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RESEARCH PAPER

Numerical Taxonomic Evaluation of Leaf Architectural Morphology of *Vitex* L. species (Lamiaceae Martinov) in Peninsular Malaysia

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Abstract

A numerical taxonomic is an algorithm method that is used to evaluate scored characters of continuous characters such as leaf architectures. Leaf architecture characters were scored from 11 *Vitex* species in Peninsular Malaysia (*V. gamosepala, V. glabrata, V. longisepala, V. millsii, V. negundo, V. pinnata, V. quinata, V. trifolia, V. vestita, Vitex* sp.1 and *Vitex* sp.2), one subspecies (*V. trifolia* subsp. *litoralis*) and two varieties (*V. negundo* var. *bicolor* and *V. negundo* var. *cannabifolia*) were investigated using herbarium voucher specimens and fresh specimens collected from the fieldwork. Species collected were from Pasoh Forest Reserve, Negeri Sembilan; Gunung Lambak Forest Reserve, Johor; Sungai Dara Forest Reserve, Perak; Proton City, Tanjong Malim, Perak; Gunung Lambak Forest Reserve, Johor; Cameron Highland, Pahang; Herb Walk Garden, Langkawi; and Kg. Pulau Belanga, Kota Bharu, Kelantan. All the studied *Vitex* species shared leaf architecture as follow: arrangement (opposite), marginal ultimate (looped), position of petiole arrangement- P.O.P.A (marginal) and petiole feature (swollen base). Specimens with single leaf type and presence of petiolule further help in distinguishing *Vitex* species. The scored data were analysed using Multi-Variate Statistical Package (MVSP) and Phylogenetic Analysis Using Parsimony (PAUP) softwares to obtain similarity values between species and their evolutionary relationship, respectively. Dendogram clades show an overall Gower General Similarity Coefficient (GGSc) value at 0.715 to 0.964 which indicate clear separation at genus and species level while pairwise distance between the in-group and out-group taxa analysed in the neighbour-joining tree was in between 0.26364 to 0.55455 showing that trees were constructed with strong bootstrap support with Consistency Index (CI) 0.6852 (CI) excluding uninformative character = 0.6000, Homoplasy index (HI) 0.3148 (HI excluding uninformative character = 0.4000), Retention Index (RI) 0.5802 and rescaled consistency index (RC) 0.3976. As a conclusion, high similarity in leaf architecture characters of all 11 species, one subspecies and two varieties of *Vitex* studied in Peninsular Malaysia suggest and support them belongs to the same genus hence supporting the taxonomic significance. This study implicate that leaf architecture morphological characters of vegetative parts can be used in identification to species, subspecies and variety level of this genus and might propose some changes to the placement of taxa hence can be devoted as an improvement for the previous *Vitex* study.

Keywords: *Vitex;* Lamiaceae; Leaf architecture morphology; Numerical analysis; Taxonomy

INTRODUCTION

The family Lamiaceae is famously known as the mint family which is in the major group of Angiosperms (The Plant List, 2010). Lamiaceae is denoted as the largest family in the order Lamiales (Stevens, 2001) with 236 genera and 7,173 species which is widely distributed globally (Harley et al., 2004). *Vitex* is one of the important genus belongs to the Lamiaceae (Corner, 1988). It is the largest genus in the subfamily Viticoideae of Lamiaceae with 25 known species worldwide (Bello et al., 2018) while species in Peninsular Malaysia was first described by Lam (1919) with 46 species, followed by Ridley (1923) with 15 species, both Kochummen (1978) and Turner (1995) recorded ten species and Bramely (2019) recorded 13 species.

Vitex species is common among Malays with the name *Leban, Haleban, Halban, Leban Pachat, Lemuning, Demundi, Muning, Lagundi, Kemuning Hitam* and *Leban Pelandok*. Commonly, *Vitex* is recognised by having trifoliate or palmate with 3-7 leaflets, where the middle is the largest (Corner, 1988) if in case when only one leaflet is present then two leaflet scars on the petiole are always clearly present (de Kok, 2007) and *Vitex* in Malaya are nearly all trees or shrubs (Henderson, 1959).

Morphology currently provides most of the characters used in constructing taxonomic systems (Samuel & Arlene, 1986). Heywood (1967) highlighted that morphological features have been studied so extensively by botanists in the various classes of plants that it might be assumed there is little left to learn. Furthermore, morphology currently provides most of the characters used in constructing taxonomic systems (Samuel & Arlene, 1986). Plant habit and leaves are among the morphological characters contributed in specific groups of plants (Gurcharan, 1999). Evidence from the external morphology provides the informative characters for plant characterization, identification, classification and relationship (Phongoudome, 2000). Hence, the use of morphological characters in taxonomic studies is frequently employed because these are most available (Radford, 1986). A few studies have been made using morphological characters in treating the taxonomy of *Vitex.* Among of them are Kurz (1877), Clarke (1885), Pearson (1912), Lam (1919), Ridley (1923), Kritikar et al. (1975), Henderson (1930; 1974), Kochummen (1978), Corner (1988) and Bramely (2021).

Numerical taxonomy was developed in the late 1950s as part of multivariate analyses and in parallel with the development of computers. Its aim was to devise a consistent set of methods for classification of organisms. Such numerical methods also promised to improve the exactitude in measuring taxonomic, phylogenetic, serological, and other form of relationship, together with other benefits that can accrue from quantitation (Sneath, 2001).

Vitex is currently placed in Lamiaceae which is accepted in Angiosperm Phylogeny Group IV, APG IV (2016). This genus is well known for the complexity in morphological characters, therefore, contributed to its taxonomic conflict worldwide especially in Southeast Asia species including Peninsular Malaysia. Furthermore, the most problematic group in the subfamily Viticoideae is the large genus *Vitex* and its related genera. At present the generic delimitation based on a number of traditionally used morphological characters is confusing and troublesome (Bramley et al., 2009). Therefore, this research attempts to provide the generic characteristics by evaluating numerical data comprehensively based on morphological characters of the leaf architecture.

MATERIALS AND METHODS

Main materials used in this study were fresh specimens and herbarium voucher specimens. Sample collection was collected during the fieldwork at the forest reserve located in Perak, Negeri Sembilan, Pahang and Langkawi as it stands for its richness in species diversity affected by its unique phytogeography position including Pasoh Forest Reserve, Negeri Sembilan; Gunung Lambak Forest Reserve, Johor; Sungai Dara Forest Reserve, Perak; Proton City, Tanjong Malim, Perak; Cameron Highland, Pahang; Herb Walk Garden, Langkawi; and Kg. Pulau Belanga, Kota Bharu, Kelantan. Sampling map location were shown in Figure 1. Fresh specimens collected on Jan 2014-May 2015 at the field were used for morphological study and herbarium voucher specimens. These voucher specimens (SMZ001 – SMZ025) were deposited in the Universiti Pendidikan Sultan Idris, UPSI herbarium. Herbarium specimens investigated were mainly from Forest Research Institute Malaysia, FRIM (KEP) and Universiti Kebangsaan Malaysia (UKMB). Specimens of *V. pinnata*, *V. negundo* L. and *V. negundo* var. *cannabifolia* were also observed virtually from Singapore Botanic Garden (SING), Royal Botanic Gardens, KEW (K), University of South Florida (USF) and New York Botanical Garden (NY). A total of 509 specimens of the 11 species, one subspecies and two varieties of *Vitex* were observed.

