Schneider *et al*., 2020).

Morphological complexity and deficiencies in herbarium material, notably the relatively few flowering specimens compared to fruiting material, have led to difficulties in species delimitation and, thus, estimating the number of species to be recognized. These estimates (Robson, 1963; Verdcourt, 2005) are due to the lack of a comprehensive global revision for the genus and that many putative new species are not considered. This has left several regional flora accounts incomplete. For example, the *Flora of Tropical East Africa* (FTEA; Verdcourt, 2005) listed 49 species, 13 of which were not formally described, and others are only known from one or few collection(s) (Verdcourt 2005). Similarly, *Trees of southern Africa* (Palgrave, 2002) listed 22 species and two additional putative new species. Although new species continue to be described, including recent discoveries such as *O. dolicharthros* F.M.Crawford & I.Darbysh from Mozambique (Crawford & Darbyshire, 2015) and two endemic species from South Africa: *O. barbertonensis* T.Shah (Shah, Burrows & Darbyshire, 2018) and *O. maguirei* K.Balkwill (Balkwill, 2020). These uncertainties in species delimitation highlight the need for a better understanding of *Ochna* and a detailed systematic revision of the genus.

In this paper, we present a taxonomically comprehensive phylogenomic study of *Ochna*, including *c*. 85% of the accepted species and multiple samples for morphologically diverse and geographically widespread species. We specifically aim: (1) to evaluate the current morphology-based infrageneric classification; (2) resolve relationships at the sectional and species level and (3) assess the species delimitation of morphologically diverse and geographically widespread species. The phylogenomic results also provide insights into ecologically interesting groups such as the fire-prone geoxylic *O. katangensis* De Wild. and large forest tree, *O. holstii* Engl., giving a better understanding of the biographical patterns in the genus and a robust framework for future analyses (Shah *et al*., unpubl. data). *Ochna* is a taxonomically challenging group; integrating phylogenomics and morphology is important for resolving its systematics.

MATERIAL AND METHODS

Taxon sampling

We included a total of 192 samples, consisting of 173 ingroup samples of *Ochna* and 19 outgroup samples. Ingroup samples included 65 species plus eight morphospecies identified by Verdcourt (2005) and a further four identified in this study, representing *c*. 85% of the total species diversity (POWO, 2019; African Plants Database, 2021) (in the Supporting Information, Table S1). Samples were taken from herbarium specimens or freshly collected silica-dried leaf material. We ensured comprehensive sampling of each section following Robson (1962b) and all major geographical regions of its distribution including continental Africa, Madagascar and Asia. For 40 *Ochna* spp., we included multiple samples reflecting their morphological and/or geographical variation. The 19 outgroup samples spanned all other subtribes, tribes and subfamilies of Ochnaceae.

DNA extraction, library preparation and sequencing

In this study, new targeted sequencing data was generated for 150 samples, 140 ingroup and ten outgroup samples. Additionally, targeted sequencing data of 33 ingroup and nine outgroup samples, generated at the Senckenberg Research Institute (Frankfurt, Germany) for the study of Schneider *et al*. (2020) are also used in this study (see voucher information in the Supporting Information, Table S1).

Molecular laboratory work to generate new targeted sequencing data was conducted at the Sackler Phylogenomic Laboratory, in the Jodrell Laboratory at the Royal Botanic Gardens, Kew. Genomic DNA was extracted from leaf tissue using a modified cetyl-tri-methylammonium bromide approach, with chloroform:isoamyl alcohol (SEVAG) and precipitation in isopropanol at –20 °C (Doyle & Doyle, 1987). The samples were purified with Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN, USA) following the manufacturer's protocol. All DNA extracts were quantified with a Quantus Fluorometer (Promega, Madison, WI, USA) and run on a 1% agarose gel to assess their average fragment size. Samples with low concentration (not visible on a 1% agarose gel) were assessed on an Agilent Technologies 4200 TapeStation System (Santa Clara, CA, USA). DNA extracts with average fragment size > 350 bp were sonicated using a Covaris M220 Focused-ultrasonicator (Covaris, Woburn, MA, USA) following the manufacturer's protocol to obtain an average fragment size of 350 bp. Dual-indexed libraries for Illumina sequencing were prepared using the NEBNEXT Ultra II DNA Library Prep Kit and the NEBNEXT Multiplex Oligos for Illumina (Dual Index Primers 1 and 2; New England BioLabs, Ipswich, MA, USA) following the manufacturer's protocol, but using half the recommended volumes. Briefly, we used 200 ng (or minimum 50 ng) of the fragmented DNA for the end-preparation reaction. Following the adapter ligation

and size-selection, the DNA fragments were amplified using eight cycles of PCR. The libraries were quantified using a Quantus Fluorometer and fragment size was assessed with TapeStation using High Sensitivity D1000 ScreenTapes. The final library size including the adapters was *c*. 500 bp on average. For targeted enrichment of nuclear loci, we followed Johnson *et al.* (2019), samples with similar library concentrations and fragment sizes were pooled and enriched with the Ochnaceae-specific probes (see Schneider *et al*., 2020, for bait design and details). We use target enrichment and custom baits (Schneider *et al*., 2020), designed using a transcriptome of *O. serrulata*, for which we expected to yield high capture success due to taxonomic proximity to the study group (Shah *et al*., 2021). Two hundred and seventy-five nuclear genes were targeted that spanned 19 398 baits, with a mean length of 2402 bp and consisted of a total of 660 730 bp (Schneider *et al*., 2020). The hybridization was performed for 24 h at 65 ˚C, followed by 12 cycles of PCR. Final products were again run on the TapeStation to assess the fragment size, so they could be pooled equimolarly for sequencing. Sequencing of library pools was performed on an Illumina HiSeqX instrument (San Diego, CA, USA) at Macrogen (Seoul, South Korea) producing 2 × 150 bp paired-end reads.

Phylogenetic analyses

The raw sequencing reads were trimmed with Trimmomatic v.0.36 (Bolger, Lohse & Usadel *et al.*, 2014) using the settings LEADING:20 TRAILING:20 SLIDINGWINDOW:4:20 MINLEN:36 to remove adapter sequences and portions of low quality. The HybPiper pipeline v.1.31 was implemented (Johnson *et al.*, 2016) using BWA v.07.17-r1188 (Li & Durbin, 2009). Mapped reads were then assembled into contigs with SPAdes v.3.13.1 (Bankevich *et al.*, 2012) and the retrieve_sequences.py script from the HybPiper suite was used with the .dna flag to produce outputs of a single sequence per gene which is selected using length, similarity and coverage. Potential paralogous exons were identified using HybPiper, the impact of paralogy was investigated in subsequent analysis by conducting analysis with and without paralogue genes, in which 19 putatively paralogous genes were removed. Furthermore, HybPiper recovered additional 'splash zones' (Dodsworth *et al*., 2019), which include non-coding intronic or flanking regions that were combined with exons to form supercontigs. AMAS (Borowiec, 2016) was used to produce summary statistics for each exon and supercontig regions, evaluating the amount of missing data and the number of potentially parsimony-informative sites (see also Supporting Information Table S2).

To examine the impact of missing data, we analysed our data under two filtering strategies: a moderate versus a stringent approach. Both approaches included removing samples that had < 25% overall mean recovery across all loci and removing sequences that had < 30% of the average sequence length for that gene or, in shorter loci, < 200 bp. A further filtering measure was implemented for the more stringent method whereby sites within genes with < 70% occupancy were omitted. After filtering, both exon and supercontig datasets were individually aligned using MAFFT v.7.305b (Katoh *et al.*, 2002) with –maxiter 1000.

For the concatenation approach, the alignments of each locus were concatenated using AMAS for the exon and supercontig datasets separately. A species tree was generated using maximum likelihood analysis with the concatenated supermatrix of exon and supercontig alignments using IQTREE v.2.0 with 1000 ultrafast bootstraps using the '-B' option (Nguyen *et al.*, 2015). The data were treated as a single partition and the optimum model was selected automatically by the program.

For the multi-species coalescent approach, individual maximum likelihood gene trees were constructed from the aligned exons and supercontig regions with IQTREE v.2.0 (Nguyen *et al.*, 2015) with 1000 ultrafast bootstraps using the '-B' option. All genes were treated as a single partition with optimum model selection implemented by the program. Internal branches with bootstrap support values < 10% were collapsed with 'nw_ed' in Newick utilities v.1.6 (Junier & Zdobnov, 2010), and long branches were removed with TreeShrink v.1.3.3 (Mai & Mirarab, 2018) to avoid poor support in the subsequent species tree analysis. Species trees were then inferred from the gene trees using ASTRAL-III v.5.6.3 (Zhang *et al.*, 2018) with the '-t 2' option providing annotation outputs for quartet support to allow visualization of the main topology, first and second alternative topologies as pie charts.

Species trees were rooted using the outgroup taxa *Touroulia guianensis* Aubl. (Ochnaceae: Quiinoideae) and *Medusagyne oppositifolia* Baker (Ochnaceae: Medusagynoideae) with 'nw_reroot' in Newick utlilites v.1.6 (Junier & Zdobnov, 2010).

All the analyses are summarized in Table 1. A consensus topology of all the datasets was constructed in R v.3.6.3 (R Core Team, 2020) using the ape v.5.3 package (Paradis, Claude & Strimmer, 2004), and congruence between the datasets was measured using the pairwise Robinsons–Foulds (RF) distances for all trees with phangorn v.2.5.5.

