



RESEARCH ARTICLE

ANATOMICAL AND MICROMORPHOLOGICAL CHARACTERISTICS OF LEAF EPIDERMIS OF SELECTED SPECIES OF *Hoya* R.BR. (APOCYNACEAE) IN PENINSULAR MALAYSIA

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Abstract. Leaf anatomy and micromorphology studies were conducted on ten selected *Hoya* species in Peninsular Malaysia, namely *H. archboldiana* C.Norman, *H. bhutanica* Grierson & D.G.Long, *H. glabra* Schltr., *H. halconensis* Kloppenb., *H. hanhiae* V.T.Pham & Aver., *H. limoniaca* S.Moore, *H. mindorensis* Schltr., *H. multiflora* Blume, *H. paziae* Kloppenb. and *H. pubifera* Elmer. This study aims to identify the common characteristics, variations, and diagnostic features of leaf anatomy and micromorphology in the studied *Hoya* species and to construct a dichotomous key for species identification. This study involved epidermal screening techniques for abaxial and adaxial epidermal observations of leaves, while micromorphological observations involved gold coating, critical point drying, and examination with a scanning electron microscope. Common characteristics observed include the presence and distribution of stomata, leaf margin patterning, and the pattern of anticlinal walls on the leaf's epidermal surface. Variable characteristics include the type of wax, cuticle engraving on the leaf epidermis, structural composition, margin, shape, size, stomatal index, trichomes, leaf margins, starch nodules, and crystals. Diagnostic features identified include the leaf epidermal wax type and starch nodules, the structure of stomata, and trichomes. A dichotomous key for species identification can be developed. This study's results demonstrated that the leaves' anatomical and micromorphological characteristics hold taxonomic significance for identifying, differentiating, and classifying the *Hoya* species studied.

Keywords: *Hoya* R.Br., taxonomy, leaf anatomy, leaf micromorphology.

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1. INTRODUCTION

Apocynaceae or commonly known as the ‘Dogbane’ family, is one of the ten largest angiosperm families, consisting of 378 genera and 5,350 species [1]. Species within this family include medium to large trees, shrubs, and mostly lianas or climbers, mainly found in pantropical regions, including Peninsular Malaysia, Borneo, Papua New Guinea, South Africa, and Madagascar. *Hoya* R.Br. of the subfamily Asclepiadoideae tribe Marsdenieae is the largest genus within Apocynaceae, with over 500 species recorded worldwide [2,3]. The first revision of this genus in Peninsular Malaysia listed 23 species [4]. Since then, multiple revisions have been made, and the genus now comprises 36 species in Peninsular Malaysia [5].

Hoya species are widely used as ornamental plants and are sold commercially at high prices due to their beautiful and fragrant waxy flowers [2,6]. Recent trends indicate that *Hoya* is in high demand for decorative and personal collections, as it is hardy and easy to care for in pots, with blooms that frequently appear [7]. *Hoya* is also widely used in traditional medicine. In Malaysia, *H. multiflora* is used to treat rheumatism [8]. Ethnobotanical studies have found that *H. diversifolia* can help with skin conditions like eczema and acne, and *H. parasitica* leaf extracts show analgesic, antidiarrheal, and antipyretic effects [8,9].

Despite its high economic value, knowledge of the number of *Hoya* species and the taxonomic relationship of this genus remains controversial. The taxonomic issues in *Hoya* have led to uncertainty on the actual number of species due to differing opinions among taxonomists [10]. Various approaches have been taken to resolve species identification based on foliar morphology and reproductive organs, but these characteristics can be difficult to obtain and may change due to environmental influences, with the main challenge of getting complete samples with fruits and flowers [11]. Biochemical and molecular methods have also been used to distinguish *Hoya* species. However, these approaches can be costly, and there are still uncertainties in some cases [12].

