TAXONOMIC IMPLICATIONS OF LEAF MICROMORPHOLOGY IN THE GENUS *HERITIERA* (STERCULIACEAE)

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Abstract. A microscopic analysis study was conducted on the leaf micromorphology of six *Heritiera* species using observation under light and scanning electron microscope. *Heritiera* is a genus under Sterculiaceae, however based on new evidences the families of the Malvales order were re-positioned into nine subfamilies. Thus, it is important to continuously document additional anatomical and micromorphological information especially with advancement in microscopy technologies. The objective of this study is to identify taxonomically significant leaf micromorphology characteristics that can be used to assist species identification and classification. Methods involved were leaf peeling and staining for observation under light microscope, whilst dehydration, critical point drying and gold coating for observation under scanning electron microscope. The results have been presented in a matrix based on four foliar epidermal characters which were epidermal anticlinal wall shape, presence of cuticular waxes, types of stomata and trichomes. Several diagnostic characters were found in this study. These diagnostic characters which can be useful in species identification are the shape of epidermal anticlinal walls in *H. elata*, cuticular stellate wax sheet in *H. littoralis* and cyclotetracytic stomata in *H. simplicifolia*. Among the 18 types of trichomes observed, nine can be used in *Heritiera* species identification. In conclusion, leaf micromorphological characteristics in *Heritiera* have significant taxonomic value which can be useful in species identification.

Keywords: Sterculiaceae, *Heritiera*, leaf micromorphology, light microscope, scanning electron microscope, trichome, cuticular wax, stomata, anticlinal wall

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Introduction

The plant anatomy field generally concerns the study of cell and tissue structures which are involved in certain functions. The development of the plant systematics has also seen the contributions from plant anatomy studies especially when combined with other plant taxonomic studies [1]. The delimitation of families within Malvales has puzzled systematists for years [2]. The Sterculiaceae family consist of approximately 60 genera (700 species) across the tropical and subtropical land but rarely found in temperate areas [3]. There are 16 genera found in Malaysia among which is the genus *Heritiera* [4-5]. In Malaysia, even if samples collected have flowers the heterogeneity of Sterculiaceae flowers and its affinity to Malvaceae and Tiliaceae made it difficult to describe them [4-6]. It is rather difficult to collect samples with flowers or fruits present, which makes identification of the specimen tougher. There are nine species of *Heritiera* found in peninsular Malaysia [5] and nine species found in Sarawak as according to [4].

The genus *Heritiera*, are good suppliers of red-brown construction timber with similar properties to the *Shorea* species in its strength and durability. Thus, the timbers of *Heritiera* are sometimes sorted and mixed-up wrongly during the process of transporting timber. The timber of *Sterculia* share the same light and pale pink colour which is a similar trait to the timber of *Heritiera* [4]. The species *H. elata* are noticeably more distinguishable in field as the trees shed all its leaves while new silvery-copper leaves emerge [5].

Based on molecular, morphology and biogeography, the core Malvales order were rearranged into nine subfamilies [7]. However, the rearrangement was still unable to explain the relationship between the taxa. Hence, there is a need for anatomical evidence in addition to leaf micromorphological characters for species identification. Leaf anatomical characteristic is essential in increasing the numbers of data sets, to link fossil record and molecular phylogenetics and phylogenomics in order to understand plant evolution [8].

Materials and Methods

Fresh and dried leaf samples (Figure 1) were used for the study. Fresh specimens were collected from the Pasoh Forest Reserve in Negeri Sembilan and forest in Langkawi Island, Kedah. Dried leaf samples were obtained from the FRIM Herbarium in Kepong (KEP) and Universiti Kebangsaan Malaysia Herbarium, in Bangi (UKMB). A list of the species studied is given in Table 1. Voucher specimens are kept at UKMB Herbarium. Fresh leaf specimens collected were fixed in AA (70% Ethanol: 30% Acetic Acid in ratio of 1:3), while dried herbarium samples were boiled. All the process involved in fixation and embedding were done through some modification [9-10]. A few pieces of leaf lamina (1 cm x 1 cm) were cut and soaked in the Jeffery solution for a few hours, until the tissues are peeled or separated and the epidermal layer was obtained. The sample sections were then rinsed with distilled water 2-3 times and were steeped in Safranin solution for approximately 5 minutes, rinsed with water, dehydrated in a series of alcohol concentrations starting from 50%, 70%, 95% and 100%. Finally, the samples were mounted on microscope slides in Euparal as permanent medium, carefully covered with slide covers, and then kept in drying oven at 40 °C for two weeks. For micromorphological characters analysis, a 1 cm x 1 cm piece of the samples were cut from similar area of each lamina sample and the small pieces were then fixed onto a stub by double sided tapes. These small pieces of the sample on the stub are then coated with a thin layer of gold to enhance and improve electrical conducting properties of the leaf lamina