RESULTS

Capture success and data quality

The capture success of the custom probe kit for *Ochna* and closely related genera of Ochnaceae is extremely high, with all 275 loci recovered. The average number

Table 1. Summary of data analysis methods and parameters used for generating eight species trees. Inferences methods are abbreviated to MSC for multi-species coalescent and ML concatenated for maximum likelihood concatenation

Filtering	Moderate				Stringent			
Region	Exon		Supercontig		Exon		Supercontig	
Paralogues	Removed		Removed	Retained	Removed		Removed	Retained
Inference	MSC	ML concat.	MSC	MSC	MSC	ML concat.	MSC	MSC

of reads per sample is 2 564 534 with a range of 89 410–12 091 738 (Table S2 in the Supporting Information). No major bias of recovery across the dataset is observed; ingroup taxa are well recovered across the genus and outgroup taxa are equally well recovered (Fig. S1; Table S1 in the Supporting Information). Some genes were not recovered at all for some ingroup, e.g. *O. multiflora* DC., *O. beirensis* N.Robson and *O. polyneura* Gilg, and for some outgroup taxa, e.g. *Testulea gabonensis* Pellegr., *Medusagyne oppositifolia* Baker and *Sauvagesia erecta* L. These taxa are phylogenetically spread across the family showing no taxonomic bias. Reads mapped on target for ingroup taxa are 85.8% and for outgroup taxa 82.9%, with an average of 83.6% reads mapped on target for all samples (Table S2 in the Supporting Information). For all ingroup taxa, the number of genes with sequences for at least 25 and 75% of the target length have median values of 275 and 274, respectively (Table S2 in the Supporting Information). Summary statistics reveal differences between datasets that underwent moderate versus more stringent filtering strategies, in addition to exon versus supercontig alignments. Under moderate filtering, 275 genes are recovered in the exon alignments and have a mean length across all loci of 2975 bp, 21.9% missing data and 27.8% potentially parsimony-informative sites, and supercontigs have an average alignment length of 33 504 bp, 81.6% missing data and 13.0% potentially parsimony-informative sites. Under more stringent filtering, 256 exons are recovered and 275 supercontigs. Exons have an average alignment length of 2426 bp, 4.5% missing data and 33.8% parsimony-informative site, and supercontig alignments have an average length of 5010 bp, 13.4% missing data and 55.0% potentially parsimony-informative sites (Table S3).

Topological impact of data filtering, paralogs and inference approach

Eight species trees were produced based on different filtering approaches, genomic regions and species tree inference methods. Overall, all species trees resolve the same higher-level relationships (Fig. S2 in the Supporting Information). Pairwise RF distances show that the removal of paralogues has minimal effect on the final tree topologies, whereas the choice of the dataset (exons only or supercontig) had the greatest impact on topology. Filtering approaches have only marginal effects on the tree topologies for the supercontig and the exon dataset (Fig. S2A in the Supporting Information). Some topological conflict is revealed at shallower evolutionary levels. A majority rule consensus tree shows conflicting topologies collapsed as polytomies (Fig S2B in the Supporting Information). The consensus tree of all eight species trees shows that there is significant conflict between topologies in the backbone of *Ochna* section *Ochna* and conflict at shallow levels. Similarly, in section *Schizanthera* there is conflict at shallow levels in both major clades of the section. No topological conflict between species trees was found in sections *Renicarpus* and *Ramistylus* (see further).

The multi-species coalescent (MSC) exon topology under a moderate filtering approach has the most robust topology due to minimal pairwise RF distance (Fig. S2A in the Supporting Information), overall support and congruence to current morphology-based species concepts. Therefore, from here, we will present the phylogenetic relationships as resolved by this approach and only refer to other topologies when relevant.

Phylogenetic relationships

Ochna is resolved as monophyletic, sister to a clade of *Brackenridgea*, *Campylospermum* and *Idertia*. In *Ochna*, four strongly supported major clades are retrieved, three of which correspond to sections as currently circumscribed, and a fourth to a new section, section *Ramistylus* T.Shah, described in the Taxonomic Treatment that follows.

Our results resolve section *Renicarpus* as monophyletic and sister to the rest of *Ochna* with strong support (1 LPP and 100% BS). In our study, section *Renicarpus* is represented by 11 species from tropical Africa and Madagascar. *Ochna multiflora*. is sister to the rest of section *Renicarpus,* which is divided into two clades: (1) a clade including two species from Madagascar and the African *O. arborea s.l.*; and (2) a larger clade with five African species. All species-level relationships in this section are resolved with strong

support, with the majority of gene trees supporting this topology (Fig. 3). For species with multiple accessions, most were retrieved as monophyletic (*O. calodendron* Gilg & Mildbr., *O. latisepala*, *O. pulchra*). The exception is *O. arborea* Burch. ex DC. *s.l.*, which is retrieved as paraphyletic, with *O. arborea* s.s. as a monophyletic group sister to a clade consisting of *O. barbertonensis* and *O. arborea* var. *oconnorii* (E.Phillips) Du Toit.

A strongly supported clade (1 LPP and 100% BS) unites all species that are sister to a section *Schizanthera* + section *Ochna* clade. This clade is here formally described as a new section, i.e. *Ochna* section. *Ramistylus*. In our study, section *Ramistylus* is represented by five accessions endemic to Mauritius, and four species from Madagascar (Fig. 3). This section is divided into two clades. All the relationships in this section received strong support and low gene tree conflict.

Section *Schizanthera* is resolved as sister to section *Ochna*. The section received strong support (1 LPP and 100% BS) and low gene tree conflict. In our study, section *Schizanthera* is represented by 17 species from tropical Africa and divided into two clades

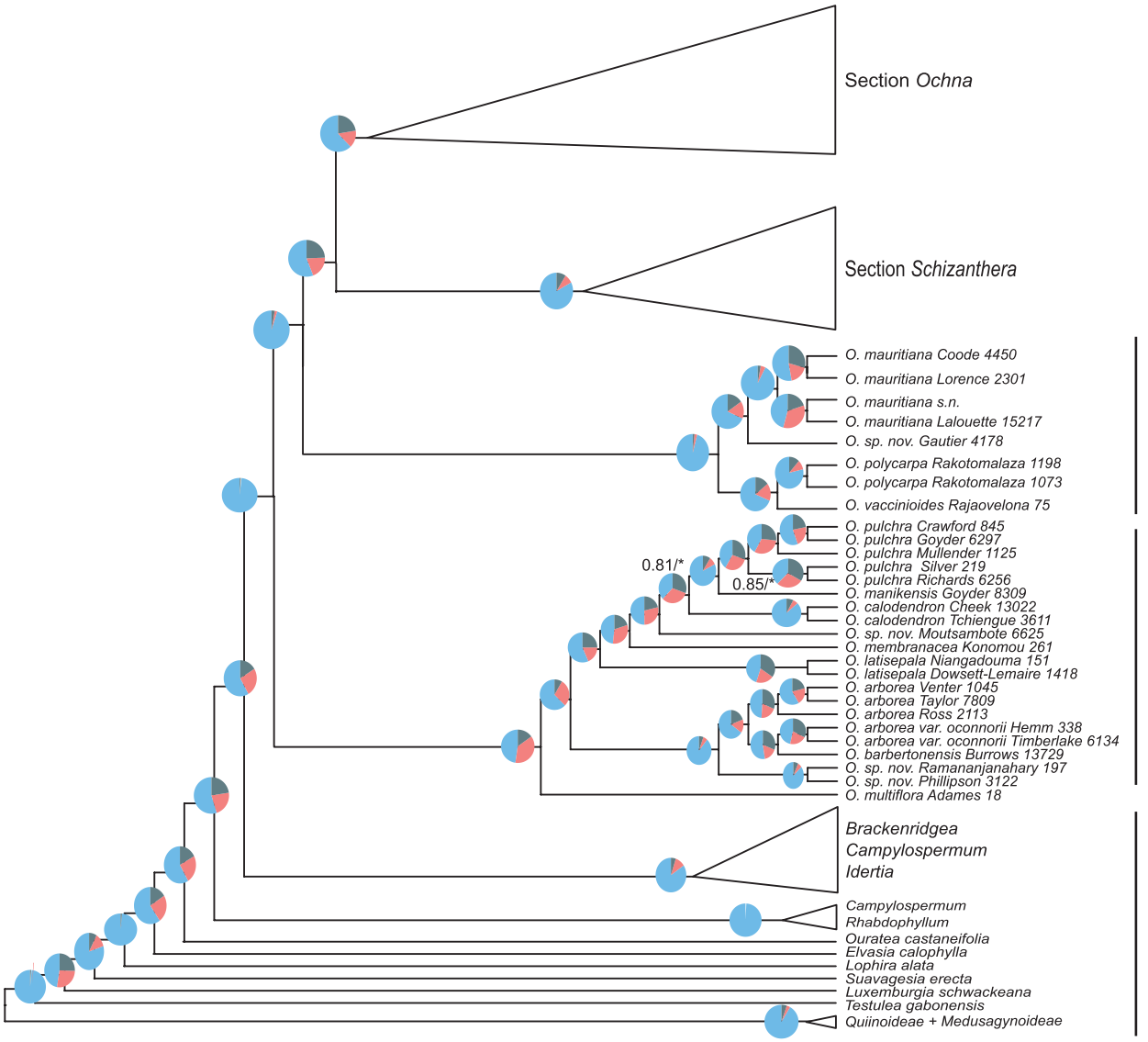

Figure 3. Multi-species coalescent inference of *Ochna* section *Renicarpus* and section *Ramistylus* from 275 exons. Relationships of species in section *Renicarpus* and section *Ramistylus*. Other sections and outgroups collapsed into triangles. Support not indicated as all nodes received support > 0.8 LPP for the multi-species coalescent approach and > 80% BS for the concatenated maximum likelihood approach. Pie charts indicate conflict in gene tree topologies as quartet support.

(Fig. 4, Clade I and II), with most species resolved as monophyletic with strong support. Clade I consists of species with a tree or shrub habit, some of which are widely distributed across eastern and southern Africa. The backbone of this clade includes multiple nodes with poor support, ranging between 0.50 and 0.75 LPP in the MSC analysis and additionally nodes with < 80% BS support from the concatenation ML tree (Fig. 4; Clade I). This is linked with short branches in the ML concatenation analysis (Fig. S3 in the Supporting Information), and a high amount of gene tree conflict. Some species within Clade I are resolved as polyphyletic, including *O. polyneura*, *O. afzelioides* N.Robson and, most notably, *O. holstii*, which appears in multiple groups across Clade I.

Clade II largely comprises species with a suffrutescent habit. Notably, *O. katangensis* and *O. confusa* Burtt Davy & Greenway are not monophyletic but together form a clade. Additionally, *O. schweinfurthiana* appears as polyphyletic but several relationships received low support. (Fig. 4; Clade II).

