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A Comparative Study on Selected Members of Families Verbenaceae and Lamiaceae with Special Emphasis on Pollen Morphology

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Abstract

Verbenaceae and Lamiaceae are two advanced members of the class Dicotyledonae. As both of them share some similarities and dissimilarities, the proper systematic placement of some members of the family Verbenaceae is still remain a topic of discussion. The present study aims to compare some selected members of Verbenaceae and Lamiaceae by giving special emphasis on their pollen morphology. The different taxa under investigation were *Vitex negundo* L., *Clerodendron infortunatum* L., *Duranta plumier* Jacq., *Lantana camara* L., *Leucas aspera* Spreng., *Plectranthus scutellarioides L.* and *Ocimum sanctum* L. Pollen morphological analysis was done using Scanning Electron Micrographs. Although these families show some similarities, they differ in many features such as phyllotaxy, inflorescence type, style, aperture number and type of pollen. By evaluating both morphological and palynological characters, the authors have the opinion that the transfer of the genus *Vitex* to Lamiaceae seem to be a wise decision while it is better to retain the genus *Clrodendrum* in Lamiaceae .

Keywords: Verbenaceae, Lamiaceae, Pollen morphology, Vitex negundo, Clerodendrum infortunatum

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1. Introduction

Plant systematic deserves more attention in the field of plant biology due to its importance in ethno botany, pharmacology and allied areas. In the present scenario, taxonomic study is not only deals with external morphology, but also aided with the comparative anatomy, embryology, cytogenetics, phytochemistry, palynology etc. Thus, in addition to the morphological characteristics, it has been aimed to compare the pollen morphological features of the families- verbenaceae and Lamiaceae. Verbenaceae and Lamiaceae have been placed under the order Lamiales by Bentham and Hooker [1].The present study discussed about the inclusion of some genera of the family Verbenaceae into Lamiaceae in the APG system of classification which were treated in Verbenaceae by Benthem and Hooker.

In the APG III classification, Verbenaceae and Lamiaceae has been placed in the order Lamiales which is included under the Asterids clade of Eudicots. Many genera which were included in Verbenaceaeby earlier classifications are treated under the family Lamiaceae in APG III classification.

Verbenaceae comprises 75 genera with over 3000 species [2]. Recent phylogenetic studies have shown that numerous genera which were traditionally classified in Verbenaceae are now in Lamiaceae (APG III-2009). The new narrowly circumscribed Verbenaceae family includes 35 genera and 1200 species (APG III). Family contains trees, shrubs and herbs notable for heads, spikes or clusters of small flowers and many of which have an aromatic smell. The distinguishing characters of this family are tubular flowers, didynamous stamen and forked or capitate stigma.

Lamiaceae is a large cosmopolitan family. The enlarged Lamiaceae contains about 236 genera and has been stated to contain 6,900 to 7,200 species (APG III), but the World Checklist lists 7,534. The earlier name was Labiatae, which was originated from the nature of corolla: with an upper lip and a lower lip. Stem quadrangular with opposite leaves. Plants are usually aromatic. Inflorescence is a Verticillaster. Stamens 4, didynamous; bicarpellary ovary which is tetra-locular with gynobasic stigma. The fruit is commonly a dry nutlet. The Lamiaceae is an economically important family.

Pollen characters are specific for different genera and may vary from species to species. Diversity of the aperture types and multiplicity of the exine sculpturing make them highly useful characters of taxonomic value [3]. From the studies on pollen conducted over years, it is now realised that the morphology of pollen as contained in the exine wall may be resolved into five groups of characters, namely: germinal aperture, exine ornamentation, exine strata, size and shape in the order of their importance. A combination of these features provides a

particular taxon an entity in itself. The application of pollen morphology in taxonomy and phylogeny are based on the above facts. In studies involving hybrids and their parents, the pollen grains have provided interesting data on the inheritance patterns, relating to exine ornamentation and the germinal aperture [4]. Based on external morphology and palynology, the taxonomic comparison of the families, Verbenaceae and Lamiaceae have been framed in the current study to determine the extent of pollen diversity within the families. The systematic relationship of the group using additional data of pollen morphology has also been discussed.

2. Material and methods

2.1. Plant material

The plants belonging to both the families were collected in and around the campus of Sree Narayana College, Cherthala, Kerala, India (**Table.1**). Photographs were taken using digital camera. The collected plants were watered and stored in polythene bags to keep the materials fresh till we reach the lab.

Table 1. List of taxa collected for present study.

