

Research Article

## Taxonomic Significance of Trichome Ultrastructure in Five Genera of Lamiaceae

Norhazila Hussin, Nor Nafizah Mohd Noor\* and Fatimah Mohamed

Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim Perak, Malaysia

\*Corresponding author: [nafizah@fsmt.upsi.edu.my](mailto:nafizah@fsmt.upsi.edu.my)

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### ABSTRACT

The trichome of 12 species from five genera of the Lamiaceae family, comprising *Petraeovitex bambusetorum*, *Petraeovitex membranacea*, *Petraeovitex wolfei*, *Petraeovitex scortechinii*, *Petraeovitex trifoliata*, *Peronema canescens*, *Congea forbesii*, *Congea griffithiana*, *Sphenodesme racemosa*, *Sphenodesme triflora*, *Sphenodesme pentandra*, and *Rothea serrata* was investigated under scanning electron microscope (SEM). This study aimed to assess their ultrastructure and ascertain their respective taxonomic value accordingly. Two types of glandular trichomes, specifically peltate and capitate, and three types of non-glandular trichomes, namely, multicellular uniseriate, stellate, and conical were thus observed. The stellate type of non-glandular trichome was solely found in *S. pentandra* among the 12 studied species, while the data further demonstrated both *Congea* species to reflect the multicellular uniseriate structure on the respective leaf surfaces, whereas *C. forbesii* depicted the longest length size among others at 600 µm. In the case of *P. canescens* and *R. serrata*, both were found to be with compact trichomes on both surfaces. Going forward, new data on trichome types, distribution, and size, particularly of these 12 studied species, would provide useful guidance for infrageneric classification.

**Keywords:** SEM, trichomes, glandular, non-glandular, Lamiaceae, ultrastructure

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### 1. INTRODUCTION

Lamiaceae is one of the largest plant families among the dicotyledons, with many of its species being highly aromatic due to the presence of external glandular structures that produce volatile oil (Giulani et al., 2008). Some genera and species in this family are known for the significant variability of secretory structures, which are responsible for the biosynthesis and accumulation of essential oils (Ascensao & Pais, 1998). The family is well acknowledged for its great economic importance, with many studies having been done to assess its various aspects, including trichome type and distribution (Ascensao et al., 1999; Satil & Kaya, 2007; Jia et al., 2013; Manan et al., 2016).

Trichomes or hairs are other forms that outgrowth from the epidermal cells of most plants. More than one type of trichomes may occur on a single plant, thereby constituting the indumentum of the said plant, and may be extremely different from each other (Mauseth, 1998). The functions of trichomes are diverse and trichome types, specifically, have been successfully

used in the classification of genera, species, and even interspecific hybrids (Metcalf & Chalk, 1950). According to Cantino (1990), trichomes are among the most useful taxonomic characters, are distributed widely over the aerial, reproductive, and vegetative parts of Lamiaceae, and ordinarily distinguished as either glandular or non-glandular in nature. He has further implemented the trichome type and distribution as the underpinning features in differentiating subfamilies in the plant family. Most works on the trichomes of Lamiaceae have been performed on species belonging to the subfamily Nepetoideae (Hallahan, 2000; Werker, 2000) and subfamily Lamioideae (Giulini & Bini, 2008). A study by Eiji and Salmaki (2006) on 49 species of *Salvia*, which is the largest genus in Lamiaceae, shows a wide distribution of subsessile glandular trichomes with short-stalked glandular trichomes were observed in most taxa and branched trichomes are present in almost all clades of Salviinae. Trichome studies of Lamiaceae species are limited, particularly in Peninsular Malaysia, with examples including a trichome study on *Vitex* by Zin et al. (2022a) and other leaf studies on *Vitex* and *Premna* by Amran et al. (2023), Zin et al. (2022b), Rahman et al. (2019), and Noor et al. (2018). Nevertheless, the family members are continuously reclassified, with a new update by Zhao et al. (2021) based on plastome phylogenomics.

Five genera investigated in this study are predominantly distributed in Peninsular Malaysia, demonstrating that morphological and anatomical studies are insufficient. In order to add value to the current body of work available on the topic matter, this study is carried out to observe the ultrastructure of leaf trichomes and to determine their taxonomic significance. The findings obtained will provide valuable information to assist the family's current classification decisively.

## 2. MATERIALS AND METHODS

All 12 selected species of Lamiaceae were collected between June 2015 and January 2018, in several places throughout Malaysia. The locality of the studied samples is shown in Table 1. All samples were deposited at the Herbarium of Universiti Pendidikan Sultan Idris (FP).

