

COMPARATIVE MICROMORPHOLOGY LEAF SURFACE OF SELECTED *HOYA* SPP. (APOCYNACEAE) FROM SARAWAK

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ABSTRACT

KHALEEDA, R. & MEEKIONG, K. 2023. Comparative micromorphology leaf surface of selected *Hoya* spp. (Apocynaceae) from Sarawak. *Reinwardtia* 22(2): 69–77. — Traditionally, morphological parameters have been used for several taxonomic and ecological identifications. The morphological characteristics alone would be difficult to distinguish the evidence for species identification. Hence, a study of leaf anatomy was conducted to compare the structural characteristics which focused on the epidermal cells, stomata, and trichomes by using a Compound Microscope and Scanning Electron Microscope (SEM). Four selected species of *Hoya* from various localities within western of Sarawak were studied: *H. caudata*, *H. coronaria*, *H. omlorii*, and *H. verticillata*. The results showed stomatal types of *Hoya* species have stephanocytic stomata, except for two species which have slightly significant differences: *H. coronaria* has anomocytic stomata and *H. omlorii* has paracytic stomata. Meanwhile, in terms of the stomatal presence, all species possessed hypostomatic stomata, only *H. verticillata* has amphistomatic stomata which stomata presence on both sides of the adaxial and abaxial surfaces. Highest stomatal density was recorded in *H. omlorii* whereas the lowest were recorded in *H. coronaria*. Stomatal index were calculated and *H. caudata* has the highest with 12.80% and *H. coronaria* has the lowest value which is 6.28%. All four species were completely absence of trichomes except for *H. coronaria* which has simple unicellular non-glandular trichomes. The result indicates that the anatomical characteristics provide additional information and could be a great assist in the distinction within *Hoya* species.

Key words: Amphistomatic, Borneo, epiphyte, systematic, wax plant.

ABSTRAK

KHALEEDA, R. & MEEKIONG, K. 2023. Perbandingan morfologi mikro permukaan daun *Hoya* spp. (Apocynaceae) terpilih dari Sarawak. *Reinwardtia* 22(2): 69–77. — Secara konvensional, parameter morfologi telah digunakan untuk mengidentifikasi secara taksonomi dan ekologi. Karakter morfologi saja sulit dipakai untuk membedakan bukti identifikasi jenis. Oleh karena itu, kajian anatomi daun telah digunakan untuk membandingkan karakter struktur sel epidermis, stomata, dan trikoma dengan menggunakan Mikroskop Majemuk dan Mikroskop Pemindai Elektron (SEM). Empat jenis *Hoya* terpilih dari berbagai lokasi di bagian barat Sarawak telah dipelajari yaitu *H. caudata*, *H. coronaria*, *H. omlorii*, dan *H. verticillata*. Hasil penelitian menunjukkan kebanyakan tipe stomata keempat jenis *Hoya* mempunyai stomata stefanositik, kecuali dua jenis yang mempunyai perbedaan yang sedikit, yaitu *H. coronaria* yang mempunyai stomata anomositik dan *H. omlorii* mempunyai stomata parasitik. Hasil pengamatan stomata menunjukkan semua jenis mempunyai stomata hipostomatik dan hanya *H. verticillata* yang mempunyai stomata amfistomatik yaitu adanya stomata pada kedua belah permukaan adaksial dan abaksial. Kerapatan stomata tertinggi terdapat pada *H. omlorii*, sedangkan yang terendah pada *H. coronaria*. Indeks stomata tertinggi pada *H. caudata* ialah 12,80% dan terendah pada *H. coronaria* yaitu 6,28%. Trikoma tidak terdapat pada keempat jenis *Hoya* yang diperiksa, kecuali pada *H. coronaria* yang mempunyai trikoma tanpa kelenjar uniselular yang sederhana. Hasil penelitian menunjukkan karakter anatomi dapat memberi informasi tambahan dan dapat menjadi alat bantu untuk membedakan jenis-jenis *Hoya*.

Kata kunci: Amfistomatik, Borneo, epifit, sistematik, tumbuhan lilin.

