



**RESEARCH ARTICLE**

**MICROSCOPY OBSERVATIONS ON LEAF EPIDERMAL MICROMORPHOLOGY OF *CONGEA* ROXB. AND *SPHENODESME* JACK (LAMIACEAE) FROM PENINSULAR MALAYSIA WITH TAXONOMIC IMPLICATIONS**

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**Abstract.** The leaf epidermal micromorphology provides important taxonomic characters for distinguishing closely related taxa within the family Lamiaceae. However, information on the micromorphological features of *Congea* and *Sphenodesme* species in Peninsular Malaysia remains limited. A micromorphological analysis of the leaf epidermis in five species belonging to the genera *Congea* and *Sphenodesme* was conducted to elucidate their generic and infrageneric relationships based on comparative epidermal traits. Microscopic observations were carried out using both light microscopy (LM) and scanning electron microscopy (SEM) to examine leaf micromorphological features, including epidermal cells, stomata, trichomes, and epicuticular wax, in *Congea forbesii*, *C. griffithiana*, *Sphenodesme racemosa*, *S. pentandra*, and *S. triflora*. The results showed that *C. forbesii* was clearly distinguished by its sinuous epidermal cells and amphistomatous leaves, in contrast to the hypostomatous condition observed in the other species. Variation in epidermal sculpturing on both leaf surfaces was recorded, with stomata predominantly flush except in *S. racemosa*, where they were slightly raised. Paracytic stomata predominated across the examined taxa, although interspecific variation in stomatal type and epidermal sculpturing provided additional diagnostic value. Trichome diversity also proved taxonomically informative, with stellate trichomes occurring uniquely in *S. pentandra*, while peltate trichomes were consistently present across all species. In addition, epicuticular wax occurred in two distinct morphological forms, namely thin rods and platelets, both contributing supplementary characters of taxonomic significance. These micromorphological traits serve as diagnostic characters for distinguishing *Congea* and *Sphenodesme*, further elucidating their taxonomic relationships within the Lamiaceae.

**Keywords:** Lamiaceae, *Congea*, *Sphenodesme*, micromorphology, leaf epidermis.

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## 1. INTRODUCTION

The genera *Congea* and *Sphenodesme*, which were formerly placed in the tribe Symphoremeeae of the family Verbenaceae, are closely related, and subsequent studies on their morphological and molecular characteristics have led to their reclassification within the family Lamiaceae [1–4].

According to the World Flora Online, fifteen accepted names are currently recognised within the genus *Congea* [5]. Initially established by Roxburgh in 1819 with the species *C. tomentosa*, described as “a native of Chittagong where it blossoms in March,” the genus was subsequently expanded to include *C. pentandra* and *C. villosa*. *Congea* is widely distributed across Asia, particularly in Southeast Asia, encompassing regions such as southern China, Myanmar, Thailand, Malaysia, and Indonesia [6]. It is estimated that there are between seven and ten species distributed across India, Bangladesh, Myanmar, Thailand, Cambodia, Laos, Vietnam, Malaysia, Sumatra, and southwestern China [1]. In Peninsular Malaysia, two species, *C. forbesii* and *C. griffithiana*, have been documented [2,7]. Species within the genus are characterised as climbing shrubs with entire leaves, bearing terminal or axillary inflorescences and capitate cymes comprising three to nine flowers [1].

The genus *Sphenodesme* comprises 22 accepted species names according to the World Flora Online [8]. It was first established by Jack in 1820, with *S. pentandra* as the initial species and the type specimen collected in Penang. Originally placed in the tribe Viticeae of the family Verbenaceae, the genus was distinguished from *Congea* of Roxburgh based on several floral characters, including “the leaves of the involucre being all distinct; the nearly regular corolla; and the five nearly equal stamens” [9]. In Peninsular Malaysia, *Sphenodesme* is represented by three species: *S. pentandra* and *S. racemosa*, which are typically confined to lowland forest habitats, and *S. triflora*, which occurs in both lowland and hill forest zones [7]. These climbing shrubs have entire leaves and frequent presence of paniculate inflorescences, which are both terminal and axillary [1]. Despite its broad distribution across tropical Asia, including India, China, Myanmar, and parts of Southeast Asia, the genus nevertheless remains geographically confined to this region [9].

