Temporal and Biogeographical History of the Sesame Family (Pedaliaceae) with a Focus on the Succulent Genera *Uncarina* and *Sesamothamnus*

By

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A dissertation submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

(Botany)

at the

UNIVERSITY OF WISCONSIN-MADISON

2018

Date of final oral examination: July 19, 2018

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ACKNOWLEDGEMENTS

I am grateful to the members of my graduate committee Dr. Ken Sytsma, Dr. Ken Cameron, Dr. Tom Givnish, Dr. Eve Emshwiller, and Dr. David Spooner: thank you for providing comments, expertise, and ideas to this project. I am also thankful to Dr. David Baum and Dr. Ellen Damschen for serving on my committee in earlier stages of my graduate career.

My colleagues in the Sytsma Lab have provided help, understanding, and friendship during my seven years at UW. Ricardo Kriebel and Chloe Drummond both helped with questions about lab and analytical techniques. I am most grateful to Jeff Rose, who has become one of my closest friends. He was always willing to explain a method or approach (sometimes multiple times) and always helped me in any way he could. I could not have hoped to have met a person like him and made such a lifelong friend.

My undergrad adviser and (still!) mentor, Dr. Emmet Judziewicz has taught me so much over the years. He saw potential in me during my time at UWSP and provided me with so many opportunities. He has pushed me to do things I never would have done and continues to be an influence on my professional career. I can only hope to be as good a botanist as he is.

Lastly, I am thankful for the love and support of my family: my wife Emily and our daughters Elizabeth and Julia. Without them, I may never have completed this difficult endeavor and it was certainly Emily who kept me motivated.

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ABSTRACT

The sesame family (Pedaliaceae) is a species-poor (~70 spp. in 14 genera), largely herbaceous family that is primarily distributed across drier regions of Africa with outliers in India, Madagascar, and Australasia. Our research investigates this little-studied and taxonomically complex family in a temporal and biogeographical context. Building on the work of previous authors, we provide a date for the origin of the family using fossil priors of its closest relatives. We show that the Pedaliaceae are a relatively young family, with a crown age of 33.4my. Our dated phylogeny shows that diversification in the family occurred during, and was most likely driven by, the worldwide increase in arid climates during the Miocene Epoch. We analyze the family in a biogeographical context using BioGeoBEARS and show that the family has a southern African origin, as has been long hypothesized. Most of its species are confined to Africa, but we show that there have been range expansions into India, Madagascar, and Australasia as well as into northern Africa. These expansions all occurred during the Miocene as well. We narrow the scope of our research onto the two woody, succulent genera *Uncarina* and *Sesamothamnus*. *Uncarina*, endemic to Madagascar, is one of the most diverse genera in the family and is distributed across the drier regions of the island. We use Next Generation sequencing to elucidate relationships within the genus and show that there is strong discordance between nDNA and cpDNA datasets. While morphological characters help link the clades in both datasets, geography has the strongest signal with clear southern and northern groups being recovered. Within *Sesamothamnus*, we use Next Generation sequencing methods to better understand and date its origin and diversification. We use these dates to place it in the context of the African Arid Corridor distributional pattern, one shared among many unrelated plants that

occupy arid areas in the north and south. Our research adds more evidence that the Miocene aridity played a large role in shaping arid region floras.

INTRODUCTION

The Pedaliaceae are a small (~70 spp. in 14 genera) but interesting family. The majority of the family is distributed across drier regions of Africa but also occurs in similar habitats in Madagascar, India, and Australasia. With the exception of three genera, the entire family is made up of herbaceous taxa. This unusual distributional pattern, array of life history traits, and propensity for arid habitats makes the family attractive for addressing several questions about plant evolution.

My first chapter dates the origin of the family and shows that it is relatively young: 33.4my. I further show that diversification in all the large genera occurred during the Miocene Epoch (5-20Mya), during a time of global aridity intensification. Indeed, most of the family occupies dry or arid regions today. I then investigate the family in a biogeographical context using BioGeoBEARS. These analyses show that the family originated in southern Africa with subsequent range expansions into east and north Africa, Madagascar, India, and Australasia. These expansions occurred during the Miocene Epoch and were probably driven by the increase in aridity during this time and a potential increase in suitable habitat.

My second chapter focuses on the genus *Uncarina*, which is endemic to Madagascar. *Uncarina* is the second largest genus in the family, after *Sesamum*, with 15 known taxa and is morphologically and taxonomically complex. Using Next Generation sequencing I generate the first taxonomically complete phylogenetic analysis for any genus in the family. *Uncarina* shows clear geographical separation of taxa in both the nuclear DNA and chloroplast DNA datasets, despite there being a large amount of discordance: species are split into southern, western, and northern clades. Morphological characters that had been previously used to hypothesize relationships in the genus are backed up by the DNA evidence.

The third chapter investigates the small genus *Sesamothamnus*, which contains six known species. This genus, like others in the family, shows a marked disjunction between arid regions of northern and southern Africa. This pattern is also shown among many unrelated arid-adapted plants and has been linked to the increased aridity that occurred during the Miocene Epoch. Using Next Generation Sequencing methods, I generate a dated phylogeny of the genus and show that it too diversified and geographically split during the Miocene. I also show that past hypotheses about relationships in the genus are largely unfounded and that geography is the strongest link between species.

Chapter 1: Temporal and Biogeographical History of the Pedaliaceae

John G. Zaborsky and Kenneth J. Sytsma developed the research questions and approach. Emily M. Lemmon and Alan Lemmon sequenced the DNA. J. Zaborsky, K. Sytsma, and Jeffrey P. Rose performed analyses. J. Zaborsky wrote the rough draft of the manuscript and K. Sytsma contributed to the revisions.

Abstract

The Pedaliaceae are a small family almost entirely restricted to Africa. While the tribal and generic relationships within the family have recently been investigated in a phylogenetic context, no studies have looked at the temporal and spatial origin of the family. Using Next-Generation Sequencing (fully sampling two of the larger genera) and already published sequences, we present a biogeographical scenario for the family and date its origin. Our results show that the Pedaliaceae originated in southern Africa during the Oligocene. Diversification and range expansions in numerous genera all appear to have happened during the Miocene. During this time, Africa and other parts of the world underwent increased aridification and this may explain the geographic patterns seen in the Pedaliaceae.

Introduction

The Pedaliaceae are a small family of mostly annual and perennial herbs and a few shrubs and small trees. The family is most famous for the oilseed crop *Sesamum indicum* L. but also includes a few species used locally as vegetable and seed crops or purported medicinal purposes. Some of the succulent species are grown as curiosities. The Pedaliaceae are almost entirely restricted to dry regions of mainland Africa with only three genera being found elsewhere: *Sesamum* L. has a few species in India, *Uncarina* Stapf. is endemic to Madagascar, and *Josephinia* Vent. has three species in Australasia (as well as one in Africa). The family is not very speciose, with only 14 genera and ~70 spp. recognized (Ihlenfeldt, 2004). The most recently described genus, *Dewinteria* van Jaarsv. & A.E. van Wyk, was established to accommodate an unusual species of *Rogeria* J. Gay ex Delile (van Jaarsveld & Van Wyk, 2007). The largest genera are *Sesamum* (~20 spp.), *Uncarina* (~14 spp.) and *Pterodiscus* Hooker (~13

spp.). All other genera in the family each contain fewer than 10 described species, with four genera being monotypic (e.g. *Dewinteria*, *Holubia* Oliv., *Pedalium* Royen ex L., *Pedaliodiscus* Ihlenf.). Despite this lack of diversity, the family has a remarkable array of dispersal strategies, employing anemochory, various forms of epizoochory, and potentially hydrochory (Ihlenfeldt, 2004). All genera have capsular fruits, often adorned with wings (*Pterodiscus, Holubia*), large hooks and spines (*Uncarina*, *Josephinia*, *Harpagophytum* DC. ex Meisn.), or they have passively dispersed seeds that are simply knocked out of the mature fruits (*Sesamum*, *Sesamothamnus* Welw.) (Fig. 1).

The Pedaliaceae are well-known for not just their varied dispersal strategies, but also their unusual trichomes. These hairs, found nowhere else in the angiosperms, have a unique construction: a unicellular to multicellular hair topped by a gland consisting of four cells. These four cells have thickened cell walls and upon contact with water and some sort of disturbance, the outer cell walls dissolve, creating a large amount of mucilage. The purpose of this mucilage is unknown but could be an herbivory deterrent. The chemical make-up of the mucilage and the phytochemistry of the plants themselves has not been studied in great detail. All members of the family have an unusual odor to the stems, leaves, and flowers, all of which are generally covered in the aforementioned mucilage glands and could be the source of the odors.

The affinities of the Pedaliaceae to other families in the Lamiales have long been controversial. Originally, the Pedaliaceae (and Martyniaceae) were included in the Bignoniaceae. Brown (1810) established the Pedaliaceae as a separate family and Kunth (1818) moved some genera of the current Martyniaceae into the Pedaliaceae. Despite others arguing for its exclusion (Link, 1829), most authors have maintained the Martyniaceae within the Pedaliaceae, considering the former a New World derivative of the latter (Cronquist, 1981). The

defense for keeping the two families together was based on the similar dispersal strategies of many of the plants' fruits, primarily ones that use large hooks and spines for epizoochory. This is despite the fact that the fruit protuberances are formed in totally different ways: in the Martyniaceae, the fruit wall collapses around the outgrowths, while in the Pedaliaceae the protuberances grow outward from the endocarp (Ihlenfeldt, 2004). Members of the Martyniaceae also lack the distinctive glandular hairs seen in the Pedaliaceae. Phylogenetic analyses focusing on the Lamiales, and Asterids in general, have shown that the Martyniaceae and Pedaliaceae, while being superficially similar, should be recognized as separate families (Refulio-Rodriguez & Olmstead, 2014). As methods and sampling have improved, many of these studies have shown a close relationship between the Acanthaceae, Martyniaceae, and Pedaliaceae (Refulio-Rodriguez & Olmstead, 2014, Gormley et al., 2015). Gormley et al. (2015), in the first phylogenetic study of the generic relationships in the family, also showed that the unusual aquatic herb *Trapella* Oliv., sometimes included in the Pedaliaceae, is actually part of the Plantaginaceae. Its inclusion in the Pedaliaceae has been viewed as dubious (Ihlenfeldt, 2004) due to its habit and geography and it was sometimes treated as the sole member of the Trapellaceae. Outside of their study, no genus of the Pedaliaceae has been studied in its entirety in a phylogenetic context and members of the family have only been used as placeholders in wider analyses of the Lamiales and angiosperms.

Ihlenfeldt (1994) presents a putative phylogeny of the Pedaliaceae based on morphological characters and the work of Gormley et al. (2015) presented that some of these relationships are true; most notably that the three recognized tribes within the family (Pedalieae, Sesameae, and Sesamothamneae) are each monophyletic. The Pedalieae (Fig. 2) is sister to the other two tribes and contains herbaceous perennials, some annuals, subshrubs, shrubs, and small

trees. Although Ihlenfeldt (1994) argues that *Sesamothamnus* (Fig. 3), the sole member of the Sesamothamneae, is the most "primitive" genus in the family (due to its bignoniaceous appearance), this is not the case. *Sesamothamnus* is a small genus of large shrubs and the Sesameae contains mostly herbaceous perennials, a few annuals, and some subshrubs. The Sesameae (Fig. 3) contains five genera (*Sesamum*, *Josephinia*, *Dicerocaryum* Bojer, *Ceratotheca* Endl., and *Linariopsis* Welw.) and Gormley et al. (2015) showed that *Sesamum* is paraphyletic, with *Ceratotheca*, *Dicerocaryum*, and *Josephinia* embedded within it (*Linariopsis* was not sampled). This was suspected by Ihlenfeldt (1994) and Abels (1975) based on the heterogeneous circumscription of *Sesamum*. Both of these authors thought that the sections of *Sesamum* were more closely related to other genera within the Sesameae than they were to each other.

Our work investigates the Pedaliaceae in a phylogenetic context with greater taxon sampling, uses an anchored phylogenomics approach, Anchored Hybrid Enrichment (AHE), is the first to date the origin of the family, and investigates the biogeographic diversification of this largely African and arid-adapted family. We aimed to elucidate when the Pedaliaceae diversified and explore the biogeographical history of the family.

Methods

Taxon Sampling in Pedaliaceae — We used a two-step sampling procedure to cover the majority of the family. First, we obtained DNA from a subset of the family using various vouchered sources. Silica-dried leaves of wild-collected specimens of each known *Uncarina* taxon, *Pterodiscus aurantiacus*, *Sesamothamnus benguellensis*, and *S. guerichii* were used for DNA extraction. Silica-dried leaves of *Uncarina* and *Sesamothamnus* species were also collected from the living collection at the Huntington Botanical Garden in San Marino, CA as

well as the private collection of Dr. Dan Mahr (UW-Madison, Entomology Dept.); only specimens of known wild heritage were collected. These included twenty accessions of *Uncarina* and eleven accessions of *Sesamothamnus*, representing all known taxa of each genus. *Ceratotheca triloba* was collected from the UW-Madison Botany Department Greenhouse. Specimens for which DNA became available formed the subset of taxa across the family for which AHE sequencing was applied to obtain around 500 single-copy nuclear genes, but also the chloroplast genome and nuclear ribosomal DNA (nrDNA) were sampled.

Second, we screened GenBank for all published sequences of Pedaliaceae to gain robust coverage for the family-wide analyses. Taxa that had sequences for up to five cpDNA regions (*matK, ndhF, pbsA, rbcL, rps16, trnL-F*) formed the second subset of taxa. A few of the downloaded sequences did not cleanly align with the other samples and were excluded; this includes the nuclear ribosomal ETS region used by Gormley et al. (2015), as it did not align with any of our large nrDNA sequences from the AHE sequencing.

DNA Extraction — DNA was extracted from silica-dried plant material using the DNeasy™ plant mini kit (Qiagen, Valencia, California) according to manufacturer's specifications.

Next Generation DNA Sequencing — We utilized an anchored phylogenomics approach, Anchored Hybrid Enrichment (AHE), using the Center for Anchored Phylogenomics at Florida State University. This method targets highly conserved "anchor" regions in the nuclear genome and generates hundreds of loci including both introns and exons (Lemmon et al., 2012). This specific pipeline has been used effectively to infer infrageneric relationships in angiosperms (Buddenhagen et al., 2016; Cardillo et al., 2017; Fragoso-Martínez et al., 2017; Mitchell et al., 2017). DNA was sonicated to a fragment size of between 200–600 bp before library preparation

and indexing following a modified protocol of Meyer and Kircher (2010). Indexed samples were pooled and enriched using the Angiosperm v.1 enrichment kit (Buddenhagen et al., manuscript). Sequencing was done on 4.5 PE150 Illumina HiSeq 2500 lanes at the Translational Science Laboratory, College of Medicine, Florida State University.

Paired reads were merged before assembly, following Rokyta et al. (2012). Reads were mapped to the probe regions using *Arabidopsis thaliana*, *Billbergia nutans*, and *Carex lurida* as references, combined with a de novo assembly approach to extend the assembly into flanking regions (Prum et al. 2015; Buddenhagen et al., 2016). Read files were traversed repeatedly until no additional mapped reads were produced and consensus sequences were calculated for each assembly cluster with contigs based on fewer than 100 reads removed. For each locus, orthology was determined following the procedures in Prum et al. (2015). Contig orthology was assessed using a pairwise distance matrix among homologs and used to cluster sequences with a neighbor-joining algorithm to assess if gene duplication occurred prior to or following the crown of the clade. Contigs suggesting duplication were removed from further analysis if they contained fewer than 32 taxa (92%). Sequences in each orthologous cluster were aligned using MAFFT v. 7.023b (Katoh and Standley, 2013), then trimmed and masked using the following procedure from Prum et al. (2015). Sites with the same character in > 50% of sequences were considered "conserved." A 20 bp sliding window was then moved across the alignment, and regions with < 13 characters matching the common base at the corresponding conserved site were masked. Sites with < 152 unmasked bases were removed. Finally, the masked alignments were inspected by eye. Regions considered obviously misaligned or likely paralogous were removed and poorly aligned sections in a given alignment were deleted.

As DNA fragments from non-targeted loci may be captured, we attempted to extract the plastid (cpDNA) genome from our reads. We used Geneious v. 10.2.3 to map all recovered forward and reverse reads to reference sequences. For the plastid genome, we used the whole plastid genome of *Sesamum indicum* (GenBank accession KCS569603) as a reference. Raw reads were trimmed and assembled using iterative refinement of up to 5 times with the default Geneious mapper and medium sensitivity. For the cpDNA, consensus sequences were generated using the strict consensus. If coverage was < 2, the consensus nucleotide was scored as a gap. Unmapped regions were treated as missing data and reads mapped to multiple positions were excluded from consensus calculations. Sequences were aligned using MAFFT with default parameters. After alignment, ambiguously aligned or called regions were removed by hand.

Constructing a Time-calibrated Phylogeny of Pedaliaceae — The taxa with AHE derived cpDNA sequences were merged with the taxa with GenBank derived sequences as one large concatenated file. Although incongruence between the nuclear and chloroplast genomes is seen within one genus (*Uncarina* – see Chapter 2), our aim here was to evaluate relationships generally above the species level and as widely-sampled as possible across the family. To ensure these goals, a concatenated alignment to cover both taxa subsets was necessary. Indeed, generic relationships (but only a subset of the total genera in the family) obtained by the AHE generated data of ca. 500 single-copy loci and of the chloroplast genome are congruent (data not shown; see Chapter 2 and 3).

The concatenated alignment for Pedaliaceae was analyzed under Bayesian Inference in BEAST to obtain both phylogenetic relationships and branch times. To do this, we enlarged the sampling of taxa to provide a larger context of the Lamiales. We sampled taxa widely across the

Lamiales using 103 species across 22 families with GenBank sequences coming primarily from the work of Refulio-Rodriguez & Olmstead (2014). BEAST 2.4.3 (Bouckaert et al., 2014) in the XSEDE interface of CIPRES (Miller et al., 2010) was used to generate a time-calibrated tree by incorporating an uncorrelated log-normal clock and a Yule speciation process. Two priors were used. First, we set the crown of Lamiales at 97 Mya (5% and 95% quantile = 94.5 and 99.5 Mya, respectively). Although considerable variation exists in this crown date among recent studies that have included at least three members of the order, the most sampled studies for the order have obtained dates for the crown spanning this prior: (105.3-)93(-80.5) Mya (Tank & Olmstead pers. comm.); ca. 97 Mya (Bremer et al., 2004); 100.6-97.5 Mya (Nylinder et al., 2012); and (104)101.6(-98.2) Mya (Roalson & Roberts, 2016). As there are no known fossils for Pedaliaceae, we used the secondarily derived date of 81.9 Mya (79.4-84.4, 95% confidence interval) for the crown of Pedaliaceae + Acanthaceae + Martyniaceae based on Acanthaceae fossils (Tripp and McDade, 2014). We ran two independent analyses of 100,000,000 generations each in BEAST, saving 10,000 trees for each run. Effective sampling of all parameters was visualized in output log files in Tracer v.1.6 (Rambaut et al., 2014). After removing 20% of samples as burn-in, independent runs were combined and a maximum clade-credibility (MCC) tree was constructed using TreeAnnotator v.1.8.4 (Bouckaert et al., 2014).

Tracking Biogeographical Shifts in Pedaliaceae — Ancestral range estimation (ARE) for Pedaliaceae was done with the BEAST tree in which all species outside Pedaliaceae were removed. We evaluated ARE with both the DEC (Ree and Smith, 2008) and DECj models in BioGeoBEARS (Matzke, 2013, 2014) in R v3.3.1. The "j" or jump parameter allows for a daughter lineage to immediately occupy via long-distance dispersal a new area that is different from the parental lineage. We explored both models to evaluate any major differences in the

inferred biogeographical history of Pedaliaceae. We identified eight geographic areas important in the context of the distributions of the family across Africa, Madagascar, India, and Australasia: (1) southern Africa, (2) Zambezian Africa, (3) Horn of Africa, (4) subhumid Madagascar, (5) dry Madagascar, (6) arid Madagascar, (7) India, (8) Australasia. We used a dispersal probability file for a single time slice for the family in which the probability of dispersal between areas was scaled from 1 to 0.1. Max range was set to 3 areas, because no individual species exceeded this biogeographical distribution. We conducted 100 biogeographical stochastic mapping (BSM) replicates in BioGeoBEARS (Matzke, 2016; Dupin et al., 2017) on the pruned BEAST tree.

Results

Dated Phylogeny of the Pedaliaceae – Our chronogram of the Pedaliaceae (Fig. 5) shows relatively young ages for the three tribes. Crown Pedalieae began diverging 27 Mya and Sesameae split from Sesamothamneae at 25 Mya. Within the Pedalieae, diversification within *Uncarina* began 9 Mya (see Chapter 2 for further discussion) while its split from the common ancestor with *Rogeria* occurred 15 Mya. The other clade in the Pedalieae contains five genera, all of which (except *Pterodiscus*) are small. This clade began diversifying 23 Mya with the lineage from which *Holubia* arose being the first to diverge. *Harpagophytum* contains two species and these diverged from one another 5 Mya. Further diversification in this clade did not happen until 11 Mya with the split of the lineages leading to *Pterodiscus* and *Pedalium* and *Pedaliodiscus*, the latter two diverged from one another relatively recently: 3 Mya. Diversification within *Pterodiscus* began occurring 9 Mya and appears to have happened quickly after that.

Although the split between the Sesameae and Sesamothamneae happened 25 Mya, crown *Sesamothamnus* only began diversifying 12 Mya (see Chapt 3 for further discussion). Diversification within the Sesameae began 21 Mya with crown group *Sesamum* sect. *Sesamopteris* diversifying relatively recently: 5 Mya. The rest of the tribe, which includes three sections of *Sesamum, Ceratotheca, Dicerocaryum*, and *Josephinia*, began diversifying 16 Mya. *Sesamum* sect. *Sesamum* diversified 8.9 Mya and the paraphyletic *Sesamum* sect. *Aptera* began diversifying 13 Mya.

Historical Biogeography of the Pedaliaceae – Using the work of previous authors (Linder et al., 2012; Zimkus et al., 2017) to partition Africa into biogeographic units, we established three regions across the continent with some modifications. We include eight worldwide regions for analyses. In Africa, we define "southern Africa" as South Africa, Namibia, Lesotho, Swaziland, Botswana, and part of Angola, largely following the work of Linder et al. (2012). We include Somalia, Ethiopia, and Djibouti in our "Horn of Africa" region. The rest of sub-Saharan Africa is lumped together into our "Zambezian" region. This was done because few taxa reach outside the Zambezian region of Linder et al. (2012) and the sparse floristic treatments focusing on the Pedaliaceae for many of the western countries. Without these works or databased specimens, it is hard to discern the natural ranges of many taxa, especially *Sesamum* and *Ceratotheca*, which are often cultivated. Within Madagascar, we delimit three regions: arid, dry, and subhumid using the recent work of Vieilledent et al. (2016). We also code India as a separate area that contains only three taxa in *Sesamum*. Australasia is defined as Australia and outlying islands, encompassing the entire known range of the three species of *Josephinia* that occur there. We manually coded geographic areas for each included taxon in our BioGeoBEARS analysis using monographic works, floristic studies, and herbarium specimens.

Based on a likelihood ratio test, the BioGeoBEARS DECj model of ancestral ranges (lnL = -110.57) was a significantly better fit (pval = 0.0003) for our data compared to DEC (lnL = -117.16). Our analysis shows a southern African origin of the family with subsequent dispersals to other parts of Africa, India, and Australasia (Fig. 7,8). The Pedalieae is primarily distributed in southern Africa, with a few genera found elsewhere. The first shift away from southern Africa in this tribe occurred in the *Pterodiscus*/*Pedalium*/*Pedaliodiscus* clade. All three genera are found throughout the Zambezian region with both *Pedalium* and *Pterodiscus* also found in the Horn of Africa. The natural range of *Pedalium murex* L. is difficult to determine due to its weedy nature and potentially water dispersed fruits. It is considered a saline soil indicator and may be native to African seashores, and potentially Madagascar (Ihlenfeldt, 1994). We follow Ihlenfeldt (1994) and tentatively coded this species as being native to the Zambezian region and the Horn of Africa. *Pterodiscus* is distributed widely across southern and eastern Africa, but many species have relatively small ranges (Ihlenfeldt, 1994). The origin of *Pterodiscus* is equivocal as to which region it arose. The disjunct distribution of some species and restricted ranges of others makes it hard to determine where this genus arose, but there have been clear shifts from southern Africa to the Horn of Africa. *Rogeria* is shown to have a southern African origin. This genus is most diverse in southwestern Africa with one species distributed in a narrow band along the southern border of the Sahara Desert (it also occurs in southwestern Africa). *Uncarina* is the only genus in this tribe to clearly be native to a region outside Africa. We recover a jump dispersal of its ancestor to the southern arid regions of Madagascar. Within *Uncarina*, we recover another shift to the dry regions of western and northern Madagascar. Within this large western/northern clade (see Chapter 2 for further details), there are two independent shifts to subhumid regions in the far northern part of the island.

The Sesameae and Sesamothamneae both have a southern African origin. Within *Sesamothamnus* there is one shift into the Horn of Africa. The paraphyletic *Sesamum* had a southern African origin with shifts to the other two African regions occurring in *Sesamum alatum* Thonn., a widespread species of *Sesamum* sect. *Sesamopteris* Endl.. The remaining sections of *Sesamum* and the other genera of the tribe also have a southern African origin. *Sesamum* sect. *Sesamum* and sect. *Chamaesesaum* Benth. have their origin in India. *Sesamum radiatum* Schumach. & Thonn. is a widespread African species that is a member of sect. *Aptera*. This species was not included in the analyses of Gormley et al. (2015) and we acquired it from GenBank. It is possible that there was a shift back to Africa from India, but it also seems plausible that this accession was identified incorrectly, and its true identity is *Sesamum indicum*. The Indian sections of *Sesamum* contain only one species each and they are both native to that country (Bedigian, 2003). The rest of the Sesameae contains species from *Sesamum* sect. *Aptera*, *Ceratotheca*, *Dicerocaryum*, and *Josephinia*. Three of the genera in this clade are not monophyletic but it clearly originated in southern Africa. *Ceratotheca sesamoides* Endl. is the most widespread species in the genus (Abels, 1975), occurring throughout Africa. *Dicerocaryum* has four species and we include two in our analyses. There was a dispersal to the Zambezian region on the stem of *Dicerocaryum zanguebarium* (Lour.) Merr. and this is the most northerly occurring species in the genus. *Josephinia* has three species that are widespread across Australasia and one in the Horn of Africa. *Josephinia eugeniae* F. Muell. is an Australasian species and range expansion occurred from southern Africa to the Horn of Africa to Australasia.

Discussion

Our phylogeny shows similar relationships among the tribes and genera to those found by Gormley et al. (2015). In both our analyses and theirs, there is strong support for the monophyly of the Pedaliaceae (Fig. 6). Gormley et al. (2015) showed strong support for the monophyly of the three tribes in their cpDNA phylogeny. However, in their analysis using ETS, they showed very weak support for the monophyly of the Pedalieae, but the other two tribes were strongly supported as monophyletic. When we tried to align their ETS sequences to our AHE data, we could not find the region. In earlier Sanger sequencing work that we performed, we were able to sequence ETS from numerous *Sesamothamnus* and *Uncarina* species. However, when we tried aligning these sequences with those generated by Gormley et al. (2015), they would not align either. It appears as if the ETS sequences used by Gormley et al. (2015) have something wrong with them or they amplified a different copy of this gene. So, we cannot say for certain if the nDNA phylogeny of Gormley et al. (2015) is accurate as we were unable to independently verify it. Morphologically, the Pedalieae are defined by an absence of short shoots, flowers in contracted dichasia or solitary in the leaf axils, stamen characters, and pollen in monads. More thorough sampling of nDNA regions within this tribe would help to understand the unusual results shown by Gormley et al. (2015). The monogeneric Sesamothamneae contains shrubs with distinct short shoots and long shoots, very reduced leaves, persistent petioles, leaves on short shoots being held in fascicles, flowers in raceme-like inflorescences, and pollen in tetrads. The Sesameae are primarily herbaceous (rarely subshrubs), lack short shoots and spines, and have flowers always borne solitary in the axils of leaves. While *Sesamum* lacks protuberances of any kind on its fruits, there is a general trend among the other genera to having outgrowths on the capsule body. In *Ceratotheca*, these are small “horns” on either carpel (Fig. 1). The same type of design is seen in *Dicerocaryum*, but the capsule body is flattened so that the horns stick

up from a flat disk (Fig. 1). In *Josephinia*, the fruit body is covered in numerous spines while in *Linariopsis* the fruit is covered in small tubercles but topped with a small mucro. With greater taxon and gene sampling, fruit evolution could be better explored in this tribe.

The Pedaliaceae originated in the Oligocene - Our fossil-calibrated phylogeny of the Lamiales (Fig. 4) shows an origin of crown Pedaliaceae at 33.4 Mya. The family is placed in a clade with the Acanthaceae and the Martyniaceae (which are sister taxa), as has been shown in other analyses of the Lamiales (Refulio-Rodriguez & Olmstead, 2014). The tribal relationships within the Pedaliaceae also match those showed by Gormley et al. (2015). We expand the sampling of the succulent genera *Pterodiscus*, *Uncarina* and *Sesamothamnus* over that in Gormley et al. (2015) for this study with full taxonomic sampling of the latter two. Within the Pedaliaceae, the diversification of the Pedalieae and the Sesameae and Sesamothamneae occurred at around the same time: 27.4 Mya and 25.2 Mya, respectively (Fig. 5). The Pedalieae split into two clades, one containing *Uncarina* and *Rogeria*, and the other made up of *Pterodiscus*, *Pedaliodiscus*, *Pedalium*, *Harpagophytum*, and *Holubia*. The former clade's two genera diverged 15 Mya with *Uncarina* further diversifying 8 Mya (see Chapter 2 for further discussion). No other sequences of *Rogeria* spp. were available for this study, so we cannot elucidate when diversification in that genus occurred. *Rogeria* is a small genus of herbs with a few species restricted to southwestern Africa and one species (*R. adenophylla* J. Gay ex Delile) that is disjunct between that region and a long, thin band that runs east to west, south of the Sahara Desert (Bedigian, 2013). The placement of *Dewinteria* also could not be ascertained due to a lack of samples.

The remainder of the Pedalieae began diversifying 23 Mya and the monotypic *Holubia* is sister to the rest of the genera. The next genus to diverge is *Harpagophytum*, which contains two

species of prostrate perennial herbs, at 21 Mya. The monotypic genera *Pedalium* and *Pedaliodiscus* diverged from their common ancestor with *Pterodiscus* 11 Mya and diversification of crown *Pterodiscus* began 9 Mya. Ihlenfeldt (2001) posited that these five genera were closely related, and his phylogeny based on morphology is confirmed by our analysis and those of Gormley et al. (2015). *Pterodiscus* species are all perennials, producing annual shoots from an underground, succulent caudex. The species are highly variable and Ihlenfeldt (2001) argued that the genus may not be monophyletic due to the fact that the "primitive" *Pt. angustifolius* Engl. has fruits that are similar to those of *Pedalium* and *Pedaliodiscus*. Even though we sample only five of the ~13 known species in *Pterodiscus*, our analyses do not support the view that the genus is not monophyletic. Increased sampling of *Pterodiscus*, especially *Pt. angustifolius*, would be ideal to better understand this genus.

The Sesamothamneae and Sesameae diverged from one another 25 Mya. The Sesamothamneae contains only one genus, *Sesamothamnus*, and has long been considered to be the most primitive genus in the family (Ihlenfeldt, 1994), with an argument sometimes being made for its inclusion in the Bignoniaceae (Bruce, 1953). Diversification of *Sesamothamnus* occurred 12.5 Mya with a split between the northern and southern species (see Chapter 3 for further discussion).

The Sesameae, as currently defined (Ihlenfeldt, 2004), contains five genera: *Sesamum*, *Dicerocaryum*, *Josephinia*, *Ceratotheca*, and *Linariopsis*. Material of *Linariopsis* was not procured for the analyses of Gormley et al. (2015) or for the present analyses. Efforts should be made to include this genus of 2-3 species in further analyses due to its apparent rarity and presence in western Africa, where few other genera are found. As shown by Gormley et al. (2015), *Sesamum* is paraphyletic with *Dicerocaryum*, *Josephinia*, and *Ceratotheca* being

imbedded within it. Our analyses also show that *Dicerocaryum* and *Ceratotheca* themselves are not monophyletic. The placement of the other *Josephinia* species could not be tested due to a lack of material. This genus has an unusual distribution: one species found in Kenya, Somalia, and Ethiopia and three species in Australia and Malaysia. This distributional pattern is unique in the family, but not unheard of in angiosperms (Goldblatt, 1981; Baum, 1995). Full sampling of this genus would greatly improve our understanding of the biogeography of the family and especially of *Sesamum*. It is also worth noting that *Josephinia africana* Vatke is rare across its range, while the Australasian taxa are wide-ranging. *Sesamum* has always been a heterogeneous assemblage of species (Ihlenfeldt & Grabow-Seidensticker, 1979; Ihlenfeldt, 1994) and is also the largest genus in the family. *Sesamum* contains four sections and we recover sect. *Sesamopteris* as monophyletic. It diverged from the remainder of *Sesamum* s.l. 20.9 Mya and diversification in that section happened 5.4 Mya. *Sesamum* sect. *Sesamum* and sect. *Chamaesesamum* are sister to one another and diverged from the remainder of the sampled taxa 15.7 Mya. *Sesamum prostratum* Retz. is the sole member of sect. *Chamaesesamum* and is endemic to India (Bedigian, 2015). It is sister to a clade containing cultivated sesame (*S. indicum*) and its wild progenitor, *S. indicum* var. *malabaricum* (Burm.) Bedigian, both of which are considered native to India (Bedigian 2003, 2014, 2015). The West African *S. radiatum* Schumach. & Thonn. is also placed in this clade, rendering *S. indicum* paraphyletic. The confident placement of *S. radiatum* into a section of the genus has been debated (Bedigian, 2010) and it could indeed be part of *Sesamum* sect. Sesamum. It is also possible that these sequences (obtained from GenBank) were from a misidentified plant and actually represent another accession of *S. indicum*.

The remainder of the Sesameae includes the three genera *Ceratotheca*, *Dicerocaryum*, and *Josephinia*, as well as species from *Sesamum* sect. *Aptera* Seidenst. (the largest section of *Sesamum*). This clade has a slightly different topology in our tree compared to that in Gormley et al. (2015) but the paraphyletic nature of *Sesamum* sect. *Aptera* is upheld. It has been argued that *Ceratotheca* and *Sesamum* sect. *Aptera* are very similar to one another with some suggesting that *Ceratotheca* should be included in *Sesamum* as its own section (reviewed in Bedigian, 2015). A new generic and sectional classification of this clade is currently underway (D. Bedigian, pers. comm.) with more thorough sampling, so we abstain from making any new combinations or assertions as to the relationships in this clade.

The majority of species in the Pedaliaceae are adapted to dry, often disturbed habitats. Many species are weedy and some are used as signs of overgrazing (*Harpagophytum*) or even saline soils (*Pedalium*) (Ihlenfeldt, 2004). Diversification within the Pedalieae and Sesameae/Sesamothamneae began in the early Miocene, 25-27 Mya. The different genera within each tribe diversified in the mid to late Miocene. The mid to late Miocene was a time of drying climates, decline in global CO_2 levels, and the rise of C_4 grasslands (Arakaki et. al, 2011). It was also a time when many succulent plant lineages saw rapid rates of diversification and speciation (Arakaki et. al, 2011). Major increases in diversification have been shown to have occurred during this time period in the Cactaceae, *Agave* (Asparagaceae), Aizoaceae: Ruschioideae, succulent *Euphorbia* spp. (Euphorbiaceae), and the Madagascan representatives of the Didiereaceae (Arakaki et. al, 2011). Many of these groups arose before the Miocene Epoch, but their diversifications took place 2.5-17 Mya. According to our analyses, the largest genera of the Pedaliaceae all diversified during this same period (Fig. 5). Arakaki et. al (2011) argue that this time frame was important worldwide since it seemingly affected so many major succulent plant

lineages. The three succulent genera of the Pedaliaceae also underwent speciation events starting 5 Mya in *Sesamothamnus*, 9 Mya in *Pterodiscus*, and 8 Mya in *Uncarina*. The largely herbaceous (rarely subshrub) *Sesamum* s.l. shows diversification of its major sections happening 5 Mya (sect. *Sesamopteris*), 9 Mya (sects. *Sesamum* and *Chamaesesamum*), and 12.8 Mya (sect. *Aptera* and related genera). Diversification of arid-adapted, non-succulent lineages also increased during this same time period (Moore & Jansen, 2006; Catalano et. al, 2008), and *Sesamum* also seems to fit this pattern.

The genera of the Pedaliaceae that are centered in Africa exhibit a distinct and interesting biogeographical pattern that may be explained by the same climatic events that seem to have shaped their diversification. As outlined by Ihlenfeldt (1994), there is a clear pattern of north/south disjunction in the family across the African continent. Some genera chiefly occur north of the equator (*Pedaliodiscus*) or south of it (*Holubia*, *Harpagophytum*, *Dicerocaryum*; Fig. 9). Others are disjunct between the south and north (*Sesamothamnus*, *Sesamum* sect. *Sesamopteris*, *Rogeria*; Fig. 10) and three groups can be considered to have relatively continuous ranges across southern/eastern/northern Africa (*Pterodiscus*, *Ceratotheca*, *Sesamum* sect. *Aptera*; Fig. 11). The north-south disjunction exhibited by some taxa in the Pedaliaceae is a common pattern in the flora of Africa (Verdcourt, 1969). Many unrelated plant groups that are adapted to arid or semi-arid habitats occur in parts of western South Africa, Namibia, and Angola with taxa also found in the Horn of Africa, sometimes extending into the Arabian Peninsula (De Winter, 1971). In many cases, there are no populations linking these regions (Thulin, 1994; Jürgens, 1997; Thulin et al., 2012). However, some genera occur in both of these regions but also link them through drier parts of eastern Africa (Holland, 1978; Bissinger et al., 2014). In both cases, it is hypothesized that the distributions of these arid-adapted plants have been shaped by the

"greening" of central and eastern Africa which created an area of inhospitable terrain for them. It has also been hypothesized that since the formation of tropical forests in central Africa, there has existed an African Arid Corridor (AAC) that linked the northern and southern arid areas (Bellstedt et al., 2012). This AAC would have cut through the eastern tropical forests as a direct route, or it may have consisted of "pockets" of drier areas that acted as stepping stones for arid-adapted plants to disperse (Bellstedt et al., 2012). Some recent phylogenetic analyses of arid-adapted plants showing this distribution pattern have shown that migrations occurred during periods when the AAC likely existed (Bellstedt et al., 2012; Bissinger et al., 2014). Periods of aridification in Africa have happened repeatedly, being caused by uplift in Eastern Africa (Sepulchre et al., 2006), intensification of the Benguela Current near southwest Africa (Siesser, 1980), and glacial/interglacial cycles in the Northern Hemisphere (Feakins et al., 2013). These events appear to have triggered diversification in various succulent plant lineages (Arakaki et al., 2011), non-succulent plants (Linder et al., 2006) and a rise in C_4 vegetation (Feakins et al., 2013). Our dated phylogeny shows that the evolutionary history of the Pedaliaceae has been shaped by these events as well.

