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Disentangling a complex genus: systematics, biogeography and bioactivity of the genus *Phyllanthus* L. and related genera of tribe Phyllanthae (Phyllanthaceae)

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CHAPTER 10

Conclusions

Chapter 10. Conclusions

General discussion

Phyllanthus L. sensu lato (s.l.) is known as a diverse clade with often difficult to distinguish species due to the small flowers and the often high resemblance in leaf shapes and sizes. In this thesis I explored the evolution of this group while also proposing an alternative classification as a solution to the apparent paraphyly (not all descendents of an ancestral species included). Initial work by Linneaus (1753, 1778) and De Jussieu (1803) already indicated a diversity of characters that were used to place the species in different genera. After Müller (11863, 1865, 1866) defined *Phyllanthus* as one genus with over 40 different sections, the biggest update to this system was implemented by Webster (1956, 1957, 1958 and thereafter). Webster focused on the West Indian and American species of *Phyllanthus*, but treatments for other floristic regions were scattered and a global inventory of the number of accepted species was overdue. The systematic history was summarised in chapter 3 in the form of a list, which included all accepted species and their infrageneric placement to that point. While Webster published many treatments for the Neotropical flora, the Palaeotropics remained understudied. The first of these groups to be treated was *Phyllanthus* subgenus *Macraea* (Wight) Jean F. Brunel (Chapter 4). Follow-up revisions that focus on other groups are now also being progressed (Wibowo et al. unpublished; Hinloopen et al. unpublished).

By increasing our understanding of the relationship between groups in tribe Phyllantheae while revising various groups to look for synapomorphies, this thesis presents another option to the apparent paraphyly of *Phyllanthus*, which has been under discussion already for several years. Also an investigation in the metabolites of several species was included as this field keeps growing and the interest in *Phyllanthus*, already substantial in traditional medicines, is increasing.

How large is the diversity of Phyllanthus and tribe Phyllantheae and how is it structured?

Our initial review of the long taxonomic history of *Phyllanthus* resulted in a list containing 880 species, which could be classified into 18 subgenera and 70 accepted sections (chapter 3). A few overlooked issues were found that needed to be addressed. *Phyllanthus* subsections *Callidisci* Jean F. Brunel and *Odontadenii* Jean F. Brunel & Jacq. Roux had been placed in a large concept of *Phyllanthus* subgenus *Phyllanthus* by Brunel (1987). However, the phylogenetic study of Kathriarachchi et al. (2006) had already shown that this subgenus was polyphyletic and a new subgenus was subsequently created with the goal of housing the African and Malagasy species in the revision work by Ralimanana & Hoffmann (2011, 2014). Their work did not take into account the subsections defined by Brunel (1987). In chapter 3 we transferred these subsections, but also specifically listed all species that we hypothesized to be included in subgenus *Afroswartziani* Ralim. & Petra Hoffm.

Other changes, in *Phyllanthus* subgenus *Gomphidium* (Baill.) G.L.Webster, were also included based on recommendations in Webster's manuscripts that were never completed or published.

Additional changes to the number of species in *Phyllanthus* came with the revision of one of the understudied subgenera (chapter 4) and the study of some poorly known species from Sulawesi (chapter 5). The revision of *Phyllanthus* subgenus *Macraea* included 24 species with indications that there might be a few more in India. It is a Palaeotropical group with the majority of its diversity in Asia and previously species of this group were only treated separately for local floras. This group of species is characterized by non-phyllanthoid branching, 3-merous flowers with three free stamens and clypeate pollen. The constituent species were expanded to include species from the Philippines and the Pacific, indicating that *P.* subgenus *Macraea* is distributed from Africa all the way to Hawai'i.

Koorders (1898) published two species of *Phyllanthus* for the island of Sulawesi, but only included a concise description, which precluded assignment to any subgenus. After studying the specimens collected by him, we lectotypified these species and also concluded that they should be placed in *Phyllanthus* subgenus *Eriococcus* (chapter 5). They are most likely related to similar looking species from the Philippines of which one appeared to be synonymous. These species likely belong to a lineage that might have originated on the mainland and dispersed through the Philippines to Sulawesi and ultimately reached the Lesser Sunda islands.