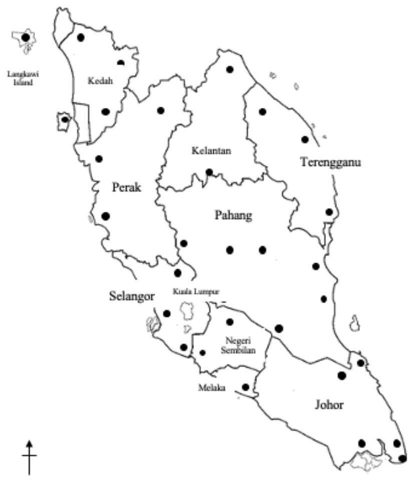

Figure 1. Sampling areas in Peninsular Malaysia

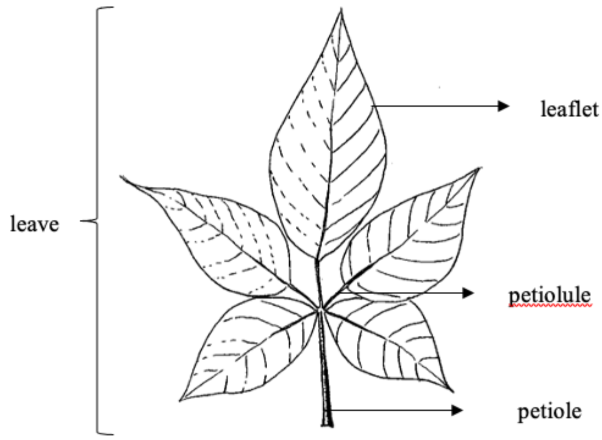

Figure 2. Leaf part to be described in morphological study

For leaf architecture morphology investigation, three replicas belonging to each species were examined. All leaf architecture characteristics were observed under standard dissecting microscope. The main including vegetative characters. All useful main characters have been tabulated (Table 1). The gross morphology of all the specimens were studied thoroughly. Figure 2 shows leaf parts described in morphological study. The collector's field notes from herbarium specimens including important characters such as habit, colour of the bark, texture and height of the tree have been recorded. Morphological study was done according to Veldkamp (1987) and a combined method of Kochummen (1978) and Corner (1988). While stem and fine leaf architecture attributes of the studied species were evaluated based on Hickey (1999) and Ellis et al. (2019). Useful characters were scored in terms of numerical data to be analysed. A total of 49 leaf morphological characters (Table 1) were subjected to numerical analyses. Two methods involved in data analysis; the software of Multi Variate Statistical Package (MVSP) and Phylogenetic Analysis Using Parsimony (PAUP). MVSP software was used to determine their similarities. All the values obtained including the Gower's General Coefficient (GGSc) values were discussed. Gower's General Coefficient (GGSc) is scored based on three categories. GGSc value above 0.65 is considered high while GGSc value between 0.3 – 0.65 is considered moderate and GGSc value lower than 0.3 is considered very low in term of similarity coefficients.

While PAUP software was used in order to analyse the phylogenetic relationship with 500 replicates for bootstrapping. Heuristic search in maximum parsimony provide a result Consistency Index (CI), Retention Index (RI) and Homoplasy Index (HI). CI, defined as measurement of homoplasy in phylogenetic tree. The index is set from 0 – 1. CI with the value below 0.5 indicate that the set of data undergoing much homoplasy. RI is another mean of measurement to calculate amount of homoplasy required by a tree. RI is used to calculate synapomorphy on the resulting cladogram. RI index were also set from 0 – 1 in which 1 represent the character fit the tree perfectly and 0 represent the character poorly. HI is determined by the equation 1 minus the Consistency Index (1 – CI).

RESULTS AND DISCUSSION

Leaf Architectural Observation

There are 49 leaf architectural characters studied in this research. Plant habit, stem and fine leaf architecture belongs to the studied species were summarised in Table 2. Most of the species collected showing the dominant vegetative characters in species identification. The most important characters in *Vitex* species are its simple and compound palmate leaves (Bramely, 2019) as illustrated in Figure 3. Furthermore, the organization and shape of leaves, leaves attachment, leaf blade, shape of apex, shape of abax, leaf margin, leaf lamina, leaf venation, leaf rank, petiole attachment, leaf sinuses, leaf length, leaf width and leaf texture are among the important characteristics in all species. The differences in morphology of the species are clearly seen within the species. This has been agreed with Nor Nafizah et al. (2018).

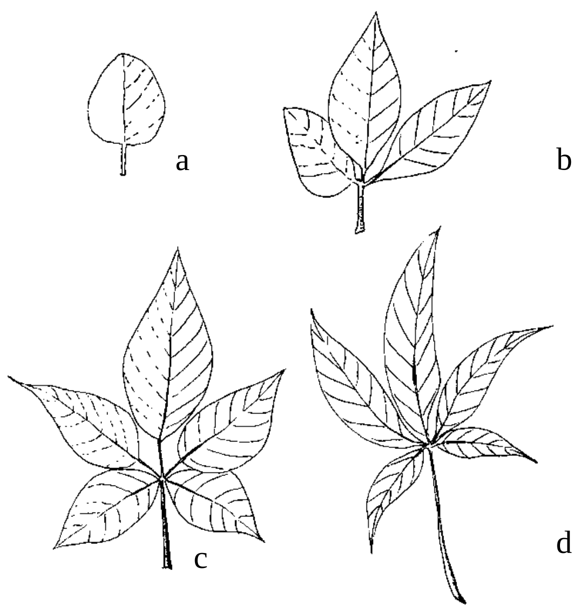

Figure 3. Different of leaves type in *Vitex,* (a) simple single leaf, (b) compound trifoliate leaf, (c) and (d) compound palmate leaf.

