

Morphometric Analysis of Genus *Premna* in India

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Abstract- In the present investigation, an attempt is made to apply numerical methods for examining morphological variation to infer phenetic relationships among *Premna* species occurring in South India. A data matrix of 50 vegetative and reproductive characters of 31 Indian taxa was prepared. A dendrogram is plotted from a distance matrix generated using an arithmetic average (UPGMA) clustering algorithm and standardized data based on the average taxonomic distance subjected to the unweighted pair-group clustering method using NTSYS Software version 2.02i.

Keywords: Morphometric Analysis, UPGMA method, Cladogram, *Premna* species, Phenetics

INTRODUCTION

Over the years, taxonomy has found important practical applications in various fields of science such as theoretical and applied biology including agriculture, evolutionary study, forestry, public health, biodiversity management and environmental issues. (Godfray, 2002). Adanson (1763) proposed that classification should be based on characters covering all aspects of plant such as leaf, flower, fruit, seed, and each character should be given equal importance. As a consequence, a mathematical approach has been established by taxonomists called Numerical Taxonomy (Sokal and Sneath, 1963). Morphological data are considered significant in systematics because variation exhibited by morphological traits is supposed to be categorized by gaps between taxa which reflect their evolutionary arrangement emerged through morphological changes (Otte and Endler, 1989).

Numerical taxonomy, also termed as morphometrics deals with grouping by numerical methods of taxonomic units into taxa on the basis of their character state (Sneath and Sokal, 1973). The modern methods of numerical taxonomy had their beginning from the contributions of Sneath (1957), and Sokal and Michener (1958) which culminated in the publication of *Principles of Numerical Taxonomy* (Sokal and Sneath, 1963), with an expanded and updated version *Numerical Taxonomy* (Sneath and Sokal, 1973). Before the development of modern methods of cladistics, the numerical methods were used for drawing phylogenetic inferences from the data analysis.

Cluster analysis and principal component analysis are two techniques commonly used in numerical classification. Cluster analysis produces a hierarchical classification of taxa based on the similarity matrix. It thus provides a logical means of expressing the relationship existing between taxa. Despite of the criticism of using cladistic analysis in taxonomy, cladistic methods have become a most useful technical tool for clarifying intrafamilial relationships. Numerical taxonomy has the power to integrate data from a variety of sources like physiology, phytochemistry, embryology, anatomy, palynology, chromosomes, ultrastructure, morphology and micromorphology. Numerical taxonomy aims at determining phenetic relationships between organisms or taxa.

The genus *Premna* belongs to family Verbenaceae which are generally trees and shrubs in habit and very rarely herbs and climbers. This genus belonging to the family *Verbenaceae* was established by Linnaeus (1771) based on *P. serratifolia*. The genus now contains about 200 species worldwide which are mainly distributed in tropical and subtropical Asia, Africa, Australia and the Pacific Island (Harley et al., 2004). A preliminary study of the genus in Thailand was first undertaken by Fletcher (1938), enumerating 30 species. Later, Moldenke (1980), The Forest Herbarium (2001) and Govaerts et al., (2008) reported the checklists of *Premna* with 39, 19 and 33 taxa, respectively. About 35 species occur in India.

In the present investigation, an attempt is made to apply numerical methods for examining morphological variation to infer phenetic relationships among *Premna* species occurring in India.

REVIEW OF LITERATURE

The genus *Premna* was described by Linnaeus (1771) for two species, *P. integrifolia* and *P. serratifolia*, which were collected by Paul Hermann in Ceylon. It was placed in "Didynamia Angiosperma", where it was retained by Murray (1774), Gmelin (1791), Schreber (1791), Persoon (1797), Willdenow (1800), Poiret (1823), Sprengel (1825, 1831), Roxburgh (1832), Blanco (1837), Dietrich (1843) and a few others. Scopoli (1777) placed it in "Personata" which was later accepted for the genus by Giseke (1792) and Batsch (1802). Gaertner (1788) recorded it in "Centuria Quarta", Jussieu (1789) in "Vitices", Necker (1790) in this "Plasyrgophyta", and Reichenbach (1828) under the tribe "Verbeneae" in the Labiatae. In 1806, de Jussieu referred it to the family Verbenaceae where it has been retained by the majority of botanists.

