

LEAF ANATOMICAL AND MICROMORPHOLOGICAL SYSTEMATIC SIGNIFICANCE OF *Schoutenia* Korth. IN PENINSULAR MALAYSIA

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Abstract. An anatomical and micromorphological study of the leaf epidermis was conducted on the *Schoutenia* from the Tiliaceae family in Peninsular Malaysia. This genus is also classified in the subfamily Dombeyoideae according to classification based on plastid *atpB* analysis and *rbcL* DNA sequence. The objective of the study is to identify similarities and variations in the anatomical and micromorphological characteristics of the *Schoutenia* taxon studied to overcome identification problems, especially for species that are difficult to distinguish due to similar morphological characteristics. Anatomical study methods involve specimen collection, sliding microtome slices, epidermal peels, tissue clarification, staining, and observation under a light microscope. The method of studying the micromorphology of the leaf epidermis involves critical point drying, gold coating, and observation under a scanning electron microscope. The results of the study show some significant characteristics for the differentiation of *Schoutenia* species studied, namely wax on the leaf epidermis, adaxial epidermal cuticle ornamentation, the external shape of midrib and leaf margins, the presence of intraxillary phloem and sclerenchyma phloem. Several identified features can be used as diagnostic features to identify the species of *Schoutenia* studied directly. Therefore, the results of the study clearly show the taxonomic value of the anatomical and micromorphological characteristics of the *Schoutenia* genus from the Malay Peninsular.

Keywords: Microscopic, systematic, taxonomic, Malvaceae

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1. INTRODUCTION

The genus *Schoutenia* Korth. is belongs to Tiliaceae and this family has been revised several times due to problems in the identification and differentiation of species, and one of them is due to the similarity of morphological characteristics. The revision was done many times by several taxonomists [1-4]. The last revision made by [4] and according to [5], the Tiliaceae family in Malaysia has 12 genera namely *Brownlowia*, *Pentace*, *Schoutenia*, *Berrya*, *Grewia*, *Microcos*, *Muntingia*, *Triumfetta*, *Corchorus*, *Colona*, *Trichospermum* and *Diplodiscus*. *Schoutenia* Korth. known by its local name as 'bayur bukit' consists of only nine species that are scattered in Southeast Asia, Cambodia, and Siam to Burma and Java and in Malaysia, there are only five species.

There are several species such as *Schoutenia corneri*, *Schoutenia furfuracea*, *Schoutenia kunstleri*, and *Schoutenia leprosula* which are endemic species in Peninsular Malaysia [6,7]. According to [8], genetic loss may occur to endemic species through deforestation. *Schoutenia* consists of small to medium-sized trees. The outer bark is varied with the wood being white to yellow-brown, and has opposite, simple leaves; asymmetrical with three leaf veins. Flowers have attached pedicels; calyx white to yellow; bell-shaped with five lobes; lacks petals for species found in Malesiana; numerous stamens usually present in five bundles; ovary has five locules. Capsule-shaped fruit, sometimes stalked. *Schoutenia accrescens* subsp. *accrescens* and *Schoutenia accrescens* subsp. *borneensis* is a medium-sized tree. It can grow up to 30 m tall, the leaves are simple and arranged alternately and inhabits lowland to montane forests.

The distribution of *S. accrescens* subsp. *accrescens* ranges from Peninsula Thailand to West Malesia, while *S. accrescens* subsp. *borneensis* and *S. accrescens* subsp. *stellata* was found in Borneo [5,9]. Kochummen [5] stated *S. accrescens* subsp. *accrescens* is suitable for use in house construction because the wood is hard, strong, and very durable. *S. kunstleri* is a native species and the native range of this species is from Peninsula Thailand to West Malesia. According to [8], most species in *Schoutenia* are ideal for house construction, hand tools, agricultural tools, and charcoal. The bark of the tree is suitable as a strong rope. *Schoutenia* is suitable for use as padding, making hand tools, flooring materials, heavy furniture, door and window frames, and any application that requires the strength of a wooden structure [10].