Foliar micromorphological characteristics, including trichome types, stomatal configuration, epidermal cell outlines, and anticlinal wall patterns, are recognised as reliable diagnostic features for species delimitation and systematic placement within Lamiaceae [10]. In *Congea*, peltate and multicellular uniseriate trichomes have been documented, whereas *Sphenodesme* exhibits a broader diversity, encompassing peltate, multicellular uniseriate, and stellate forms that contribute to interspecific differentiation [11]. Although detailed investigations of these genera remain limited, taxonomic studies and ongoing revisions of Lamiaceae, which include *Congea*, *Sphenodesme*, and other taxa formerly classified within Verbenaceae, continue to refine their systematic placement [3,4]. This study examines micromorphological traits, including epidermal cell shape, stomatal features, trichomes, and epicuticular wax, to evaluate their taxonomic significance and aid species identification within these genera.

## 2. MATERIALS AND METHODS

Specimens of two *Congea* species and three *Sphenodesme* species were collected from various localities across Peninsular Malaysia, as detailed in Table 1. Field collection followed standard botanical procedures, with notes taken on locality, habitat, and general morphological features observed in the field. Fresh leaves intended for SEM observation were gently cleaned and lightly sprayed with 70% ethanol before being pressed in newspapers and dried at approximately 40–45 °C to preserve surface morphology for voucher preparation. The procedures for voucher preparation were followed as described in [12]. Leaves designated for LM observation were stored in 70% ethanol prior to epidermal preparation to prevent tissue hardening. Voucher specimens of all studied taxa have been deposited in the Herbarium of Universiti Pendidikan Sultan Idris (FP), Malaysia.

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

| Taxa  | Voucher no. | Locality   |
|---|-------------|--|
| <i>Congea forbesii</i> King & Gamble            | NHH013      | Selangor, Kepong, FRIM                           |
| <i>Congea griffithiana</i> Munir                | NHH094      | Kedah, Kuala Nerang, Bukit Janing Forest Reserve |
| <i>Sphenodesme racemosa</i> (C. Presl) Moldenke | NHH007      | Selangor, Kepong Botanical Garden                |
|   | NHH034      | Pahang, Jerantut, Yong Forest Reserve            |
| <i>Sphenodesme triflora</i> Wight               | NHH010      | Kedah, Langkawi                                  |
|   | NHH061      | Pahang, Tasik Cini                               |
| <i>Sphenodesme pentandra</i> Jack               | NHH058      | Pahang, Jengka, Sg. Tekam                        |

The micromorphological characteristics of the leaf epidermis in *Congea* and *Sphenodesme* were studied using light microscopy (LM) and scanning electron microscopy (SEM), following standard procedures [13,14]. Light microscopy was used to observe stomata, trichomes, and other epidermal cells, while SEM provided high-resolution images of the leaf surface. These techniques enabled a thorough analysis of both abaxial and adaxial leaf surfaces.

For LM, the epidermis was prepared by soaking the middle section of each leaf in bleach for 15–20 minutes. The softened tissue was gently scraped on a white tile to isolate the epidermal layer and then stained with Safranin 'O'. The samples underwent graded dehydration (50%, 70%, 95%, and 100% ethanol, followed by 100% ethanol and xylene). The stained and dehydrated specimens were mounted with Eupharal and left to dry on a slide warmer before being observed under a light microscope. For SEM, freshly collected leaves were cleaned with 70% ethanol and oven-dried at 40 °C for a week. Small sections (0.5 cm × 0.5 cm) from the mid-lamina were prepared from both leaf surfaces. These sections were mounted on stubs with adhesive tape, gold-coated, and examined under SEM to capture high-resolution images.

