

## A study on phylogeny of phyllanthaceae using morphological features

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### Abstract

The Phyllanthaceae family of plant contains around 6000 species in 59 accepted genera. It is one of the five subfamilies of Euphorbiaceae *sensu lato* and is now a family on its own after the latter is split into five family. The family is represented by herb, shrubs and trees. Mesquite software running Zephyr package to use PAUP algorithm is used to describe the species relationship and morphological phylogeny. 31 contrasting binary character states that can be used to define the individual species were selected and the matrix from 30 collected taxa were analysed by random taxon addition followed by a tree bisection reconnection, all the characters are unweighted and are unordered. Furthermore, jackknife-analysis with 50 replicates of the characters were also performed. The analysis results a tree with consistency index of 0.39 and a retention index of 0.79 with tree length of 76 steps. The analysis shows the two tribes Antidesmatoideae and Phyllanthoideae in different clade. The resultant trees show support for various subfamily and tribe while there are some contrasting results.

**Keywords:** mesquite, paup, phyllanthaceae, phylogeny, zephyr

### Introduction

Phyllanthaceae is a family of flowering plants that was recognized earlier as a sub-family in the Euphorbiaceae family. The family shows vast range in morphological features and rangers from herbs, shrub and trees. Phyllanthaceae are a pantropic family about 2000 species in 60 genera that is morphologically diverse. The family was separated from Euphorbiaceae together with Picrodendraceae and Putranjivaceae and Euphorbiaceae *s.s.* as a result of molecular work done (Savolainen *et al.*, 2000<sup>[11]</sup>; APG II, 2003)<sup>[14]</sup>. As part of a study on the systematics of Euphorbiaceae, molecular systematics of Phyllanthaceae were mostly studied. *s.l.* (Rosabelle *et al.*, 2005)<sup>[9]</sup>.

Recent study in the family shows the divergence form the euphorbiaceae in a number of variable possibility and was further classified as a different family in the Angiosperm phylogeny Group III. The family is divided into two sub family only i.e. Antidesmatoideae and Phyllanthoideae, the later holding a greater number of genera than the former. Molecular study by using *rbcL* (Wurdack *et al.*, 2004)<sup>[19]</sup> *matK* and *PhyC* sequence data (Samuel *et al.*, 2005)<sup>[10]</sup> are essentially important and support grouping of genera within phyllanthaceae that differ from those proposed in Webster classification. The studies found two major family clades that matched two different morphological characters. One clade predominantly has fascicular inflorescences, while the other clade mainly has elongated axes of inflorescence. Nevertheless, anatomical characters of wood, morphological characters of flower, and fruits were often found to be homoplastic. (Hasendra *et al.*, 2005).

Pax (1924)<sup>[7]</sup> first portrayed evolutionary relationships between Phyllanthoideae in intuitive phylograms. In his study of Flueggea, Webster (1984)<sup>[17]</sup> seems to have been the first to perform a cladistic analysis of any Phyllanthaceae (and any Euphorbiaceae). Levin (1986) research on Phyllanthoideae is the most widely published study to date, as well as the revolutionary in its explanation

of the utility of foliar morphology in relationship resolution. Phyllanthoideae (as classified under Euphorbiaceae *s.l.*) are known to be the most primitive Euphorbiaceae *s.l.* subfamily from which the others are derived or to which they are related (Webster, 1994)<sup>[18]</sup>. The Phyllanthoideae subfamily was proposed by Simpson and Levin (1994)<sup>[12]</sup> as paraphyletic evidenced by plesiomorphic characters.

To evaluate species relationship on a morphological level, we use certain character that are either contrasting to one another or have two or three sets of characters that can be categorized into two sets. On selection of character emphasis was given first to characters that defines and differentiate a particular genera, tribe or sub-family and secondly contrasting characters that were present on a number of plants was chosen, and the rest of the characters are added in order to have more accurate result as we conclude more the characters used better will be the resultant tree. 30 taxa were collected and in addition study of literature was used. We refrain for giving weights to certain characters during PAUP analysis because whether these characters were derived or ancestral was not clearly defined for a few of character.

