# A COMPREHENSIVE TRICHOME INVESTIGATION OF *Vitex* L. (LAMIACEAE MARTINOV) IN PENINSULAR MALAYSIA AND ITS TAXONOMIC SIGNIFICANCE

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**Abstract**. The micromorphology and anatomy of trichomes of the leaves of 11 species of the genus *Vitex* (*V. negundo, V. glabrata, V. gamosepala, V. longisepala, V. millsii, 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 light and scanning electron microscopy which were collected from Peninsular Malaysia. Data were gained both from herbarium and field study. Two basic types of trichomes were identified; non-glandular and glandular trichomes. The non-glandular trichomes are subdivided into simple and branched trichomes ($N_1$, $N_2$, $N_3$, $N_4$, $N_5$, $N_6$, and $N_7$) while glandular trichomes are subdivided into two subtypes; stalked and sessile ($G_1$, $G_2$, $G_3$, $G_4$, $G_5$ and $G_6$). Trichome distribution and cover within the species provide valuable characters for comparison hence have taxonomic value. Furthermore, trichomes show considerable variation among the species. The diversity of the characters produced an interspecific variation among the genus members, hence can be used to delineate one species from another. The presence of $G_2$, $N_6$ and $N_7$ types of trichome helps to differentiate the species, therefore verifying them belongs to the same genus hence proving the taxonomic significance. This study implicates that leaf trichome characters can be used in identification of species, subspecies and variety of this genus. The new data might propose some changes to the placement of taxa hence can be devoted as an improvement for the previous study.

**Keywords:** *Vitex* sp., trichomes, lamiaceae, leban, taxonomy

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## Introduction

Lamiaceae is the largest family in the order Lamiales [1] with 236 genera and 7,173 species which is widely distributed globally [2]. *Vitex* is one of the important genus belongs to the Labiatae [3] which currently group into Lamiaceae [4]. There are 250 known *Vitex* species worldwide with 25 species occurring in Southeast Asia [5], while species in Peninsular Malaysia was first described by Lam [6] with 46 species, followed by Ridley [7] with 15 species, both Kochummen [8] and Turner [9] recorded ten species and Bramely [10] recorded 13 species. The most important characters in *Vitex* species are its simple and palmate leaves [10] arranged oppositely and *Vitex* in Malaya are nearly all trees or shrubs [11].

Trichomes are the common characters in the family Lamiaceae [12] which are widely distributed over the aerial reproductive and vegetative parts of plants of Lamiaceae and are usually identified as glandular and non-glandular trichomes [13,14]. Trichomes are well known in Lamiaceae and other related families such as Verbenaceae and Scrophulariaceae as one of the important characters which has the taxonomic value as well as contributing implication in systematics and phylogenetics [13,15-19]. Furthermore, trichome micromorphology has been suggested to be useful in the phylogeny reconstruction [16,20] and systematics of Lamiaceae [13]. Moreover, there are a number of research on comprehensive trichome micromorphology of Lamiaceae been done all over the world including *Phlomoides* [15], *Leucas* [21], *Salvia* [22] and *Vitex* [23]. However, there are only a few research contributed with detailed analysis of trichomes in *Vitex* especially in Peninsular Malaysia.

To date, a list of taxonomic studies have been conducted on *Vitex* in Peninsular Malaysia have been recorded by many researchers [3,7,8,10,24-27] but none of them comprehensively classify the importance of trichomes in the taxonomy of this genus. Thus, a comprehensive study which includes morphology, anatomy and micromorphology of trichomes in *Vitex* of Peninsular Malaysia is still poorly documented. Therefore, this study focuses not only on the morphological character of trichomes, but also its anatomical and micromorphological characters which proved that it can be used as a taxonomic marker.

## Materials and Methods

Trichomes of 11 species of the genus *Vitex* (*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. Herbarium specimens were investigated mainly in Forest Research Institute Malaysia (KEP) and Universiti Kebangsaan Malaysia (UKMB). Specimens were also observed virtually from KEW (K), Singapore Botanic Garden (SING), Royal Botanic Gardens, University of South Florida (USF) and New York Botanical Garden (NY) for the purpose of species identification. A total of 509 specimens of *Vitex* were observed. At least five specimens of each species were analysed to ensure certainty of trichome characters of one species among different specimens. While fresh samples were collected through a series of fieldwork in Peninsular Malaysia. Species were collected at Pasoh Forest Reserve, Negeri Sembilan; Sungai Dara Forest Reserve, Perak; Proton City, Tanjong Malim, Perak; Gunung Lambak Forest Reserve, Johor; Cameron Highland, Pahang; Herb Walk Garden, Langkawi; and Kota Bharu, Kelantan. Voucher

herbarium prepared were deposited at Universiti Pendidikan Sultan Idris herbarium (Voucher number: SMZ001 – SMZ0052).

Herbarium and field samples were studied morphologically by following Veldkamp [28] and a combined method of Corner [3] and Kochummen [8]. The surface of the leaves was examined with an Olympus S2 111 stereo microscope. Full attention was paid to the type, density, shape and size of trichomes. All terminology attributes of the studied species were evaluated based on Hickey [29] and Ellis [30]. While for anatomical study, the dissections of petiole, lamina and midrib of the leaflets were done using a microtechnique procedure based on modification of reported methods by Nor Nafizah [31], Cutler [32] and Johansen [33].