sample. This was continued with critical point drying and gold coating step was conducted as follows - the samples on stub were coated with a sputter coater and examined under a variable pressure scanning electron microscope (Leo 1450 VP Zeiss) and the micromorphological characteristics were analysed accordingly.

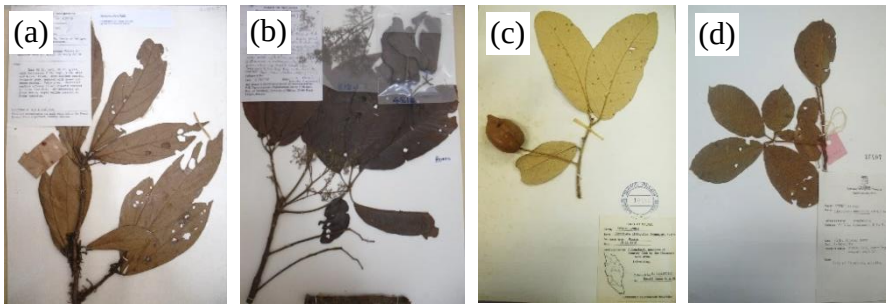

Figure 1. Herbarium samples (a) *H. elata,* (b) *H.javanica,* (c) *H. littoralis* and (d) *H. sumatrana.*

Results and Discussion

Vast study had been conducted on leaf micromorphological characteristics as it has shown significant taxonomic value and adds valuable data to currently available morphological characters use in species identification. Especially with presence of diagnostic characters such as presence of four different types of trichomes on the epidermis of *Lepisanthes fruticosa* [11], presence of papillose epidermal cells in certain species of *Parashorea* (Dipterocarpaceae) [12] and combination of several leaf micromorphological characteristics such as stomatal complexes and epidermal cell characteristics in *Drypetes* and *Putranjiva* that brought taxonomic and nomenclature revision [13].

In this study, several leaf epidermal characteristics such as shape of the epidermal anticlinal cell walls, occurrence of different types of wax, trichomes and stomata were found useful in *Heritiera* species identification. The epidermal cell wall shape is widely studied due to the consistency of the characteristic within taxa [12, 14-15].

Shape of the epidermal anticlinal cell walls. In this study, all the species had straight to curved anticlinal wall on both adaxial and abaxial leaf surface, except for *H. elata* whereby the adaxial anticlinal wall was sinuous and abaxial anticlinal wall was curved to wavy (Table 2 and Figure 2). [16] in his study on the Korean Piperales, *Piper kadsura* was observed to have a unique periclinal cell wall shape - tuberculate and thick cell walls on the adaxial surface, thus the species was easily distinguishable. Leaf epidermal characteristics are becoming more recognised as a reliable source for authentication and identification of leaf species especially in the medicinal plant field. This demonstrates how epidermal surface of the leaves have taxonomically informative characteristics especially when used with a combination of other leaf epidermal characters [17]. Some studies have also considered that epidermal cell shape is an environmental adaptation, as terrestrial species have sinuous walls while xerophytic plants have straight walls [18].