INTRODUCTION

The genus *Hoya* belongs to the family Apocynaceae (subfamily Asclepiadoiceae) which is known as the wax-plant, wax-vine, wax-flower, or simply *Hoya*. *Hoya* R.Br. is a large and complex genus with approximately 350–450 species (Rodda, 2015). The Indomalaysian-Australian-Western Pacific Region is home to this plant (Rodda &

Simonsson, 2011), followed by Philippines, Borneo, and New Guinea which serving as diversity hotspots (Cabactulan *et al.*, 2017). Recent study by Rahayu (2021) revealed that Indonesia leads the most diverse *Hoya* which recorded with more than 110 species. Therefore, multiple papers describing new *Hoya* species have been published previously (Lamb *et al.*, 2014; Rodda, 2015) resulting increasing the number of *Hoya* taxa occurring in Borneo

which updated up to 85 species and four subspecies (Rodda & Rahayu, 2022). Most of the descriptions of these species are mainly focused on the morphological characteristics where Lamb & Rodda (2016) has listed a preliminary checklist consisting of 32 species occurring in Sarawak alone. *Hoya* is highly appreciated for its horticultural values and also believed to have a potential in medicinal uses (Rahayu, 2011; Silalahi *et al.*, 2015). As ornamental plants, conservation and sustainable commercialization of these native species are important in the country of origin because it facilitates reintroductions if wild populations decline in numbers for any reason.

Stomata are important structures in plant biology and have been the subject of extensive research to understand their regulation, response to environmental and their role in plant physiology and adaptation. According to Cowan & Farquhar (1977) differences in stomatal characteristics may indicate adaptive relates to the environmental conditions in which plants grow, given the critical function that stomata play in managing the conflicting demands of carbon uptake and regulating the amount of water loss from the leaf. Stomata and epidermal cell characteristics have also been extensively employed as taxonomic data to support plant grouping, for instance in *Hoya* species (Hakim *et al.*, 2013), orchid species (Rompas *et al.*, 2011) and *Begonia* species (Efendi, 2019). Therefore, various studies have proven that stomatal density with light environment (James & Bell, 2000) and carbon dioxide concentration (Hetherington & Woodward, 2003) as well as stomatal size and shape (Jordan *et al.*, 2015), have been identified as adaptive or beneficial under specific conditions. As such, this study was carried out to differentiate the micromorphology features of leaves surfaces within four *Hoya* species that may contribute to a beneficial enhancement for species delimitation and useful parameter for taxonomic difficult in systematic investigations and species management between *Hoya* genus.

MATERIALS AND METHODS

Samples collection. This study were involved a field surveys and laboratory works. The field survey comprises a convenience sampling where subjected to a small scale of several areas in Western Sarawak: Kuching, Kota Samarahan, and Serian Divisions respectively (Fig. 1). Global Positioning System (GPS) was used to record coordinates localities for every species collected which adapted to different types of environments and habitats. A total of four species were successfully collected: *H. caudata*, *H. coronaria*, *H. omlorii*, and *H. verticillata* as shown in Table 1. The identification was done and compared based on specimen in Herbarium of Sarawak (SAR).

Anatomical preparation. A modification of Cutler's method (1978) in preparing a cuticular epidermal cell was applied. A free-hand sectioning was employed to gather the structural organization of the leaf of *Hoya* species. A fresh and matured leaves were taken randomly from all parts of the leaves of each species. For comparison and consistency, at least 3 replicates of approximately 1 cm² of fresh samples were used. Each leaf part was subjected to a cuticle epidermal clearing using 15% nitric acid (NHO₃). The leaf was slowly heated under hotplate until the adaxial and abaxial surfaces of the leaf were split completely. The samples were removed by using a brush, placed into a petri dish and washed with distilled water. All the mesophyll were gradually washed and became a clean piece of tissue. The clean tissues were placed in a petri dish containing sodium hypochloride (NaOCl) until decolourized. The tissues were then washed again with distilled water and transferred into another petri dish containing safranin and staining for 3–5 minutes. The tissues were then dehydrated using a series of ethanol (50%, 60%, 70%, 80%, 90%, and 100%). The tissues were then placed on a clean slide and glued with Canada balsam. Labelled the slides accordingly and dried using the slide drier (at 60°C) overnight until the slides were completely dried and ready for observation. The specimens then were observed under compound microscope. The cell arrangement was observed and images taken. Each of the *Hoya* species was used to compare their differences in the cell structure.