### Materials and methods:

**Plant collection and identification:** 30 plant species were collected from different parts in Aizawl district, Mizoram, India. The morphological features used in the current research are noted and in addition certain literature were consulted for various genera and their characters. Identifications were done with the help of published papers, identification keys and various flora book.

**Cladistic analysis:** For conducting cladistic analysis 30 plant species belonging to the family euphorbiaceae was selected with 31 characters states were coded as binary that represents various characters that are carefully selected so that there could be clear distinction in the genera, sub families and clades. All 31 characters states are unordered and unweighted. Gaps are treated as missing. The analysis

was conducted in Mesquite software (Maddison and Maddison, 2018) [6, 5]. The binary character sets were analysed by Zephyr (Maddison and Maddison, 2018) [6, 5] package that runs on the Mesquite software itself. The matrix was analysed first jack-knife analysis with 50 replicates resulting in a number of trees, from which consensus tree was made. The data obtained was again analysed on PAUP4 (Swofford, 2003) [13] using 100 random taxon addition followed by branch swapping using tree bisection reconnection algorithm in the PAUP4 heuristic search program, and obtained 1274 trees. The tree is unrooted and uses midpoint method and there is no specific outgroup designated for the family. The tree is edited with Adobe Photoshop CS3.

Characters used in cladistic methods:

Habit: Phyllanthaceae shows variation in growth form trees, shrubs or lianas (rarely) or herbs, (Watson & Dallwitz, 1992) [16] deciduous or evergreen, monoecious or dioecious.

Leaves: Alternate or opposite, simple (pinnately compound in the case of *Bischofia javanica*); stipules present or rarely absent; petiole usually present or sometimes absent, venation mostly pinnate.

Flowers: Unisexual, sepals 4 to 6, size ranges from a few mm to a few cm, distinct or connate basally; petals absent or present 4 or 45 if present, mostly free, nectary present or absent, stamens 2 to many, distinct or connate, pistil 1, [2–3]–10[–15]–carpellate, [2–]3–10[–15]–locular, ovules 2 per locule; styles 2 to many, distinct or connate, unbranched or bifid; stigmas 2 to many.

Fruits: Fruits are either capsule or drupe, dehiscent or indehiscent. Only two characters are taken for this category in order to reach fast and conclusive results.

Characters and characters states used in Cladistic analysis of Phyllanthaceae.

1. Habit Herbaceous = 0; Woody = 1
2. Sexuality Monoecious = 0; Dioecious = 1
3. Leaf Shedding Evergreen = 0; Deciduous = 1
4. Longevity Annual = 0; Perennial = 1
5. Branching Monopodial = 0; Sympodial = 1
6. Leaf size Small/ Minute = 0; Macrophyll = 1
7. Venation Pinnate = 0; Palmate = 1
8. Pedicel Absent = 0; Present = 1
9. Stipules Absent = 0; Present = 1
10. Phyllotaxy Alternate = 0; Opposite = 1
11. 11. Leaf Simple = 0; Compound = 1
12. Foliaceous Bract Absent = 0; Present = 1
13. Flower Solitary = 0; Inflorescence = 1
14. Inflorescence Axillary = 0; Terminal = 1
15. Petals Apetalous = 0; Petalous = 1
16. Flower size Conspicuous = 0; Showy = 1
17. Inflorescence Spike/raceme = 0; Glomerules/fascicle = 1
18. Floral disc Absent = 0; Present = 1
19. Inflorescence axis Micro = 0; large = 1
20. Aestivation Imbricate = 0; Valvate/ Induplicate = 1
21. Involucre Absent = 0; Present = 1
22. Floral bract Absent = 0; Present = 1
23. Flower on inflorescence Male and Female = 0; Separate = 1
24. Nectar glands Absent = 0; Present = 1
25. Ovary Bilocular/Trilocular = 0; >4 locular = 1
26. Style Bifid = 0; Undivided = 1
27. Calyx/Corolla Free = 0; Connate = 1
28. Filaments Distinct / Free = 0; Connate = 1
29. Stamen number 1-5 = 0; >5 = 1