Baffling (1830) split the Verbenaceae into two sections:- Viticeae and Verbeneae, with *Premna* in the section Viticeae. This section was accepted for the genus by Spach (1840). In 1836, Endlicher divided the family into three tribes:- Lippieae, Lantaneae and Aegiphileae, with *Premna* in the tribe Lantaneae. This tribe was accepted for the genus by Meisner (1840), Endlicher (1841), Brongniart (1843), Dietrich (1843) and Walpers (1845). Schauer (1847) classified the Verbenaceae into three new tribes:- Verbeneae, Viticeae and Avicenniae, with *Premna* in the tribe Viticeae. The genus was retained in the new tribe by Walpers (1852), Miguel (1858), Bentham (1870), Bentham & Hooker (1876), Bailey (1883, 1901, 1913), Hooker (1885), Durand (1888), King & Gamble (1909), Ewart & Davies - (1917), Fletcher (1938), Lemée (1943) and a few others. Schauer (1847) subdivided the tribe Viticeae into three subtribes:- Symphoremeeae, Caryopterideae and Viticeae, with *Premna* in the subtribe Viticeae. He also split the genus into two sections:- Gumira and Premnos, based chiefly on their calyx being regularly 4- or 5-toothed. The subtribe Viticeae

was later accepted for the genus by Miguel (1858) and Bentham (1870), and the division of the genus into two sections was adopted by Miguel (1858), H J Lam (1919), Moldenke (1959, 1971) and a few others. In 1895, Briquet reclassified the Verbenaceae and upgraded the tribe Viticeae to a subfamily Viticoideae. The latter consisted of four tribes:- Callicarpeae, Tectoneae, Viticeae and Clerodendreae, with *Premna* in the tribe Viticeae. This classification was adopted by Dalla Torre & Harms (1904), H.J. Lam (1919), Gardner (1931), June 11 (1934), Moldenke (1959, 1971) and Melchior (1964). In the same treatment, Briquet (1895) subdivided the genus into five sections: *Holopremna*, *Odontopremna*, *Gumira*, *Premnos* and *Holochiloma*, each characterised chiefly by the size and number of their calyx-lobes. These sections were adopted by Dalla Torre & Harms (1904). The majority of botanists, however, have not divided the genus into sections, but have retained it in the Verbenaceae without reference to any subfamily or a tribe. In the present work, Briquet's (1895) classification of the Verbenaceae is followed in retaining *Premna* in the tribe Viticeae. The subgeneric sections proposed for the genus, however, are not accepted because of the unreliability of characters used.

A brief history of the genus *Premna* is given in the taxonomic revision of the Indian Verbenaceae by Rajendran and Daniel (2002). The first report of the genus *Premna* (Linnaeus, 1771) was based on the material (*Premna serratifolia*) collected by Konig from peninsular India (Nicolson *et al.*, 1988). In the beginning of 18th century; Willdenow (1800) described *Premna tomentosa* based on Klein's collection from India. Rottler (1803) described *Premna corymbosa* based on his collection at Tempakkam near Madras. He also specified about *Premna serratifolia* in his work. Jussieu (1806) described a species, *Premna flavescens* from Madras, which is now treated as a synonym of *Premna tomentosa*. Roth (1821), reported *Premna mollissima*, which is now treated as a variety under *Premna latifolia* Roxb. Roxburgh (1832) described 11 species of *Premna* from India. Graham (1839) reported four species viz., *Premna cordifolia*, *Premna integrifolia*, *Premna scandens* and *Premna nimmoniana* from western India. Walpers (1845) described 11 species of *Premna* from India in his monograph. In another study, Schauer (1847) included 25 species from India of which 9 were new and they were based on Wallich's numerical list. From peninsular India, Wight (1849) reported *Premna glaberrima* as a new species. Clarke (1885) described *Premna bengalensis*, *Premna coriacea*, *Premna khasiana*, *Premna milleflora* and *Premna villosa* as new species. Parkinson (1922) reported *Premna integrifolia* and *Premna divaricata* from the Andaman Islands. In another study, Rao (1986) reported the occurrence of *Premna coriacea*, *Premna parasitica*, *Premna pubescens* and *Premna pyramidatus* in Andaman Islands. Deshpande (1961) reported a new species, *Premna resinosa* to the Indian flora. According to Santapau and Henry (1973), there are 25 species of *Premna* in India. However, according to Moldenke (1980), there are 31 species and 11 varieties of *Premna* in India. In the taxonomic revision of Indian Verbenaceae, Rajendran and Daniel (2002), recognized 31 species and 6 varieties of *Premna*. Of these three species, viz., *Premna balakrishnanii*, *Premna debiana* and *Premna mundanthuraiensis* are new species reported from India. Recently, PrabhuKumar *et al.* (2013) reported the discovery of a new species *Premna rajendranii* from Western Ghats (Chinnar and Madukkarai) of Kerala. Apart from this, a research team comprising Robi, Augustin, Sasidharan and Udayan (2013) rediscovered an endemic and rare species of *Premna* viz., *Premna paucinervis* (C. B. Clarke) Gamble from the Vagamon hills along South Western ghats of Kerala after a lapse of 140 years of its original type collection by R.H. Beddome (1872) from Anamalayas, Western Ghats (Tamilnadu).