Table 1. A complete list of the *Heritiera* species studied

Species	Specimen code	Locallity	Collection date	Collector name	Source
Heritiere albiflora (Ridley) Kosterm.	S 33292	-	-	-	-
	FDS 24503	-	-	-	-
H. elata Ridley	S.F.N 37731	-	-	-	Herbarium specimen
	KEP 93316	-	-	-	Herbarium specimen
	FRI 9912	-	-	-	Herbarium specimen
	NBM 15	Pasoh F.R., Jelebu, Negeri Sembilan, Malaysia	23.12.2010	Abu Husin, H.	Fresh specimen
	NBM 09	Pasoh F.R., Jelebu, Negeri Sembilan, Malaysia	23.12.2010	Abu Husin, H.	Fresh specimen
	PW 131	Pasoh F.R., Jelebu, Negeri Sembilan, Malaysia	13.02.2001	Wilkie, P.	Herbarium specimen
	PW132	Pasoh F.R., Jelebu, Negeri Sembilan, Malaysia	13.02.2002	Wilkie, P.	Herbarium specimen
H. javanica (Bl.) Kosterm.	KL 4818	Gua Musang, Kelantan, Malaysia	06.10.1998	Teo, L.E.	Herbarium specimen
	NBM 03-L	Pulau Langkawi, Kedah, Malaysia	23.01.2011	Sani Miran	Fresh specimen
	NBM 11-L	Pulau Langkawi, Kedah, Malaysia	23.01.2011	Sani Miran	Fresh specimen
H. littoralis Dryand.	GF 265	-	-	-	-
	NBM 12-L	Pulau Langkawi, Kedah, Malaysia	23.01.2011	Sani Miran	Fresh specimen
	NBM 14-L	Pulau Langkawi, Kedah, Malaysia	23.01.2011	Sani Miran	Fresh specimen
H. simplicifolia (Mast.) Kosterm.	KEP 98192	-	-	-	-
	FRI 21139	Malaysia	15.05.1974	Ng, F.S.P.	Herbarium specimen
	NBM 06	Pasoh F.R., Jelebu, N. Sembilan, Malaysia	23.12.2010	Abu Husin, H.	Fresh specimen
H. sumatrana (Miq.) Kosterm.	FRI 69762	-	-	-	-
	SFN 21447	-	-	-	-

Table 2. Types of anticlinal wall shape in six *Heritiera* species studied

Species	Adaxial anticlinal wall shape	Abaxial anticlinal wall shape
H. albiflora	straight to curved shape	straight to curved shape
H. elata	sinuous	curved to wavy
H.javanica	straight to curved shape	straight to curved shape
H. littoralis	straight to curved shape	straight to curved shape
H. simplicifolia	straight to curved shape	straight to curved shape
H. sumatrana	straight to curved shape	straight to curved shape

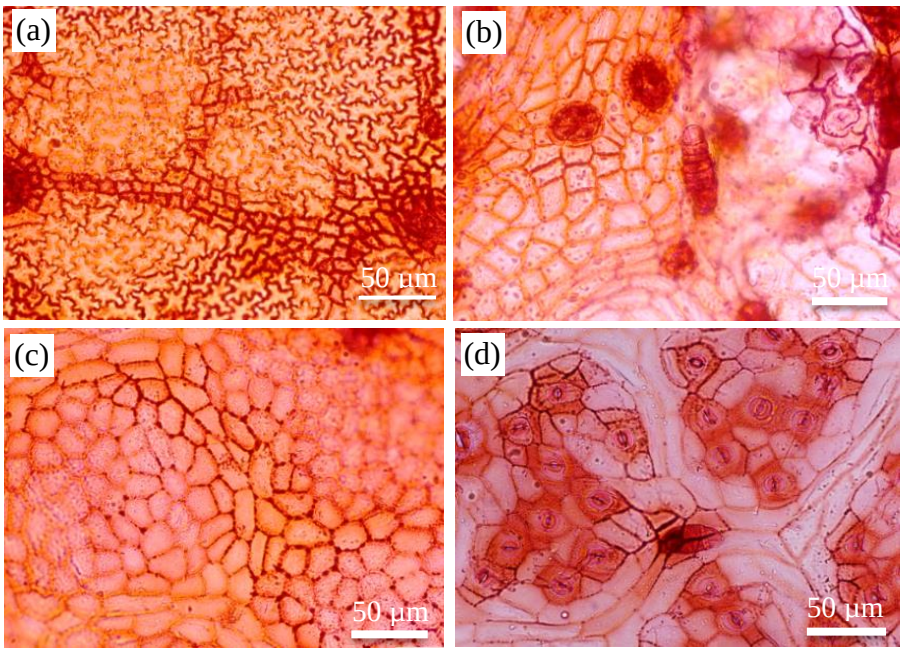

Figure 2. Anticlinal wall shape in *H. elata* (a) adaxial surface: sinuous, (b) abaxial surface: curved to wavy. Anticlinal wall shape in *H. javanica* (c) abaxial and (d) adaxial surface, both showing straight to curved shape.