Determination stomatal density and index.

The determinations were done according to Salisbury (1927) methods. The stomatal index (SI %) was determined using the formula $(S/S+E) \times 100$, where the S and E stand for the number of stomata and epidermal cells, respectively. Whereas, the stomatal density was obtained by using formula as described by Wilmer (1983 as cited in Hakim *et al.*, 2013).

$$\text{Stomatal density} = \frac{\Sigma \text{ Stomata}}{\text{Field of view area (mm}^2\text{)}}$$

Stomatal index =

$$\frac{\Sigma \text{ Stomata}}{\Sigma \text{ stomata} + \Sigma \text{ epidermis cell}} \times 100\%$$

Scanning Electron Microscope (SEM). The middle part of the samples was cut into 5 mm² and cleaned thoroughly with distilled water for the Scanning Electron Microscopes (SEM). The samples were then fixed with 4% gluteraldehyde overnight before being dehydrated with a series of ethanol 50%, 60%, 70%, 80%, 90%, and 100%. The



Fig. 1. The study sites of *Hoya* species within Western of Sarawak.

Table 1. Localities and ecology of *Hoya* species in Western of Sarawak.

No	Species	Localities	Altitude	Habitat
1	<i>H. caudata</i>	Padawan, Kudung	8 m	MDF
2	<i>H. coronaria</i>	Siburan, Serian	19 m	MDF
3	<i>H. omlorii</i>	Kota Samarahan	17 m	Mangrove Forest
4	<i>H. verticillata</i>	Bako NP, Kuching	38 m	Kerangas Forest

Notes. MDF: Mixed Dipterocarp Forest, NP: National Park.

samples underwent Critical Point Drying (CPD) to make sure the tissues were totally dried. Both surfaces of dried samples were fixed with double-sided adhesive tape on stub and labelled accordingly. A Sputter Coater was used to coat the leaf specimen with platinum. The samples were analysed under JSM – 6390 LA Analytical Scanning Electron Microscope (SEM) and the images were captured at various magnifications to examine the characteristics and to obtain the measurement of parameters.

RESULTS

Epidermal cells

The examination of the epidermal cells of four species studied revealed a similarity in the shape and the type of anticlinal cell wall. Three species including *H. caudata*, *H. coronaria*, and *H. verticillata* shared the same characteristic of the epidermal cell which appeared as a thin and straight

cell wall with a polygonal shape of five to eight sides on both abaxial and adaxial surfaces. Nonetheless, only *H. omlorii* exhibited a characteristic and showed a different shape on the abaxial surfaces where the anticlinal cell wall arrangement was slightly undulate compared to the actual polygonal shapes (Fig. 2F).

Stomata

From the observation, leaves of *H. caudata*, *H. omlorii*, and *H. coronaria* are generally found hypostomatic, with the distribution distance between the stomata varies in some species where much closer to each other and some species vice versa. With an exception of all four species, *H. verticillata* observed has an amphistomatic leaves with the presence of stomata on both abaxial and adaxial surfaces. However, the stomata distribution on the adaxial surfaces is not as dense as on the abaxial surfaces (Figs. 2G & 2H). This may be considered a distinct character for species identifi-

Table 2. Comparison of the leaf anatomical characters of *Hoya* species.