**Table 1.** Collection data for the studied sample

Taxa	Voucher No.	Locality
<i>Petraeovitex bambusetorum</i> King & Gamble	NHH 001	Pahang, Lipis, Ulu Jelai Forest Reserve
<i>Petraeovitex membranacea</i> var. <i>malesiana</i> Munir	NHH 004	Kedah, Langkawi, Gunung Machinchang
<i>Petraeovitex wolfei</i> J. Sinclair	NHH 091	Pahang, Jerantut, Sungai Tekai Forest Reserve
<i>Petraeovitex scortechinii</i> King & Gamble	NHH 097	Negeri Sembilan, Lembah Jemaloi
<i>Petraeovitex trifoliata</i> Merr.	NHH 022	Sarawak, Marudi, Taman Negara Mulu
<i>Peronema canescens</i> Jack	NHH 052	Pahang, Jerantut, Yong Forest Reserve
<i>Congea forbesii</i> King & Gamble	NHH 013	Selangor, Kepong, FRIM
<i>Congea griffithiana</i> Munir	NHH 094	Kedah, Kuala Nerang, Bukit Janing Forest Reserve
<i>Sphenodesme racemosa</i> (C. Presl) Moldenke	NHH 007	Selangor, Kepong Botanical Garden, FRIM
<i>Sphenodesme pentandra</i> Jack	NHH 058	Pahang, Jengka, Sg. Tekam
<i>Sphenodesme triflora</i> Wight	NHH 010	Kedah, Langkawi, Gunung Machinchang
<i>Rothea serrata</i> (L.) Steane & Mabb.	NHH 049	Pahang, Raub, Tersang

Scanning electron microscope (SEM) preparation: Collected fresh leaves were washed with alcohol 70% and dried at 40°C in an oven for one week. The mid-lamina of each dried leaf was cut into small sizes and the sections of both adaxial and abaxial surfaces were prepared. The sections were mounted on electron microscope stubs by using double-sided adhesive tape,

whereby the stubs were then coated with gold before being subjected to observation under the SEM (Hitachi SU-8020). The method was done by following Kim et al. (2011). Both the leaf surfaces were examined and the micrographs of trichomes were captured. The SEM operated at an accelerating voltage of 15 kV and was done at FSM Labs, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris.

### 3. RESULTS AND DISCUSSION

Findings of the trichome types, size, and distribution are recorded, summarised, and then tabulated in Table 2.