Fresh samples were fixed with Formaldehyde Alcohol Acetic Acid (FAA) solution for 24 hours, while leaves from herbarium specimens were boiled 5 – 10 minutes, followed by fixing in the same solution as fresh leaves. Direct sectioning using a microtome at 2 µm thick was done. Sections were then bleached with Clorox for five minutes followed by gently and thoroughly rinsing with distilled water for a 3 – 5 times before staining in a few drops of Safranin and Alcian Blue. The sample was soaked for 3 – 5 minutes and rinsed in distilled water to clear off color. The sections were then underwent an ascending alcohol series, 50 %, 70 %, 95 % and 100 %, 2 – 5 minutes for each for the purpose of dehydration, then 1:1 Xylene + alcohol for 3 – 5 minutes and then rinsed with pure Xylene. The sample was then mounted onto slides with cover slips by using Canada Balsam and kept in 60 °C. Microimages were captured using Nikon Eclipse 2000-U microscope fitted with image analyzer. Anatomical descriptions were made according to Metcalfe and Chalk [18,34] while anatomical slides prepared were kept at Plant Microtechnique Laboratory, Centre for Biodiversity and Conservation, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris for future references.

For the scanning electron microscopy (SEM), samples from the leaves were cut with size 0.3 to 0.5 $cm^3$ using knife and then were mounted directly onto stub by using double sided tape and gold-coated. After that, the samples were examined at different several magnifications and the best were selected normally at 500 to 2 000 magnification to observe the trichome clearly. The SEM micrographs were taken at an accelerating voltage of 10–15 kV. The type of trichome was described and classified. The general classification scheme and the terminology were done according to Metcalfe et. al. [34] and Payne [35].

## Results and Discussion

### *Types of Trichome in Vitex*

The basic types of the observed trichomes were illustrated in Figure 1. The characteristic features and their distribution among the studied species were summarized in Table 1. Selected SEM and light microscopy (LM) micrographs of trichome types were presented in Figures 2 – 5. Morphological, micromorphological and anatomical observations of trichomes of *Vitex* in Peninsular Malaysia shows variation from unicellular to multicellular, conical to elongated, smooth to ridges, curved at apices to blunt and glabrous to very dense. Trichomes were present on both abaxial and adaxial epidermises of the leaves, petiole and stalk of all species examined.

There were 13 types of trichomes identified in this research. Each of them was categorized into two; glandular and non-glandular trichomes, supporting the classification of trichomes in Lamiaceae which was done by Cantino [13]. Based on the variation observed, glandular trichome can be subdivided into two subtypes: stalked (*V. longisepala, V. gamosepala* and *V. trifolia* subsp. *litoralis*) and sessile (*V. millsii, V. negundo, V. vestita, V. negundo* var *cannabifolia, V.* sp.1 and *V.* sp.2). There were two types of stalked glandular trichome observed in this study; $G_1$ and $G_2$. Stalked glandular trichome can be short with a capitate head. The heads of the glandular trichome were either bicellular; $G_1$ (*V. longisepala, V. gamosepala* and *V. trifolia* subsp. *litoralis*) or multicellular, $G_2$ (*V. vestita, V. negundo*, *V. millsii, V. negundo* var *cannabifolia, V. negundo* var *bicolor, V.* sp.1 and *V.* sp.2). While non-glandular trichomes were divided into two subtypes: unbranched; $N_1$, $N_2$, $N_3$, $N_4$ and $N_5$ and branched trichomes; $N_6$ and $N_7$.