Sl. No.	Name of taxon	Family	Place of collection	Remarks
1.	*Clerodendrum infortunatum* L.	Verbenaceae	S.N. College Campus, Cherthala	Flowering shrub with quadrangular hairy stem and pinkish white flowers.
2.	*Duranta plumieri*Jacq.	Verbenaceae	S.N. College Campus, Cherthala	Flowering shrub with quadrangular hairy stem and lavender flowers.
3.	*Lantana camara* L.	Verbenaceae	Kanjikuzhi, Cherthala	Perennial shrub with quadrangular hairy stem and umbellate cyme inflorescence.
4.	*Vitex negundo* L.	Verbenaceae	S.N. College Campus, Cherthala	Aromatic small tree with quadrangular and tomentose stem
5.	*Leucas aspera,* Spreng.	Lamiaceae	S.N. College Campus, Cherthala	Aromatic annual herb with pubescent quadrangular stem
6.	*Ocimum sanctum* L.	Lamiaceae	Kanjikuzhi, Cherthala	Perennial herb with typical aromatic smell
7.	*Plectranthus scutellarioides*L.	Lamiaceae	Kanjikuzhi, Cherthala	Aromatic perennial succulent herb with thyrsus inflorescence.

2.2. Palynology

For pollen morphological studies, pollen grains were collected from mature flower buds. Pollen grains were examined using both light microscopy (LM) and Scanning Electron Microscopy (SEM). The terminology used to categorize pollen grains according to shape and size was that used by Walker and Doyle [5] (**Table2** and **3**).

For SEM, pollen samples were washed with distilled water, dehydrated in an ethanol series and air dried on Aluminium stubs. Then, sputter coated with Gold–Palladium by a Quorum SC7620 sputter coater. Subsequently these were examined and photographed with a TESCAN VEGA 3 SBH scanning electron microscope operated at 8-10 V. Pollen shape, pollen diameter and exine sculpturing were examined. This work was carried out in Physics Research Laboratory, Maharajas College, Ernakulam, Kerala. India.

Table 2. Pollen size classes according to Walker and Doyle [5].

Sl. No.	Size classes	Longest axis
1.	Minute grain	<10µm
2.	Small grain	10-24µm
3.	Medium sized grain	25-49µm
4.	Large grain	50-99µm
5.	Very large grain	100-199µm
6.	Gigantic grain	>200µm

Table 3. Pollen shape classes based on the P/E ratios as recognized by Walker and Doyle [5]. (*P-Polar diameter; E-Equatorial diameter)

Sl.No.	Shape classes	P/E* x 100
1.	Per oblate	< 50
2.	Oblate	50-75
3.	Sub oblate	75-88
4.	Oblate spheroidal	88-100
5.	Spheroidal	100
6.	Prolate spheroidal	100-114
7.	Sub prolate	114-133
8.	Prolate	133-200
9.	Perprolate	> 200

3. Results

Morphological characters of the families, Verbenaceae and Lamiaceae and key to genera are well described previously [6] [7].

Verbenaceae

***Vitex negundo*L.**

Pollen morphology: Pollengrains in monads, isopolar, 3 zonocolpate, small sized grains, grain size ranges 22.80 x 16.58µm, pollen shape prolate. Exine ornamentation microreticulate (**Figure 1a and b**).