Section *Ochna* is the most species-rich section of *Ochna*. In our study, it is represented by *c*. 36 species, most of them from tropical Africa, except some species united in two Madagascan clades and two Asian radiations. The section is strongly supported (1 LPP and 100% BS) and broadly divided into three main clades with levels of gene tree conflict similar across the section. Clade I (Fig. 5), sister to the rest of the section, is strongly supported and consists of

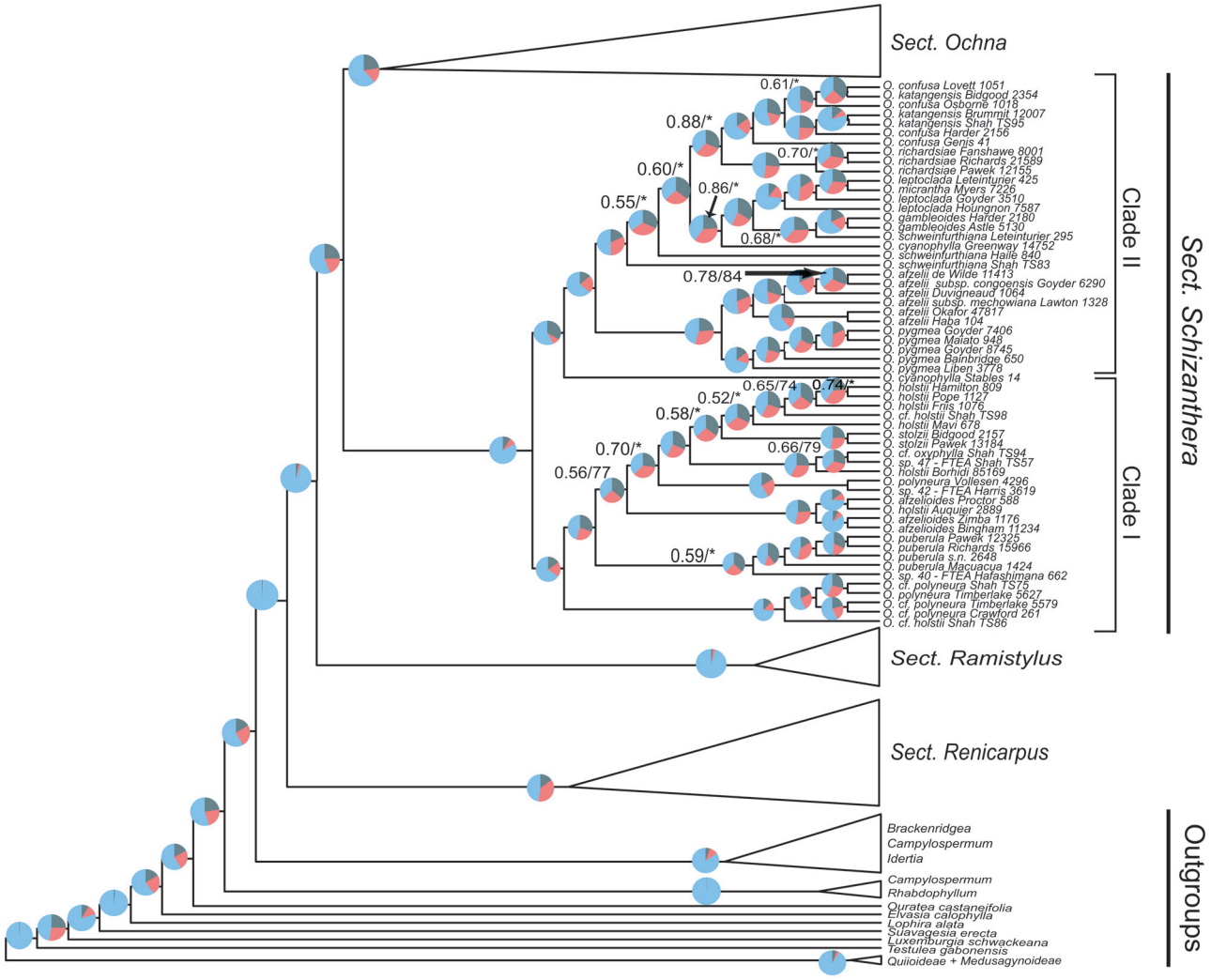

Figure 4. Multi-species coalescent inference of *Ochna* section *Schizanthera* from 275 exons. Relationships of species in section *Schizanthera* displayed. Other sections and outgroups collapsed into triangles. Support only indicated for nodes that received poor support < 0.8 LPP for the multi-species coalescent approach and < 80% BS for the concatenated maximum likelihood approach, * indicating full support. Pie charts indicate conflict in gene tree topologies as quartet support.

O. staudtii Gilg. Clade II (Fig. 5) consists of species predominantly from eastern Africa and one accession from Asia. Some backbone nodes in this clade are poorly supported, notably, multiple accessions of *O. ovata* F.Hoffm. show that this taxon is not monophyletic respective to several other species in Clade II, including *O. leucophloeos* Hochst. ex A.Rich., *O. monantha* Gilg, *O. hackarsii* Robyns & Lawalrée and *O. glauca* I.Verd., which are embedded between accessions of *O. ovata*. A morphospecies listed as 'sp. 17' in FTEA (Verdcourt, 2005) is resolved as sister to an Asian species, *O. lanceolata* Spreng. Clade III (Fig. 5) is the most species-rich clade, in which there are four notable groups (Fig. 5). Sister to the rest of the Clade III is a monophyletic group consisting of *O. serrulata*. Embedded in Clade III is a small group of Asian species including *O. integerrima* (Lour.) Merr. and *O. obtusata* DC., which are sister to a larger group that is less well supported in several of the deeper nodes under the MSC inference. In this larger group, all species relationships are resolved as monophyletic including one clade from Madagascar. Finally in Clade III, is a group of species from tropical Africa and one group from Madagascar, all species were resolved as monophyletic except for *O. kirkii* Oliv. and *O. macrocalyx* Oliv.

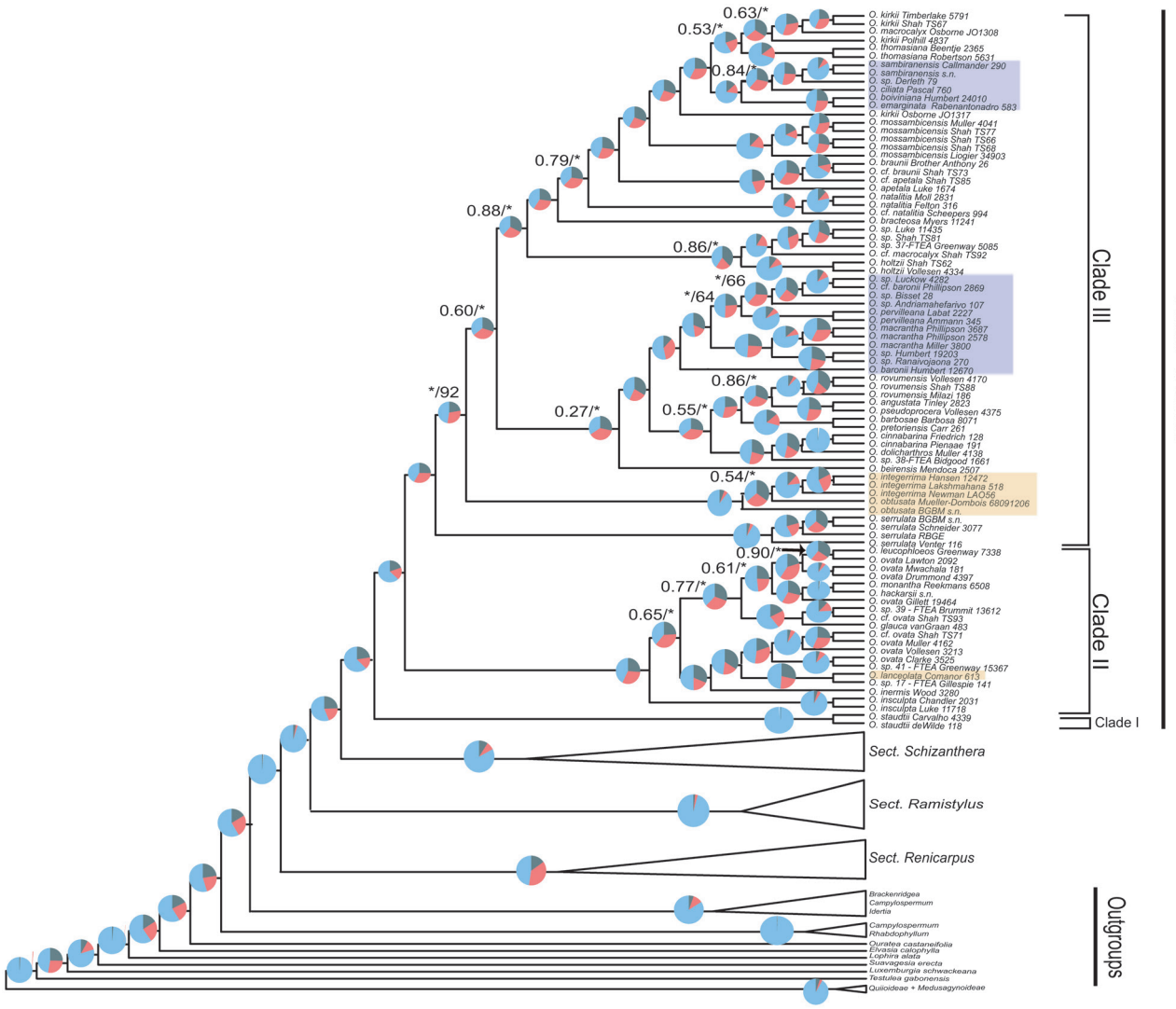

Figure 5. Multi-species coalescent inference of *Ochna* section *Ochna* from 275 exons. Relationships of species in section *Ochna* displayed. Other sections and outgroups collapsed into triangles. Support only indicated for nodes that received poor support < 0.8 LPP for the multi-species coalescent approach and < 80% BS for the concatenated maximum likelihood approach, * indicating full support. Pie charts indicate conflict in gene tree topologies as quartet support. Shaded purple clades represent species from Madagascar and shaded orange clade represent species from Asia.

On close inspection of the species relationships resolved in the phylogenomic results, 27 samples required name changes because of voucher specimens being previously misidentified. Figures 3–5 include updated, re-identified and accepted names. Furthermore, 11 samples, previously unplaced in the infrageneric classification due to deficient herbarium material have now been placed for the first time; and seven clades in which up to 30 taxa require further taxonomic revision and detailed study (Fig. 6).