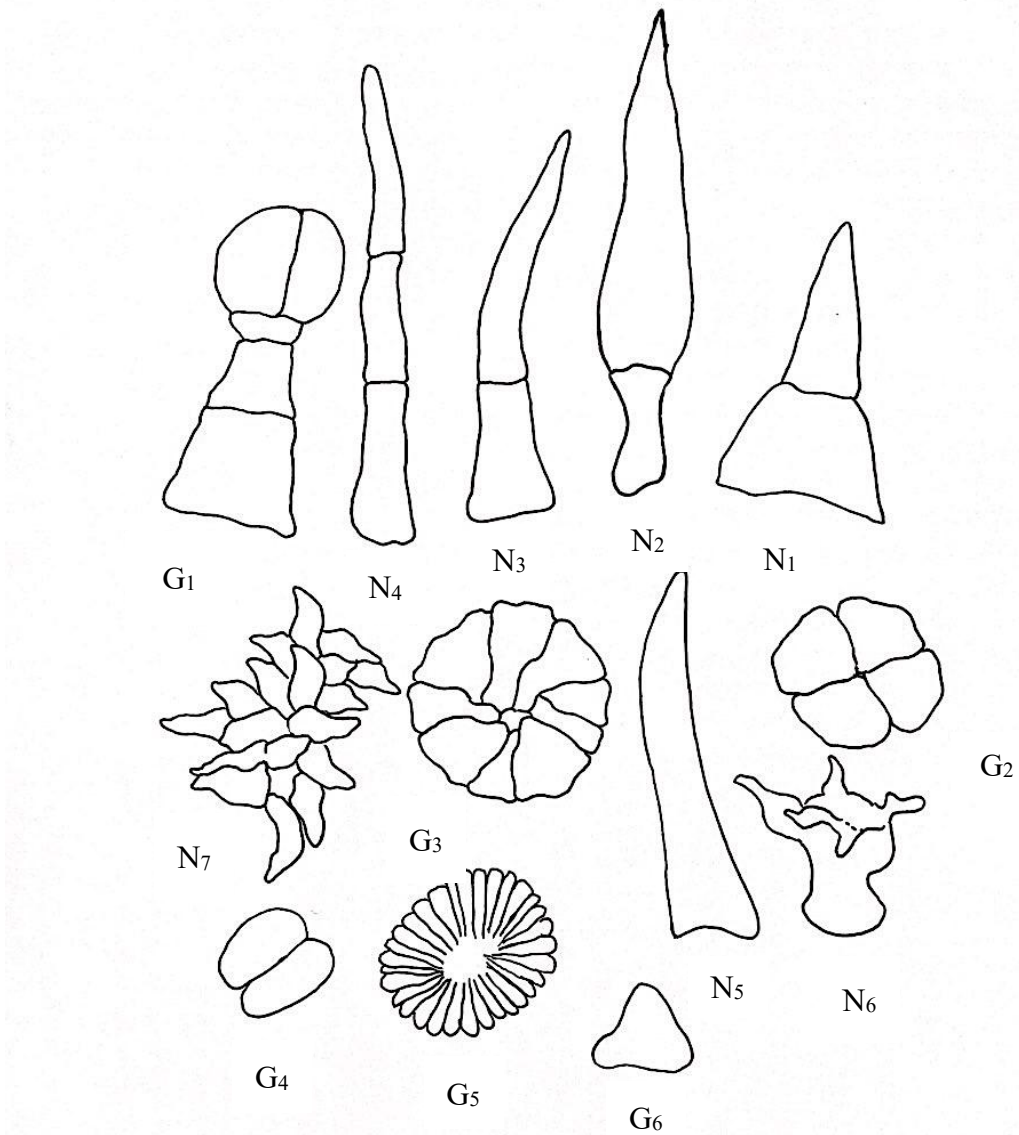


**Figure 1:** Types of trichome identified in *Vitex* sp. Glandular trichomes; $G_1$, $G_2$ (stalked), $G_3$, $G_4$, $G_5$ and $G_6$ (sessile), Non-glandular trichomes; $N_1$, $N_2$, $N_3$, $N_4$, $N_5$ (unbranched), $N_6$ and $N_7$ (branched) [18,34]

**Table 1:** The main types of trichomes presence and some of the characteristics features in investigated *Vitex* species

| Species | Surface | Density | | Size range | Glandular trichome | | | | | | Non-glanduar trichome | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Adax | Abax | | $G_1$ | $G_2$ | $G_3$ | $G_4$ | $G_5$ | $G_6$ | $N_1$ | $N_2$ | $N_3$ | $N_4$ | $N_5$ | $N_6$ | $N_7$ |
| *V. pinnata* L. | Papillate | Dense | Dense | 6 – 44µm | + | - | + | + | + | + | - | - | - | + | + | - | - |
| *V. glabrata* R. Br. | Smooth | Glabrous | Sparse | 43 – 80µm | - | - | - | - | + | - | + | + | - | - | - | - | - |
| *V. quinata* (Lour.) | Smooth | Sparse | Very dense | 33 - 114µm | + | - | - | - | + | - | + | + | + | - | - | - | - |
| *V. longisepala* King & Gamble | Papillate | Dense | D ense | 8 - 117µm | + | - | + | + | - | - | + | - | + | + | + | - | - |
| *V. trifolia* L. | Papillate | Dense | Very dense | 40 - 85µm | + | - | - | - | - | - | - | + | + | - | - | - | - |
| *V. trifolia* subsp. *litoralis* Steenis | Smooth | Dense | Very dense | 35 - 75µm | + | - | - | - | - | - | - | + | + | - | - | - | - |
| *V. gamosepala* Griff. | Papillate | Sparse | Sparse | 45 - 89µm | - | - | + | - | - | - | + | - | - | + | - | - | - |
| *V. millsii* M.R. Hend. | Papillate | Sparse | Sparse | 74 - 112µm | + | - | + | - | + | - | + | - | + | + | - | - | - |
| *V. vestita* Wall. Ex Schauer | Papillate | Dense | Very dense | 38 - 576µm | + | - | + | + | - | - | + | - | + | + | + | + | + |
| *V. negundo* L. | Papillate | Sparse | Very dense | 18 - 113µm | + | - | - | - | - | - | + | + | + | + | - | - | - |
| *V. negundo* var. *bicolor* (Willd.) H.J. Lam | Papillate | Sparse | Very dense | 15 - 110µm | + | - | - | - | - | - | + | + | + | + | - | - | - |
| *V. negundo* var. *cannabifolia* (Siebold & Zucc.) Hand.-Mazz. | Papillate | Sparse | Sparse | 25 - 109µm | - | + | - | - | - | - | + | + | + | + | - | - | - |
| *V.* sp.1 | Smooth | Sparse | Dense | 70 - 135µm | - | - | + | - | - | - | + | - | + | - | - | - | - |
| *V.* sp.2 | Smooth | Sparse | Dense | 28 - 201µm | + | - | + | - | - | - | + | - | + | - | - | - | - |

### *Trichome Description*

The trichome characters which found to have a taxonomic value in this study were presence of glandular trichome and non-glandular, presence of branched trichomes (dendroid or peltate), number of cells (unicellular or multicellular), density of trichomes (abaxial and adaxial surface), size of trichomes (glabrous, sparse, dense or very dense) and surface of the leaves (smooth or papillate). There are 13 types of trichome characters identified in this study (Figure 1) which were described based on [12,13]. All of these trichome types are found to be useful and valuable taxonomically especially in delineating the species, further aid the identification of the species. Type of trichome shows significant variability among different species, but is constant among different populations of one species, and therefore, affords valuable characters in delimitation of sections and species [15].

The trichome varies from a simple unbranched trichome, single cell, into scales and dendritic branched trichomes. The glandular trichome is observed in six different types. The $G_1$ type has a simple, short, thin capitate (Figure 2(1)). It is an unbranched trichome which is observed in *V. pinnata, V. glabrata, V. longisepala, V. trifolia, V. milsii, V. vestita, V. negundo, V. negundo* var. *bicolor* and *V.* sp.2. The $G_2$ type is a multicellular scale, rounded with four cells divided (Figure 2(2)). This was observed only in *V. negundo* var. *cannabifolia.* Type $G_3$ is a glandular trichome, multicellular with more than four cells divided (Figure 2(3)). This type of trichome was observed in *V. pinnata, V. glabrata, V. longisepala, V. gamosepala, V. millsii, V. vestita, Vitex* sp.1 and *Vitex* sp.2. Type $G_4$ is a bicellular scale, oval with two cells divided (Figure 2(4)) observed in *V. pinnata, V. longisepala* and *V. vestita.* While type $G_5$ is subsessile glandular trichome, rounded with more than ten cells (Figure 2(5)) observed in *V. pinnata, V. glabrata, V. quinata* and *V. millsii.* Type $G_6$ is a unicellular triangle shaped trichome (Figure 2(6)) which was observed only in *V. pinnata.* The result has supported the findings of [13].

