Spring 5-2017

Phylogenetic Relationships of the Genera of *Achariaceae* Based on Analyses of Morphological and DNA Data

Corey L. Pagart
University of Southern Mississippi

The University of Southern Mississippi

Phylogenetic Relationships of the Genera of Achariaceae Based on Analyses of Morphological and DNA Data

by

Corey Pagart

A Thesis
Submitted to the Honors College of
the University of Southern Mississippi
in Partial Fulfillment
of the Requirements for the Degree of
Bachelor of Science
in the Department of Biological Sciences

May 2017

Approved by

Mac H. Alford, Ph.D., Thesis Adviser
Associate Professor of Biological Sciences

Janet Donaldson, Ph.D., Chair
Department of Biological Sciences

Ellen Weinauer, Ph.D., Dean
Honors College

Abstract

Achariaceae are a mostly tropical family of flowering plants consisting of about 29 genera and 150 species of trees and shrubs. Although they are closely related to passionflowers (Passifloraceae), violets (Violaceae), and willows and cottonwoods (Salicaceae), phylogenetic relationships of the genera remain unclear because the only studies have been focused on particular genera or had limited sampling. Few studies of the family in general have been conducted, except for some on species that produce chaulmoogra oil, a commonly used historical treatment for leprosy. For my study, I investigated the relationships of the genera within the family using morphological and molecular data. For morphology, I created a data matrix of 37 features, and for DNA, I collected data from one plastid region (*ndhF*) and one nuclear region (GBSSI). Phylogenetic analyses of these data indicate that the tribes within the family are not monophyletic and that the family consists of two major, fairly well-supported clades. As hypothesized in a previous study, the genera of Phyllobotryeae (two or three) are related to genera of Achariaceae, and not to Salicaceae, and one genus, *Ahernia*, is more closely related to Salicaceae. Thus, Achariaceae now consist of 32–33 genera.

Key Terms: Achariaceae, chaulmoogra oil, Flacourtiaceae, nuclear GBSSI, plastid *ndhF*, phylogenetic systematics

Acknowledgments

I would like to thank John Marshall Diffey for assistance with lab work. I would like to thank Dr. Mac H. Alford for his support and for mentoring throughout this project. I would like to thank the Honors College for their enduring contribution to my education.

Table of Contents

List of Figures

List of Tables

Chapter 1: Introduction

Flacourtiaceae have long been a catch-all family of plants. If there were no clear affinities of a newly discovered flowering plant, it was commonly placed here. There have even been a few prominent botanists who coined sayings such as “If you don’t know what family it is, try Flacourtiaceae or Euphorbiaceae” (Gentry, 1993), and “When in doubt, put it in Flacourtiaceae” (Williams, 1965). Within recent years, however, this family has been subdivided into many more families, including but not limited to Salicaceae, Samydaceae, and Achariaceae, as it has become more and more evident from analyses of DNA sequence data that this group did not represent closest relatives.

Unfortunately, however, very few people specialize in this group or even have a desire to research it, as many of the included plants have little economic importance. One of the most noteworthy species is the chaulmoogra tree (*Hydnocarpus wightiana*), which has historically been the source of chaulmoogra oil used to combat leprosy (Kerr, 1925). However, with the advent of antibiotics, even this plant has faded into obscurity, and thus, much of the family remains understudied. There is an issue with this mindset, however. If only because of the chaulmoogra tree, further research is a necessity, as in recent years antibiotic resistant strains of bacteria have become more prevalent. If this trend keeps up, we may need alternative treatments to formerly easily controlled diseases such as leprosy. Having a more complete understanding of the relationships among these plants would make such an endeavor all the more possible.

Additionally, it is beneficial to study these plant groups simply because we know so little about them. Perhaps their economic importance has not yet been discovered, or perhaps they have genetic similarity to a group we had not even considered, which could open up new doors in developing and utilizing these plants. In addition, there is a lot of morphological variation in this small family: vines, shrubs, and trees; different kinds of anthers (male parts); extra parts attached to the petals in some; and some with flowers positioned on the leaves. Determining relationships among the genera may help us to understand how these different life forms and structures evolved.

For this project, I will primarily be inferring the phylogenetic relationships of the genera now placed in Achariaceae, a family originally consisting of three small genera from southern Africa but now also including genera from the former catch all family Flacourtiaceae (Table 1). This will involve the use of morphological characteristics, such as the presence or absence of certain key features, as well as DNA features obtained via PCR. PCR stands for polymerase chain reaction, and it is a method that uses a small amount of DNA and replicates it into many. Additionally, this method has become cheaper and more reliable over the years, to where it is feasible to use it in just about any lab setting with only a few tools. By observing these two main features of plants and comparing the data, I hope to discover some relationships that can give better insight into the evolution of these genera of the once large Flacourtiaceae.

Some may question the need for systematics—that is, the categorization of living things based on their relationships—but its value should not be underestimated. The phylogenetic relationships between organisms can offer great insight to not only the history of life as we know it, but also into more applicable solutions to problems that may

seem unrelated at first glance. A plant that was formerly disregarded and has a genetic similarity to an effective medicinal plant might prove to be even more effective in being used as a treatment with just a little genetic modification. Yew trees, for example, are currently being used to combat cancer, and this application was only recently discovered (Baloglu et al., 2001). While my results may not directly cause any impact, it is a valuable first step to potentially identifying new, valuable products.