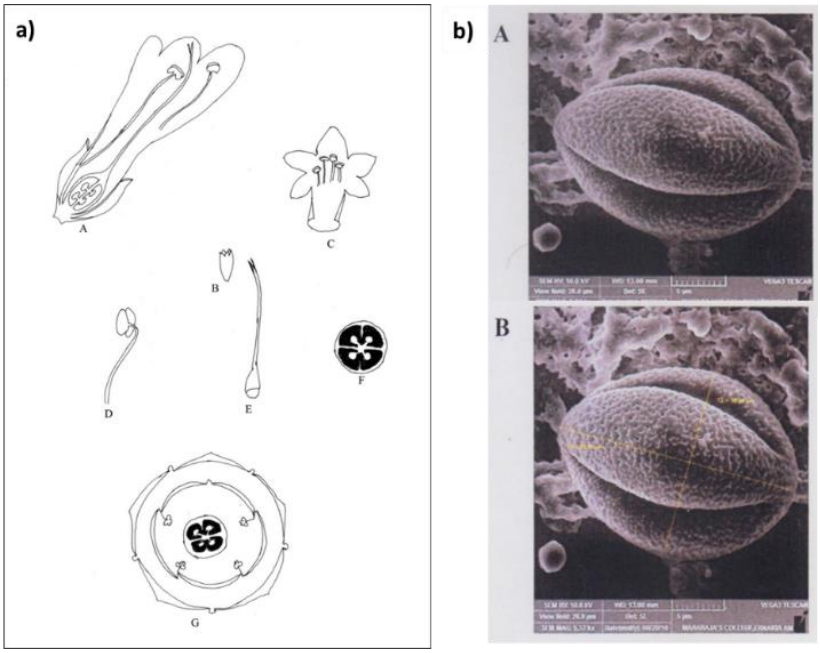

Figure 1a. *V. negundo L.* A) L.S. of flower, B) calyx, C) corolla split open, D) stamen, E) gynoecium, F) C.S of ovary and G) floral diagram; **b)** SEM images of A) pollen grains of *V. negundo* L. and B) portion enlarged.

***Clerodendrum infortunatum*L.**

Pollen morphology: Pollen grains in monads, 3 zonocolpate, large sized grains, grain size ranges (47.15 x 49.47µm). Grain oblate spheroidal. Exine ornamentation spinate (**Figure 1a** and **b**).

***Duranta plumieri* Jacq.**

Pollen morphology: Pollen grains in monads, isopolar, 3 zonocolporate, medium sized grains, grain size ranges (37.52 x 19.57 µm) grain prolate. Exine ornamentation pislate (**Figure 3a** and **b**).

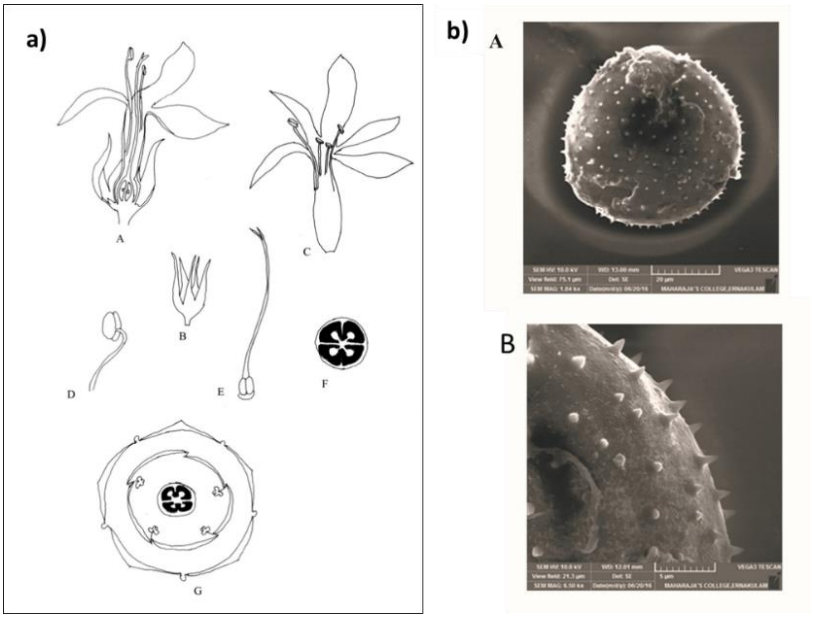

Figure 2a. *C. infortunatum* L. A) L.S. of flower, B) calyx, C) corolla split open, D) stamen, E) gynoecium, F) C.S of ovary and G) floral diagram; **b)** SEM images of A) Pollen grains of *C. infortunatum* L. and B) Portion enlarged.