Species	<i>H. caudata</i>	<i>H. coronaria</i>	<i>H. omlorii</i>	<i>H. verticillata</i>
Type of anticlinal cell wall	Straight	Straight	Slightly undulate	Straight
Presence of stomata	Hypostomatic	Hypostomatic	Hypostomatic	Amphistomatic
Position of stomata	Raised	Same level	Same level	Raised
Type of stomata	Stephanocytic	Anomocytic	Paracytic	Stephanocytic
Size of stomata (μm)	30.05–34.48 × 27.67–33.67	32.76–38.18 × 27.95–31.51	27.29–29.01 × 15.45–20.88	26.60–36.66 × 30.67–34.96
Stomatal density (mm^2)	148.15	83.95	187.65	9.88 (Adaxial) & 167.90 (Abaxial)
Stomatal index (%)	12.80	6.28	11.56	10.34
Presence of trichomes	Absent	Present	Absent	Absent
Presence of ornamentation	Papillae	Absent	Absent	Papillae

cation within the genus. Thus, it appears that plants with hypostomatic leaves have to face habitats with water stress which need to withstand and thrive in harsh environment. The stomatal types in this study showed a substantial variation among the four *Hoya* species. *H. coronaria* was noticed as anomocytic stomata while *H. omlorii* has paracytic stomata with two subsidiary cells, one with a short cell on each side of the guard cells and one with a wide cell at each pole. The stomata are randomly and evenly distributed on the abaxial leaves surfaces. The biggest stomata were noticed in *H. coronaria*, which is 32.76–38.18 μm length and 27.95–31.51 μm width. Contrasted with *H. omlorii* which recorded to be the smallest stomata cells compared to the others species measuring 27.29–29.01 μm length and 15.45–20.88 μm width respectively. Figures 2E & 2F illustrated the stomata on abaxial surfaces of the species examined.

Stomatal density & stomatal index

Stomatal density for the abaxial part of four *Hoya* leaves, the highest record was observed in *H. omlorii*, which is 187.65 mm^2 and the lowest stomatal density was 83.95 mm^2 which shown in *H. coronaria*. Whereas, *H. verticillata* which presence stomata on both of the leaves surfaces represent 9.88 mm^2 and 167.90 mm^2 on adaxial and abaxial respectively (Table 1). Although *H. verticillata* was considered as amphistomatic stomata, but the stomatal distribution on the adaxial surfaces are scarce compared to the abaxial surfaces. Stomatal index shows the ratio between the number of stomata with the number of the stomata and the epidermal cells. From the observation, the highest stomatal index was shown in *H. caudata* with value of 12.80% and the lowest stomatal index was found in *H. coronaria* with 6.28% as shown in Table 1.

Trichome

The presence of trichomes only observed in *H. coronaria*, where the trichomes occur singly, unicellular with non-glandular, long and acute apical cells on both sides of the leaves epidermis.

Papillae

Papillae ornamentation was observed in *H. caudata* and *H. verticillata*. However, only on the abaxial surfaces of both species showed abundance density of papillae that raised directly over the cells (Figs. 2B & H).

DISCUSSION

Epidermal cells

According to Salasiah & Meekiong (2018), a polygonal are the two-dimensional shapes with straight lines which are consist of rectangular, pentagonal, hexagonal, heptagonal and octagonal on the cells. The majority of anticlinal cell walls of dicotyledon epidermal cells are straight, curved, or sinuous in shape (Cutler *et al.*, 2008). However, the distinct characters of anticlinal cell wall shown in *H. omlorii* are believed to be taxonomically valuable in separating species within this genus which also found was reported by Fontenelle *et al.* (1994) to identify species of *Eugenia* (Myrtaceae) and by Moraes *et al.* (2009) to distinguish *Simira sampaiouana* (Rubiaceae) one species to another by using the outline of the anticlinal cell wall characteristics.

Stomata

Stomata occurred either on both sides of surfaces amphistomatic or only on the upper surfaces hyperstomatic or more commonly on the lower surfaces hypostomatic of leaves (Serna *et al.*, 2002; Parveen *et al.*, 2007). Mbagwu *et al.* (2008) report-