### 3. RESULTS AND DISCUSSION

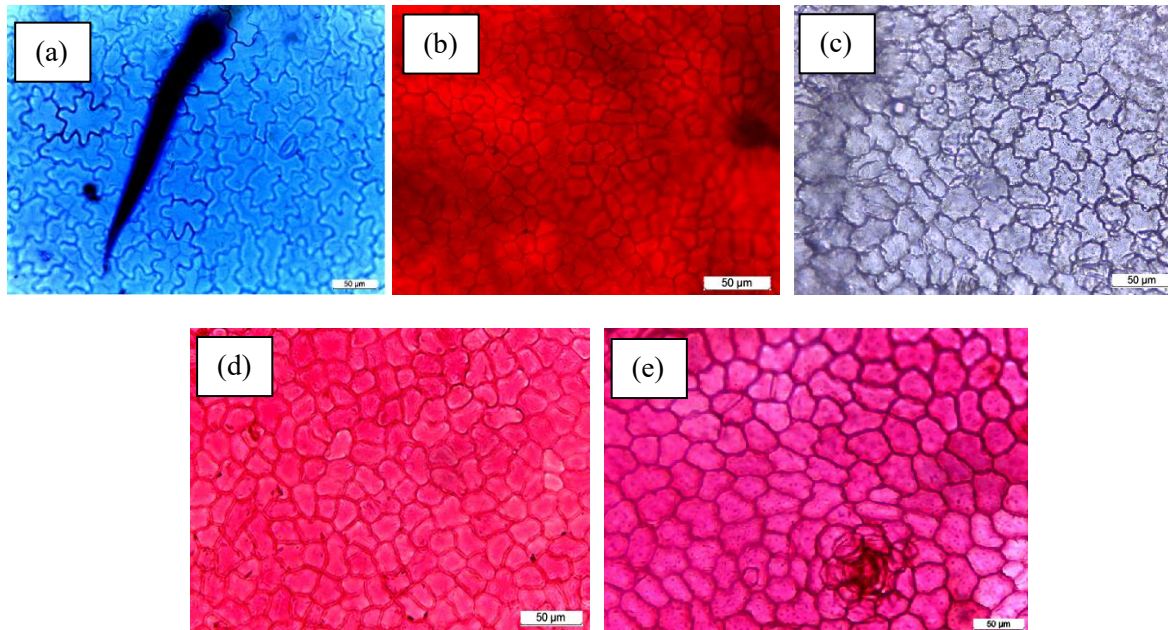
The analysis of epidermal anticlinal wall shapes revealed the presence of both sinuous and straight-to-curved configurations among the studied species (Table 2). Notably, *Congea forbesii* was unique in exhibiting a sinuous anticlinal wall, while the other four species displayed straight to curved shapes. This morphological distinction serves as a critical diagnostic feature, enabling the differentiation of *Congea forbesii* from its congeners.

**Table 2:** LM observations of the epidermal characteristics and stomata on the leaf surfaces.

| No. | Species                      | Epidermal anticlinal wall Shape | Stomata  |                       | Trichomes         |           |                                      |
|-----|------------------------------|---------------------------------|----------|-----------------------|-------------------|-----------|--------------------------------------|
|     |                              |                                 | Adaxial  | Abaxial               | Number of stomata | Size (µm) |                                      |
| 1.  | <i>Congea forbesii</i>       | Sinuous                         | Diacytic | Diacytic, Paracytic   | 1 (ad), 25 (ab)   | 40-48     | Peltate/<br>Multicellular uniseriate |
| 2.  | <i>Congea griffithiana</i>   | Straight to curved              | -        | Paracytic             | 23 (ab)           | 23-29     | Peltate/<br>Multicellular uniseriate |
| 3.  | <i>Sphenodesme racemosa</i>  | Straight to curved              | -        | Paracytic             | 18 (ab)           | 24-30     | Peltate<br>Multicellular uniseriate  |
| 4.  | <i>Sphenodesme triflora</i>  | Straight to curved              | -        | Branchparacytic       | 13 (ab)           | 25-46     | Peltate                              |
| 5.  | <i>Sphenodesme pentandra</i> | Straight to curved              | -        | Anomocytic, Paracytic | 18 (ab)           | 30-42     | Peltate/<br>Stellate                 |

Note: ad = adaxial surface; ab = abaxial surface.