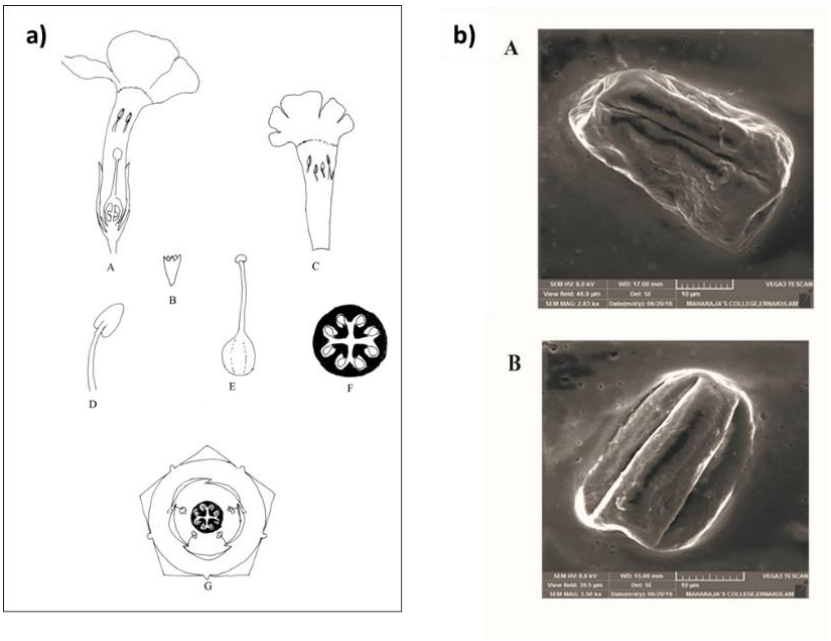

Figure 3a) *D. plumier* Jacq. A) L.S. of flower, B) calyx, C) corolla split open, D) stamen, E) gynoecium, F) C.S of ovary, G) floral diagram; **b)** SEM images of A) pollen grains of *D. plumier* Jacq. and B) portion enlarged.

***Lantana camara* L.**

Pollen morphology: Pollengrains in monads, isopolar, 3 zonocolporate, medium sized grains, grain size ranges (27.93 x 26.65µm.), pollen shape prolate spheroidal. Exine ornamentation pislate (**Figure 4a** and **b**).

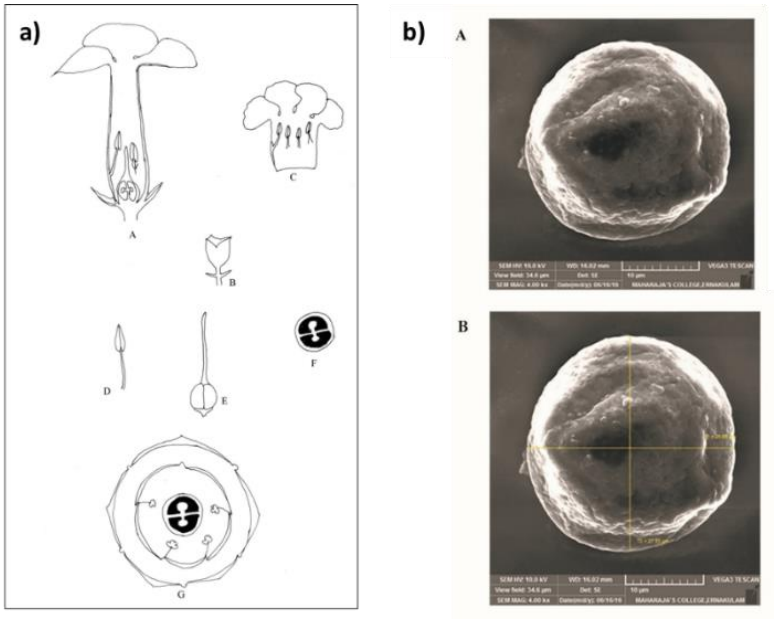

Figure 4a) *L. camara* L. A) L.S. of flower, B) calyx, C) corolla split open, D) stamen, E) gynoecium, F) C.S of ovary and G) floral diagram; **b)** SEM images of A) pollen grains of *L.camara* L. and B) portion enlarged.

Lamiaceae

***Leucas aspera* Spreng.**

Pollen morphology: Pollengrains in monads; 3 zonocolpate; medium sized grains; grain size ranges (29.07x21.70µm.), pollen shape prolate, exine ornamentation reticulate (**Figure 5a** and **b**).

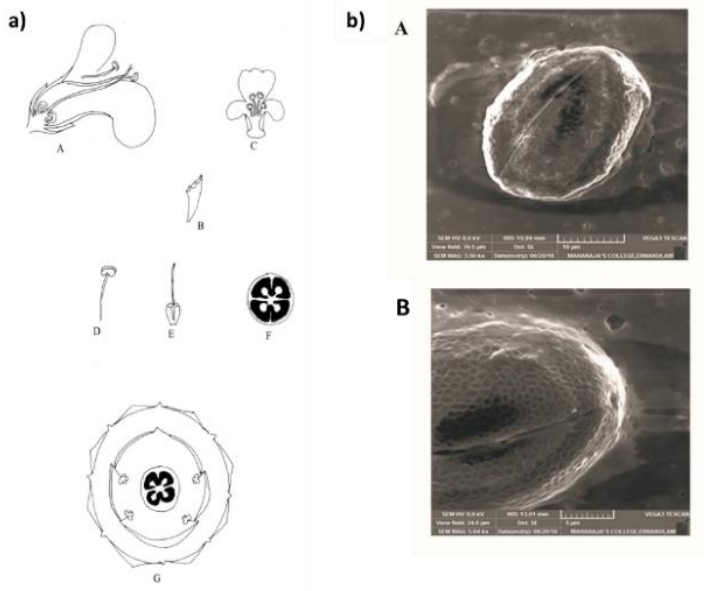

Figure 5 a) *L. aspera* Spreng. A) L.S. of flower, B) calyx, C) corolla split open, D) stamen, E) gynoecium, F) C.S of ovary, G) floral diagram; and **b)** SEM images of A) pollen grains of *L. aspera* Spreng. and B) portion enlarged.