Species from this genus still have identification problems if they are without flowers and fruits, and most of the time these species are found without flowers and fruits. Plant anatomy is very important in plant taxonomy as the anatomical characteristics of vegetative and floral parts of flowering plants can successfully solve taxonomic problems and elucidate of phylogenetic relationships [10,11]. Besides that, they also stated that anatomical studies are sometimes very helpful in identifying the fragmentary material without flowers and fruits, and at the same time, plant anatomy is essential to the study of plant systematics, physiology, paleobotany, ecology, evolutionary biology, seeds development and science of developmental genetics [11-13]. Therefore, the rationale of this study is to identify similarities, variations, and diagnostics of the anatomical and micromorphological characteristics of the leaves of the *Schoutenia* genus to be used as alternative species identification characteristics.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh mature leaves of five selected taxon were collected from FRIM Herbarium (Table 1). For these investigations, mature leaves were regarded as vegetative leaves harvested from the third and fourth nodes of the plant from the apex.

2.2 Scanning Electron Microscopy

Fresh leaves, 5-8 mm in length, were removed from the aerial part of the plant. Both abaxial and adaxial surfaces were observed under a TM-1000 Tabletop Microscope from Hitachi High-Technologies.

2.3 Light Microscopy

Fixation, embedding, and sectioning were performed following the method described by [11]. The sectioning was done using a sliding microtome, and sections were stained in Safranin and Alcian green and dehydrated, then mounted in Euparal. Photographs of sections were taken using a Leitz Diaphlan polarizing microscope and images were processed using Analysis Docu Software.

Table 1: List of species and specimens studied

Species and herbarium specimens code	Location	Collector, date of collection
<i>S. accrescens</i> subsp. <i>accrescens</i> SFN 35774, KEP 50830, KEP 78797	Telok Bahang Forest Reserve, Penang, Malaysia Hotel Craig, Penang, Malaysia Bukit Lagong Forest Reserve, Gombak, Selangor, Malaysia	Yahya, 26.06.1938 Symington, CF 08.04.1940 Kochummen, KM 24.09.1957
<i>S. accrescens</i> subsp. <i>borneensis</i> S 32039, FRI 7923	Tanjung Kedurong, Bintulu, Sarawak Labis Forest Reserve, Mersing, Johor, Malaysia Sanggau, Kalimantan	Ilias, Peter, 25.09.1972 Cockburn PF, 26.03.1968 Elsener PA, 13.01.1972
<i>S. accrescens</i> subsp. <i>stellata</i> S 15779, S 15852, S 36904	Semengoh Forest Reserve, Kuching, Sarawak, Borneo Labang Forest Reserve, Bintulu, Sarawak, Malaysia Gunung Buri, Samarahan, Sarawak, Malaysia	Galau, 1961 Ilias Paie, 04.12.1961 Martin PJ, 21.09.1975
<i>S. kunstleri</i> Connors SN	Bukit Kajang, Selangor, Malaysia	E.J.H. Corner, 01.11.1935
<i>S. leprosula</i> KEP 110422, RC 160, FRI 27450	Labis Forest Reserve, Ulu Endau, Johor, Malaysia Tenggeroh Forest Reserve, Mersing, Johor, Malaysia Endau Rompin, Mersing, Johor, Malaysia	Ogata K, 28.03.1968 . Chan Y.C, 26.08.2004 Ng FSP, 30.07.1985

3. RESULTS AND DISCUSSION

3.1 Common Anatomical and Micromorphological Characteristic

Stomata are present hypostomatically and heterostomatically with the presence of parasitic and anisocytic stomata. Stomata have clear, raised stomatal margins and no cuticular striations. The shape of the guard cell is round. All species have sinuous 90° curved anticlinal cell walls on the adaxial epidermal. Multicellular peltate glandular trichomes are present and trichomes are only observed on the abaxial surface of the leaf epidermis. The majority of venation in the leaf lamina is closed and the minority is opened. Mucilage vessels are found

in the cortex parenchyma and the vascular bundles, solitary or druses, and lamellar collenchyma cells in the adaxial or abaxial vascular bundles of the midrib. The outer shape of the petiole is round, and the pattern of vascular bundles with a closed vascular system and with one oval-shaped continuous ring of intraxylary phloem, medullary sclerenchyma cells, and crystals of either single or druses types were also observed. Solitary crystals and druses are observed on the lamina. The presence of mucilage cells and vessels on the cortex and vascular bundles of midrib, and lamellar collenchyma cells on the adaxial side of vascular bundles can also be a common feature of *Schoutenia*. Clusters of sclerenchyma cells are present near vascular bundles of midrib in all *Schoutenia* species studied.