Epidermal cuticular wax. The cuticular waxes observed through the scanning electron microscope showed high varieties within the taxa studied. There were six types of cuticular waxes observed in this study which are crust-like, film-like, crustose, sheet-like, granule and stellate wax sheet (Table 3). Cuticular waxes presence and their various types imparts additional information for classification and can be useful as systematic evidence to support existing morphological and anatomical data [19]. The presence of crustose (AD-adaxial) and film-like wax (AB-abaxial) was observed in *H. javanica* and *H. simplicifolia*. Other species showed the presence of crust-like (AD) and film-like (AB) wax type in *H. albiflora*, a combination of crustose and sheet-like wax on both leaf surfaces of *H. elata*, and a combination of crustose and granule wax was observed on both leaf surfaces of *H. sumatrana*. Stellate wax sheet was observed present only in *H. littoralis* along with crust-like, granule (AD) and crustose wax (AB) which can be useful in species identification (Figure 3).

Table 3. Types of epidermal cuticular wax in six *Heritiera* species studied

Species	Adaxial epidermis	Abaxial epidermis
H. albiflora	Crust-like	Film-like
H. elata	Crustose, sheet-like	Crustose, sheet-like
H.javanica	Crustose	Film-like
H. littoralis	Crust-like, granule, Stellate sheet	Crustose
H. simplicifolia	Crustose	Film-like
H. sumatrana	Crustose, granule	Crustose, granule

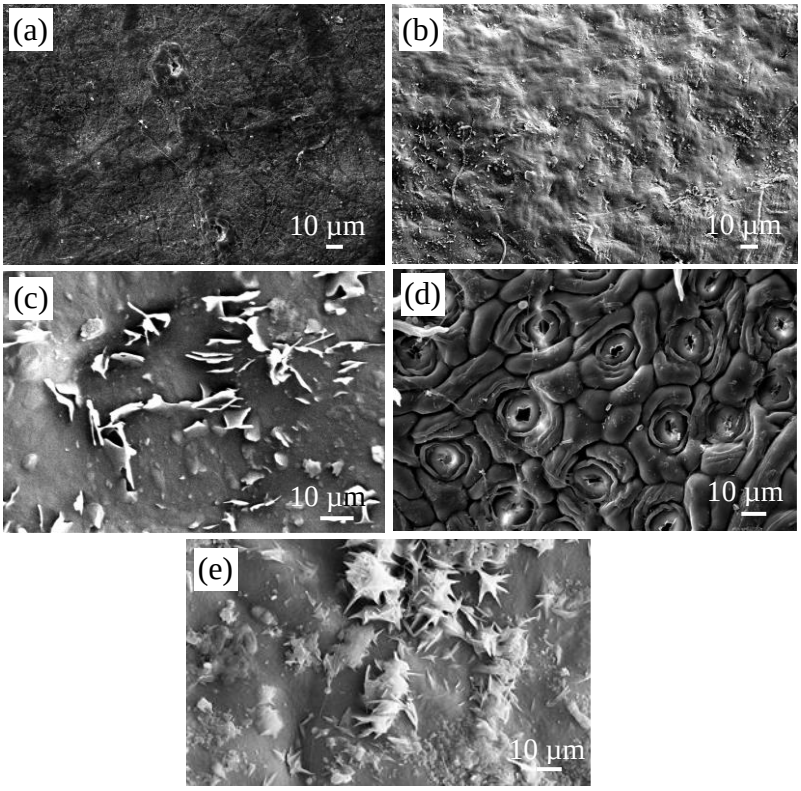

Figure 3. Cuticular waxes (a) Crust-like in *H. littoralis*, (b) crustose on adaxial surface of *H. javanica* and *H. simplicifolia* (c) sheet-like on abaxial surface of *H. elata* (d) stellate wax sheet and granule in *H. littoralis* and (e) film-like in *H. albiflora*.