**Table 2.** Trichome type, size, and distribution data

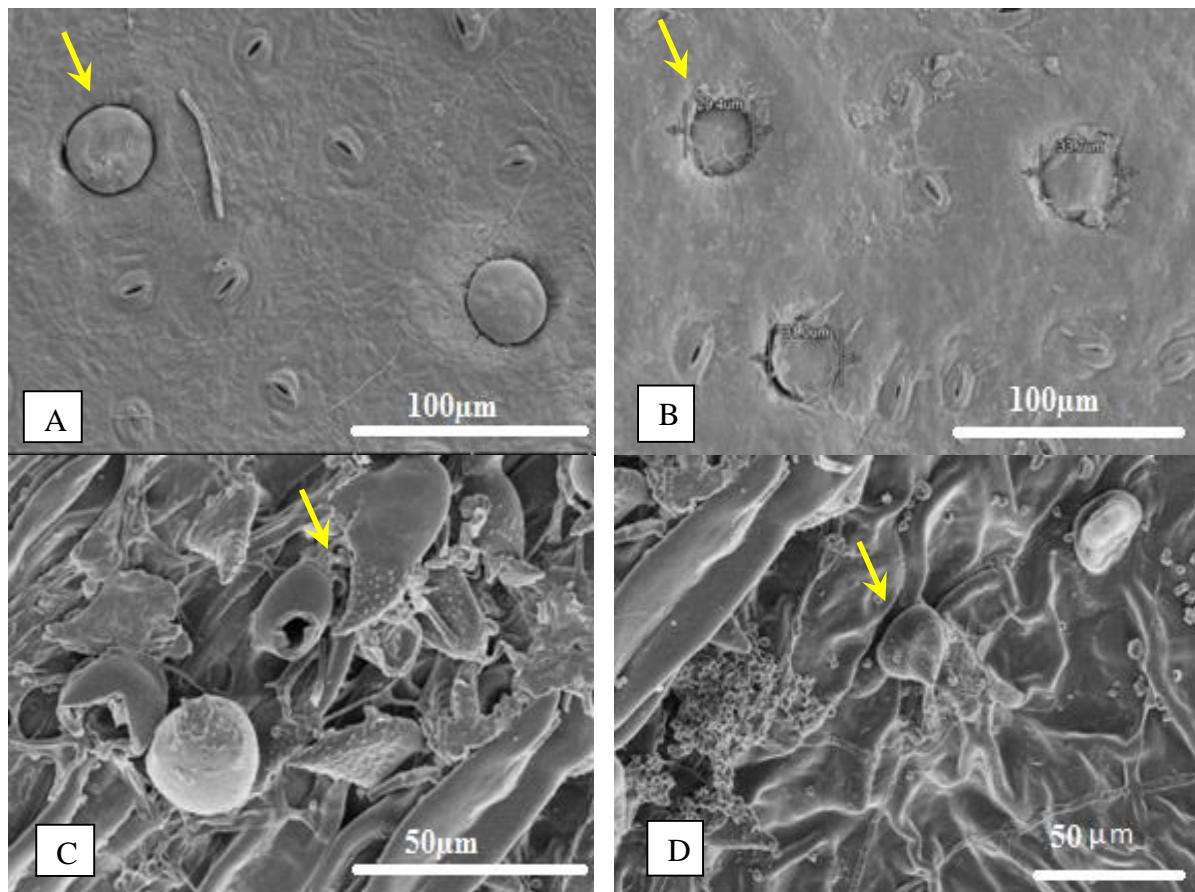
No	Species	Leaf Surface	Glandular Trichomes			Non-Glandular Trichomes		
			Type	Size (µm)	Distribution	Type	Size (µm)	Distribution
1	<i>Petraeovitex bambusetorum</i> King & Gamble	Adaxial	Peltate	30±20	Evenly	-	-	-
		Abaxial	Peltate	29±5	Evenly	-	-	-
2	<i>Petraeovitex membranacea</i> var. <i>malesiana</i> Munir	Adaxial	Peltate	30±3	Sparsely	-	-	-
		Abaxial	Peltate	36±2	Evenly	-	-	-
3	<i>Petraeovitex wolfei</i> J. Sinclair	Adaxial	Peltate	20±3	Sparsely	-	-	-
		Abaxial	Peltate	36±3	Sparsely	-	-	-
4	<i>Petraeovitex scortechinii</i> King & Gamble	Adaxial	Peltate	30±4	Sparsely	-	-	-
		Abaxial	Peltate	20±3	Sparsely	-	-	-
5	<i>Petraeovitex trifoliata</i> Merr.	Adaxial	-	-	-	Conical	17±3	Sparsely
		Abaxial	Peltate	40±8	Sparsely	Conical	22±3	Sparsely
6	<i>Peronema canescens</i> Jack	Adaxial	Capitate	15±2	Sparsely	Conical	20±2	Densely
		Abaxial	Peltate	33±3	Sparsely	Multicellular uniseriate	100±10	Densely
7	<i>Congea forbesii</i> King & Gamble	Adaxial	Peltate	30±4	Evenly	Multicellular uniseriate	400±40	Evenly
		Abaxial	Peltate	25±5	Evenly	Multicellular uniseriate	600±50	Evenly
8	<i>Congea griffithiana</i> Munir	Adaxial	Peltate	35±4	Sparsely	Multicellular uniseriate	201±5	Sparsely
		Abaxial	Peltate	43±5	Evenly	Multicellular uniseriate	400±20	Densely
9	<i>Sphenodesme racemosa</i> (C. Presl) Moldenke	Adaxial	-	-	-	-	-	-
		Abaxial	Peltate	30±3	Sparsely	-	-	-
10	<i>Sphenodesme triflora</i> Wight	Adaxial	Peltate	20±6	Sparsely	-	-	-
		Abaxial	Peltate	20±6	Sparsely	Multicellular uniseriate	80±20	Sparsely
11	<i>Sphenodesme pentandra</i> Jack	Adaxial	Peltate	35±6	Sparsely	Stellate	60±10	Sparsely
		Abaxial	Peltate	25±4	Sparsely	Stellate	60±10	Sparsely
12	<i>Rothea serrata</i> (L.) Steane & Mabb.	Adaxial	-	-	-	Conical	20±8	Densely
		Abaxial	-	-	-	Multicellular uniseriate	150±25	Densely

The adaxial and abaxial SEM micrographs of all studied species are shown in Figures 1 to 5. The types of trichome identification and terminology used were based on recognition and classification by Freitas et al. (2014), Dmitruk and Weryszko (2010), Ascenco (1999), and Payne (1978). Two forms of glandular trichomes were found in this study: peltate (Figure 1A)

and capitate (Figure 1B), as well as three types of non-glandular trichomes: multicellular uniseriate (Figure 2C), stellate (Figure 4D), and conical (Figure 5B). Based on morphological characteristics, glandular trichomes are generally classified as capitate or peltate (Werker, 1993; Ascensao et al., 1999) and can be divided according to their head size and stalk length (Mannethody & Purayidathkandy, 2018). According to Jia et al. (2013), the peltate trichome consists of a basal cell, a stalk cell, and a multicellular head, whereas the capitate trichome comprises a basal cell, a stalk cell, and a unicellular head. In many Lamiaceae species, these two main types of glandular trichomes are commonly found (Werker et al., 1993; Werker, 2000). In contrast, non-glandular trichomes can be described as follows: multicellular trichomes depict a single vertical row of cells or have more than one vertical row of cells, while their stellate counterparts are star-shaped and have several arms arising from a common base (Simpson, 2019). Another shape of this type of trichome is conical, which reflects enlarged bases and shortly pointed tips (Raman, 2017).