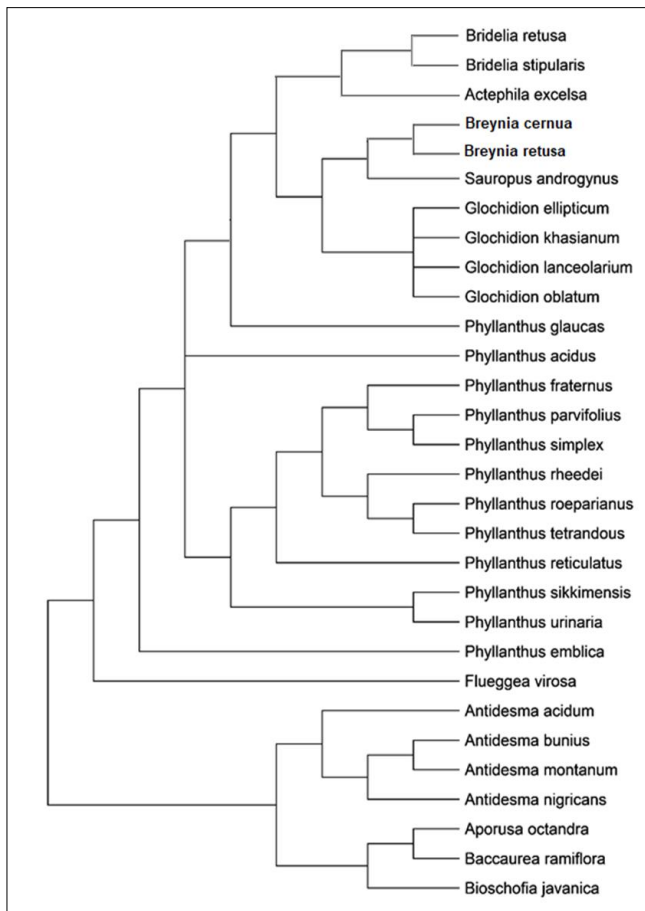
30. Fruit Dehiscence = 0; Indehiscence = 1