Biogeography linked to Miocene aridity – The Pedaliaceae clearly have a southern African origin (Fig. 7, 8), as has long been hypothesized (Ihlenfeldt, 1994). Ihlenfeldt (1994) argued this fact based on the diversity of several genera being highest in the region (e.g. *Sesamum*, *Rogeria*) and that many (in his view) "primitive" taxa occur there (e.g. *Sesamothamnus*). We show that no one genus can clearly be considered the earliest diverging, but rather that the three tribes began diversifying around the same time. The Pedalieae are clearly southern African in origin and have expanded their ranges to northern and eastern Africa as well as Madagascar. The *Pedalium*/*Pedaliodiscus*/*Pterodiscus* clade originated in southern

Africa but has diversified into two other regions. *Pedalium* itself is more widespread, occurring in Madagascar, India, and elsewhere, but whether these populations are native is questionable. Wider sampling from regions outside mainland Africa of this weedy species would help to elucidate its provenance and may show an interesting biogeographical history. *Uncarina* is the only genus in the family that is endemic to Madagascar. We show that its common ancestor with *Rogeria* was southern African and there was a jump to the arid regions of Madagascar. *Uncarina* itself shows a clear split between the arid region species and the dry region species, with two dispersals to the subhumid regions of the northern part of the island and one back to the arid region. This apparent dispersal back to the arid region could be an artifact of the unusual cpDNA sequences in the genus (see Chapter 2 for further discussion).

Sesamothamnus had a southern African origin and subsequent dispersal to the Horn of Africa. Two species occur in the latter region and four in the former. For further discussion of this genus' history, see Chapter 3. The historical biogeography of the Sesameae is complicated by the paraphyly of *Sesamum*, the cultivation history of *Sesamum*, and incomplete sampling. We show that this tribe arose in southern Africa and had numerous dispersals to other parts of Africa, as well as India and Australasia. *Sesamum* sect. *Sesamopteris* is a southern African section with *S. alatum* having dispersed to other regions of Africa. This species is widespread, perhaps due to cultivation, and is weedy (Bedigian, 2003). *Sesamum* sect. *Sesamum* contains the oilseed crop *S. indicum* and its progenitor *S. indicum* var. *malabaricum* (Burm.) Bedigian. Bedigian (2014, and sources therein) showed that *S. indicum* var. *malabaricum*, is the progenitor of the cultivated sesame and that it originated in India. Both varieties are native to India, but the typical variety has been spread widely around the world as a crop plant. In addition to these taxa, the lone species of *Sesamum* sect. *Chamaesesamum*, *S. prostratum*, is endemic to India. *Sesamum* shows

one dispersal to India from southern Africa and further diversification there into two distinct sections. The identity of our accession of *S. radiatum* is questionable (see above) but it does seem possible that there could have been a dispersal back to Africa from India. Either way, these results beg the question as to how *Sesamum* reached India. Long-distance dispersal of the small seeds could be possible, but we cannot discount range expansion of some ancestral species through eastern and northern Africa into the Arabian Peninsula and eastward with subsequent extinction in the former regions. Increased sampling of *Sesamum* with multiple accessions from all known species would greatly improve our understanding of this ancient crop and its relatives. The paraphyletic *Sesamum* sect. *Aptera* is difficult to interpret with our moderate sampling. This section has been considered taxonomically problematic (Ihlenfeldt & Grabow-Seidensticker, 1979; Bedigian, 2010) and only two of the ~13 species in the section are in this clade. The other genera in this clade also complicate the issue as their diversity was not well-sampled. Neither *Dicerocaryum* (4 spp.) or *Ceratotheca* (5 spp.) are monophyletic and we only sampled two species from each genus. Range expansion occurred along the branch leading to *C. sesamoides* as well as *D. eriocarpum*. *Josephinia* shows range expansion to the Horn of Africa as well as Australasia. This genus has an unusual distribution pattern with one species in Africa and three in Australasia. The species in Australasia appear to be wide-ranging while the African species has a somewhat restricted range. Only *J. eugeniae* has ever been sequenced so dating the divergence within this genus and examining its dispersal history or direction is hampered. Again, more collections and better data would help understand these issues.

Regardless of the complicated nature of *Sesamum* s.l., an interesting pattern is discernible across the family's biogeographical history. As shown in the dated phylogeny of the family above and in the individual genera *Uncarina* (Chapter 2) and *Sesamothamnus* (Chapter 3), the

increased aridity of the Miocene Epoch has had a profound impact on the Pedaliaceae. The family contains many weedy annual species that are adapted to dry and arid conditions. The perennial species also show many adaptations to these conditions as well: tuberous roots, stem succulence, and dry deciduousness. Examining the BioGeoBEARS tree (Fig. 7,8) it can be seen that range expansion events that resulted in lineages leaving southern Africa happened during a relatively short period during the Miocene. The major expansions include *Sesamothamnus* moving north, *Sesamum* moving to India, the origin and subsequent diversification of the *Pedalium*/*Pedaliodiscus*/*Pterodiscus* clade, and the ancestor of *Uncarina* moving into Madagascar. These events all happened between ~15-10 Mya. It is not hard to imagine that during the Miocene, as the African Arid Corridor may have formed and reformed, that ancestral lineages within the Pedaliaceae moved north out of southern Africa and diversified in new regions. Some of these taxa moved north and then westward across Africa as well (e.g., *Sesamum*, *Ceratotheca*, *Rogeria*) now existing in a narrow band running along the southern border of the Sahara Desert. Many arid-adapted lineages have been shown to have diversified during the Miocene across both Africa (Bellstedt et al., 2012; Bissinger et al., 2014) and other parts of the world (Arakaki et al., 2011). Yet another example can now be added to this list of taxa whose evolutionary history was shaped by this pivotal time period in Earth's history.

It is clear that the Pedaliaceae, despite being largely restricted to Africa, have an interesting and varied biogeographical history. Arid habitats have helped to shape its morphological and distributional history with still much to be learned. Incorporating more samples of the large and complex genera *Pterodiscus* and *Sesamum* s.l. should greatly increase our understanding of the phenomena seen in the family. These analyses may also help clarify the long history of *Sesamum indicum* cultivation and its domestication, as well as that of other

lesser-known *Sesamum* crops. Unfortunately, some of this work is hampered by political strife in the Horn of Africa, which greatly limits access to wild habitats for collections. Hopefully future researchers will be able to gain access to the regions and plants once more and further develop our understanding of this remarkable African family.

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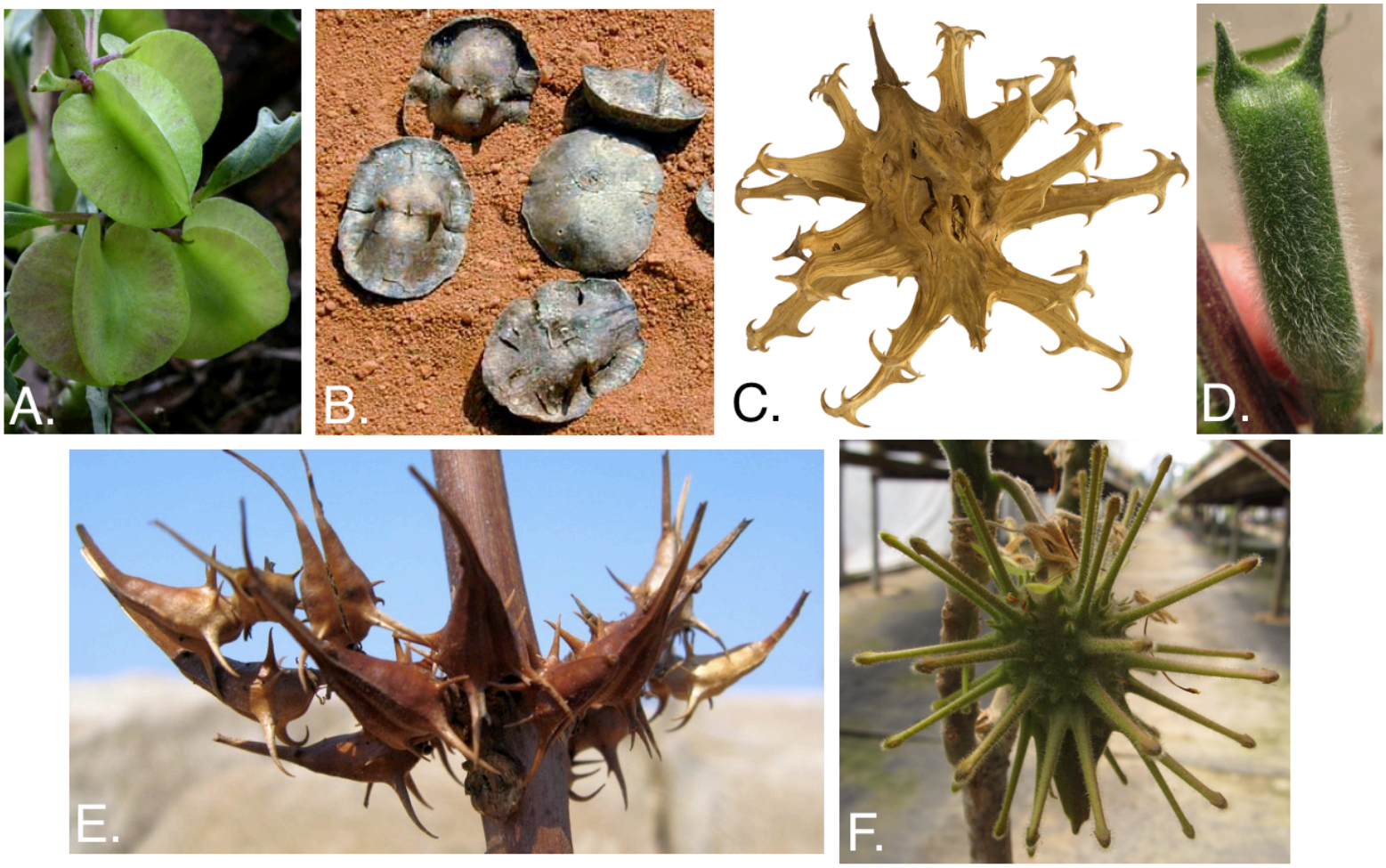

Fig. 1. Fruit diversity in the Pedaliaceae. A. Anemochorous fruits of *Pterodiscus ngamicus* ©BT Wursten. B. Epizoochorous fruits of *Dicerocaryum eriocarpum* © ethnopharmacologia.org. C. Epizoochorous fruit of *Harpagophytum procumbens* © R. Culos. D. Immature capsule of *Ceratotheca triloba*. E. Mature capsules of *Rogeria adenophylla* © N. Juergens. F. Epizoochorous fruit of *Uncarina roeoesliana*.

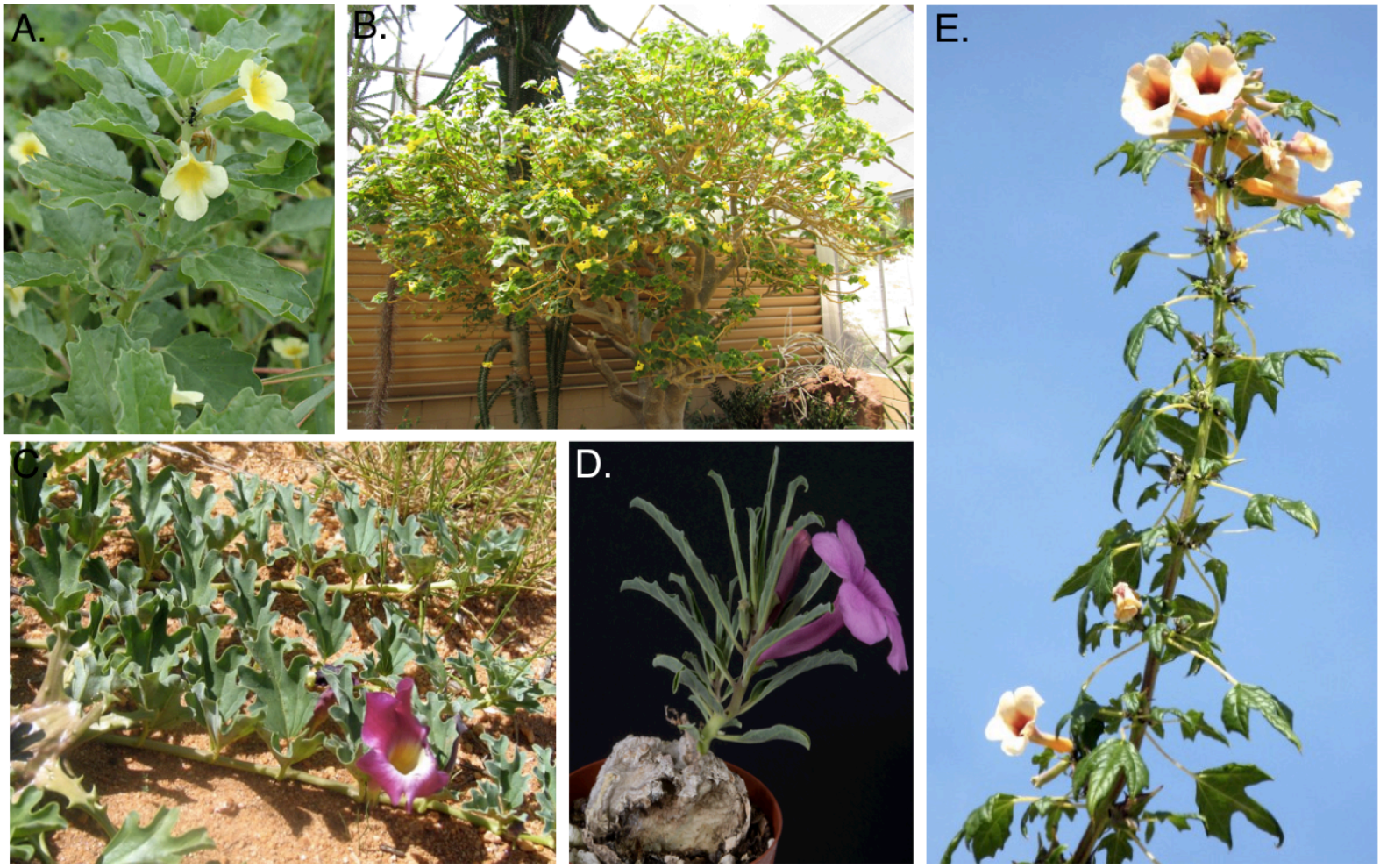

Fig. 2. Morphological diversity in the tribe Pedalieae. A. *Pedalium murex* ©M. Schmidt. B. *Uncarina grandidieri* C. *Harpagophytum procumbens* © N. Juergens. D. *Pterodiscus speciosus* © M. Massara. E. *Rogeria adenophylla* © N. Juergens.

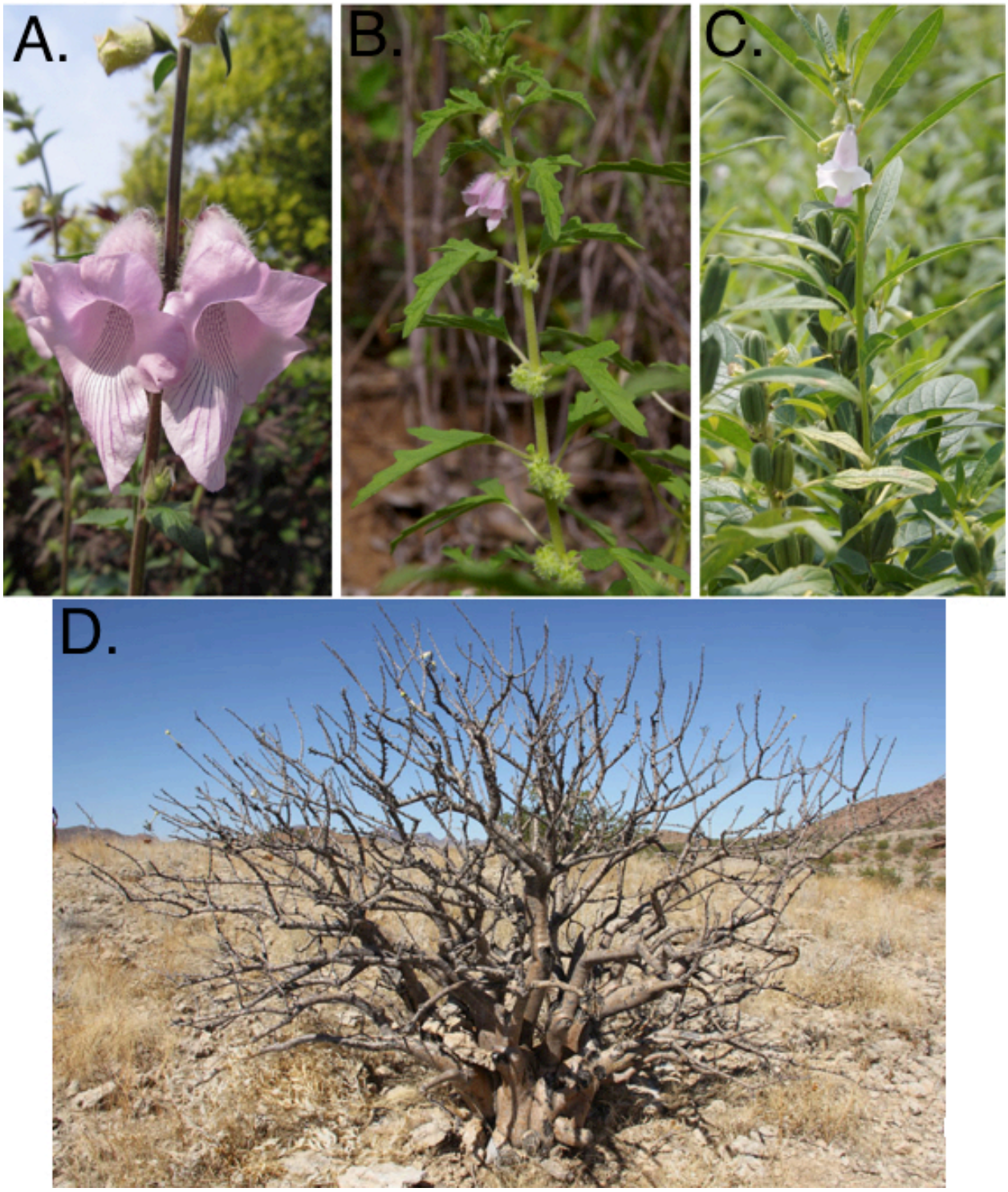

Fig. 3. Morphological diversity in the tribes Sesameae (A-C) and Sesamothamneae (D). A. *Ceratotheca triloba*. B. *Josephinia eugeniae* © R. Cumming. C. *Sesamum indicum* © Fl. Of Bangladesh. D. *Sesamothamnus benguellensis* © D. Mahr.

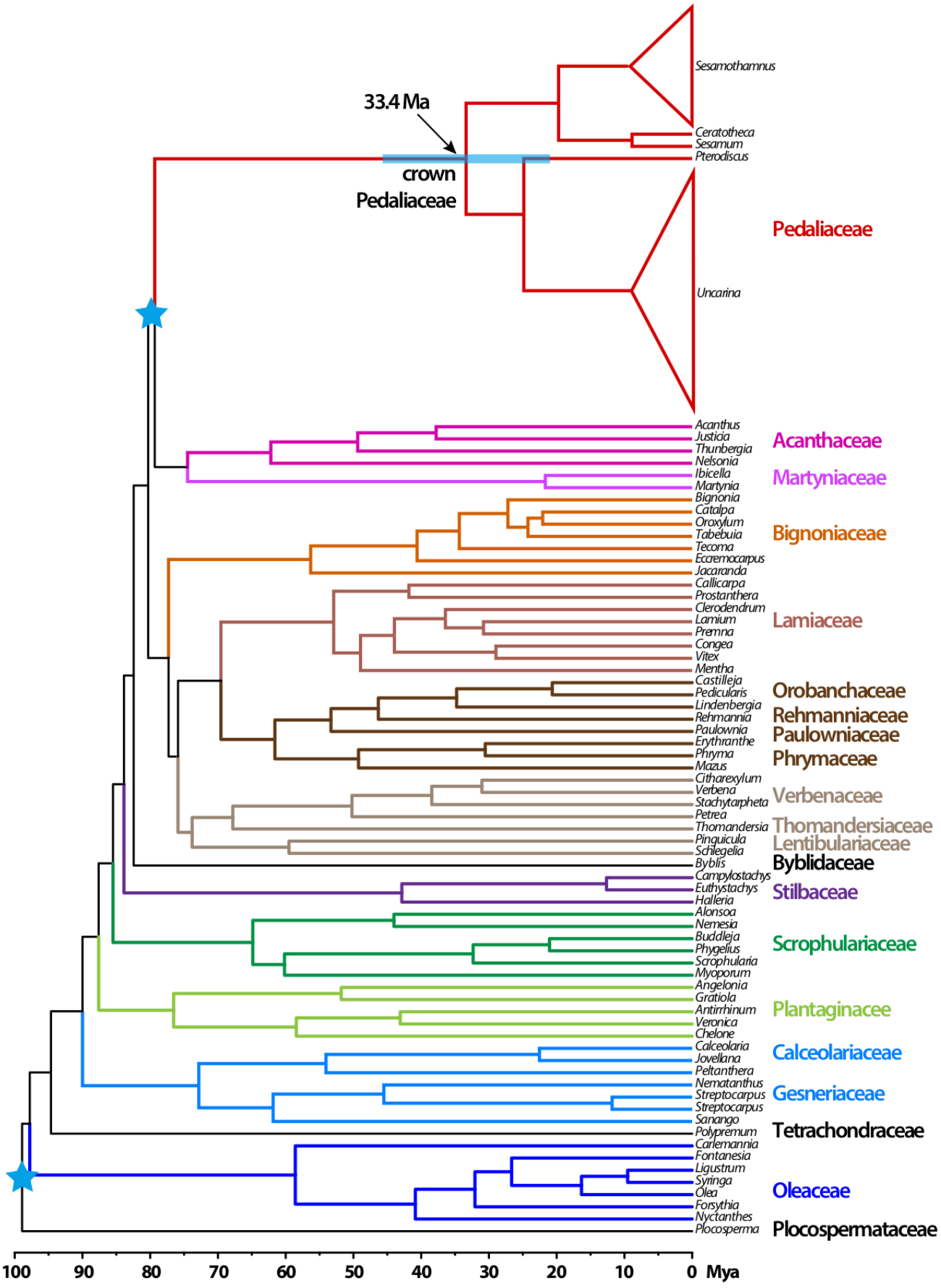
33.4 Ma
crown
Pedaliaceae
Sesamothamnus
Ceratotheca
Sesamum
Pterodiscus
Uncarina
Pedaliaceae
Acanthus
Justicia
Thunbergia
Nelsonia
Ibicella
Martynia
Acanthaceae
Martyniaceae
Bignonia
Catalpa
Oroxylum
Tabebuia
Tecoma
Eccremocarpus
Jacaranda
Bignoniaceae
Callicarpa
Prostanthera
Clerodendrum
Lamium
Premna
Congea
Vitex
Mentha
Lamiaceae
Castilleja
Pedicularis
Lindenbergia
Rehmannia
Paulownia
Erythranthe
Phryma
Mazus
Orobanchaceae
Rehmanniaceae
Paulowniaceae
Phrymaceae
Citharexylum
Verbena
Stachytarpheta
Petrea
Thomandersia
Pinguicula
Schlegelia
Verbenaceae
Thomandersiaceae
Lentibulariaceae
Byblis
Byblidaceae
Campylostachys
Euthystachys
Halleria
Stilbaceae
Alonsoa
Nemesia
Buddleja
Phygelius
Scrophularia
Myoporum
Scrophulariaceae
Angelonia
Gratiola
Antirrhinum
Veronica
Chelone
Plantaginacee
Calceolaria
Jovellana
Peltanthera
Calceolariaceae
Nematanthus
Streptocarpus
Streptocarpus
Gesneriaceae
Sanango
Polypremum
Tetrachondraceae
Carlemannia
Fontanesia
Ligustrum
Syringa
Olea
Forsythia
Oleaceae
Nyctanthes
Plocosperma
Plocospermataceae
100
90
80
70
60
50
40
30
20
10
0 Mya

Fig. 4. Chronogram of the Lamiales showing origin and relationships of the Pedaliaceae. (above)

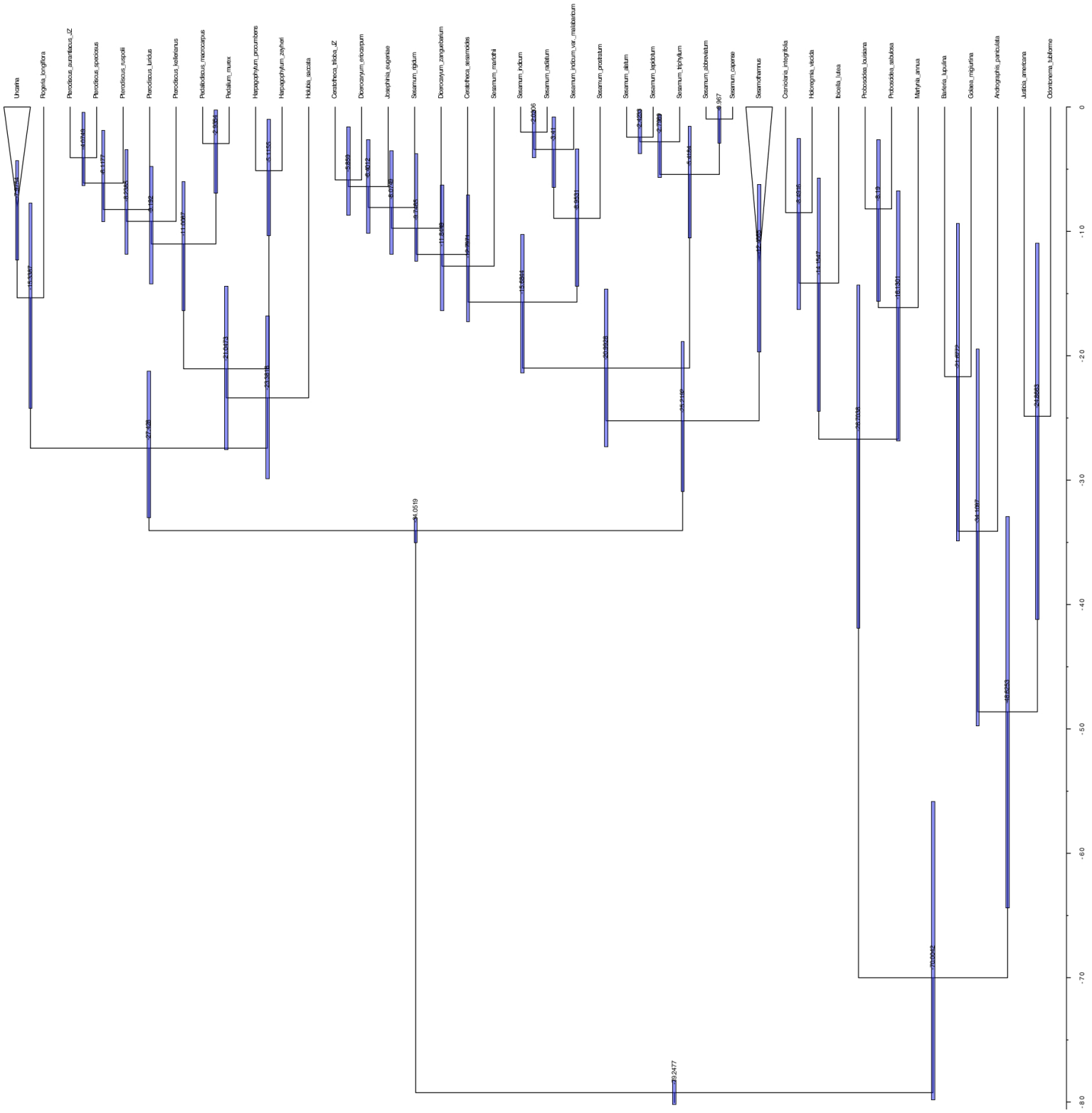

Fig. 5. Chronogram of the Lamiales clade of Acanthaceae, Martyniaceae, and Pedaliaceae. Ages at nodes are in Mya. (above)

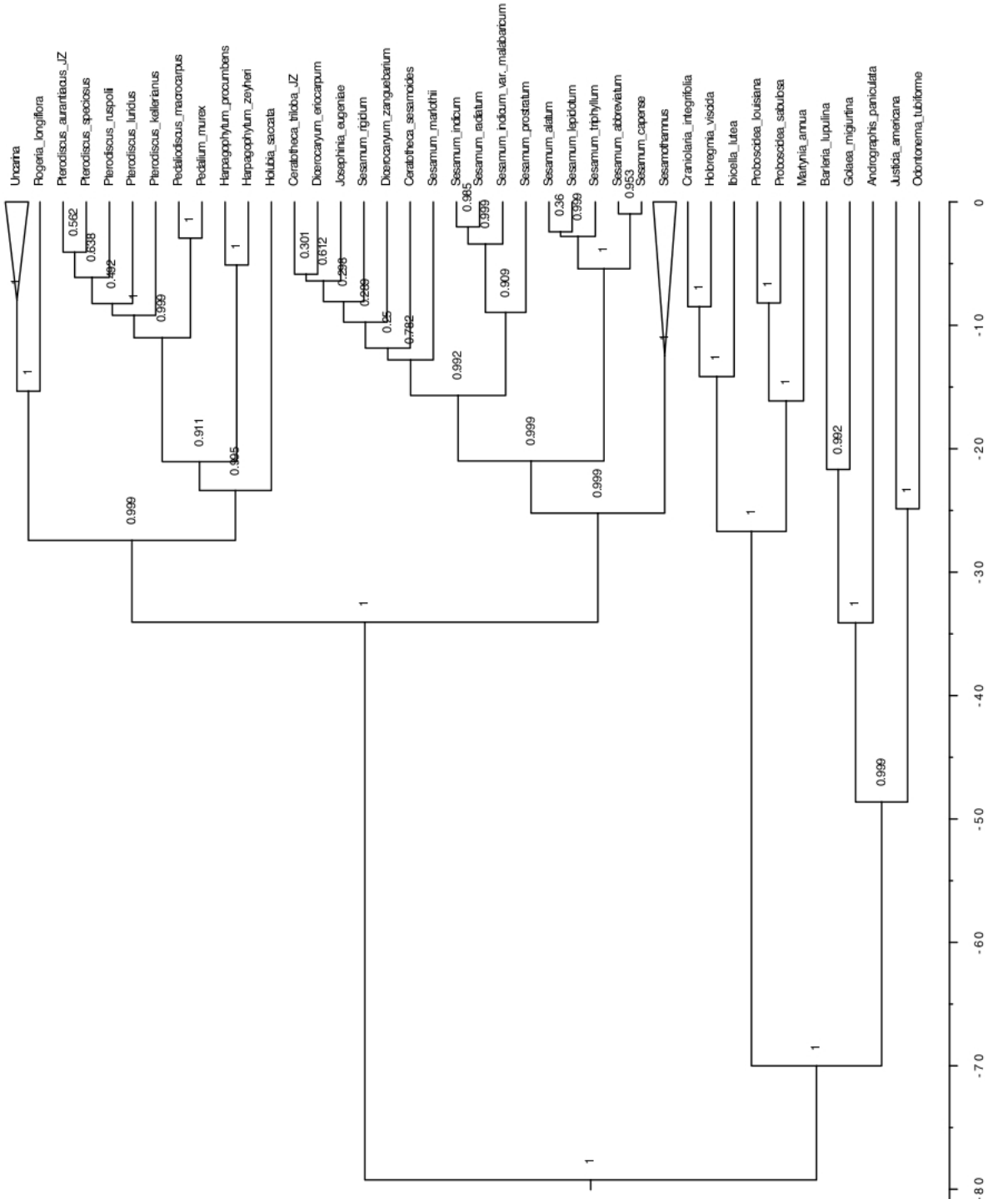

Fig. 6. Bayesian phylogeny of the Lamiales clade of Acanthaceae, Martyniaceae, and Pedaliaceae. Numbers at nodes indicate posterior probability.

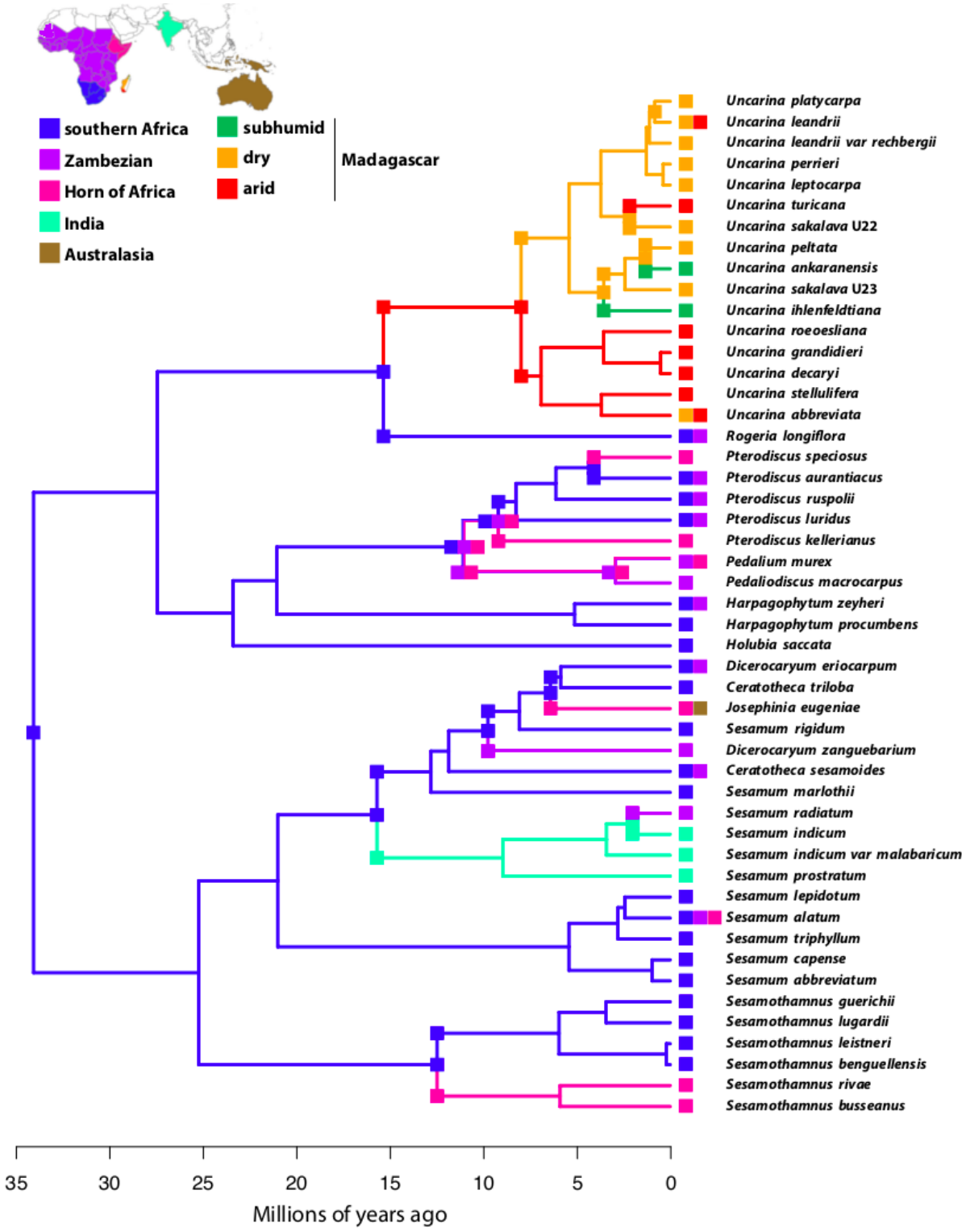

Fig. 7. Most likely ancestral range of sampled genera in Pedaliaceae in our DECj BioGeoBEARS model. Geographic areas used in this analysis are color-coded in the map legend.