An overview of the genus *Premna* with special reference to its taxonomic history, classification and distribution in the tropical and subtropical regions is presented in this section. The genus, from its first report in 1771, was under the confused category due to its indistinguishable taxonomic characters and diverse morphological features. Presently, it is one of the largest genera of the family Verbenaceae with two hundred species worldwide which are mainly distributed in tropical and subtropical Asia, Africa, Australia and the Pacific Islands (Harley *et al.*, 2004).

The various *Premna* species are well known for their medicinal properties and have been used in Indian traditional system of medicine especially for diarrhea, stomach and hepatic disorders. Phytochemical work on this genus has resulted in the isolation of more than hundred secondary metabolites reported from the different species of this genus. The isolated compounds include iridoid and their glycosides diterpenoids, sesquiterpenoids triterpenoids, flavonoids, isoflavones, lignans, xanthones and other classes of compounds. The various biological activities including antioxidant, antibacterial, anti-inflammatory, cytotoxic and hepatoprotective have been displayed both at extract and pure compound level. (Rekha *et al.*, 2015). This genus also can be used traditionally in treating various ailments like rheumatism, asthma, dropsy, cough, fever, boils and scrofulous disease's. The different parts of the plant like leaves, stem, stem barks, root, root barks and wood have been used for extraction purpose (Atul Kabra *et al.*, 2015). *Premna serratifolia* is specific to anemia, weak digestion, piles, constipation, amavata and accumulation of fluid in the tissues or in body cavities and also effective to reduce serum cholesterol level and eliminating poison/ heavy metals from the body parts. *Premna serratifolia* is traditionally used in the treatment for beriberi, vaginal irritation, to relieve headache and as eye lotion (Dassanayake and Fosberg, 1980; Padua, 1999).

MORPHOMETRIC ANALYSIS

A total of 50 diagnostic characters related to habit, stem, leaf, flower, fruit and seed were chosen and scored for each OTU (Table 1). Both qualitative and quantitative characters were converted into binary states. A data matrix of 50 vegetative and reproductive characters of 31 Indian taxa was prepared. A dendrogram is plotted from a distance matrix generated using an arithmetic average (UPGMA) clustering algorithm and standardized data based on the average taxonomic distance subjected to the unweighted pair-group clustering method using NTSYS Software version 2.02i.