Table 1. Characteristics and Representation of the Morphological Data

Key	Character	Character no.
1. General feature		
a) Habit	Leaf shrub (1) Creeping shrub (2) Small tree (3) medium-sized tree (4) Large tree (5)	1
b) Type of leaves	Simple (1) Compound (2)	2
c) Leaves organization	Simple (1) Trifoliate (2) Palmate (3)	3
d) Leaves attachment	Opposite (1)	4
e) Leaflets	Present (1)	5
f) Number of leaflets	1 (1) 3 (2) 3-5 (3) 5 (4)	6
g) Leaf blade	Elliptic (1) Orbicular (2)	7
h) Leaf apex	Acute (1) Accuminate (2) Attenuate (3) Obtuse (4)	8
i) Leaf base	Acute (1) Cuneate (2) Obtuse (3)	9
j) Leaf margin	Entire (1) Serrate (2)	10
k) Leaf lamina	Symmetry (1)	11
l) Hair	Present (1)	12
• **Abax hair**	Present (1)	13
• **Adax hair**	Present (1)	14
2. Venation		
a) Leaf venation	Pinnate (1)	15
b) 2nd Vein distance	0 – 1 cm (1) 2 – 3 cm (2) 4 – 5cm (3) More than 5 cm (4)	16
c) Inter 2nd vein	Weak (1) Strong (2)	17
d) 3rd vein category	Random reticulate (1) Mixed opp/alt (2)	18
e) 3rd Vein course	Sinuous (1) Straight (2)	19
f) 3rd Vein angle variability	Inconsistent (1) Uniform (2)	20
g) No of lateral vein	5 – 7 (1) 8 – 10 (2) 11 – 13 (3) More than 13 (4)	21
h) Vein spacing	Irregular (1) Uniform (2)	22
i) 4th Vein category	Regular polygonal reticulate (1) Opposite percurrent (2) Alternate percurrent (3)	23
j) 5th Vein category	Dichotomizing (1) Regular polygonal (2)	24
k) Agrophic	Not applicable (1) Simple agrophic (2)	25
l) Marginal ultimate	Looped (1)	26
m) Intercostal area	Irregular (1) Regular (2)	27
n) Free ending veins	Absent (1) Present (2)	28
o) Leaf rank	1^r (1) 2^r (2) 3^r (3) 4^r (4)	29
3. Petiole and stem feature		
a) Blade petiole separation	Good (1)	30
b) Petiole feature	Base swollen (1)	31
c) Position of petiole attachment	Marginal (1)	32
d) Petiole outline	Square (1) Rounded (2)	33
e) Petiole hair	Present (1)	34
f) Petiole length	0 – 2 cm (1) 2 – 4 cm (2) 4 – 6 cm (3) 6 – 8 cm (4) More than 8 cm (5)	35
g) Petiolule	Absent (1) Present (2)	36
h) Stem shape	Square (1) Rounded (2)	37
4. Serration and sinusses		
a) Serration type	Absent (1) present (2)	38
b) Leaf sinuses	Absent (1) Present (2)	39
c) Teeth order	Absent (1)	40
d) Teeth/cm	Absent (1)	41
e) Tooth spacing	Not applicable (1)	42
f) Tooth shape	Not applicable (1)	43
g) Sinus shape	Not applicable (1)	44
5. Special feature		
a) Stipule shape	Absent (1)	45
b) Leaf length (Plastic Character)	0 – 5 cm (1) 5 – 10 cm (2) 10 – 15 cm (3) 15 – 20 cm (4) More than 20 cm (5)	46
c) Leaf width (Plastic Character)	0 – 2 cm (1) 3 – 5 cm (2) 6 – 8 cm (3) More than 8 cm (4)	47
d) Length between leaf attachment	0 – 2 cm (1) 2 – 4 cm (2) 4 – 6 cm (3) 6 – 8 cm (4) More than 8 cm (5)	48

Diagnostic Characters

The leaves of *Vitex* consist of simple leaf, compound trifoliate leaf and compound palmate leaf (Figure 3) which arranged oppositely. Species of *Vitex* which have simple single leaves are very rare and only found in *V. trifolia* subsp. *litoralis. Vitex* commonly have trifoliate or palmate leaflets, which may range 3–5 leaflets. The size of the leaf blade are varies (3 cm×1.3 cm to 15.5 cm×7.5 cm). Trifoliate leaves usually larger in the middle and the rest are approximately equal in size while palmately compound leaves have one largest middle leaflets, two lateral ones small and two lower ones much smaller than others. In the other case, there is some species has all leaves same in size, for instance *V. negundo* and *V. negundo* var. *bicolor* (Phongoudome, 2000). The growth form of *Vitex* varies from creeping shrub to large tree up to approximately 30 m tall which was found in *V. glabrata*. While *V. pinnata* and *V. quinata* grow as medium-large tree with 20 m and 25 m tall respectively. Nevertheless, there are records from KEP herbarium specimen showed that *V. glabrata* grows with 10 m tall and 35 cm girth, means that there were species of *V. glabrata* grow as small tree. The small tree species like *V. gamosepala, V. millsii, V. vestita, V. longisepala, V.* sp1 and *V.* sp2 reach a height up to 10 m tall and 25 cm girth. The shrubs including creeping shrubs species like *V. negundo, V. trifolia, V. negundo* var. *cannabifolia, V. trifolia* subsp. *litoralis* and *V. negundo* var. *bicolor* can reach up to 2 m tall. *V. trifolia* and *V. ovata* are both creeping shrub, but these two species can be distinguished by the presence of drooping branches in *V. trifolia.* Furthermore, since *V. ovata* is a synonym of *V. trifolia* subsp. *litoralis, V. ovata* is a special species in the genus *Vitex* which is known as creeping *Vitex* because of its long runners and rooting from the stem (Henderson, 1974).

Another special characteristic was bark, especially for the species that habit as tree. Although the bark characteristics is not important in determining the species, but the variation of its colour distinctly vary from grey to brown. *V. longisepala* for instance have brown outer bark colour while *V. glabrata, V. pinnata* and *V. vestita* presence with grey outer bark. Among these three species with grey bark, *V. pinnata* has a little bit differ which can be distinguished by its pale yellowish grey bark. Most of the species have smooth surface of bark. The branching varies from monopodial in shrubs and small trees to lush sympodial in larger trees.

Numerical Analysis

The characteristics and representation of the leaf architecture morphological data were important in order to create numerical data analysis. 49 characters of the gross morphology (Table 1) had been used in order to create the analysis. The characters involved are including the characters of petiole, stem, venation, serration and special feature. Leaf architecture numerical dendogram analysis obtained from Multi-Variate Statistical Package (MVSP) is shown in Figure 4. Morphological dendogram obtained in this study shows an overall GGSc value at 0.741 to 0.963. The data shows a clear morphological separation in both genus and species level. Based on the data obtained, genus *Vitex* is divided into two clusters (Figure 4). Cluster I consist of only *V. pinnata*, while cluster II was further divided into two sub clusters. Sub cluster I consist of *V.trifolia* subsp. *litoralis*, while sub cluster II consists of *V. negundo* var. *bicolor, V. negundo, V. negundo* var. *cannabifolia, V. trifolia, V.* sp.2, *V.* sp.1, *V. millsii, V. gamosepala, V. vestita, V. longisepala, V. quinata* and *V. glabrata.* Both cluster I and II shows high similarities indicating that this taxon shared many characteristics. The highest GGSc value recorded was scored by the cluster II with the value of 0.766. Multiple characters are shared by the members in the genus *Vitex* in comparison to one outgroup genus used in this study *(Tectona grandis).* This includes leaf arrangement (opposite), 3rd vein category (random reticulate), 3rd vein angle variability (inconsistent), 3rd vein course (sinuous), 4th vein category (regular polygonal reticulate), marginal ultimate (looped margin), position of petiole arrangement (marginal) and petiole feature (swollen base). These characters are described as the delineating factor separating the genus *Vitex* from the out group used in this study.