Table 1. Genera of Achariaceae, with distribution and numbers of species. Composition is based on Chase et al. (2002), except for Phyllobotryeae, which they classified as part of tribe Scolopieae of family Salicaceae. *=Merged by Hul, 1991.

Tribe	Genus	Number of Species	Distribution
Acharieae (the original family Achariaceae)	*Acharia*	1	southern Africa
	Ceratiosicyos	1	southern Africa
	Guthriea	1	southern Africa
Phyllobotryeae	*Phyllobotryon**	3	tropical Africa
	*Phylloclinium**	2	tropical Africa
	Mocquerysia	1	tropical Africa
Pangieae	*Baileyoxylon*	1	Australia
	Chiangiodendron	1	Mexico
	Chlorocarpa	1	Sri Lanka
	Eleutherandra	1	Malesia
	Gynocardia	1	southeast Asia
	Hydnocarpus	~40	southeast Asia

	Kiggelaria	1	southern Africa
	Pangium	1	Malesia
	Ryparosa	18	Malesia
	Scaphocalyx	2	Malay Peninsula
	Trichadenia	2	Sri Lanka and Malesia
Lindackerieae	*Buchnerodendron*	2	central and eastern Africa
	Caloncoba	10	Africa
	Camptostylus	3	western and central Africa
	Carpotroche	11	tropical Americas
	Grandidiera	1	eastern Africa
	Lindackeria	13	tropical Americas and Africa
	Mayna	6	tropical Americas
	Peterodendron	1	eastern Africa
	Poggea	4	Africa
	Prockiopsis	1	Madagascar
	Xylotheca	3	eastern Africa
Erythrospermeae (*Ahernia* moved to Salicaceae)	*Dasylepis*	6	tropical Africa
	Erythrospermum	4	Indo-Pacific
	Rawsonia	2	tropical Africa

	Scottellia	3	tropical Africa

Chapter 2: Literature Review

Systematics is the study of the relationships of things, and in biology, this means the study of the relationships of living things. Within systematics is a field known as phylogenetics. Phylogenetics is described as the study of evolutionary (or historical, or genealogical) relationships—regardless of the type of data used to infer these relationships (morphological, DNA, fossil, etc.). Phylogenetics is an essential part of taxonomy, the classification of living things, as it is very data-oriented and organizes the commonality between all life. As one might guess, this field has recently become more prominent as analyses of DNA and genetic information have become more and more accurate, inexpensive, and prevalent. However, shifting from older systems that rely solely on morphology to a more genetically based form of taxonomy has taken time, and it takes a while for data to be gathered and processed. This is especially true for groups that have been used as "junk bins" and "catch-alls," as few people are available or wish to sort through them, and even worse is when the group or family does not have any immediate economic importance for people and is solely tropical, such as the group studied here, Achariaceae.

Flacourtiaceae were once the family for plants that had no obvious affinity to any other plants. As such, it functioned as a junk category and had become less of a family and more of a trash bin. Inevitably, this led to a call to revise the family, and so it was rearranged when DNA data became available. When Flacourtiaceae were split, the vast majority of plants were moved to Salicaceae (>80%), with a much smaller number placed

into Achariaceae (Chase et al., 2002). Between the small number of species and their seeming economic insignificance, very little has been done with this family. However, at least one species has once been very valuable to humanity, and that is the chaulmoogra tree (*Hydnocarpus wightiana*). Historically, it has been used as an important treatment for leprosy (Kerr, 1925). While not necessarily a cure, the oil yielded from its seeds was able to treat the symptoms well. However, antibiotics treat leprosy much more effectively. The problem lies in their potential overuse. Although leprosy has been basically eliminated from many developed nations, chaulmoogra oil remains an option should a resistant strain arise, both as an immediate treatment and as a system worth studying for his mechanism of control. However, a study of relationships has a broader impact than just providing a background for future studies of potential medicines. Since the evolutionary relationships of these plants have not been deeply analyzed (Keating, 1973), the study may yield some unexpected results or point to new avenues of research.

One of the methods which will be used to determine the evolutionary relationships of these plants is PCR. Formerly, this process was quite expensive, as the machines, chemicals, and primers were still relatively expensive or difficult to design. Today, however, this approach has become far more streamlined, which has made it more accurate and affordable (Buerki and Baker, 2016). By using this method, I hope to obtain some genetic evidence of this family's relationships that have not been looked into.

This method is not without its flaws, however. In plant cells, there are 3 sources of DNA: mitochondrial, nuclear, and plastid (chloroplast). Mitochondria and plastids have DNA that is similar to that of bacteria (Margulis, 1971). While these organelles are essential to complex life, they also lead to complications at times when studying DNA.

Since obtaining specific parts from a single cell is difficult, DNA is often extracted all together, which gives mixed results when analyzing PCR amplified results (Yen et al., 2014). However, with some work, these obstacles may be overcome.