Three key stomatal characteristics: type, number, and size were evaluated through LM, while additional features, including stomatal sculpturing type and their level, were examined using scanning electron microscopy (SEM). Figure 1 shows the epidermal features on the adaxial surfaces of the five species. The results revealed that four of the five species are hypostomatous, with the exception of *Congea forbesii*, which exhibits amphistomatous leaves. This finding contradicts earlier reports that described *C. forbesii* as hypostomatous [15]. However, in the present study, stomata were detected sparsely on the adaxial surface, in contrast to their dense distribution on the abaxial surface.

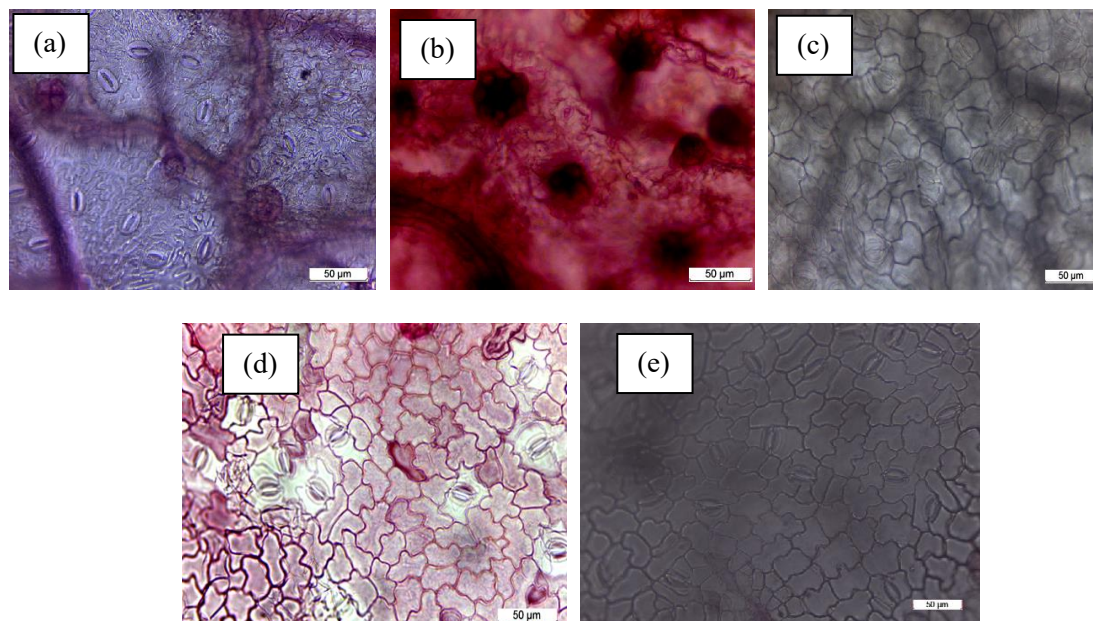


**Figure 1:** Light microscopy (LM) micrographs of the epidermal anticlinal wall shapes on the adaxial surfaces of five species: (a) sinuous anticlinal wall in *Congea forbesii*; (b)-(e) straight to curved anticlinal walls in (b) *Congea griffithiana*, (c) *Sphenodesme racemosa*, (d) *Sphenodesme triflora*, and (e) *Sphenodesme pentandra*