The infrageneric framework outlined in chapters 2–5 provided the best hypothesis to test for monophyletic groups as potential candidates to be integrated in a re-classification of *Phyllanthus* s.l. A phylogenetic study of five molecular markers found several problems with the previous system, most commonly due to monospecific taxa or small sections, which were found to be embedded in other groups (chapter 7). Some subgenera like *Phyllanthus* subgenus *Gomphidium* and *Kirganelia* were found to be polyphyletic and the paraphyly of *Phyllanthus* was again confirmed. *Phyllanthus* s.l. appeared to consist of eight separate clades with *Glochidion* closely related to a paraphyletic *Phyllanthus* subgenus *Phyllanthodendron*. A new classification has to correct for these findings, but could focus on these clades as potentially separate genera.

Medicinal properties of Phyllanthus

Species of *Phyllanthus* s.l. are used in various traditional medicines. Plant metabolomics using Proton nuclear magnetic resonance (HNMR) combined with anti-bacterial and anti-fungal testing provided a strong method to study these effects and potentially find bioactive compounds, which could be targets for future studies. Unfortunately our results were minimal as only a few extracts of species were found to possess antibacterial effects. Most likely due to concentration issues we could not determine which compounds were responsible for these effects. A

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survey across the literature showed that more and more compounds are discovered in tribe Phyllanthae every year and potentially a study of the related species suggested by our phylogenetic work, can identify new candidates for future work.

Monophyletic or paraphyletic, large versus small genera

The paraphyletic nature of *Phyllanthus* s.l. was one of the main focuses of this thesis. After indications from Wurdack et al. (2004) and confirmation by Kathriarachchi et al. (2006), a new classification of tribe Phyllanthae sought to combine *Phyllanthus* with *Breynia*, *Sauropus* and *Glochidion* (Hoffmann et al. 2006). *Phyllanthus* would be monophyletic when combining all embedded groups, but also by creating a new classification by dividing the genus into smaller genera based on clades and recognisability. However, creating one giant genus would temporarily solve the problem and identifications issues would just be pushed to subgeneric levels. A broader treatment of *Phyllanthus* would still result in a few hundred species needing to be transferred, while *Phyllanthus* becomes a giant genus with no apparent synapomorphies. Christenhusz (2020) advocated for a conservative approach in naming with increasingly larger genera on the basis that it would be more stable as it would be easier to maintain monophyly. As long-standing genera have large bodies of literature attached to them, dividing genera into new groups would ignore the relations between clades and their evolutionary history. Then what to do with paraphyletic groups? Christenhusz (2020) indicated that these should not be accepted, but instead create larger groups and look for similarities between the clades (giving an example of birds and reptiles). Applying this to *Phyllanthus*, would result in a group with no distinct synapomorphy and the characters that differ between clades are equal or larger than the difference between *Phyllanthus* s.l. and *Flueggea* Willd., *Margaritaria* L.f. and the rest of tribe Phyllanthae. The alternative approach presented here, might not work in other genera, but actively avoiding one side of the argument is not good science.

In chapter 9 we present our classification of tribe Phyllanthae with 18 genera based on the results from our phylogenetic and morphological work. A new phylogeny is presented by combining the contemporaneous studies of Chapter 7 and Falcón et al. (2020). *Phyllanthus* s.s. is restricted to mostly the Neotropics while the genera *Cathetus* Lour., *Cicca* L., *Dendrophyllanthus* F.Muell., *Embllica* Gaertner, *Kirganelia* A.Juss, *Moeroris* Raf., *Nellica* Raf. and *Nymphanthus* Lour. are reinstated and *Lysiandra* (F.Muell.) R.W.Bouman, I.Telford & J.J.Bruhl is raised to the level of genus. Each genus is (re-)described while taking into account all previously defined subgroupings of which not all are retained. Especially a number of monospecific sections are subsumed in larger groups. While we present a more conservative approach in the number of subgenera and (sub)sections of the various genera, we feel that this classification

Morphological evolution

The large morphological diversity found in *Phyllanthus* s.l. could already be seen in the large number of subgenera and sections. The flowers show large differences between taxa while there are also some interesting oddities. While studying the phylogeny of tribe Phyllanthaeae, it became apparent that some morphological features thought to be indicative of common ancestry, were actually the result of convergent evolution (chapter 7).