***Plectranthus scutellarioides*L.**

Pollen morphology: Pollengrains in monads; 6 zonocolpate; medium sized grains; grain size ranges (28.77x21.49 µm.), pollen shape prolate. Exine ornamentation microreticulate (**Figure 6a** and **b**).

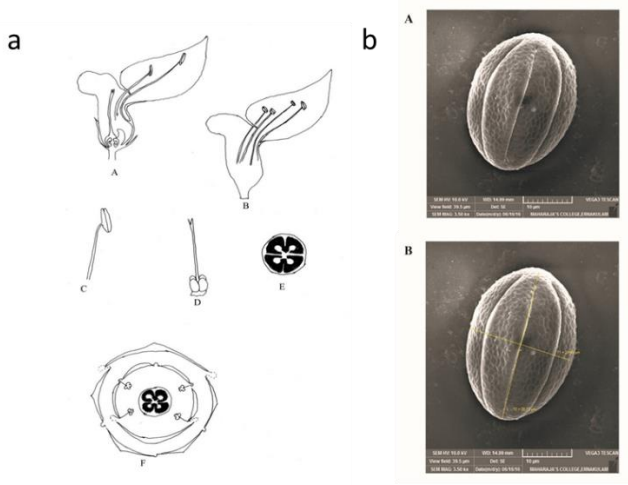

Figure 6a) *P. scutellarioides*L. A) L.S. of flower, B) corolla split open, C) stamen, D) gynoecium, E) C.S of ovary, F) floral diagram and **b)** SEM images of A) Pollen grains of *P. scutellarioides* L. and B) portion enlarged.

***Ocimum sanctum* L.**

Pollen morphology: Pollengrains in monads; 6 zonocolpate; medium sized grains; grain size ranges (28.77x21.49µm.), pollen shape prolate. Exine ornamentation lophoreticulate (**Figure 7a** and **b**).

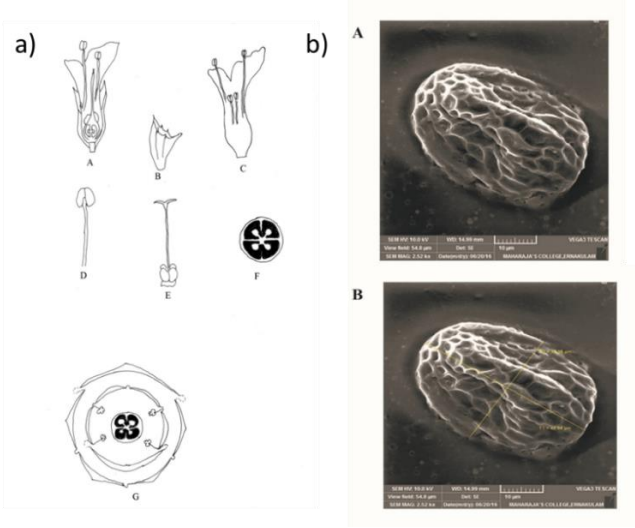

Figure. 7a) *O. sanctum* L. A) L.S. of flower, B) calyx, C) corolla split open, D) stamen, E) gynoecium, F) C.S of ovary, G) floral diagram and **b)** SEM images of A) pollen grains of *O. sanctum* L. and B) portion enlarged.