Anatomical and micromorphological characteristics play an important role in plant taxonomy by providing additional information to help in species identification and classification [13]. Plant anatomy involves examining the structure of tissues and cells of plant organs using a light microscope (LM), while plant micromorphological studies allow for micro-level analysis of plant structures such as pollen, leaves, tissues, and seeds using a scanning electron microscope (SEM) [14]. Previous studies have shown that leaf epidermal characteristics such as stomata, trichomes, and cell structure are useful in identifying plant species, especially when other parts like flowers or fruits are not available [15].

Previous studies on *Hoya* species have highlighted the significance of anatomical and micromorphological characteristics for species identification. Hafiz et al. [16] studied various *Hoya* species and identified common anatomical features such as epidermal layers, stomatal size and types, and the presence of trichomes, which provided insights into species relationships. Salas et al. [17] studied the leaves of *H. incrassata* and *H. soligamiana*, focusing on the abaxial and adaxial epidermal surfaces, venation, and lamina. The anatomical differences between the two species were found in the type of stomata where *H. incrassata* has sunken cyclocytic stomata while *H. soligamiana* has actinocytic stomata. A study by Basir et al. [18] discovered specific trichome characteristics from three *Hoya* species, namely *H. cagayanensis*, *H. lacunose*, and *H. coriacea*. This study proved that anatomical and micromorphological characteristics of plant structures such as trichomes can provide diagnostic characteristics to differentiate closely related *Hoya* species. Despite these studies, research on the anatomy and micromorphology of *Hoya* remains limited, with many species yet to be fully described. Therefore, the objective of this study was to identify the general, variation and diagnostic characteristics of leaf anatomy and micromorphology of the studied *Hoya* species. The results of this study were used to construct a dichotomous key for species identification.

2. MATERIALS AND METHODS

This study involved ten species from the genus *Hoya* from Peninsular Malaysia (Table 1). Study specimens were preserved using AA fixing solution (concentrated Acetic Acid: Alcohol 70 %) with a ratio of 1:3 for 48 hours. The epidermal scraping method was used to obtain the adaxial and abaxial epidermal layers of leaves. The staining process was done using Safranin and Alcian Blue dye solutions. The dehydration process used 95 % and 100 % alcohol before being dripped with Euparal mount on the slide and dried in a 50 °C oven for a week. Observation of anatomical features was performed under an Olympus BX43 microscope. The leaf micromorphology study method was performed on a leaf lamina section measuring 1cm³, fixed with Glutaraldehyde 4 % for 12 to 24 hours at 4 °C before being washed with 0.1 M Phosphate Buffer solution for 10 minutes, performed three times. The dehydration process was done by soaking the tissue in a series of Acetone 35, 50, 75, 95, and 100 %. Samples were subjected to critical drying point and plated with gold before being observed under a scanning electron microscope. Analysis and comparison of data were performed for the construction of a dichotomous key for species identification. The study method of leaf anatomy and micromorphology is according to the method of Noraini et al. [19].

Table 1: List of *Hoya* species studied

Species	Code	Locality	Date
<i>H. archboldiana</i>	H23	Bangi Botanical Garden UKM	28.12.2020
<i>H. bhutanica</i>	H24	Bangi Botanical Garden UKM	28.12.2020
<i>H. glabra</i>	H26	Bangi Botanical Garden UKM	28.12.2020
<i>H. halconensis</i>	H25	Bangi Botanical Garden UKM	28.12.2020
<i>H. hanhiae</i>	H12	Bangi Botanical Garden UKM	28.12.2020
<i>H. limoniaca</i>	H9	Bangi Botanical Garden UKM	28.12.2020
<i>H. mindorensis</i>	H27	Bangi Botanical Garden UKM	28.12.2020
<i>H. multiflora</i>	H8	Bangi Botanical Garden UKM	28.12.2020
<i>H. paziae</i>	H49	Bangi Botanical Garden UKM	05.01.2021
<i>H. pubifera</i>	H7	Bangi Botanical Garden UKM	28.12.2020