The rest other seven types are non-glandular trichomes. The non-glandular trichome identified in this study varies from a single-celled to multi-celled which is common in *Vitex.* The $N_1$ type is an unbranched trichome with short and thin prickles (Figure 2(7) and (8)). This $N_1$ type is a multicell which was observed in *V. glabrata, V. quinata, V. longisepala, V. gamosepala, V. millsii, V. vestita, V. negundo, V. negundo* var. *cannabifolia, V. negundo* var. *bicolor, V.* sp.1 and *V.* sp.2. The $N_2$ type is a simple multicellular unbranched trichome, with a Christmast tree feature (Figure 3(9)) which were observed in *V. glabrata, V. quinata, V. trifolia, V. negundo, V. negundo* var. *bicolor* and *V. negundo* var. *cannabifolia.* The $N_3$ type has a simple long multicellular trichome with two cells divided (Figure 3(10)), observed in *V. quinata, V. longisepala, V. trifolia, V. millsii, V. vestita, V. negundo, V. negundo* var. *bicolor, V. negundo* var. *cannabifolia, V.* sp.1 and *V.* sp.2. Besides that, the $N_4$ type has long multicellular trichomes with three cells divided (Figure 3(11)). Species with this type of trichome were observed in *V. pinnata, V. quinata, V. gamosepala, V. millsii, V. vestita, V. negundo, V. negundo* var. *bicolor* and *V. negundo* var. *cannabifolia.* The $N_5$ type has simple, long and thin unicellular trichome (Figure 3(12)) which was observed in *V. pinnata, V. longisepala* and *V. vestita.* The $N_6$ type is a sessile, non-glandular scale with multicelled (Figure 3(13)). This type of trichome was only observed in *V. vestita*. The $N_7$ type is a sessile, non-glandular scale with multicelled (Figure 3(14)). This type of trichome was also observed only in *V. vestita.* Based on the size, sessile glandular trichome type are the shortest (from 5.59 μm) in *V. trifolia,* while longest are $N_4$ type which were found in *V. vestita* (up to 576 μm). Figure 5 and 6 shows the comparison of trichomes between LM and SEM micrographs of trichome types present in *Vitex* species.