31. Fruit Capsule/Drupe = 0; Berry = 1

**Results**

**Table 1:** Data matrix of character states used in the cladistic analysis. (“?” denotes missing characters).

Taxon Number	0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2
Species Name	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1
Antidesma acidum	1 1 0 1 1 1 0 1 1 0 0 0 1 1 0 0 0 1 1 1 0 1 1 ? 0 0 1 0 1 1 1
Antidesma bunius	1 1 0 0 1 1 0 1 1 0 0 1 1 1 0 0 0 1 1 1 0 1 1 ? 0 0 1 0 0 1 1
Antidesma montanum	1 1 0 0 1 1 0 1 1 0 0 1 1 1 0 0 0 1 1 1 0 1 1 ? 0 0 1 0 1 1 1
Antidesma nigricans	1 1 0 ? 0 1 ? 1 1 0 0 0 1 1 0 0 0 1 1 1 0 1 1 ? 0 0 1 0 1 1 1
Aporosa octandra	1 0 0 1 1 1 0 1 1 0 0 1 0 1 0 0 0 0 1 1 1 1 1 1 1 0 0 1 ? 0 1 0
Baccaurea ramiflora	1 1 0 1 1 1 0 1 1 1 0 1 1 1 0 0 0 0 1 1 0 0 1 0 0 0 1 0 1 1 0
Bischofia javanica	1 1 0 1 1 1 0 1 1 1 0 1 1 0 0 0 0 1 1 0 0 1 ? 0 0 0 0 1 1 1
Actephila excelsa	1 0 0 1 1 1 0 1 1 1 0 1 0 0 1 1 1 1 0 0 0 0 0 0 0 1 0 0 1 0 0
Breynia cernua	1 1 0 1 1 1 0 1 1 0 0 0 1 0 1 0 1 1 0 0 0 0 ? 0 1 0 1 0 0 1
Breynia retusa	1 1 0 1 1 1 0 1 1 0 0 0 1 0 1 0 1 1 0 0 0 0 ? 0 1 0 1 0 0 1
Bridelia stipularis	1 ? 0 1 1 1 0 0 0 0 0 0 1 0 1 0 1 1 0 0 0 0 ? 0 1 0 1 0 0 1
Bridelia retusa	1 1 0 1 1 1 0 1 1 0 0 0 1 0 1 0 1 1 0 0 0 0 ? 0 1 0 1 0 0 1
Flueggea virosa	1 0 0 1 1 0 0 1 1 0 0 0 1 1 0 0 1 1 0 0 0 0 0 0 0 0 1 1 1 1 0
Glochidion ellipticum	1 0 0 1 1 1 0 1 1 0 0 0 1 0 0 1 0 0 0 0 0 0 ? 1 1 0 1 0 1 0
Glochidion khasianum	1 0 0 1 1 1 0 1 1 1 0 0 0 1 0 0 1 0 0 0 0 0 ? 1 1 0 1 0 1 0
Glochidion lanceolarium	1 0 0 1 1 1 0 1 1 0 0 0 1 0 0 1 0 0 0 0 0 0 ? 1 1 0 1 0 1 0
Glochidion oblatum	1 0 0 1 1 1 0 1 1 0 0 0 1 0 0 1 0 0 0 0 0 0 ? 1 1 0 1 0 1 0
Phyllanthus acidus	1 0 0 1 1 0 0 1 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0 0 0 0 1 0 0
Phyllanthus emblica	1 0 0 1 1 0 0 1 1 0 0 0 1 1 0 0 1 1 0 0 0 0 0 0 0 0 0 0 1 0 0
Phyllanthus fraternus	0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 1 1 0 0 0 0 ? 0 0 0 0 1 0 0
Phyllanthus glaucus	1 0 0 1 0 1 0 1 ? 0 0 0 0 1 0 0 1 1 0 0 0 0 ? 0 0 0 0 1 0 0
Phyllanthus parvifolius	0 0 1 0 0 0 0 1 1 0 0 0 0 1 0 0 1 1 0 0 0 0 ? 0 0 0 0 1 0 0
Phyllanthus reticulatus	1 0 0 1 1 0 0 1 1 0 0 0 0 0 0 0 1 1 0 0 0 0 0 1 0 0 0 0 1 0 0
Phyllanthus rheedei	0 0 0 1 0 0 0 1 0 0 0 0 0 0 0 0 1 1 0 0 0 0 ? 0 0 0 0 1 0 0
Phyllanthus roeparianus	0 0 0 ? 0 0 0 1 0 0 0 ? 1 0 0 1 1 0 0 0 0 ? 0 0 0 0 1 0 0
Phyllanthus sikkimensis	1 0 0 0 1 0 0 1 1 0 0 0 0 1 0 0 1 1 0 0 0 0 ? 0 0 0 0 1 0 0
Phyllanthus simplex	0 0 1 0 0 0 0 1 1 0 0 0 0 0 0 0 1 1 0 0 0 0 ? 0 0 0 0 1 0 0
Phyllanthus tetrandrus	0 0 1 1 0 0 0 1 0 0 0 ? 1 0 0 1 1 0 0 0 0 ? 0 0 0 0 1 0 0
Phyllanthus urinaria	0 0 0 0 1 0 0 1 1 0 0 0 0 1 0 0 1 1 0 0 0 0 0 1 0 0 0 0 1 0 0
Sauropus androgynus	1 1 0 1 1 1 0 1 1 0 0 1 0 0 0 0 1 0 0 0 0 0 ? 0 1 1 1 0 0 0