DISCUSSION

Methods of phylogenetic reconstruction

The custom probe kit for *Ochna* enabled extremely efficient gene capture with an average of 85.5% reads mapped on target and all targeted loci recovered. This is comparable to other studies using custom probe kits such as Soto Gomez *et al.* (2019) with an average of over 96.5% recovered reads for *Dioscorea* Plum. ex L. per sample, and 90% of the target per sample for Annonaceae (Couvreur *et al.*, 2019). Our gene capture is significantly higher than studies using universal probe kits for species-level phylogenetic analyses as in *Nepenthes* L. (Murphy *et al.*, 2020) which recovered 59% of the target length. This is an expected result as samples included in our study are likely to share more genes present in the custom probe kit developed with *O. serrulata* (Shah *et al.*, 2021).

We conducted data analyses in eight different ways to understand which factors may be more influential on species tree topology and overall support, and to produce a more robust phylogenetic hypothesis (Herrando-Moraira *et al.*, 2018; Gardner *et al.*, 2020). The recovery of paralogous genes and non-coding regions is common when using targeted sequencing, although largely dependent on the probe kit used. The presence of paralogues is an indication of gene duplication, and their inclusion in phylogenetic analysis is known to affect species tree inferences (Fernández, Gabaldón & Dessimoz, 2019). However, Gardner *et al.* (2020) showed inclusion of paralogous genes as separate alignments can reduce topological disagreement when a genome duplication is known. In our study, the inclusion or removal of paralogous genes did not affect the overall species relationships. This is probably due to only 15 genes receiving paralogue warnings, not holding enough weight to make a difference to the data, with strong recovery in over 200 other genes. Furthermore, this may suggest there is a low chance of gene duplication events having occurred in this lineage in the recent past (Johnson *et al.*, 2016; Fernández *et al.*, 2019). Moreover, we tested the impact of data filtering, of which the consequences are still under debate. It is argued that the removal of missing data may result in better phylogenetic resolution due to removal of phylogenetic noise. On the other hand, more stringent data filtering may reduce phylogenetic support through the loss of informative sites and phylogenetic signal (Ranwez & Chantret, 2020; Shah *et al.*, 2021). For the supercontig dataset, differences in filtering thresholds made little impact on the topology, probably due to the huge amount of data when including coding and non-coding regions. The greatest differences between species tree topology are between the supercontig and exon datasets, similar to the findings of Gardner *et al.* (2020) and Kuhnhäuser *et al.* (2021). This is probably because supercontigs include intronic regions, which contain more variable, rapidly evolving sites (Dodsworth *et al.*, 2019). Notably the total length of supercontig alignments was considerably larger than total exon length, with many missing data. This was significantly reduced under the stringent filtering approach. Another notable difference in the final species trees is between the MSC versus the ML concatenation phylogenetic inference of the exon datasets, which is expected due to inherent differences in the algorithmic approach of each method. Overall, despite the differences in species trees, we find a surprising degree of congruence between the final topologies, providing more certainty to the relationships inferred here and the overall phylogenetic hypothesis.

Insights into phylogenetic relationships

Our phylogenetic results provide a robust phylogenomic framework for a global taxonomic revision of *Ochna*. There are 80 accepted species of *Ochna*, 65 of which are included in this study. A further 12 putative new species were identified and eight unidentified samples. Relationships in each section are discussed next.

Comparing our phylogenomic results with the currently accepted infrageneric classification, we find support for the three sections proposed by Robson (1962b). However, our results confirm the presence of a fourth major clade, as first highlighted by Schneider *et al.* (2020), requiring recognition of a new fourth section. Section *Renicarpus* is sister to the rest of *Ochna*, followed by sect. *Ramistylus* which is sister to section *Schizanthera* + section *Ochna*.

Section *Renicarpus*

This section is defined by species with reniform drupelets that are attached centrally. Although fruit morphology is consistent in the group, the species vary in habit and ecology and are distributed widely across tropical Africa and Madagascar. The species resolved in this section are largely consistent with those recognized by Robson (1963) and Verdcourt (2005).

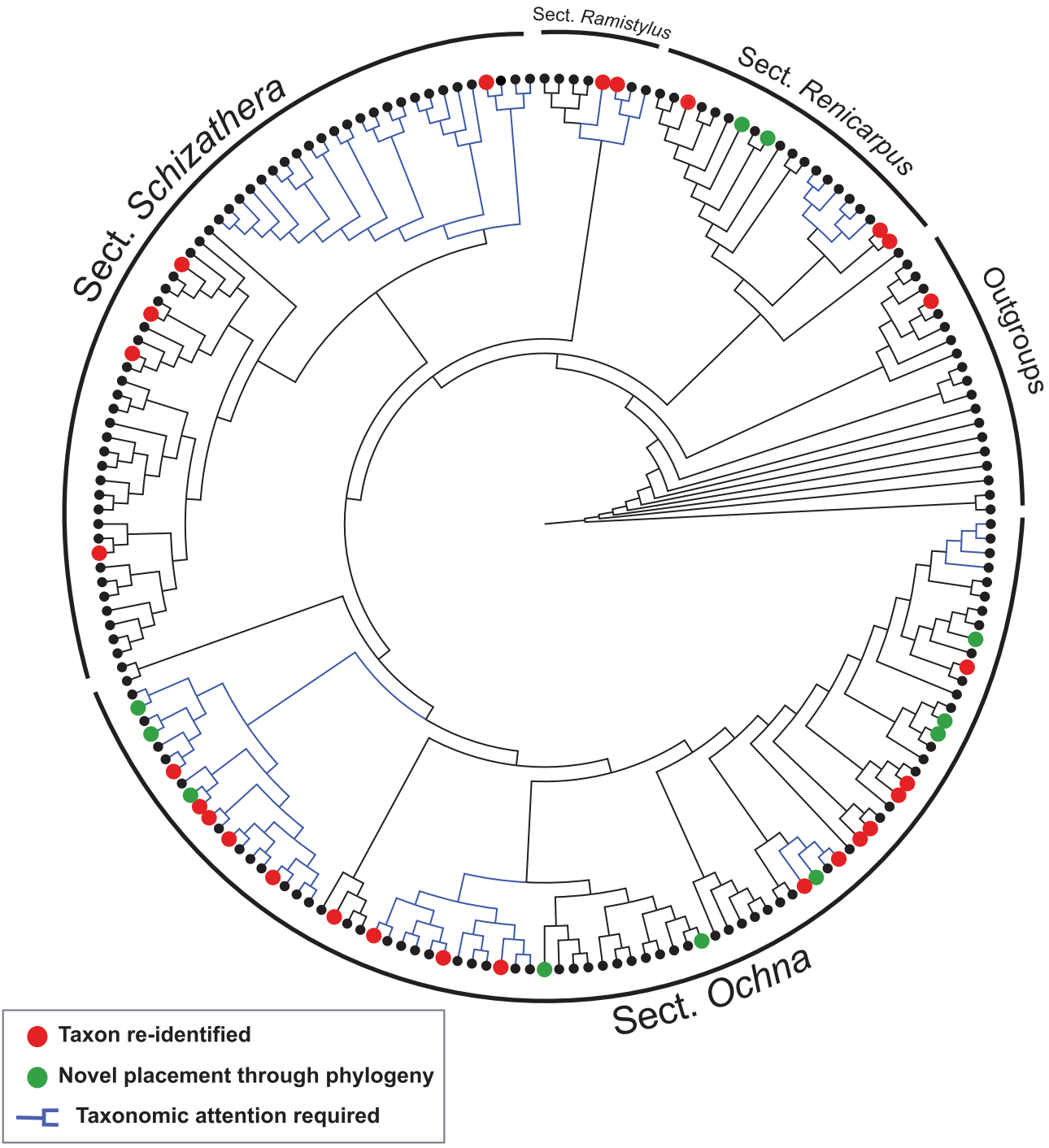

Figure 6. Overview of phylogenetic relationships and taxonomic queries. Phylogenetic tree showing species that required secondary identification and renaming indicated in red, species that received novel placement indicated in green, and clades or groups requiring further taxonomic study indicated in blue. All tips reflect actual species in this study.

The group includes rainforest tree species, such as *O. calodendron* and *O. multiflora*; species of fire-prone woodlands, such as *O. pulchra*, and savanna grassland species, *O. barbertonensis* and *O. arborea*. This section was previously only known from continental Africa, but a novel finding of this study is that this section also includes two samples from Madagascar. For these samples, due to voucher specimens only having immature drupes, reniform fruits were not confirmed, but the diagnostic central drupe attachment was

visible which is characteristic for this section. Furthermore, the specimens do all have conspicuously elongate racemose inflorescences. The latter character is also largely present in other species of this section, which could represent an additional synapomorphy for the group, although further study is needed. Anther dehiscence in this group is known to be variable, but among the species in this section only *O. multiflora* and *O. calodendron* are known to have longitudinal dehiscence; anther dehiscence is still unknown for the two species from Madagascar.

Our results show that *O. arborea* is paraphyletic, with *O. arborea* sister to a clade with *O. barbertonensis* and *O. arborea var. oconnorii*. Given that *O. barbertonensis* is morphologically different from *O. arborea s.s.*, with the former being a geoxylic suffrutex up to 0.2 m tall and the latter a shrub or tree, 3–9 m tall, it is not surprising that *O. arborea* was not considered as a likely confusion species when describing *O. barbertonensis* (Shah *et al.*, 2018). Our results suggest *O. arborea s.l.* may encompass more than one species. This finding agrees with previous treatments recognizing *O. arborea* var. *oconnorii* at species level, *O. oconnorii* E.Phillips (Phillips, 1922; Robson, 1963), and is reinforced by differences in morphology (I. Darbyshire, pers. obs.) and habitat: *O. arborea var. oconnorii* being a species of seasonal wet forest and *O. arborea s.s.* a lowland species occurring in drier habitats. In contrast, *O. barbertonensis* only occurs on clay-loam soil of the fire-prone Barberton montane grasslands at the border of South Africa and Eswatini.

The findings of Schneider *et al.* (2020) suggested that section *Renicarpus* may be polyphyletic due to the placement of a sample identified as *O. arborea* in section *Schizanthera* in their results. This placement was unexpected as this species is characterized by having poricidal anthers disagreeing with the morphological circumscription of section *Schizanthera*, which has anthers with longitudinal dehiscence. Including multiple samples of *O. arborea* in our study shows that although *O. arborea s.l.* was resolved as paraphyletic, all samples of the species were retrieved in section *Renicarpus*. The erroneous placement of *O. arborea* in Schneider *et al.* (2020) probably resulted from specimen misidentification.