The identification and classification of stomatal types followed the criteria proposed in previous studies [13]. In *Congea forbesii*, two stomatal types namely diacytic and paracytic were observed. However, a previous study documented a broader range of stomatal types in this species, including anomocytic (the most common type), as well as actinocytic, anisocytic, and paracytic stomata [15]. Additionally, *Sphenodesme pentandra*, which was included in Cantino's study [15], exhibited similar results in this research, being documented as possessing hypostomatous leaves with anomocytic and paracytic stomata. This combination provides a diagnostic marker within *Sphenodesme*, as the coexistence of two stomatal types is not shared by the other studied species. All five species investigated exhibited paracytic stomata, with *Congea griffithiana*, *Sphenodesme racemosa*, and *Sphenodesme triflora* each exhibiting only a single stomatal type. *Congea griffithiana* and *Sphenodesme racemosa* possess paracytic stomata, whereas *Sphenodesme triflora* is characterised by branchparacytic stomata, in which two subsidiary cells are positioned laterally alongside the guard cells without completely enclosing them [13]. *Sphenodesme pentandra*, in turn, exhibits a combination of anomocytic and paracytic stomatal types. The observed consistency in stomatal type across these species indicates commonalities among them within the same subfamily.

The average number of stomata ranged from 1 to 25 per 50 mm<sup>2</sup>. Stomatal distribution was evaluated by counting the number of stomata within a standard frame of 250 × 200 µm. Based on the number observed, three distinct distribution patterns were identified on the leaf surfaces: sparse (0–10 stomata), moderate (11–20 stomata), and dense (21–30 stomata). *Congea* species exhibited a dense stomatal distribution on the abaxial surface, while all three *Sphenodesme* species showed a moderate distribution on the same surface.

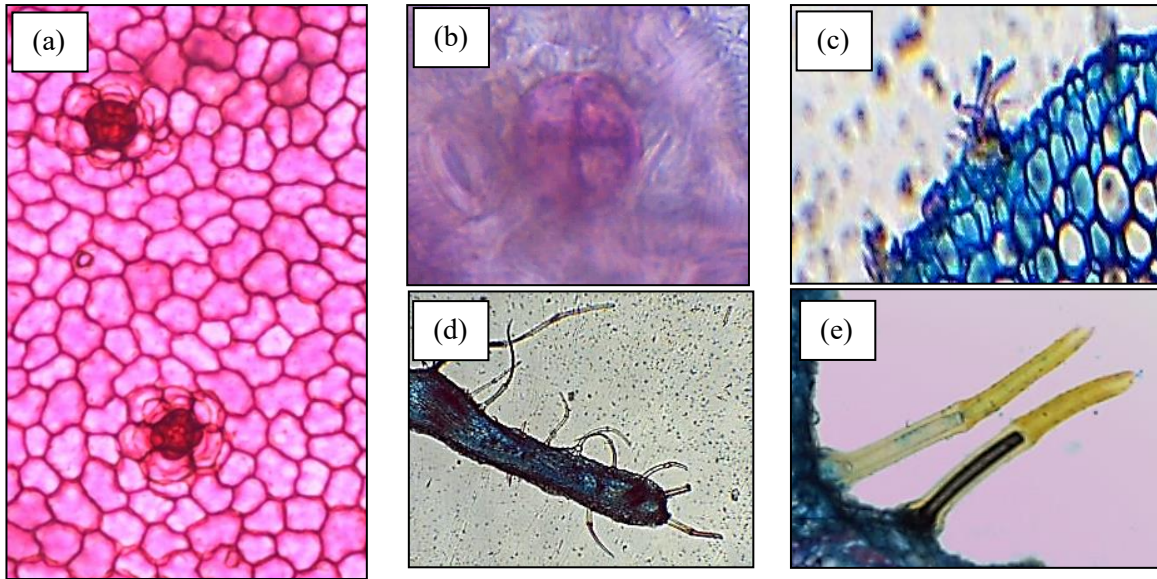
Figure 2 shows the abaxial epidermal surfaces of the five species, illustrating considerable variation in stomatal size, which ranged from 24 to 48  $\mu\text{m}$ . *Congea forbesii* exhibited the largest stomata (40–48  $\mu\text{m}$ ), whereas *C. griffithiana* possessed the smallest (23–29  $\mu\text{m}$ ).