### 3.1. *Petraeovitex* Oliv.

In this genus, two types of trichomes were observed, namely, peltate and conical. All five species showed the existence of peltate trichome, which is a type of glandular trichome (Figures 1A-1B).



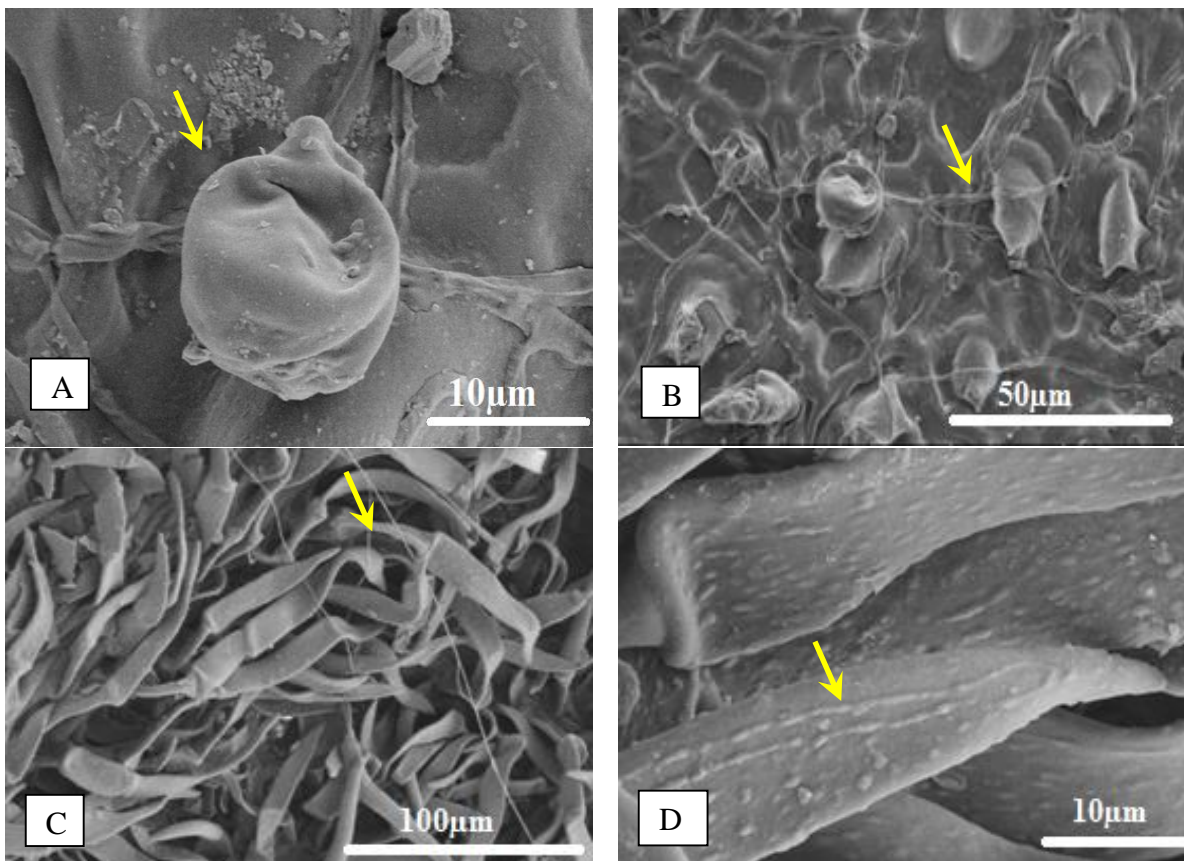
**Figure 1.** Trichomes found on the genus *Petraeovitex*: (A-B) Sunken peltate glandular trichomes on the adaxial surface; (C-D) Conical trichomes on the abaxial surface of *P. trifoliata*

This type of trichome has an average size of 25 μm. It occurs on both surfaces of a leaf, except for *P. trifoliata*, where it is only present on the abaxial surface. Figures 1A and 1B show sunken peltate glandular trichomes, which are found in species of *Petraeovitex* in this study.