Section *Ramistylus*

Our results reveal a new section in the genus unknown from previous taxonomic work (Fig. 3). This new section consists of species from Madagascar and Mauritius and can be split into two clades. The first clade includes two species from Madagascar and the second clade includes *O. mauritiana* and another potentially new species from Madagascar. *Ochna mauritiana*, previously thought to be in section *Schizanthera* (Richardson, 1979), is endemic to Mauritius and occurs in high-elevation wet forest and ericoid scrub but is also known from lower-elevation forests. *Ochna polycarpa* Baker and *O. vaccinioides* Baker are known from wet forest and sclerophyllous vegetation, respectively (Perrier de la Bâthie, 1951). Diagnostically important morphological characters for this group are difficult to identify, largely due to the lack of flowering and fruiting material present in the few herbarium specimens available from Madagascar and the Mascarene Islands. Some of the characteristics are coriaceous leaves with conspicuous finely reticulate venation, a deeply divided style and anthers with apically biporose dehiscence, but over time splitting open and becoming seemingly longitudinally dehiscent (Fig. 7; Table 2; see Taxonomic Treatment next). This last character appears to be an intermediate state between the poricidal and longitudinal dehiscence types observed elsewhere in the genus. The new section is formalized next.

Section *Schizanthera*

This section includes two major clades (Fig. 4). Species in Clade I (Fig. 4) occur in less fire-prone habitats including dry forest and montane wet forest environments, although some species such as *O. puberula* Robson and *O. afzelioides* are from fire-prone miombo woodland. In Clade I (Fig. 4), our results indicate potential taxonomic issues for several species, with several nodes in the backbone poorly supported with high amounts of gene tree conflict. Potential reasons for this include incomplete lineage sorting (ILS), hybridization, recombination and gene duplication events (Degnan & Rosenberg, 2006, 2009; Roch & Warnow, 2015). The most likely hypothesis, although not well documented for *Ochna*, is the possibility of hybridization or ILS. Broad sampling of *O. holstii* revealed it to be polyphyletic in its current circumscription. This is not surprising as several authors, including Robson (1963) and Verdcourt (2005), noted that the species is extremely variable in morphology and habitat, though previous attempts at separation have failed (Robson, 1963). Furthermore, the species has a broad distribution overlapping with other species here retrieved in Clade I. Embedded in the *O. holstii* clade are *O. polyneura*, *O. oxyphylla* Robson, *O. stolzii* Enlg., *O. afzelioides* and *O. puberula,* which also require taxonomic revision due to cases of paraphyly and uncertain species limits (Shah *et al.*, unpubl. data). Our results show that for groups such as this, an integrated approach with the use of molecular and morphological data is necessary to successfully decipher species concepts. Additionally, nested in Clade I are two species undescribed in FTEA (Verdcourt, 2005), for which we now have an initial

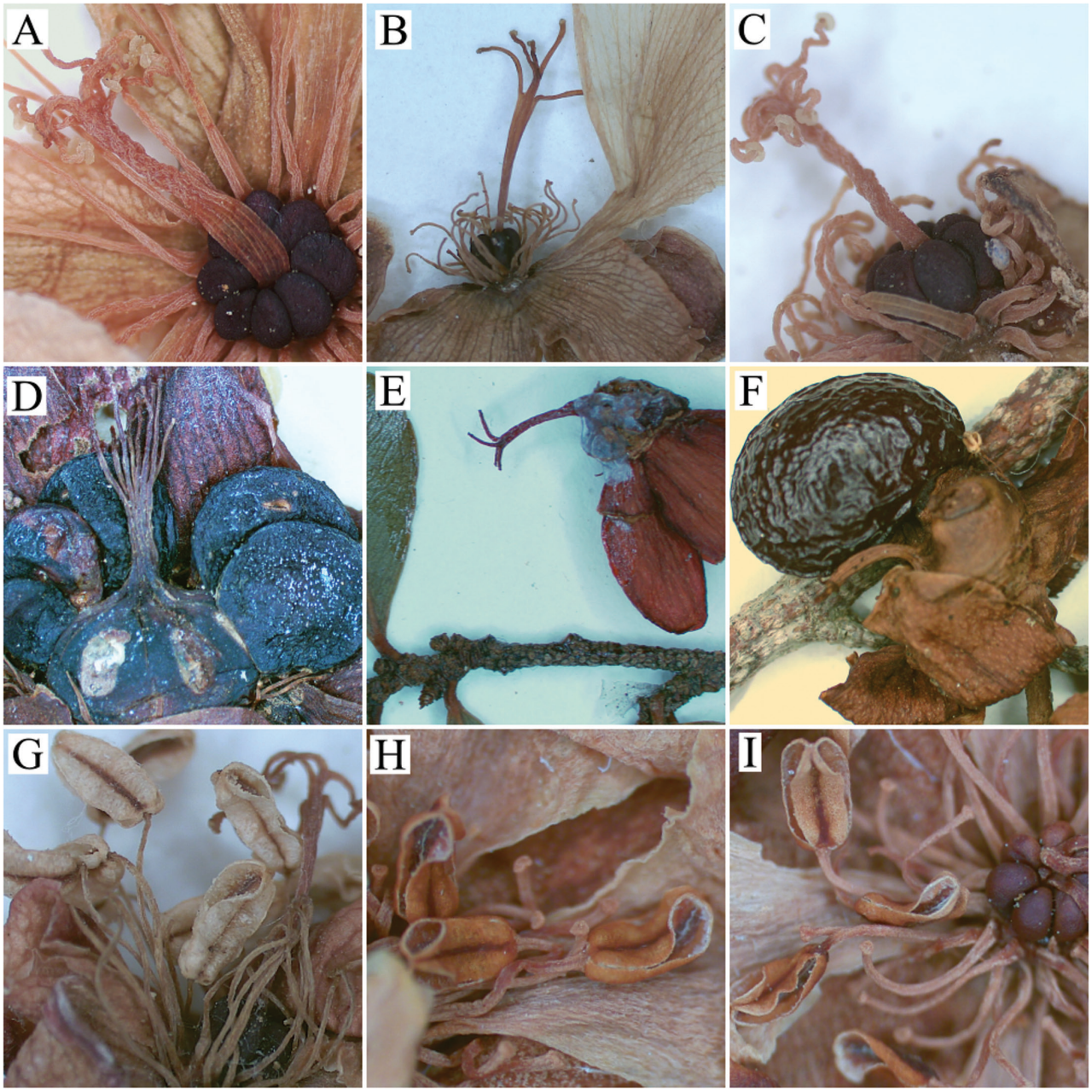

Figure 7. Diagnostic characters for *Ochna* section *Ramistylus.* A–E, Deeply branched style. A–C, Branched style in flower of *O. mauritiana.* D, E, Branched style in fruit of *O. polycarpa.* F, Drupe shape of *O. polycarpa* somewhat reniform attached between base and middle. G–I, Anther dehiscence in *O. mauritiana* showing poricidal anthers splitting open into longitudinal dehiscence.

understanding of their relationships. These are the first insights into undescribed species from the region, in which the phylogenetic relationships can provide a starting point for a taxonomic assessment of these morphologically challenging species.

Clade II is composed of several species from fire-prone savanna woodland or wooded grassland environments and include a number of geoxylic suffrutices such as *O. katangensis* and *O. richardsiae* N.Robson, and tree and shrub taxa, such as *O. afzelii* and *O. gambleoides* N.Robson (Fig. 4). In Clade II, our results show that specimens of *O. confusa* Burtt Davy & Greenway and *O. katangensis* are intermingled. The two species are morphologically similar, with overlapping distributions and occurrence in similar habitats. The difficulty in distinguishing between

Table 2. Comparison of diagnostic morphological characters for each infrageneric section of the genus *Ochna*

Character	Section *Ramistylus*	Section *Renicarpus*	Section *Schizanthera*	Section *Ochna*
Habit (m)	Shrubs or trees, 3.0–7.0 m tall	Geoxylic suffrutices, shrubs, trees, 0.3–10.0(–12.0) m tall	Geoxylic suffrutices, shrubs, 0.1–7.0 m tall or trees, 3.0–27.0 m tall	Geoxylic suffrutices, shrubs, trees, 0.5–10.0 m tall
Inflorescence arrangement	Simple or compound raceme, 2– to 10– (to 13–) flowered	Simple or compound elongate racemose	Variable; fasciculate, umbellate, solitary	Variable- paniculate, fasciculate, umbellate, solitary, sometimes racemose
Anther dehiscence	Apically biporate, later splitting longitudinally	Both longitudinal and apically biportate	Longitudinal	Apically biporate
Style branching	Prominently branched	Variable, usually free at apex	United to apex	Shortly branched
Style branch length (mm)	At least 1.0, up to 3.5	Always < 3.0	N/A	<0 .5
Drupe shape	Ellipsoid, sub-globose or somewhat reniform	Reniform	Ellipsoid to sub-globose	Ellipsoid to sub-globose
Drupe attachment	At base to between centre and base	At centre	± at base	± at base

these species could lead to misidentification. However, the morphological and ecological similarities combined with our molecular phylogenetic results suggest *O. confusa* and *O. katangensis* may represent a single species. Similarly, *O. micrantha* Schweinf. & Gilg, only known from Sudan, is embedded in the widely distributed *O. leptoclada*, which occurs throughout central, eastern and southern Africa. *O. micrantha* may therefore be synonymous with *O. leptoclada*. We also show the widely distributed tree species *O. afzelii* is monophyletic, with subspecies *O. afzelii* subsp. *mechowiana* (O.Hoffm.) N.Robson and *O. afzelii* subsp. *congoensis* (Tiegh.) N.Robson embedded in the group.