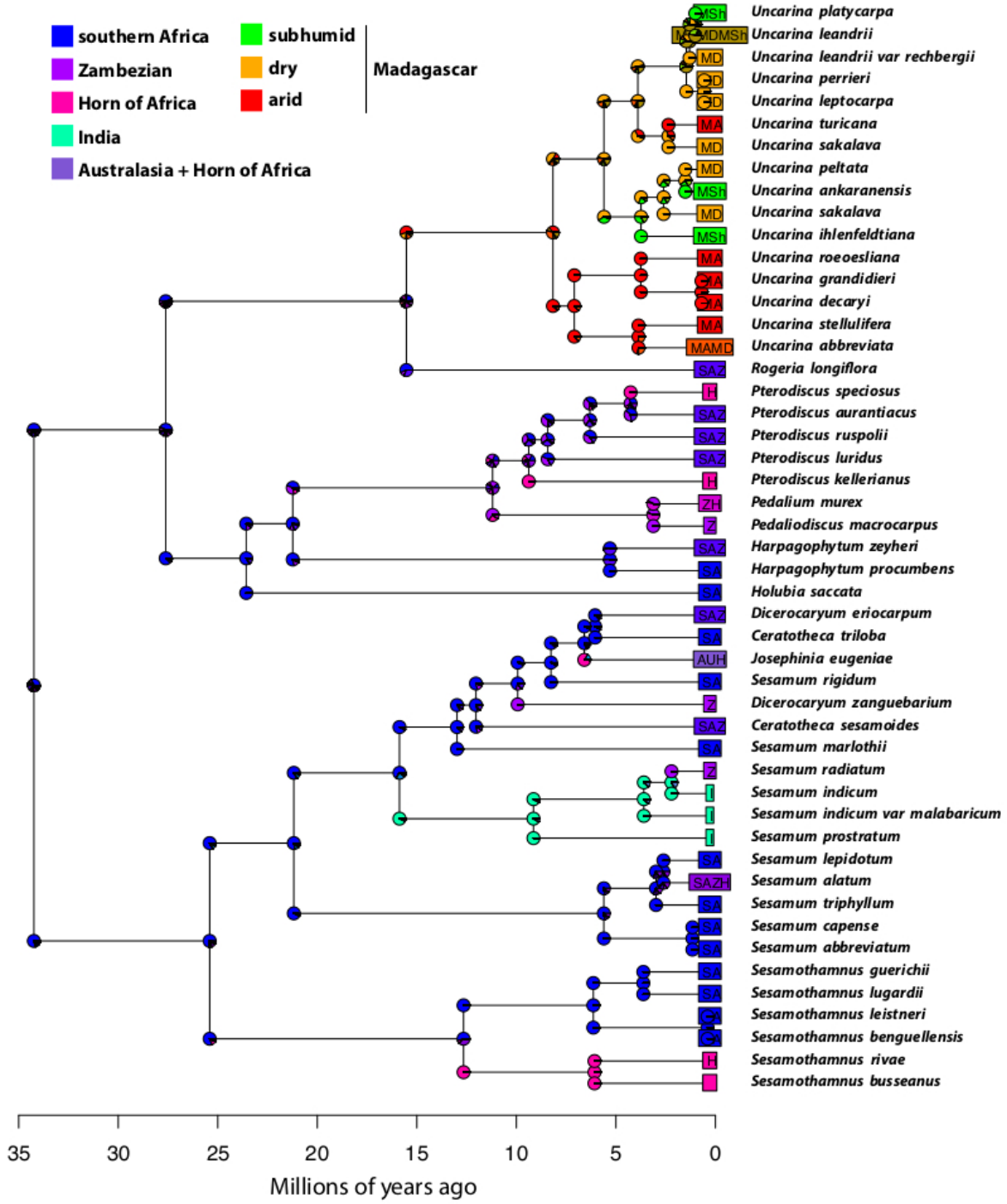

Fig. 8. Most likely ancestral range of sampled genera in Pedaliaceae in our DECj BioGeoBEARS model. Pies at each node show the likelihood of each region being the ancestral.

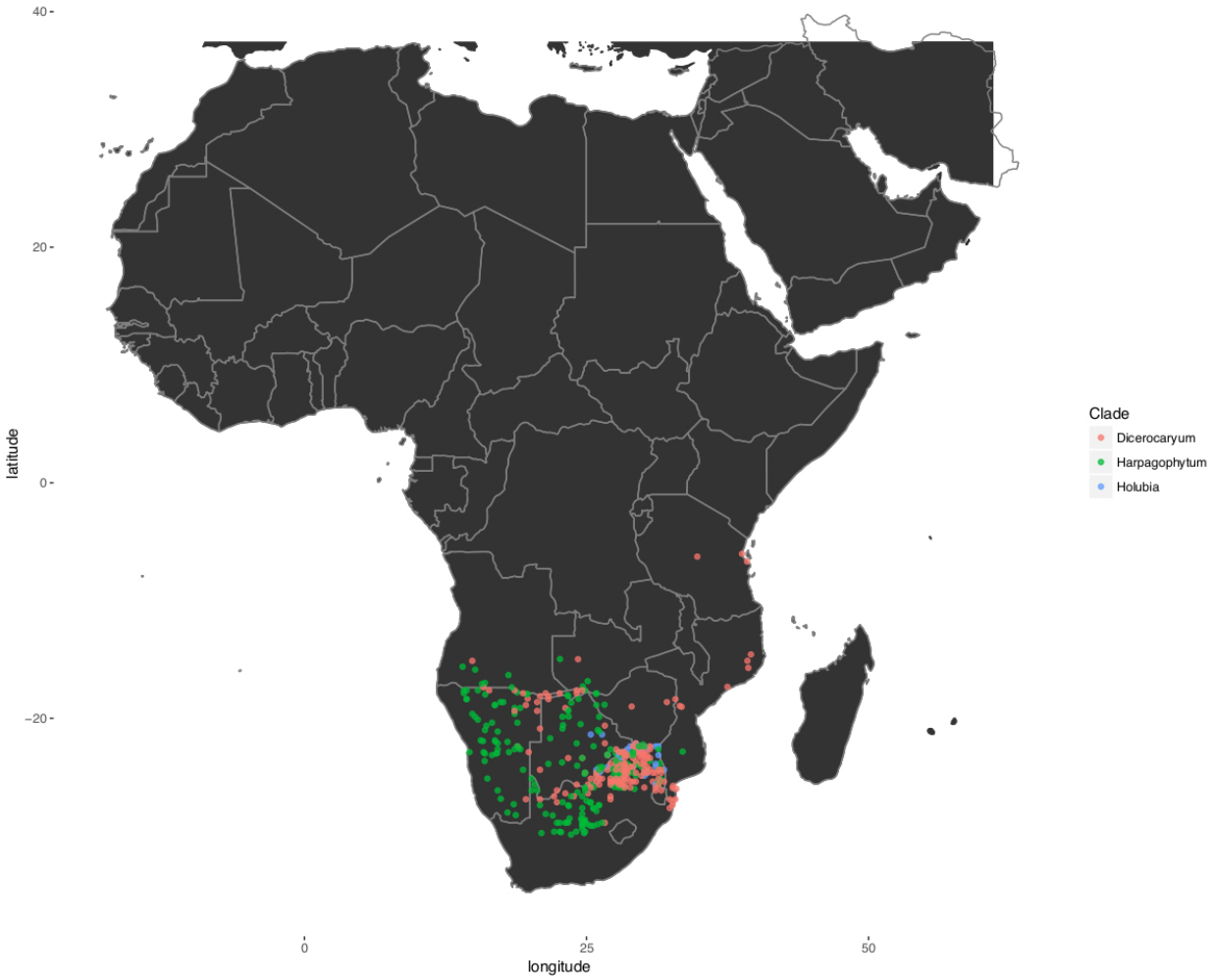

Fig. 9. Distributional range of three Pedaliaceae genera that occur chiefly south of the Equator. Specimen records taken from GBIF.

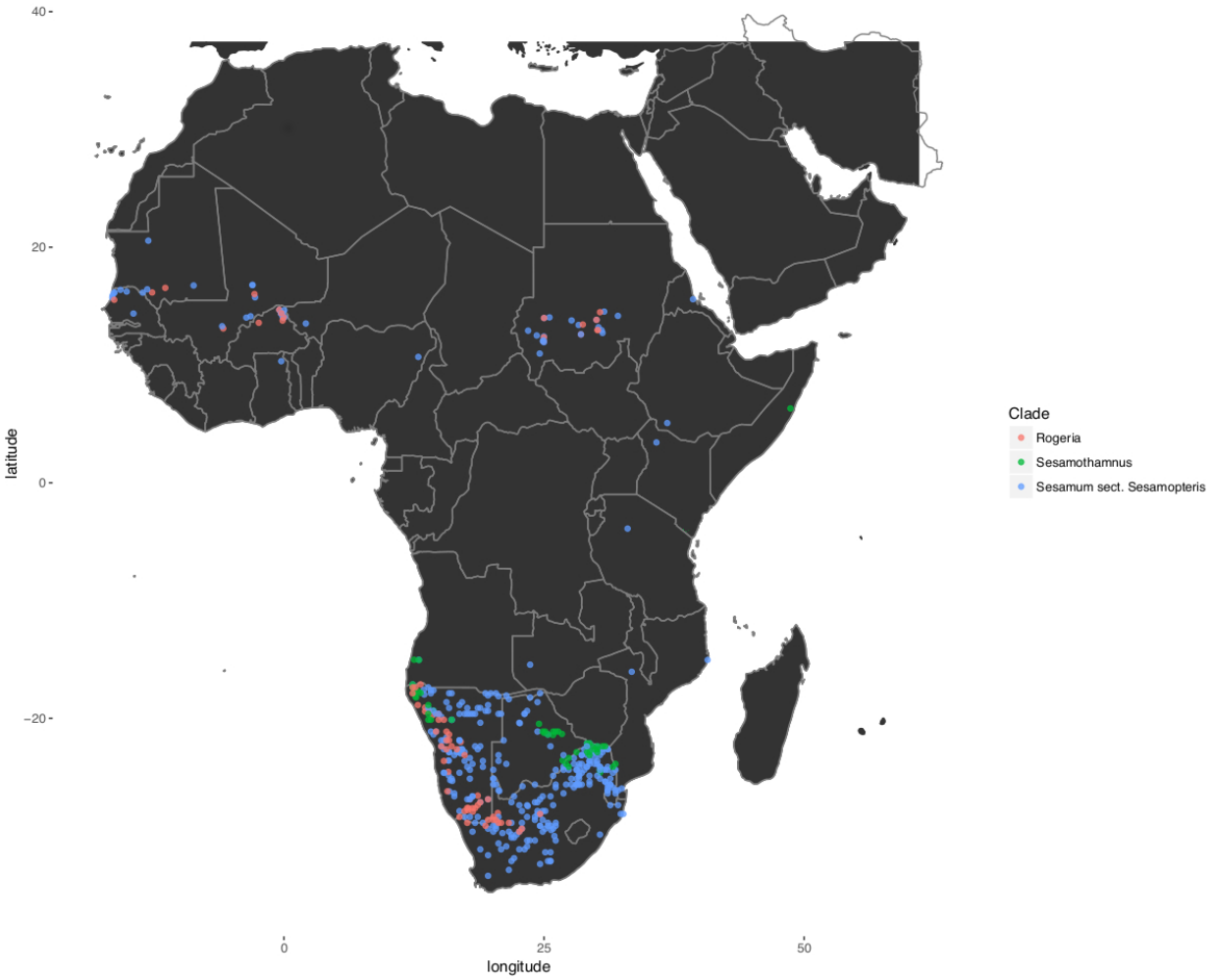

Fig. 10. Distributional range of three Pedaliaceae taxon groups that have a disjunct distribution from southern Africa to northern Africa. Specimen records taken from GBIF.

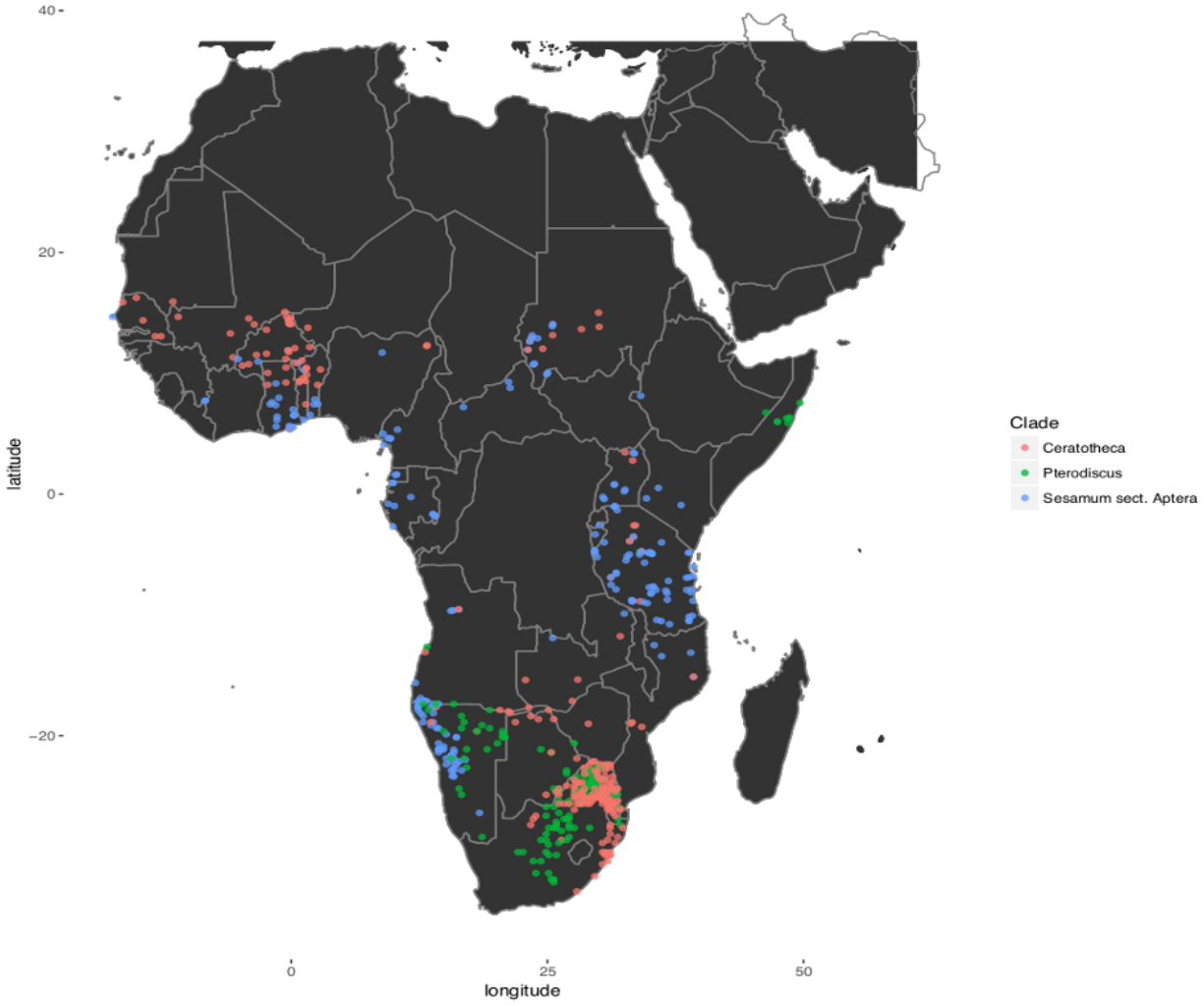

Fig. 11. Distributional range of three Pedaliaceae taxon groups that have a relatively continuous range across Africa. Specimen records taken from GBIF.

Taxon	atpB	matK	ndhF	psbB_psbT	rbcL	rps16	rps4	trnL-F	trnV-atpE
Acanthus_montanus	HQ384715.1	HQ384511.1		HQ384599.1			HQ385052.1		HQ412860.1
Alonsoa_meridionalis	HQ384737.1	HQ384534.1		HQ384623.1			HQ385077.1	HQ412948.1	HQ412883.1
Angelonia_angustifolia	HQ384740.1	HQ384537.1		HQ384626.1			HQ385080.1		HQ412886.1
Antirrhinum_majus								KJ161974.1	KJ161967.1
Bignonia_capreolata	HQ384723.1	HQ384518.1		HQ384607.1	HQ384884.1	HQ385160.1	HQ385060.1		HQ412867.1
Buddleja_davidii	HQ384734.1	HQ384530.1	HQ384835.1	HQ384619.1		HQ385169.1	HQ385073.1		HQ412879.1
Byblis_liniflora	HQ384703.1	HQ384500.1	HQ384816.1	HQ384586.1	HQ384870.1		HQ385039.1		
Calceolaria_mexicana				HQ384632.1			HQ385086.1		
Calceolaria_sp._Clark_6747	HQ384746.1	HQ384541.1			HQ384899.1				HQ412891.1
Callicarpa_mollis	HQ384701.1	HQ384498.1		HQ384584.1	HQ384868.1	HQ385145.1	HQ385037.1	HQ412928.1	HQ412847.1
Campylostachys_cernua		HQ384527.1	HQ384833.1	HQ384616.1	HQ384891.1	HQ385168.1	HQ385070.1		HQ412876.1
Carlemannia_tetragona	HQ384757.1	HQ384548.1		HQ384643.1		HQ385182.1	HQ385097.1	HQ412957.1	HQ412899.1
Castilleja_linariifolia	HQ384707.1	HQ384504.1	HQ384819.1				HQ385043.1		HQ412852.1
Catalpa_aff._speciosa_Olmstead_88-003	HQ384724.1	HQ384519.1		HQ384608.1	HQ384885.1	HQ385161.1	HQ385061.1	HQ412940.1	HQ412868.1
Ceratotheca_triloba_JZ		XXXX	XXXX		XXXX	XXXX		XXXX	
Chelone_glabra	HQ384738.1	HQ384535.1	HQ384837.1	HQ384624.1	HQ384895.1	HQ385172.1	HQ385078.1	HQ412949.1	HQ412884.1
Citharexylum_berlandieri	HQ384712.1	HQ384507.1	HQ384822.1	HQ384595.1	HQ384876.1	HQ385151.1	HQ385048.1	HQ412932.1	HQ412856.1
Clerodendrum_trichotomum	HQ384695.1	HQ384492.1		HQ384578.1	HQ384865.1	HQ385140.1	HQ385031.1		HQ412842.1
Congea_tomentosa	HQ384702.1	HQ384499.1	HQ384815.1	HQ384585.1	HQ384869.1		HQ385038.1	HQ412929.1	HQ412848.1
Eccremocarpus_scaber	HQ384728.1	HQ384523.1		HQ384612.1		HQ385165.1	HQ385065.1	HQ412943.1	HQ412872.1
Erythranthe_guttata	KJ161980.1	KJ161979.1	KJ161984.1	KJ161983.1	KJ161981.1	KJ161978.1	KJ161985.1	KJ161975.1	KJ161968.1
Euthystachys_abbreviata		HQ384526.1					HQ385069.1		HQ412875.1
Fontanesia_phillyreoides	HQ384753.1	HQ384545.1		HQ384639.1	HQ384902.1		HQ385093.1		HQ412896.1
Forsythia_sp._Reeves_11	HQ384754.1	HQ384546.1	HQ384843.1	HQ384640.1	HQ384903.1	HQ385181.1	HQ385094.1	HQ412955.1	HQ412897.1
Gratiola_floridana	HQ384741.1	HQ384538.1	HQ384839.1	HQ384627.1		HQ385174.1	HQ385081.1		HQ412887.1
Halleria_lucida	HQ384732.1	HQ384528.1		HQ384617.1			HQ385071.1		HQ412877.1
Ibicella_lutea	HQ384731.1	HQ384525.1	HQ384832.1	HQ384615.1	HQ384890.1	HQ385167.1	HQ385068.1	HQ412945.1	HQ412874.1

Jacaranda_mimosifolia	HQ384729.1			HQ384613.1	HQ384888.1		HQ385066.1		
Jovellana_violacea	HQ384747.1	HQ384542.1		HQ384633.1		HQ385178.1	HQ385087.1	HQ412953.1	HQ412892.1
Justicia_americana		HQ384510.1	HQ384825.1	HQ384598.1		HQ385154.1	HQ385051.1	HQ412935.1	HQ412859.1
Lamium_purpureum	HQ384696.1	HQ384493.1		HQ384579.1		HQ385141.1	HQ385032.1		HQ412843.1
Ligustrum_vulgare	HQ384750.1	HQ384543.1		HQ384636.1	HQ384901.1	HQ385180.1	HQ385090.1		HQ412894.1
Lindenbergia_philippensis	HQ384708.1			HQ384590.1					
Martynia_annua	HQ384730.1	HQ384524.1		HQ384614.1	HQ384889.1	HQ385166.1	HQ385067.1	HQ412944.1	HQ412873.1
Mazus_reptans	HQ384705.1	HQ384502.1	HQ384817.1	HQ384588.1	HQ384872.1	HQ385147.1	HQ385041.1		HQ412850.1
Mentha_spicata	HQ384698.1	HQ384495.1	HQ384813.1	HQ384581.1			HQ385034.1		
Myoporum_mauritianum		HQ384532.1	HQ384836.1	HQ384621.1	HQ384894.1		HQ385075.1	HQ412947.1	HQ412881.1
Nelsonia_sp._Harris_5722	HQ384717.1	HQ384513.1	HQ384827.1	HQ384601.1	HQ384879.1	HQ385155.1	HQ385054.1	HQ412936.1	HQ412862.1
Nematanthus_hirsutus	HQ384743.1	HQ384540.1	HQ384840.1	HQ384629.1	HQ384897.1	HQ385176.1	HQ385083.1	HQ412951.1	HQ412889.1
Nemesia_fruticans	HQ384736.1	HQ384533.1		HQ384622.1		HQ385171.1	HQ385076.1		HQ412882.1
Nyctanthes_arbor-tristis	HQ384755.1	HQ384547.1		HQ384641.1			HQ385095.1		HQ412898.1
Olea_europaea	HQ384752.1			HQ384638.1			HQ385092.1		
Oroxylum_indicum	HQ384725.1	HQ384520.1		HQ384609.1	HQ384886.1	HQ385162.1	HQ385062.1	HQ412941.1	HQ412869.1
Paulownia_tomentosa				HQ384592.1		HQ385149.1	HQ385045.1		HQ412854.1
Pedicularis_groenlandica	HQ384706.1	HQ384503.1	HQ384818.1	HQ384589.1	HQ384873.1	HQ385148.1	HQ385042.1	HQ412930.1	HQ412851.1
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Petrea_kohautiana	HQ384714.1	HQ384509.1	HQ384824.1	HQ384597.1	HQ384877.1	HQ385153.1	HQ385050.1	HQ412934.1	HQ412858.1
Phryma_leptostachya	HQ384710.1			HQ384593.1			HQ385046.1		
Phygelius_capensis	HQ384735.1	HQ384531.1		HQ384620.1	HQ384893.1	HQ385170.1	HQ385074.1		HQ412880.1
Pinguicula_moranensis	HQ384704.1	HQ384501.1		HQ384587.1	HQ384871.1	HQ385146.1	HQ385040.1		HQ412849.1
Plocosperma_buxifolium	HQ384756.1		HQ384844.1	HQ384642.1	HQ384904.1		HQ385096.1	HQ412956.1	
Polypremum_procumbens	HQ384749.1			HQ384635.1		HQ385179.1	HQ385089.1	HQ412954.1	HQ412893.1
Premna_odorata	HQ384697.1	HQ384494.1	HQ384812.1	HQ384580.1	HQ384866.1	HQ385142.1	HQ385033.1	HQ412925.1	HQ412844.1
Prostanthera_calycina	HQ384700.1	HQ384497.1	HQ384814.1	HQ384583.1	HQ384867.1	HQ385144.1	HQ385036.1	HQ412927.1	HQ412846.1
Pterodiscus_aurantiacus_JZ		XXXX	XXXX		XXXX	XXXX		XXXX	

Rehmannia_elata	HQ384709.1	HQ384505.1	HQ384820.1	HQ384591.1	HQ384874.1		HQ385044.1		HQ412853.1
Sanango_sp._Bremer_5350	HQ384745.1			HQ384631.1			HQ385085.1		
Schlegelia_fuscata	HQ384718.1	HQ384514.1	HQ384828.1	HQ384602.1	HQ384880.1	HQ385156.1	HQ385055.1		
Scrophularia_californica	HQ384733.1	HQ384529.1	HQ384834.1	HQ384618.1	HQ384892.1		HQ385072.1	HQ412946.1	HQ412878.1
Sesamothamnus_benguellensis_S01		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_busseanus_S05		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_guerichii_S02		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_guerichii_S11		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_guerichii_S12		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_leistneri_S06		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_lugardii	HQ384720.1	HQ384516.1	HQ384830.1	HQ384604.1	HQ384881.1	HQ385158.1	HQ385057.1	HQ412938.1	HQ412864.1
Sesamothamnus_lugardii_S04		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_lugardii_S10		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_lugardii_S14		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_rivae_S07		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamothamnus_rivae_S08		XXXX	XXXX		XXXX	XXXX		XXXX	
Sesamum_indicum	HQ384721.1			HQ384605.1	HQ384882.1	HQ385159.1	HQ385058.1		HQ412865.1
Stachytarpheta_cayennensis	HQ384713.1	HQ384508.1	HQ384823.1	HQ384596.1		HQ385152.1	HQ385049.1	HQ412933.1	HQ412857.1
Streptocarpus_holstii	HQ384742.1	HQ384539.1		HQ384628.1	HQ384896.1	HQ385175.1	HQ385082.1	HQ412950.1	HQ412888.1
Streptocarpus_ionanthus	HQ384744.1		HQ384841.1	HQ384630.1	HQ384898.1	HQ385177.1	HQ385084.1	HQ412952.1	HQ412890.1
Syringa_vulgaris	HQ384751.1	HQ384544.1		HQ384637.1			HQ385091.1		HQ412895.1
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Tecoma_stans	HQ384727.1	HQ384522.1		HQ384611.1		HQ385164.1	HQ385064.1		HQ412871.1
Thomandersia_laurifolia	HQ384719.1	HQ384515.1	HQ384829.1	HQ384603.1		HQ385157.1	HQ385056.1	HQ412937.1	HQ412863.1
Thunbergia_alata	HQ384716.1	HQ384512.1	HQ384826.1	HQ384600.1	HQ384878.1		HQ385053.1		HQ412861.1
Uncarina_abbreviata_U27		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_ankaranensis_U02		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_decaryi_U28		XXXX	XXXX		XXXX	XXXX		XXXX	

Uncarina_grandidieri	HQ384722.1	HQ384517.1	HQ384831.1	HQ384606.1	HQ384883.1		HQ385059.1	HQ412939.1	HQ412866.1
Uncarina_grandidieri_U30		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_ihlenfeldtiana_U06		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_ihlenfeldtiana_U31		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_leandrii_U07		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_leandrii_U08		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_leandrii_var_rechbergeri_U09		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_leandrii_var_rechbergeri_U41		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_leptocarpa_U12		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_peltata_U32		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_perrieri_U18		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_platycarpa_U34		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_roeoesliana_U20		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_roeoesliana_U21		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_sakalava_U22		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_sakalava_U23		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_stellulifera_U43		XXXX	XXXX		XXXX	XXXX		XXXX	
Uncarina_turicana_U45		XXXX	XXXX		XXXX	XXXX		XXXX	
Verbena_bracteata	HQ384711.1	HQ384506.1	HQ384821.1	HQ384594.1	HQ384875.1	HQ385150.1	HQ385047.1	HQ412931.1	HQ412855.1
Veronica_persica	HQ384739.1	HQ384536.1	HQ384838.1	HQ384625.1		HQ385173.1	HQ385079.1		HQ412885.1
Vitex_agnus-castus	HQ384699.1	HQ384496.1		HQ384582.1		HQ385143.1	HQ385035.1	HQ412926.1	HQ412845.1

Appendix 1: List of taxa and GenBank accession numbers used in the Lamiales-wide dating analysis. Sequences newly generated for this study represented by "XXXX".

Taxon	matK	ndhF	psbA	rbcL	rps16	trnL-F
Andrographis_paniculata	NC_022451.2	NC_022451.2	NC_022451.2	NC_022451.2	NC_022451.2	NC_022451.2
Antirrhinum_majus	AF051978.1	L36392.1	HM152925.1	GQ997015.1	AJ431054.1	AJ492270.1
Barleria_lupulina	NA	JQ712671.1	KT161362.1	NA	EU529010.1	AF289758.1
Buddleja_davidii	HQ384530.1	L36394.1	MF348398.1	AJ001757.1	HQ385169.1	AF380861.1
Catalpa_speciosa	HE967371.1	DQ411407.1	HE966548.1	MG222435.1	AJ609197.1	FJ870023.1
Ceratotheca_sesamoides	NA	KJ743169.1	NA	NA	NA	KJ743180.1
Ceratotheca_triloba	JQ024946.1	KJ743150.1	NA	AY919277.1	AF482534.1	KJ743178.1
Ceratotheca_triloba_JZ	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Craniolaria_annua	NA	NA	JN686593.1	NA	NA	KJ743198.1
Craniolaria_integrifolia	NA	JN686625.1	JN686599.1	NA	JN686536.1	JQ322788.1
Dicerocaryum_eriocarpum	NA	KJ743172.1	NA	NA	NA	KJ743186.1
Dicerocaryum_zanguebarium	NA	NA	KT717163.1	NA	NA	NA
Fraxinus_excelsior	AM933427.1	JN591031.1	GU120316.1	KP088611.1	NA	AF231830.1
Fraxinus_excelsior_var._diversifolia	NA	NA	NA	NA	AF225238.1	NA
Golaea_migiurtina	NA	NA	NA	NA	EU529022.1	EU528921.1
Harpagophytum_procumbens	KT717097.1	KJ743153.1	KT717143.1	KT717172.1	JN686541.1	KJ743203.1
Harpagophytum_zeyheri	KT717111.1	KJ743154.1	KT717155.1	KT717180.1	NA	KJ743192.1
Holoregmia_viscida	NA	KJ743168.1	JN686598.1	NA	JN686505.1	KJ743190.1
Holubia_saccata	NA	KJ784300.1	NA	NA	NA	KJ743197.1
Ibicella_lutea	HQ384525.1	HQ384832.1	JN686601.1	HQ384890.1	JN686537.1	JQ322787.1
Ibicella_parodii	NA	KJ743159.1	NA	NA	NA	KJ743207.1
Josephinia_eugeniae	NA	KJ743151.1	KT717162.1	NA	NA	KJ743199.1
Justicia_americana	HQ384510.1	HQ384825.1	MF348382.1	L14401.1	HQ385154.1	HQ412935.1
Martynia_annua	HQ384524.1	JN686624.1	JN686602.1	HQ384889.1	JN686535.1	HM216649.1
Ocimum_basilicum	AY177670.1	KT210254.1	MF348819.1	KP172036.1	FJ593338.1	FJ593458.1
Odontonema_tubiforme	JQ586417.1	NA	NA	JQ590071.1	DQ059215.1	AF063127.1
Olea_europaea	AJ429335.1	AF130163.1	FJ493289.1	DQ673304.1	AJ431047.1	HQ117895.1

Oroxylum_indicum	GQ434292.1	AF102635.1	JN406947.2	HQ384886.1	JN686524.1	FJ870048.1
Pedaliodiscus_macrocarpus	NA	KJ743158.1	KT717139.1	NA	NA	KJ743196.1
Pedalium_murex	NA	KJ743156.1	KT717140.1	NA	NA	KJ743194.1
Phryma_leptostachya	AJ429341.1	AJ429118.1	HQ596786.1	AF190438.1	AJ431053.1	DQ532466.1
Proboscidea_altheifolia	MF963699.1	NA	JN686591.1	MF963355.1	NA	NA
Proboscidea_fragrans	AJ429334.1	NA	JN686595.1	NA	AJ431046.1	NA
Proboscidea_louisiana	AF531809.1	KJ743160.1	JN686600.1	L01946.2	JN686538.1	JQ322786.1
Proboscidea_parviflora	NA	KJ743161.1	NA	NA	NA	KJ743206.1
Proboscidea_parviflora_subsp._gracillima	NA	NA	JN686596.1	NA	NA	JQ322790.1
Proboscidea_parviflora_subsp._sinaloensis	NA	NA	JN686607.1	NA	NA	NA
Proboscidea_parviflora_var._hohokamiana	NA	NA	JN686597.1	NA	NA	NA
Proboscidea_parviflora_var._parviflora	NA	NA	JN686604.1	NA	NA	NA
Proboscidea_sabulosa	NA	JN686628.1	JN686605.1	NA	JN686539.1	JQ322791.1
Proboscidea_triloba_subsp._diversifolia	NA	NA	JN686594.1	NA	NA	NA
Proboscidea_triloba_subsp._triloba	NA	NA	JN686608.1	NA	NA	JQ322789.1
Pterodiscus_aurantiacus	NA	KJ743157.1	KT717141.1	NA	NA	KJ743205.1
Pterodiscus_aurantiacus_JZ	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Pterodiscus_kellerianus	NA	KJ743175.1	NA	NA	NA	KJ743195.1
Pterodiscus_luridus	NA	NA	NA	NA	NA	NA
Pterodiscus_ruspolii	NA	NA	NA	NA	NA	KJ743204.1
Pterodiscus_speciosus	NA	NA	KT717142.1	NA	NA	NA
Rogeria_adenophylla	NA	NA	KT717167.1	NA	NA	NA
Rogeria_longiflora	NA	KJ743155.1	NA	NA	NA	KJ743193.1
Salvia_rosmarinus	FR719112.1	NA	FJ513141.1	KM360960.1	AJ505425.1	AY570465.1
Sesamothamnus_benguellensis_S01	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamothamnus_busseanus_S05	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamothamnus_guerichii_S02	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamothamnus_guerichii_S11	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX

Sesamothamnus_guerichii_S12	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamothamnus_leistneri	NA	KJ743173.1	NA	NA	NA	KJ743187.1
Sesamothamnus_leistneri_S06	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamothamnus_lugardii	HQ384516.1	HQ384830.1	NA	HQ384881.1	HQ385158.1	HQ412938.1
Sesamothamnus_lugardii_S04	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Sesamothamnus_lugardii_S10	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamothamnus_lugardii_S14	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamothamnus_rivae_S07	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamothamnus_rivae_S08	XXXX	XXXX	XXXX	XXXX	XXXX	XXXX
Sesamum_abbreviatum	NA	KJ743170.1	NA	NA	NA	KJ743182.1
Sesamum_alatum	NA	KJ743152.1	NA	NA	NA	KJ743201.1
Sesamum_capense	NA	KJ743171.1	NA	NA	NA	KJ743185.1
Sesamum_indicum	AJ429340.1	L36413.1	EU531713.1	HQ384882.1	HQ385159.1	AF067067.1
Sesamum_indicum_var._malabaricum	NA	KJ743164.1	NA	NA	NA	KJ743177.1
Sesamum_lepidotum	NA	KJ743167.1	NA	NA	NA	KJ743183.1
Sesamum_marlothii	NA	NA	NA	NA	NA	KJ743181.1
Sesamum_prostratum	NA	KJ743165.1	NA	NA	NA	KJ743179.1
Sesamum_radiatum	NA	JN686631.1	KT717165.1	NA	JN686542.1	NA
Sesamum_rigidum	NA	KJ743163.1	NA	NA	NA	KJ743176.1
Sesamum_triphyllum	NA	KJ789853.1	KT717161.1	NA	JQ322784.1	KJ743202.1
Trapella_sinensis	KX526744.1	KJ743162.1	NA	KX527437.1	JN686513.1	KJ743188.1
Uncarina_abbreviata_U27	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_ankaranensis_U02	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_decaryi_U28	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_grandidieri	HQ384517.1	HQ384831.1	NA	HQ384883.1	FN794094.1	HQ412939.1
Uncarina_grandidieri_U30	XXXX	XXXX	KT717166.1	XXXX	XXXX	XXXX
Uncarina_ihlenfeldtiana_U06	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_ihlenfeldtiana_U31	XXXX	XXXX	NA	XXXX	XXXX	XXXX

Uncarina_leandrii_U07	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_leandrii_U08	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_leandrii_var_rechbergeri_U09	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_leandrii_var_rechbergeri_U41	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_leptocarpa_U12	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_peltata_U32	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_perrieri_U18	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_platycarpa_U34	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_roeoesliana_U20	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_roeoesliana_U21	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_sakalava_U22	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_sakalava_U23	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_stellulifera_U43	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Uncarina_turicana_U45	XXXX	XXXX	NA	XXXX	XXXX	XXXX
Utricularia_vulgaris	AF531831.1	JQ712680.1	NA	JN890555.1	NA	JQ728994.1
Verbena_bracteata	HQ384506.1	MG051528.1	NA	HQ384875.1	HQ385150.1	MG051605.1

Appendix 2: List of taxa and GenBank accession numbers used in the dating analyses focusing on Acanthaceae, Martyniaceae, and Pedaliaceae. Sequences newly generated for this study represented by "XXXX".

Chapter 2: Systematics and Phylogenetics of the genus *Uncarina* (Pedaliaceae)

John G. Zaborsky and Kenneth J. Sytsma developed the research questions and approach. Emily M. Lemmon and Alan Lemmon sequenced the DNA. J. Zaborsky, K. Sytsma, and Jeffrey P. Rose performed analyses. J. Zaborsky wrote the rough draft of the manuscript and K. Sytsma contributed to the revisions.

Abstract

Uncarina (Pedaliaceae) is a small genus endemic to Madagascar. Its taxonomic history has been confusing due to fragmentary specimens and high morphological variability. A classification system within the genus has never been published, however indumentum characters and fruit characters have been used to postulate some species relationships. We are the first to perform a phylogenetic analysis of the genus and use Next-Generation Sequencing to do so. We sample every known taxon with multiple accessions of some taxa. Our results show that there is a clear biogeographical signal within the genus with clades in both nDNA and cpDNA datasets representing northern, western, and southern species. Within these clades, we show that indumentum characters are often best for understanding the relationships recovered. We also show that a cryptic species exists within the genus and encourage more thorough sampling and collecting to explore the full range of diversity the genus presents. Our dated phylogenies show that *Uncarina*, like numerous arid-adapted plant lineages, diversified during the Miocene Epoch, when global climatic conditions were quite dry and arid in Madagascar and across Africa.

Introduction

Uncarina (Baill.) Stapf (Pedaliaceae) is a genus of small trees and shrubs endemic to Madagascar. The genus is large, by the standards of the family, and contains 14 known taxa. *Uncarina* is also noteworthy in the Pedaliaceae because all of its taxa are woody and exhibit some degree of stem succulence: the majority of the family is made up of herbaceous taxa. While the Pedaliaceae are almost entirely restricted to mainland Africa (Ihlenfeldt, 1994),

Uncarina is the only genus that is native to Madagascar. Members of the genus are often grown by succulent plant enthusiasts because of their swollen stems.