Table 2. Leaf Architecture of *Vitex* L. in Peninsular Malaysia

Species	**Habit**	**Types of leaves**	**Leaves organization**	**Leaves attachment**	**Leaflets**	**No of leaflets**	**Leaf blade**	**Leaf apex**	**Leaf base**	**Leaf margin**	**Leaf lamina**
V. pinnata L.	Medium sized tree	Compound	Palmate	Opposite	Present	5	Elliptic	Acute	Acute	Entire	Symmetry
V. glabrata	Large tree	Compound	Palmate	Opposite	Present	5	Elliptic	Acuminate	Acute	Entire	Symmetry
V. quinata	Medium sized tree	Compound	Trifoliate	Opposite	Present	3	Elliptic	Obtuse	Acute	Entire	Symmetry
V. longisepala	Medi um sized tree	Compound	Trifoliate	Opposite	Present	3	Elliptic	Acuminate	Acute	Entire	Symmetry
V. trifolia	Leaf Shrub	Compound	Trifoliate	Opposite	Present	3	Elliptic	Acuminate	Acute	Entire	Symmetry
V. trifolia subs. litoralis	Creeping shrub	Simple	Simple	Opposite	Present	1	Orbicular	Obtuse	Obtuse	Entire	Symmetry
V. gamosepala	Small tree	Compound	Trifoliate	Opposite	Present	3	Elliptic	Acuminate	Acute	Entire	Symmetry
V. millsii	Small tree	Compound	Trifoliate	Opposite	Present	3	Elliptic	Acuminate	Acute	Entire	Symmetry
V. vestita	Small tree	Compound	Trifoliate	Opposite	Present	3	Elliptic	Acuminate	Acute	Entire	Symmetry
V.negundo var. cannabifolia	Shrub	Compound	Palmate	Opposite	Present	3-5	Elliptic	Attenuate	Cuneate	Serrate	Symmetry
V. negundo L.	Shrub	Compound	Palmate	Opposite	Present	5	Elliptic	Attenuate	Cuneate	Entire	Symmetry
V. negundo var. bicolor	Shrub	Compound	Trifoliate	Opposite	Present	3	Elliptic	Attenuate	Cuneate	Entire	Symmetry
V. sp. 1	Small tree	Compound	Trifoliate	Opposite	Present	3	Elliptic	Acuminate	Acute	Serrate	Symmetry
V. sp. 2	Small tree	Compound	Trifoliate	Opposite	Present	3	Elliptic	Acuminate	Acute	Serrate	Symmetry

Table 2 (cont). Leaf Architecture of *Vitex* L. in Peninsular Malaysia

Species	**Hair**	**Abax hair**	**Adax hair**	**Leaf venation**	**2nd Vein distance**	**Inter 2nd vein**	**3rd vein category**	**3rd Vein course**	**3rd Vein angle variability**	**No of lateral vein**
V. pinnata	Present	Present	Present	Pinnate	2-3.5 cm	Strong	Mixed opp/alt	Straight	Uniform	13-18
V. glabrata	Present	Present	Present	Pinnate	3-4.5 cm	Strong	Random reticulate	Sinuous	Inconsistent	10-12
V. quinata	Present	Present	Present	Pinnate	3-3.5 cm	Strong	Random reticulate	Sinuous	Inconsistent	9-11
V. longisepala	Present	Present	Present	Pinnate	4.5-5 cm	Strong	Random reticulate	Sinuous	Inconsistent	5-7
V. trifolia	Present	Present	Present	Pinnate	2.5-3.5 cm	Strong	Random reticulate	Sinuous	Inconsistent	5-7
V. trifolia subs. *litoralis*	Present	Present	Present	Pinnate	0.5-1.5 cm	Strong	Mixed opp/alt	Straight	Inconsistent	5-6
V. gamosepala	Present	Present	Present	Pinnate	4-5 cm	Strong	Random reticulate	Sinuous	Inconsistent	8-9
V. millsii	Present	Present	Present	Pinnate	2.5-3.5 cm	Strong	Random reticulate	Sinuous	Inconsistent	5-7
V. vestita	Present	Present	Present	Pinnate	3-3.5 cm	Strong	Random reticulate	Sinuous	Inconsistent	7-8
V.negundo var. cannabifolia	Present	Present	Present	Pinnate	0.5-1 cm	Strong	Mixed opp/alt	Sinuous	Inconsistent	5-9
V. negundo	Present	Present	Present	Pinnate	1.5-2 cm	Weak	Mixed opp/alt	Sinuous	Inconsistent	11-17
V. negundo var. *bicolor*	Present	Present	Present	Pinnate	1.5-3 cm	Weak	Random reticulate	Sinuous	Inconsistent	8-11
V. sp.1	Present	Present	Present	Pinnate	2.5-4 cm	Strong	Random reticulate	Sinuous	Inconsistent	7-8
V. sp. 2	Present	Present	Present	Pinnate	3.5-4 cm	Strong	Random reticulate	Sinuous	Inconsistent	5-7

Table 2 (cont). Leaf Architecture of *Vitex* L. in Peninsular Malaysia

Species	Vein spacing	4th Vein category	5th Vein category	Agrophic	Marginal ultimate	Intercostal area	Free ending veins	Leaf rank	3rd Vein angle variability	Blade petiole separation
V. pinnata	Uniform	Opposite percurrent	Regular polygonal reticulate	Not applicable	Looped	Regular	Absent	4r	Uniform	Good
V. glabrata	Irregular	Regular polygonal reticulate	Dichotomizing	Simple agrophic	Looped	Irregular	Present	4r	Inconsistent	Good
V. quinata	Irregular	Regular polygonal reticulate	Dichotomizing	Simple agrophic	Looped	Irregular	Present	4r	Inconsistent	Good
V. longisepala	Irregular	Regular polygonal reticulate	Dichotomizing	Simple agrophic	Looped	Irregular	Present	4r	Inconsistent	Good
V. trifolia	Irregular	Regular polygonal reticulate	Dichotomizing	Simple agrophic	Looped	Irregular	Present	1r	Inconsistent	Good
V. trifolia subs. *litoralis*	Irregular	Regular polygonal reticulate	Dichotomizing	Simple agrophic	Looped	Irregular	Present	1r	Inconsistent	Good
V. gamosepala	Irregular	Regular polygonal reticulate	Dichotomizing	Not applicable	Looped	Irregular	Present	1r	Inconsistent	Good
V. millsii	Irregular	Regular polygonal reticulate	Dichotomizing	Not applicable	Looped	Irregular	Present	4r	Inconsistent	Good
V. vestita	Irregular	Regular polygonal reticulate	Dichotomizing	Simple agrophic	Looped	Irregular	Present	4r	Inconsistent	Good
V.negundo var. cannabifolia	Irregular	Regular polygonal reticulate	Dichotomizing	Simple agrophic	Looped	Irregular	Present	4r	Inconsistent	Good
V. negundo	Irregular	Alternate percurrent	Dichotomizing	Simple agrophic	Looped	Irregular	Present	3r	Inconsistent	Good
V. negundo var. *bicolor*	Irregular	Alternate percurrent	Dichotomizing	Simple agrophic	Looped	Irregular	Present	3r	Inconsistent	Good
V. sp.1	Irregular	Regular polygonal reticulate	Dichotomizing	Not applicable	Looped	Irregular	Present	4r	Inconsistent	Good
V. sp. 2	Irregular	Regular polygonal reticulate	Dichotomizing	Nor applicable	Looped	Irregular	Present	4r	Inconsistent	Good