3.2 Variation of Leaf Anatomical and Micromorphological Characteristics

The variation of anatomical and micromorphological characteristics of the leaves obtained from the study can be used for the construction of a dichotomous key for the identification and differentiation of *Schoutenia* species.

3.3 Stomata and Anticlinal Wall Patterns of Leaf Epidermis

Schoutenia accrescens subsp. *accrescens* shows a clear but not raised stomatal frame while for other species with a less clear stomatal frame. There is a difference in the anticlinal wall pattern of the abaxial epidermis, *S. accrescens* subsp. *accrescens* has a 45° sinuous anticlinal wall pattern, while 30° is observed in *S. accrescens* subsp. *borneensis*, *S. kunstleri* and *S. leprosula*. The anticlinal wall pattern of the abaxial epidermis shows a sinuous pattern in all species except curved to wavy in *S. accrescens* subsp. *stellata*.

3.4 Leaf Venation

S. kunstleri and *S. leprosula* have a complete venation of the leaf margin and the position is right on the leaf margin leaf while in *S. accrescens* subsp. *accrescens*, *S. accrescens* subsp. *borneensis* and *S. accrescens* subsp. *stellata* are incomplete. Leaf venation arrangement is proven to have taxonomic value for the differentiation of *Schoutenia* taxa.

3.5 Outline Shape and Presence of Sclerenchyma Cells on Leaf Margins

The outline shape of leaf margins is rarely used in systematic studies but has been proven to have taxonomic value for species identification and classification of the genus *Alpinia* (Zingiberaceae) [12]. Table 2 shows the variation of the outline shape of leaf margin. In *Schoutenia* there are two outline shapes of leaf margin, namely Shape 1 and Shape 2.

Table 2: Variation of the outline shape of leaf margin

Shape	Diagram	Species
Shape 1: Curved 5 ° - 10 ° towards the abaxial direction of the leaf; rounded end.		<i>S. accrescens</i> subsp. <i>accrescens</i> <i>S. accrescens</i> subsp. <i>stellata</i> <i>S. kunstleri</i>
Shape 2: Straight or nearly straight; rounded end.		<i>S. accrescens</i> subsp. <i>borneensis</i> <i>S. leprosula</i>

The presence of clusters of sclerenchyma cells can be seen at the tip of the leaf margin of *S. accrescens* subsp. *accrescens* and *S. accrescens* subsp. *borneensis*. The results of this study show that the outline shape of the leaf margin and the presence of clusters of sclerenchyma cells at the tip of the leaf margin can be used in distinguishing species.

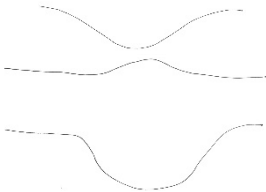
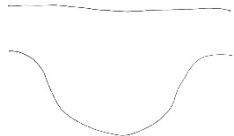
3.6 Layers of Epidermal Cells and Chlorenchyma Cells on the Leaf Lamina

The height ratio to the width of epidermal cells in *Schoutenia* are 1:1, 1:2, and 3:1. *S. leprosula* can be distinguished by a 1:1 ratio, *S. accrescens* subsp. *stellata* with 3:1 while other species 1:2. The number of palisade mesophyll cell layers of *S. accrescens* subsp. *accrescens* are three, *S. accrescens* subsp. *borneensis* 3-4 while other species have two layers. For the number of sponge mesophyll cells, *S. accrescens* subsp. *accrescens* and *S. accrescens* subsp. *borneensis* has one layer while the others have 2-3 cell layers. The presence of the number of palisade mesophyll cell layers also sometimes has a certain taxonomic value but is rarely used in systematic studies of anatomy because it often changes according to habitat and environment [13-14].