The genus is confined to arid and semi-arid regions of Madagascar, most commonly occurring in dry spiny forest and western dry forest. *Uncarina* species occur from the far northern tip of Madagascar near Antsiranana, along the western extent of the island into the south, reaching as far east as Ambovombe. The two pink-flowered species, *U. abbreviata* (Baill.) Ihlenf. & Straka and *U. stellulifera* Humbert, grow along the western coast with only a small region of range overlap near the Fiherenana and Onilahy Rivers. Three closely-related species, *U. decaryi* Humbert *ex* Ihlenf., *U. grandidieri* (Baill.) Ihlenf. & Straka, and *U. roeoesliana* Rauh, all occur in the southern part of the island. All three species grow sympatrically, with *U. roeoesliana* having a very restricted range. In the north, *U. peltata* Stapf has a wide range with most collections coming from the northern tip of the island but it is also found at a few stations farther to the southwest. The rare *U. ankaranensis* Ihlenf. is restricted to the Ankarana Reserve in northern Madagascar and its range overlaps with that of *U. peltata*. *Uncarina perrieri* Humbert is almost entirely restricted to the northern portion of Melaky Region with a few outliers to the south and one collection from the border with Boeny Region. *Uncarina leptocarpa* (Decne.) Ihlenf. & Straka grows throughout the southwestern portion of Melaky Region into Menabe Region, as far south as the city of Morondava. *Uncarina ihlenfeldtiana* Lavranos is a narrow endemic, being known only from Mandritsara. *Uncarina leandrii* Humbert is the only species with a described intraspecific taxon: var. *rechbergeri* Lavranos. This species occurs in southern Madagascar, just slightly to the north of the extent of the range of *U. decaryi* and *U. grandidieri*. It is also disjunct to the northwest near Morondava, Masoarivo, and Antsakoabe (Melaky). The distribution of var. *rechbergeri* overlaps with that of

the typical variety and is known from near Tsimafana, Marofandilia, and Berevo (Lavranos, 1995). *Uncarina platycarpa* Lavranos is known only from the type locality, in Mahajanga. *Uncarina sakalava* Humbert is distributed in Melaky Region with one collection from Boeny Region. *Uncarina turicana* Lavranos has a narrow distribution, being known only from southwestern Toliara.

Uncarina species all share simple, palmately-lobed leaves that are highly variable in the degree of lobing and shape of lobes across species, with both of these features varying within an individual throughout the growing season. Like all other members of the family, the leaves of *Uncarina* are covered by unusual glandular hairs, as well as simple hairs. These glandular hairs, unique among angiosperms, have a head that is made of four cells. When the cells are ruptured in the presence of water and some sort of disturbance, they release a large amount of mucilage (Ihlenfeldt, 2004a). The purpose of this mucilage is unknown, but it may act as an herbivory deterrent. The shape of the head of these hairs and the distribution of the hairs across leaf surfaces has been used taxonomically in *Uncarina* (Ihlenfeldt, 2002). Recently described species (e.g. *U. ankaranensis* and *U. ihlenfeldtiana*) have included SEM photos of leaf surfaces (Ihlenfeldt, 2004b; Lavranos, 2004) to better illustrate these mucilage glands. However, no genus-wide study has been done using SEM to investigate and document the ultrastructure of the mucilage glands.

Flower color in *Uncarina* is fairly uniform, with most taxa possessing yellow corollas that often have red stripes down the throats. One species, *U. leptocarpa*, has white corollas and two species, *U. abbreviata* and *U. stellulifera*, have bright pink corollas. Gamo et al. (2006) investigated the pollination ecology of seven *Uncarina* taxa and showed that two genera of bees (Hymenoptera: Apidae) are involved in the transfer of pollen. They observed that *U. leptocarpa*

and *U. roeoesliana* are pollinated by bees of the genus *Amegilla*. The other species investigated (*U. abbreviata*, *U. decaryi*, *U. grandidieri*, *U. leandrii* var. *rechbergeri*, and *U. stellulifera*) are all pollinated by bees of the genus *Macrogalea*. Interestingly, both genera of bees pollinated flowers in the same way: the bee crawled into the corolla tube upside down, then shook its body back and forth (from the back of the tube to the front) until pollen was dehisced. Despite observations of butterflies and sunbirds visiting the flowers, these bees were the only animals that effected pollination.

The Pedaliaceae are famous for having highly diverse and unusual fruit dispersal modes. Many genera have capsular fruits which are adorned with sharp outgrowths to aid in epizoochory (Ihlenfeldt, 2004a). *Uncarina* has fruits of this type that, depending on the species, possess long prickles adorned with hooked tips or short prickles that can be hooked or unhooked. Midgley & Illing (2009) proposed that *Uncarina* fruits were dispersed by the extinct elephant birds *Mullerornis* and *Aepyornis*. They argue that because they witnessed fruits lying ripe underneath one tree, and not attached to the tree, that they must not be dispersed by lemurs. They go on to posit that the branches of *Uncarina* trees are too narrow for lemurs to climb which would prevent them from reaching fruits at the tips of branches and getting them entangled in their fur. Therefore, they postulate that the fruits must be 'trample-burs' that were formerly dispersed on the feet of the 350-500kg elephant birds. Their argument fails to take into account that the fruits of *Uncarina* are very fragile and the spines and fruit body itself can be easily crushed, even with bare hands (pers. obsv.). It seems implausible that the fruits could get stuck around the toes of these giant birds and be carried long distances without immediately being destroyed. Despite what they claim, there are reports in the literature of ring-tailed lemurs having *Uncarina* fruits tangled in their fur (Crowley, 2011). It is highly unlikely that elephant birds were dispersers of

Uncarina fruits, at least as trample-burs. It is plausible that the fruits could have become entangled in the feathers of these birds and they could have been dispersed in the fur of extinct lemurs as well.

While a few species of *Uncarina* have been included in ordinal analyses of the Lamiales (Refulio-Rodriguez & Olmstead, 2014), no genus-wide analysis has ever been published. The Pedaliaceae was recently investigated in a phylogenetic context, primarily looking at the relationships between genera (Gormley et al., 2015). Their paper was the first family-wide phylogeny and showed that the Pedaliaceae and the Martyniaceae are monophyletic entities, respectively. These two families were long thought to be closely related, with the Martyniaceae being considered a New World derivative of the Old World Pedaliaceae (Cronquist, 1981). Gormley et al. (2015) also showed that the genus *Trapella* Oliv., which had long been included in the Pedaliaceae, is in fact a member of the Plantaginaceae. The family-wide analysis of Gormley et al. (2015) included only one species of *Uncarina* and resolved it as sister to the genus *Rogeria* J. Gay ex Delile. These two genera share an unusual inflorescence character: in all members of the family (except *Rogeria* and *Uncarina*) the flowers are borne in small dichasia of 3-9 flowers that are reduced to the first (central) flower so that it appears that the flowers are borne singly (Ihlenfeldt, 2004). *Rogeria* and *Uncarina* do not have the other flowers of the dichasia reduced. Reto Nyffeler (University of Zurich) investigated species relationships in *Uncarina* using ITS and the chloroplast marker *trn*L-F but never published the results (R. Nyffeler, pers. comm.). Sampling all known taxa and using Next-Generation sequencing, our study is the first to examine relationships within a single genus of the Pedaliaceae. Our study aims to elucidate species relationships within the genus and test and discover morphological traits that can be used to better understand the taxonomy of the genus.

Methods

Sampling — Silica-dried leaves of wild-collected specimens of each known *Uncarina* taxon were sent to me by a private collector in Switzerland: Walter Röösli. Mr. Röösli has made many collecting trips to Madagascar and discovered many new taxa as a result. Silica-dried leaves were also collected from the living collection at the Huntington Botanical Garden in San Marino, CA. Leaves were only collected from specimens of known wild heritage. Twenty accessions of *Uncarina* were used in this study, representing all known taxa. Outgroup genera in Pedaliaceae are described in detail in Chapter 1.

SEM Photography — SEM was utilized for the examination of adaxial and abaxial leaf trichomes of all known taxa (14 species, 1 variety) using silica-dried, wild-collected specimens. Photos were taken with a FEI Quanta Environmental Scanning Electron Microscope at 20kV. Specimens did not undergo any special treatment before being photographed. One specimen per taxon was examined due to limited availability of tissue.

DNA Extraction — DNA was extracted from silica-dried plant material using the DNeasy™ plant mini kit (Qiagen, Valencia, California) according to manufacturer's specifications.

DNA Sequencing — We utilized an anchored phylogenomics approach, Anchored Hybrid Enrichment (AHE) using the Center for Anchored Phylogenomics at Florida State University, to obtain 513 single-copy nuclear loci as well as the entire chloroplast genome. The nuclear data matrix had only 4.2% missing data. This method targets highly conserved "anchor" regions in the nuclear genome and generates hundreds of loci including both introns and exons (Lemmon et al. 2012). This specific pipeline has been used effectively to infer infrageneric relationships in

angiosperms (Buddenhagen et al., 2016; Cardillo et al., 2017; Fragoso-Martínez et al., 2017; Mitchell et al., 2017). DNA was sonicated to a fragment size of between 200–600 bp before library preparation and indexing following a modified protocol of Meyer and Kircher (2010). Indexed samples were pooled and enriched using the Angiosperm v.1 enrichment kit (Buddenhagen et al., manuscript). Sequencing was done on 4.5 PE150 Illumina HiSeq 2500 lanes at the Translational Science Laboratory, College of Medicine, Florida State University.

Paired reads were merged before assembly, following Rokyta et al. (2012). Reads were mapped to the probe regions using *Arabidopsis thaliana*, *Billbergia nutans*, and *Carex lurida* as references, combined with a de novo assembly approach to extend the assembly into flanking regions (Prum et al. 2015; Buddenhagen et al., 2016). Read files were traversed repeatedly until no additional mapped reads were produced and consensus sequences were calculated for each assembly cluster with contigs based on fewer than 100 reads removed. For each locus, orthology was determined following the procedures in Prum et al. (2015). Contig orthology was assessed using a pairwise distance matrix among homologs and used to cluster sequences with a neighbor-joining algorithm to assess if gene duplication occurred prior to or following the crown of the clade. Contigs suggesting duplication were removed from further analysis if they contained fewer than 32 taxa (92%). Sequences in each orthologous cluster were aligned using MAFFT v. 7.023b (Katoh and Standley, 2013), then trimmed and masked using the following procedure from Prum et al. (2015). Sites with the same character in > 50% of sequences were considered "conserved." A 20 bp sliding window was then moved across the alignment, and regions with < 13 characters matching the common base at the corresponding conserved site were masked. Sites with < 152 unmasked bases were removed. Finally, the masked alignments were inspected by

eye. Regions considered obviously misaligned or likely paralogous were removed and poorly aligned sections in a given alignment were deleted.

As DNA fragments from non-targeted loci may be captured, we attempted to extract the plastid (cpDNA) genome from our reads. We used Geneious v. 10.2.3 to map all recovered forward and reverse reads to reference sequences. For the plastid genome, we used the whole plastid genome of *Sesamum indicum* L. (GenBank accession KCS569603) as a reference. Raw reads were trimmed and assembled using iterative refinement of up to 5 times with the default Geneious mapper and medium sensitivity. For the cpDNA, consensus sequences were generated using the strict consensus. If coverage was < 2, the consensus nucleotide was scored as a gap. Unmapped regions were treated as missing data and reads mapped to multiple positions were excluded from consensus calculations. Sequences were aligned using MAFFT with default parameters. After alignment, ambiguously aligned or called regions were removed by hand.

Phylogenetic Analyses — The concatenated AHE dataset was used to estimate a phylogeny under the GTRGAMMA model implemented in RAxML v8.1.21 (Stamatakis, 2014; with default parameters), with the GTR model and branch lengths being allowed to vary across loci. One hundred bootstrap replicates were collected to estimate phylogenetic support. In addition, the species tree was estimated under the coalescent model as implemented by ASTRAL-II (v.4.9.7, Mirarab & Warnow, 2015), using bootstrapped gene trees estimated under the GTRGAMMA model in RAxML v8.1.21 (Stamatakis, 2014).

Aligned plastid genomes were analyzed using a Bayesian framework in MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003) implemented in the CIPRES Science Gateway (Miller et al., 2010). Default parameters were used, set to the GTRGAMMA model, temp=.3, and the program was run for 5,000,000 generations sampling every 10,000.

We looked to examine if any of the topological discordance between the nDNA tree and cpDNA tree could be explained by incomplete lineage sorting (ILS) alone. We used a modified approach of Olave et al. (2017) which takes a given species tree and simulates gene trees using the program MS (Hudson, 2002). The number of extra lineages between the species tree and gene trees is then calculated using the function deep_coal_count in Phylonet v. 2.4 (Than & Nakhleh, 2009). We used the ASTRAL tree to simulate gene trees in MS for the cpDNA tree. To account for the faster coalescence time of organellar genomes, branch lengths in the species tree were divided by a factor of two before simulating these gene trees to account for the faster coalescent times of uniparentally inherited genes. We generated 3,000 gene trees simulated under a coalescent process with no gene flow. Extra lineages were counted on the 3,000 simulated gene trees, the majority-rule gene trees from Dataset 2 from loci without any missing sequences, and our organellar genome trees. If there was evidence for hybridization in our gene trees, we would expect our observed gene trees to have more extra lineages than the simulated trees.

Dating the Diversification of Uncarina — As the size of the final AHE dataset precluded dating in BEAST (Drummond et al., 2012), we utilized penalized likelihood as implemented in treePL (Smith and O'Meara, 2012) on our best ML trees. This program has been shown, however, to estimate divergence dates similar to those of BEAST (Lagomarsino et al., 2016). The nuclear AHE tree and the derived plastid genome tree were examined separately. We used nodal dates for the crown and stem of *Uncarina* and the outgroup root all derived from a fossil-calibrated analysis of Pedaliaceae in the larger framework of the order Lamiales (see Chapter 1).

Results

Nuclear single copy loci phylogenetic tree – 513 single copy loci from the nuclear genome were effectively sampled across *Uncarina*. The nDNA tree (Fig. 9) shows strong support for the monophyly of *Uncarina* with a bootstrap value of 100. All internal nodes are highly supported with bootstrap values of 97-100. The pink-flowered species ("Pink" in Fig. 10), *U. abbreviata* and *U. stellulifera*, are resolved as sister to the rest of the genus. The remainder of the genus is split into two clades that are well-defined biogeographically: a southern species clade and a northern/western species clade. The former includes *U. turicana*, *U. decaryi*, *U. grandidieri*, and *U. roeoesliana*. In this clade, *U. turicana* is sister to the remainder of the clade. *Uncarina decaryi* and *U. grandidieri* are sister to one another, with *U. roeoesliana* sister to them. These species all occur in the southern part of Madagascar, with only *U. turicana* occurring to the northwest ("Southern" in Fig. 10).

The northern/western species clade is split into two separate clades itself, one containing species from far northern, northwestern, and midwestern Madagascar ("Northern" in Fig. 10) and the other containing a few species with primarily western ranges ("Western" in Fig. 10). In the northern clade, the rare *U. ankaranensis* is sister to the wide-ranging *U. peltata*. These taxa are sister to a clade containing *U. perrieri* and *U. leptocarpa* and the rare and recently described *U. ihlenfeldtiana.*

The western species clade shows a paraphyletic *U. sakalava*. This species has a disjunct range in Madagascar (Fig. 11) and sample U23 came from the northern portion of the range, while U22 came from the southern portion of the range. Upon examining the leaf material of these accessions more closely, U22 corresponds to the typical *U. sakalava* and U23 does not closely match any described species. This sample must represent a cryptic, undescribed species.

The rare *U. platycarpa*, which is found in the northern part of Madagascar, is sister to *U. leandrii*. *Uncarina leandrii* and its variety *rechbergeri* form a monophyletic clade with clear separation between the typical variety and var. *rechbergeri*.

Chloroplast genome phylogenetic tree is discordant with the nuclear tree - The cpDNA tree (Fig. 12) has a much different topology than the nuclear tree. However, there is still a strong biogeographical signal. This tree has high support with a posterior probability of 1 for nearly every node. Two large clades are present, one consisting of southern species and the other of northern and western species (for ranges of each clade, see Fig. 13). In the southern clade, *U. roeoesliana* is sister to the remainder of the species. *Uncarina decaryi* and *U. grandidieri* form a clade sister to *U. abbreviata* and *U. stellulifera*. In the northern/western clade, there are two distinct clades present. A small clade of only four northwestern taxa is sister to the rest of the remaining species. The former clade forms a grade with *U. sakalava* (U23) diverging first, then an accession of *U. ihlenfeldtiana*, then *U. peltata*. These taxa grade into a clade of *U. ankaranensis* and another accession of *U. ihlenfeldtiana*. The rare *U. ihlenfeldtiana* is polyphyletic in this tree but monophyletic in the nuclear tree.

The "western clade" has a grade of *U. turicana*, *U. sakalava* (U22), and one accession of *U. leandrii* var. *rechbergeri*. The rare *U. platycarpa* is sister to *U. perrieri,* and these form a clade with a grade of *U. leptocarpa* and *U. leandrii* accessions. Fig. 14 shows the discordance between the nuclear and chloroplast trees. There is discordance in all three geographical clades, but it is most pronounced in the northern/western clades.

Fig. 17 shows the results of the ILS test. Our results show that the discordance between the nuclear and chloroplast phylogenies cannot be entirely explained by ILS. Some other process must be affecting the topology of the chloroplast tree, such as ancient hybridization. It is

also possible that the extraction of the cpDNA from the AHE contained erroneous calls in certain parts of the genome. These problems will continue to be addressed.

Uncarina *diversified in the Miocene* - The chronogram produced with TreePL for the nDNA (Fig. 15) shows that *Uncarina* diverged from its common ancestor with *Pterodiscus* Hook. around 27.3 Mya. *Uncarina* began to speciate at 11.2 Mya with the lineage that led to the pink-flowered species diverging first. The split between the southern and northern/western species occurred at 10.5 Mya. Diversification of the southern species happened at 7.3 Mya. The northern/western species began diversifying slightly earlier, at 8.4 Mya. The cpDNA chronogram (Fig. 16) shows an earlier split of the ancestor with *Pterodiscus*, at 32.1 Mya. The divergence between the northern/western and southern clades occurred at 29.7 Mya. The southern clade began diversifying at 28.2 Mya. The two main clades within the northern/western clade diverged at 29.4 Mya. The smaller clade of northern species began its diversification at 26.5 Mya. We do not further examine the dates generated using the cpDNA tree as the unusual branch lengths and topology may be giving incorrect dates and relationships (see Discussion).

SEM analysis of leaf surfaces – Numerous photographs of each taxon were captured and exemplars are presented here. Fig. 1a-c show the abaxial (1a) and adaxial (1b-c) leaf surfaces of *U. abbreviata*. Stellate mucilage glands can be seen in all three photos, a distinctive character of this species, which it shares with the closely-related *U. stellulifera*. The wrinkled adaxial epidermis also has numerous stomata present. Fig. 1d-f show the adaxial (1d) and abaxial (1e-f) leaf surfaces of *U. ankaranensis*. An unusual reticulate pattern can be seen on the cells of the mucilage glands. Reduced glands can also be seen on the tips of the adaxially-borne simple hairs.

Fig. 2a-c show the adaxial (2a) and abaxial (2b-c) leaf surfaces of *U. decaryi*. The abaxial surface is covered in mucilage glands, which give the leaves their distinctive white appearance. These photos can be compared to the closely-related *U. grandidieri* in Fig. 2d-f. Fig. 2d shows the abaxial leaf surface with mucilage glands with simple hairs interspersed. A single mucilage gland is picture in Fig. 2e.

Fig. 3a-c shows the adaxial (3a) and abaxial (3b-c) leaf surfaces of *U. ihlenfeldtiana*. The abaxially-borne, simple hairs are thick and interwoven. Fig. 3d-f show the adaxial (3d) and abaxial (3e-f) leaf surfaces of *U. leandrii* var. *leandrii*. The sparsely hairy adaxial leaf surface has a unique, wrinkled surface that was also seen in other species in this study. Note the copious mucilage glands along a vein and the numerous stomata on the abaxial surface seen in Fig. 3e.

Fig. 4a-c show the adaxial (4a) and abaxial (4b-c) leaf surfaces of *U. leandrii* var. *rechbergeri*. The abaxial leaf surface has sparse mucilage glands (4b) and dense simple hairs (4c). Fig. 4d-f show the adaxial (4d) and abaxial (4e-f) leaf surfaces of *U. leptocarpa*. This specimen has abundant stomata on its abaxial surface as can be seen in Fig. 4f.

Fig. 5a-c show the adaxial (5a) and abaxial (5b) leaf surfaces, and the leaf edge (5c) of *U. peltata*. The adaxial leaf surface is sparsely-covered by simple hairs with reduced glandular heads whereas the abaxial leaf surface has numerous, short mucilage glands. The leaf edge in this specimen had a thick layer of long, simple hairs with reduced glandular heads, like those seen on the adaxial leaf surface. Fig. 5d-f show the adaxial (5d) and abaxial (5e-f) leaf surfaces of *U. perrieri*. Both leaf surfaces have a bare appearance with scattered mucilage glands.

Fig. 6a-c show the adaxial (6a) and abaxial (6b-c) leaf surfaces of *U. platycarpa*. Note the similarity of the abaxially-borne, long, simple hairs of this species with those seen in *U. leandrii* var. *rechbergeri* (Fig. 4b). Fig. 6d-f show the adaxial (6d) and abaxial (6e-f) leaf

surfaces of *U. roeoesliana*. The long-stalked mucilage glands that are unique in the genus (and the family) can be seen in Fig. 6e-f.

Fig. 7a-c show the adaxial (7a) and abaxial (7b-c) leaf surfaces of *U. sakalava*. This specimen shows an interesting feature in that many of its mucilage glands appear collapsed. The four-celled heads on many glands have a shrunken appearance, perhaps caused by the silica gel drying procedure. This phenomenon was also seen in *U. stellulifera* and *U. peltata*. Fig. 7d-f show the adaxial (7d) and abaxial (7e-f) leaf surfaces of *U. stellulifera*. The thick covering of long-armed, stellate hairs that immediately distinguish this species from all others can be seen in Fig. 7e-f. Compare these to the closely-related *U. abbreviata* (Fig 1a-c). Note also the shrunken appearance of the adaxial hairs. Fig. 8a-c show the adaxial (8a) and abaxial (8b-c) leaf surfaces of *U. turicana*. Note the abaxial veins (Fig 8c) bearing simple hairs and mucilage glands, a distinctive characteristic of this species.

Discussion

Nuclear genes provide a comprehensive scenario of Uncarina *diversification* - The nuclear tree (Fig. 9) shows strong support for a monophyletic *Uncarina*. When more than one accession was available, this tree shows strong support for the monophyly of each sampled taxon, except *U. sakalava*. The earliest diverging taxa are the pink-flowered *U. abbreviata* and *U. stellulifera*. These two species are the only ones in the genus that possess pink flowers, mucilage glands with a stellate head (Fig. 1a-1c, 7d-7f), and occurrence of false septa in the fruits. These species' leaf morphology is also similar: both possess simple, sometimes shallowly-lobed, triangular to ovate leaves. The two species occur sympatrically in southwestern Madagascar. They are believed to hybridize in the wild and have been artificially crossed in the

horticultural trade (T. Harvey, pers. comm.). Interestingly, these two species differ quite markedly in their fruit morphology: *U. abbreviata* has a relatively broad, winged capsule body with spines to 25mm while *U. stellulifera* has a narrow, unwinged capsule body with a long beak and spines to 30mm.

The remainder of the genus is split into two well-supported clades: one consisting of four species mainly from southern Madagascar and the other containing species from western and northern Madagascar. In the southern clade, *U. decaryi*, *U. grandidieri*, and *U. roeoesliana* form a clade sister to *U. turicana*. The latter species occurs to the west of the three former species but all four are located in either dry forest or spiny forest, as mapped in Vieilledent et al. (2016). *Uncarina turicana* was described in 1999 and is known from only a small area (Ihlenfeldt, 2002). It shares many features with a diverse array of species in the genus: arborescent habit (shared with *U. abbreviata* and *U. grandidieri*), exclusively barbed spines on the fruits (*U. peltata* and *U. leandrii*), and fruits with narrow wings (*U. abbreviata* and *U. leandrii*). Its leaves resemble those of *U. grandidieri* in having relatively broad, shallowly-lobed blades. *Uncarina turicana* is sister to a group of species that have long been hypothesized to be closely related. *Uncarina roeoesliana*, the smallest species in the genus, is sister to *U. decaryi* and *U. grandidieri*. *Uncarina roeoesliana* is unique in having a large underground tuber that produces a single stem to 1.5m; all other members of the genus are trees or multi-stemmed shrubs. This species has a very restricted range and is found on compacted sand dunes in extreme southern Madagascar. The leaves are deeply lobed, like some forms of *U. decaryi*, and possess mucilage glands that are long-stalked, a feature unique in the genus and the family (Fig. 6d-f). All three of these taxa have glandular hairs with heads that are slightly stellate (Fig. 2, 6d-f), unlike *U. turicana*, where the heads are more rounded (Fig. 8), like those seen in the northern and western

clades. In addition, these three species have fruits that are extremely similar to one another, so much so that Ihlenfeldt (2002) claims they cannot be told apart without leaf material or plant habit being examined. *Uncarina decaryi* and *U. grandidieri* both form small trees to 3m tall but differ markedly in their leaves. *Uncarina grandidieri* has leaves with only slight to moderate lobing, while *U. decaryi* leaf blades are quite variable, being either slightly to moderately lobed, trilobate, or nearly digitate with five large, obtuse lobes. Both species have two (sometimes indistinct) lobes at the base of the blade. In addition to the more variable blade shape of *U. decaryi*, that species also has distinctly different colors to its abaxial (greyish green) and adaxial blade (green) surfaces, owing to the presence of a dense covering of short mucilage glands on the abaxial surface. The abaxial and adaxial leaf surfaces of *U. grandidieri* are only slightly different in their greenish coloring. The ranges for *U. decaryi* and *U. grandidieri* are quite large and sympatric; not surprisingly, they have been suspected to hybridize (Humbert, 1971). Further studies would benefit from the inclusion of more accessions of each species taking into account their full distribution and variability.

The northern clade includes five species that are primarily distributed in northern and northwestern Madagascar (Fig. 10), with *U. leptocarpa* and *U. perrieri* occurring farther south, but along the western coast of the island. *Uncarina peltata* and *U. ankaranensis* form a clade that is sister to the remainder of the northern clade. *Uncarina ankaranensis* is recently described and known only from the Ankara Massif in far northern Madagascar (Fig. 11). It resembles *U. peltata* in having adaxial leaf blades covered with simple hairs and abaxial surfaces with mucilage glands almost exclusively along the veins (Fig. 1d-f, 5a-c). The two species are also similar in seed structure, the orientation of the flowers, and curvature of the corolla tube. *Uncarina ankaranensis* differs markedly from *U. peltata*, and all other *Uncarina* species, in its

low-lying, shrubby growth form, with many branches descending down to ground level. *Uncarina peltata* has a fairly large range throughout northern Madagascar, exclusively within the dry forest zone. There are two localities for this species farther south that are quite disjunct from the majority of its range and could represent misidentifications.

The recently described *U. ihlenfeldtiana* has an extremely restricted range in northern Madagascar, being known only from near Mandritsara (Fig. 11). The altitudinal location of this species is unique in the genus, ranging from 700-1000m. This species was originally cited as a (fragmentary) paratype of *U. sakalava* by Humbert (1962), but he expressed doubts as to whether it was truly the same entity as the holotype. Its range is far outside that of *U. sakalava*, which occurs farther west and south (Fig. 11). Lavranos (2004) relays the efforts of Walter Röösli and Ralph Hoffmann to locate this paratype taxon and collect more material that ultimately proved to be a new species. *Uncarina ihlenfeldtiana* differs from *U. sakalava* in having leaf blades with abaxial surfaces covered in dense simple hairs. The former species also has only shallowly lobed leaves, while the latter has leaves that are deeply lobed. The orientation of the flowers of both species is quite different: the limb of the flower in *U. sakalava* is held in such a way that it opens upward, while in *U. ihlenfeldtiana*, it is oriented parallel with the ground. Overall, the fruits of *U. ihlenfeldtiana* are much smaller than those of *U. sakalava* and they have shorter spines across the bodies.

The remaining species of the northern clade, *U. leptocarpa* and *U. perrieri*, have been considered to be closely related (Ihlenfeldt, 2002) based on leaf and indumentum morphology and the nuclear data supports this. *Uncarina leptocarpa* is unusual in the genus in having white flowers. Both species have pentagonal leaves with dark green adaxial surfaces and prominent white to reddish veins. The leaves have only indistinct, acute lobing and both surfaces have a

sparse indumentum (Fig. 4d-f, 5d-f). The ranges of these two species overlap slightly with *U. leptocarpa* occurring farther south than any other species in this clade.

The western clade (Fig. 10) contains five taxa with the most notable feature being the paraphyly of *U. sakalava*. This species has a disjunct distribution (Fig. 11) with collections from northwestern Madagascar as well as farther south, near Bekopaka and Ambinda in Melaky Region. Humbert (1962), in his original description of the species, expressed concerns that one of his paratypes, *Perrier* 15083 (P), might represent a different taxon. It was not until Walter Röösli and Ralph Hoffmann explored the locality from which this paratype came that it was confirmed that it represented a new species: *U. ihlenfeldtiana*. The U23 accession of *U. sakalava* was collected in the northern part of its range, near the locality of *Perrier* 8456 (P), which was also designated as a paratype and is figured in Humbert (1962). The holotype was collected in the southern portion of the species' range, ~65mi north of where the U22 accession was collected. Upon examining the leaves (the only material we have), the U22 accession more closely matches the species description in Humbert (1962). This accession has leaves with adaxial surfaces covered with mucilage glands and numerous simple hairs. The abaxial surfaces have a sparser indumentum with mucilage glands borne primarily along the veins (Fig. 7a-c). The leaves of the U23 accession have an adaxial surface almost exclusively of mucilage glands and abaxial surface densely covered in mucilage glands; they are not just confined to the veins. An SEM photo of the abaxial leaf surfaces of what appears to be this accession (they have the same collection number) is featured in Lavranos (2004). Whether the U23 accession is the same taxon as the paratype *Perrier* 8456, is unknown. I have not been able to secure a loan of this taxon from P to better understand what is going on. However, it is clear that another cryptic species exists within *U. sakalava* and that, perhaps, Humbert too broadly defined the species or

selected paratypes from too broad a geographic range. Unfortunately, *Uncarina* specimens are often fragmentary, (e.g. *Perrier* 8456, consisting of just a leaf and a fruit) which makes accurate identification and even species descriptions very difficult. More collecting of this species is needed and perhaps more accessions of it should be included in future phylogenetic work.

The last three taxa in the western clade all share similar fruit morphology and two of them have only recently been described (Lavranos, 1995). *Uncarina platycarpa* occurs in far northern Madagascar and is apparently quite rare. The typical variety of *U. leandrii* occurs in central-western Madagascar, with its range ending just north of the spiny forest. *Uncarina leandrii* var. *rechbergeri* is sympatric with the typical variety and differs mainly by possessing a dark red throat in the corolla tube and having yellowish leaf veins, which are reddish in var. *leandrii*. *Uncarina platycarpa* differs from *U. leandrii* in having larger, wider fruits, a rusty-red corolla throat, shorter petioles, and hairier leaf blades (Lavranos, 1995).

Uncarina *exhibits discordance between the plastome and nuclear genomes* — The cpDNA tree (Fig. 12) shows a much different topology than the nuclear tree. The Next Generation cpDNA and nuclear phylogenies for the family Pedaliaceae as a whole (see Chapter 1) and the genus *Sesamothamnus* (see Chapter 3), are completely congruent. Thus, *Uncarina* exhibits an unusual and strong discordance between the two genomes and we are evaluating three possible phenomena as causes for this discordance. First, the cp plastome in *Uncarina* has some unusual sequence structures scattered around the ca. 140 kb molecule and will require future plastid-wide evaluation of possible inversions and duplications that may be impacting topology and branch lengths. Once these structural features are verified, a more accurate set of phylogenetic analyses will be possible to determine if incomplete lineage sorting (ILS) and/or hybridization can be tested in the context of a rapidly diverging lineage (e.g., Jakob and Blattner,

2006; Folk et al., 2017; Kamneva and Rosenberg, 2017). Despite the incongruence, there is still a strong biogeographical signal of northern/western and southern species' clades perhaps suggesting that early hybridization within geographically delimited clades has occurred. Unlike the nuclear tree, *U. abbreviata* and *U. stellulifera* are not sister to the remainder of the genus, but rather are embedded in the southern clade, sister to both *U. decaryi* and *U. grandidieri*. These are, together, sister to *U. roeoesliana*. As stated above, *U. abbreviata* and *U. stellulifera* have mucilage glands with stellate heads (Fig. 1a-c, 7d-f). The heads on the other three species of the southern clade are only slightly stellate (Fig. 2, 6d-f); the heads on the remainder of the species in the genus are rounded (Fig. 1d-f, 3-7c, 8). *Uncarina abbreviata* and *U. stellulifera* share a suite of morphological characters and *U. decaryi*, *U. grandidieri*, and *U. roeoesliana* also share much in common with each other. The ranges of these two groups of species overlap only slightly. There are (unpublished) reports of *U. stellulifera* being able to cross with *U. grandidieri* via hand-pollination (T. Harvey, pers. comm.). The resulting offspring produce peach-colored flowers, quite unlike the pink and yellow flowers of the respective parents, or anything else in the genus. Absent from the southern clade is *U. turicana*. This species shares much in common with other taxa in the genus, is poorly known, and occurs in only a small geographic area. Interestingly, while it shares a growth habit similar to other southern clade species, its fruits and mucilage glands share more in common with species of the northern and western clades and the cpDNA dataset places it in that clade.

The northern clade of the cpDNA tree is relatively similar to the northern clade of the nuclear tree but excludes *U. perrieri* and *U. leptocarpa* and includes the U23 accession of *U. sakalava* (which was collected in the northern portion of the species' range). Strikingly, *U. ihlenfeldtiana* is polyphyletic here, which is quite strange since this species is only known from a

very small area. One of the accessions, U06, is sister to the equally restricted *U. ankaranensis*. These two species are separated geographically by a large area of inhospitable humid forest. It is possible that *U. ankaranensis* once had a larger range south of its current location which enabled it to cross with *U. ihlenfeldtiana* and the former went extinct in the shared range, or vice versa. These three species share a suite of leaf characters: veins sunken into the blade, adaxial surfaces sparsely covered with simple hairs and lacking mucilage glands, abaxial surfaces covered by long simple hairs which conceal the mucilage glands that are primarily arranged along the veins. The fruits of *U. peltata* are quite different from those of the other two species, having shorter spines and a more prominent beak; this species has the widest range of any of the northern clade taxa and is highly variable (Ihlenfeldt, 2002).

The remainder of the genus in the cpDNA tree shows a great deal of discordance from the nuclear tree (Fig. 14). *Uncarina leandrii* is paraphyletic with *U. platycarpa*, *U. perrieri* and *U. leptocarpa* embedded within it. *Uncarina turicana* is sister to the *U. leandrii* clade and *U. sakalava* U22. *Uncarina turicana* is placed in the southern clade in the nuclear analysis but here it groups with species from central-western, southwestern, and northwestern Madagascar. As stated above, it shares both fruit and mucilage gland features in common with taxa in this clade. The next diverging taxon in this clade is the U22 accession of *U. sakalava*. This accession comes from the more southern portion of the species' range, not too far from the type locality. The remainder of the clade is three species embedded within *U. leandrii* (and its variety *rechbergeri*). In this clade, *U. perrieri* and *U. platycarpa* are sister to one another and in turn are sister to the remainder of the taxa. Despite the topological incongruence and paraphyly of *U. leandrii* in this clade, there are a few morphological features shared among these taxa. All members of this clade are dwarf trees 2.5-3m in height. The leaf blades of all of the taxa are

dark green in color with the adaxial surfaces only sparsely covered with mucilage glands. The lobing of the leaves varies among the species, but the lobes are always acute. Since the ranges of many of these taxa overlap, the incongruence and paraphyly may be the result of chloroplast capture. In addition to the biogeographical signal seen in the cpDNA and nDNA trees, there is perhaps a chemical signal as well. Yamazaki et al. (2007) examined the external and internal flavonoids of 12 *Uncarina* taxa: *U. grandidieri, U. decaryi, U. abbreviata, U. turicana, U. stellulifera, U. sakalava, U. perrieri, U. platycarpa, U. leptocarpa, U. peltata, U. leandrii*, and *U. leandrii* var. *rechbergeri*. They showed that five of these taxa have nearly the exact same flavonoid composition: *U. grandidieri, U. decaryi, U. turicana, U. abbreviata,* and *U. stellulifera* (only *U. stellulifera* was missing four of the nine shared flavonoids). The first three species are part of the same clade in the nDNA tree while in the cpDNA tree all but *U. turicana* share a clade. One could posit that this particular flavonoid composition could have been present early on in the history of the genus and subsequently changed in the western/northern clades. Examining the flavonoid composition of *U. roeoesliana* would add an important piece to this hypothesis. Yamazaki et al. (2007) also showed that some northern and western taxa share some of these same flavonoids, but their composition is otherwise quite different. A more thorough sampling of the flavonoids within *Uncarina* would add more data to better understand its evolutionary history.