**Figure 2:** Light microscopy (LM) micrographs showing epidermal anticlinal wall shapes on the abaxial surfaces of five species: (a) sinuous anticlinal wall in *Congea forbesii*; (b)-(e) straight to curved anticlinal walls in (b) *Congea griffithiana*, (c) *Sphenodesme racemosa*, (d) *Sphenodesme triflora*, and (e) *Sphenodesme pentandra*

Trichomes serve as valuable taxonomic characters for species identification and classification, in addition to their protective role in plants [13]. Light microscopy (LM) observations revealed three trichome types, namely peltate, multicellular uniseriate, and stellate, following the terminology and morphological descriptions outlined by a previous researcher [16]. Within the genus *Congea*, both *C. forbesii* and *C. griffithiana* exhibit a combination of peltate and multicellular uniseriate trichomes. Figure 3 shows the trichomes, with peltate trichomes (Figure 3(a) and (b)) composed of a basal cell embedded in the epidermis, a short stalk cell, and a large, spherical secretory head consisting of four secretory cells arranged in a single layer. Multicellular uniseriate trichomes (Figure 3(d) and (e)) consist of two or more cells aligned in a single vertical row. In the genus *Sphenodesme*, trichome diversity is more variable. *S. racemosa* exhibits both peltate and multicellular uniseriate trichomes, whereas *S. triflora* displays only peltate trichomes. *S. pentandra*, on the other hand, possesses both peltate and stellate trichomes. The stellate trichomes (Figure 3(c)), which are star-shaped, have several arms radiating from a common base. This unique occurrence of stellate trichomes in *S. pentandra* represents a distinctive diagnostic character within the genus.

High-resolution SEM observations were conducted on the leaf surfaces of five species, focusing on three main aspects of sculpturing. The micromorphological characteristics of *Congea* and *Sphenodesme* species reveal notable distinctions across primary sculpturing, stomatal sculpturing, and epicuticular wax deposition. The findings are summarised in Table 3.

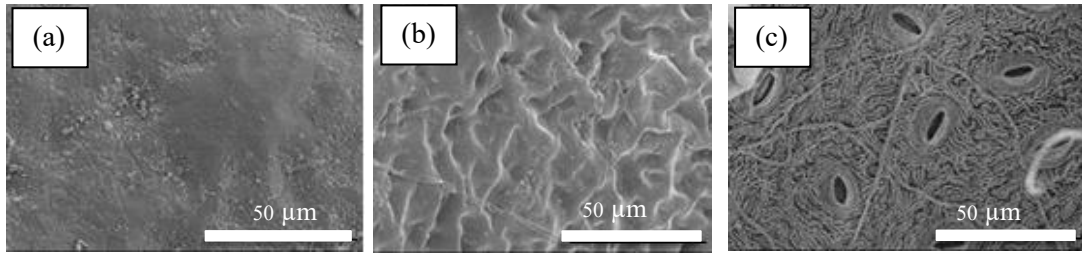


**Figure 3:** Light microscopy (LM) micrographs showing trichome types in Lamiaceae species: (a) peltate trichomes on the adaxial surface of *Sphenodesme pentandra*, (b) peltate trichomes on the abaxial surface of *Congea forbesii*, (c) stellate trichome on the petiole of *S. pentandra*, (d) multicellular uniseriate trichomes on the margin of *C. forbesii* and (e) multicellular uniseriate trichomes on the midrib of *Sphenodesme racemosa*.