Section *Ochna*

This species-rich section is represented in our study by 36 accepted species and numerous unnamed species. Species in this section are defined by poricidal anther dehiscence. They occur in a broad range of habitats, including *O. insculpta* Sleumer and *O. kirkii* from evergreen forest, *O. mossambicensis* Klotzsch from deciduous woodland and *O. dolicharthros* from open grassland habitats. The section encompasses two separate lineages from Madagascar and two from Asia, with all other species from tropical Africa. In Clade II (Fig. 5), *O. ovata,* a widespread and polymorphic species occurring in a range of woodland habitats, is found to be paraphyletic, forming two clades. Other species embedded in *O. ovata* include *O. leucophloeos*, *O. hackarsii*, *O. monantha* and *O. glauca*. Furthermore, in this '*O. ovata* complex' a sample from Sri Lanka identified as *O. lanceolata* is resolved as sister to an undescribed species listed as 'sp. 17' in FTEA (Verdcourt, 2005) known only from the Lamu Archipelago in Kenya. The voucher specimens for each accession show differences in the inflorescences with *O. lanceolata* having simple inflorescences with one to free flowers, and sp. 17 having condensed four- to eight-flowered fascicles, with the latter distinctly flowering without leaves. Despite these differences, the two species share similarities in habit, leaf shape and habitat preferences. However, more adequate material is required to determine the status of this taxon. Nonetheless, the presence of this Asian group embedded in an African clade highlights an interesting distribution for the genus. Overall, voucher material for specimens resolved in this clade show significant morphological overlap, suggesting the need for a more detailed taxonomic assessment of this group.

In Clade III of section *Ochna*, the polyphyly of *O. macrocalyx* (Fig. 5) is an unexpected result, given that this species has distinctive, large persistent sepals when in fruit making species identification easy. The species is known from tropical east and southern Africa from open woodland, grassland and rocky slopes.

However, Verdcourt (2005) discusses variation in carpel number among specimens of *O. macrocalyx* that makes confident identification difficult and requires further field and taxonomic work. This is further corroborated by the present findings. Furthermore, in Clade III, one of the potential accessions for *O. macrocalyx* is sister to three unknown species including an undescribed taxon listed in FTEA (Verdcourt, 2005) as 'sp. 37', all requiring further research. In Clade III (Fig. 5) the two radiations from Madagascar reveal numerous unknown taxa and some that are polyphyletic. With these findings, together with the discovery of the new section from Madagascar and Mauritius, and the occurrence of species from Madagascar in section *Renicarpus* first shown here, we recommend a taxonomic revision of *Ochna* from Madagascar and the Mascarene Islands, as already suggested by Callmander & Phillipson (2012). Most other species included in this section were resolved as monophyletic.

Importance of integrated systematics

The advent of phylogenetic methods and the ability to obtain DNA sequence data from hundreds of species has led to an increase in molecular phylogenetic analyses across all organisms (Hinchliff *et al.*, 2015; Soltis *et al.*, 2018). In plants, high-throughput sequencing has transformed our understanding of plant diversity and evolutionary history (Lemmon & Lemmon, 2013; McKain *et al.*, 2018; Soltis *et al.*, 2018; Dodsworth *et al.*, 2019). Despite these advances, it is important to recognize issues relating to reliability of phylogenetic results. The discussion about specimen identification and importance of voucher material is not new (Pleijel *et al.*, 2008; Funk, Edwards & Keeley, 2018). However, it is vital to reignite the conversation in light of the phylogenomic era we are currently in due to the increasing ease of generating densely sampled phylogenetic trees. The phylogenetic results of our study required careful inspection and thorough name checking. In *Ochna*, specimen misidentification is common due to the morphological plasticity, limited informative characters and insufficient fertile herbarium material. This has led to numerous samples revealing apparent paraphyly or phylogenetic placement requiring re-identification. Of course, cases of paraphyly and polyphyly may be attributed to complex biological processes such as hybridization, introgression and ILS. However, it is important to rule out potential misidentification first (Collins & Cruickshank, 2012). Equally, the phylogenetic tree was able to place samples previously unidentified due to insufficient herbarium material, providing a starting point for taxonomic investigation for several suspected new species to science, including eight of the morphospecies listed in FTEA (Verdcourt, 2005). Our results show that molecular and morphological data go hand-in-hand for the systematics of *Ochna*, of which up to seven clades require detailed taxonomic revision, including two major species complexes (*O. holstii* and *O. ovata*) and a full revision for species of Madagascar and the Mascarene Islands.

CONCLUSIONS

Our results show that *Ochna* is a diverse genus with a distribution spanning tropical Africa, multiple radiations in Madagascar and two radiations in Asia. We highlight numerous clades and species in the genus requiring detailed taxonomic work. Ideally, the genus requires a comprehensive global revision, but a full monographic treatment demands dedicated time from specialist taxonomists which is undervalued and poorly funded today. To tackle this understudied yet sizeable genus, we highlight critical areas as starting points toward a full taxonomic revision. It is evident that *Ochna* presents taxonomic difficulties due to morphological plasticity and herbarium specimens often lacking crucial morphological characters needed for species identification. Many specimens remain unidentified or misidentified, and many taxa remain incompletely circumscribed or not yet formally described. In this case, molecular data are extremely useful for placing poorly known groups and unravelling cryptic species. In *Ochna*, our molecular data has shed light on the phylogenetic relationships of numerous potentially new species and has provided an important starting point for investigating closely related species in the genus. Moreover, it has allowed us to begin to understand the morphological variation in widespread species such as *O. holstii*, which probably represents more than one species, and several non-monophyletic species, including *O. katangensis*, *O. confusa* and *O. ovata*. This also emphasizes the need for targeted field work to collect flowering material crucial for species delimitation and detailed taxonomic work.

Taxonomic Treatment

All accepted species and the section to which they belong are listed in the Supporting Information (Table S4).

***Ochna* L.,** Sp. P1. 1: 513. 1753.; Gen. P1. ed. 5: 229. 1754.

Type: Ochna jabotapita L. (lectotype, designated by N. Robson, Taxon, 11: 48-52. 1962).

1. *Ochna* section *Renicarpus* N. Robson Bol. Soc. Brot. sér. 2, 36: 12. 1962.

Type: Ochna multiflora DC.

= *Discladium* Tiegh. in Bull. Mus. Hist. Nat. (Paris) 8: 214. 1902.

Type: Discladium squarrosum Tiegh. (=Ochna jabotapita L.).
= *Diporochna* Tiegh. in J. Bot. (Morot) 16: 181. 1902.

Type: Diporochna membranacea (Oliver) Tiegh. (=Ochna membranacea Oliv.).
= *Porochna* Tiegh. in Bull. Mus. Hist. Nat. (Paris) 8: 214. 1902.

Type: Porochna membranacea Tiegh. (=Ochna membranacea Oliv.).
= *Pleodiporochna* Tiegh. in Ann. Sci. Nat., Bot., sér. 8, 18: 58. 1903.

Type: Pleodiporochna buettneri (Engl. & Gilg) Tiegh. [=*Ochna latisepala* (Tiegh.) Bamps].
= *Pentochna* Tiegh. in Ann. Sci. Nat., Bot., sér. 9, 5: 161. 1907.

Type: Pentoochna ramose Tiegh. (=*Ochna multiflora* DC.).

Description: Plants geoxylic suffrutices, shrubs or trees, 0.3–10.0(–12.0) m tall. Leaf shape variable, 2–20 cm long. Inflorescence simple racemes or compound elongate panicles; petals yellow; anther dehiscence longitudinal or biporose, styles usually free at apex. Drupelets reniform, attached centrally on the concave side.

Species included: Accepted species eight (Table S4 in the Supporting Information), and three species not formally described, two from Madagascar and one from Congo.

Distribution: Tropical Africa and Madagascar.

Notes: Our results revealed three undescribed species in this section. Further work is needed to fully circumscribe the species in section Renicarpus, particularly for Madagascar where the presence of this section is documented for the first time here.

2. *Ochna* section *Ramistylus T.Shah sect. nov.* (Table 2; Fig. 7)

Type: Ochna mauritiana Lam.
= *Polyochnella* Tiegh. in Bull. Mus. Hist. Nat. (Paris) 8: 547. 1902.

Type: Polyochnella mauritiana (Lam.) Tiegh. (=*Ochna mauritiana* Lam.).

Description: Shrubs or trees, 3–6 m tall. Bark grey-brown, smooth, branches pale brown to grey, lenticellate, sometimes extremely densely with individual lenticels swollen. Leaves alternate, mature leaves thick and coriaceous, obovate to ovate or elliptic, 0.8–8.5(–10.0) cm long, 0.4–4.5 cm wide, rounded to acute at apex, cuneate to rounded at base, margins serrate or sparsely serrate to entire, sometimes revolute, lateral veins numerous with prominent, dense reticulate tertiary vernation, particularly adaxially; leaves sometimes immature or absent at flowering; petiole 0.5–5.0 mm long; stipules semi-persistent, prominent, up to 5–6 mm long. Flowers two to ten (to 13) in simple or compound condensed racemose or corymbose inflorescences. Sepals five, oblong, persistent. Petals five, yellow or white. Anthers dehiscing by apical pores which soon split open longitudinally to form slits. Carpels five to eight (to ten), styles fused at the base and prominently branched at the apex, branches five to ten, often twisted, at least 1.0 mm long, up to 3.5 mm long. Drupelets ellipsoid to sub-globose or partially reniform (only immature fruits seen), attached at base or sometimes in between base and centre.

Species included: Known species four (Table S4 in the Supporting Information), suspected additional species include *O. andravinensis* Baill., *Ochna comorensis* Baill., *O. humblotiana* Baill., *O. madagascariensis* DC., *O. thouvenotii* (H.Perrier) Callm. & Phillipson.

Distribution: Madagascar and Mauritius.

Notes: The genus *Polyochnella* was based on division of the style with more than five branches, however, anther dehiscence was not specified in the protologue. Furthermore, 13 out of the 14 species assigned to that genus by van Tieghem (1902) are not placed in this new section. Most of these now fall in section *Schizanthera*, which is characterized by having a capitate style, whereas one species is now placed in section *Renicarpus*. Only *O. mauritiana* is retained in the new section. To avoid future confusion, and for the reasons stated previously, we have chosen to provide a new sectional name for the novel section here described rather than changing the status of *Polyochnella*.

3. *Ochna* section *Schizanthera* Engl. Bot. Jahrb. Syst. 17(1–2): 75. 1893.

Type: Ochna schweinfurthiana Hoffm.
= *Ochnella* Tiegh. in Bull. Mus. Hist. Nat. (Paris) 8: 214. 1902.

Type: Ochnella leptoclada (Oliv.) Tiegh. (=*Ochna leptoclada* Oliv.).
= *Biramella* Tiegh. in J. Bot. (Morot) 17: 96. 1903.

Type: Biramella holstii Tiegh. (=*Ochna holstii* Engl.).
= *Proboscella* Tiegh. in J. Bot. (Morot) 17: 4. 1903.

Type: Proboscella hoepfneri Tiegh. (=*Ochna pygmaea* Hiern).