The specific type of branching found in the majority of species of tribe Phyllanthaeae, the so called phyllanthoid branching, sets it apart of other members of the Phyllanthaceae. It is likely that this branching system is involved with energy management and maximizing photosynthetic potential. How this system exactly works and whether nutrients flows are reversed is not known. The ability to shed whole branchlets and have leaves on the main stem reduced to scales provides the plants with a great system for energy management and to prevent light competition between its own branchlets while keeping a slim habit.

Staminate flowers usually show little difference between species in a particular clade, but they are invaluable for distinguishing genera. Early diverged clades in *Phyllanthus* s.l. have trimerous flowers with two perianth whorls and this trait seems to be conserved in parts throughout the phylogeny. Pentamerous flowers originated independently at least six times. In contrast pistillate flowers show more differences in the shape of the style and stigmas related to different pollination systems and these can be used to differentiate species.

Capsules are common fruits in the Phyllanthaceae family, but several instances are found in tribe Phyllanthaeae, where berries have evolved.

Dispersal history

The reconstruction of *Phyllanthus* showed that it likely originated somewhere in the Early Eocene and possibly in the boreotropics. Early dispersals to North America, Africa and Asia have lead to the present day distribution of the reinstated *Nellica*, a clade sister to the rest of *Phyllanthus* s.l. The diversification of *Flueggea* and *Margaritaria* is of a more recent date, which suggests that their pantropical distribution is also an effect of more recent long-distance dispersals than a boreotropical origin. Initial results indicated that boreotropical dispersal might have influenced the distribution of several clades, with *Nellica* present in North America, Africa and Asia, while some clades only show major distribution centers in Asia and Africa (*Cathetus* and *Moeroris*). Subsequent dispersal out of Africa to the Neotropics are inferred to have occurred during the Oligocene, which lead to *Phyllanthus* sensu stricto (s.s.; here the only clade retained as *Phyllanthus*, chapter 9). Within *Phyllanthus* s.s. several independent diserspals northwards are found to Mexico, but also to the West Indies. From Africa, one clade also dispersed to Australia, which lead to *Cicca* and the very diverse *Dendrophyllanthus*. *Cicca* has a wide distribution with a few species in Asia to more diverse clades in Madagascar, Africa and again some species in the Neotropics, which seems to be the result of

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more recent dispersals. In mainland Asia, several groups diverged to form *Emblica*, *Glochidion*, *Breynia* (more explored in van Welzen et al. 2015) and with one dispersal event, which lead to *Synostemon* in mainly Australia.

Diversification of tribe Phyllanthae

Species diversity within *Phyllanthus* s.l. shows an uneven pattern, with some highly speciose clades seeming to be very young, while others that diverged earlier do not include as many species. A diversification analysis on the dated tree of chapter 8 showed two possible shifts in speciation. One was for the clade comprising the reinstated genera *Cicca* and *Dendrophyllanthus*, while the other occurred for the genus *Glochidion*. A high rate of speciation of tribe Phyllanthae had previously been related to a pollination mutualism with parasitic moths, but this pattern was not observed in all clades where this system has been inferred. Other factors such as dispersal strategy are proposed to have affected potential speciation rates, but a larger ecological study is necessary to understand this. As the pollination system is also found to vary between genera and also species, subtle nuances could also still have an effect. Highest speciation rates and the strongest shift was still found for *Glochidion* subgenus *Glochidion* and not as much in the former *Phyllanthodendron* attributed species. Species placed here in *G.* subgenus *Pseudoactephila* and *G.* subg. *Phyllanthodendron* were hypothesized to be pollinated perhaps by flies and some characters like the inflated fruits have previously been attributed as a defense against herbivory and seed predation (Kato & Kawakita 2017). The pollination mutualism with moths is hypothesized to have evolved from an original parasitic system (Kato & Kawakita 2017) and perhaps parasitism is present in more clades in the tribe, with some species evolving defences against this.