**Table 3:** SEM observations of leaf surface sculpturing and epicuticular wax characteristics

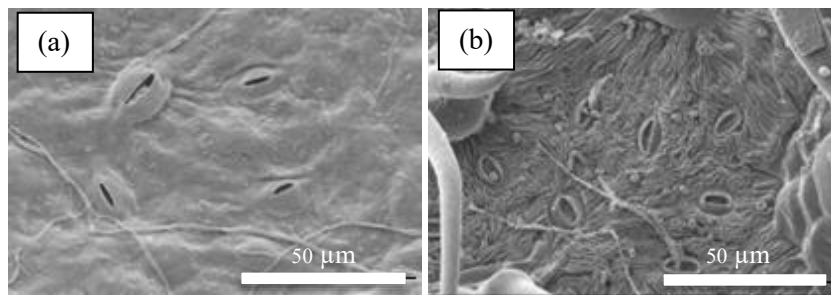
| No. | Species                      | Primary sculpturing |         | Stomatal sculpturing |                       | Epicuticular wax |           |
|-----|------------------------------|---------------------|---------|----------------------|-----------------------|------------------|-----------|
|     |                              | Adaxial             | Abaxial | Type                 | Position to Epidermis | Thin rods        | Platelets |
| 1.  | <i>Congea forbesii</i>       | Type 1              | Type 3  | Type 2               | Same level            | +                | -         |
| 2.  | <i>Congea griffithiana</i>   | Type 2              | Type 3  | Type 2               | Same level            | +                | -         |
| 3.  | <i>Sphenodesme racemosa</i>  | Type 1              | Type 2  | Type 2               | Slightly raised       | +                | +         |
| 4.  | <i>Sphenodesme triflora</i>  | Type 1              | Type 1  | Type 1               | Same level            | +                | +         |
| 5.  | <i>Sphenodesme pentandra</i> | Type 1              | Type 1  | Type 1               | Same level            | +                | +         |

Three types of primary leaf sculpturing were identified, as shown in Figure 4. These types are described as follows: Type 1 – a relatively smooth surface with tiny hollow parts on the surface; Type 2 – broad protrusions clustered together, forming a reticulate pattern; and Type 3 – closely packed, uniformly shaped protrusions densely arranged. In *Congea* species, primary sculpturing patterns exhibit variation between adaxial and abaxial surfaces, with *C. forbesii* displaying Type 1 on the adaxial and Type 3 on the abaxial surface, while *C. griffithiana* presents Type 2 on the adaxial and Type 3 on the abaxial. This differentiation contrasts with the uniform Type 1 sculpturing observed on both adaxial and abaxial surfaces of *Sphenodesme* species, except for *S. racemosa*, which shows Type 2 sculpturing on the abaxial surface.



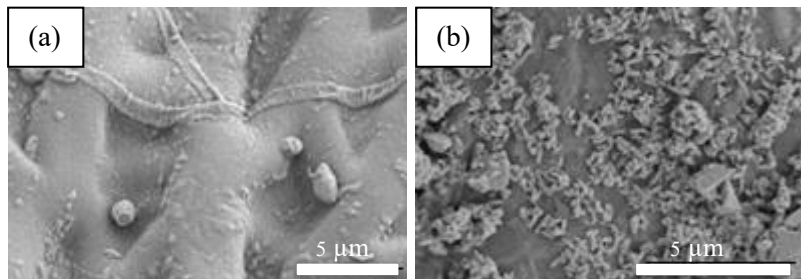
**Figure 4:** SEM micrographs depicting primary sculpturing types: (a) Type 1 on the adaxial surface of *Sphenodesme triflora*, (b) Type 2 on the adaxial surface of *Congea griffithiana*, and (c) Type 3 on the abaxial surface of *Congea forbesii*

Two types of stomatal sculpturing have been identified: Type 1, characterised by the absence of prominent protrusion flanges or surrounding depressions, and Type 2, marked by multiple protrusion flanges running parallel towards both poles of the stomata (Figure 5). The stomatal level classification follows the descriptions by Pratt [17], who identified three levels relative to the surrounding epidermal cells: raised, sunken, and same level as the other epidermal cells. Stomatal sculpturing further highlights interspecific variations. Both *Congea forbesii* and *C. griffithiana* exhibit Type 2 stomatal sculpturing that is positioned at the same level as the epidermis. Conversely, *Sphenodesme* species show distinct patterns; *S. racemosa* is characterised by slightly raised Type 2 stomatal sculpturing, while *S. triflora* and *S. pentandra* display Type 1 at the same level as the surrounding epidermal cells.