3. RESULTS AND DISCUSSION

The results of the study showed that there were two common characteristics of leaf anatomy and micromorphology for the ten *Hoya* species studied, namely the presence and distribution of stomata and the anticline wall pattern on the epidermal surface of the leaf (Figure 1(a) and (b)). All the *Hoya* species examined share the same stomatal type, classified as hypostomatic. The term hypostomatic refers to the presence of stomata exclusively on the abaxial epidermal surface. Similarly, a previous study by Salas et al. [17] on ten *Hoya* species, including *H. bilobata*, *H. diversifolia*, *H. dolichosparte*, *H. kuhlii*, *H. lacunosa*, *H. latifolia*, *H. micrantha*, *H. oblanceolata*, *H. purpureofusca*, and *H. verticillata*, also reported stomata restricted to the abaxial epidermis.

The hypostomatic stomatal arrangement is advantageous for plants as it helps reduce transpiration rates. Additionally, the distribution of stomata on the adaxial epidermal surface in all species studied was random. This study also showed that the anticline wall pattern on both the abaxial and adaxial epidermis of the leaf was straight to curved. This feature is a general feature of the genus and cannot be used to differentiate the *Hoya* species studied.

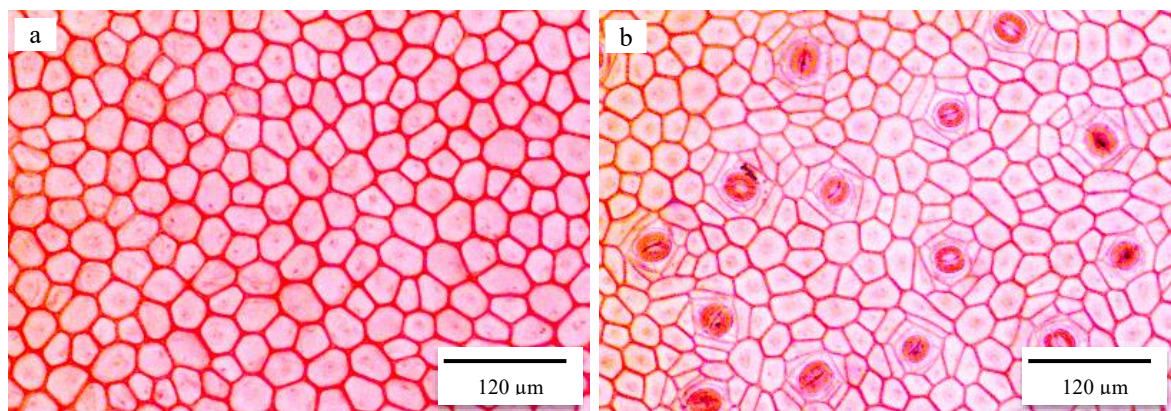


Figure 1: LM micrographs showing the epidermal surface: (a) Adaxial epidermal surface and (b) Abaxial epidermal surface of *H. limoniaca*. Scale bar: 120µm

Variations in anatomical and micromorphological characteristics can be seen in the type of wax, cuticular carving, type of stomata, structure of the stomata, epidermal cells or subsidiary cells encapsulating the stomata, presence of stripes, frames, margins, shape, size, and index of the stomata, presence of trichomes, crystals and nodules starch.

This study identified two stomatal positions, which were superficial and parafacial. Superficial stomata are raised above the epidermal layer, while parafacial stomata are partially raised or positioned at the same level as the epidermis. The findings showed that five *Hoya* species, *H. archboldiana*, *H. bhutanica*, *H. multiflora*, *H. paziae*, and *H. pubifera*, possessed parafacial stomata which are partially raised or level with the epidermis (Figure 2(a) to (b) and (h) to (i)). On the other hand, superficial or raised stomata were observed in *H. halconensis*, *H. hanhiae*, *H. limoniaca* and *H. mindorensis* (Figure 2(d) to (g)). These results contrast with previous studies by Basir et al. [18], who reported sunken stomata in *H. incrassata* and *H. soligamiana*. According to Noraini et al. [19], stomatal positioning on the epidermal surface is associated with the plant's environment or habitat. Many prior studies also demonstrated the taxonomic value of stomatal positioning in species differentiation, identification, and classification.