Another method I will be using to help better understand this family's relationships is observing its morphology (Bernhard, 1999). Morphology is often indicative of common ancestry, though not always. Organisms sometimes undergo what is called convergent evolution, which means that although organisms may not be related, they will evolve the same characteristics due to environmental pressures or other such factors. In spite of this, using a mixture of DNA and morphological techniques can provide semi-independent data to infer an organism's evolutionary history, which is the objective for this project (Sanchez, 2015). Since DNA provides one line of (genotypic) evidence that organisms are related, we can observe the features that are similar and different and find the branching points for evolving particular traits.

Chapter 3: Materials and Methods

The methods I used to study Achariaceae are two-fold—both morphological characteristics as well as genetic information. This involves using scientific literature and museum specimens available to me to identify the characteristics of a variety of specimens, and PCR to identify the genetic similarity of the subjects in question. Following these data collection steps, the goal was to utilize the information gathered to form a theoretical phylogenetic tree. This was accomplished by using software capable of finding the most likely possibility of evolutionary pathways, and it used the data gathered to come to these conclusions.

For morphological characteristics, identifying a variety of features that can be applied across the family was the key. Basic features that can be used in dichotomous keys such as leaf venation and patterns help, but a wider range is necessary to truly narrow down the differentiation of families and species. These features were extracted from scientific literature on Achariaceae species (Hul, 1991, 1995; Jessup, 1982; Killick, 1976; Perrier de la Bâthie, 1946; Sleumer, 1954, 1975, 1980; Verdcourt, 1996; Yang and Zmarzty, 2007), as well as available museum specimens, which includes USM's herbarium (USMS). Using this information, I kept a log of what characteristics define each species and prepared those data for entry into WinClada (Nixon, 2002).

Before I was done with data collection, however, I also gathered the genetic information for these plants. The methodology behind this approach is that of DNA extraction, PCR, and sequencing. DNA had been extracted for a previous study using the Qiagen DNeasy mini-kit (Qiagen, Inc., Valencia, CA) or purchased from the Missouri

Botanical Garden or the Royal Botanic Gardens, Kew (U.K.). Using Taq polymerase and primers following the procedure outlined in Samarakoon et al. (2013), I used small samples from the plants I am studying to amplify common regions of their DNA. The regions chosen for amplification were plastid *ndhF* and nuclear GBSSI. This decision was made so that both nuclear and plastid data could be compared, and these regions have previously been useful for studies in related families (Shaw et al., 2014; Small et al., 1998; Williams, 2012). Once the DNA from the PCR was amplified (=many copies made), an agarose gel was run, followed by analysis of the products stained with ethidium bromide by UV spectroscopy. This was largely a verification step, as the essential data came later from the sequencing; however, if the gel showed no product, the product was the wrong size, or several products were produced by the same primers, we had to return to the original DNA or PCR stage to check for potential errors. The amplified regions we tested are known, so the band lengths that were expected are known, and as long as the gel was clear, the DNA could be used for sequencing. DNA was then purified and sent to MWG Operon in Louisville, KY, for sequencing. They returned to us computer files that provided the text sequence as well as a color-coded chromatogram that the machine interpreted. We use the program Sequencher (Spurr, 1992) for "cleaning" the sequences, that is, cutting the hard-to-interpret beginnings and ends and double-checking the computer's assignment of letters, and then we exported the sequences to ClustalX (Thompson et al., 1997, 1998) to "align" the sequences, that is, to make sure the same parts of the DNA are placed together. One sequence (*Hydnocarpus* sp. 1) was obtained from GenBank (https://www.ncbi.nlm.nih.gov/nuccore/), an online source of previously sequenced DNA regions. The aligned sequences were then input

into WinClada (Nixon, 1999, 2002), much like the physical characteristics, and the program calculated the shortest, or most parsimonious, tree or trees. If there were multiple trees, a consensus tree was calculated that represented the relationships found in all of the most parsimonious trees. To test how strongly the data support each part of the relationships, the bootstrap statistical test was used (Felsenstein, 1985). Basically, this technique takes a subset of the data many times and re-analyzes it. How often groups appear in these re-analyses leads to the bootstrap number (0%=never; 100%=every time). For this project, the bootstrap was run for 500 replications. The consistency index (CI) and retention index (RI) were also calculated; these give an idea of how much of the data agree with each other (Kluge and Farris, 1969; Farris, 1989).

Difficulties lay in these methods, but they were not insurmountable. On the morphological end, finding "the perfect specimen" was difficult. Features of one specimen may have been damaged or missing. Literature often contained only some of the required parameters for study. Genetically, PCR is a very effective way of amplifying DNA, but that could be a problem in itself. Chloroplast and mitochondrial DNA can get mixed in and interfere with results, and the tendency of plants to hybridize or become polyploid could be an issue. Regardless, these issues can be avoided by altering which chemicals and mixtures will be used in the PCR, so they are not a large problem.