**Fig 1:** A cladogram showing relationship of Phyllanthaceae.

The analysis yields an unrooted tree with treelength of 69, consistency index of 0.4348 and a retention index of 0.8178. One of the character state is constant and parsimony uninformative (7. Venation) and 3 characters states are not constant but parsimony uninformative (8. Pedicel, 11. Leaf type and 21. Involucre), these uninformative characters are because there is only one Taxon each that match for these characters in their respective cases and all the other taxa have contrasting feature. The resultant trees show a clear distinction in the two subfamilies, Phyllanthoideae and Antidesmatoideae and are separated in two Clades that represents the former and the later which is supported by the recent APG III system of classification (APG III, 2009) [2]. *Flueggea* is a sister taxon to *Phyllanthus* and forms a polyphyletic group under the tribe Phyllanthae. *Phyllanthus* is polyphyletic from our findings, and occurs in clade with *Antidesma*, *Breynia*, *Sauropus* and *Glochidion*. *Baccaurea ramiflora* and *Aporosa octandra* are sister taxon accordingly in tribe Scepae (Hoffmann *et al*, 2006) [3]. *Sauropus*, *Glochidion* and *Breynia* forms a monophyletic group but clarification could be made according to classification proposed by Webster. The close relationship between *Phyllanthus* and other genera like *Sauropus*, *Glochidion* and *Breynia* are in question according to our findings, *Sauropus* and *Glochidion* however form a close relationship with other genera *Bridelia* and *Actephila* while *Phyllanthus* forms closer relation to *Flueggea*.

### Discussion

Phyllanthaceae is family of plants comprising of trees, shrubs and herbs and a predominantly found in tropical

region, the family is grouped into 2 monophyletic subfamilies Phyllanthoideae and Antidesmatoideae.

*Phyllanthus* is one of the largest genera in the family, the genus *Phyllanthus* have close association with other genera like *Breynia*, *Sauropus* and *Glochidion*. Hoffmann *et al* (2006) [3] proposed that *Sauropus*, *Breynia*, and *Glochidion* be included in *Phyllanthus* in order to prevent paraphyly of the genera. However according to Van Welzen *et al* (2014) [15], because there is no morphological feature that defines the extended *Phyllanthus*, he suggested dividing *Phyllanthus* into > 20 smaller genera.

From our finding the genus *Phyllanthus* is not a monophyletic genus because *P. glaucus* and *P. acidus* diverge from the main group. *Phyllanthus glaucus* forms closer relationship with *Breynia* in an entirely different clade. Whether to incorporate three genus *Glochidion*, *Sauropus* and *Breynia* into *Phyllanthus* cannot be accomplish by taking factors such as 'morphology of flower and phyllanthoid branching' at least from our finding. However, *P. glaucus* placed in closer association with *Breynia*, *Sauropus* and *Glochidion* could also means that these four genera have closer relationship with one another as suggested by Hoffman *et al*, 2006 [3].

Based on morphology and molecular data, the *Sauropus* genus and the closely related genus *Breynia* have recently been thoroughly reviewed (Pruesapan *et al*. 2008, 2012; van Welzen *et al*. 2014) [15].

Van Welzen *et al*. 2014 [15] expanded *Breynia* to include *Sauropus*, based on the results of well-sampled phylogenetic analysis. This is supported from our finding as *Breynia* and *Sauropus* forms a monophyletic group, from morphological observation alone, one could simply conclude that these two genera are extremely similar with one another. The three species??

All species under the sub family Antidesmatoideae form a single clade according to our findings. The genus *Antidesma* is a monophyletic genus. All species under the sub family are in order according to the latest classification systems (APG II 2003, APG III 2006) as *Aporosa* and *Baccaurea* are group together representing tribe Scepae and *Bischofia* in different group and a sole genus of tribe Bischofieae.

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