The shape of stomata, either round, elliptical, oblong, or angular, observed under a scanning electron microscope, has been proven to hold taxonomic significance [13] and has been shown to assist in distinguishing plant species. This study revealed that round stomata were present on the abaxial epidermis of *H. archboldiana*, *H. bhutanica*, *H. glabra*, *H. paziae* and *H. pubifera* (Figure 2(a) to (c) and (i)). Meanwhile, elliptical stomata were observed in *H. limoniaca*, *H. hanhiae*, *H. halconensis*, *H. mindorensis*, and *H. multiflora* (Figure 2(d) to (g)).

This study identified various types of trichomes present in the examined *Hoya* species and each with distinct characteristics. *Hoya bhutanica* exhibited simple multicellular trichomes with pointed tips and echinate ornamentation (Figure 4(a)), while *H. paziae* displayed similar trichomes but with elongated blunt tips (Figure 4(g)). *Hoya pubifera* was the only species to feature simple unicellular trichomes with thin walls exclusively on the abaxial epidermal surface, making this a unique identifying trait (Figure 4(h)). Glandular trichome bases were found in *H. glabra*, *H. hanhiae* and *H. mindorensis* (Figure 4(b) to (f)), consistent with the findings of Salas et al. [17], who reported glandular trichomes in *H. bilobata* and non-glandular trichomes in other species. *H. hanhiae* and *H. mindorensis* featured lithops papilla trichomes with dense coverage, while those on *H. hanhiae* had striations (striae) (Figure 4(c) and (d)), whereas those on *H. mindorensis* were without striae (Figure 4(e) and (f)). These distinctive trichome types and structures provide valuable diagnostic features for differentiating the studied *Hoya* species. The observations from this study also revealed the absence of trichomes on the epidermal surface of *H. archboldiana*, *H. halconensis*, and *H. multiflora*. The lack of trichomes on these surfaces may be attributed to the thickness of the wax layer as suggested by Noraini et al. [19]. The

findings of this study indicated that druse crystals are present only on the abaxial epidermis of *H. hanhiae* and *H. paziae* (Figure 3(a) and (b)). In contrast, no crystals were observed in the other species studied. This discovery aligns with the work of Konyar et al. [14], who noted the presence of crystals within poisonous plants in the Apocynaceae family. Additionally, the study identified densely packed starch grains on both adaxial and abaxial epidermal surfaces exclusively in *H. halconensis* (Figure 3(c)). This feature serves as a diagnostic characteristic that distinctly differentiates this species from the other species examined.

On the adaxial epidermal surface, *H. bhutanica*, *H. hanhiae*, *H. glabra*, *H. mindorensis*, *H. multiflora*, and *H. paziae* exhibited a crustose and granular wax layer similar to *H. limoniaca* (Figure 5(c)), with flake-like wax present only in *Hoya glabra* (Figure 5(a)). *H. halconensis* displayed a film and scaly wax layer (Figure 5(b)) while *H. pubifera* featured crustose and granular wax (Figure 5(d)). *H. archboldiana* has only verrucate wax on its surface (Figure 5(e)). On the abaxial epidermal surface, a crustose and granular wax layer was observed in *H. mindorensis* and *H. paziae* similar to *H. glabra* (Figure 5(f)) while a film and granular wax layer similar to *H. multiflora* (Figure 5(g)) appeared in *H. archboldiana*, *H. bhutanica*, *H. halconensis*, *H. hanhiae*, and *H. pubifera*. A warty and granular layer was found only in *H. limoniaca* (Figure 5(h)). According to Hafiz et al. [16], crustose wax is a common characteristic in many species within the Asclepiadaceae family which is also known as Apocynaceae.