According to Werker (1993), the glandular hairs become sunken upon the maturation of the leaf, while Jia (2013) has also reported that they sink gradually before finally sinking under the epidermis completely in the maturing of *Thymus quinquecostatus* leaves. The latter study has further claimed that most prior works reported that this type of trichome would fall off from the mature leaves rather than sink under the epidermis. Another type of trichome found in this genus is the conical type, which is a type of non-glandular trichome (Figure 1C-D). Among the five species, this trichome was only found in *P. trifoliata*, sparsely distributed on the abaxial surface. The average length of these trichomes is 20  $\mu\text{m}$ . Figures 1C and 1D both show conical trichomes, which are uniseriate and approximately conical in shape.

### 3.2. *Peronema* Jack

In this study, four types of trichomes were observed on both surfaces of *Peronema*, a monotypic genus, namely, capitate, peltate, conical, and multicellular uniseriate as found in *P. canescens*. For the capitate trichome, a sparse distribution is seen on the adaxial surface with an average size of 15  $\mu\text{m}$  (Figure 2A).



**Figure 2.** Trichomes found on genus *Peronema* (*P. canescens*): (A) Capitate trichome; (B) Conical trichomes on adaxial; (C) Multicellular uniseriate trichomes densely distributed on abaxial; and (D) Papillose on the multicellular uniseriate trichomes

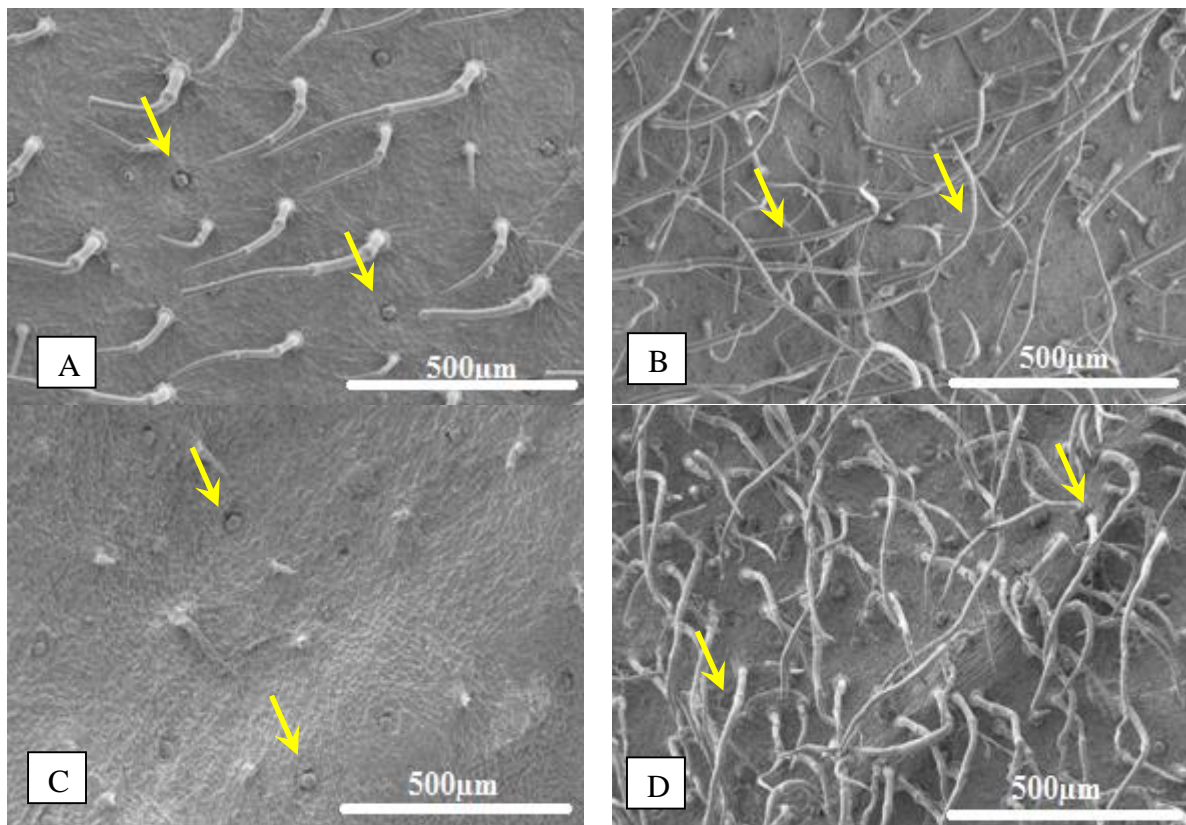
Another glandular trichome, peltate, was found to be sparsely distributed on the abaxial surface and an average size of 33  $\mu\text{m}$ . For the non-glandular trichomes, the conical and multicellular uniseriate types are densely distributed, with the former found adaxial-side with an average size of 20  $\mu\text{m}$  (Figure 2B). The multicellular 3-celled uniseriate type of trichomes had an average length size of 100  $\mu\text{m}$ , with papillose found on their surfaces. The data obtained



parallel to those of Agustin et al. (2022), which has stated that capitate trichomes and trichomes shaped like thorns (which refer to conical trichomes) could be found on *P. canescens*.