Table 2. Samples utilized for DNA work. (Outgroups from other families=*)

Species	Voucher	Alford DNA Collection Number
**Hybanthus concolor*	Alford 3056 (BH)	89
**Rinorea pubiflora*	Alford 3134 (BH)	147
**Paropsia madagascariensis*	Zyhra 949 (WIS)	J-3761
Acharia tragodes	Missouri Botanical Garden	MO-32
Caloncoba echinata	Lewis 01-112 (FTG)	50
Caloncoba welwitschii	Walters 898 (MO)	133
Camptostylus mannii	Missouri Botanical Garden	MO-1121
Carpotroche longifolia	Alford 3117 (BH)	98
Ceratiosicyos laevis	Chase 811 (?)	811
Dasylepis seretii	Harris 5503 (K)	5850
Erythrospermum sifarii	Hoffmann 393 (K)	15931
Grandidiera boivinii	Robertson s.n.	141
Guthriea capensis	Abbott 6071 (?)	813
Gynocardia odorata	Chase 1279 (K)	1279
Hydnocarpus sp. 1	GenBank: AY425058	n/a
Hydnocarpus sp. 2	Middleton 2260 (GH)	172
Kiggelaria africana	Alford 3028 (BH)	51
Lindackeria dentata	Stone et al. 3258 (MO)	135
Lindackeria paludosa	Alford 3110 (BH)	99
Mayna odorata	Missouri Botanical Garden	MO-152A
Pangium edule	Chase 1285 (K)	1285
Peterodendron ovatum	Missouri Botanical Garden	MO-5022
Phyllobotryon spathulatum	Cheek s.n. (K)	13382
Phylloclinium paradoxum	Bidgood et al. 2787 (K)	11360
Poggea gossweileri	Missouri Botanical Garden	MO-12194
Prockiopsis hildebrandtii	Missouri Botanical Garden	MO-936
Rawsonia lucida	Salazar 326 (BH)	164
Ryparosa javanica	Chase 1287 (K)	1287
Scaphocalyx spathacea	Missouri Botanical Garden	MO-2081
Scottellia klaineana	Harris 4076 (K)	5849
Trichadenia zeylanica	Chase 1289 (K)	1289
Xylotheca tettensis	Salazar 300 (BH)	165

Chapter 3: Results

Morphology

A matrix of 37 morphological, anatomical, and chemical characters was generated. After comparing data across genera, many of these characters did not seem appropriate for phylogenetic analysis because they were continuous, hard to define, or lacking for many genera. However, several characters were fairly straight-forward and have been used in previous classifications.

1. Habit: herbaceous vs. woody
2. Leaf venation: pinnate vs. palmate
3. Inflorescence location: axillary/terminal/cauliflorous vs. on the leaves/petioles
4. Sepals: free vs. fused
5. Petals: with basal scales vs. without scales
6. Petals: equaling sepals in number and slightly larger than sepals vs. numerous and much longer than sepals
7. Fruits: smooth vs. winged/bristly

Although these features were not used in phylogenetic analysis itself, they were used to assess their congruence with the phylogenies obtained from DNA data. These are represented by colors in the figures, with blue representing those with scales or appendages on the petals, green representing those with herbaceous habit and palmate venation, purple representing fused sepals, orange representing numerous, long petals and bristly/winged fruits, and pink representing those with flowers positioned on the petioles or leaves.

Phylogenetic Analyses

Variable DNA data were obtained for plastid *ndhF* and nuclear GBSSI regions, although one other region was tested (plastid *trnH-psbA*). The *trnH-psbA* region, although highly variable, had extensive homopolymer regions at two or more locations in the small region, making it impossible to obtain complete, "clean" sequences, even when sequenced from both directions.

For plastid *ndhF*, 31 individuals were successfully sequenced, yielding an aligned data matrix of 737 DNA base-pairs (bp). Of these, there were 134 potentially informative substitutions. Phylogenetic analysis of this region using parsimony yielded 71 most parsimonious trees of length 274, CI of 0.62, and RI of 0.82 (Figure 1).

For nuclear GBSSI, 18 individuals were successfully sequenced here, yielding an aligned data matrix of 768 bp. Of these, 134 were potentially informative substitutions. Phylogenetic analysis of this region using parsimony yielded 48 most parsimonious trees of length 320, CI of 0.63, and RI of 0.68 (Figure 2).

Finally, data from plastid *ndhF* and nuclear GBSSI were combined into one matrix and simultaneously analyzed. This analysis resulted in 99 most parsimonious trees of length 509, CI of 0.65, and RI of 0.79 (Figure 3).