**Figure 5:** SEM micrographs illustrating types of primary sculpturing: (a) Type 1 on the abaxial surface of *Sphenodesme triflora* and (b) Type 2 on the abaxial surface of *Congea griffithiana*

In addition to primary and stomatal sculpturing, SEM was utilised to examine epicuticular wax, which occurred in two forms: thin rods and platelets, the latter exhibiting a flake-like appearance, as shown in Figure 6.



**Figure 6:** SEM micrographs of epicuticular wax: (a) thin rod on the adaxial surface of *Congea griffithiana* and (b) platelets on the adaxial surface of *Sphenodesme pentandra*

The composition of epicuticular wax is similarly differentiated between the genera. Thin rods (Figure 6(a)) are consistently present across all studied species, resembling the rodlets or threads described by Barthlott. However, platelets are absent in *Congea* species but are present in all

*Sphenodesme* species. These platelets, defined as flat crystalloids with entire margins and a regular shape, typically measure 1–3  $\mu\text{m}$  [18]. A recent study reported that in certain Lamiaceae taxa, the leaf surface is characterised by epicuticular waxes, which vary in shape from particles to granules and exhibit differing densities [19].

The presence of paracytic stomata as the predominant type in both genera aligns with findings from related studies on Lamiaceae micromorphology [20]. However, *Congea forbesii*'s amphistomatous leaves and unique sinuous epidermal cell shapes serve as distinguishing features, separating it from its congeners, which are all hypostomatous. The analysis also reveals that *Congea forbesii* possesses a unique combination of stomatal types, suggesting an adaptation or evolutionary trait specific to its ecological niche. In contrast, *Sphenodesme* species exhibit more consistent stomatal and epidermal characteristics across their species, reinforcing the coherence within this genus. The sculpturing patterns and epicuticular wax types further illustrate important taxonomic markers between the two genera. While *Congea* species display a mixture of sculpturing types across both leaf surfaces, *Sphenodesme* species exhibit relatively uniform sculpturing, particularly on their adaxial surfaces. Epicuticular wax formations, such as thin rods common to all species and platelets exclusive to *Sphenodesme*, contribute further insights into genus-level differentiation. Collectively, these micromorphological variations offer a solid basis for species identification within *Congea* and *Sphenodesme*, thereby enriching the micromorphological data on the Lamiaceae family and aiding in the systematic positioning of these genera within the family.

#### 4. CONCLUSIONS

This study highlights the significance of micromorphological traits of the leaf epidermis in *Congea* and *Sphenodesme* species from Peninsular Malaysia, revealing distinctive features that are crucial for taxonomic classification. Key findings indicate that *Congea forbesii* possesses unique characteristics, such as sinuous epidermal cells and amphistomatous leaves, which effectively distinguish it from other species. The study identified predominant stomatal types, with paracytic stomata being the most common across the genera, while variations in stomatal density and size further contribute to species differentiation. Additionally, the analysis of trichomes reveals that all species exhibit peltate trichomes, with *Congea forbesii*, *Congea griffithiana*, and *Sphenodesme racemosa* also showing multicellular uniseriate trichomes, and *Sphenodesme pentandra* uniquely possessing stellate trichomes. The analysis of leaf sculpturing patterns and epicuticular wax forms provides further insights into the morphological diversity within these genera. Overall, the results underscore the value of micromorphological characteristics in enhancing our understanding of the taxonomy and classification of *Congea* and *Sphenodesme*, supporting their reclassification within the Lamiaceae family. Future research incorporating molecular techniques could further elucidate the phylogenetic relationships among these species, enriching the taxonomic framework of this intriguing group.

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#### Author Contributions

Norhazila Hussin collected the specimens, conducted the laboratory work, analysed and interpreted the data, and drafted the manuscript. Nor Nafizah Mohd Noor conceptualised and designed the study, supervised the research, and provided critical revisions. Fatimah Mohamed assisted with data interpretation and contributed to manuscript editing. All authors 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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