### 3.3. *Congea* Roxb.

Two species of this genus, *C. forbesii* and *C. griffithiana*, possessed both glandular and non-glandular trichomes, namely, peltate and multicellular uniseriate types. These trichomes are found on both leaf surfaces (Figure 3). Peltate trichomes were evenly distributed on both surfaces of *C. forbesii*, with the average size of 30  $\mu\text{m}$  on the adaxial and 25  $\mu\text{m}$  on the abaxial. For *C. griffithiana*, however, the peltate trichomes were also evenly distributed on both surfaces with the trichome size of 35  $\mu\text{m}$  on the adaxial and 43  $\mu\text{m}$  on the abaxial. Meanwhile, the multicellular uniseriate trichomes, which are 3-celled, were evenly distributed on both adaxial and abaxial surfaces of *C. forbesii*. The length of this type of trichome found on the abaxial was longer (600  $\mu\text{m}$ ) than on the adaxial (400  $\mu\text{m}$ ). For *C. griffithiana*, the multicellular uniseriate was 3 to 5-celled in arrangement and sparsely distributed on the adaxial (201  $\mu\text{m}$ ) while densely distributed on the abaxial (400  $\mu\text{m}$ ).

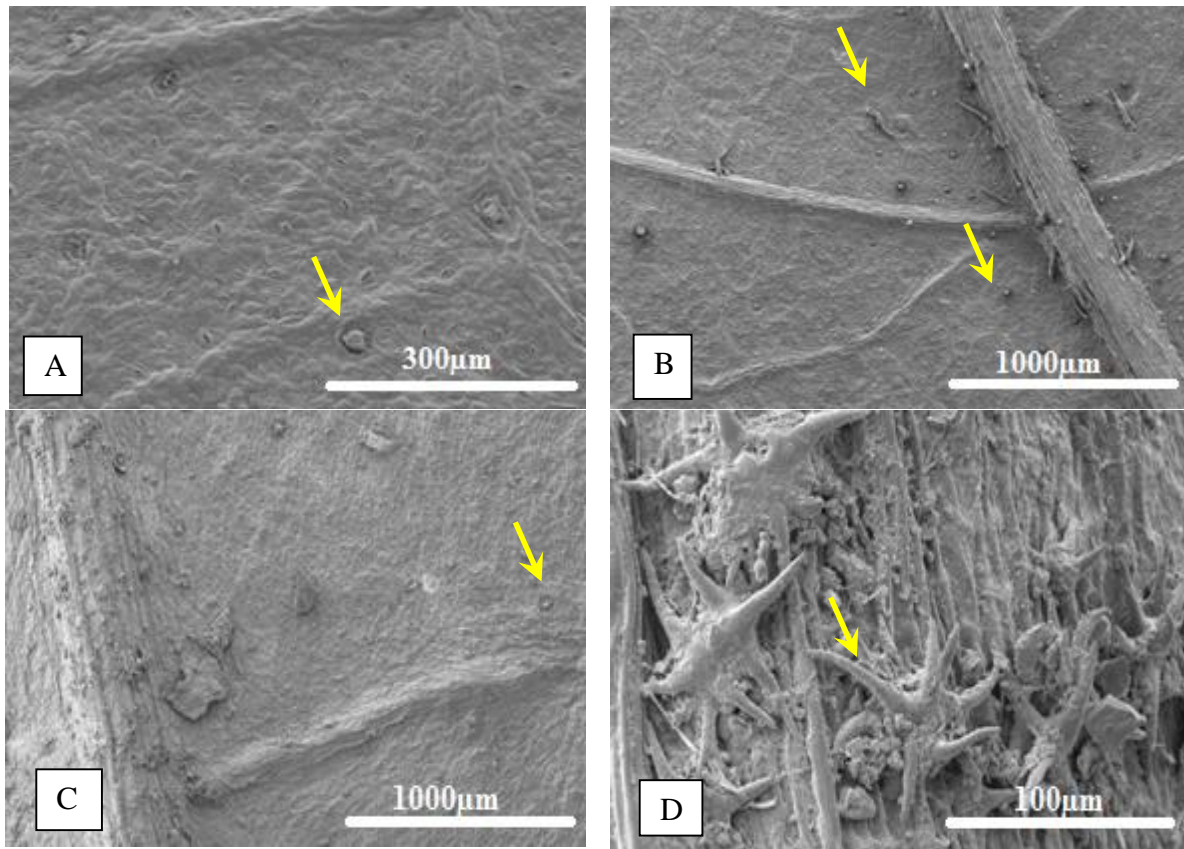


**Figure 3.** Arrows show: (A) Peltate trichomes on the adaxial of *C. forbesii*; (B) Multicellular uniseriate trichomes on the abaxial surface of *C. forbesii*; (C) Peltate trichomes on the adaxial of *C. griffithiana*; and (D) Multicellular uniseriate trichomes on the abaxial side of *C. griffithiana*