Future perspectives

This thesis investigated the global evolutionary patterns of *Phyllanthus* and how to translate these in a monophyletic classification at generic level. Studying other factors such as flower development and differences in ecology, could provide more insight in the evolution of the diverse tribe Phyllanthae. Especially ecological information on many species is lacking and as in many plant families, the life histories of the plants have barely been studied.

The pollination mutualism with parasitic moths has, since its first publication (Kato 2003), garnered much attention as an ideal study system for co-evolution and co-diversification of two groups. While the diversification rates for tribe Phyllanthae were studied here in relation to this mutualism, the amount of data on other ecological factors and variations in this system is very limited. Other ecological factors such as dispersal mechanics are also a field that presents interesting opportunities within tribe Phyllanthae. The phylogeny showed several shifts in fruit type, which probably indicate differences in dispersal vectors, but which vectors or how this is associated with speciation has not been studied. An

interesting system could be in the genus *Cicca* where the fruits are capsules in the Malagasy clades, while in *C.* subgenus *Cicca* different types are found from small berries to apple-like fruits and inflated capsules that are a contender for the biggest fruits in the tribe.

As the speed and data originating from molecular tools is rapidly increasing, future studies might implement a much broader sampling of the genome of the various genera in tribe Phyllanthae. Initial studies have already sequenced the plastid genome of species of *Breynia* (Cai et al. 2019; Zhou et al. 2020), *Glochidion* (Cheon et al. 2019) and *Flueggea* (Wang et al. 2020) and as more information becomes available, it will be possible to study other aspects then phylogenetics such as genome duplication patterns or the evolution of gene function and composition. Indications of whole genome duplications in Phyllanthaceae have been found by Cai et al. (2019), but they only included two species of this family. Chromosome counts from Miller & Webster (1966) found that the base number in Phyllanthaceae (then Euphorbiaceae subfamily Phyllanthoideae) was $x = 13$ (also in Webster & Ellis 1962). The same base number has been found in species of *Flueggea*, *Margaritaria*, *Kirganelia*, *Nellica*, *Cathetus* (Webster & Ellis 1962; Hans 1973), while higher chromosome numbers are found in *Moeroris*, *Phyllanthus* s.s., *Breynia* and *Synostemon* (Webster & Ellis 1962; Miller & Webster 1966). This indicates at least one possible duplication or hybridisation event within tribe Phyllanthae with possibly more since some clades have species that are tetraploids (Hans 1973).

New taxonomic studies will continue on tribe Phyllanthae, however to prevent overlooked species, revision work should focus on separate taxa instead of local floras. Some taxa are in dire need of an updated treatment, like the reinstated genus *Emblica*, where its constituent species have always been scattered over several subgeneric groups. By understanding more of the phylogenetic context of each genus, it will be easier to make comparisons between groups of species to find more diagnostic characters between them. Following our new classification of tribe Phyllanthae, several genera are already geographically separated and once the genus is known, species identification becomes more narrow in this framework. Hopefully this will aid future taxonomists with their understanding of these awesome plants.

Final remarks

My journey with *Phyllanthus* has been long and interesting. Not all experiments succeeded and naturally not all species could be exhaustively treated. Still, I hope that the work presented in this thesis has demonstrated how wonderful the plants that made up *Phyllanthus* s.l. are. The species in the tribe Phyllanthae display an enormous variation in habit, flowers and fruit morphology, which is only indicative of a long and intricate evolutionary history and likely partly due to the moth pollination. I have attempted to redefine *Phyllanthus* s.l. and create a classification

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that shows this history, with reinstated genera that show where clades diverged, but which sometimes also showed convergent evolution. A new view on *Phyllanthus* and its related genera creates the way to actively compare clades and study how these evolved to lead to the diversity we see today.