3.7 Outline Shape, Vascular Bundle Pattern, Intraxylary Phloem, Sclerenchyma Cells, Phloem Sclerenchyma Cells, Lamella Collenchyma Cells and Types of Trichomes on Midribs

There are five outline shapes of midrib found in this study, namely Shape 1, Shape 2, Shape 3, Shape 4, and also Shape 5 as shown in Table 3. Azahana et al. [15] stated that the outline shape of leaf bones and petioles can be used in species identification which this fact is proven from the study on the genus *Pandanus* (Pandanaaceae).

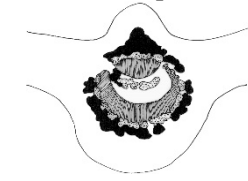
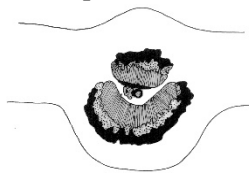
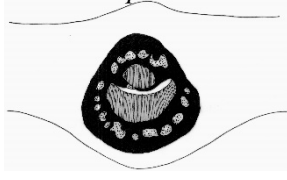
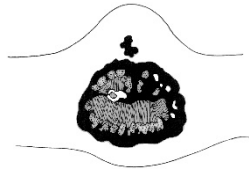
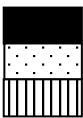
Table 3: Outline the shape of midribs

Form	Diagram	Species
Shape 1 Adaxial surface: hump Abaxial surface: arc		<i>S. accrescens</i> subsp. <i>borneensis</i>
Shape 2 Adaxial surface: hump Abaxial surface: convex		<i>S. leprosula</i>
Shape 3 Adaxial surface: convex Abaxial surface: convex		<i>S. kunstleri</i>
Shape 4 Adaxial surface: convex Abaxial surface: arc		<i>S. accrescens</i> subsp. <i>stellata</i>
Shape 5 Adaxial surface: flat Abaxial surface: arc		<i>S. accrescens</i> subsp. <i>accrescens</i>

The presence of clusters of sclerenchyma cells surrounding the vascular bundles of

midribs can be used to differentiate *Coelostegia* species [16]. In this study clusters of sclerenchyma cells on the adaxial part of the midrib in this study were observed in *S. leprosula*. In *S. accrescens* subsp. *accrescens* and *S. accrescens* subsp. *stellata*, the clusters of sclerenchyma cells on the adaxial side of the midribs project to touch the adaxial epidermis (Table 4). The absence of phloem sclerenchyma cells in *S. leprosula* can be used as a diagnostic feature for this species. In plant identification often involves a combination of macroscopic and microscopic features, including leaf shape, bark texture, flower morphology, and wood anatomy. Therefore, this finding shows that the absence of phloem sclerenchyma cells can be a valuable clue, but it's essential to consider other characteristics as well to identify plant species with certainty.

Table 4: Midrib main vascular bundle pattern

Pattern	Species	
Pattern 1 Primary vascular bundle continuous; arc-shaped to a 'U' shape. There is an additional vascular bundle on the adaxial side of the main vascular bundle.	<i>S. accrescens</i> subsp. <i>borneensis</i>	<i>S. accrescens</i> subsp. <i>stellata</i>
		
Pattern 2 Primary vascular bundle continuous; arc-shaped to 'U' shape. There are several medullary vascular bundles on the adaxial side.	<i>S. kunstleri</i>	<i>S. leprosula</i>
		
	<i>S. accrescens</i> subsp. <i>accrescens</i>	
		
	Keywords:  Sclerenchyma cells Phloem Xylem	

3.8 Wax Types and Cuticular Ornamentation Patterns

The wax found on the surface of the leaf epidermal has a different chemical content depending on the type of wax which contains various chemical compounds [17]. Table 5 and Figure 1 show the variation in the type of wax present on the leaf surface of *Schoutenia* taxon studied. Nurshahidah et al. [18] stated that cuticle ornamentation patterns are a good supporting feature and have taxonomic value for species identification. Pattern 2 can be used as a diagnostic feature for *S. kunstleri*. [19] also stated that the pattern of cuticle ornamentation either for adaxial or abaxial is one of the features to distinguish *Pentace* species (Malvaceae). This shows that this feature has a high taxonomic value for the differentiation of *Schoutenia* species.