### 3.4. *Sphenodesme* Jack

Three types of trichomes were found in the three species of this genus (i.e. *S. racemosa*, *S. triflora*, and *S. pentandra*), specifically the peltate, multicellular uniseriate, and stellate types. Peltate trichome (Figure 3A) is sparsely distributed in *S. racemosa* with an average size of 30  $\mu\text{m}$ , while it is 20  $\mu\text{m}$  in *S. triflora* and 35  $\mu\text{m}$  in *S. pentandra*. The trichome could be seen on

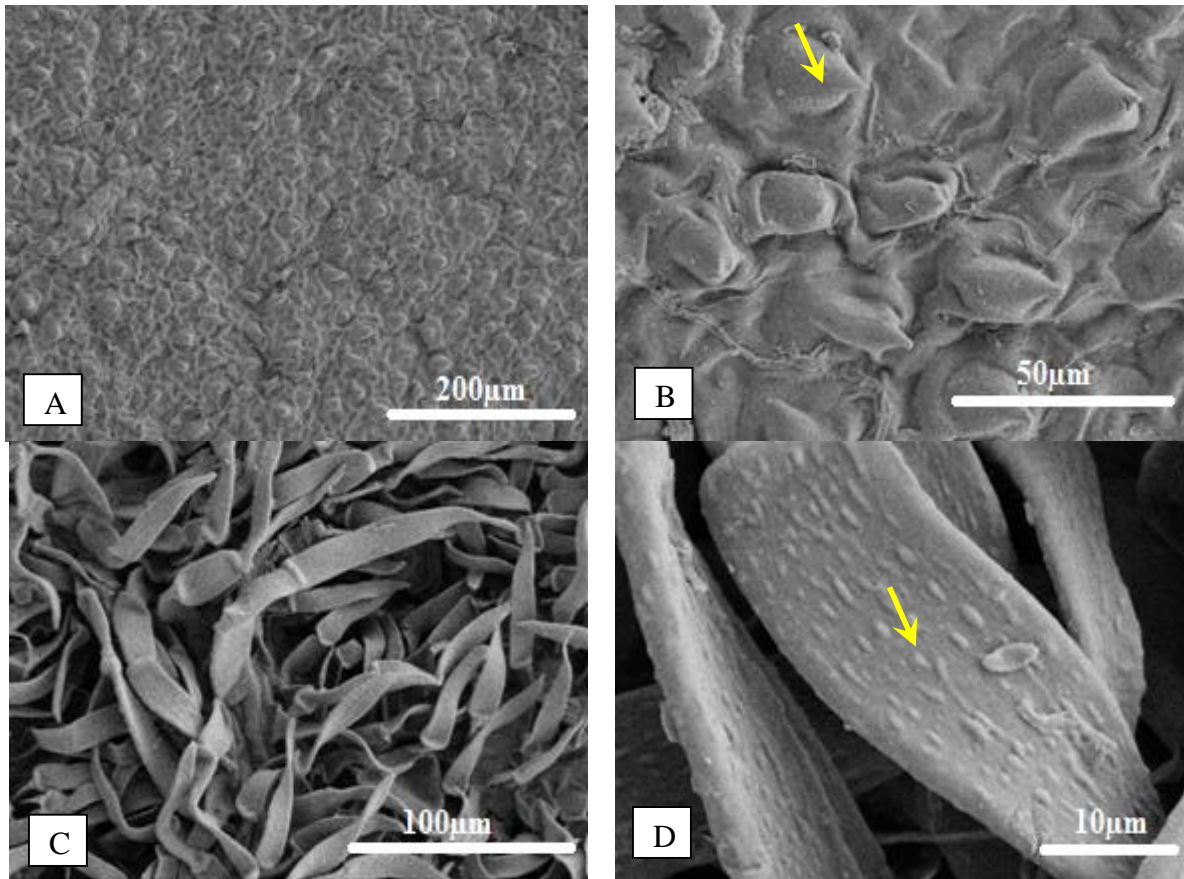
both surfaces of the leaf, except for *S. racemosa*, where it only occurred on the abaxial side. Meanwhile, two types of non-glandular trichomes were observed, namely, multicellular uniseriate and stellate. The multicellular uniseriate trichomes of 80  $\mu\text{m}$  average size are found on the abaxial surface of *S. triflora* (Figure 3B), which is 2-celled and sparsely distributed. The stellate trichomes, however, are found on both surfaces of *S. pentandra* with an average size of 60  $\mu\text{m}$  (Figure 3D).