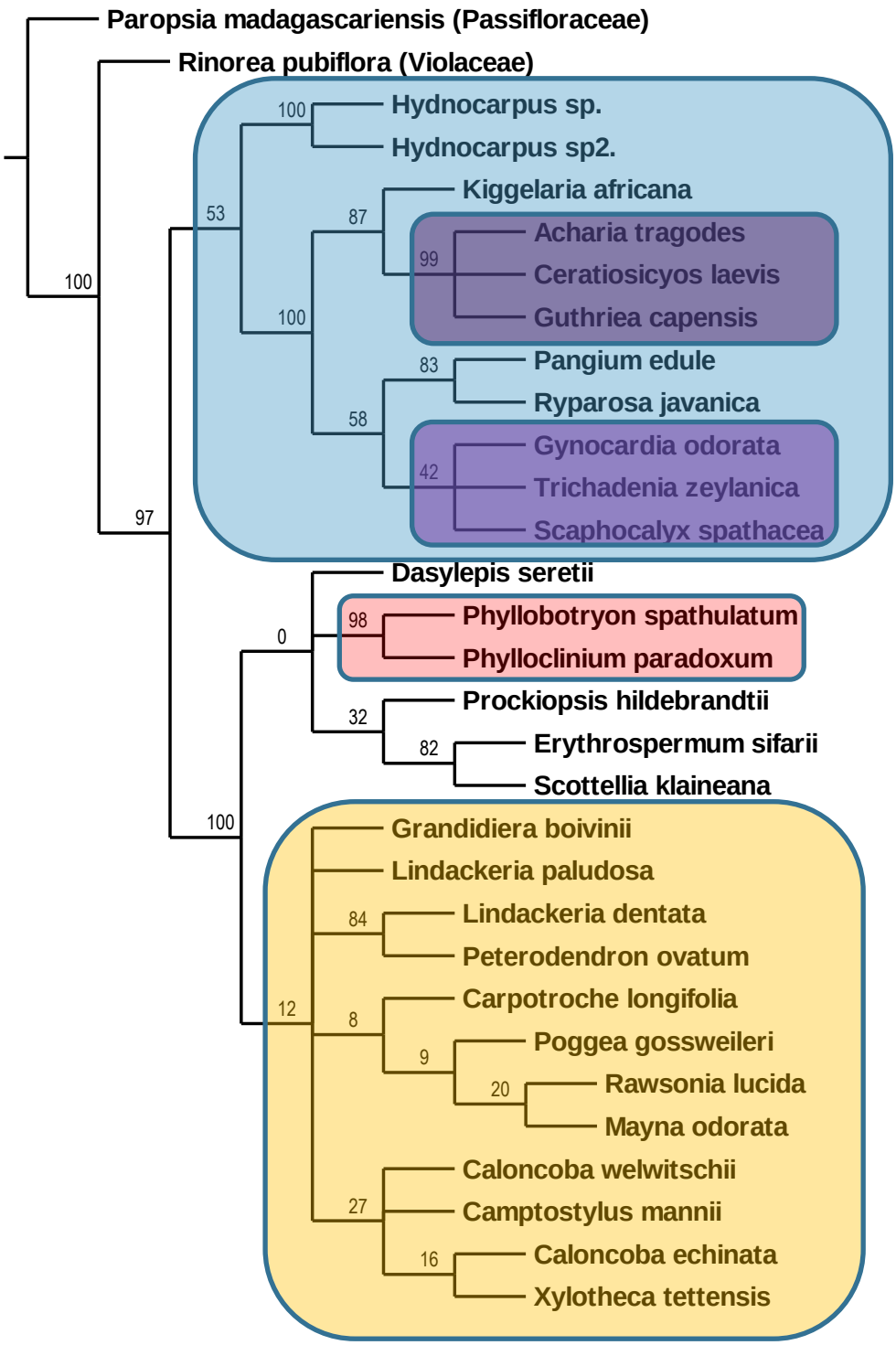

Figure 1. Strict consensus tree of 71 most parsimonious trees obtained from parsimony analysis of plastid *ndhF* data. Bootstrap values are given above the branches. Color-coded clades are discussed in the text. L=274, CI=0.62, RI=0.82.