Table 5: Wax types and cuticle ornamentation patterns on the adaxial and abaxial epidermis

Species	Types of Waxes on the Epidermis		Cuticular ornamentation	
	Adaxial	Abaxial	Adaxial	Abaxial
<i>S. accrescens</i> subsp. <i>accrescens</i>	Crusted	Thin film layer	Pattern 3	Pattern 4
<i>S. accrescens</i> subsp. <i>borneensis</i>	Crustose	Thin film layer	Pattern 1	Pattern 4
<i>S. accrescens</i> subsp. <i>stellata</i>	Crustose	Thin film layer	Pattern 3	Pattern 4
<i>S. kunstleri</i>	Crustose Granules	Crustose Granules	Pattern 2	Pattern 4
<i>S. leprosula</i>	Crusted	Thin film layer	Pattern 1	Pattern 4

Keywords:

Crustose=rough but not eared. Granular=granular, seeded, knurled. Crust = rough and eared. A thin layer of film or thin layer of wax that looks smooth. Pattern 1= cuticle ornamentation is clear, anticlinal walls raised, and periclinal walls sunk. Pattern 2: cuticular ornamentation is less clear, the anticlinal wall rises and the periclinal wall sinks. Pattern 3: cuticular ornamentation indistinct, anticlinal wall, and periclinal wall indistinguishable. Pattern 4: cuticular ornamentation indistinct, anticlinal walls and periclinal walls indistinguishable due to the presence of very dense trichomes.

3.9 Trichomes

On the adaxial and abaxial leaf epidermal surfaces variety of trichomes are found including coiled trichomes (Table 6). The presence of trichomes, especially the rare trichomes, is very important in taxonomy where the presence of these trichomes is important for classifying genera and species and also for analyzing hybrid variation between species [19-20].

Table 6: Types of trichomes

Types of trichomes	Species
Leaf lamina	
Armed (2 – 4 arms)	<i>S. accrescens</i> subsp. <i>stellata</i>
Cushioned stellate (long arms, thick walls)	<i>S. accrescens</i> subsp. <i>accrescens</i>
Coiled cushion stellate (long arm, thin wall)	<i>S. accrescens</i> subsp. <i>accrescens</i>
Flat cushioned stellate (long arm, thin wall)	<i>S. accrescens</i> subsp. <i>borneensis</i>
Coiled flat cushioned stellate (long arm, thin wall)	<i>S. accrescens</i> subsp. <i>stellata</i>
Porate stellate	<i>S. accrescens</i> subsp. <i>accrescens</i>
Flat lepidote stellate	<i>S. kunstleri</i> , <i>S. leprosula</i>
Multicellular peltate glandular	<i>S. accrescens</i> subsp. <i>accrescens</i> , <i>S. accrescens</i> subsp. <i>borneensis</i> , <i>S. accrescens</i> subsp. <i>stellata</i> <i>S. kunstleri</i> , <i>S. leprosula</i>
Midribs	
Capitate glandular trichomes	<i>S. accrescens</i> subsp. <i>borneensis</i>
Stellate trichomes	<i>S. accrescens</i> subsp. <i>accrescens</i> , <i>S. accrescens</i> subsp. <i>stellata</i>
Serrated trichomes	<i>S. leprosula</i>
Petioles	
Stellate	<i>S. accrescens</i> subsp. <i>accrescens</i> , <i>S. accrescens</i> subsp. <i>borneensis</i> , <i>S. accrescens</i> subsp. <i>stellata</i> <i>S. kunstleri</i>
Coiled stellate	<i>S. accrescens</i> subsp. <i>accrescens</i> , <i>S. accrescens</i> subsp. <i>borneensis</i> , <i>S. accrescens</i> subsp. <i>stellata</i>
Lepidote	<i>S. leprosula</i>