Table 2 (cont). Leaf Architecture of *Vitex* L. in Peninsular Malaysia

Species	Petiole feature	Position of petiole attachment (P.O.P.A)	Petiole outline	Petiole hair	Petiole length	Petiolule	Stem shape	Serration type	Leaf sinuses	Teeth order
V. pinnata	Base swollen	Marginal	Square	Present	6-13 cm	Present	Square	Absent	Absent	Absent
V. glabrata	Base swollen	Marginal	Rounded	Present	11-16 cm	Present	Rounded	Absent	Absent	Absent
V. quinata	Base swollen	Marginal	Rounded	Present	7-12 cm	Present	Square	Absent	Absent	Absent
V. longisepala	Base swollen	Marginal	Rounded	Present	5-6 cm	Present	Rounded	Absent	Absent	Absent
V. trifolia	Base swollen	Marginal	Rounded	Present	1-1.5 cm	Present	Square	Absent	Absent	Absent
V. trifolia subs. *litoralis*	Base swollen	Marginal	Rounded	Present	0.5-1 cm	Absent	Square	Absent	Absent	Absent
V. gamosepala	Base swollen	Marginal	Rounded	Present	5-6.5 cm	Present	Rounded	Absent	Absent	Absent
V. millsii	Base swollen	Marginal	Rounded	Present	3.5-7 cm	Present	Square	Absent	Absent	Absent
V. vestita	Base swollen	Marginal	Rounded	Present	9.5-10 cm	Present	Square	Absent	Absent	Absent
V.negundo var. cannabifolia	Base swollen	Marginal	Rounded	Present	3.5-5 cm	Present	Square	Present	Present	Absent
V. negundo	Base swollen	Marginal	Rounded	Present	6-9 cm	Present	Square	Absent	Absent	Absent
V. negundo var. *bicolor*	Base swollen	Marginal	Rounded	Present	3-4.5 cm	Present	Square	Absent	Absent	Absent
V. sp.1	Base swollen	Marginal	Rounded	Present	4-7.5 cm	Present	Square	Present	Present	Absent
V. sp.2	Base swollen	Marginal	Rounded	Present	6-7 cm	Present	Square	Present	Present	Absent

Table 2 (cont). Leaf Architecture of *Vitex* L. in Peninsular Malaysia

Species	Teeth (cm)	Tooth spacing	Tooth shape	Sinus shape	Stipule shape	Leaf length	Leaf width	Length between leaf attachment	Leaf texture
V. pinnata	Absent	Not applicable	Not applicable	Not applicable	Absent	9-13 cm	4-5.5 cm	4-9.5 cm	Coriaceous
V. glabrata	Absent	Not applicable	Not applicable	Not applicable	Absent	19-23 cm	6-9 cm	12-15 cm	Coriaceous
V. quinata	Absent	Not applicable	Not applicable	Not applicable	Absent	13-15 cm	3.5-5 cm	3-4.5 cm	Chartaceous
V. longisepala	Absent	Not applicable	Not applicable	Not applicable	Absent	10-12 cm	3.5-4.5 cm	6.5-9 cm	Coriaceous
V. trifolia	Absent	Not applicable	Not applicable	Not applicable	Absent	3.5-4 cm	1.5-1.7 cm	3.5-7 cm	Coriaceous
V. trifolia subs. *litoralis*	Absent	Not applicable	Not applicable	Not applicable	Absent	2-2.7 cm	1.3-2 cm	1.5-2.5 cm	Coriaceous
V. gamosepala	Absent	Not applicable	Not applicable	Not applicable	Absent	12-14 cm	4.5-6.5 cm	3-3.5 cm	Coriaceous
V. millsii	Absent	Not applicable	Not applicable	Not applicable	Absent	9-10 cm	4-5 cm	5-5.5 cm	Hairy
V. vestita	Absent	Not applicable	Not applicable	Not applicable	Absent	12.5-15.5 cm	6-7.5 cm	8-8.5 cm	Coriaceous
V.negundo var. cannabifolia	Absent	Not applicable	Not applicable	Not applicable	Absent	3-5 cm	1.3-1.5 cm	3-3.5 cm	Coriaceous
V. negundo	Absent	Not applicable	Not applicable	Not applicable	Absent	8-14 cm	1.5-2.5 cm	4.5-7 cm	Coriaceous
V. negundo var. *bicolor*	Absent	Not applicable	Not applicable	Not applicable	Absent	3.5-5.5 cm	1.2-1.8 cm	3-5.5 cm	Coriaceous
V. sp. 1	Absent	Not applicable	Not applicable	Not applicable	Absent	9-11 cm	3.5-4 cm	3-5.5 cm	Hairy
V. sp. 2	Absent	Not applicable	Not applicable	Not applicable	Absent	8-8.5 cm	2.5-3.5 cm	4-5 cm	Coriaceous

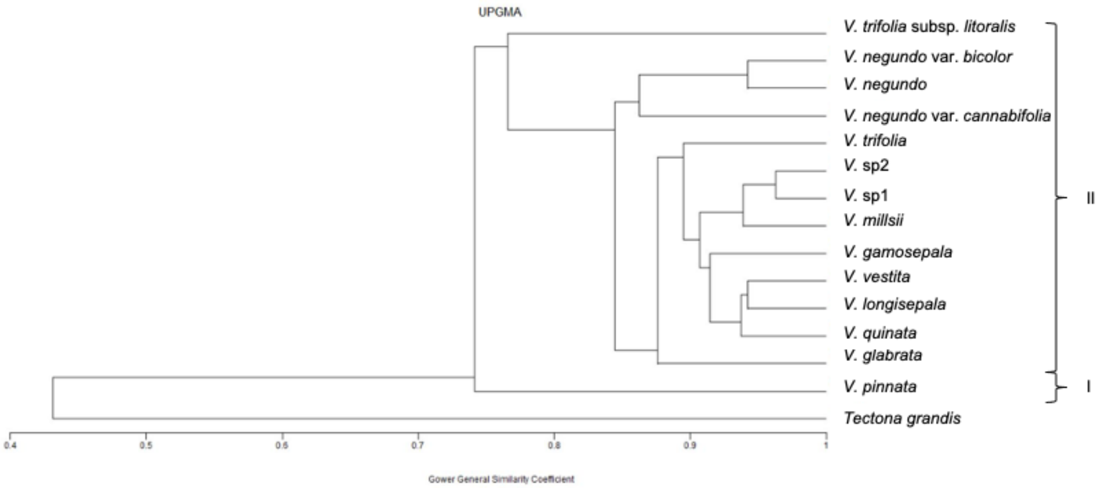

Figure 4. Dendogram or cladogram based on UPGMA for leaf architectural morphological characteristics of *Vitex*

Figure 5 shows a cladogram of parsimonious tree based on morphological character. Out of 49 characters tested, all characters are of type 'unord' and have equal weight. 20 variable characters are parsimony uninformative, while 6 characters are constant and the rest 23 characters are parsimony-informative characters (Table 3). The parsimony informative characters were used as a guideline for clustering studied species into respected taxa or separating it from known out group taxa.