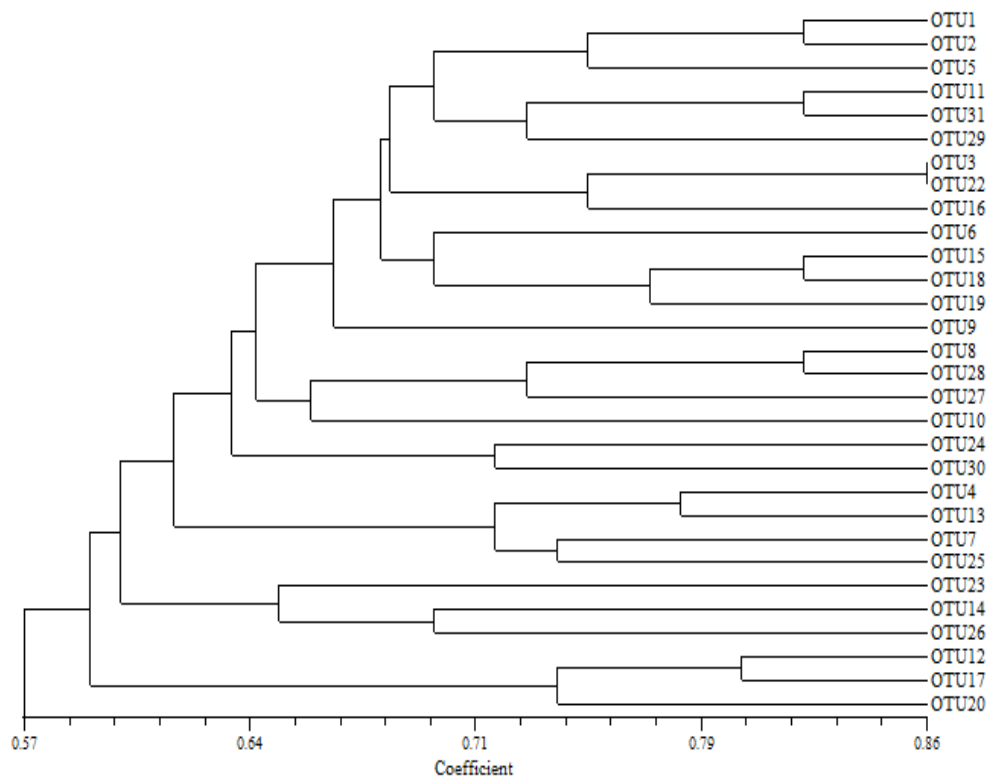
Table 1. Morphological characters and their state used in the numerical analysis.

1	Plants herb (0) Plants shrub or tree (1)	2	Plants more than 2cm(0) plants less than 2cm(1)
3	Plants not epiphytic(0) Plants epiphytic(1)	4	Plants common (0) Plants rare(1)
5	plants scandent(0) Plants erect (1)	6	Plants not stellate tomentose(0) Plants stellate tomentose(1)
7	Branches and branchlets sub terete(0) Branches and branchlets not sub terete(1)	8	Branches and branchlets villous(0) Branches and branchlets glabrous (1)
9	Leaves petiolate(0) Leaves sessile(1)	10	Petioles glabrous(0) Petioles pubescent(1)
11	Leaves glabrous(0) Leaves pubescent(1)	12	Leaves serrated (0) Leaves entire(1)
13	Leaves chartaceous(0) Leaves subcoriaceous(1)	14	Leaves cuneate at base(0) Leaves not cuneate at base(1)
15	Leaves elliptic(0) Leaves not elliptic(1)	16	Internodes 0.5-6.5cm long(0) Internodes not 0.5-6.5 long(1)
17	Nodes annulate(0) Nodes not annulate(1)	18	Inflorescence corymb or panicle(0) Inflorescence not corymb or panicle (1)
19	Petioles canaliculated(0) Petioles not canaliculated(1)	20	Bracts numerous(0) Bracts not numerous(1)
21	Bracts foliaceous(0) Bracts not foliaceous(1)	22	Bractlets present(0) Bractlets not present(1)
23	Bracts linear(0) Bracts not linear(1)	24	Bracts acuminate(0) Bracts not acuminate(1)
25	Peduncles obtusely 4 angular(0) Peduncles obtusely 4 angular(1)	26	Peduncles sulcate between angles (0) Peduncles not sulcate between angles(1)
27	Flowers pedicelate(0) Flowers sessile(1)	28	Flowers not foetid(0) Flowers foetid(1)
29	Calyx 3x2mm(0) Calyx 3x2mm(1)	30	Calyx copular(0) Calyx campanulate(1)
31	Calyx pubescent(0) Calyx glabrous(1)	32	Calyx toothed(0) Calyx not toothed(1)
33	Corolla infundibular(0) Corolla subinfundibular(1)	34	corolla two lipped(0) corolla not two lipped(1)
35	Corolla creamy or white(0) Corolla not creamy or white(1)	36	Corolla tube densely hairy at throat(0) Corolla tube densely hairy at throat(1)
37	Anthers divaricate(0) Anthers not divaricate(1)	38	Filaments slender(0) Filaments not slender(1)
39	Ovary truncate (0) Ovary truncate(1)	40	Ovary glabrous(0) Ovary glabrous(1)
41	Anthers globose(0) Anthers not globose(1)	42	Ovary obovoid(0) Ovary obovoid(1)
43	Style slender(0) Style not slender(1)	44	Stigma 2 lobed (0) Stigma not 2 lobed(1)
45	Stigma lobes equal(0) Stigma lobes not equal(1)	46	Plants bearing fruits(0) Plants not bearing fruits(1)
47	Drupes obovoid(0) Drupes not obovoid(1)	48	Fruiting calyx copular(0) Fruiting calyx copular(1)
49	Drupes black when mature(0) Drupes not black when mature(1)	50	Fruiting calyx glabrous(0) Fruiting calyx not glabrous(1)