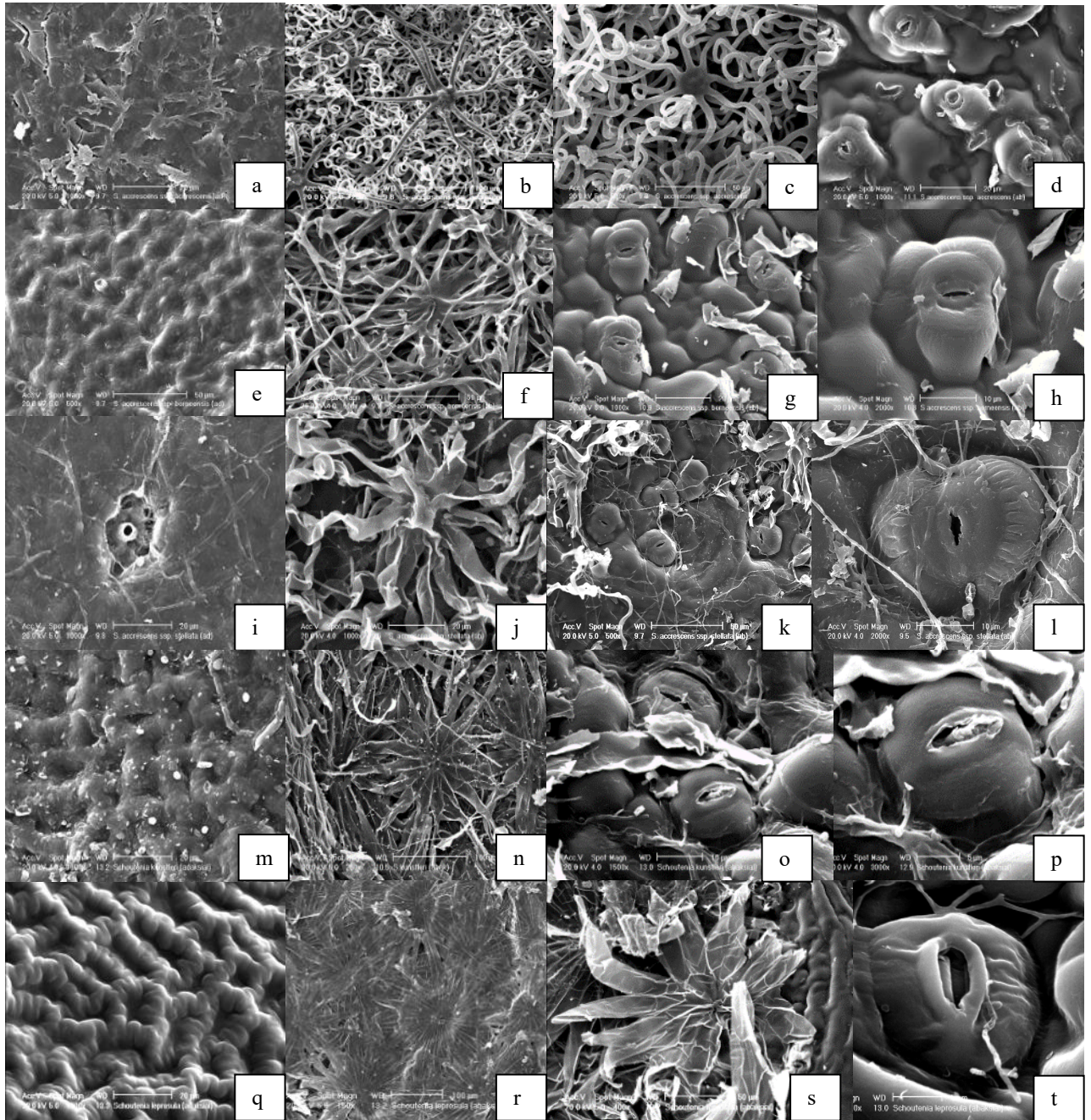


Figure 1: FESEM micrographs of *Schoutenia accrescens* subsp. *accrescens*: (a) Scaly wax, cuticle ornamentation indistinct, (b) Coiled cushion stellate trichomes and porate stellates, (c) Cushioned stellate trichomes coiled, (d) Anisocytic and parasitic stomata. *S. accrescens* subsp. *borneensis*, (e) Crustose wax and cuticle ornamentation clear, (f) Flat cushioned stellate trichomes, (g) and (h) Stomata of anisocytic type and parasitic type. *S. accrescens* subsp. *stellata*, (i) Crustose wax and cuticle ornamentation indistinct, (j) Coiled flat cushioned stellate trichomes, (k) Thin film of wax and thin coiled flat cushioned stellate trichomes, (l) Parasitic type of stomata. *S. kunstleri*, (m) Crustaceous wax and granules, cuticle ornamentation less obvious, (n) Flat lepidote stellate trichomes, (o) and (p) Stomata. *S. leprosula*, (q) Cuticle ornamentation clear, rugulate shape, (r) and (s) Flat lepidote trichomes (long arms, thin walls) and (t) Parasitic stomata

3.10 Diagnostic of Leaf Anatomical and Micromorphological Characteristics

Some species have diagnostic anatomical and micromorphological characteristics that can be identified directly through these characteristics. Table 7 shows the anatomical and micromorphological diagnostic features found in the study. Dichotomy key to the identification of the *Schoutenia* taxon is constructed using diagnostic and variation of leaf anatomical and micromorphological characteristics.

Table 7: Diagnostic of leaf anatomical and micromorphological characteristics

Species	Diagnostic features
<i>S. accrescens</i> subsp. <i>accrescens</i>	Anticlinal wall sinuous 45°
<i>S. accrescens</i> subsp. <i>accrescens</i>	Adaxial surface of petiole: hump Abaxial surface of midrib: arc
<i>S. accrescens</i> subsp. <i>Stellata</i>	Adaxial surface of petiole: convex Abaxial surface of midrib: arc
<i>S. leprosula</i>	Adaxial surface of petiole: hump Abaxial surface of midrib: convex
<i>S. accrescens</i> subsp. <i>Stellata</i>	Lamellar collenchyma cells are observed on the petioles on the adaxial and abaxial sides of the petiole vascular bundle

3.11 Dichotomy Key to Identification of *Schoutenia*