4. Discussions

In the present investigation, a comparative study on morphological characteristics with special emphasis on pollen grains had been made for seven different taxa belonging to families Verbenaceae and Lamiaceae. The different taxa under investigation are *V. negundo* L., *C. infortunatum* L., *D. plumieri* Jacq., *L. camara* L., *L. aspera* Spreng., *P. scutellarioides* L. and *O. sanctum* L. It has been observed that all the taxa studied possess a quadrangular hairy stem and a bicarpellary gynoecium. In Verbenaceae, the habit of taxa under study varied from shrubs to trees, while in Liliaceae all taxa were herbs (**Table 4** and **5**). The phyllotaxy was found to be opposite, decussate for both two families. The leaves were simple or compound in Verbenaceae, while all taxa under Lamiaceae possess simple leaves. The inflorescence was found to be different cymose (dichacial cyme, umbellate cyme, and cymose panicle) or racemose in Verbenaceae, while it was thyrsus or verticillaster in Lamiaceae. All the taxa under study in Verbenaceae and Lamiaceae possess bilipped corolla. All the taxa under observation possess epipetalous and didynamous stamens. Anther were in rose and dorsifixed or basifixed in the members of Verbenaceae, while introse and dorsifixed in Lamiaceae. Gynoecium was bicarpellary, superior, for all taxa under present investigation [8] (Figure 1-7).

Pollen grains form a unique entity with regard both to its form and function. Although tiny in size, it serves as the sole medium through which the entire male genetic attribute are transmitted to the next generation. For the discharge of this vital function, the pollen grains are endowed with special structural features which make them least influenced by changing ecological conditions. The structural features of pollen grains are regarded as a more dependable tool in studies of comparative morphology that leads to conclusion in plant taxonomy, phylogeny and evolution than those of other vegetative characters [9-11].

Pollen grains consist of an outer exine and an inner intine; both together constitute the protective pollen wall. Of the layers the outer coat or exine possesses characteristic features that are of diagnostic value. The unique architectural features of exine are genetically controlled and stable. The exine of pollen grain embodies morphological characters which are broadly categorized into aperture, exine ornamentation, pollen size and shape in the order of importance in its application in plant taxonomy and phylogeny [12, 13].

Table 4. Morphological characters of Verbenaceae.

Morphological features	***Vitex negundo*L.**	***Clerodendrum infortunatum* L.**	***Durantaplumieri* Jacq.**	***Lantana camara*L.**
Habit	Small tree	Shrub	Shrub	Shrub
Stem	Quadrangular hairy	Quadrangular hairy	Quadrangular hairy	Quadrangular hairy
Phyllotaxy	Opposite	Opposite,decussate	Opposite, decussate	Opposite, decussate
Leaf	Compound, trifoliate; Leaf blade lanceolate, Hairy.	Simple; Leaf blade cordate, crenate, acuminate, hairy.	Simple, ovate, glabrous, Acute	Simple, Leaf blade ovate, serrate, Acute, Hairy.
Inflorescence	Cymose panicle	Dichasial cyme	Raceme	Umbellate cyme
Calyx	Tubular, toothed	Gamosepalous	Tubular	Tubular
Corolla	Violet, small, tubular, 2/3bilipped, quincunsial aestivation	Pinkish white, bilipped, quincunsial aestivation	Light blue, Tubular, 2/3 bilipped, quincunsial aestivation	Yellow, pink or red,4/1 bilipped, Quincunsialaestivation
Androecium	Stamens epipetalous, didynamous; anther introse, Dorsifixed	Stamens epipetalous, didynamous; anther introse, Dorsifixed	Stamens epipetalous, didynamous; anther introse, Basifixed	Stamens epipetalous, didynamous; anther introse, Basifixed
Gynoecium	Bicarpellary, syncarpous superior; style filiform; stigma bifid.	Bicarpellary, syncarpous superior; style filiform; stigma bifid.	Bicarpellary syncarpous superior; style simple; stigma lobed.	Bicarpellary syncarpous superior; style simple; stigma globular.

Pollen unit is the grouping in which pollen is found at maturity within the anther [5]. Different types of pollen unit occurring in angiosperm families are monads, dyads, tetrads, polyads, massulae and pollinia. Monads are considered as the simplest in evolutionary line and polyads are the most advanced. Presently all of the taxa belonging to family Verbenaceae and Lamiaceae possess grains as monads.

Apertures are delimited, thin walled areas in the exine. Functionally they are meant for protection, ion exchange and germination [14]. Aperture morpho-form provides one of the best taxonomic characters especially at the higher level of taxonomy. The aperture may either in the form of a furrow (colpus- colpate grain) or circular (pore-porate grain). Phylogenetically the colpate condition is primitive in angiosperms. Here, the aperture is a wide-open furrow in which the ectocolpium and endocolpium are congruent. The colpate,

porate and pororate are evolved conditions formed by the reduction and modification of the size of the ecto and endocolpium. According to Nair [15], as regard to position of the pollen aperture, proximal position is considered to be most primitive while others such as distal, zonal and global are the derived and advanced conditions. In the presently investigated species of the family Verbenaceae, the pollen grains were found to possess Zonocolpate aperture. The number of aperture is found to 3 except *Plectranthus* and *Ocimum* (**Table 6**). With regard to position, all the species show zonal aperture. All the taxa studied in the family Verbenaceae show common apertural form 3-zonocolporate or 3-zonocolpate. In the members of Lamiaceae except *Leucas*, pollen grains were found to possess zonocolpate aperture. The number of aperture in Lamiaceae is found to vary from 3 to 6, of which 6 aperturate grains were common. Among all the taxa studied in the family Lamiaceae, the common apertural form is 6-zonocolpate (Table 6).