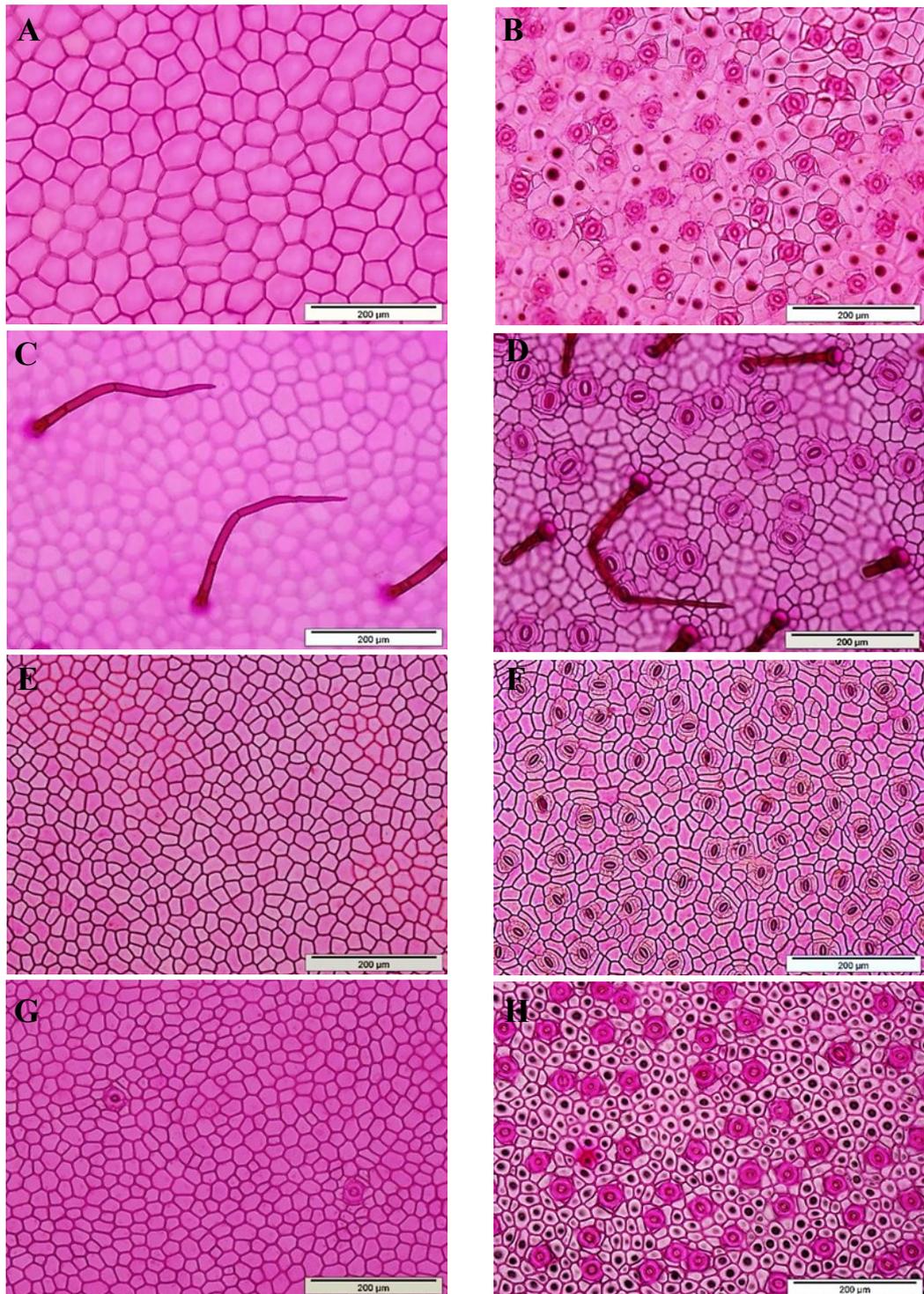


Fig. 2. Comparison leaf epidermis of four *Hoya* species by using compound microscope. A-B. The adaxial and abaxial of *H. caudata*; C-D. The adaxial with unicellular trichome and abaxial of *H. coronaria*; E-F. Adaxial and abaxial surfaces of *H. omlorii*; G-H. The adaxial with rarely presence of stomata and abaxial surfaces of *H. verticillata*.