1. The abaxial epidermal anticlinal wall is curved to wavy *S. accrescens* subsp. *stellata*
1. Abaxial epidermal anticlinal wall sinuous 30 ° or sinuous 45 °2
2. Laminar epidermal cells with a height-to-width ratio of 1:1..... *S. leprosula*
2. Laminar epidermal cells with a height-to-width ratio of 1:2 3
3. The pattern of the main vascular bundles of the midrib is arc-shaped to U-shaped, continuous with several groups of vascular bundles on the adaxial side of the main vascular bundle*S. accrescens* subsp. *accrescens*
3. The pattern of the main vascular bundle of the midrib is arc to U-shaped continuous with one vascular bundle on the adaxial part of the main vascular bundle..... 4
4. Flat cushioned stellate trichomes (long arms, thin walls) present on the epidermal surface of the lamina..... *S. accrescens* subsp. *borneensis*
4. Flat lepidote stellate trichomes (long arms, thin walls) present on the epidermal surface of the lamina..... *S. kunstleri*

4. CONCLUSIONS

This study successfully proved that leaves anatomical and micromorphological characteristics are useful for distinguishing *Schoutenia* species. Several species of this study can be identified through their diagnostic anatomical and micromorphological characteristics. The diagnostic characteristics that can be used for species identification are the type of leaf lamina venation, the pattern of anticlinal walls on the epidermal surface, the type of stomata, the pattern of petiole vascular tissue arrangement, the pattern of medullary vascular bundles on the petiole, the outline shape of the midrib, the outline shape of the leaf margin, the presence of lamellar collenchyma cells on petioles and also trichome types. The results obtained successfully prove that the anatomical and micromorphological characteristics of leaves can be used as supporting data for species identification in *Schoutenia*. This study of leaf anatomy and micromorphology on most *Schoutenia* species is useful to help solve problems in species identification, especially for incomplete and perfect specimens.

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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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