**Figure 4.** Trichomes on the *Sphenodesme*: (A) Peltate trichomes; (B) Peltate and multicellular filiform trichomes on the abaxial surface of *S. triflora*; (C) Peltate trichomes on the abaxial side of *S. pentandra*; and (D) Stellate trichomes on the adaxial side of *S. pentandra*

### 3.5. *Rothea* Raf.

Two types of non-glandular trichomes, namely, the conical and multicellular uniseriate types, were observed in *R. serrata*, which is the only *Rothea* species found in Peninsular Malaysia. The conical trichomes show an average length size of 20  $\mu\text{m}$  and are densely distributed on the adaxial surface (Figure 5A-B). The 3-celled multicellular trichomes, meanwhile, have an average length size of 150  $\mu\text{m}$  and are found densely distributed on the abaxial side (Figure 5C). This finding is partly similar to that recorded by Phumprasert (2018), which has identified two types of trichomes in *R. serrata*, which are multicellular uniseriate and capitate glandular trichomes. In this study, however, capitate trichomes were not detectable under SEM, possibly due to the density of multiseriate uniseriate ones present on the surface (Figure 5C). Meanwhile, Phumprasert's (2018) study did not record the presence of any conical trichomes, which are conversely documented in this study as densely distributed on the adaxial surface (Figure 5A).



**Figure 5.** Trichomes of *Rothea* (*R. serrata*): (A-B) Conical trichomes densely distributed on the adaxial side; (C) Multicellular uniseriate trichomes densely distributed on the abaxial side; and (D) Papillose on the multicellular uniseriate trichomes

Lamiaceae family members have reported the presence of both peltate and capitate glandular trichomes, as well as non-glandular ones (unicellular to multicellular, uniseriate, unbranched) on their stems, inflorescence axes, leaf blades, petioles, and calyces (Kahraman et al., 2010; Kaya et al., 2007). The results showed that the diagnostics characteristics of several species could be identified. For example, the data showed that non-glandular trichome of the stellate type was only found only in *S. pentandra* while absent in other species. Another *Sphenodesme* species, namely, *S. triflora*, possessed multicellular uniseriate trichomes that were 2-celled in contrast to other species being 3 to 5-celled in nature. Furthermore, both *Congea* species investigated demonstrated multicellular uniseriate trichomes on both leaf surfaces, whereas this type could be found on only on one surface of other species. The finding also showed that *P. trifoliata* could be distinguished from other species in its genus due to the presence of conical trichomes. For the glandular type of trichomes, meanwhile, capitate trichomes were only found on the adaxial side of *P. canescens*, which was not present on both surfaces of other species. Nevertheless, the study by Phampuraset (2018) has detected this particular trichome in *R. serrata*.

The lack of information on the trichomes of the studied species has been addressed. The data presented in this study is helpful and could increase the value of trichomes in taxonomic classification, specifically their type, size, and distribution. The study displayed the dominant type of glandular trichomes, namely, peltate, while the non-glandular counterpart was the multicellular uniseriate type. For other Lamiaceae species, two types of glandular trichomes, namely, peltate and capitate, and the multicellular type of non-glandular trichomes are dominant (Kaya et al., 2007). Both glandular and non-glandular trichomes are distributed uniformly on



the leaf surface in almost all species. Contrarily, Choi and Ekim (2013) on three *Menthae* species (Lamiaceae) have reported that trichome distribution is not uniformly distributed over the leaf surfaces. Nevertheless, previous studies by Salmaki et al. (2009) and Giuliani et al. (2008) have highlighted the usefulness of trichomes in achieving the taxonomic character identification of Lamiaceae members.

#### 4. CONCLUSION

The investigation of trichomes in this study targeted the leaf parts and the resulting information offered would underpin their importance in taxonomy classification. The findings gained, in particular non-glandular trichomes, provide valuable data on species identification for the studied species. Trichome characteristics, namely, type, size, and distribution, as demonstrated in this study, successfully contribute to the identification and infrageneric classification. Further trichome studies focusing on other plant parts are undoubtedly necessary further onwards for all species known, spanning their stems, rachis, calyces, and corolla. In particular, future investigations on other aspects of micromorphological studies such as leaf sculpturing and stomata involving a wide range of species in the Lamiaceae family could provide enhanced knowledge in taxonomy studies in the context of this plant family accordingly.

#### Declaration of Interest

The authors declare that there is no conflict of interest.

#### Acknowledgment

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