The temporal context of Uncarina *diversification* — The chronogram based on nDNA shows that *Uncarina* split from its common ancestor with *Pterodiscus* 27.3 Mya. *Uncarina* began diversifying around 11Mya with the pink-flowered *U. stellulifera* and *U. abbreviata* diverging first, followed by a radiation of the remainder of the genus. The southern clade began diversifying 7.27Mya. The northern clade split from the western clade at 8.37Mya with the

former diversifying at 7.14Mya and the latter diversifying at 5.0Mya. These clade radiations all occurred around 7-8Mya, during the late Miocene. This was a time of global decline in temperatures, greater rainfall seasonality, and the spread of grasslands (Zachos et al., 2001; Bobe, 2006). The rise of grasslands dominated by C_4 grasses is believed to have been caused by the global increase in aridity during the late Miocene (Edwards et al., 2010). An analysis of the grass flora of Madagascar showed that C_4 lineages were present before- and diversified during- the expansion of these grasslands on the island (Hackel et al., 2018). Many recent studies have shown that arid-adapted and succulent lineages diversified during this same time period (Arakaki et al., 2011; Bellstedt et al., 2012; Bissinger et al., 2014). *Uncarina* also appears to have diversified during the late Miocene, when climatic conditions were dryer and adds yet another example of this phenomenon. The nDNA chronogram shows a crown age of 11.18Mya with subsequent diversification occurring 10-3Mya. If the dates in the cpDNA chronogram are correct, then diversification of plastid DNA occurred during the Oligocene and early Miocene. Much of Madagascar was covered by subarid to arid habitats (similar to those seen in the south today) from the late Cretaceous to the early Palaeocene (Buerki et al., 2013). This extensive habitat started a gradual decline as conditions became suitable for the formation and expansion of humid and dry forests as Madagascar moved northward to its current position. Despite the discrepancy in dates, it is clear that *Uncarina* diversified in Madagascar during a time when new habitat types were becoming available for it to colonize. The dry forests that are occupied by the northern and western clades are more extensive than the spiny forests occupied by the southern clade(s) and there are more taxa present in the former, with many of them having restricted ranges. It is plausible to imagine the ancestor of *Uncarina* reaching Madagascar from Africa, speciating in the southern arid regions as they contracted (with niches being opened by possible

extinction of other groups), then becoming adapted to the northern and western dry forests as they were forming. Species would subsequently move northward and speciate even further. Regardless of the DNA organelle analyzed, all the species arose during an 8-10my timeframe (Fig. 15, 16), so, *Uncarina* underwent very rapid speciation. The high level of restricted range endemics in the north may be due to specialization to specific soil types (Ihlenfeldt, 2002) or is merely an artifact of the extensive habitat destruction that has befallen this region (Ihlenfeldt, 2010). Ihlenfeldt (2010), argues that *Uncarina* is a very old genus because of its endemicity and lack of relatives in Africa. Gormley et al. (2015) showed that *Uncarina* is sister to *Rogeria* (endemic to Africa) and they do in fact share some specialized characters (Ihlenfeldt, 2004a). Our dates show that *Uncarina* is not that old of a genus and that Ihlenfeldt's (2010) idea that the genus radiated only in the last 100,000 years, is false.

Mucilage gland morphology helps to interpret phylogeny - The mucilage glands of *U. abbreviata* and *U. stellulifera* are very similar to one another as can be seen in Fig. 1a-c and Fig. 7d-f. These species are closely related, sharing pink flowers in addition to stellate mucilage glands. Their fruits are considerably different however (Ihlenfeldt, 2002). The "arms" of each mucilage gland are longer and thicker in *U. stellulifera* than in *U. abbreviata*.

Uncarina decaryi, U. grandidieri, and *U. roeoesliana* are closely-related, sympatric species found in southern Madagascar. The first two are quite common with the latter being rare (Ihlenfeldt, 2002). *Uncarina decaryi* has an abaxial leaf surface covered in mucilage glands which give the leaves a white appearance. These glands are similar to those of *U. grandidieri* but differ in the size of the four apical cells. Those of *U. grandidieri* are shorter and more rounded, somewhat resembling a button-tucked pillow. Simple hairs with reduced heads can be seen on this specimen of *U. grandidieri* in Fig. 2d. *Uncarina roeoesliana* is unique in the genus

(and the family) in having mucilage glands borne on long stalks. These can be seen in Fig. 6e-f. The heads of these glands (at least in this specimen) appear closer to those seen in *U. grandidieri* than in *U. decaryi*. These glands are thick, covering most of the abaxial leaf surface. The mucilage glands and simple hairs of *U. turicana* (Fig. 8) do not show clear affinities with other species. This species has a rather narrow range (Ihlenfeldt, 2002) in southern Madagascar. Live leaves of *U. turicana* are much stickier than those of any other species (pers. obsv.) perhaps due to the simple hairs' reduced glandular heads.

Figs. 1e, 5b, and 7c show similarities in the mucilage gland heads of the *U. ankaranensis*, *U. peltata*, and *U. sakalava*. All three have similar reticulation patterns, perhaps caused by the silica gel drying process. Of note is the fact that the mucilage glands of both *U. peltata* and *U. sakalava* collapsed in some cases. The mucilage glands of the northwestern and central-western species (*U. perrieri, U. platycarpa, U. leandrii*) are quite similar to one another. Arrangement of mucilage glands and simple hairs are one character used to differentiate these species.

Uncarina perrieri, U. platycarpa, U. leptocarpa and *U. leandrii* are represented in Figs. 5d-f, 6a-c, 4d-f, and 3d-f, respectively. These species have close affinities with one another and some have overlapping ranges (*U. leandrii* and *U. leptocarpa*). *Uncarina leandrii* (Fig. 3d-f) and its variety *rechbergeri* (Fig. 4a-c), are differentiated primarily by foliar vein color and flower throat color, but these specimens show a marked difference in indumentum. The specimen of *U. leandrii* var. *rechbergeri* used has a dense abaxial indumentum of long, simple hairs, as seen in the specimen of *U. platycarpa*. The specimen of *U. leandrii* has sparse long, simple hairs and they are borne primarily along the abaxial veins. *Uncarina perrieri* and *U. leptocarpa* have sparse mucilage glands on the abaxial leaf surfaces, which are more numerous in *U. leptocarpa* and are found mainly along the veins.

It appears that *Uncarina* is a relatively young (late Miocene) and quickly speciating genus. There is a wide range of often variable characters that define each species, but these also link them. While our nDNA and cpDNA phylogenies show a large amount of discordance, there is a clear biogeographical signal that the southern species are closely related, as are the northern/western species. These geographically-linked clades (in both datasets) also have morphological characters that can help us understand their evolutionary history. Unfortunately, *Uncarina* specimens are often fragmentary and poorly preserved, causing misidentifications and taxonomic uncertainty. Therefore, more thorough collecting and increased sampling will help us gain a better understanding of the relationships and diversity within the genus, especially concerning the existence of cryptic species, such as in *U. sakalava*. Such an endeavor is imperative considering the extent to which Madagascar is undergoing deforestation and habitat destruction. Our work shows that there is still diversity to be discovered in yet another Madagascar endemic.

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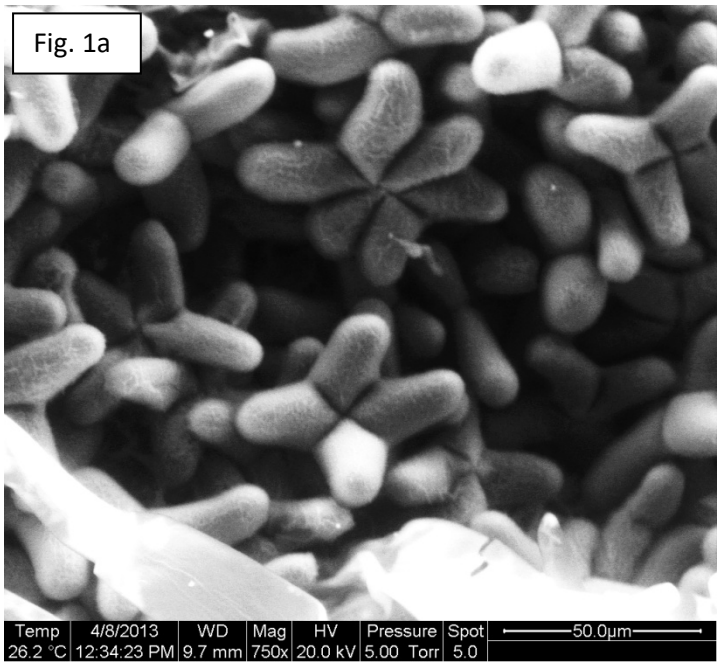
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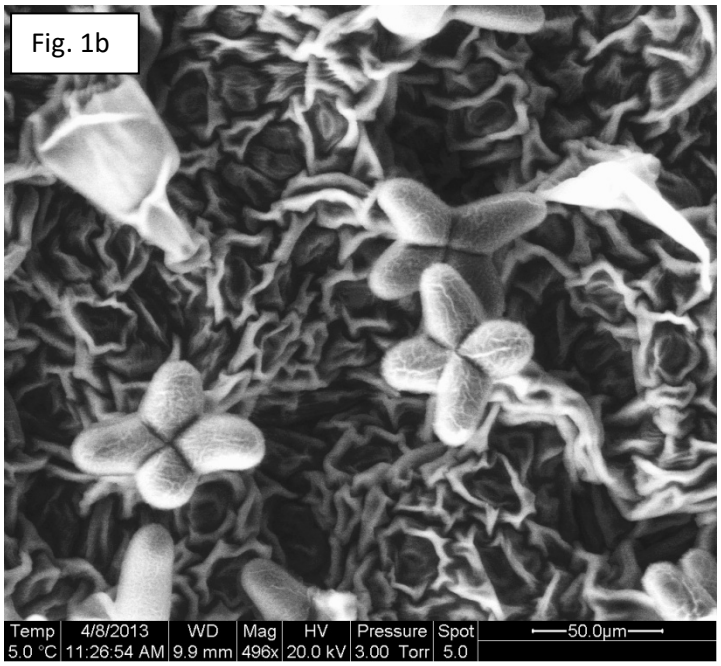
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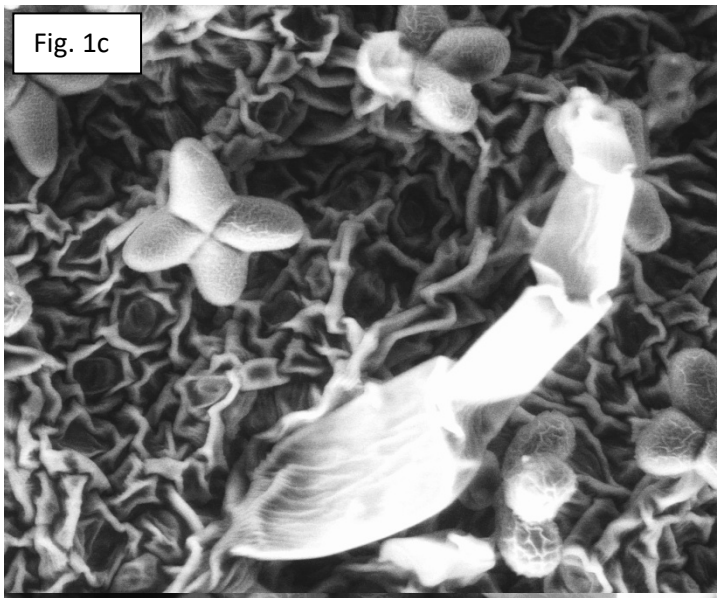
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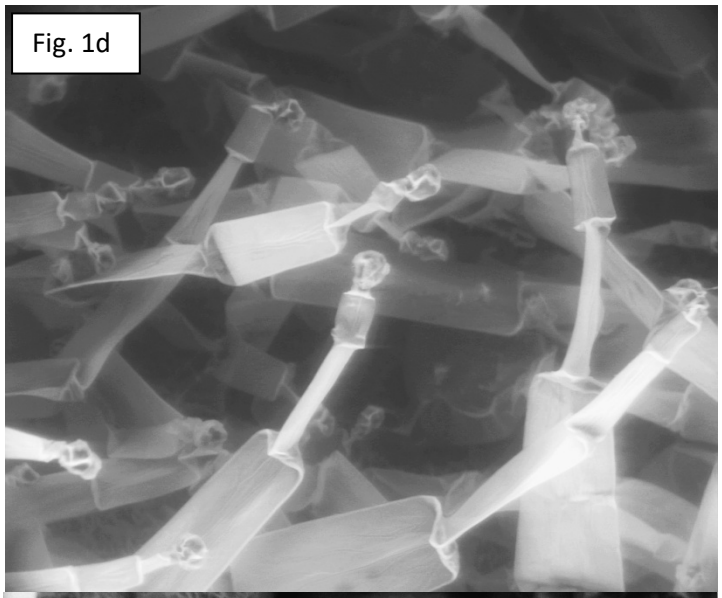
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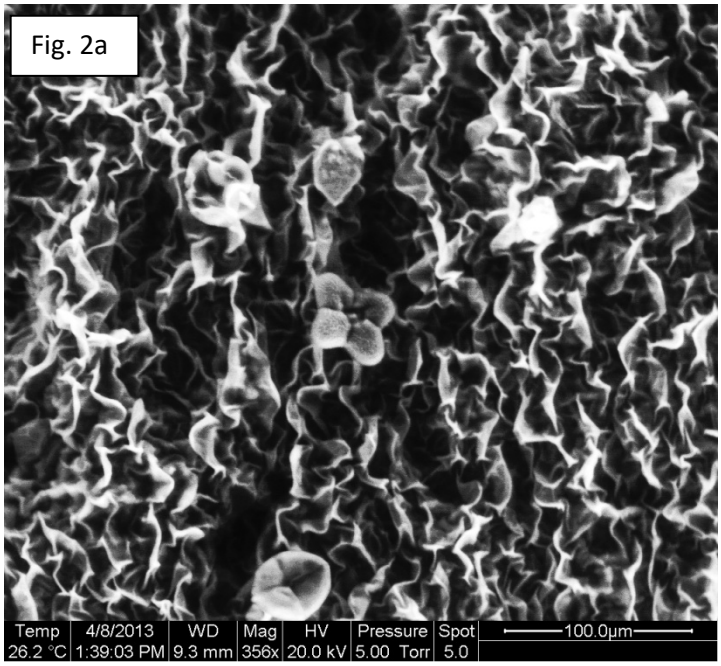
Fig. 2a



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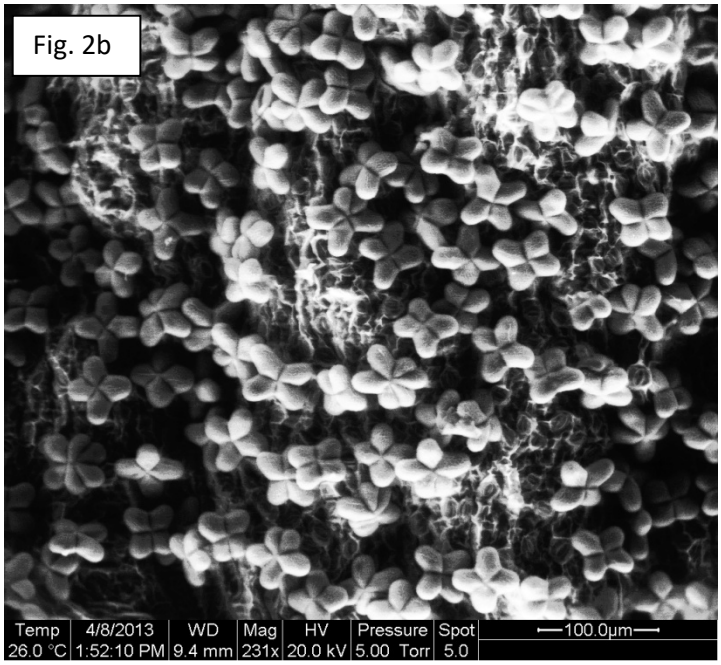

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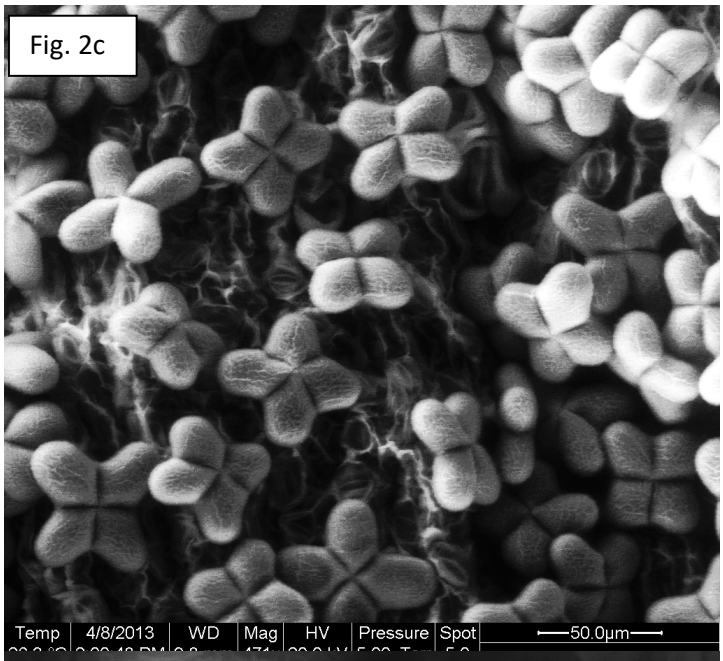

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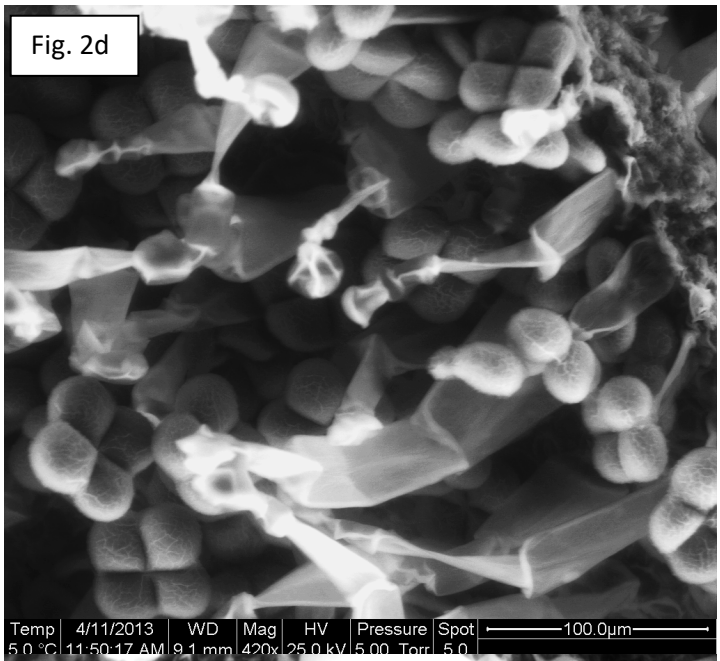

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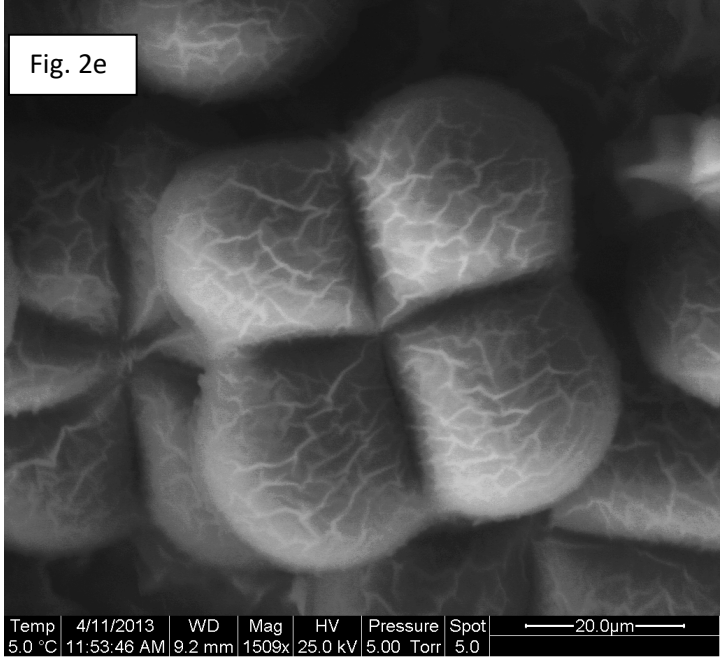

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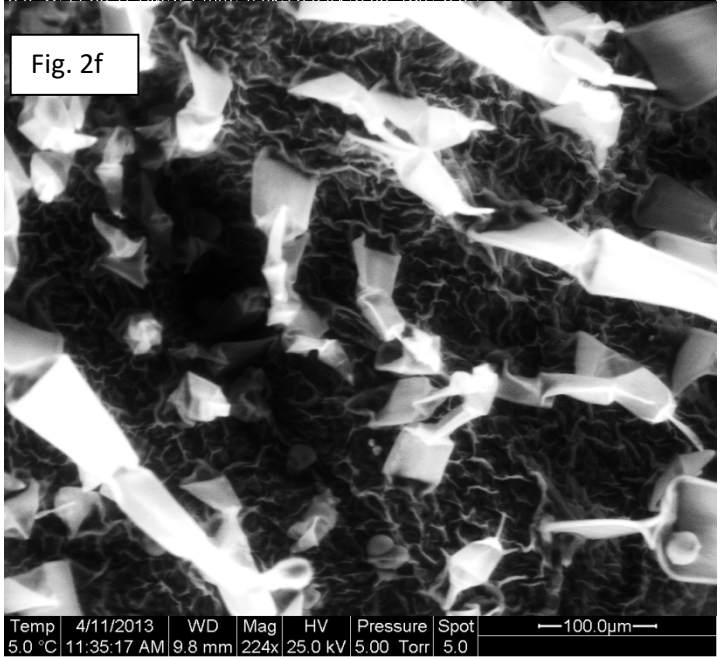

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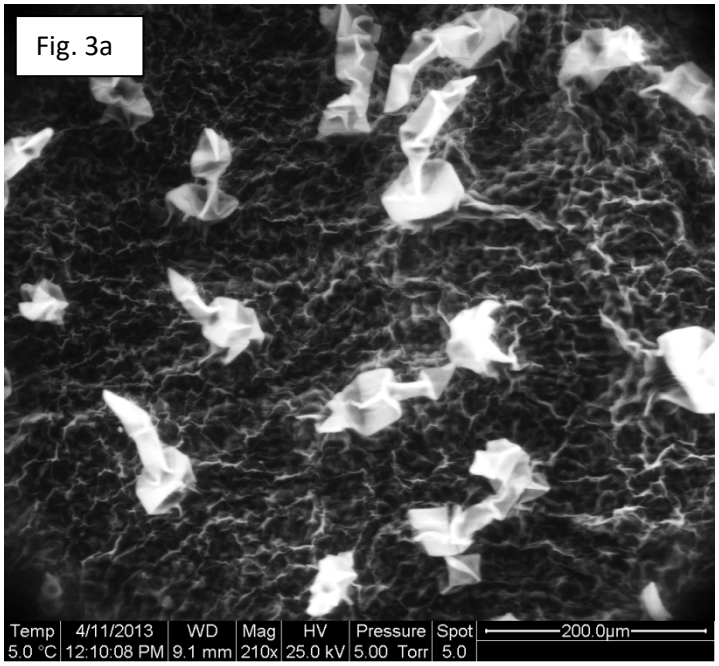

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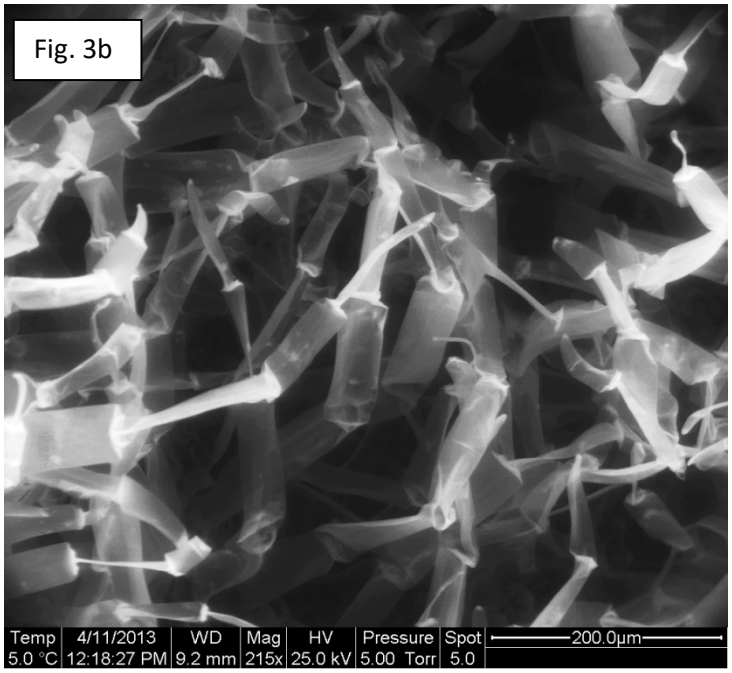

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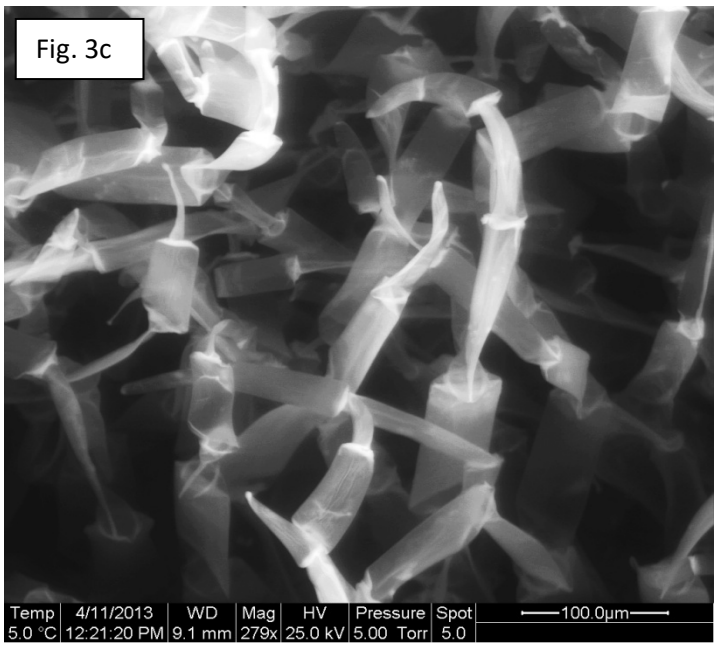

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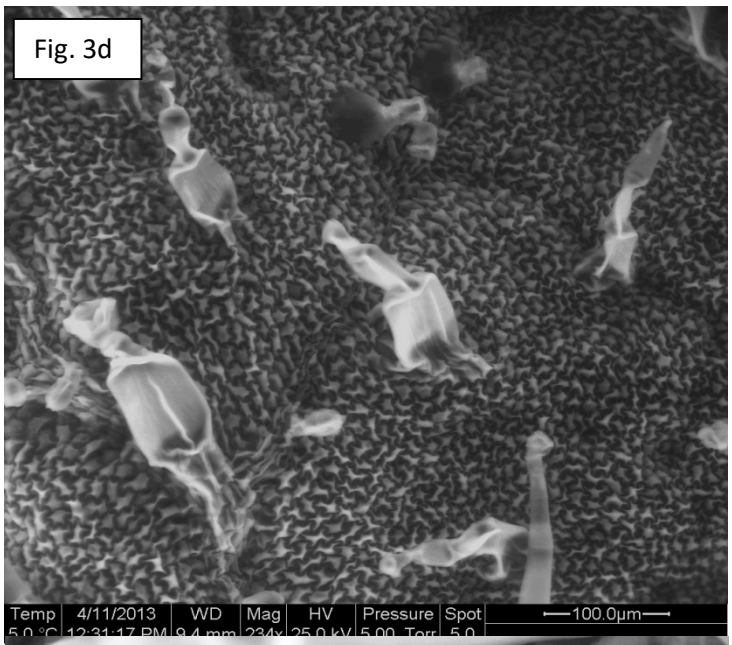

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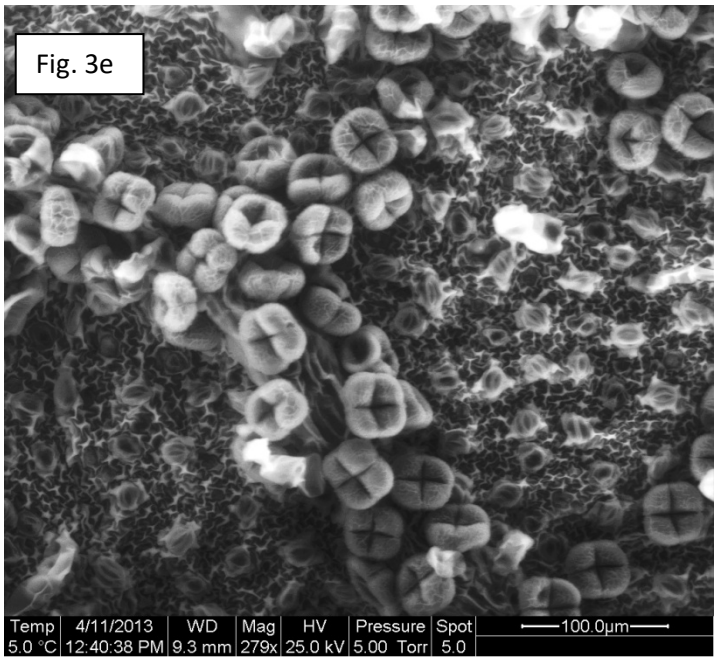
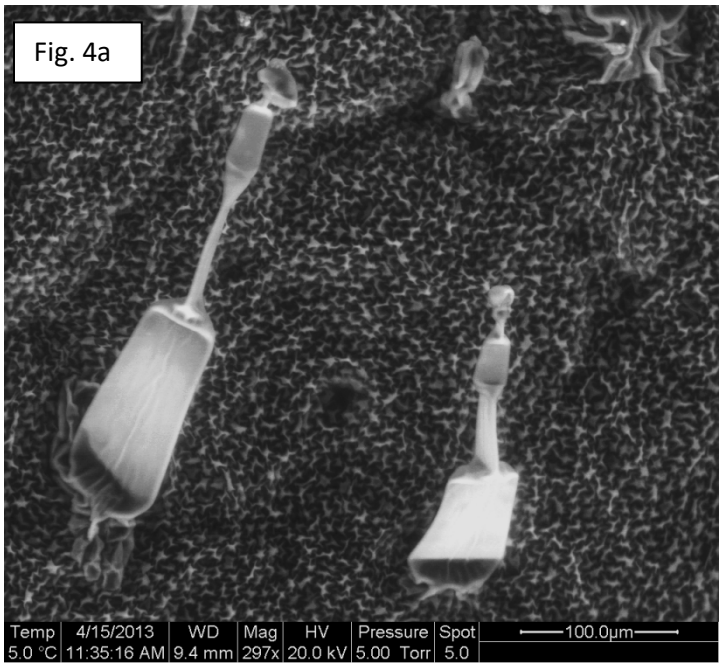


Fig. 3f

Fig. 4a



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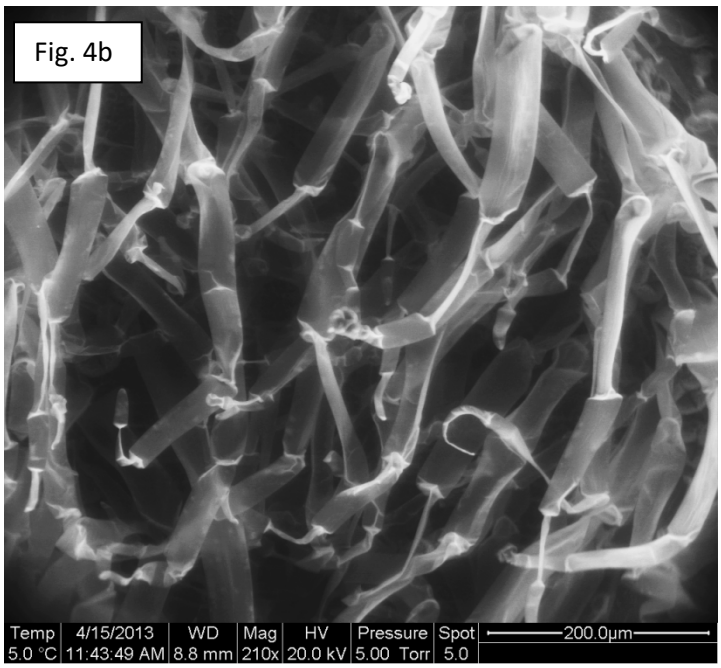

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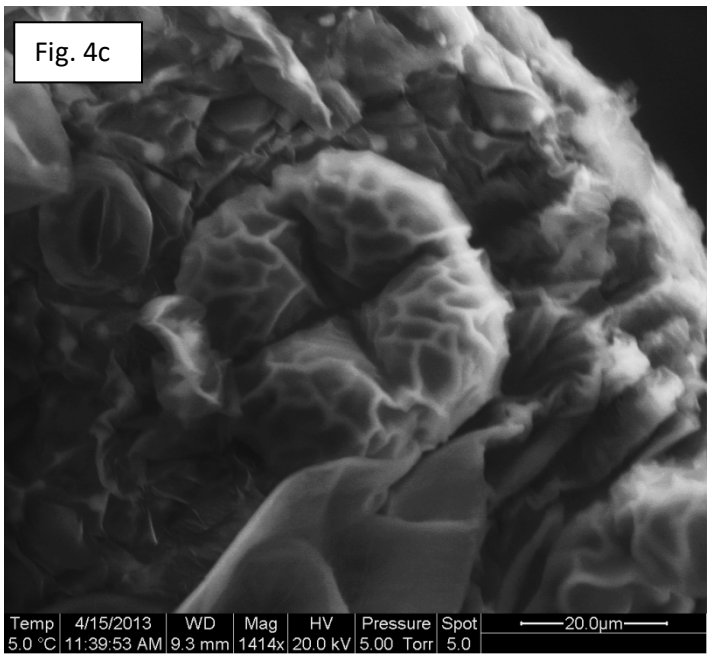

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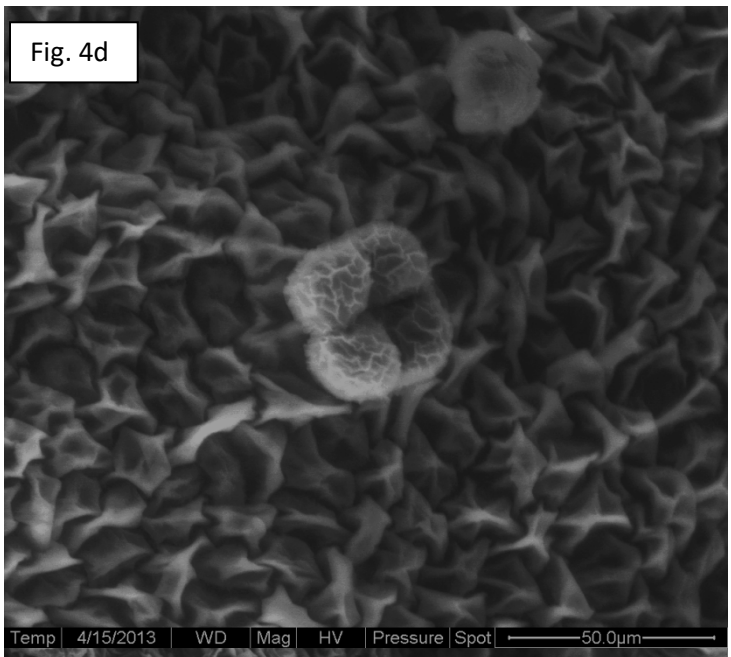


Fig. 4e

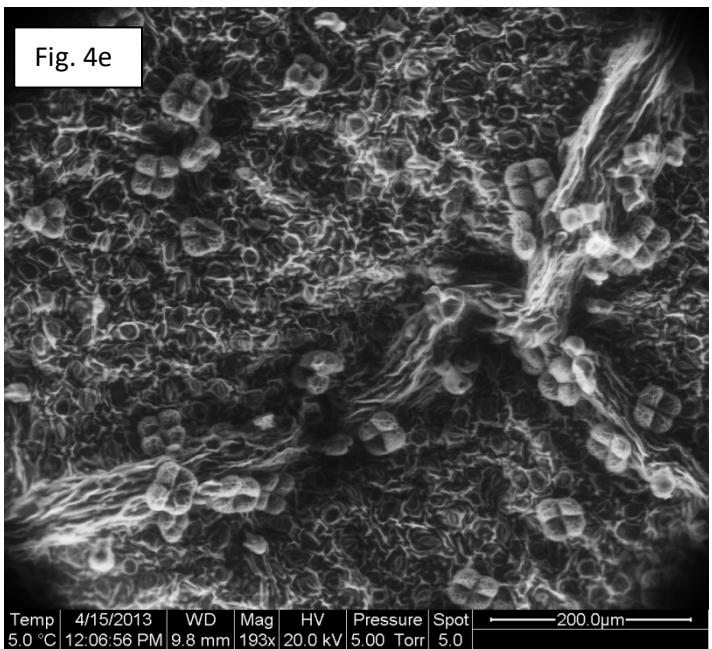
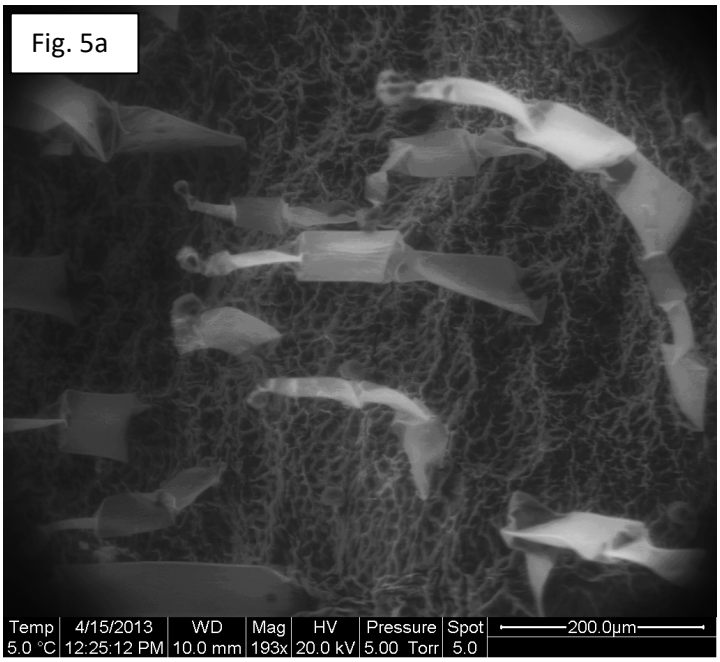