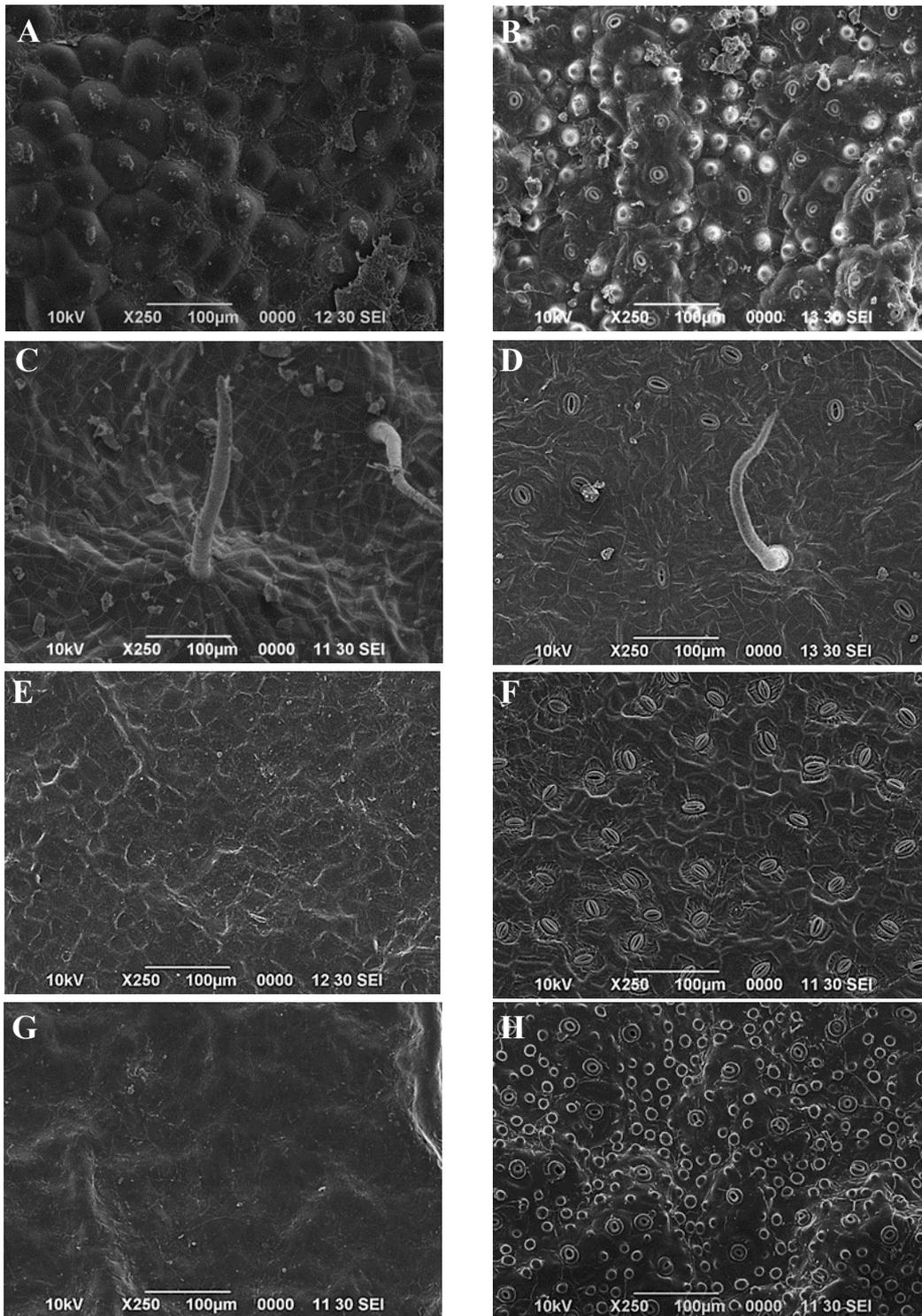


Fig. 3. Comparison leaf epidermis of four *Hoya* species by using Scanning Electron Microscope (SEM). A-B. The adaxial and abaxial surface with raised stomata of *H. caudate*. C. The adaxial surface with unicellular trichome of *H. coronaria*. D. Stomata and trichomes of *H. coronaria*. E-F. Adaxial surface and same level of stomata of *H. omlorii*. G-H. The adaxial surface and raised stomata and abundance papillae of *H. verticillata*.