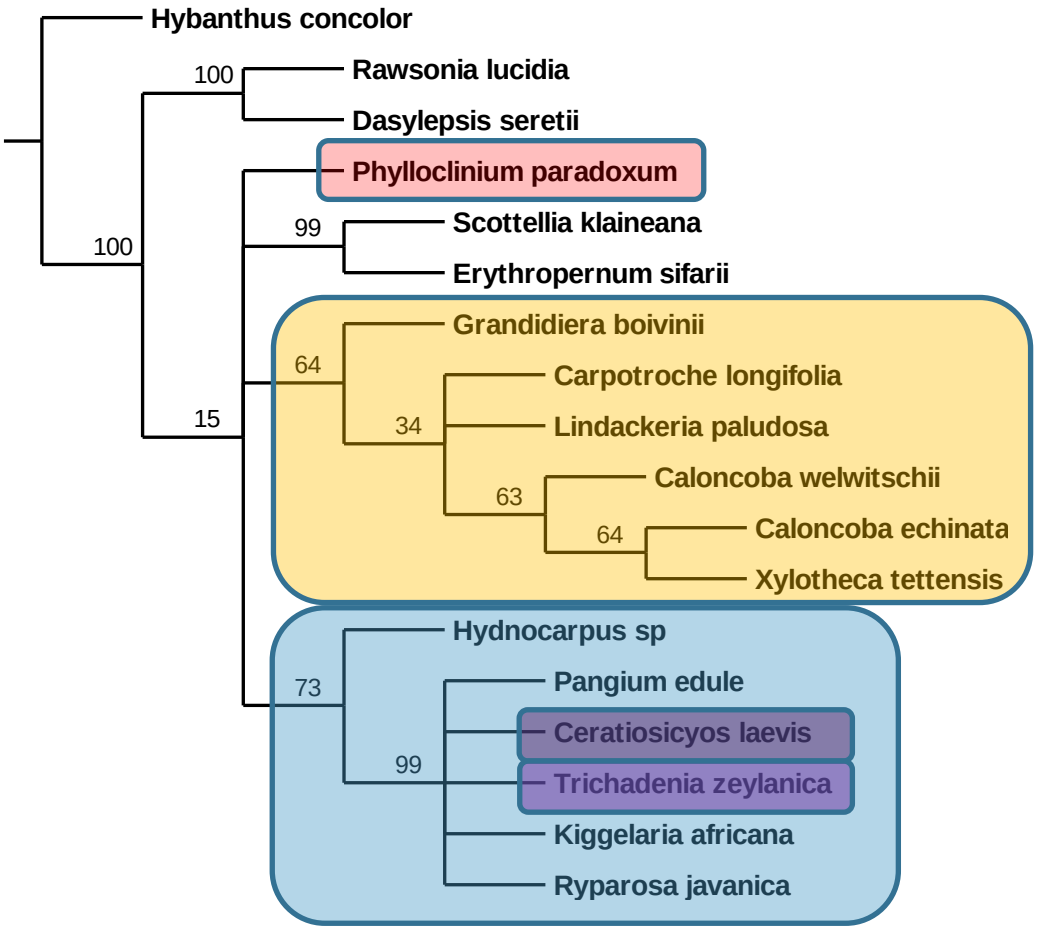

Figure 2. Strict consensus tree of 48 most parsimonious trees obtained from parsimony analysis of nuclear GBSSI data. Bootstrap values are given above the branches. Color-coded clades are discussed in the text. L=320, CI=0.63, RI=0.68.

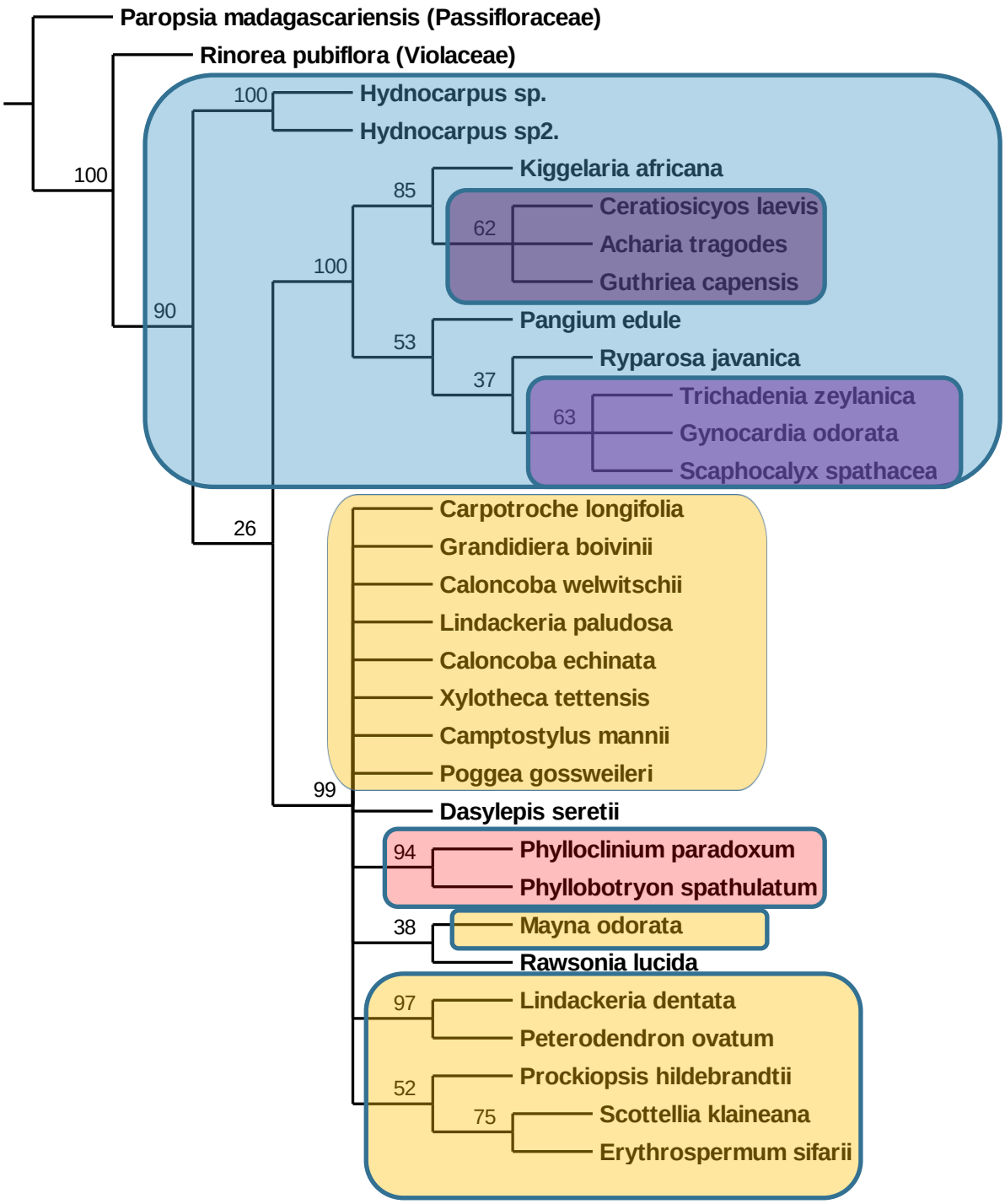

Figure 3. Strict consensus tree of 99 most parsimonious trees obtained from parsimony analysis of nuclear GBSSI data combined with plastid *ndhF* data. Bootstrap values are given above the branches. Color-coded clades are discussed in the text. L=509, CI=0.65, RI=0.79.