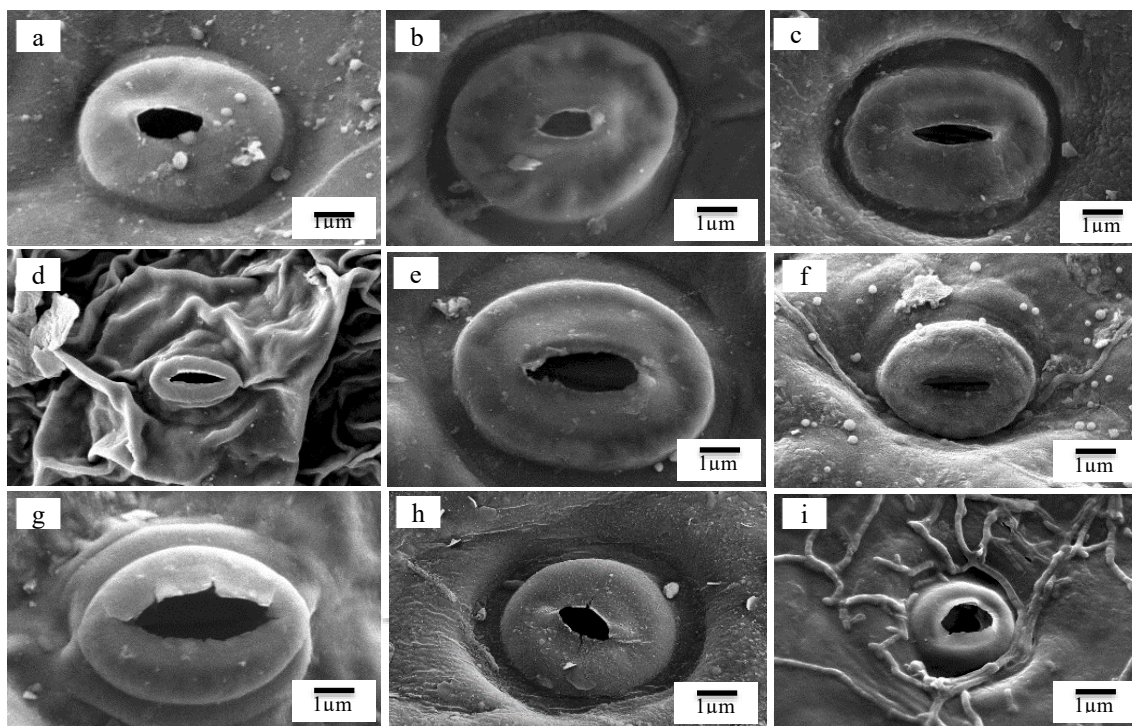


Figure 2: SEM micrographs showing the stoma of the studied *Hoya* species of (a) *H. archboldiana*, (b) *H. bhutanica*, (c) *H. glabra*, (d) *H. halconensis*, (e) *H. hanhiae*, (f) *H. limoniaca*, (g) *H. mindorensis*, (h) *H. paziae* and (i) *H. pubifera*. Scale bar: 1µm

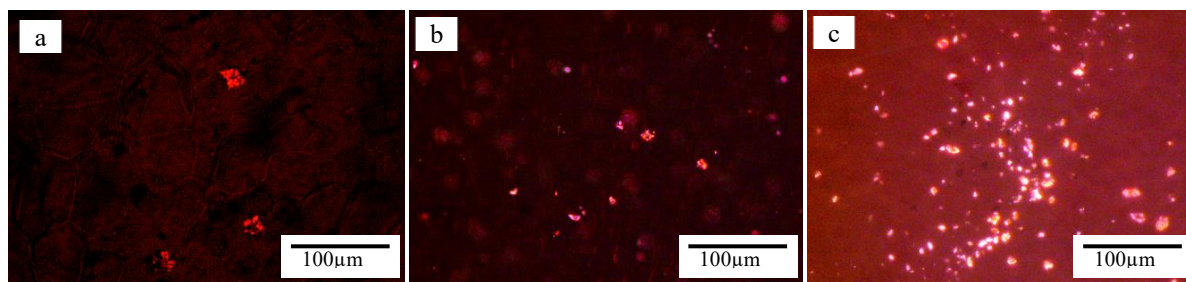


Figure 3: LM micrographs showing the ergastic substances of the studied *Hoya* species; (a) *H. archboldiana* (druse), (b) *H. bhutanica* (druse), and (c) *H. glabra* (starch). Scale bar: 100µm.