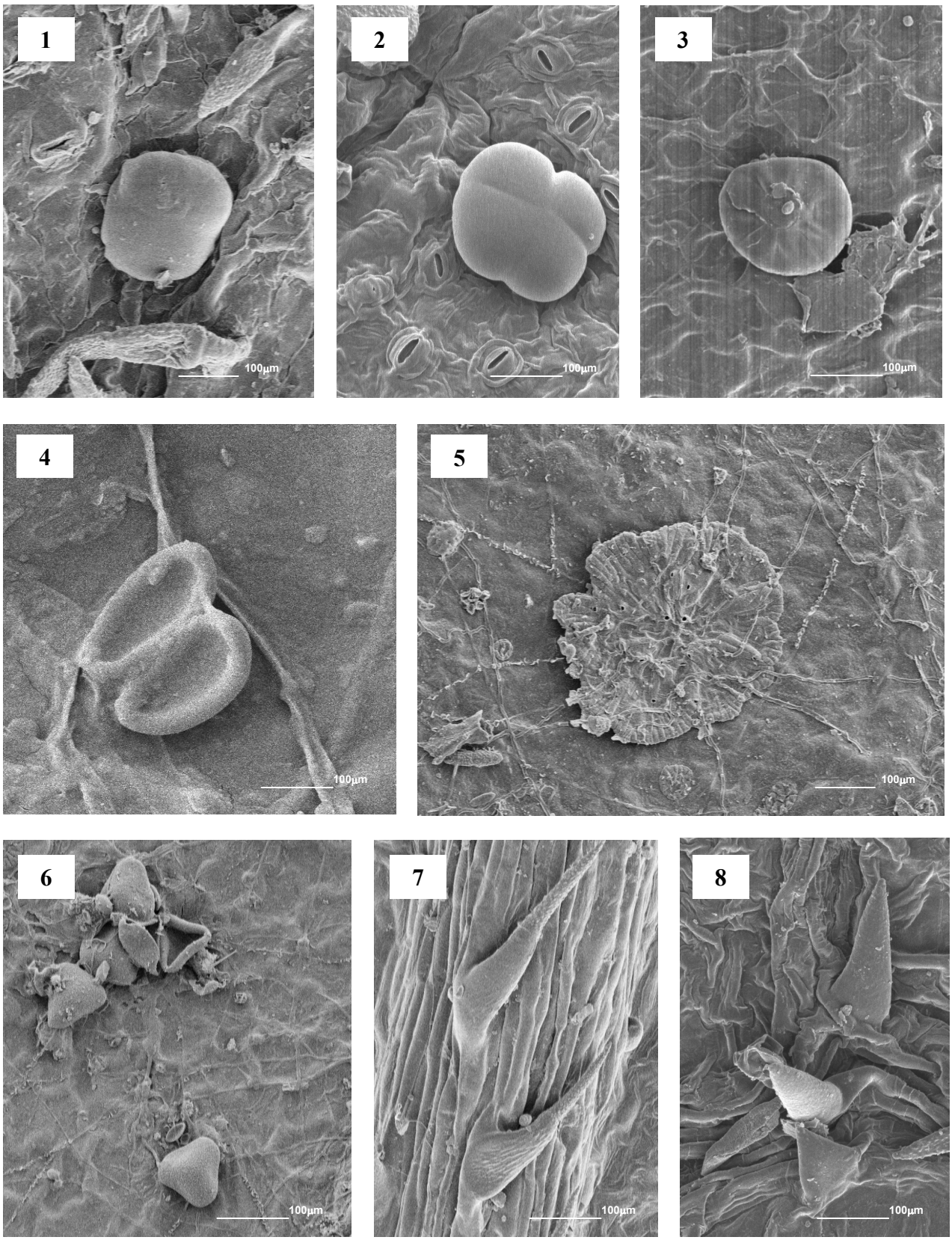


**Figure 2:** SEM micrographs of *Vitex* sp. in Peninsular Malaysia: (1) *V. quinata,* (2) *V. negundo* var. *cannabifolia,* (3) *V. gamosepala,* (4) *V. longisepala*, (5, 6) *V. pinnata,* (7) *V.* sp. 2 and (8) *V. negundo* var. *cannabifolia.* Scale = 100µm

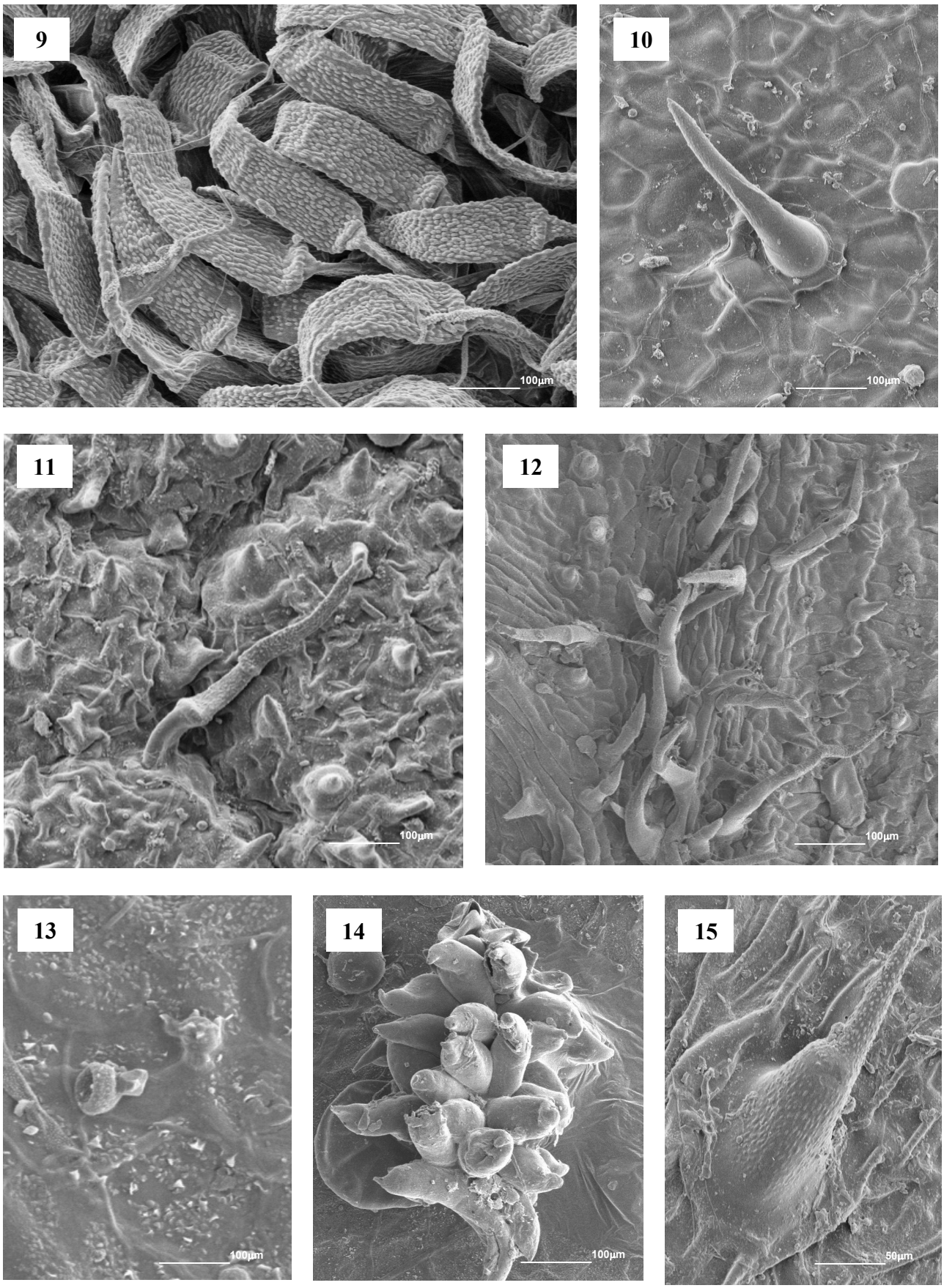


**Figure 3:** SEM micrographs of *Vitex* sp. in Peninsular Malaysia: (9) *V. vestita,* (10) *V. longisepala,* (11, 12) *V. negundo*, (13, 14) *V. vestita* and (15) *V.* sp. 2. Scale: (9 – 14) = 100µm, (15) = 50µm