Fig. 4f

Fig. 5a



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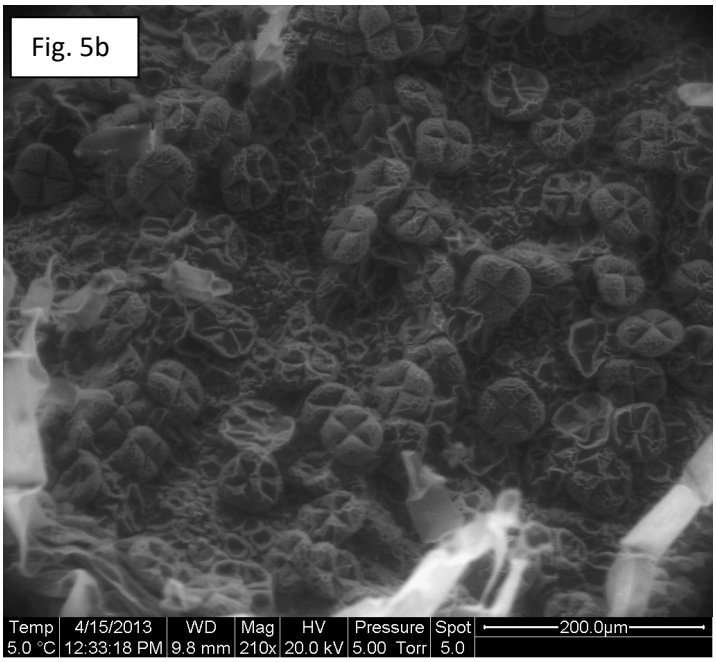


Fig. 5c

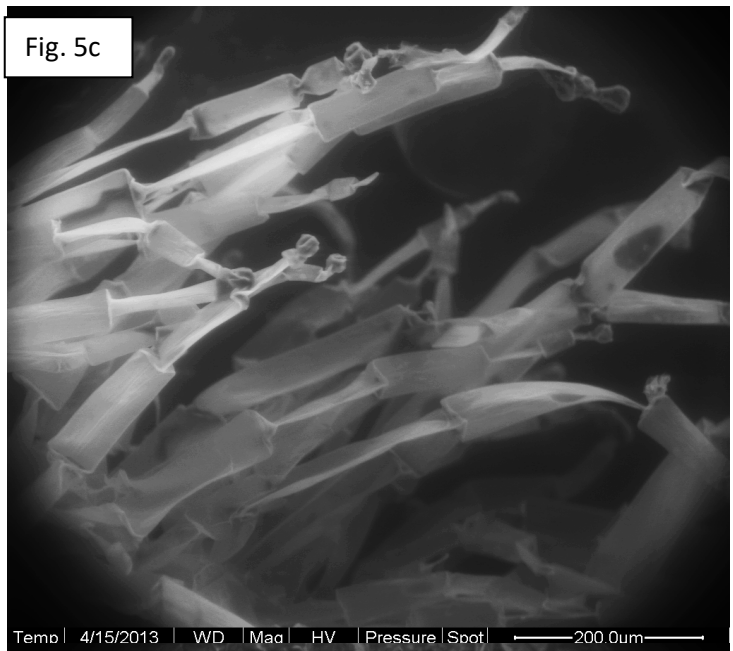


Fig. 5d

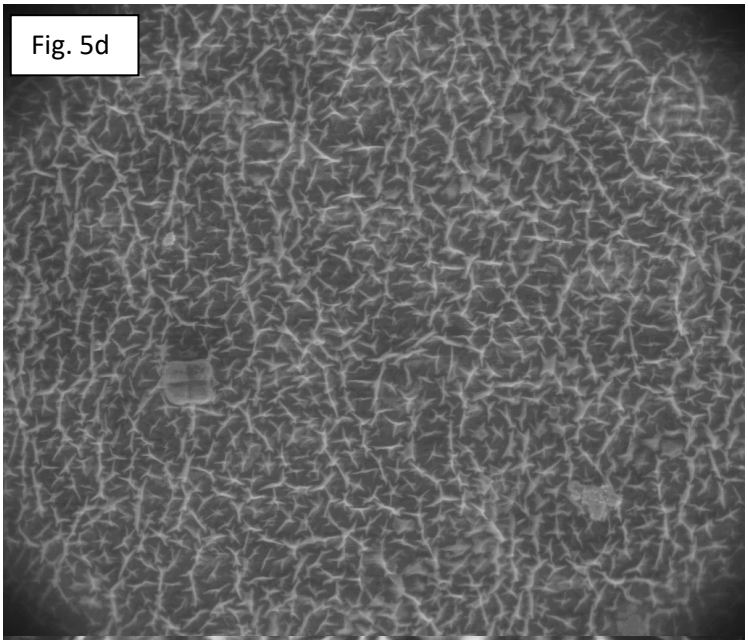


Fig. 5e

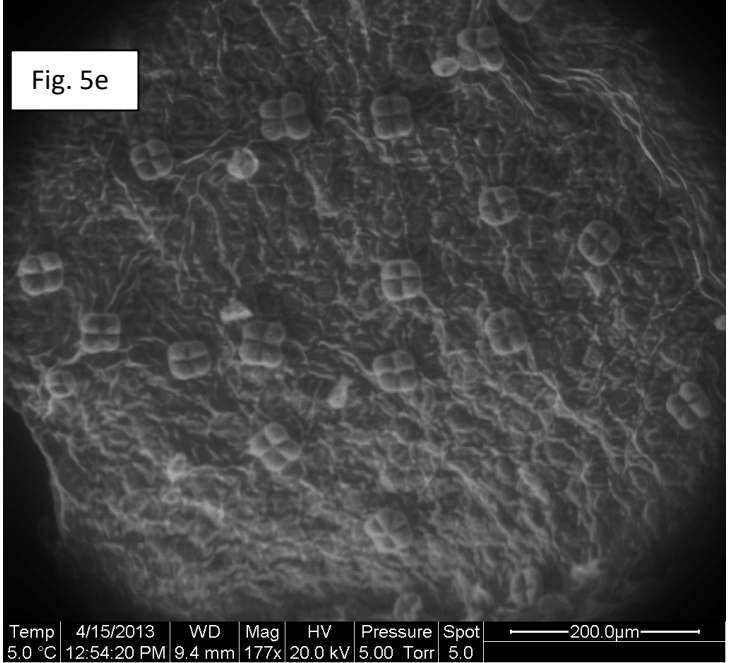


Fig. 5f

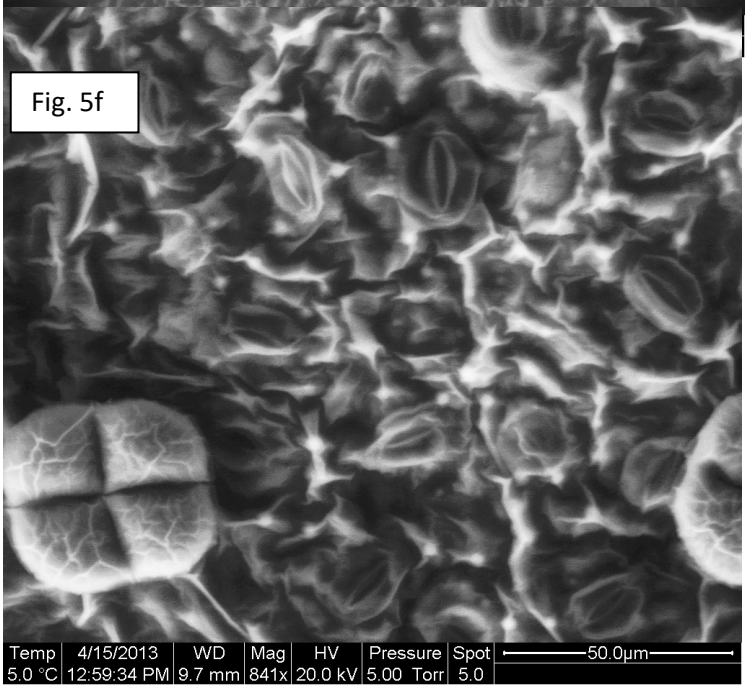


Fig. 6a

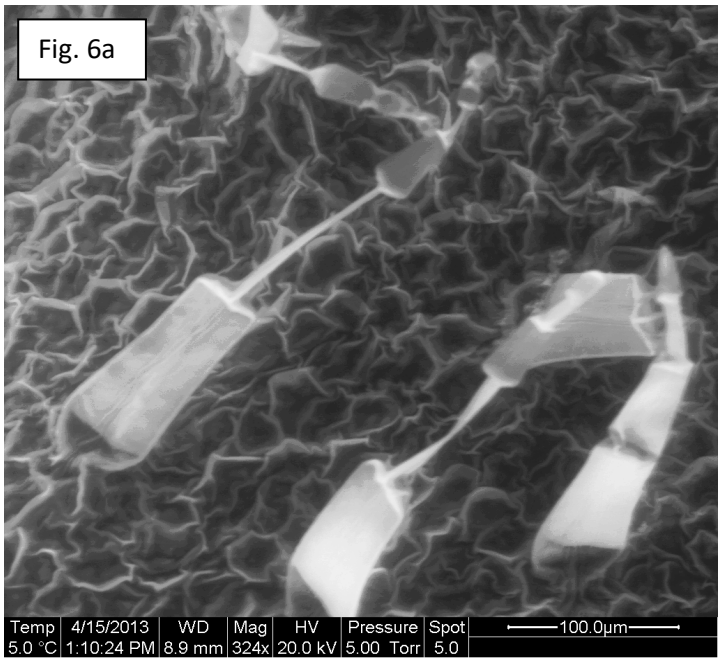


Fig. 6b

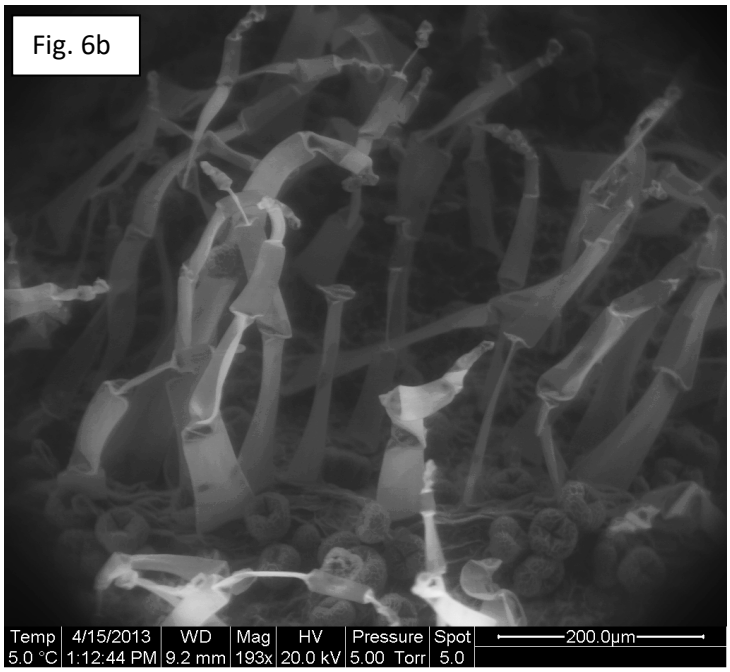


Fig. 6c

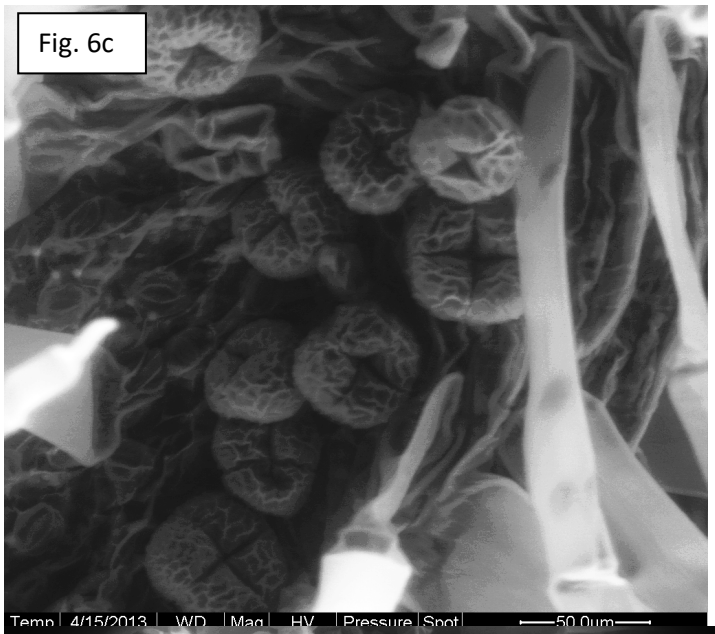


Fig. 6d

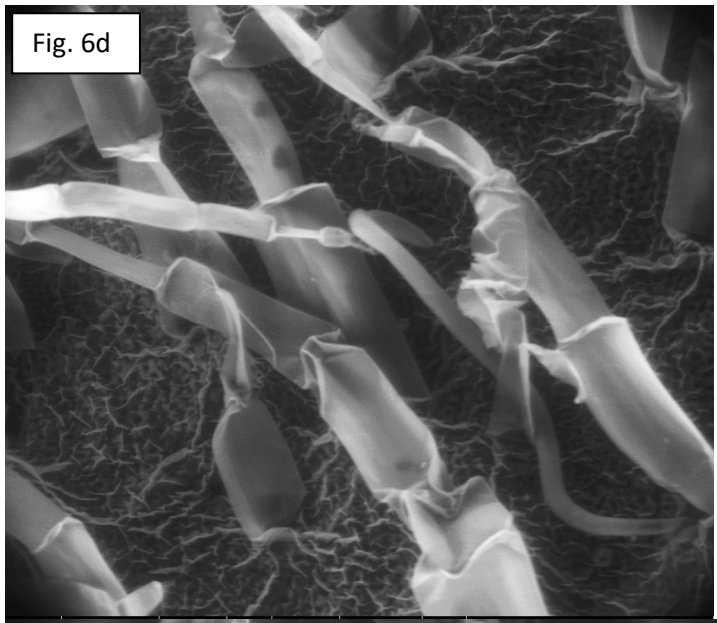


Fig. 6e

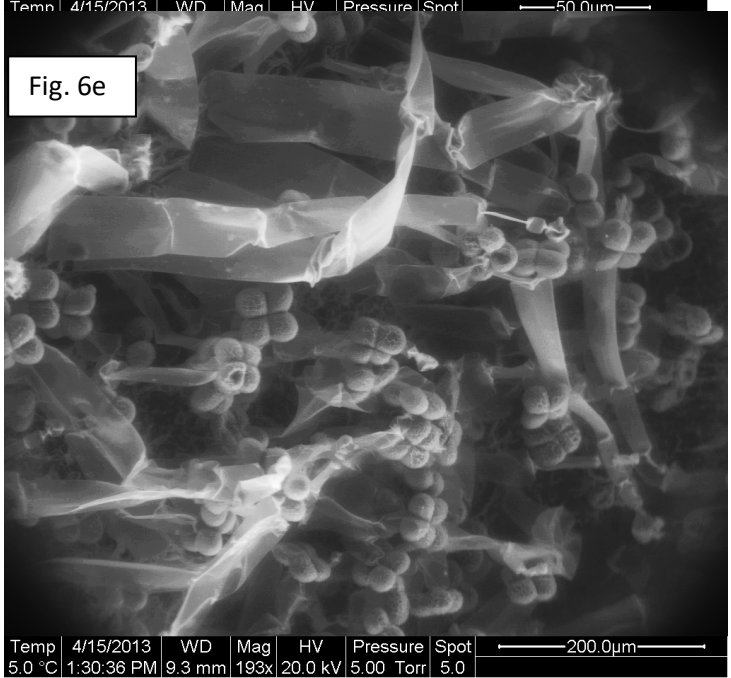
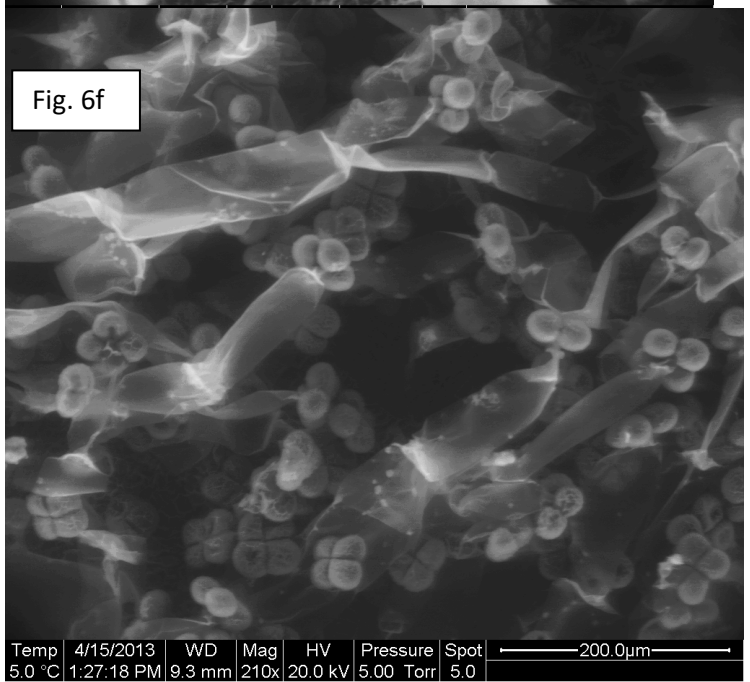


Fig. 6f



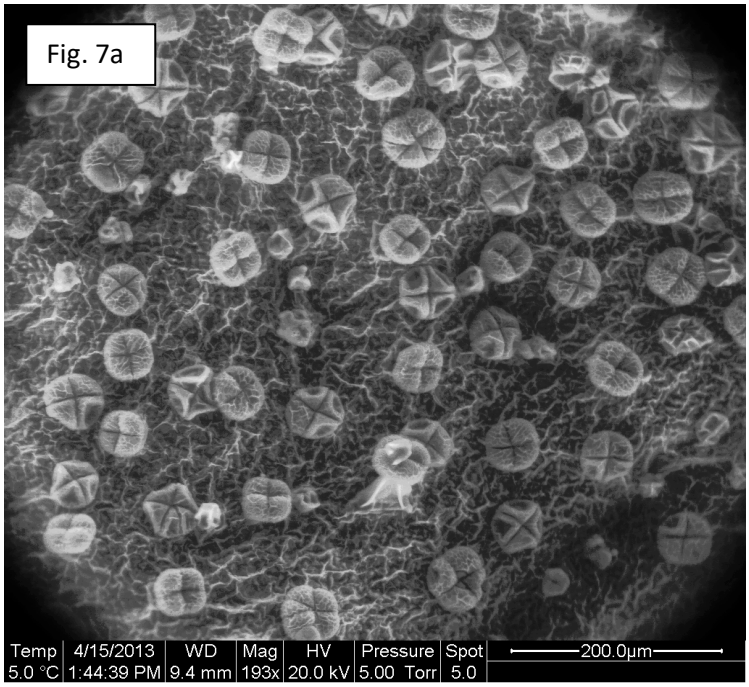


Fig. 7a
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Pressure 5.00 Torr
Spot 5.0
200.0µm

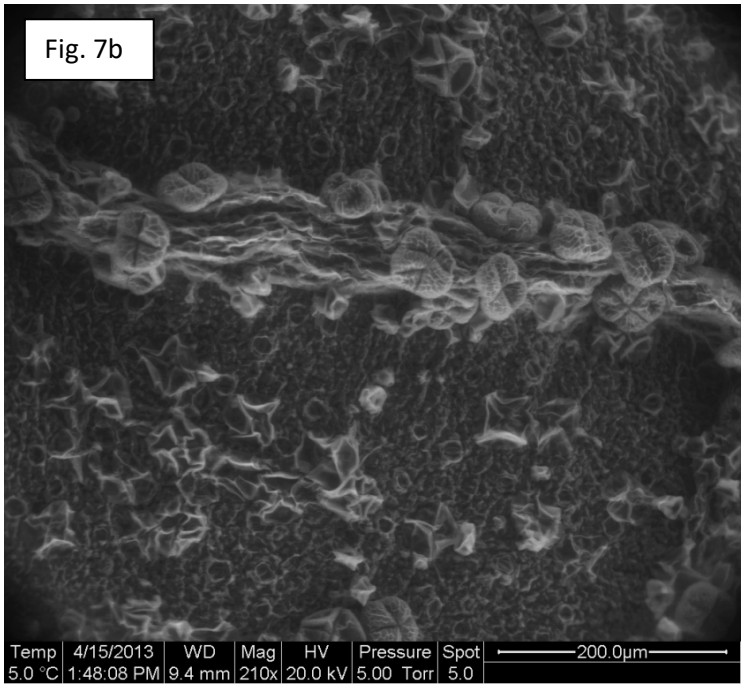
Fig. 7b
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Spot 5.0
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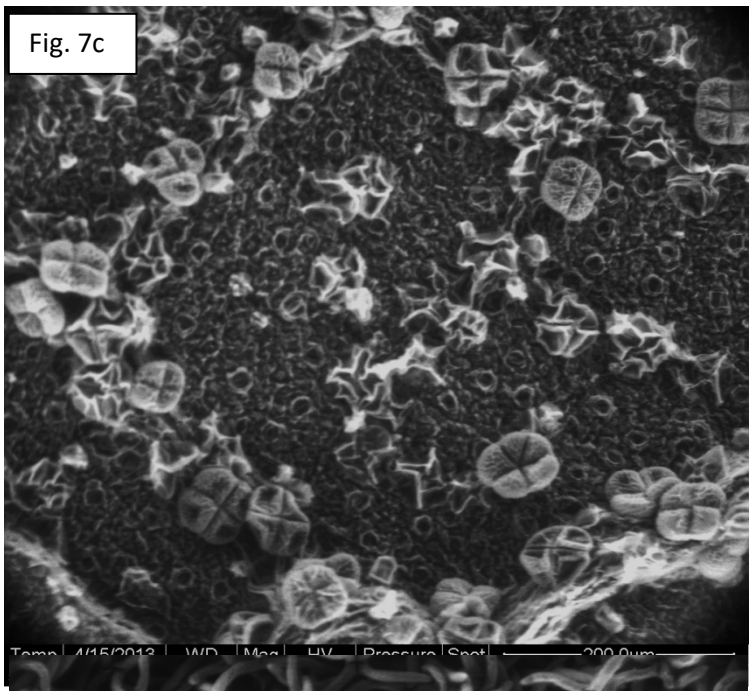
Fig. 7c

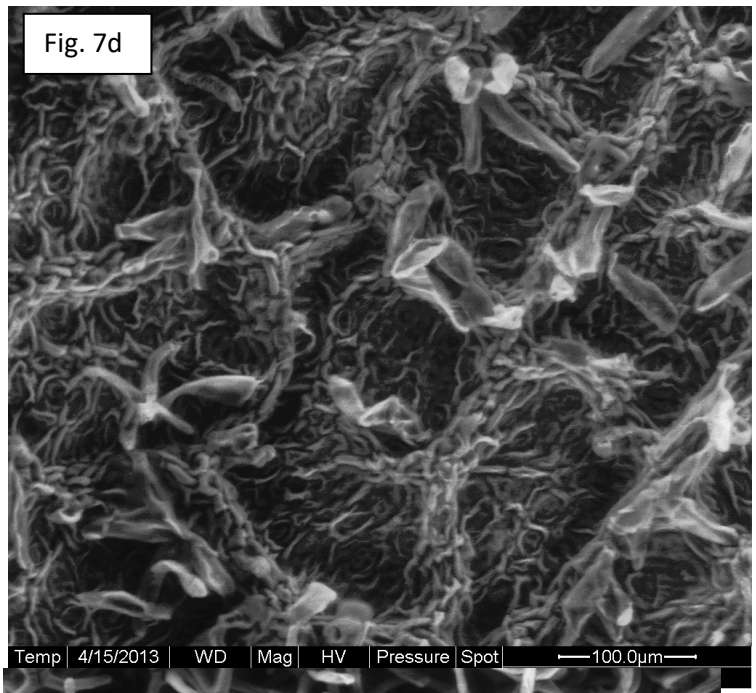
Fig. 7d
Temp
4/15/2013
WD
Mag
HV
Pressure
Spot
100.0µm

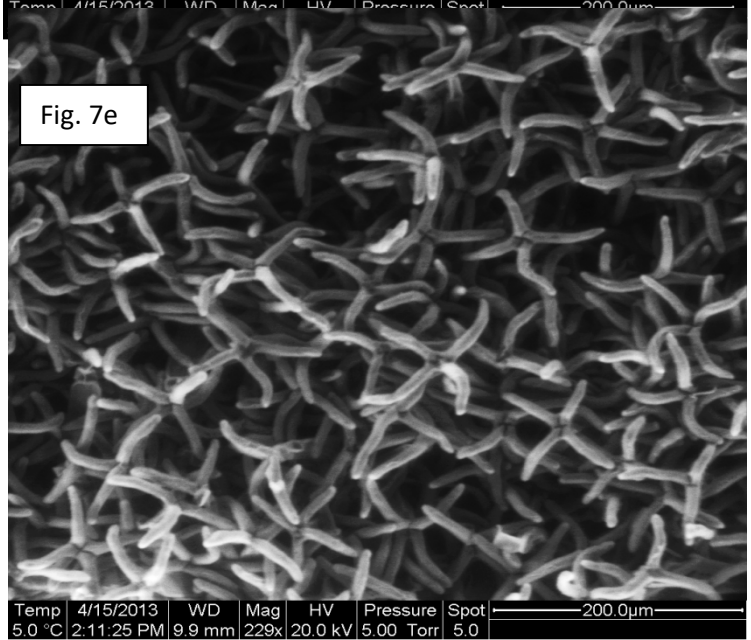
Fig. 7e
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Spot 5.0
200.0µm

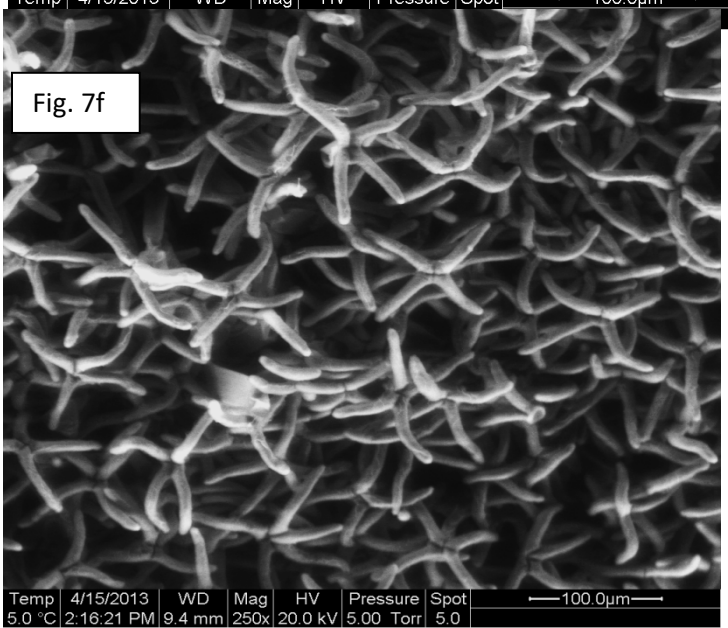
Fig. 7f
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WD 9.4 mm
Mag 250x
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Pressure 5.00 Torr
Spot 5.0
100.0µm

Fig. 8a

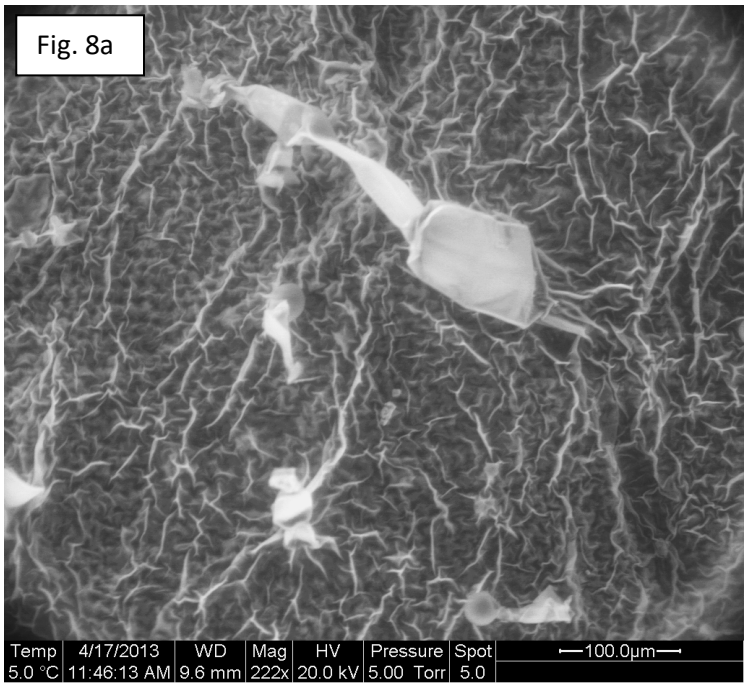

Fig. 8b

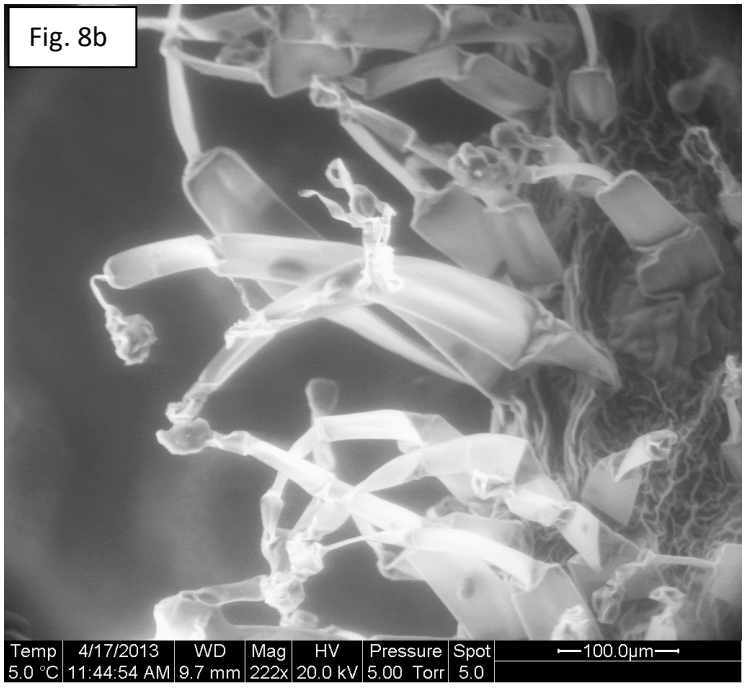

Fig. 8c

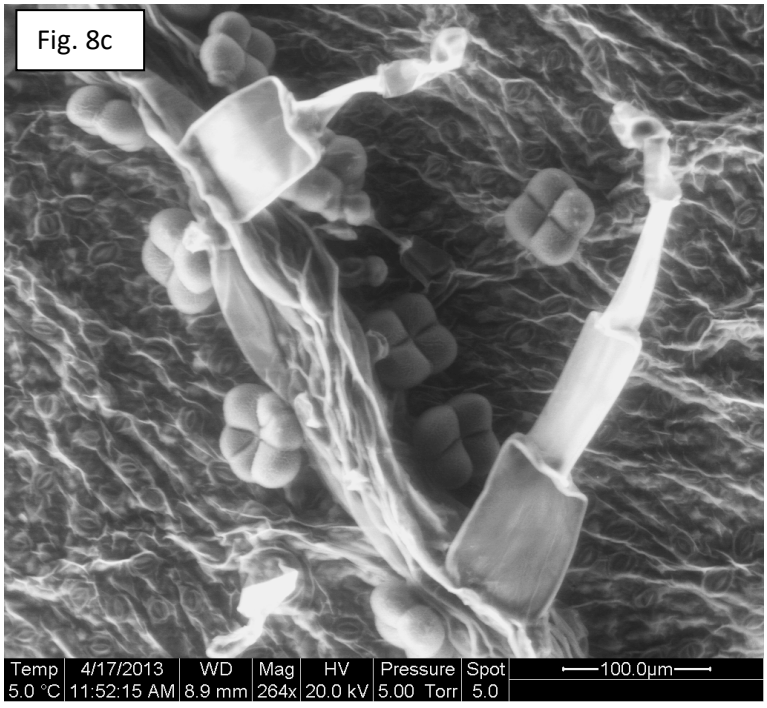

Fig. 1a: *Uncarina abbreviata* abaxial leaf surface with dense stellate mucilage glands. **1b, 1c:** Adaxial leaf surface of *U. abbreviata* showing sparse mucilage glands and simple hairs. **1d:** Adaxial leaf surface of *U. ankaranensis* showing dense simple hairs. **1e, 1f:** Abaxial leaf surface of *U. ankaranensis* showing close-up views of mucilage glands.

Fig. 2a: Adaxial leaf surface of *U. decaryi* showing sparse mucilage glands. **2b, 2c:** Abaxial leaf surface of *U. decaryi* showing dense covering of mucilage glands. **2d:** Abaxial leaf surface of *U. grandidieri* showing dense covering of mucilage glands with interspersed simple hairs. **2e:** Close-up of abaxial mucilage gland of *U. grandidieri*. **2f:** Adaxial leaf surface of *U. grandidieri* showing many simple hairs.

Fig 3a: Adaxial leaf surface of *U. ihlenfeldtiana* with sparse simple hairs. **3b, 3c:** Abaxial leaf surface of *U. ihlenfeldtiana* showing thick, tangled, simple hairs. **3d:** Adaxial leaf surface of *U. leandrii* var. *leandrii* with sparse simple hairs. **3e:** Abaxial leaf surface of *U. leandrii* var. *leandrii* showing mucilage glands along veins and numerous stomata. **3f:** Close-up of mucilage gland of *U. leandrii* var. *leandrii.*

Fig 4a: Adaxial leaf surface of *U. leandrii* var. *rechbergeri* with sparse simple hairs. **4b:** Abaxial leaf surface of *U. leandrii* var. *rechbergeri* with thick simple hairs. **4c:** Close-up of abaxial mucilage gland of *U. leandrii* var. *rechbergeri* (note stomate). **4d:** Adaxial leaf surface of *U. leptocarpa* showing sparse mucilage glands. **4e, 4f:** Abaxial leaf surface of *U. leptocarpa* showing mucilage glands primarily along veins (note numerous stomata).

Fig 5a: Adaxial leaf surface of *U. peltata* showing sparse covering of simple hairs. **5b:** Abaxial leaf surface of *U. peltata* with numerous mucilage glands and stomata (note collapsed glands). **5c:** Leaf edge of *U. peltata* with thick simple hairs. **5d:** Adaxial leaf surface of *U. perrieri* showing sparse mucilage glands and intricate patterning of epidermis. **5e:** Abaxial leaf surface of *U. perrieri* showing sparse mucilage glands. **5f:** Close-up of abaxial mucilage gland and stomata of *U. perrieri.*

Fig 6a: Adaxial leaf surface of *U. platycarpa* showing sparse simple hairs. **6b, 6c:** Abaxial leaf surface of *U. platycarpa* showing simple hairs with mucilage glands. **6d:** Adaxial leaf surface of *U. roeoesliana* showing long simple hairs. **6e, 6f:** Abaxial leaf surface of *U. roeoesliana* showing long-stalked mucilage glands.

Fig 7a: Adaxial leaf surface of *U. sakalava* showing dense covering of mucilage glands. **7b, 7c:** Abaxial leaf surface of *U. sakalava* showing numerous mucilage glands, primarily along veins. **7d:** Adaxial leaf surface of *U. stellulifera* showing sparse stellate mucilage glands. **7e, 7f:** Abaxial leaf surface of *U. stellulifera* with dense stellate mucilage glands.

Fig 8a: Adaxial leaf surface of *U. turicana* showing sparse simple hairs. **8b, 8c:** Abaxial leaf surface of *U. turicana* showing simple hairs and scattered mucilage glands.