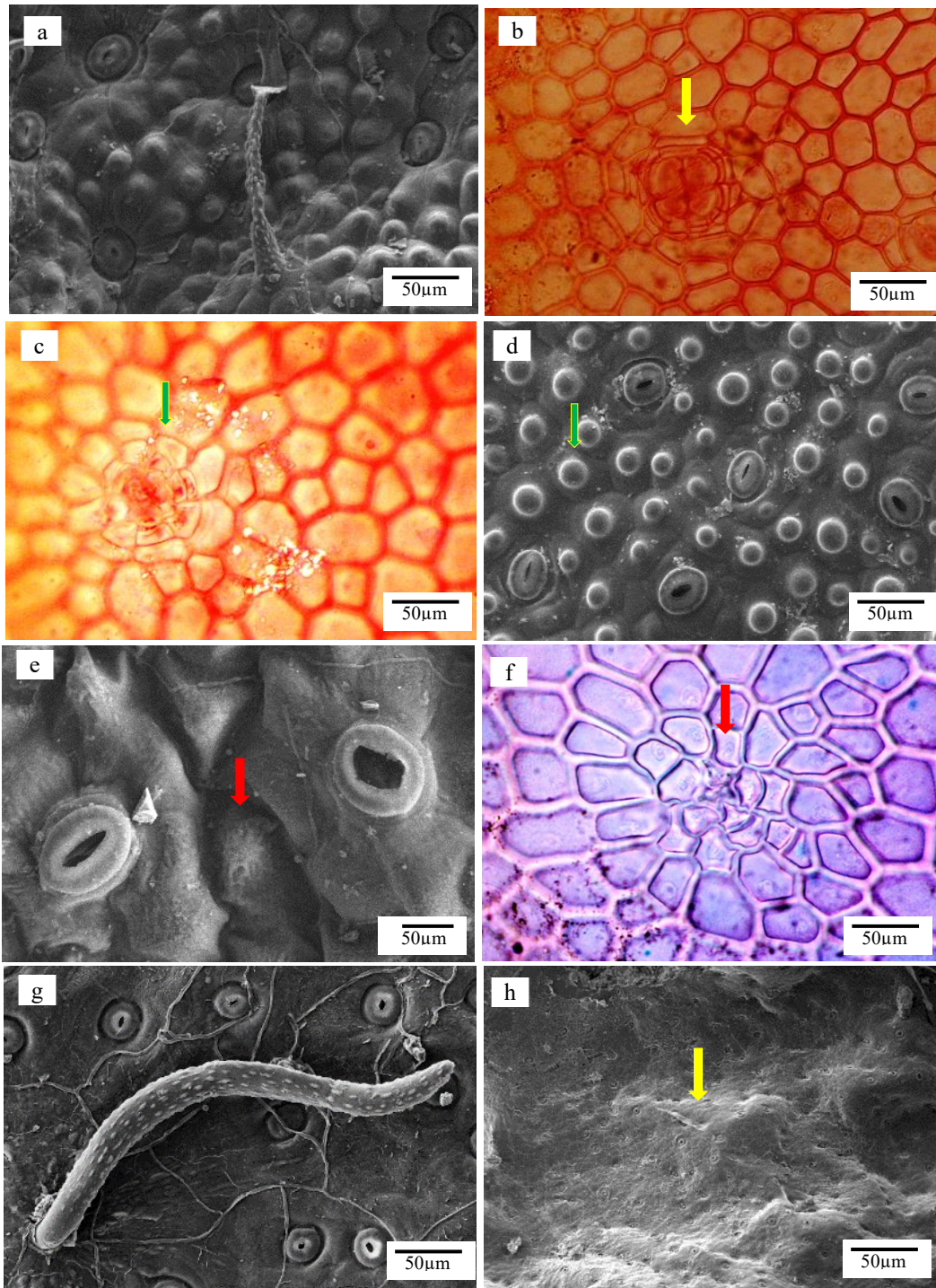


Figure 4: LM and SEM micrographs showing the trichome variations in the *Hoya* species studied; (a) *H. bhutanica* (achinate sculpture, multicellular simple trichome), (b) *H. glabra* (multicellular peltate glandular trichome), (c) to (d) *H. hanhiae* (base of multicellular peltate glandular trichome and lithops papilla trichome), (e) to (f) *H. mindorensis* (lithops papilla trichome and base of trichome), (g) *H. paziae* (achinate sculpture, multicellular simple trichome) and (h) *H. pubifera* (unicellular simple trichome). Scale bar: 50µm.