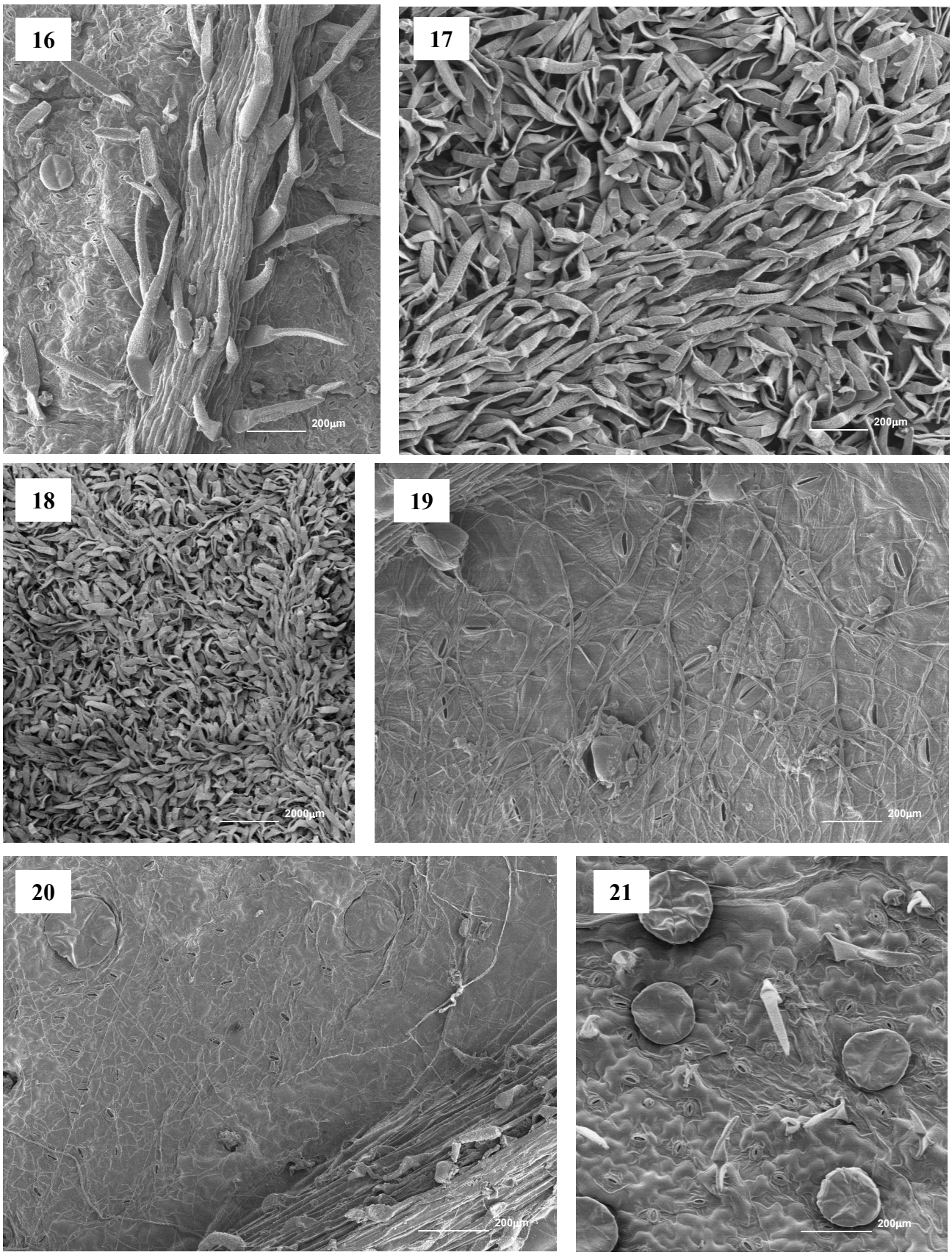


**Figure 4:** SEM micrographs of *Vitex* sp. in Peninsular Malaysia: (16) *V. negundo* var. *cannabifolia,* (17) *V. negundo,* (18) *V. negundo* var. *bicolor,* (19) *V. pinnata* and (20) *V. glabrata,* (21) *V.* sp.2. Scale = 200µm