Table 5. Morphological characters of Lamiaceae.

Morphological features	***Leucas aspera* Spreng.**	***Plectranthusscutellarioides*L.**	***Ocimum sanctum* L.**
Habit	Shrub	Shrub	Shrub
Stem	Quadrangular hairy	Quadrangular hairy	Quadrangular hairy
Phyllotaxy	OppositeDecussate	OppositeDecussate	Opposite
Leaf	Simple, linear, lanceolate, crenate, Acute; hairy.	Simple, ovate,Crenate, acuminate;Hairy	Simple, ovate, Serrate, acute;Hairy
Inflorescence	Verticillaster	Thyrrsus	Thyrrsus
Calyx	Tubular, 8-10 ribbed, hairs in teeth;oblique ends.	¼ bilipped, hairy	¼ bilipped, hairy
Corolla	White,2/3bilipped; imbricate aestivation.	Pale blue, ¼ bilipped; imbricate aestivation	Purple, ¼ bilipped ; imbricate aestivation
Androecium	Stamens epipetalous, didynamous; anther introse, Dorsifixed	Stamens epipetalous, didynamous; anther introse, Dorsifixed	epipetalous, didynamous; anther introse, Dorsifixed
Gynoecium	Bicarpellary, syncarpous superior; style long, gynobasic; stigma bifid	Bicarpellary, syncarpous superior; style gynobasic;stigma bifid.	Bicarpellary, syncarpous superior; style gynobasic; stigma bifid

Table 6. Pollen morphology of Verbenaceae and Lamiaceae.

Name of taxon	**Aperture morphotype**	**Shape**	**Pollen diameter (mean)**		**Size**	**Exine ornamentation**
			Polar (µm)	Equatorial (µm)		
Vitex negundo L.	3 Zonocolpate	Prolate	22.80	16.58	Small	Micro reticulate
Clerodendrum infortunatum L.	3 Zonocolpate	Oblate Spheroidal	47.15	49.47	Large	Spinate
Durantaplumi -eri Jacq.	3 zonocolporate	Prolate	37.52	19.57	Medium	Pislate
Lantana camara L.	3 Zonocolpate	Prolate Spheroidal	27.93	26.65	Medium	Pislate
Leucas aspera Spreng.	3 zonocolporate	Prolate	29.07	21.70	Medium	Reticulate
Plectranthus scutellarioides L.	6 zonocolpate	Prolate	28.77	21.49	Medium	Micro reticulate
Ocimum sanctum L.	6 Zonocolpate	Prolate	43.94	30.98	Medium	Lipho reticulate

The sculpturing (ornamentation) on the outer surface of exine is of considerable phylogenetic importance. Generally, exine sculpturing is of two broad categories- the exerscence type and depression type. The exerscence type (spinulose, spinose, baculate, clavate, verrucate, tuberculate and granulate) which is less specialized, while the latter is advanced (psilate, reticulate, foveolate, scrobiculate, fossulate and striate) [16]. A critical observation of the scanning electron micrographs of the exine surface of different Verbenaceae members revealed less diversity. All the pollen grains investigated have depression type of ornamentation except *C. infortunatum* L. It have spinate ornamentation. Among the different depression types of ornamentation, the most predominantly occurring type is psilate. *D. plumier* Jacq. and *L. camara* L. also possess psilate type of ornamentation. The ornamentation variations of different taxa are shown in Table 6. According to Woodehouse [17], pollen grains with thick and heavily ornamented exine have been considered to be primitive, while those with thin unornamented (psilate) or lightly ornamented exine are

advanced. Bentham and Hooker [1] considered these two families as advanced families in Dicotyledonae based on the morphological characters. In the present investigation, the pollen morphology was also analysed. On the basis of both, these can be considered as advanced among the class Dicotyledonae.

