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

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SUMMARY

In the past 50 years, plant systematics has undergone a revolution with the advent and rapid accessibility of molecular techniques, which greatly influenced how we study and classify taxa. While many organism groups and theories have been proven accurate, some appeared to be more complex than previously thought. The plant genus *Phyllanthus* (Phyllanthaceae tribe Phyllantheae) is a good example of a group of species with varying views on its taxonomy and it has always been treated as closely related to the genera *Glochidion*, *Breynia* (including *Sauropus*) and *Synostemon*. Previous studies found the latter genera to have developed within *Phyllanthus* and a proposal was made to merge all, thereby creating one giant genus with over 1200 species with an enormous variation in morphological features. To study the evolutionary history and possibilities in classification, we explored this genus in greater detail. A number of species of *Phyllanthus* are also used in traditional medicine and two well-known examples (*P. emblica* and *P. acidus*) are species grown for their their edible fruits that contain high numbers of vitamin C.

The genus *Phyllanthus* was first described by Linnaeus in 1753 and with subsequent publications over the past 250 years, this group has grown to include over 800 species. Some have been treated as separate genera by some authors, but in the 19th century clusters of similar species were united in one *Phyllanthus* and grouped in a classification below the genus level consisting of over 40 sections. From the 1950s onwards, the relation between these sections was organized in several subgenera. This classification was based on anatomy, which was expanded with the incorporation of various morphological features including habit, branching system and pollen morphology. However, taxon recognition often focused on specific regions instead of complete (sub) groups and in chapter 3 we compiled a checklist that incorporated all the subgroups that have been defined and placed almost all species in this framework. Almost all 800 species were in the framework, based on morphological features and past classifications, and it resulted in the recognition of 18 subgenera, 70 sections and 14 subsections. The various subgenera presented strong candidates for a new classification on genus level of *Phyllanthus* and the related genera. We followed up the checklist with the publication of a new species (chapter 2), a study on *P.* subgenus *Macraea* (chapter 4) which included species never placed in that group before and a study of the species described by Koorders for the island of Sulawesi (chapter 5).

The results from previous phylogenetic studies had included around 10% of the species of *Phyllanthus*. In our phylogenetic study based on five markers (chapter 6), we included species from all subgenera and 53 of the 70 sections, while discussing patterns of morphological evolution and several instances of convergent

evolution. Characters such as the specialized branching system present in the majority of species, has been lost in several clades independently. Other features such as the loss of the nectar disk, or the simplified style and stigma morphology that is associated with a specific pollination system involving mutualistic moths is also indicated to have evolved several times. By comparing the phylogeny with the framework outlined in the checklist of chapter 3, we found multiple instances of paraphyletic subgroups often related to small (usually monospecific) taxa nested in other groups. However, the backbone phylogeny showed good support between major clades that are often morphologically distinct.

In addition to their diversity and evolution, *Phyllanthus* is also an interesting group with regards to the medicinal usage of several species. Species of *Phyllanthus* are used in various ways and extractions are also sold as tablets with vitamin supplements, to help kidney and liver function or to combat inflammation. A few species are grown in botanical gardens throughout Europe, which provided a great opportunity to study their antimicrobial and antifungal effects. In chapter 6 we sampled several species from the living collections of the Hortus botanicus Leiden and we used Proton Nuclear Magnetic Resonance (^{1}H NMR) to study their metabolite profiles. This was correlated with the results from a screening for bioactivity against *Escherichia coli* and *Staphylococcus aureus* and followed by a targeted approach using High Performance Thin Layer Chromatography (HPTLC). Indications were found for significant activity against gram-negative bacteria for *P. arbuscula, P. muellerianus, P. tenellus and P. urinaria*, but we were unable to identify the compounds that underly this. Future studies would need a larger sample set, but our results indicate that their focus could be especially on the phenolics produced by these species.

Following the results from our phylogenetic work, it was necessary to create a new classification for *Phyllanthus* and its related genera. While previous studies had proposed to merge everything, we decided to divide *Phyllanthus* into smaller segregates (Chapter 8). We reinstated the genera *Cathetus*, *Cicca*, *Dendrophyllanthus*, *Emblica*, *Kirganelia*, *Moeroris*, *Nellica* and *Nymphanthus* while *Lysiandra* was raised to the generic level. This retained the genera *Glochidion*, *Breynia* and *Synostemon* while creating a classification based on recognisable monophyletic groups. More than 600 species were transferred to another genus in this new classification. Several paraphyletic sections and subgenera are expanded to include the species nested within them. Both solutions to the paraphyly of *Phyllanthus* are valid, union of all or separation, but by dividing *Phyllanthus* into smaller groups as proposed here, the evolutionary history of the group is reflected in a much better way.

Using the phylogeny from chapter 5 we had previously looked at various morphological evolutionary patterns, but barely touched on the subject of how the whole tribe became so diverse and how it dispersed through time. While a broad treatment of *Phyllanthus*, considered it to be a pantropical genus, the reinstated segregate genera are often restricted to one or two continents. In chapter 9 we explain how the current distribution came to be and whether there have been shifts in its diversification. Traditionally the pantropical distribution of the clade has been attributed to a Gondwanan origin, but we found that tribe Phyllantheae probably originated during the Late Palaeocene close to the Palaeocene-Eocente Thermal Maximum (PETM). The genera *Nellica* and *Cathetus* indicate early dispersals between Africa and Asia, with the former genus also being present in North America. This distribution is consistent with a possible boreotropical origin followed by subsequent extinction in Europe. Fossil pollen findings in Europe also indicate that *Flueggea* or a related genus was present there at the time. Within clades/genera we found that their distribution is often the result of a limited number of dispersal events. While studying diversification rate shifts in the tribe, we found that the pollination mutualism present in several clades is not always consistent with an increase in diversification. In some clades, this did not lead to a higher rate of diversification and this is possibly caused but not fully explained by a difference in dispersal vectors/mechanisms.

The evolution of tribe Phyllantheae is explored here in relation to its systematics and dispersal history, but as outlined in chapter 10, many questions remain to be answered. Future studies can explore the evolution of specific clades and how the species diverged and adapted to different conditions. Especially the pollination mutualism with moths present in several clades provides a great opportunity to study how both pollinator and plant are locked in an evolutionary struggle and how they adapt to each other. In this thesis I have expanded upon our knowledge of the phylogeny of *Phyllanthus* and its related genera while trying to provide a system for all species that were attributed to it. While this classification may change again in the future, the tribe remains an interesting study group.