DISCUSSION

Morphometric analysis

A morphometric study on *genus Premna* is first of its kind in India. A cladogram is plotted from a distance matrix generated using an arithmetic average (UPGMA) clustering algorithm and standardized data based on the average taxonomic distance subjected to the unweighted pair-group clustering method using NTSYS Software version 2.02i. The cladogram obtained evidently demonstrated that 31 taxa used in the morphometric analysis descend under 8 distinct clades portraying the inter relationships among the species.



Clade 1 represents 3 species of *Premna* which are herbs characterised by anthers divaricate, anthers not globose, ovary glabrous, petioles canaliculated, drupes obovoid that include *p.herbacea*, *p.macrophylla*, *p.nana*. Clade I is further subdivided into four sub-clades. Subclade Ia is represented by two taxa *P. herbacea* and *P. macrophylla* which can be distinguished by petioles pubescent, bracts foliaceous, nodes annulate.

The clade II is represented by 3 taxa which are shrubs *viz.* *P. punduana*, *P. khasiana*, *P. resinosa* in which later two species are characterized by leaves subcoriaceous, branches and branchlets are terete and glabrous. *P. punduana* is usually distinguished by its densely hairy throat of corolla.

Clade III is represented by 4 taxa *P. bracteata*, *P. interrupta*, *P. debiana*, and *P. recemosa* is characterised by sessile flowers, internodes 0.5-6.5cm long. Among which *two* diverges separately and can be distinguished by its fruits while the other two having fruits.

The clade IV is represented by two *P. pyramidata*, *P. tomentosa* which are characterized by their stellate tomentose nature. They have some other common characters like nodes annulate, bracts foliaceous, calyx campanulate etc.

Clade V contains 4 taxa which are scandent shrubs *P. divaricata* and *P. scandens* are closely related and similar in their reproductive characters. *P. rubens* is usually distinguished by pubescent petioles and small bracts. *P. glaberrima* is distinguished by truncate ovary.

Clade VI is represented by a single species *P. esculenta* is distinguished by its small size.

Clade VII is represented by 7 taxa which are characterized by their petiolate, pubescent, chartaceous leaves, pubescent calyx. Out of this 7 *P. milleflora* and *P. latifolia* show some similarities like leaves cuneate at base, peduncles subterete, calyx 3x2mm. Then *P. lucidula*, *P. pinguis*, *P. bengalensis* are together form a subclade which are characterized by their annulate nodes, linear bracts, ovary not obovoid. *P. mundanthuraiensis* is distinguished by villous branch and branchlets. *P. corymbosa* is separated by its scandent nature.

Clade VIII represented by 6 taxa *P. serratifolia*, *P. wighiana*, *P. hamiltonii*, *P. coriacea*, *P. barbata*, *P. balakrishnanii* which are characterized by their obtusely 4 angular peduncles, pubescent calyx, canaliculate petiole. *P. wighiana*, *P. hamiltonii* show some similarities like foetid flowers, globose anthers, entire leaves. *P. barbata*, *P. balakrishnanii* also have some similarities. Their branches and branchlets are glabrous, leaves serrated, linear bracts, obovoid drupes. *P. coriacea* is a scandent, epiphytic plant.

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