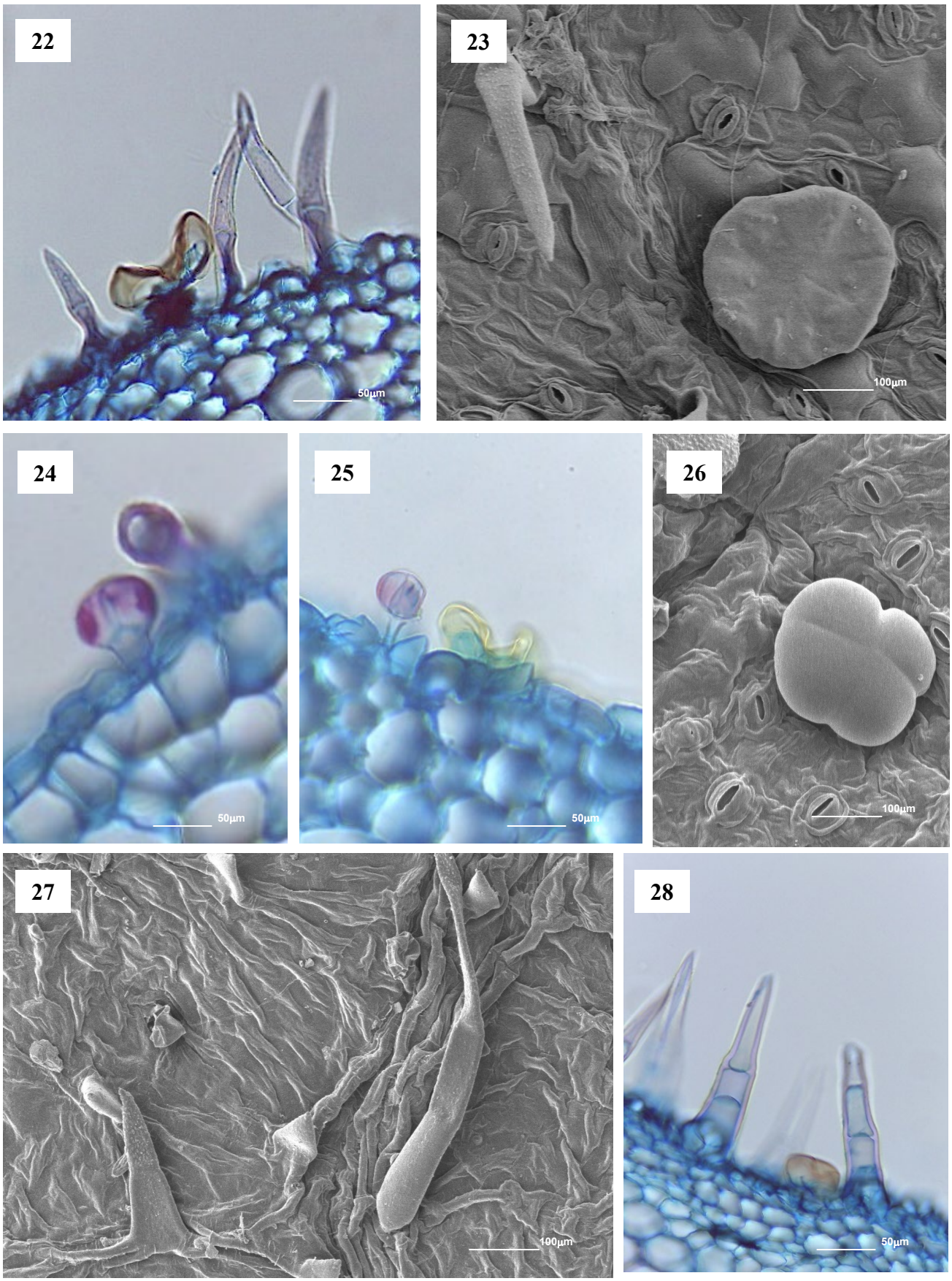


**Figure 5:** (22, 24, 25, 28) LM micrographs, (23, 26, 27) SEM micrographs of *Vitex* sp. in Peninsular Malaysia (22) *V. millsii,* (23) *V. gamosepala,* (24) *V.* sp. 2, (25) *V.* sp. 1 (26) *V. negundo* var. *cannabifolia*, (27) *V. millsii.* Scale: (22, 24, 25, 28) = 50µm, (23, 26, 27) = 100µm