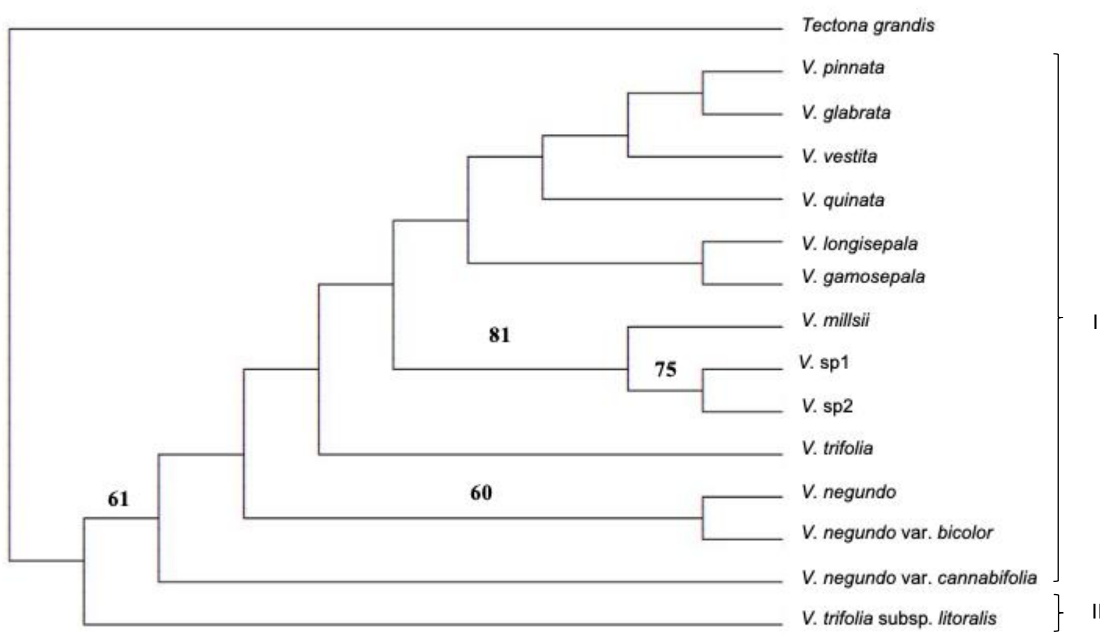

Figure 5. A cladogram of parsimonious tree based on leaf architectural morphological character

The heuristic search in maximum parsimony reveal a total of 10 parsimonious tree with the tree length of 108 step with Consistency Index at 0.6852 (CI excluding uninformative character = 0.6000), Homoplasy index (HI) recorded 0.3148 (HI excluding uninformative character = 0.4000), Retention Index (RI) recorded at 0.5802 and rescaled consistency index (RC) at 0.3976. CI shows a low homoplasy occur in the data set and a relatively high RI (0.52) indicate that the tree represented the data slightly higher than medium. This might be due to the large amount of characters shared by all the genus members. In addition, multi characters do also shared by majority of the group members hence resulting in a low homoplasy occur in the data set. Major clade of maximum parsimony was grouped into two large clusters with three subclusters. Bootstrap values support most of the branch. This includes the cluster for *V. millsii*, *V.* sp.1 and *V.* sp.2 (81%), *V. negundo* and *V. negundo* var. *bicolor* (60%).

While Figure 6 shows that the neighbour-joining tree has a minimum evolution score 2.06960 in accordance to the branch length between the in-group and out-group taxa. Pairwise distance between the in-group was between 0.02355 to 0.26486. The most minimum value (0.0235) was belongs to the node 16 which connected the species of *V.* sp1 and *V.* sp2. The neighbour-joining trees were constructed with weak/strong bookstrap support. The major clade branched into two clusters. Cluster I consist of *V. pinnata, V. quinata, V. vestita, V. longisepala, V. gamosepala, V. millsii, V.* sp.1, *V.* sp.2, *V. trifolia, V. negundo, V. negundo* var *bicolor* and *V. negundo* var. *cannabifolia.* While cluster II only consist of *V. trifolia* subsp. *litoralis.*

Cluster II constructed and supported bootstrap value at 62% showing a close phylogenetic relationship. A few inner branch also supported by the bootstrap value. *V.* sp1 and *V.* sp2 have a bootsrap support of 69% while sub-cluster of *V. trifolia* subsp. *litoralis* has 62% of bootsrap value. This shows a good relationship between the characters shared among the species in the genus.

Table 3. Summary of morphological characters status of all *Vitex* species.

Character	Type	Status	Weight	States
1	Unord	-	1	12345
2	Unord	U	1	12
3	Unord	-	1	123
4	-	UC	1	1
5	-	UC	1	1
6	Unord	-	1	1234
7	Unord	U	1	123
8	Unord	-	1	1234
9	Unord	-	1	123
10	Unord	-	1	123
11	Unord	U	1	12
12	-	UC	1	1
13	Unord	U	1	12
14	Unord	U	1	13
15	Unord	U	1	12
16	Unord	-	1	123
17	Unord	-	1	12
18	Unord	-	1	124
19	Unord	-	1	12
20	Unord	U	1	12
21	Unord	-	1	1234
22	Unord	U	1	123
23	Unord	-	1	1234
24	Unord	U	1	12
25	Unord	-	1	12
26	Unord	U	1	14
27	Unord	-	1	12
28	Unord	U	1	12
29	Unord	-	1	134

30	Unord	U	1	12
31	-	UC	1	1
32	Unord	U	1	12
33	Unord	U	1	124
34	Unord	U	1	12
35	Unord	-	1	1345
36	Unord	-	1	12
37	Unord	-	1	124
38	Unord	U	1	12
39	Unord	-	1	12
40	Unord	U	1	12
41	Unord	U	1	12
42	Unord	U	1	13
43	Unord	U	1	13
44	-	UC	1	1
45	-	UC	1	1
46	Unord	-	1	1235
47	Unord	-	1	1234
48	Unord	-	1	12345
49	Unord	-	1	12

Key to abbreviation: UC = constant characters, U = parsimony uninformative characters, - = parsimony informative characters