Description: Plants geoxylic suffrutices, shrubs, 0.1–7.0 m tall or trees, 3–27 m tall. Leaves variable in shape and size, between 1.5–13.0(–21.0) cm long. Inflorescence fasciculate, umbellate or solitary, petals yellow; anthers with longitudinal dehiscence; styles more or less united, stigmas capitate. Drupelets ellipsoid to sub-globose, attached at the base.

Species included: 17 accepted species (Table S4 in the Supporting Information), and three species not formally described.

Distribution: Tropical Africa with a centre of diversity in east Africa.

4. *Ochna* section *Ochna*

Type: Ochna jabotapita L.
= *Diporidium* H.L.Wendl. in Beitr. Bot. 2: 24. 1825.
Lectotype: *Diporidium atropurpureum* (DC.) Wendl. (designated by Kanis, 1968) (=*Ochna atropurpurea* DC.).
= *Heteroporidium* Tiegh. in Bull. Mus. Hist. Nat. (Paris) 8: 378. 1902.

Type: Heteroporidium abyssinicum Tiegh. (lectotype designated here) (=*Ochna inermis* (Forssk.) Schweinf.).
= *Ochnella* Tiegh. in Bull. Mus. Hist. Nat. (Paris) 8: 214. 1902.

Type: Ochnella leptoclada (Oliv.) Tiegh. (=*Ochna leptoclada* Oliv.).
= *Pleopetalum* Tiegh. in Bull. Mus. Hist. Nat. (Paris) 9: 163. 1903.

Type: Pleopetalum lucidum (Lam.) Tiegh. (=*Ochna obtusata* DC.).
= *Polythecanthum* Tiegh. in Ann. Sci. Nat. Bot. ser. 9, 5: 160, 175. 1907.
Lectotype: *Polythecanthum thorelii* (Tiegh.) Tiegh. (designated by Kanis, 1968) [=*Ochna integerrima* (Lour.) Merr.].
= *Polythecium* Tiegh. in Bull. Mus. Hist. Nat. (Paris) 8: 377. 1902.
Lectotype: *Polythecium ciliatum* (Lam.) Tiegh. (designated by Kanis, 1968) (=*Ochna ciliata* Lam.).

Description: Plants geoxylic suffrutices, shrubs or trees, 0.5–10 m tall. Leaves variable in shape and size, between (1–)2 and 12(20) cm long. Inflorescence fasciculate, umbellate, solitary or rarely racemose; anthers with apically biporate dehiscence; style shortly branched at the apex. Drupelets ellipsoid to sub-globose, attached at the base.

Species included: 46 accepted species (Table S4 in the Supporting Information), and 12 species not formally described.

Distribution: Tropical Africa, Madagascar and Asia.

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DATA AVAILABILITY

Raw sequence data for Shah extracted samples (Table S1 in the Supporting Information) are available from National Center for Biotechnology Information under the Bioproject number PRJNA735954: https://www.ncbi.nlm.nih.gov/. Raw sequence data for Schneider extracted samples (Table S1 in the Supporting Information) are available from GenBank SRA 661 under the Bioproject number PRJNA602196: http://www.ncbi.nlm.nih.gov/bioproject/602196 662 (Schneider *et al.*, 2020).

REFERENCES

Abdullahi MI, **Musa AM**, **Haruna AK**, **Pateh UU**, **Sule IM**, **Abdulmalik IA**, **Abdullahi MS**, **Abimiku AG**, **Iliya I. 2014.** Isolation and characterization of an anti-microbial biflavonoid from the chloroform-soluble fraction of methanolic root extract of *Ochna schweinfurthiana* (Ochnaceae). *African Journal of Pharmacy and Pharmacology* **8:** 93–99.

African Plant Database. 2021. Conservatoire et Jardin botaniques de la Ville de Genève and South African National Biodiversity Institute, Pretoria. Version 3.4.0. Available at: http://africanplantdatabase.ch [Accessed 05 March 2021].

Amaral MCE, **Bittrich V. 2014.** Ochnaceae. In: Kubitzki K, ed. *Families and genera of vascular plants. Vol. 11*. Berlin, Heidelberg: Springer Verlag, 253–268.

Balkwill K. 2020. *Ochna maguirei* (Ochnaceae), a new species with corky bark from northern South Africa. *South African Journal of Botany*. **133:** 298–306.

Bamps P, **Farron C. 1967.** *Flore du Congo, du Rwanda et du Burundi: Spermatophytes. Ochnaceae, genres* Idertia, Rhabdophyllum *et* Campylospermum. Brussels: Jardin Botanique National de Belgique.

Bandi AKR, **Lee DU**, **Tih RG**, **Gunasekar D**, **Bodo B. 2012.** Phytochemical and biological studies of *Ochna* species. *Chemistry & Biodiversity* **9:** 251–271.

Bankevich A, **Nurk S**, **Antipov D**, **Gurevich AA**, **Dvorkin M**, **Kulikov AS**, **Lesin VM**, **Nikolenko SI**, **Pham S**, **Prjibelski AD**, **Pyshkin AV**, **Sirotkin AV**, **Vyahhi N**, **Tesler G**, **Alekseyev MA**, **Pevzner PA. 2012.** SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology: A Journal of Computational Molecular Cell Biology* **19:** 455–477.

Bissiengou P. 2014. *Systematics, evolution and historical biogeography of the family Ochnaceae with emphasis on the genus Campylospermum.* PhD thesis, Wageningen University.

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

Borowiec ML. 2016. AMAS: A fast tool for alignment manipulation and computing of summary statistics. *PeerJ* **4**: e1660.

Callmander MW, **Phillipson PB. 2012.** Notes on the genus *Ochna* L. (Ochnaceae) in Madagascar. *Candollea* **67:** 142–144.

Christenhusz MJM, **Fay MF**, **Chase MW. 2017.** *Plants of the World: an illustrated encyclopedia of vascular plants*. Kew: Kew Publishing; Chicago: University of Chicago Press.

Collins RA, **Cruickshank RH. 2012.** The seven deadly sins of DNA barcoding. *Molecular Ecology Resources* **13**: 969–975.

Couvreur TLP, **Helmstetter AJ**, **Koenen EJM**, **Bethune K**, **Brandão RD**, **Little SA**, **Sauquet H**, **Erkens RHJ. 2019.** Phylogenomics of the major tropical plant family Annonaceae using targeted enrichment of nuclear genes. *Frontiers in Plant Science* **9:** 1941.

Crawford FM, **Darbyshire I. 2015.** *Ochna dolicharthros* (Ochnaceae): a new species from northern Mozambique. *Kew Bulletin* **70:** 2.

Degnan JH, **Rosenberg NA. 2006.** Discordance of species trees with their most likely gene trees. *PLoS Genetics* **2:** e68.

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

Dodsworth S, **Pokorny L**, **Johnson MG**, **Kim JT**, **Maurin O**, **Wickett NJ**, **Forest F**, **Baker WJ. 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, Botanical Society of America* **19:** 11–15.

Du Toit PCV, **Obermeyer AA. 1976.** Ochnaceae. In: Ross JH, ed. *Flora of southern Africa*. Pretoria: Botanical Research Institute, 1–12.

Engler A. 1893. Ochnaceae Africanae. Botanische Jahrbücher für Systematik, *Pflanzengeschichte und Pflanzengeographie* **33:** 75–82.

Fernández R, **Gabaldón T**, **Dessimoz C. 2019.** Chapter 2.4. Orthology: definitions, inference, and impact on species phylogeny inference. In: Scornavacca C, Delsuc F, Galtier N, eds. *Phylogenetics in the genomic era*. No commercial publisher: Authors open access book, 2.4:1–2.4:14.

Frost P. 1996. The ecology of Miombo woodlands. In: Campbell B, ed. *The Miombo in transition: woodlands and welfare in Africa*. Bogor: Centre for International Forestry Research, 11–57.

Funk VA, **Edwards R**, **Keeley S. 2018.** The problem with(out) vouchers. *Taxon* **67:** 3–5.

Gardner EM, **Johnson MG**, **Pereira JT**, **Shafreena A**, **Puad A**, **Arifiani D**, **Wickett NJ**, **Zerega NJC. 2020.** Paralogs and off-target sequences improve phylogenetic resolution in a densely sampled study of the breadfruit genus (*Artocarpus*, Moraceae). *Systematic Biology* **70:** 1–18.

Gosper CR, **Vivian-Smith GA**, **Hoad K. 2006.** Reproductive ecology of invasive *Ochna serrulata* (Ochnaceae) in south-eastern Queensland. *Australian Journal of Botany* **54:** 43–52.

Herrando-Moraira S, **Calleja JA**, **Carnicero P**, **Fujikawa K**, **Galbany-Casals M**, **Garcia-Jacas N**, **Im HT**, **Kim SC**, **Liu JQ**, **López-Alvarado J**, **López-Pujol J**, **Mandel JR**, **Massó S**, **Mehregan I**, **Montes-Moreno N**, **Pyak E**, **Roquet C**, **Sáez L**, **Sennikov A**, **Susanna A**, **Vilatersana R. 2018.** Exploring data processing strategies in NGS target enrichment to disentangle radiations in the tribe Cardueae (Compositae). *Molecular Phylogenetics and Evolution* **128:** 69–87.

Hinchliff CE, **Smith SA**, **Allman JF**, **Burleigh JG**, **Chaudhary R**, **Coghill LM**, **Crandall KA**, **Deng J**, **Drew BT**, **Gazis R**, **Gude K**, **Hibbett DS**, **Katz LA**, **Dail H**, **Iv L**, **Mctavish EJ**, **Midford PE**, **Owen CL**, **Ree RH**, **Rees JA**, **Soltis DE**, **Williams T**, **Cranston KA**, **Crandall KA**, **Cranston KA. 2015.** Synthesis of phylogeny and taxonomy into a comprehensive tree of life. *Proceedings of the National Academy of Sciences, USA* **112:** 12764–12769.

Johnson MG, **Gardner EM**, **Liu Y**, **Medina R**, **Goffinet B**, **Shaw AJ**, **Zerega NJC**, **Wickett NJ. 2016.** HybPiper: extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. *Applications in Plant Sciences* **4:** 1600016.