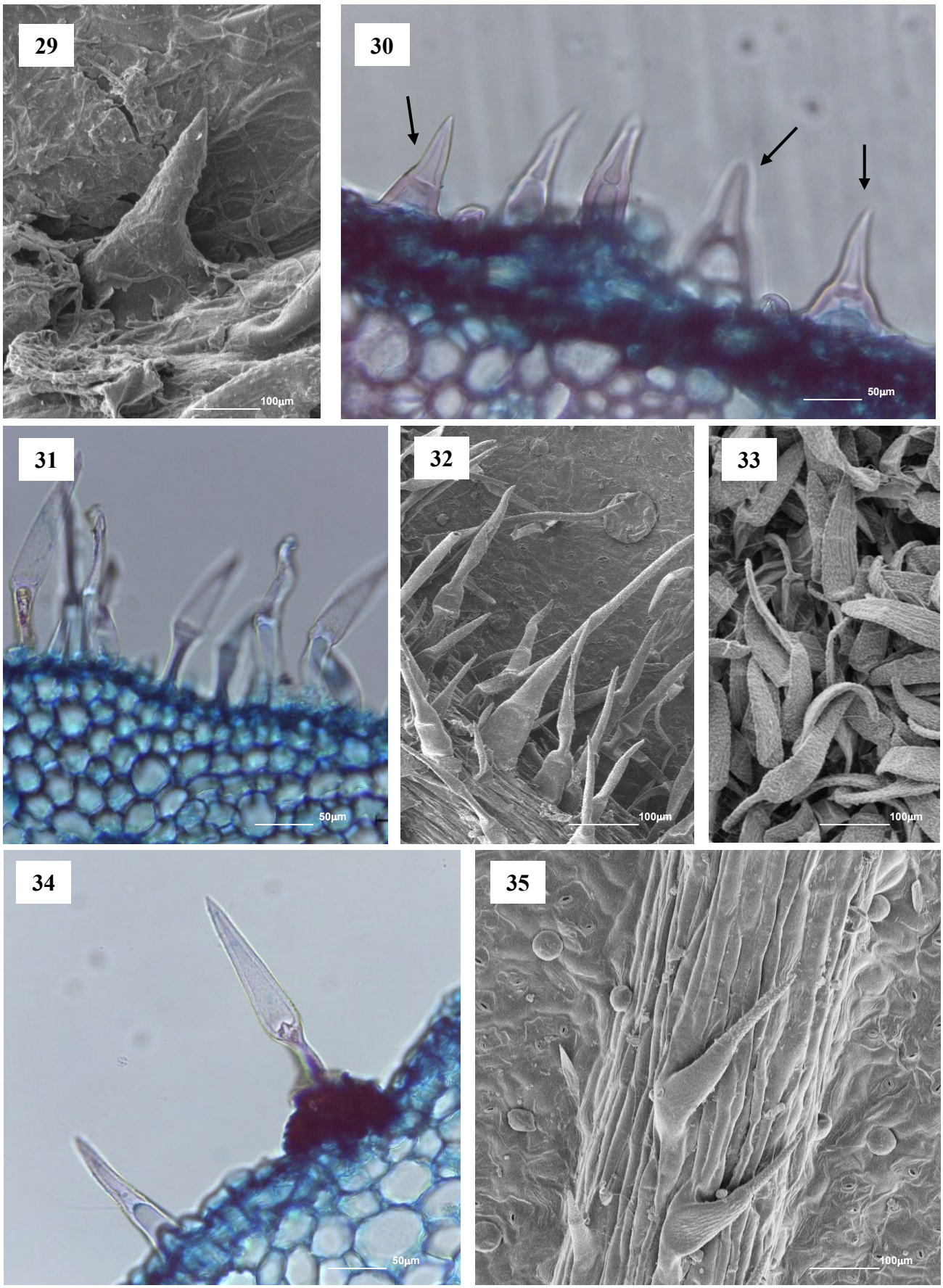


**Figure 6:** (30, 31, 34) LM micrographs, (29, 32, 33, 35) SEM micrographs of *Vitex* sp. in Peninsular Malaysia, (29, 30) *V. millsii,* (31, 32) *V. negundo* var. *cannabifolia,* (33) *V trifolia* and (34, 35) *V. millsii.* Scale: (22, 24, 25, 28) = 50µm, (23, 26, 27) = 100µm

***Diagnostic Characters***

The classification of trichomes presented in this study significantly shows the importance of trichome characters within the genus and family. Analysed images obtained support the general classification and description of the trichome in the genus *Vitex* in Peninsular Malaysia which have been updated by Bramely [10]. Furthermore, these findings are consistent with the previous studies in Lamiaceae confirming the importance of trichome characters in taxonomic identification at different infrageneric levels [36]. Moreover, morphological and trichomes distribution also have an important taxonomic significance at species level. The presence of trichome in each of the *Vitex* species proved that trichome characters have important taxonomic value. This has been agreed by Seyedi and Salmaki [15] that trichome morphology is more valuable to separate the species within sections. Furthermore, types of trichome show considerable variability among species in different sections or series, and therefore, affords valuable characters in delineating of sections and species [14].

Glandular trichomes are one of the important taxonomic characters in Lamiaceae [13,37]. Glandular trichomes have taxonomic value at both specific and subspecific and also can be considered as phylogenetically informative characters [20]. For example, the finding in this study shows that the most common types of trichome shared with almost all species of *Vitex* are type $G_1$ and $G_3$. Moreover, [38,39] successfully divided two sections involving Lamiaceae species based on the presence of glandular trichomes. Furthermore, Nor Nafizah et al. [40] agreed that some limited characters are found to be useful in delineating *Vitex* species. Therefore, type $G_2$ trichome in this study can only be found in *V. negundo* var. *cannabifolia* hence can be used as limited character to delineate the species in the genus.

Besides that, the identification of non-glandular trichomes proved the important of these characters within the genus. Variation in non-glandular trichome characters appears to have specific value, especially to separate different species within the genus [14]. For instance, there are types of trichomes which are $N_6$ and $N_7$ only present in *V. vestita* which made these characters to be used in delineating the species. On the other hand, the most common types of trichome characters shared by *Vitex* species are $N_1$ and $N_3$. Obviously, this has supported the acceptance of *V. negundo* var. *cannabifolia* as a variety of *V. negundo* L. instead of *V. cannabifolia* before this because of it sharing most common characters, not only morphologically, but anatomically and micromorphologically. While there are two unidentified *Vitex* sp.; *V.* sp.1 and *V.*sp.2 which are found to share most of the common trichome characters shared with other *Vitex* species, hence supporting their belongings in this genus.

On the other hand, trichome distribution on both abaxial and adaxial epidermises within the species provide diagnostic characters for comparison. It was shown that trichomes are generally more commonly distributed on the abaxial than the adaxial surface of the leaves supporting research conducted by Celep et al. [41]. As shown in Table 1, in all species investigated, $N_1$, $N_2$, $N_3$ and $N_4$ types occur in almost all species and their distribution differs in each of the species. $N_1$ and $N_3$ were the most frequent presents. In most species, non-glandular trichomes are mainly found on the abaxial surfaces, especially denser on the leaf margin (Figure 3(16)). In some other species including *V. negundo, V. negundo* var. *bicolor, V. negundo* var. *cannabifolia, V. trifolia, V. trifolia* subsp. *litoralis* Steenis and *V. quinata* non-glandular trichomes are distributed over the epidermis as densely as those along the veins (Figure 3(17) and (18)). While *V. glabrata* has the least distribution of trichome on both the abaxial and adaxial surface of the leaves.

## Conclusions

Since trichomes were present in plants in a great variety of forms, and are sometimes very complex structurally, the trichome in *Vitex* can be denoted as a diagnostic characteristic from this study. In summary, all the evidence gathered supported that *Vitex* is a genus of Lamiaceae family and therefore, the present study clearly revealed that the diversity of the trichomes in *Vitex* species can be used in identifying the species hence contributing in solving the taxonomic problem. The diversity of the characters produced an interspecific variation among the genus members, hence can be used to delimit one species from another. There are certain trichome characters in detail that are found to be useful in delineating the *Vitex* species. *V. negundo* var. *cannabifolia* for instance is the only species in the members that have the $G_2$ type of trichome. High similarity in trichome morphological, anatomical and micromorphological characters of all 11 species, one subspecies and two varieties of *Vitex* studied suggest and support that they belong to the same genus. Non-glandular and glandular trichomes are very helpful to differentiate the species. Hopefully, this new findings of $G_2$, $N_6$ and $N_7$ types of trichome in *Vitex* sp. can be useful in solving placement of problematic members in the family. 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 *V.* sp.1 and *V.* sp.2 are the varieties as they shared the most common trichome characters in *Vitex* species. If none of the current known species resemble the collected unknown species, it is great to recommend that the species to be viewed as a new member of the genus.

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## Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

## Disclosure of conflict of interest

The authors have no disclosures to declare.

## Compliance with ethical standards

The work is compliant with ethical standards.

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