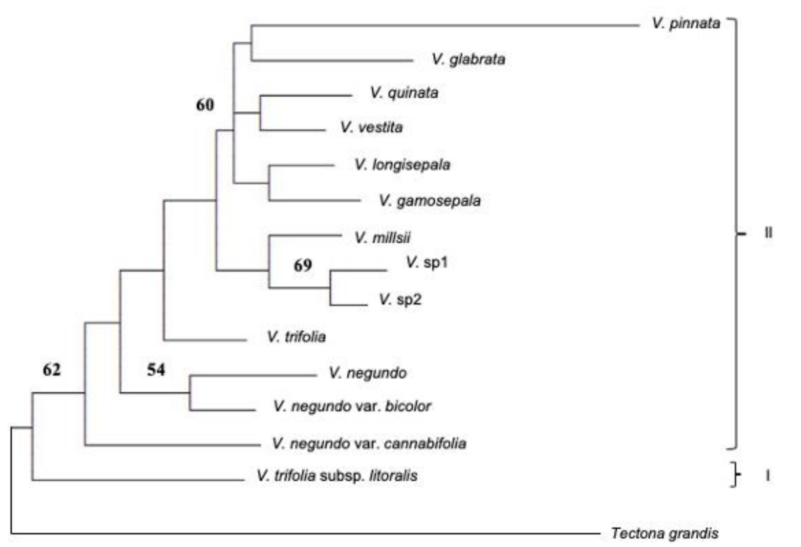

Figure 6. Neighbour-joining tree based on morphological characters (Number above branch indicates bootstrap value)

Genus Description

Vitex L., Sp. Pl. (1753) 638; H.J. Lam, Verbenaceae Malayan Archipel. (1919) 164; in H. J. Lam & Bakh., Bull. Jard. Bot. Buitenzorg Ser. 3, 3 (1921) 47; Harley et al. in Kubitzki, Fam. Gen. Vasc. Pl. 7 (2004)195; de Kok Kew Bull (2007) 587; 63 (2008) 17; Bramely et al., Taxon 58 (2009) 500; Tree Fl. Sabah & Sarawak 7 (2011) 77; Bramely, Flora Malesiana (2019) 397. – Type: *Vitex agnus-castus* L. Trees, shrub, rare lianas. **Leaves** (1-)3-(-5)-palmate, decussate; leaflets elliptic, apex rounded to acuminate, base cuneate, sometimes oblique, margin entire, seldom toothed or lobed; adaxial glabrous except on the veins; abaxial glabrous (except on the veins) to tomentose; venation pinnate, usually prominent below and sunken above; hairs simple, multicellular; petiole round to quadrate in cross section. **Distribution:** *Vitex* sp. are distributed throughout the Peninsular Malaysia. **Habitat and ecology:** *Vitex* sp. are common in swamps and primary rainforests, and often in secondary rainforest or coastal area. **Vernacular names:** Leban, Haleban, Lemuni, Legundi, Lagundi

Key to Species

1. Leaves compound; trees; leaf blade elliptic, 3rd vein course sinuose, petiolule present …………...... 2
   Leaves simple; creeping shrub; leaf blade orbicular, 3rd vein course straight, petiolule absent ……………………………………………………………………….. ***Vitex trifolia* subsp. *litoralis***
2. Leaves palmate, five leaflets…………..…………………………………........................………. 3
   Leaves trifoliate, three leaflets ………………………………………….…………………………….. 6
3. Uniform vein spacing, regular polygonal reticulate of 5th vein category, not applicable agrophic, regular intercostal area, absent of free ending veins, uniform 3rd vein angle variability, square petiole outline ……………………………………………………………………………………..….. ***Vitex pinnata***
   Irregular vein spacing, dichotomizing of 5th vein category, simple agrophic, irregular intercostal area, free ending veins present, inconsistent 3rd angle variability, rounded petiole outline …………..……. 4
4. Large tree, leaf apex acuminate, leaf base acute, 2nd vein distance 3-5 cm, 3rd vein category random reticulate, petiole length 11-16 cm, stem shape rounded, leaf length 19-23 cm, leaf width 6-9 cm ………………………………………………………………………………...……. ***Vitex glabrata***
   Shrub, leaf apex attenuate, leaf base cuneate, 2nd vein distance 0.5 – 2 cm, 3rd vein category mixed opposite or alternate, petiole length 3-9 cm, stem shape square, leaf length 3-14 cm, leaf width 1.3-2.5 cm …………………………………………………………………………………………..……. 5
5. Leaflet margin serrate, strong inter 2nd vein, number of lateral vein 5-9, 4th vein category regular polygonal reticulate, 4r leaf rank, petiole length 3.5-5 cm, serration present, leaf sinuses present, leaf length 3-5 cm, leaf width 1.3-1.5 cm, length between leaf attachment 3-3.5 cm ………………………………………………………………..… ***Vitex negundo* var. *cannabifolia***
   Leaflet margin entire, weak inter 2nd vein, number of lateral vein 11-17, 4th vein category alternate percurrent, 3r leaf rank, petiole length 6-9 cm, serration absent, leaf sinuses absent, leaf length 8-14 cm, leaf width 1.5-2.5 cm, length between leaf attachment 4.5-7 cm ……………………………………………………………………………..………... ***Vitex negundo***
6. Shrub, leaf apex attenuate, leaf base cuneate, inter 2nd vein weak, 4th vein category alternate percurrent, 3r leaf rank …………………………………………………………..…. ***Vitex negundo* var. *bicolor***
   Tree, leaf apex obtuse/acuminate, leaf base acute, inter 2nd vein strong, 4th vein category regular polygonal reticulate, 4r or 1r leaf rank ………………………………………………………..……... 7
7. Leaf apex obtuse, leaf texture chartaceous, yellow – blue corolla colour, leaf length 13-15 cm ……………………………………………………………………………………..….. ***Vitex quinata***
   Leaf apex acuminate, leaf texture coriaceous or hairy, yellow or blue corolla colour, leaf length less than 13 cm …………………………………………..…………………………………….………. 8
8. Serrate leaf margin ……………………………………..……………………………….....…. 9
   Entire leaf margin ………………..……………………..………………………………..……. 10
9. Leaves texture hairy, number of lateral vein 7-8, leaf length 9-11 cm, leaf width 3.5-4 cm ………………………………………………………………………………………..….. ***Vitex* sp.1**
   Leaves texture coriaceous, number of lateral vein 5-7, leaf length 8-8.5, leaf width 2.5-3.5 cm …………………………………………... ***Vitex* sp.2**
10. Longer sepal, tree, calyx apex acuminate ………………………………...……….. ***Vitex longisepala***
    Shorter sepal, small tree, calyx apex acute …………………………………………….……………. 11
11. Leaf hairy, leaf length 9-10 cm, length between leaf attachment 5-5.5 cm, calyx type campanulate, agrophic not applicable ...…………………….. ***Vitex millsii***
    Leaf coriaceous, leaf length 3-4 cm or 12-15 cm, length between leaf attachment 3-8 cm, calyx type cupulate, simple agrophic ……………………...………………………………………….………… 12
12. Petiole length 1-1.5 cm, leaf length 3.5-4 cm, leaf width 1.5-1.7 cm, inflorescence type cincinnus, terminal inflorescence insertion, corolla pale blue ……………………………………..…. ***Vitex trifolia***

Petiole length 5-10 cm, leaf length 12-15.5 cm, leaf width 4.5-7.5 cm, inflorescence type compound, corolla yellow – pale pink ……………….…………………………………………………….. 13