Chapter 4: Discussion

DNA data, especially from *ndhF*, which has more sampled species (Figure 1), indicate that there are two well-supported clades within the family. One clade (in blue) is distinguished by having scales/appendages on the petals. Within that group, there are two morphologically distinct sub-clades, one characterized by palmate venation and herbaceous habit (in green), the other by having fused sepals (in purple). The other clade has no apparent morphological character support, but one large sub-clade is distinguished by having long and numerous petals and bristly or winged fruits (in orange). Unfortunately, bootstrap support for that clade is really low (12%). A small, but interesting and well-supported, clade is distinguished by producing its flowers on its leaves (in pink). Some scientists recognize three genera in that group (*Phyllobotryon*, *Phylloclinium*, and *Mocquerysia*: Lemke, 1988), and others (Hul, 1991) recognize two, with one of the genera divided into two subgenera corresponding to the genera in the other system (*Phyllobotryon* subg. *Phyllobotryon*, *Phyllobotryon* subg. *Phylloclinium*, and *Mocquerysia*). For simplicity, I recorded each group as its own genus. In the *ndhF* tree, the tribe Erythrospermeae appears in several places. *Rawsonia* is more closely related to a different tribe; *Erythrospermum* and *Scottellia* are closely related; and *Dasylepis* is separate from both groups. Except for the close relationship of *Erythrospermum* and *Scottellia*, the other relationships have very poor bootstrap support and thus provide us with little confidence about their relationships.

The data from *ndhF* and GBSSI do not agree on the placement of Erythrospermeae. *Rawsonia* and *Dasylepis* are both placed in Erythrospermeae with

Erythrospermum and *Scottellia* by Lemke (1988) and Chase et al. (2002), but *Rawsonia* has long petals much like genera in Lindackerieae. Interestingly, *ndhF* data place it with Lindackerieae, but GBSSI data place *Rawsonia* and *Dasylepis* together outside both Lindackerieae and Erythrospermeae. The *ndhF* data have weak bootstrap support, but the GBSSI data have strong bootstrap support, at least for placing *Rawsonia* and *Dasylepis* together (100% bootstrap) and for placing *Scottellia* and *Erythrospermum* together (99% bootstrap, also 82% bootstrap in *ndhF*). Whether or not all four genera belong together is not resolved here.

The results indicate that the other two tribes as defined by Lemke (1988) are also not monophyletic. Lemke (1988) did not include *Acharia*, *Ceratiosicyos*, or *Guthriea* in the family at that time, and he divided the ones he did include according to presence/absence of cyclopentenyl fatty acids, wood characters, sepal/petal differentiation, and petal scales. Based on the results here in all three analyses, Acharieae are nested within Lemke's (1988) and Chase et al.'s (2002) Pangieae (mostly genera marked in blue in Figures 1–3), and there is poor support for a clade containing the genera of Lindackerieae (referred to as Oncobeae in Lemke [1988]).

Many of the relationships in the tree have low bootstrap values, meaning that the data supporting those branches in the tree are not strong, coupled with differences between *ndhF* and GBSSI. When plastid and nuclear data are analyzed together (Figure 3), many branches are not resolved at all (see orange, for example), and many others have low bootstrap values. The relationships in Figure 3, though, should be considered in the light that fewer GBSSI sequences were obtained (Figure 2) and thus that analysis had a lot of missing data. Only six relationships in the family have 85% or greater bootstrap

support in the combined analysis. Among these, the results indicate that *Kiggelaria* and other Pangieae are close relatives to the Acharieae (*Acharia, Ceratiosicyos*, *Guthriea*), which makes sense since they share scales on the petals, that the two species of *Hydnocarpus* are close relatives, that the two genera/subgenera of Phyllobotryeae are closely related, and that *Scottellia* and *Erythrospermum* are closely related.

Additional results from GBSSI would be helpful in resolving these issues, but DNA from many of the species did not amplify for GBSSI. Since there is poor bootstrap support in the Lindackerieae using both *ndhF* and GBSSI data, another DNA region needs to be found that will provide suitable variation among those species. I tried the plastid *trnH-psbA* region, which was highly variable, but it had so many repeats (homopolymer regions) that alignment was impossible for almost all species. Further research with variable regions lacking repeats would likely provide the needed data for resolving relationships in the family.

Literature Cited

Baloglu, E., D. Kingston, P. Patel, S. Chatterjee, and S. Bane. 2001. Synthesis and microtubule binding of fluorescent paclitaxel derivatives. *Bioorganic and Medicinal Chemistry Letters* 11: 2249–2252.

Buerki, S., and W. Baker. 2016. Collections-based research in the genomic era. *Biological Journal of Linnean Society* 117: 5–10.

Bernhard, A. 1999. Floral structure and development of *Ceratiosicyos laevis* (Achariaceae) and its systematic position. *Botanical Journal of the Linnean Society* 131: 103–113.

Chase, M., Zmarzty, S., Lledó, M., Wurdack, J., Swensen, S., and Fay, M. 2002. When in doubt, put it in Flacourtiaceae: A molecular phylogenetic analysis based on plastid *rbcL* DNA sequences. *Kew Bulletin* 57: 141–181.