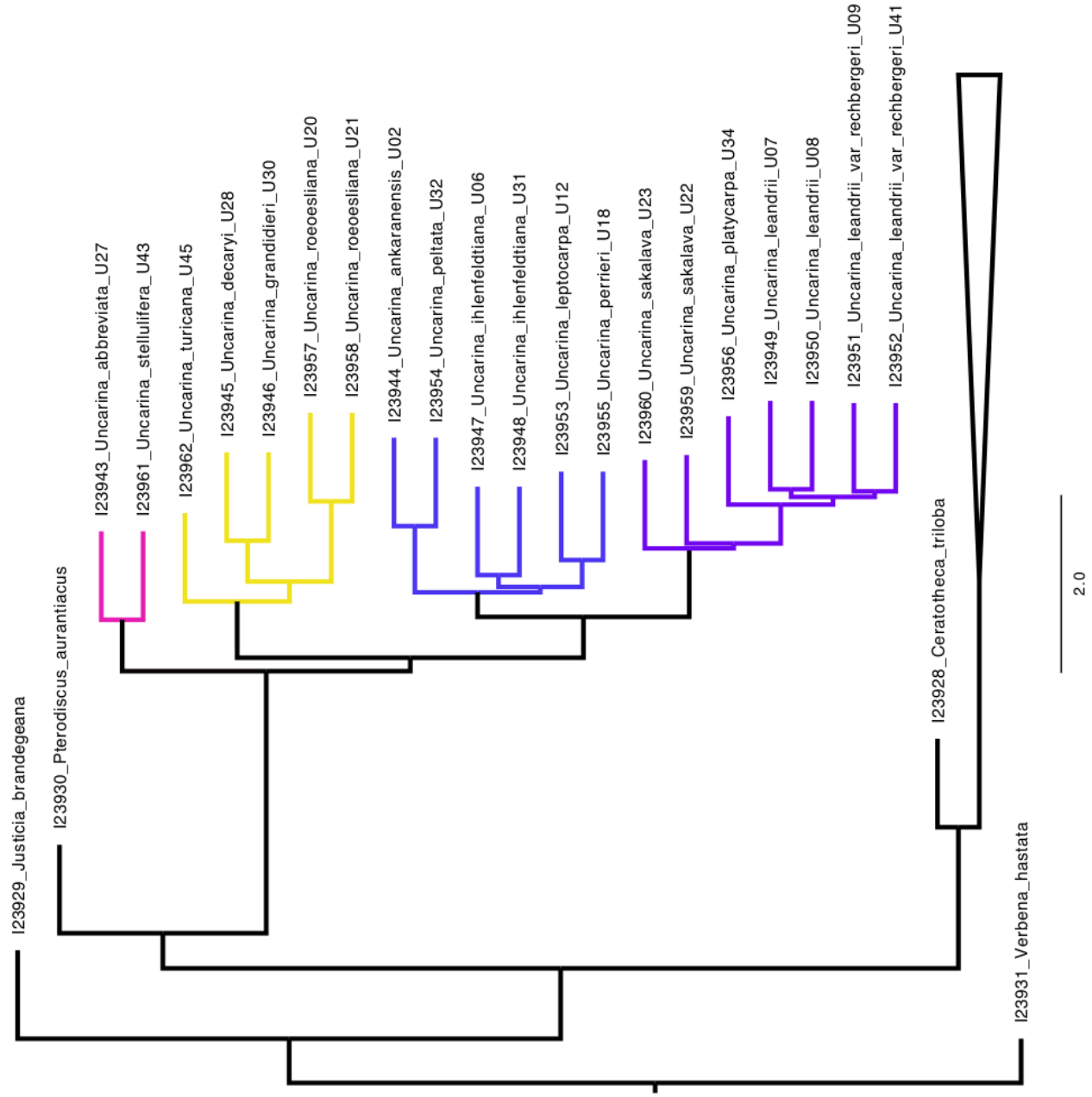

Fig. 9 Phylogeny of *Uncarina* based on nDNA. Support values at nodes represent bootstrap support. Clades correspond to those in Fig. 10: pink = Pink clade, yellow = Southern clade, blue = Northern clade, purple = Western clade

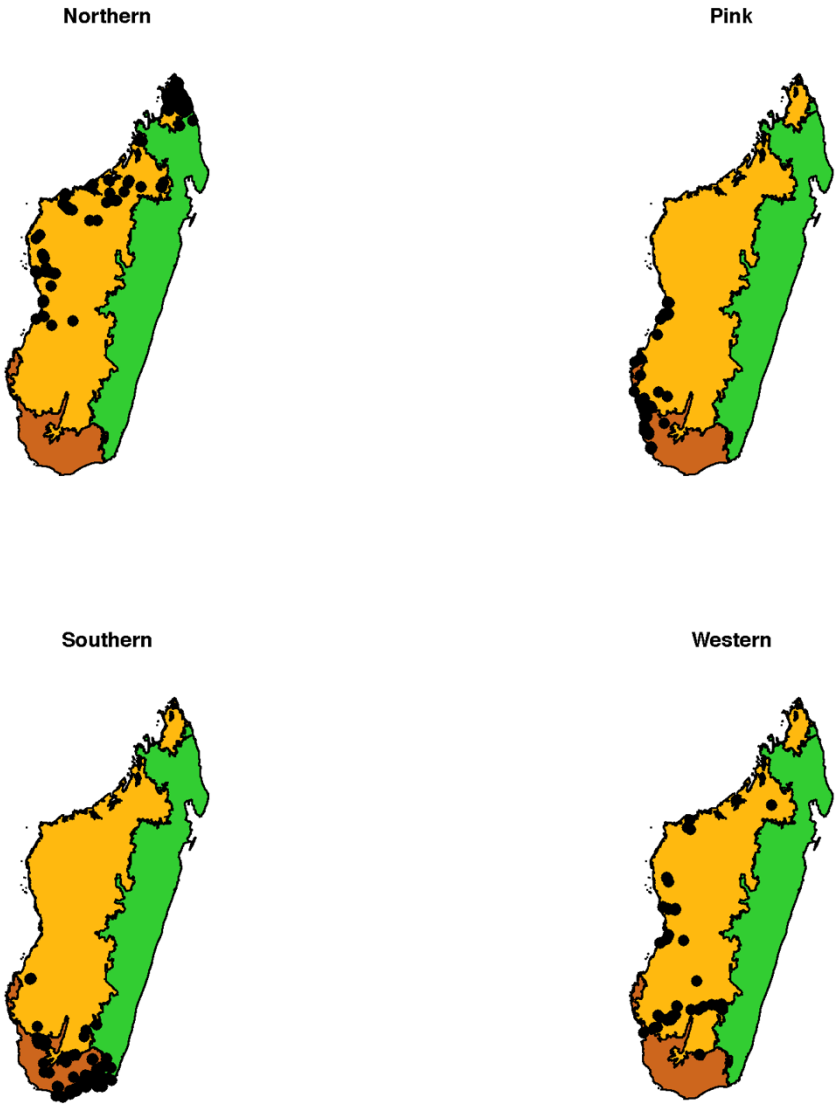

Fig. 10 Distribution maps of *Uncarina* species clades based on nDNA. Colored regions correspond to ecoregions as defined in Vieilledent et al. (2016): green=moist forest, yellow=dry forest, orange=spiny forest.

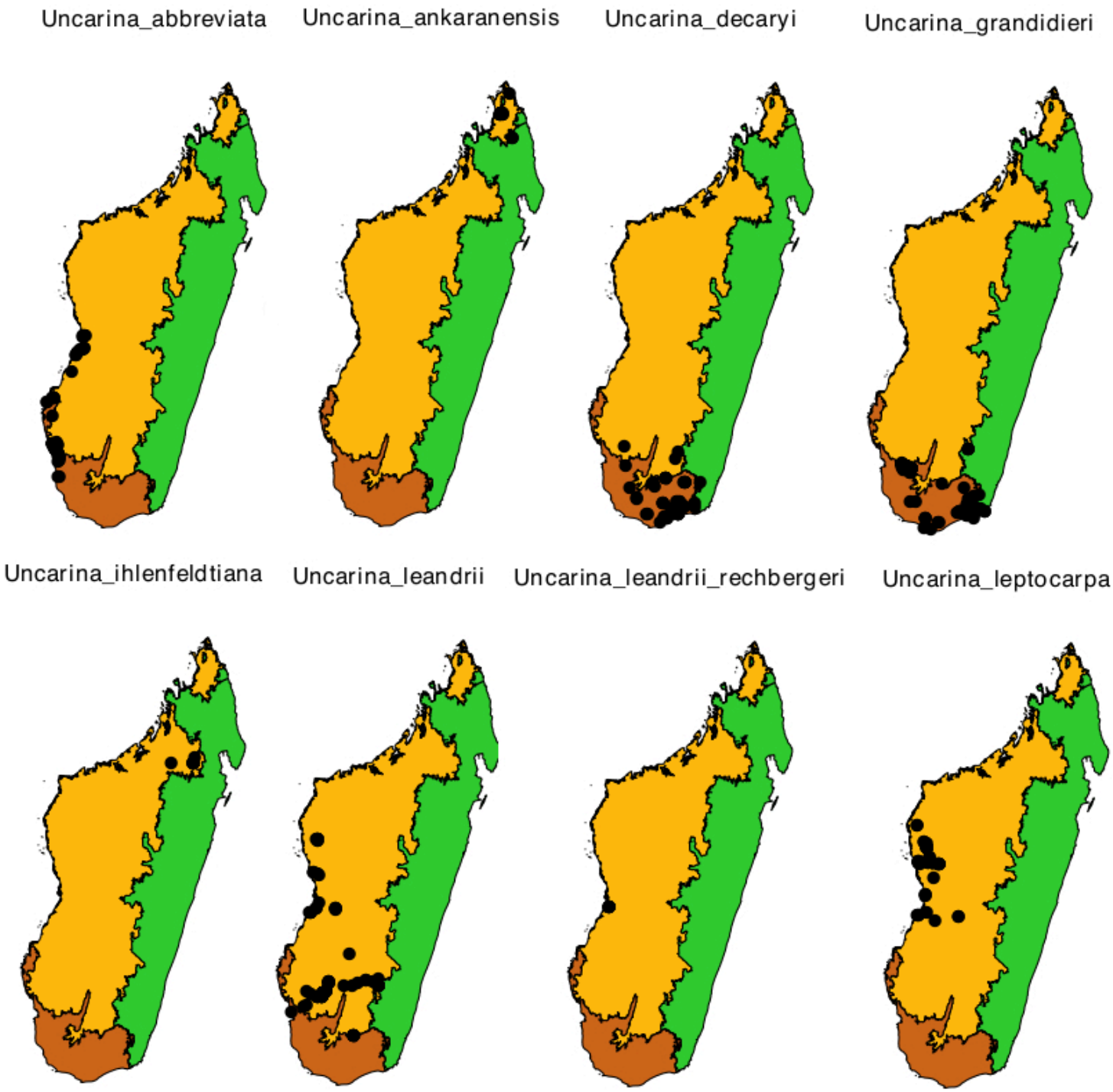

Fig. 11 Distribution maps of each *Uncarina* species. Each dot represents one unique specimen. Specimen records taken from MO and P databases. Colored regions correspond to ecoregions as defined in Vieilledent et al. (2016): green=moist forest, yellow=dry forest, orange=spiny forest.

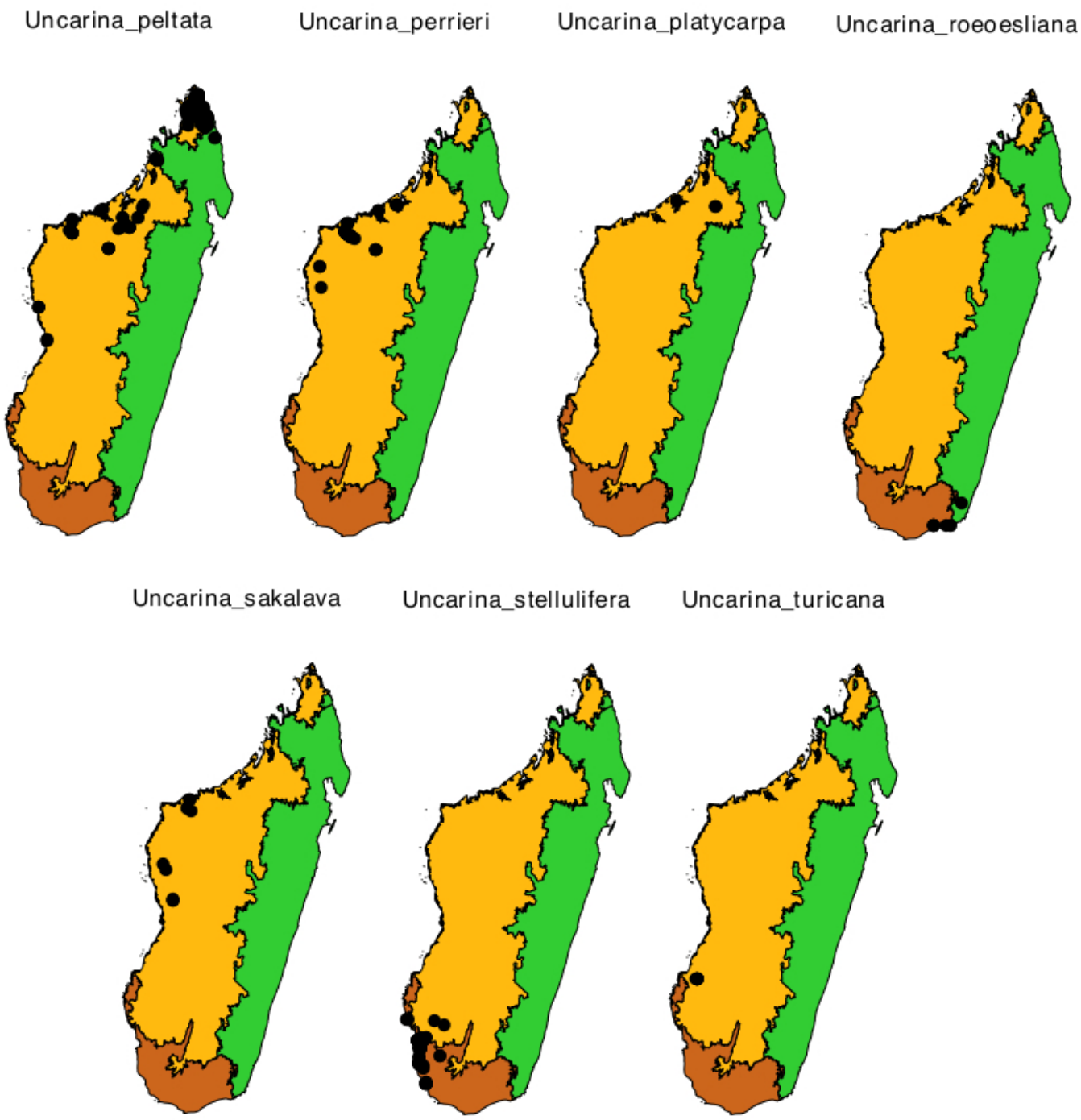

Fig. 11 (cont'd) Distribution maps of each *Uncarina* species. Each dot represents one unique specimen. Specimen records taken from MO and P databases. Colored regions correspond to ecoregions as defined in Vieilledent et al. (2016): green=moist forest, yellow=dry forest, orange=spiny forest.

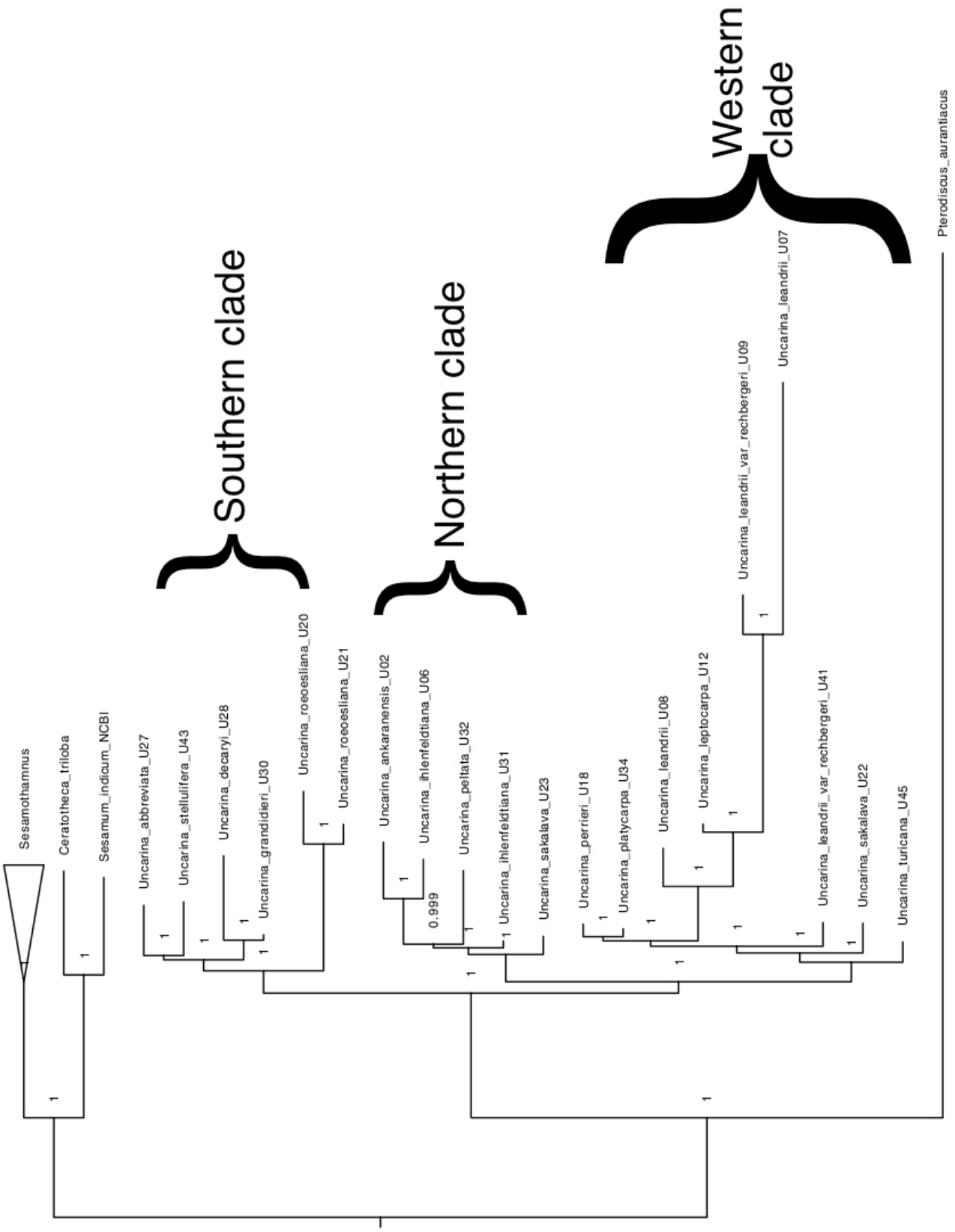

Fig. 12 Phylogeny of *Uncarina* based on cpDNA. Support values at each node are posterior probabilities. Clades correspond to those in Fig. 13.

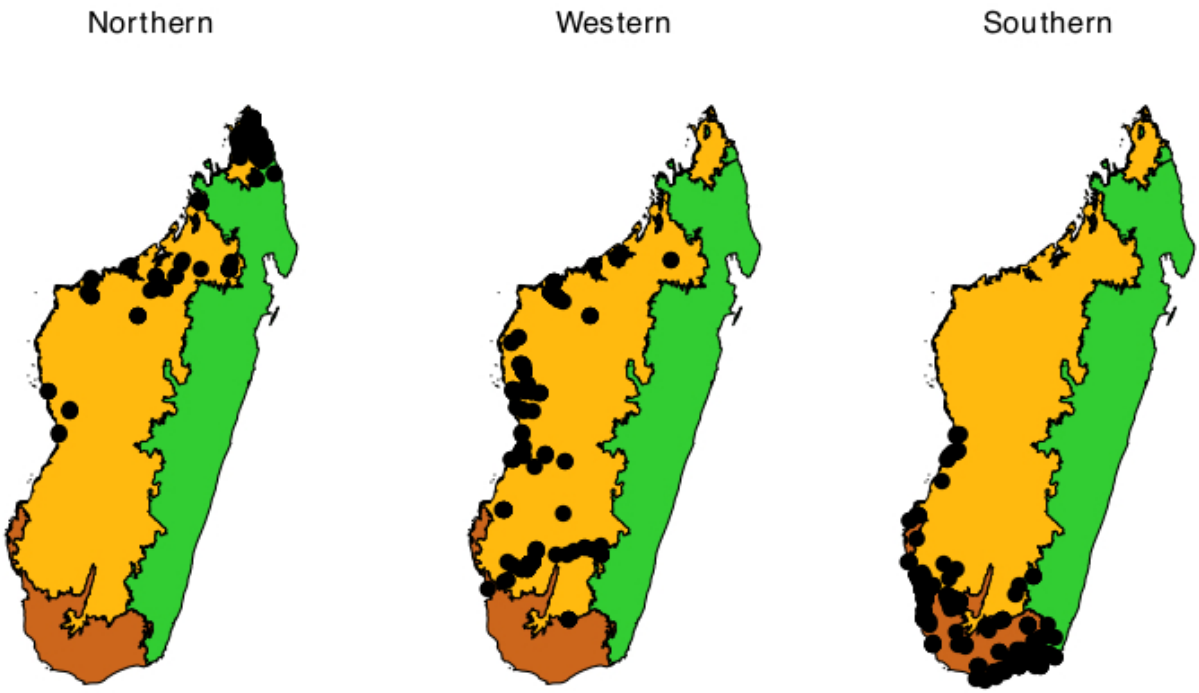

Fig. 13 Distribution maps of *Uncarina* species clades based on cpDNA. Colored regions correspond to ecoregions as defined in Vieilledent et al. (2016): green=moist forest, yellow=dry forest, orange=spiny forest.

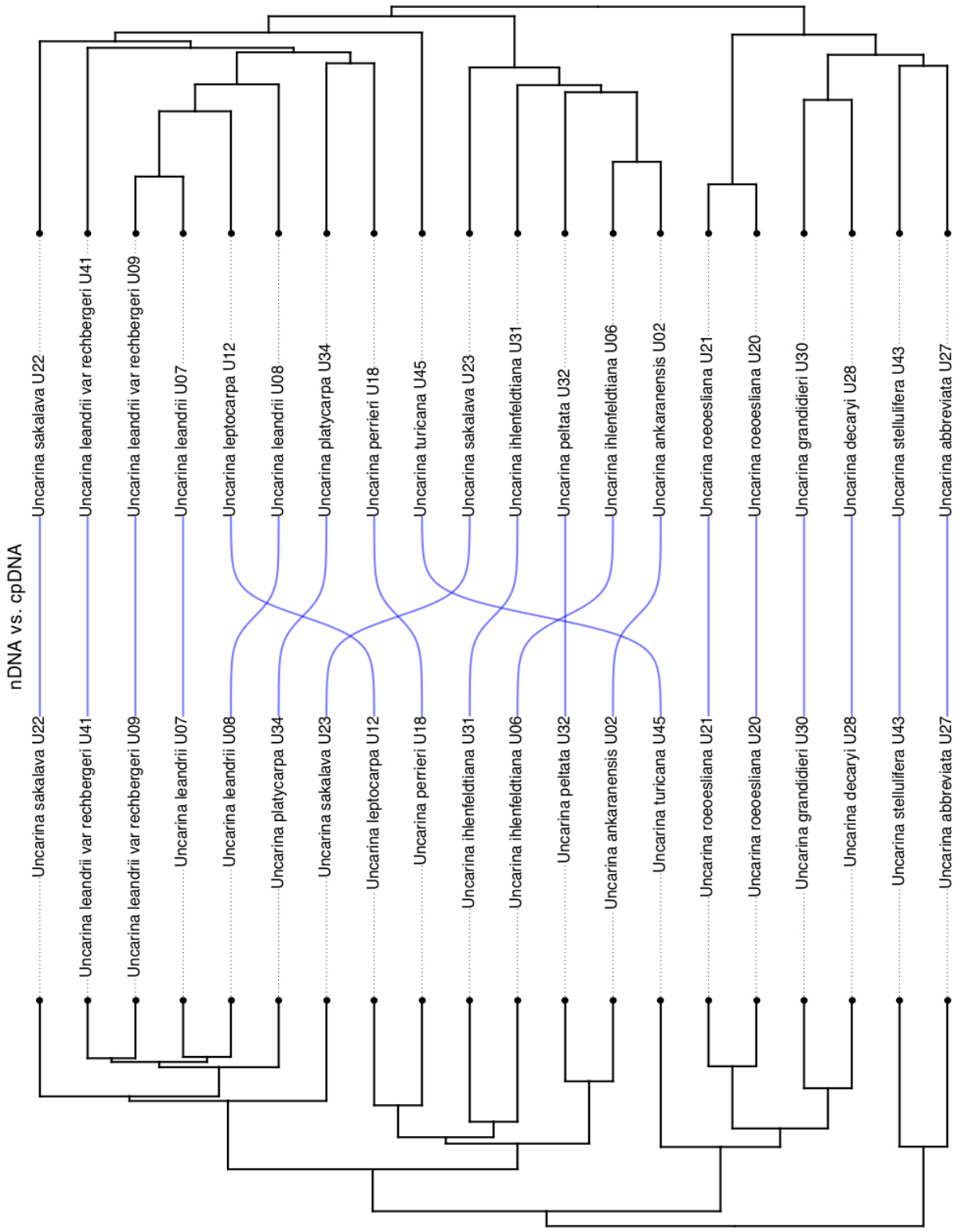

Fig. 14 Phylogenies of *Uncarina* based on nDNA (left) and cpDNA (right) illustrating discordance between datasets.

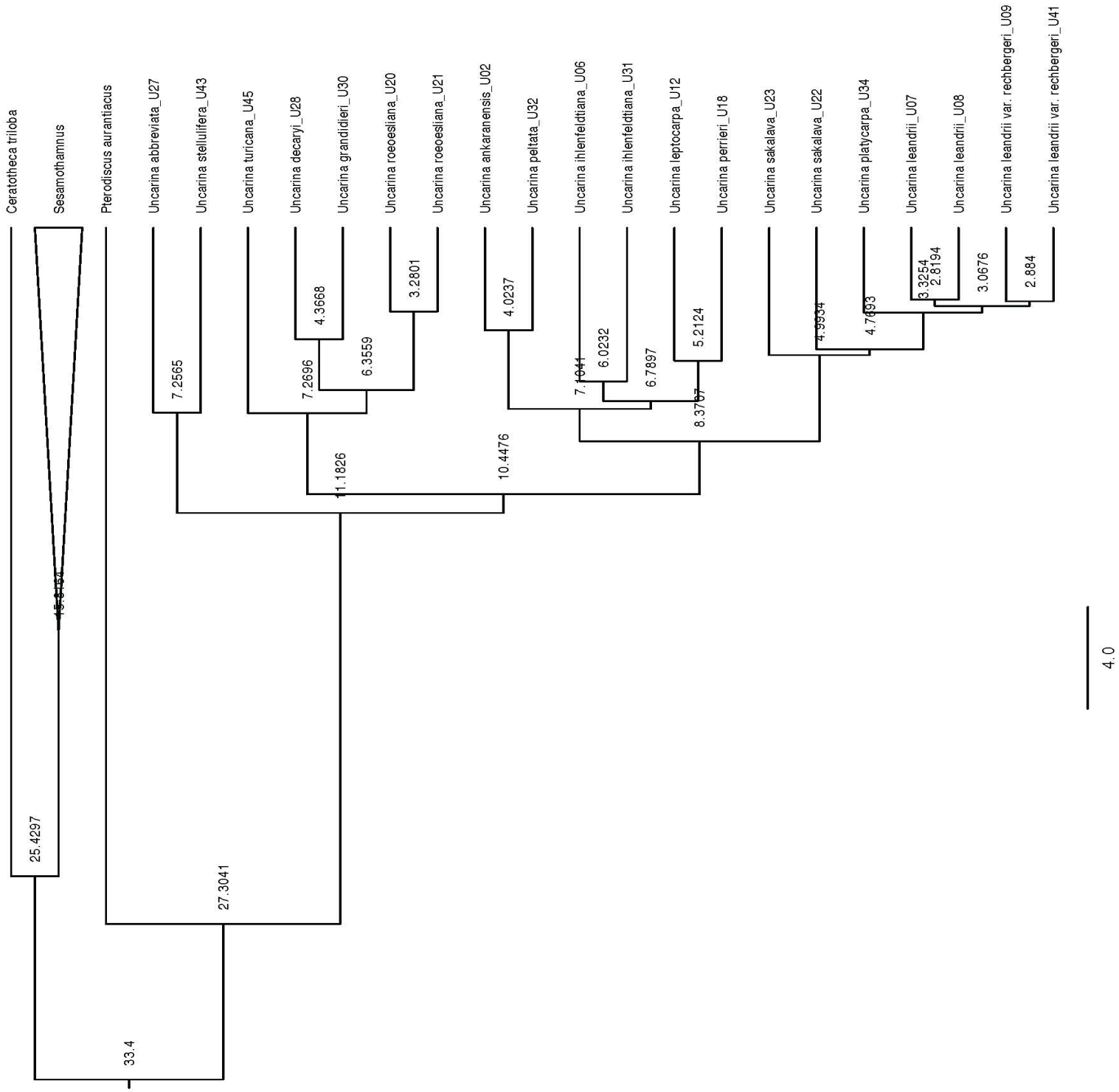

Fig. 15 Chronogram of *Uncarina* based on nDNA. Numbers at nodes are in mya.

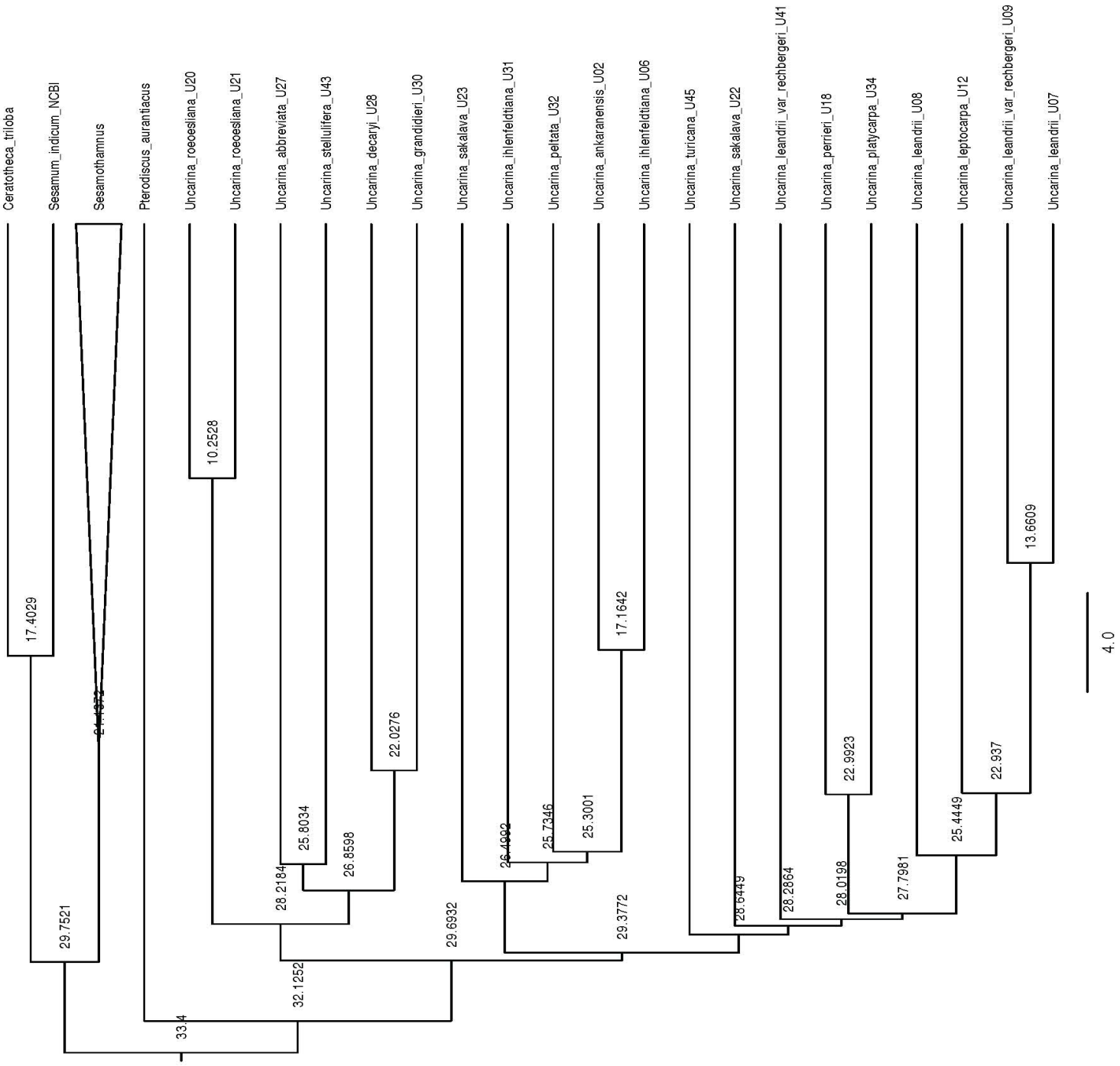

Fig. 16 Chronogram of *Uncarina* based on cpDNA. Numbers at nodes are in mya.

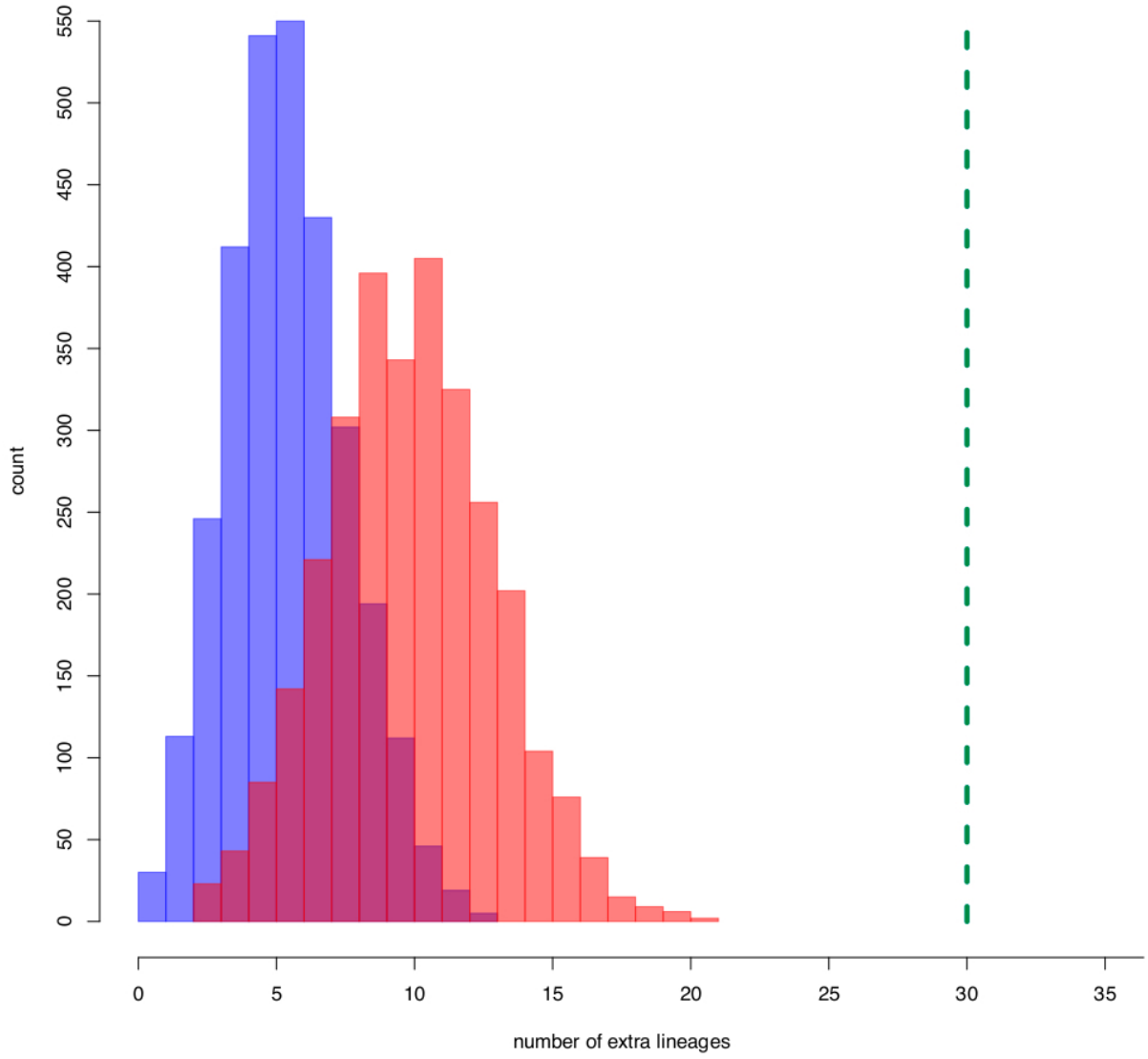

Fig. 17. Histogram of the expected number of lineages in the organellar genome (blue) and nuclear genome (orange). The dashed green line represents the number of observed extra lineages in the cp genome of *Uncarina*.

Chapter 3: Systematics and Phylogenetics of the genus *Sesamothamnus* (Pedaliaceae):

Evaluating the "African Arid Corridor" Hypothesis

John G. Zaborsky and Kenneth J. Sytsma developed the research questions and approach. Emily M. Lemmon and Alan Lemmon sequenced the DNA. J. Zaborsky, K. Sytsma, and Jeffrey P. Rose performed analyses. J. Zaborsky wrote the rough draft of the manuscript and K. Sytsma contributed to the revisions.

Abstract

Sesamothamnus (Pedaliaceae) is a small genus of shrubs with a disjunct distribution between southwestern and northeastern and east-central Africa. It has long been used as an exemplar of this disjunction pattern, one displayed by other genera in the Pedaliaceae as well as numerous unrelated families. Our research is the first to examine the genus in a phylogenetic context and the first to do so using Next-Generation sequencing methods. We generated a dated phylogeny of the genus as well, showing that it diversified during the Miocene Epoch, like numerous other arid-adapted and succulent lineages. Our analyses show a clear distinction between the southern lineages and northeastern species in the genus, as has been postulated in the past. We also show that past hypotheses about the age of the genus, relationships of some species, and which species are most "primitive" are completely unfounded.

Introduction

Sesamothamnus Welw. (Pedaliaceae) is a small genus of large shrubs native to dry and arid regions of northeastern and southern Africa. It is the sole member of the tribe Sesamothamneae. Only five species have been formally described although a sixth has been known since 1957 and has even been brought into cultivation. However, for reasons unknown, it has never been validly published. This species has the nomen nudum *S. 'leistneri'* (Ihlenfeldt, 2010). *Sesamothamnus* is unusual among other members of the Pedaliaceae in being woody, while the majority of the family is herbaceous. Along with *Uncarina* Stapf. and *Pterodiscus* Hook., *Sesamothamnus* is the only other genus in the family that has developed stem succulence. The four southern species have developed succulence to a greater degree than the two northeastern species (Ihlenfeldt, 2002). All members of the genus have large white or yellow

flowers, some of which have pronounced nectar spurs. Sphingophily has been hypothesized to be the pollination syndrome for all species in the genus and this has recently been confirmed for *S. lugardii* N.E. Br. ex Stapf. (Johnson & Raguso, 2016). The species without long spurs (that also possess equal thickening of the ovary base) were assigned their own genus by Engler: *Sigmatosiphon* (Bruce, 1953). However, Bruce (1953) determined that these species are not amply distinct from the other members of *Sesamothamnus* and should be included within it.

The genus is interesting in possessing a branching system that produces long shoots with spines and short shoots that arise from the axils of these spines. The spines are derived from leaf petioles and form when the lamina and a thin section of adaxial petiole tissue abscise. The remaining petiole tissue hardens, forming the spine. This method of spine formation is convergent with that of another arid-adapted genus of trees and shrubs: *Fouquieria* Kunth (Fouquieriaceae), an observation noted here for the first time.

Sesamothamnus has an interesting distribution within continental Africa (Fig. 1). Two species (*S. rivae* Engl. and *S. busseanus* Engl.) are confined to northeastern Africa, occurring in Ethiopia, Somalia, Kenya, and Tanzania. The remainder of the genus is disjunct to southern and southwestern Africa. *Sesamothamnus 'leistneri'* and *S. guerichii* (Engl.) E.A. Bruce are endemic to northwestern Namibia. *Sesamothamnus benguellensis* Welw. is also found in northwestern Namibia, extending into southern Angola. *Sesamothamnus lugardii* occurs in Zimbabwe, Botswana, and northeastern South Africa. The distributional pattern displayed by *Sesamothamnus* occurs in numerous other arid-adapted plant groups (Verdcourt, 1969; De Winter, 1971; Thulin, 1994; Jürgens, 1997). This pattern is hypothesized to be the result of climatic shifts that caused aridification across Africa, which in turn allowed migration of plants and animals between the arid regions of the south and north (Bellstedt et al., 2012). As the

climate changed and mountains were erected, the interior of Africa became wetter and thus inhospitable to arid-adapted plants, isolating populations in the north and south (Bellstedt et al., 2012). It is hypothesized that an "African Arid Corridor" (AAC) existed between the two regions either once or repeatedly during the Miocene, Pliocene and/or Pleistocene during these times of climatic aridity. The AAC either acted as a direct, continuous route in which taxa migrated north and south, or as a "stepping stone" that allowed for long-distance dispersal across smaller areas.