13. Agrophic absent, petiole length 5-6.5 cm, rounded stem shape, length between leaf attachment 3-3.5 cm ……………………………....………………………………………….... ***Vitex gamosepala***

Agrophic simple, petiole length 9.5-10 cm, square stem shape, length between leaf attachment 8-8.5 cm ………………………………….………………………………………………… ***Vitex vestita***

CONCLUSION

In summary, all the evidences gathered supported that *Vitex* is a genus of Lamiaceae family. All the *Vitex* species in this study were recognised either by having 1(-3)-5-foliolate palmate leaves, where the middle is the largest. The findings of variations in leaf architecture of *Vitex* sp. can be useful in solving placement of problematic members in the family. The diversity of the characters produced an interspecific variation among the genus members, hence can be used to delineate one species from another. *V. trifolia* subsp. *litoralis* for instance is the only species in the members with opposite one leaves, while the others have either three or five leaflets. This presentation of leaves lead to the observation of petiole and petiolule belongs to the members. Dendogram based on UPGMA for morphological characters resolving *V. trifolia* subsp. *litoralis* earliest as it was delimited into an only species with single leaf. Besides, two unknown *Vitex* species are collected during this research, named *V*. sp.1 and *V*. sp.2. It is suggested for further studies to identify and establish the species by conducting a research based on their DNA to confirm that either these two unknown species are the varieties as they have been supported by the highest bootstrap value which is 69%. If none of the current known species resemble the collected unknown species, it is great to recommend that the species to be viewed as new members of the genus.

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REFERENCES

Angiosperm Phylogeny Group IV (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society, 181*(1), 1-20.

Bello, M.O., Ahmed, A.Z., Sinmisola, A., Pius, S.F., Bello, E.O., Usman, L.A. & Olobunmi, S.O. (2018). The genus *Vitex:* An Overview of iridoids as chemotaxonomic marker. *Beni-Suef University Journal of Basic and Applied Science, 7,* 414-419.

Bramley, G.L.C. (2019). *Flora Malesiana*, Series I: Volume 23. Lamiaceae. National Park Board Singapore.

Bramley, G.L.C., Forest, F., & de Kok, R.P.J. (2009). Troublesome tropical mints: Re-examining generic limits of Vitex and relations (Lamiaceae) in South East Asia. *Taxon*, *58*, 500-510.

Clarke, C.B. (1885). Verbenaceae. In J.D. Hooker (Ed.). *The Flora of British India*. London: L. Reeve & Co.

Corner, E.J.H. (1988). *Wayside Tree of Malaya.* Vol. 1, 3rd ed., Kuala Lumpur: United Selangor Press.

de Kok, R.P.J. (2007). The genus Vitex L. (Lamiaceae) in New Guinea and the South Pacific Islands. *Kew Bulletin*, *62,* 587-603.

Ellis, B., Douglas, C.D, Hickey, L.J., John, D.M., Kirk, R.J., Peter, W. & Scott, L.W. (2019). *Manual of Leaf Architecture-morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperms by Leaf Architecture Working Group. 65p.* Washington D.C.: Cornell University Press

Gurcharan, S. (1999). *Plant Systematics*. (I. Science Publishers, Ed.). USA.

Harley, R.M., Atkins, S., Budntsev, P.D., Cantino, P.D., Conn, B.J., Grayer, R., Harley, M.M., De Kok, R., Kresstovskaja, T., Morales, R., Paton, A.j., Ryding, O. & Upson, T. (2004). Labiatae. In K. Kubitzki (Ed.). *The Families and Genera of Vascular Plant: Flowering Plant-Dicotyledon*, Germany: Springer Verlag.

Henderson, M.R. (1930). Additions to the flora of the Malay Peninsula. *Gardens' Bulletin. Singapore*, *5*, 96.

Henderson, M.R. (1959). *Malayan Wild Flowers Dicotyledons.* Kuala Lumpur: Caxton Press Ltd.

Heywood, V.H. (1967). *Plant Taxonomy*. London and Beccles: William Clowes and Sons Ltd.

Hickey, L.J. (1999). *Manual of Leaf Architecture - morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperm by Leaf Architecture Working Group*. *65p.* Washington, DC.

Kirtikar, K.R. & Basu, B.D. (1975). *Indian medicinal plants* (MLS Bisherg Singh, mahendral pal singh, new canaught place, dehra dun) 2nd Edition, Reprint.

Kochummen, K.M. (1978). Verbenaceae. In F. S. P. Ng (Ed.), *Tree Flora of Malaya*. 3rd ed. Malaysia: Forest Research Institute Malaysia.

Kurz, S. (1877). *Forest Flora of British Burma.* Vol.II. Government. Printing Press.

Lam, H.J. (1919). *Verbenaceae of the Malay Archipelago*. Groningen: Ly. M. DE. Waal.

Linnaeus, C. (1753). *Didynamia Angiosperma. Species Plantarum*. Reprinting in London.

Nor Nafizah, M.N., Fatimah, M., Norhayati, D., Hasimah, A., Mazatul Azrin, R. & Hilda, E. (2018). Comparative anatomical study and morhology observation of three medicinal *Vitex* species (Lamiaceae). *Malayan Nature Journal*, *70*(3), 285-296.

Pearson, H.H.W. (1912). Verbenaceae. In W. T. Thiselton-Dyer (Ed.), *Flora Cappensis.* London: Lovell Reeve & Co.

Phongoudome, C. (2000). *A Systematic Study on Vitex Tourn (Verbenaceae) in Sabah and Sarawak, Malaysia*. Universiti Putra Malaysia.

Radford, A.E. (1986). *Fundamentals of plant sytematics.* New York: Haper & Row Publishers, Inc.

Ridley, H.N. (1923). Verbenaceae. In *Flora of the Malay Peninsula* (pp. 2:630–636). London: Reeve and Co. Ltd.

Samuel, B.J. & Arlene, E.L. (1986). *Plant Systematics*. United States: McGraw-Hill, Inc.

Sneath, P.H.A. (2001). *Numerical Taxonomy.* In: Boone D.R., Castenholz R.W., Garrity G.M. (eds) Bergey's Manual of Systematic Bacteriology. Springer, New York, NY.

Soepadmo, E., Saw, L.G., Chung, R.C.K. & Kiew, R. (2011). *Tree Flora of Sabah and Sarawak*, Volume 7. Sabah Forestry Department, Forest Research Institute Malaysia (FRIM), Sarawak Forestry Department, Malaysia.

Stevens, P.F. (2001). Angiosperm Phylogeny Website. Retrieved November 03, 2014, from http://www.mobot.org/MOBOT/research/APweb/

The Plant List (2010). Lamiaceae. Retrieved from http://www.theplantlist.org/browse/A/Lamiaceae/

Turner, I.M., 1995. A Catalogue of the vascular plants of Malaya. *Gardens' Bulletin. Singapore*, *47*(1), 256-265.

Veldkamp, J.F. (1987). Manual for the description of flowering plant. In E. F. de Vogel (Ed.), *Manual of Herbarium Taxonomy.* Jakarta: UNESCO.