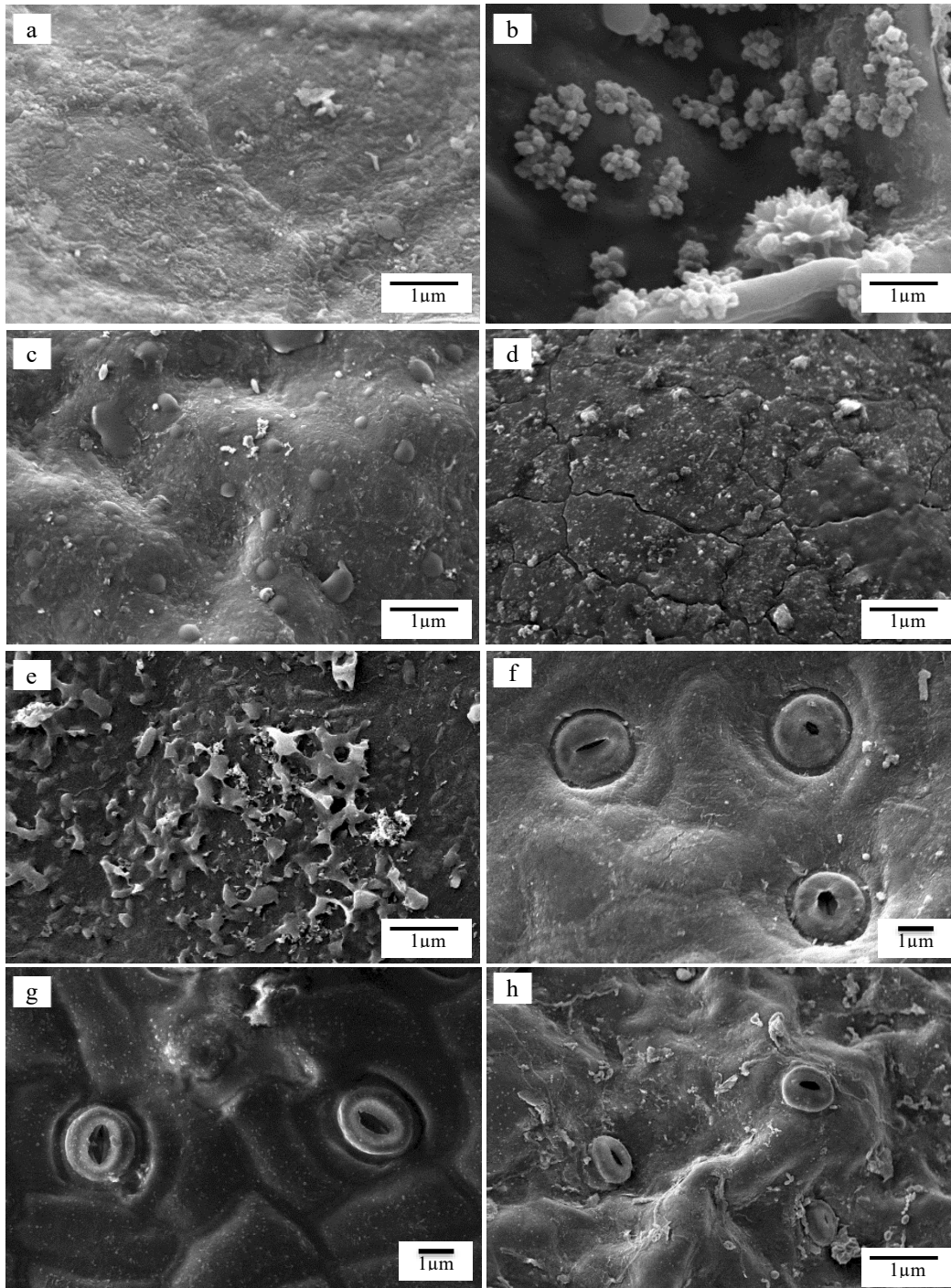


Figure 5: SEM micrographs of the studied *Hoya* species. Adaxial surfaces; (a) *H. glabra* (crustose, granule and flake), (b) *H. halconensis* (film and crystalloid), (c) *H. limoniaca* (crustose and granule), (d) *H. pubifera* (warty and granule) and (e) *H. archboldiana* (verrucate). Abaxial surfaces; (f) *H. glabra* (crustose and granule), (g) *H. multiflora* (film and granule), and (h) *H. limoniaca* (warty and granule). Scale bar: 1µm.

A dichotomous key for identifying the studied species has been developed using a combination of anatomical and micromorphological characteristics of the leaves obtained in this study. Incorporating additional anatomical features, such as the petiole, leaf veins, lamina, and leaf margins, can further enhance the key's effectiveness in identifying and differentiating the studied *Hoya* species.

A dichotomous key for identifying the studied species:

- 1 The stoma frame is submerged; different wax types on the abaxial and adaxial epidermal surfaces.....2
- 1 The stoma frame is raised; the same type of wax on the abaxial and adaxial epidermal surfaces..... *H. mindorensis*
- 2 Starch nodules are absent; cuticular projection does not form a ridge on the anticline wall....3
- 2 Starch nodules are present; projections of the cuticle forming a ridge on the anticline wall..... *H. halconensis*
- 3 Stoma located superficially.....4
- 3 The stoma is located parafacially.....5
- 4 Epidermis glabrous; the cuticular engraving is not clear, anticline and pericline walls cannot be distinguished on the abaxial epidermal surface..... *H. limoniaca*
- 4 Lithops papilla trichomes (without striations) are present; Cuticle engraving is clear, the anticline wall is sunken, and the pericline is raised on the abaxial epidermis.....*H. hanhiaie*