Farris, J. S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417–419.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39(4): 783–791.

Gentry, A. H. 1993. *A Field Guide to the Families and Genera of Woody Plants of Northwest South America (Colombia, Ecuador, Peru) with Supplementary Notes on Herbaceous Taxa*. Chicago: University of Chicago Press.

Hul, S. 1991. Révision des Flacourtiaceae–Phyllobotryoneae d'Afrique. *Bulletin du Muséum National d'Histoire Naturelle. Section B, Adansonia: Botanique Phytochemie* 4^{e} sér., 13: 155–165.

Hul, S. 1995. Flacourtiaceae. *Flore du Gabon*, treatment 34. Paris: Muséum National d'Histoire Naturelle.

Jessup, L. W. 1982. Flacourtiaceae. Pp. 66–84 in *Flora of Australia*, vol. 8. Canberra: Australian Government Publishing Service.

Keating, R. 1973. Pollen morphology and the relationships of the Flacourtiaceae. *Annals of the Missouri Botanical Garden* 60: 273–305.

Kerr, I. 1925. The chaulmoogra treatment of leprosy: at the hospital for lepers, Dichpalli, Hyderabad state. *The Lancet* 206: 373–375.

Killick, D. J. B. 1976. Flacourtiaceae (including Samydaceae). Pp. 53–92 in *Flora of Southern Africa*, vol. 22 (J. H. Ross, ed.). Pretoria, South Africa: Botanical Research Institute.

Kluge, A. G., and J. S. Farris. 1969. Quantitative phyletics and the evolution of Anurans. *Systematic Zoology* 18: 1–32.

Lemke, D. E. 1988. A synopsis of Flacourtiaceae. *Aliso* 12(1): 29–43.

Margulis, L. 1971. *Origin of Eukaryotic Cells*. New Haven, CT: Yale University Press.

Nixon, K. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.

Nixon, K. C. 2002. WinClada, version 1.00.08. Ithaca, New York. www.cladistics.com

Perrier de la Bâthie, H. 1946. Flacourtiacées. Pp. 1–131 in *Flore de Madagascar et des Comores* (H. Humbert, ed.), Family 140. Tananarive: Imprimerie Officielle.

Samarakoon, T., S. Y. Wang, and M. H. Alford. 2013. Enhancing PCR amplification of DNA from recalcitrant plant specimens using a trehalose-based additive. *Applications in Plant Sciences* 1(1): 1200236.

Sanchez, E. 2015. Parametric and non-parametric species delimitation methods result in the recognition of two new Neotropical woody bamboo species. *Molecular Phylogenetics and Evolution* 93: 261–273.

Shaw, J., H. L. Shafer, O. R. Leonard, M. J. Kovach, M. Schorr, and A. B. Morris. 2014. Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: the tortoise and the hare IV. *American Journal of Botany* 101(11): 1987–2004.

Sleumer, H. O. 1954. Flacourtiaceae. Pp. 1–106 in *Flora Malesiana*, Series 1 (C. G. G. J. van Steenis, ed.) Noordhoff-Kilff N.V., Djakarta.

Sleumer, H. O. 1975. Flacourtiaceae. Pp. 1–68 in *Flora of Tropical East Africa* (R. M. Polhill, ed.). London: Whitefriars Press Ltd.

Sleumer, H. O. 1980. Flacourtiaceae. *Flora Neotropica* 22: 1–499.

Small, R. L., J. A. Ryburn, R. C. Cronn, T. Seelanan, and J. F. Wendel. 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear *Adh* sequences for phylogeny reconstruction in a recently diverged plant group. *American Journal of Botany* 85(9): 1301–1315.

Spurr, N. and S. Bryant. 1992. Sequencher (a software package for managing and building contigs). Gene Codes, Ann Arbor, MI. *Trends in Biotechnology* 10: 450.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.

Thompson, J., F. Jeanmougin, M. Gouy, D. Higgins, and T. Gibson. 1998. Multiple sequence alignment with ClustalX. *Trends in Biochemical Sciences* 23: 403–405.

Verdcourt, B. 1996. Flacourtiaceae. Pp. 199–235 in Dassanayake et al. (eds.), *Revised Handbook Flora of Ceylon*, vol. 10.

Williams, C. 2012. A phylogeny of Samydaceae based on nuclear GBSSI and EMB2765 DNA sequences. Honors Thesis, University of Southern Mississippi.

Williams, L. O. 1965. The story of two sterile specimens. *Annals of the Missouri Botanical Garden* 52: 485–486.

Yang, Q.-E. and S. Zmarzty. 2007. Flacourtiaceae. In: Wu, Z., P. Raven, and D. Hong, eds. *Flora of China (Clusiaceae through Araliaceae)*, vol. 13. Missouri Botanical Garden Press, St. Louis.

Yen, H. C., S. L. Li, W. C. Hsu, and P. Tang, P. 2014. Interference of co-amplified nuclear mitochondrial DNA sequences on the determination of human mtDNA heteroplasmy by using the Surveyor nuclease and the WAVE HS system. *PLoS One* 9(3): e92817. doi:10.1371/journal.pone.0092817