Johnson MG, **Pokorny L**, **Dodsworth S**, **Botigué LR**, **Cowan RS**, **Devault A**, **Eiserhardt WL**, **Epitawalage N**, **Forest F**, **Kim JT**, **Leebens-Mack JH**, **Leitch IJ**, **Maurin O**, **Soltis DE**, **Soltis PS**, **Wong GKS**, **Baker WJ**, **Wickett NJ. 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.

Junier T, **Zdobnov EM. 2010.** The Newick utilities: high-throughput phylogenetic tree processing in the UNIX shell. *Bioinformatics* **26:** 1669–1670.

Kanis A. 1968. A revision of the Ochnaceae of the Indo-Pacific area. *Blumea* **16:** 1–82.

Katoh K, **Misawa K**, **Kuma K**, **Miyata T. 2002.** MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30:** 3059–3066.

Kokwaro JO. 1976. *Medicinal plants of east Africa*. Nairobi: East African Literature Bureau.

Kuhnhäuser BG, **Bellot S**, **Couvreur TLP**, **Dransfield J**, **Henderson A**, **Schley R**, **Chomicki G**, **Eiserhardt WL**, **Hiscock SJ**, **Baker WJ. 2021.** A robust phylogenomic framework for the calamoid palms. *Molecular Phylogenetics and Evolution* **157:** 107067.

Lemmon EM, **Lemmon AR. 2013.** High-throughput genomic data in systematics and phylogenetics. *Annual Review of Ecology, Evolution, and Systematics* **44:** 99–121.

Li H, **Durbin R. 2009.** Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25:** 1754–1760.

Linnaeus C. 1737. *Genera plantarum*. Leiden: Conrad Wishof.

Linnaeus C. 1747. *Flora Zeylanica.* Stockholm.

Linnaeus C. 1752. *Genera plantarum.* Halle.

Linnaeus C. 1754. *Genera plantarum*, ed. 5. Stockholm.

Mai U, **Mirarab S. 2018.** TreeShrink: fast and accurate detection of outlier long branches in collections of phylogenetic trees. *BMC Genomics* **19:** 272.

McKain MR, **Johnson MG**, **Uribe-Convers S**, **Eaton D**, **Yang Y. 2018.** Practical considerations for plant phylogenomics. *Applications in Plant Sciences* **6:** e1038.

Murphy B, **Forest F**, **Barraclough T**, **Rosindell J**, **Bellot S**, **Cowan R**, **Golos M**, **Jebb M**, **Cheek M. 2020.** A phylogenomic analysis of *Nepenthes* (Nepenthaceae). *Molecular Phylogenetics and Evolution* **144:** 106668.

Nguyen LT, **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.

Oliver D. 1868. Ochnaceae. In: *Flora of Tropical Africa*. Ashford: Reeves, L. & Co, 315–322.

Palgrave KC. 2002. *Trees of southern Africa. New edition revised and updated by Meg Coates Palgrave.* Cape Town: Struik.

Paradis E, **Claude J**, **Strimmer K. 2004.** APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20:** 289–290.

Perrier de la Bâthie H. 1941. Révision des Ochnacées de la région malgache. *Notulae Systematicae (Paris)* **10:** 333–369.

Perrier de la Bâthie H. 1951. Ochnacées. In: *Flore de Madagascar Comores*. Paris: Muséum national d'histoire naturelle, 1–19.

Perry LM, **Metzger J. 1980.** *Medicinal plants of east and southeast Asia: attributed properties and uses.* Cambridge: MIT Press.

Phillips EP. 1922. The genus *Ochna*. *Bothalia* **1:** 87–96.

Pleijel F, **Jondelius U**, **Norlinder E**, **Nygren A**, **Oxelman B**, **Schander C**, **Sundberg P**, **Thollesson M. 2008.** Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution* **48:** 369–371.

POWO. 2019. *Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew*. Available at: http://www.plantsoftheworldonline.org/ [Accessed 05 March 2021].

R Core Team. 2020. *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org/ [Accessed 20 January 2021].

Ranwez V, **Chantret N. 2020.** Strengths and limits of multiple sequence alignment and filtering methods. In: Scornavacca C, Delsuc F, Galtier N, eds. *Phylogenetics in the genomic era. Author open access book*. 2.2:1–2.2:36. Available at: https://hal.archives-ouvertes.fr/hal-02535389v2.

Richardson IBK. 1979. Ochnacées. In: Antoine R, Brenan JPM, Mangenot G. *Flore des Mascareignes: La Réunion, Maurice, Rodrigues, Vol. 67*, 1–4. *Balsaminacées à 68. Burséracées.* Le Reduit: Mauritius Sugarcane Industry Research Institute.

Robson NK. 1963. Ochnaceae. In: Exell AW, Fernandes A, Wild H, ed. *Flora Zambesiaca*. London: Flora Zambesiaca Managing Committee, 225–251.

Robson NKB. 1962a. The author and typification of the genus *Ochna*. *Taxon* **11:** 48–52.

Robson NKB. 1962b. New and little known species from the Flora Zambesiaca area. *VI. Boletim da Sociedade Broteriana* **32:** 151–173.

Roch S, **Warnow T. 2015.** Points of View on the robustness to gene tree estimation error (or lack thereof) of coalescent-based species tree methods. *Systematic Biology* **64:** 663–676.

Rutherford MC. 1983. Growth rates, biomass and distribution of selected woody plant roots in *Burkea africana-Ochna pulchra* savanna. *Vegetatio* **52:** 45–63.

Rutherford MC, **Panagos MD. 1982.** Seasonal woody plant shoot growth in *Burkea africana—Ochna pulchra* savanna. *South African Journal of Botany* **1:** 104–116.

Schneider JV, **Bissiengou P**, **Amaral MdCE**, **Tahir A**, **Fay MF**, **Thines M**, **Sosef MSM**, **Zizka G**, **Chatrou LW. 2014.** Phylogenetics, ancestral state reconstruction, and a new infrafamilial classification of the pantropical Ochnaceae (Medusagynaceae, Ochnaceae *s.str.*, Quiinaceae) based on five DNA regions. *Molecular Phylogenetics and Evolution* **78:** 199–214.

Schneider JV, **Jungcurt T**, **Cardoso D**, **Amorim AM**, **Töpel M**, **Andermann T**, **Poncy O**, **Berberich T**, **Zizka G. 2020.** Phylogenomics of the tropical plant family Ochnaceae using targeted enrichment of nuclear genes and 250+ taxa. *Taxon* **70:** 48–71.

Schneider JV, **Paule J**, **Jungcurt T**, **Cardoso D**, **Amorim AM**, **Berberich T**, **Zizka G. 2021.** Resolving recalcitrant clades in the pantropical Ochnaceae: insights from comparative phylogenomics of plastome and nuclear genomic data derived from targeted sequencing. *Frontiers in Plant Science* **12:** 105.

Shah T, **Burrows J**, **Darbyshire I. 2018.** A new species of *Ochna* (Ochnaceae) from the Barberton Mountains of Mpumalanga, South Africa. *Phytotaxa* **374:** 241–248.

Shah T, **Schneider JV**, **Zizka G**, **Maurin O**, **Baker W**, **Forest F**, **Brewer GE**, **Savolainen V**, **Darbyshire I**, **Larridon I. 2021.** Joining forces in Ochnaceae phylogenomics: a tale of two targeted sequencing probe kits. *American Journal of Botany*. **108:** 1201–1216.

Soltis DE, **Moore MJ**, **Sessa EB**, **Smith SA**, **Soltis PS. 2018.** Using and navigating the plant tree of life. *American Journal of Botany* **105:** 287–290.

Soto Gomez M, **Pokorny L**, **Kantar MB**, **Forest F**, **Leitch IJ**, **Gravendeel B**, **Wilkin P**, **Graham SW**, **Viruel J. 2019.** A customized nuclear target enrichment approach for developing a phylogenomic baseline for *Dioscorea* yams (Dioscoreaceae). *Applications in Plant Sciences* **7:** e11254.

Sprengel KPJ. 1826. *Caroli Linnaei systema vegetabilium, 16th edn*: 713. Göttingen: Dietrichian Library.

Starr F, **Starr K**, **Loope L. 2003.** Ochna thomasiana. Maui: US Geological Survey, Biological Resources Division, Haleakala Field Station. Available at: http://www.hear.org/starr/hiplants/reports/pdf/ochna_thomasiana.pdf.

van Tieghem P. 1902. Sur les Ochnacées. *Annales des sciences naturelles huitième série. Botanique tome* **16:** 161–416.

Verdcourt B. 2005. Ochnaceae. In: Beentje HJ, Ghazanfar SA, ed. *Flora of tropical East Africa*. Kew: Royal Botanic Gardens.

White F. 1983. *The vegetation of Africa. Natural Resources Research No 20.* Paris: UNESCO.

Zhang C, **Rabiee M**, **Sayyari E**, **Mirarab S. 2018.** ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* **19:** 153.

SUPPORTING INFORMATION

Figure S1. Gene recovery of *Ochna* species and outgroups. Table showing recovery of loci from the custom bait kit (Schneider *et al*., 2020) of 275 nuclear loci as a proportion of the target length. Darker colour indicates higher gene recovery.

Figure S2. A, Principal component analysis of Robinson–Foulds (RF) distances between eight species trees. F1 indicates datasets undergone moderate filtering and F2 indicates datasets undergone stringent filtering. Where not indicated, paralogues were removed. Yellow represents supercontig datasets, green represents exon dataset, with star symbols representing multi-species coalescent (MSC) inference and circles representing maximum likelihood concatenation (ML concat) inference. B, Tree summarizing species tree topologies. Majority rule consensus tree of eight species tree topologies with conflicting nodes collapsed into polytomies highlighted in red. Labels to the right denote infrageneric sections; section *Renicarpus* abbreviated as St. *Reni* and section *Ramistylus* abbreviated to St. *Rami*.

Figure S3. Phylogenetic tree inferred through maximum likelihood concatenation. Exon dataset with moderate filtering estimated with IQTREE. Branch labels indicate bootstrap values as a percentage.

Table S1. Voucher information and capture success for each sample

Table S2. Target enrichment and gene recovery efficiency for each sample

Table S3. Summary statistics generated with AMAS for A, Moderate filtered exons; B, Moderate filtered supercontigs; C, Stringent filtered exons and D, Stringent filtered supercontigs. Column L: AD count of the number of characters present for that gene

Table S4. List of accepted species in this genus from POWO (2019) and African Plants Database (2021), indication of their respective section and if included in the present study