- 5 Stoma round shape; tetracytic or/and staurocytic type stomata6
- 5 Elliptical stoma; amphiparasitic type stomata..... *H. multiflora*
- 6 Druse absent; granular wax on the abaxial epidermal surface.....7
- 6 Druse present; granular wax on the abaxial epidermal surface.....*H. paziaie*
- 7 Film-type wax layer and granules on the abaxial epidermal surface.....8
- 7 Crustose-type wax layer and granules on the abaxial epidermal surface.....*H. glabra*
- 8 Cuticle carving indistinct, anticline and pericline walls indistinguishable; no cuticular striation on the subsidiary cells.....9
- 8 Cuticle carving is clear, the anticline wall sinks and the pericline rises; cuticular striation on subsidiary cells.....*H. bhutanica*
- 9 Crusted and granular waxy layer on the surface of the adaxial epidermis.....*H. pubifera*
- 9 Verrucate waxy layer on the adaxial epidermal surface.....*H. archboldiana*

4. CONCLUSIONS

Common features among species support their grouping within the same genus and family. In contrast, variations and diagnostic features are essential for differentiation and species identification, making them particularly suitable for constructing identification keys for *Hoya* species. Diagnostic features, which hold the highest taxonomic value, can be directly used for species identification. The dichotomous key for identifying the studied *Hoya* species can be developed using seven varied anatomical and micromorphological characteristics, along with four diagnostic features identified in this study. In conclusion, anatomical and micromorphological features demonstrate significant taxonomic value for the studied *Hoya* species.

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Author Contributions

All authors contributed to 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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