The pollen size and shape are the tertiary characters which are of little importance in applied taxonomy due to their least significance in palynological considerations. The size and shape may be affected by process of acetolysis, so this cannot be considered as a reliable character [18]. But size and shape are considered in many families either in generic or species level for correct placement in the group. Pollen size classes were suggested by Walker and Doyle (**Table 2**). In the present study, all taxa of both families possess medium sized grains except *V. negundo* L. (small size) and *C. infortunatum* L. (large size). The average size of the pollen in Verbenaceae varied from 17-23 µm in *Vitex negundo* L. to 50-48 µm in *C. infortunatum* L. In *Lantana camara* L. the size of the pollen was observed to be 28-27 µm, whereas 20-38 µm in *D. plumieri* Jacq. The average size of the pollen in Lamiaceae varied from 21-29 µm in *P. scutellarioides L*. to 31-44 µm in *O. sanctum* L. In *Leucas aspera* Spreng., the size of the pollen was observed to be 22-29 µm. (**Table 6**).

Based on the shape, the pollen grains can be grouped into peroblate, oblate, sub oblate, oblate-spheroidal, spheroidal, prolate, sub prolate, euprolate and perprolate (**Table 3**). During the present study the shape of the pollen grains was found to be prolate in *V. negundo* L oblate spheroidal in *C. infortunatum* L., prolate in *D. plumieri* Jacq. and prolate spheroidal in *Lantana camara* L. All the taxa under study in Lamiaceae possess prolate shaped pollen grains (**Table 6**).

In APG III, *C. infortunatum* L. and *V. negundo* L. were shifted to family Lamiaceae. *Vitex negundo* L. showed similarity with Lamiaceae in morphology such as aromatic quadrangular hairy stem; tubular bilipped corolla, epipetalous, didynamous stamen, dorsifixed anther, and bifid stigma. It differs from Lamiaceae only in its habit and trifoliate leaves [19] (**Table 4**). Pollen morphology of *V. negundo* L. Has also showed similarity with Lamiaceae members. They have monad pollen grains, aperture type was colpate, pollen shape prolate and exine ornamentation was reticulate (**Table 6**). Based on the above observations, the authors agrees with the transfer of the genus *V. negundo* L. to the family Lamiaceae as per APG III [20].

Even though *C. infortunatum* L. showed some similarity with members of Lamiaceae in aromatic nature, quadrangular hairy stem, simple leaves, epipetalous, didynamous stamen,

and bifid stigma, it differ in the type of inflorescence and many pollen morphological characters such as aperture number (6 in Lamiaceae and 3 in *C. infortunatum* L.),shape of pollen (prolate in Lamiaceae and in *C. infortunatum* it is oblate spheroidal), size of pollen (medium sized grains in Lamiaceae and large sized grains in *C. infortunatum* L) and exine ornamentation (Lamiaceae possess reticulate type and *C .infortunatum* L. have excrescence - spinate type) (**Table 4 and 6**). Since the genus show more similarity to Verbenaceae than Lamiaceae, the authors suggest that it is better to maintain the genus *C. infortunatum* in the Verbenaceae family itself.

D. plumieri Jacq., *L. camara* L. showed similarity with Lamiaceae in morphological characters like aromatic shrub, quadrangular hairy stem, leaves simple; corolla tubular, bilipped; stamens epipetalous and didynamous. It showed difference in type of inflorescence and stigma. (**Table 4 and 5**). Pollen morphology of these two genera also differs from Lamiaceae such as difference in aperture number (6 in Lamiaceae and 3 in *D. plumieri*Jacq. and *L. camara* L.), shape of pollen (prolate in Lamiaceae and in *L. camara* L. possess spheroidal), exine ornamentation [21] (Lamiaceae possess reticulate type and these two genera have depression-pislate type) (**Table 6**).

5. Conclusions

The present work discussed the comparison of morphological and palynological characteristics of some selected members of Verbenaceae and Lamiaceae. The different taxa under investigation were *V. negundo* L., *C. infortunatum*L., *Duranta plumier* Jacq., *Lantana camara* L., *Leucas aspera* Spreng., *P. scutellarioides L.* and *O. sanctum* L. Pollen morphological analysis was done using scanning electron micrographs. Although these families showed some similarities, they differ in many features such as phyllotaxy, inflorescence type, style, aperture number and type of pollen. By evaluating both morphological and palynological characters, the authors have the opinion that the transfer of the genus *Vitex* to Lamiaceae as per APG III classification is justifiable, and it is better to retain the genus *Clerodendrum* in the family Verbenaceae as per Bentham and Hooker's classification.

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