ed that species with hypostomatic leaves adapt this strategy to allow efficient gases exchange while minimize the water loss since the limited stomatal occur at the lower part of leaves which can caused by the convection movements or current of air that could remove water vapour from the leaves surfaces. According to Mott *et al.* (1982), the greater number of stomata in amphistomatic leaves, resulting a greater capacity to absorb carbonic acid in order to achieve a high rate of photosynthesis. Generally, plants with amphistomatic leaves are correlated with habitats without water stress and considered as sun shade plants (Rao & Rammaya, 1981). A stephanocytic type were clearly observed in *H. caudata* and *H. verticillata* where the stomata were surrounded by four or more weakly differentiated subsidiary cells forming a broader, less clear ring and less elongated (Baranova, 1987). Variations of stomata within the Apocynaceae family have also been reported by Salas *et al.* (2018) recently who stated that actinocytic stomata in *H. incrassata* and cyclocytic stomata in *H. soligami-ana*. Nisa *et al.* (2019) advocated this statement with an investigation of the stomatal multiplicity in the family Apocynaceae, revealing a different stomatal types such as paracytic, anisocytic, tetracytic, actinocytic, anomocytic, laterocytic, stephanocytic, pseudoholoparacytic, and brachyparahexacytic.

Stomatal density & stomatal index

The density of stomata on plant surfaces can vary depending on factors such as species, environmental conditions, and plant adaptation to specific habitats. As such, plants in arid environments may have fewer stomata or specialized adaptations to minimize water loss, while plants in humid environments may have more stomata to maximize gas exchange. The higher the size of the stomata, the lower stomatal density, vice versa, the lower the size of stomata, was resulting the higher stomatal density on the species. However, stomatal density can influence a plant's ability to regulate gas exchange and water loss (Chaves *et al.*, 2016). According to Miskin *et al.* (1972), plants with a high stomatal density will have a higher transpiration rate than plants with low stomatal density which closely associated with photosynthetic and growth characteristics in plants. Wilmer (1983) reported that, stomatal density to be said has correlation and also effected by the size of the stomata. As reported by Mulyani (2006) the lower stomatal density, with high number of epidermal cells, will result in a low value of stomata index. Contradictly, higher stomatal density, with low number of epidermal cells, will affect a higher value of stomatal index. The differences in the stomatal index were doubt could be related to physiological reactions to various environmental conditions (Adegbite, 2008; Aworinde *et al.*, 2012). The distribution

and frequency of stomata were considerable as taxonomic significance, though sometimes may be connected with the ecology of the species (Stace, 1965).

Trichomes

Ghahreman *et al.* (1999) mentioned that this characteristic is also believed to be a useful indicator to differentiate between the genus. Furthermore, Gabr *et al.* (2015) investigated species within Apocynaceae family showed 15 different types of trichomes variations with glandular hairs and non-glandular hairs. Densely trichomes characters are always associated with protection and adaptation to high light intensity (Ichie *et al.*, 2016).

Papillae

Papillae are small projections found on the external walls of epidermal cells (Moraes *et al.*, 2011). According to Judd *et al.* (2008), the occurrence of papillae is an important systematic character. Although many theories have been proposed, Patterson (1964) and Proctor (1981) conclude that the function of papillae remains unclear, ranging from light and temperature control for adaptation to xerophytic conditions.

CONCLUSION

The study highlighted that *Hoya* can be differentiated despite of several shared characteristics. The micro-morphology studies of *Hoya* species leaf surfaces proved useful for the Apocynaceae family's systematics, providing important distinguishing features such as the different types of epidermal cells, differences in stomatal type, the stomatal density and index, the presence of trichrome and the presence of papillae. The different features of leaves anatomy studies of *Hoya* species act as complement for existing morphological data, supplementary information that should be utilised and implemented for species delimitation in the future.

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