While a few species of *Sesamothamnus* have been included in ordinal analyses of the Lamiales (Refulio-Rodriguez & Olmstead, 2014), no genus-wide analysis has ever been published. The Pedaliaceae was recently investigated in a phylogenetic context, primarily looking at the relationships between genera, by Gormley et al. (2015). Their family-wide analysis included two species of *Sesamothamnus* and resolved them as monophyletic and sister to the tribe Sesameae, which is made up largely of the paraphyletic genus *Sesamum* L. but also includes *Ceratotheca* Endl., *Josephinia* Vent., and a handful of other small genera. Almost no systematic work has been carried out on *Sesamothamnus* aside from one study looking at the pollen morphology of *S. lugardii* (Suarez-Cervera et al., 1992). Our phylogenetic study is the first to include all known taxa of *Sesamothamnus* and use Anchored Hybrid Enrichment Next-Generation sequencing methods. We aim to resolve the species relationships within *Sesamothamnus* and investigate its historical biogeography in the context of the climatic history of African arid regions.

Methods

Sampling — Silica-dried leaves from cultivated specimens of each known *Sesamothamnus* taxon were collected from the personal living collection of Dr. Dan Mahr (UW-Madison, Entomology Dept.) and the living collection at the Huntington Botanical Garden in San Marino, CA. Silica-dried leaves were also collected from two wild individuals in Africa by Dr. Mahr. Eleven accessions of *Sesamothamnus* were used in this study, representing all known taxa. Outgroup genera in Pedaliaceae are described in detail in Chapter 1.

DNA Extraction — DNA was extracted from silica-dried plant material using the DNeasy™ plant mini kit (Qiagen, Valencia, California) according to manufacturer's specifications.

DNA Sequencing — We utilized an anchored phylogenomics approach, Anchored Hybrid Enrichment (AHE) using the Center for Anchored Phylogenomics at Florida State University, to obtain 495 single-copy nuclear loci as well as the entire chloroplast genome. The nuclear data matrix had only 5.7% missing data. This method targets highly conserved "anchor" regions in the nuclear genome and generates hundreds of loci including both introns and exons (Lemmon et al. 2012). This specific pipeline has been used effectively to infer infrageneric relationships in angiosperms (Buddenhagen et al., 2016; Cardillo et al., 2017; Fragoso-Martínez et al., 2017; Mitchell et al., 2017). DNA was sonicated to a fragment size of between 200–600 bp before library preparation and indexing following a modified protocol of Meyer and Kircher (2010). Indexed samples were pooled and enriched using the Angiosperm v.1 enrichment kit (Buddenhagen et al., manuscript). Sequencing was done on 4.5 PE150 Illumina HiSeq 2500 lanes at the Translational Science Laboratory, College of Medicine, Florida State University.

Paired reads were merged before assembly, following Rokyta et al. (2012). Reads were mapped to the probe regions using *Arabidopsis thaliana*, *Billbergia nutans*, and *Carex lurida* as references, combined with a de novo assembly approach to extend the assembly into flanking regions (Prum et al. 2015; Buddenhagen et al., 2016). Read files were traversed repeatedly until no additional mapped reads were produced and consensus sequences were calculated for each assembly cluster with contigs based on fewer than 100 reads removed. For each locus, orthology was determined following the procedures in Prum et al. (2015). Contig orthology was assessed using a pairwise distance matrix among homologs and used to cluster sequences with a neighbor-joining algorithm to assess if gene duplication occurred prior to or following the crown of the clade. Contigs suggesting duplication were removed from further analysis if they contained fewer than 32 taxa (92%). Sequences in each orthologous cluster were aligned using MAFFT v. 7.023b (Katoh and Standley, 2013), then trimmed and masked using the following procedure from Prum et al. (2015). Sites with the same character in > 50% of sequences were considered "conserved." A 20 bp sliding window was then moved across the alignment, and regions with < 13 characters matching the common base at the corresponding conserved site were masked. Sites with < 152 unmasked bases were removed. Finally, the masked alignments were inspected by eye. Regions considered obviously misaligned or likely paralogous were removed and poorly aligned sections in a given alignment were deleted.

As DNA fragments from non-targeted loci may be captured, we attempted to extract the plastid (cpDNA) genome from our reads. We used Geneious v. 10.2.3 to map all recovered forward and reverse reads to reference sequences. For the plastid genome, we used the whole plastid genome of *Sesamum indicum* L. (GenBank accession KCS569603) as a reference. Raw reads were trimmed and assembled using iterative refinement of up to 5 times with the default

Geneious mapper and medium sensitivity. For the cpDNA, consensus sequences were generated using the strict consensus. If coverage was < 2, the consensus nucleotide was scored as a gap. Unmapped regions were treated as missing data and reads mapped to multiple positions were excluded from consensus calculations. Sequences were aligned using MAFFT with default parameters. After alignment, ambiguously aligned or called regions were removed by hand.

Phylogenetic Analyses — The concatenated AHE dataset was used to estimate a phylogeny under the GTRGAMMA model implemented in RAxML v8.1.21 (Stamatakis, 2014; with default parameters), with the GTR model and branch lengths being allowed to vary across loci. One hundred bootstrap replicates were collected to estimate phylogenetic support. In addition, the species tree was estimated under the coalescent model as implemented by ASTRAL-II (v.4.9.7, Mirarab & Warnow, 2015), using bootstrapped gene trees estimated under the GTRGAMMA model in RAxML v8.1.21 (Stamatakis, 2014).

Aligned plastid genomes were analyzed using a Bayesian framework in MrBayes 3.2.6 (Ronquist and Huelsenbeck, 2003) implemented in the CIPRES Science Gateway (Miller et al., 2010). Default parameters were used, set to the GTRGAMMA model, temp=.3, and the program was run for 5,000,000 generations sampling every 10,000.

Dating the Diversification of Sesamothmanus — As the size of the final AHE dataset precluded dating in BEAST (Drummond et al., 2012), we utilized penalized likelihood as implemented in treePL (Smith and O'Meara, 2012) on our best ML trees. This program has been shown, however, to estimate divergence dates similar to those of BEAST (Lagomarsino et al., 2016). The nuclear AHE tree and the derived plastid genome tree were examined separately. We used nodal dates for the crown and stem of *Sesamothmanus* and the outgroup root all derived

from a fossil-calibrated analysis of Pedaliaceae in the larger framework of the order Lamiales (see Chapter 1).

Results

The nDNA phylogeny (Fig. 2) shows strong support for the monophyly of *Sesamothamnus* with a bootstrap value of 100. All clades and species are recovered with bootstrap values of 100 as well. There is a clear split between the southern and northern species in the tree with the northern *S. busseanus* and *S. rivae* sister to a clade of the southern species. In the southern clade, the Namibian endemic, *S. 'leistneri'* and the Angolan/Namibian *S. benguellensis*, form a clade sister to a clade of the Namibian endemic *S. guerichii* and the southern African *S. lugardii*. The cpDNA phylogeny (Fig. 3) shows the exact same topology as the nDNA phylogeny and also shows strong support for the relationships. As with the nDNA, a clear distinction exists between a northern species clade and a southern species clade. The northern clade contains *S. rivae* and *S. busseanus* as sisters. In the southern clade, *S. guerichii* and *S. lugardii* are recovered as sister species with both species being monophyletic. These two are in turn sister to a clade containing *S. 'leistneri'* and *S. benguellensis*.

The nuclear (Fig. 4) and chloroplast (Fig. 5) dated phylogenies of *Sesamothamnus* show similar dates for the various diversification events in the genus, with the dates for the chloroplast tree being slightly older. The split between *Sesamothamnus* and *Ceratotheca* Endl. and *Sesamum* L. occurred at 25 Mya or 30 Mya. Diversification in *Sesamothamnus* (the split between the northern and southern species), occurred at 16 Mya or 21 Mya. The split between the two northern species occurred between 14.5 and 14.0 Mya. The two clades within the

southern clade split from each other 11 Mya or 14 Mya. The split between *S. lugardii* and *S. guerichii* occurred at 9.8 Mya or 12.9 Mya.

Discussion

The AHE data for ca. 500 single-copy genes and the derived plastid genomes provide strong and consistent relationships within *Sesamothamnus*. This is in contrast to the strong discordance between the two genomes in *Uncarina* (Pedaliaceae) – see Chapter 2. Thus, the emerging phylogenetic framework for *Sesamothamnus* offer detailed insight into the relationships, temporal diversification, and biogeography of this African genus.

Phylogenetic Relationships and Morphological Evolution within Sesamothamnus — The northern species of *Sesamothamnus* are both distributed throughout Kenya, Tanzania, Somalia, and Ethiopia (Ihlenfeldt, 2004). These two species differ from the southern species in having less swollen and succulent stems (Ihlenfeldt, 2004). They occupy bushland and grassy woodlands, usually on rocky soils, throughout their ranges. The two species differ from each other most markedly in their corolla lobes: the lobes are fringed (except the basal one) in *S. busseanus* while in *S. rivae* all the lobes have entire margins. Both species also share leaves that are only sparsely glandular on the adaxial surfaces, white flowers, and slender nectar spurs. These two species have been long hypothesized to be closely related due to similarities in flower structure, leaf surfaces, and distribution (Ihlenfeldt, 2010).

The southern clade is split into two smaller clades, one containing the Namibian endemic *S. guerichii* and the southern African *S. lugardii*; the ranges of these two species do not overlap. *Sesamothamnus guerichii* has the largest range of any of the Namibian species. The flowers of these two species are quite different, with the corollas of *S. lugardii* having a short nectar spur

(to 1.5cm), while those of *S. guerichii* have only a short sac-like protrusion at the base. *Sesamothamnus guerichii* is also unique in possessing yellow flowers. The anthers distinctly protrude from the mouth of the corolla tube in *S. guerichii* while in *S. lugardii* they are included. Both species are similar in overall appearance, being large, spiny shrubs to 5m with swollen branches. They also share leaves that are similar in size and shape and whose surfaces are densely covered with mucilage glands.

The undescribed *S. 'leistneri'* differs from its sister species, *S. benguellensis*, and the sympatric *S. guerichii*, in having a more treelike growth form with branches forming higher up on the trunk rather than near the base and in possessing peeling bark. Since the species has never been formally described, little data exist in the literature to gain a better understanding of the shared characters between *S. 'leistneri'* and *S. benguellensis*. Most sources list the salient differences between the two species, which seem to be numerous (Mannheimer & Curtis, 2009). In addition to the listed vegetative characters, *S. 'leistneri'* has corolla tubes that lack nectar spurs, while *S. benguellensis* has nectar spurs to 3.7mm in length. More collections and a formal description of this species are sorely needed.

The relationships we recover in the genus do not match the hypotheses put forward by Ihlenfeldt (2010). He argued that a putative phylogeny of the genus was difficult, due to the high similarity of numerous character states among the taxa. Indeed, without flowers many of the species are difficult to tell apart. Ihlenfeldt (2010) argued that the lack of a nectar spur was a primitive character and that the sac-like protrusion was an intermediate between the two extremes. Our analyses show that the possession of a nectar spur has most likely been lost twice: in *S. 'leistneri'* and *S. guerichii*. *Sesamothamnus guerichii* has only a small sac-like protrusion at the base of the corolla tube while *S. 'leistneri'* lacks a spur altogether. These two taxa are not

closely related, despite having completely sympatric ranges. One could posit that the two have simply converged upon a short nectar spur for some particular pollinator, rather than this being a primitive character. *Sesamothamnus guerichii* is also unique in having yellow flowers, while all other species have white flowers.

Sesamothamnus 'leistneri' presents an interesting puzzle in the genus. Ihlenfeldt (2010) speculated that this species could be the most primitive one in the genus. He explained that it possesses many "primitive" characters: large, treelike growth form, spurless flowers, sparse covering of mucilage glands on the leaves, and a "relict" distribution. We show that this species is not the earliest-diverging, but rather the most recently-arisen and that it is clearly embedded within the clade of southern species. This is in spite of Ihlenfeldt (2010) believing it was more closely related to the northern species due to its shared foliar character: sparse mucilage glands. Our dated phylogenies show that *S. 'leistneri'* split from its common ancestor with *S. benguellensis* between 1.5-3.3mya. Its restricted distribution may be due to its young age or some other ecological factor. It is also unique in flowering during the rainy season, when leaves are fully developed; all other species flower *before* the onset of the rainy season, when they are completely leafless. Certainly, more collections, detailed ecological studies, and a formal description are needed to further investigate the unusual characteristics of this species and to better understand its placement within the genus.

Biogeographical Patterns within Sesamothamnus — A complex distributional pattern involving many unrelated, arid-adapted plant taxa exists across southern, eastern, and northern Africa (Verdcourt, 1969; De Winter, 1971; Thulin, 1994; Jürgens, 1997). Some of these groups show a continuous distribution, occurring in a "bow" from southern Africa, through the eastern regions, and into northeastern Africa, sometimes extending into the Arabian Peninsula. Genera

with this distribution often termed the "African Arid Corridor" pattern include *Aloe* L. (Asphodelaceae), succulent members of *Euphorbia* L. (Euphorbiaceae), stapeliads (Apocynaceae), and *Kalanchoe* Adans. (Crassulaceae). Many other genera show a disjunct distribution between the northeast and southwest with one-to-few species occurring in each region including *Sesamothamnus*, *Adenium* Roem. & Schult. (Apocynaceae), *Kissenia* R.Br. ex Endl. (Loasaceae), *Salvia* L. (Lamiaceae), and *Wellstedia* Balf.f. (Boraginaceae), among others (Jürgens, 1997). In rare cases, the disjunction is displayed within a species, such as *Tribulocarpus dimorphanthus* Pax ex S.Moore (Aizoaceae). The disjunct pattern is common in the Pedaliaceae, with some genera also displaying the continuous pattern (Ihlenfeldt, 1994).

General worldwide cooling occurred during the early Miocene which triggered a slow aridification across Africa (Zachos et al., 2001) expanding habitat into which many lineages diversified. Aridification in the southwest was intensified by the establishment of the Benguela current during the Miocene, as well (Dupont et al., 2011). In East Africa, two periods of uplift followed: one ca. 20 Mya (Grove, 1983) and another ca. 5 Mya (Partridge & Maud, 2000). These areas would have been less likely to have experienced increased aridification, but they would have influenced the aridity of the southern regions (Partridge & Maud, 2000). It is hypothesized that these aridification events allowed these various lineages to expand their ranges and move north and/or south. As climates became less dry, their ranges were "pinched off" and isolated from one another. Aridification is hypothesized to have occurred repeatedly after the Miocene, most notably during the Pleistocene glaciation events (Bellstedt et al., 2012). These cycles of increased aridity are thought to have "reunited" the ranges of these arid-adapted taxa through an "African Arid Corridor" (AAC) in eastern Africa (Bellstedt et al., 2012). Taxa that were otherwise isolated due to inhospitable wetter habitats are believed to have been able to

move freely through this corridor, possibly via long-distance dispersal. While the cyclical climatic events did not break up the range of many genera, it is argued that the most arid-adapted (and perhaps oldest) lineages saw their ranges become much contracted and extremely disjunct (Jürgens, 1997).

Some of these taxa have recently been investigated in a phylogenetic context to better understand the timing of their diversification. To fit the AAC hypothesis, these studies must demonstrate sister relationships of the north and south (and mid-continent) taxa and that their separation must date to the mid-Miocene. Klak et al. (2017) showed a late Miocene origin for *Tribulocarpus dimorphanthus* (Aizoaceae). This species is disjunct between the north and south, and Thulin et al. (2012) showed that it is paraphyletic, with the southern populations being sister to a clade of the northern populations and the strikingly different congener *T. retusus* (Thulin) Thulin & Liede, a Somalian endemic. Bellstedt et al. (2012) identified at least three migrations through the AAC in the Zygophylloideae (Zygophyllaceae), all occurring during periods of aridification. *Gisekia* L. (Gisekiaceae), a genus of weedy herbs adapted to open habitats, most likely arose in southern Africa in the Miocene and expanded northward via the AAC (Bissinger et al., 2014). The genus *Moringa* Adans. (Moringaceae) has a handful of species in northeast Africa, one in southern Africa, two in Madagascar, one in the Arabian Peninsula, and two in India; Olson (2002) showed that the southern African species was sister to the northeastern species. In a dated phylogeny of the Caricaceae (to which the Moringaceae are sister), Carvalho & Renner (2012) recovered a crown age for *Moringa* that showed it arose in the mid-Miocene, although they had poor sampling of the genus. Many other arid-adapted lineages also diversified in the Miocene. Widespread in Africa, the diversity of *Aloe* also shows a connection to the Miocene aridity. Grace et al. (2015) showed that *Aloe* arose in southern Africa during the mid-

Miocene and spread northward with subsequent diversifications during the Pliocene. Arakaki et. al (2011) showed that many succulent groups diversified during this time period, including the Didiereaceae, Aizoaceae: Sesuvioideae, and the Cactaceae. They hypothesized that these simultaneous bursts of diversification in succulent and arid-adapted lineages are linked to a global period of climatic dryness.

Arid-adapted lineages were not the only ones to be affected by the increased aridification of this period. Pokorny et al. (2015) showed that numerous unrelated lineages that make up the so-called "Rand Flora" had their ranges contracted by the aridification during the Miocene. The Rand Flora taxa sometimes show a similar disjunction pattern to those of the AAC, but these taxa are adapted to subhumid and subarid conditions. The open habitats that were created by the increased aridity also created new habitat for forest-adapted lineages to diversify into, as shown in the Melastomateae (Melastomataceae), with at least 12 shifts from closed to open habitats occurring in the tribe (Veranso-Libalah et al., 2018).

Sesamothamnus, displaying the clear-cut disjunction, shows a Miocene origin for the genus (Fig. 4-5). Diversification within the genus also occurred throughout the Miocene, with the split between *S. 'leistneri'* and *S. benguellensis* happening during the late Pliocene. Our analyses add more data to a growing body of evidence that the worldwide Miocene aridification events drove diversification in numerous arid-adapted and succulent plant taxa and influenced the evolutionary history of groups that are not adapted to these conditions. *Sesamothamnus* is a poorly studied genus but interesting genus that warrants more taxonomic study. Its isolated position within the Pedaliaceae, biogeography, pollination biology, and vegetative morphology make it an ideal genus for further investigations.

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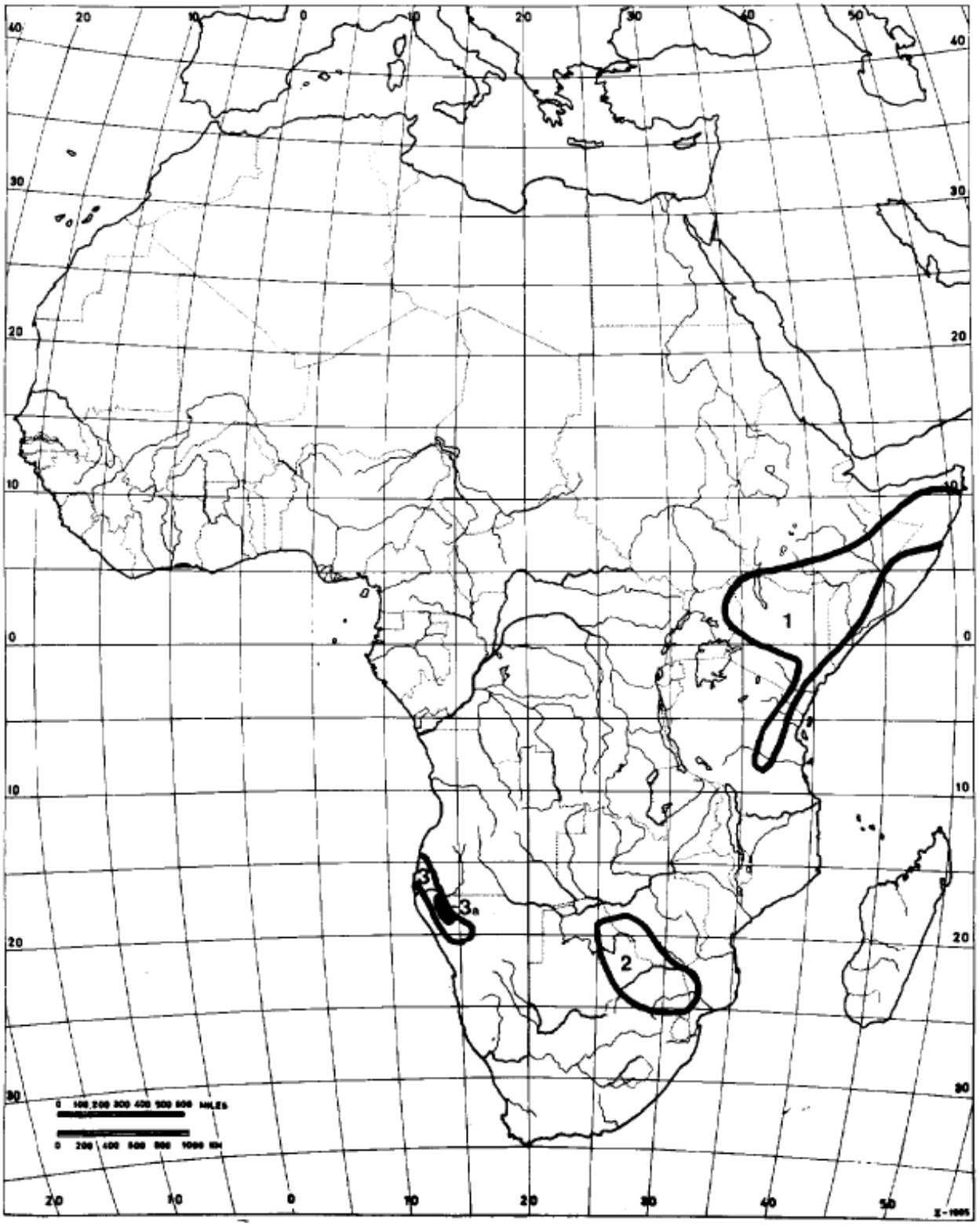

Fig. 1. Map of the distribution of *Sesamothamnus* (taken from Ihlenfeldt, 1994). 1: *S. rivae* and *S. busseanus*. 2: *S. lugardii*. 3a: *S. 'leistneri'*. 3. *S. guerichii* and *S. benguellensis*.

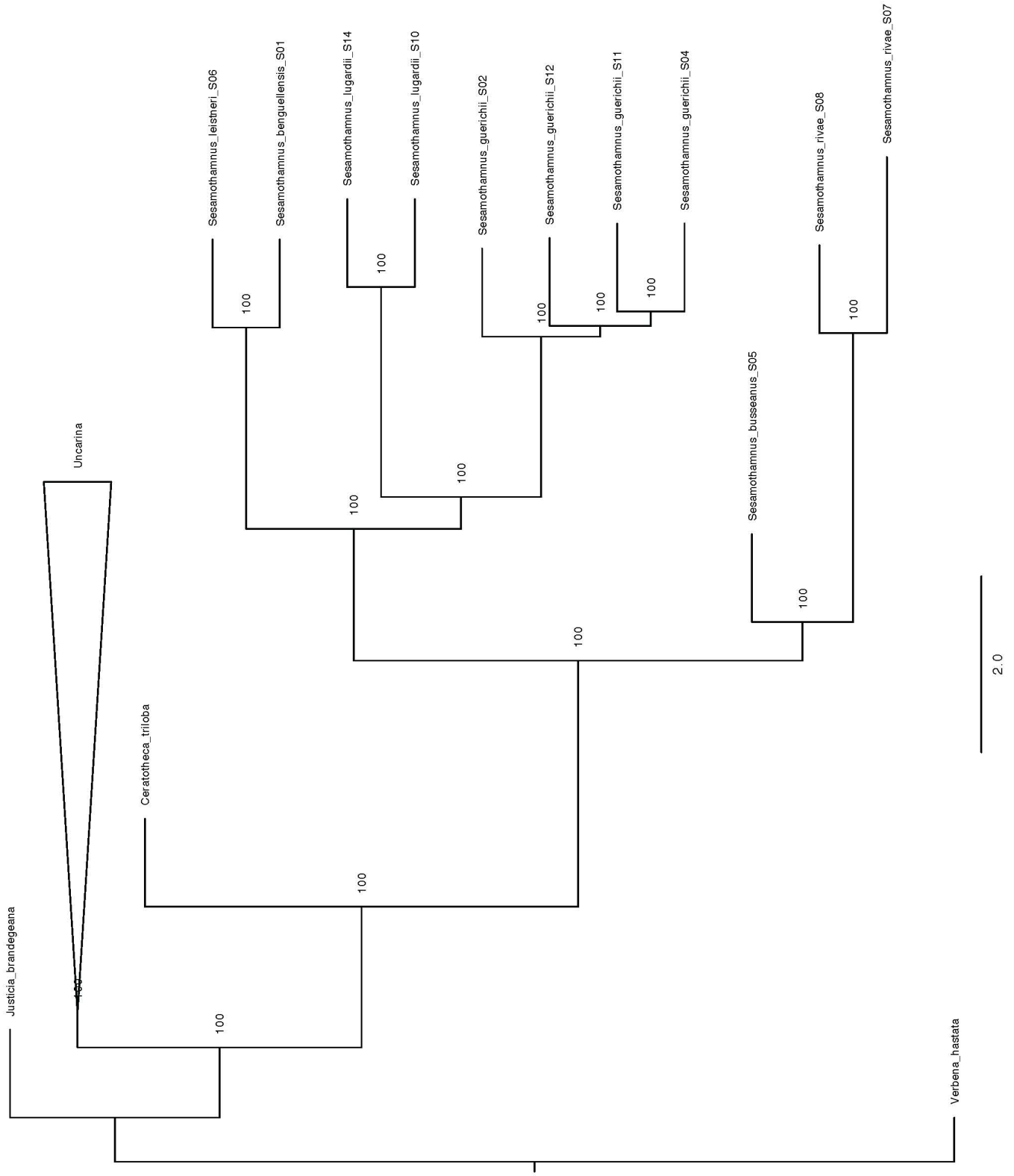

Fig. 2. Phylogeny of *Sesamothamnus* based on nrDNA generated in Astral. Bootstrap values are displayed at each node

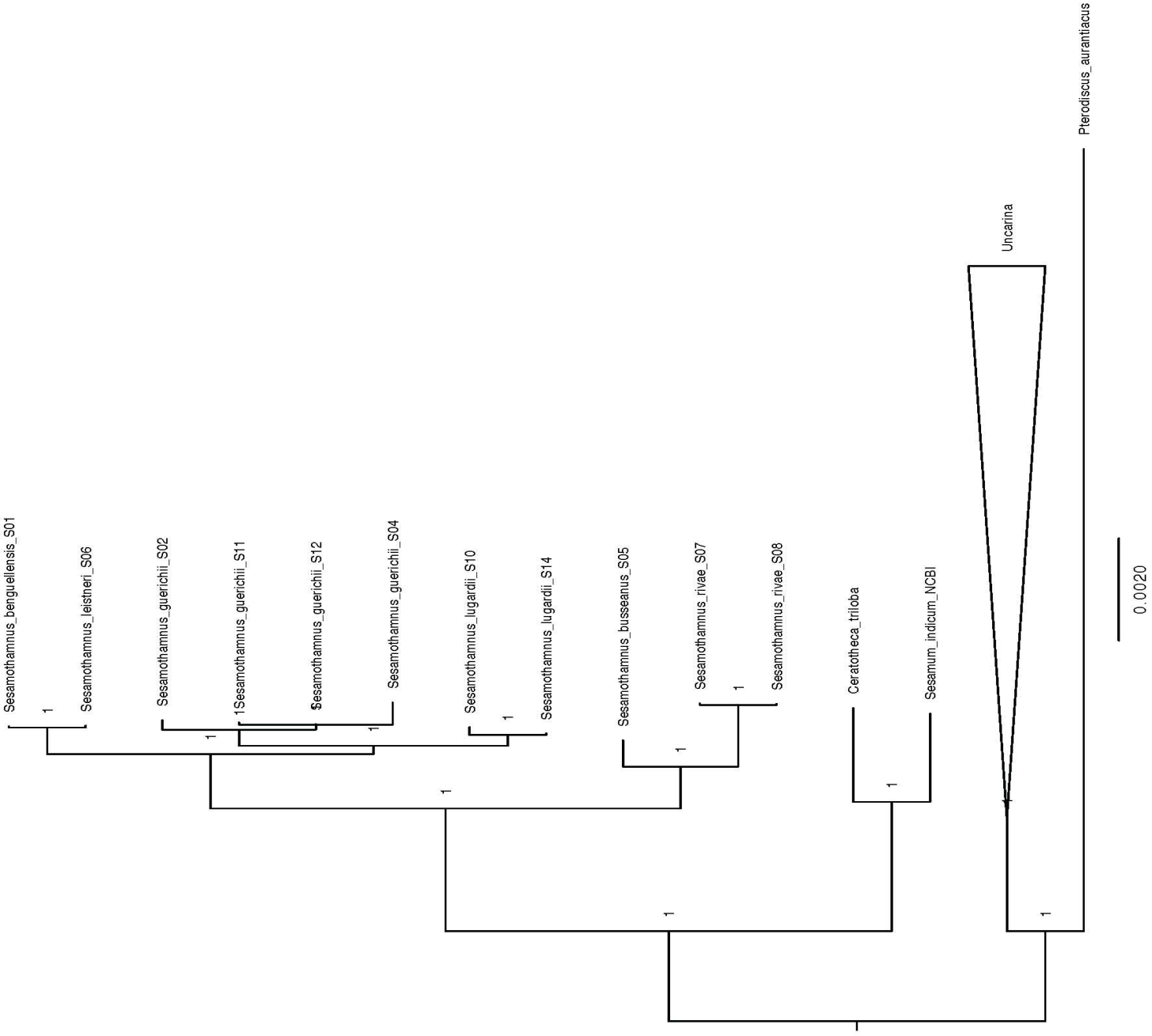

Fig. 3. Phylogeny of *Sesamothamnus* based on cpDNA generated in MrBayes. Posterior probabilities are displayed at each node.

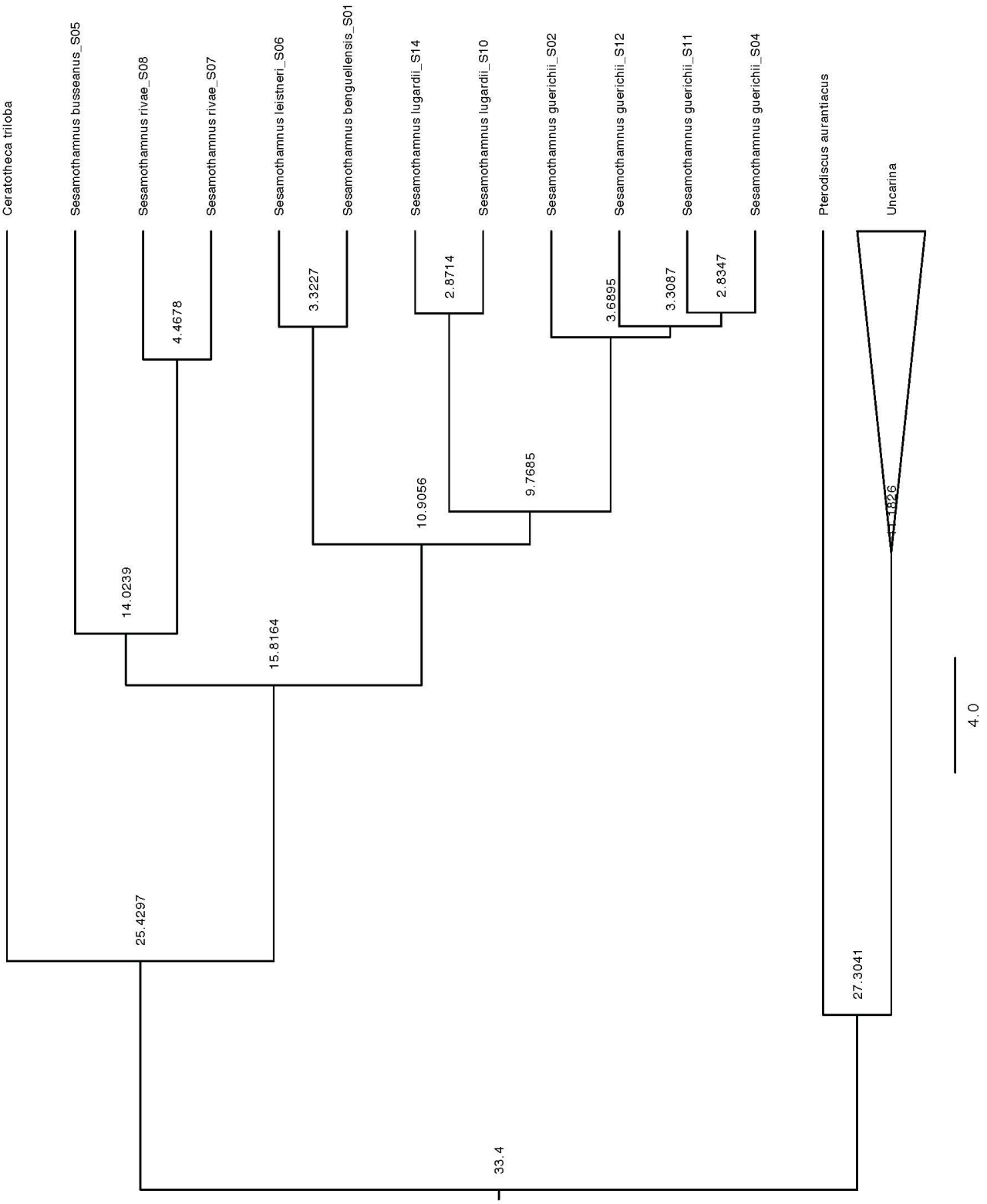

Fig. 4. Dated phylogeny of *Sesamothamnus* based on nDNA generated in ChronoPL. Dates at nodes are in mya.

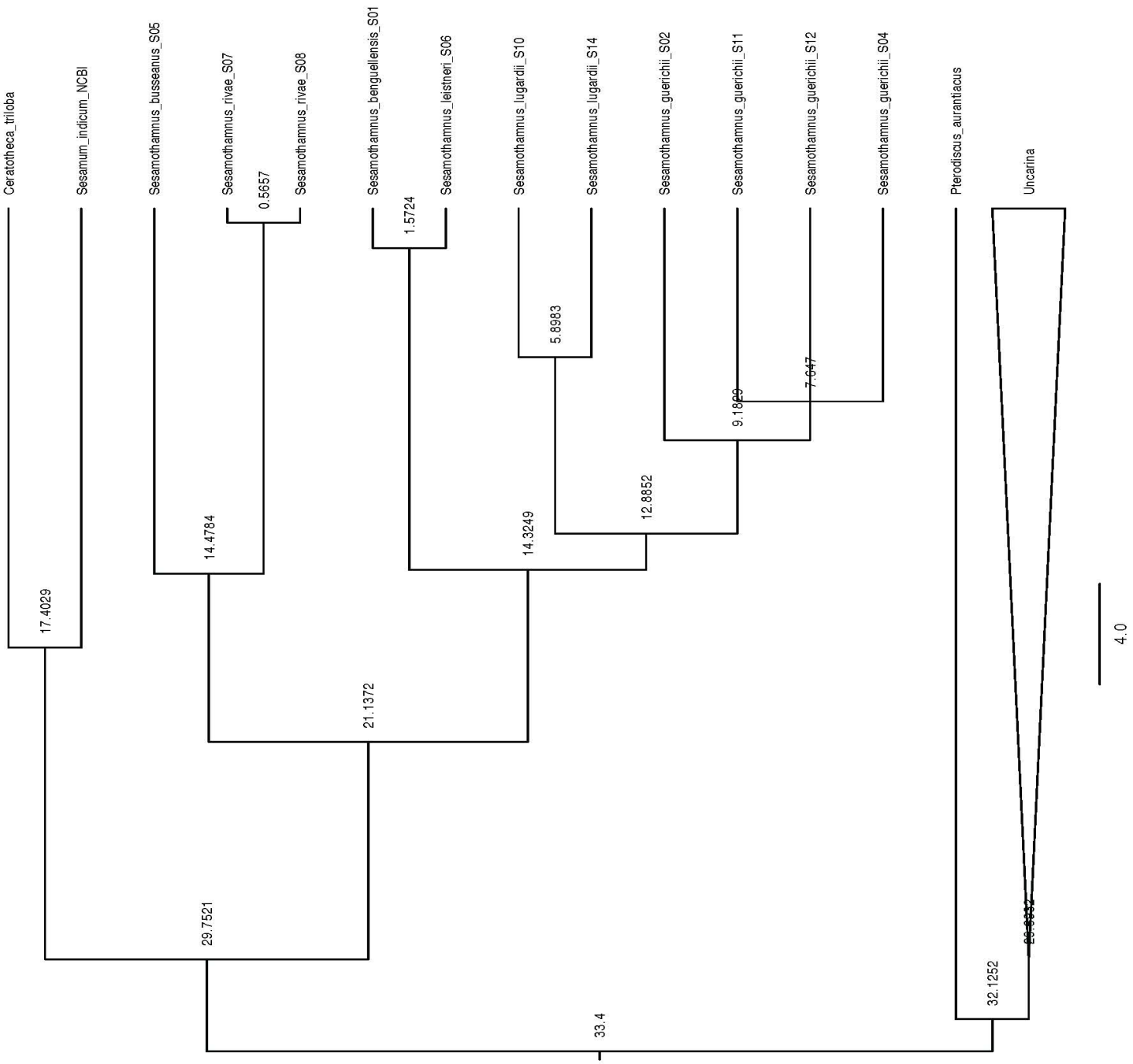

Fig. 5. Dated phylogeny of *Sesamothamnus* based on cpDNA generated in ChronoPL. Dates at nodes are in mya.