Trichomes. The trichomes found in this study can be categorised as non-glandular and glandular trichomes. Among the 18 types of trichomes observed were simple unicellular, 2-armed cushion, 4-armed cushion, stellate-rotate (radii webbed 15-30%), stellate-lepidote (radii webbed 30-50%), dentate-lepidote (radii webbed 50-80%), lepidote (radii webbed 80-100%), stellate-porate, cushioned stellate (groove armed, thin-walled), cushioned stellate (long armed, thick-walled), cushion stellate (short armed, thick-walled), peltate glandular (multicellular terminal, thick-walled), peltate glandular (multicellular terminal, thin-walled), capitate glandular (multicellular terminal, unicellular short stalk), clustered capitate glandular (multicellular terminal, unicellular short stalk), digitiform, scale and tufted (multiangular) (Figure 4).

H. albiflora has six types of trichomes - stellate-rotate (radii webbed 15-30%), cushion stellate (short armed, thick-walled), peltate glandular (multicellular terminal, thick-

walled), peltate glandular (multicellular terminal, thin-walled), capitate glandular (multicellular terminal, unicellular short stalk), clustered capitate glandular (multicellular terminal, unicellular short stalk), *H. elata* has four types of trichomes - stellate-lepidote (radii webbed 30-50%), dentate-lepidote (radii webbed 50-80%), capitate glandular (multicellular terminal, unicellular short stalk) and scale, *H. javanica* has five types of trichomes - simple unicellular, stellate-rotate (radii webbed 15-30%), peltate glandular (multicellular terminal, thin-walled), capitate glandular (multicellular terminal, unicellular short stalk) and tufted (multiangular), *H. littoralis* has seven types of trichomes - simple unicellular, 2-armed cushion, stellate-lepidote (radii webbed 30-50%), dentate-lepidote (radii webbed 50-80%), lepidote (radii webbed 80-100%), digitiform and scale, *H. simplicifolia* has six types of trichomes - stellate-rotate (radii webbed 15-30%), stellate-lepidote (radii webbed 30-50%), cushioned stellate (groove armed, thin-walled), capitate glandular (multicellular terminal, unicellular short stalk), digitiform and tufted (multiangular), *H. sumatrana* has six types of trichomes - simple unicellular, 4-armed cushion, stellate-porate, cushioned stellate (long armed, thick-walled), capitate glandular (multicellular terminal, unicellular short stalk) and digitiform (Table 4).

Nine of these trichomes identified were only found in one species thus trichome type is a diagnostic characteristic for the species studied. The trichomes - cushion stellate (short armed, thick-walled), peltate glandular (multicellular terminal, thick-walled) and clustered capitate glandular (multicellular terminal, unicellular short stalk) were found only in *H. elata*. The trichomes - 2-armed cushion and lepidote (radii webbed 80-100%) were only found in *H. littoralis*, meanwhile cushioned stellate (groove armed, thin-walled) trichome was only found in *H. simplicifolia.* The 4-armed cushion trichome, stellate-porate, and cushioned stellate (long armed, thick-walled) were only present in *H. sumatrana* (Table 4).

There are plenty of similarities in the trichomes types of the Sterculiaceae and Malvaceae - among these trichomes are simple unicellular, uniseriate, glandular, tufted, stellate and peltate [20]. Furthermore, he found that the scale trichomes were observed in multi layers in *H. littoralis*, but with advancement in technology, many studies have shown that the scales are better identified as stellate hairs as these trichomes body have rays or arms that are attached on their side looking webbed-like [21] and similar result was observed in this study. Besides that, trichomes are present not only on leaves but on sepal, petals and even on young stems [22]. It was observed that non-glandular trichomes were present in all six species of *Heritiera* studied, but the trichomes present in each species was of various types - tufted (multiangular) in *H. borneensis* and *H. javanica*, rotate in *H. simplicifolia* and peltate in *H. littoralis* [23].

Types of stomata. In this study *H. javanica* and *H. littoralis* were found with only one type of stomata (homostomatic) which were anisocytic and anomocytic accordingly. *H. albiflora, H. elata, H. simplicifolia* and *H. sumatrana* were found to have more than one type of stomata (heterostomatic) (Table 5). The different types of stomata present in each species can be used to identify the species in this study and the presence of cyclotetracytic stomata in *H. simplicifolia* is a diagnostic characteristic for the species (Figure 5).

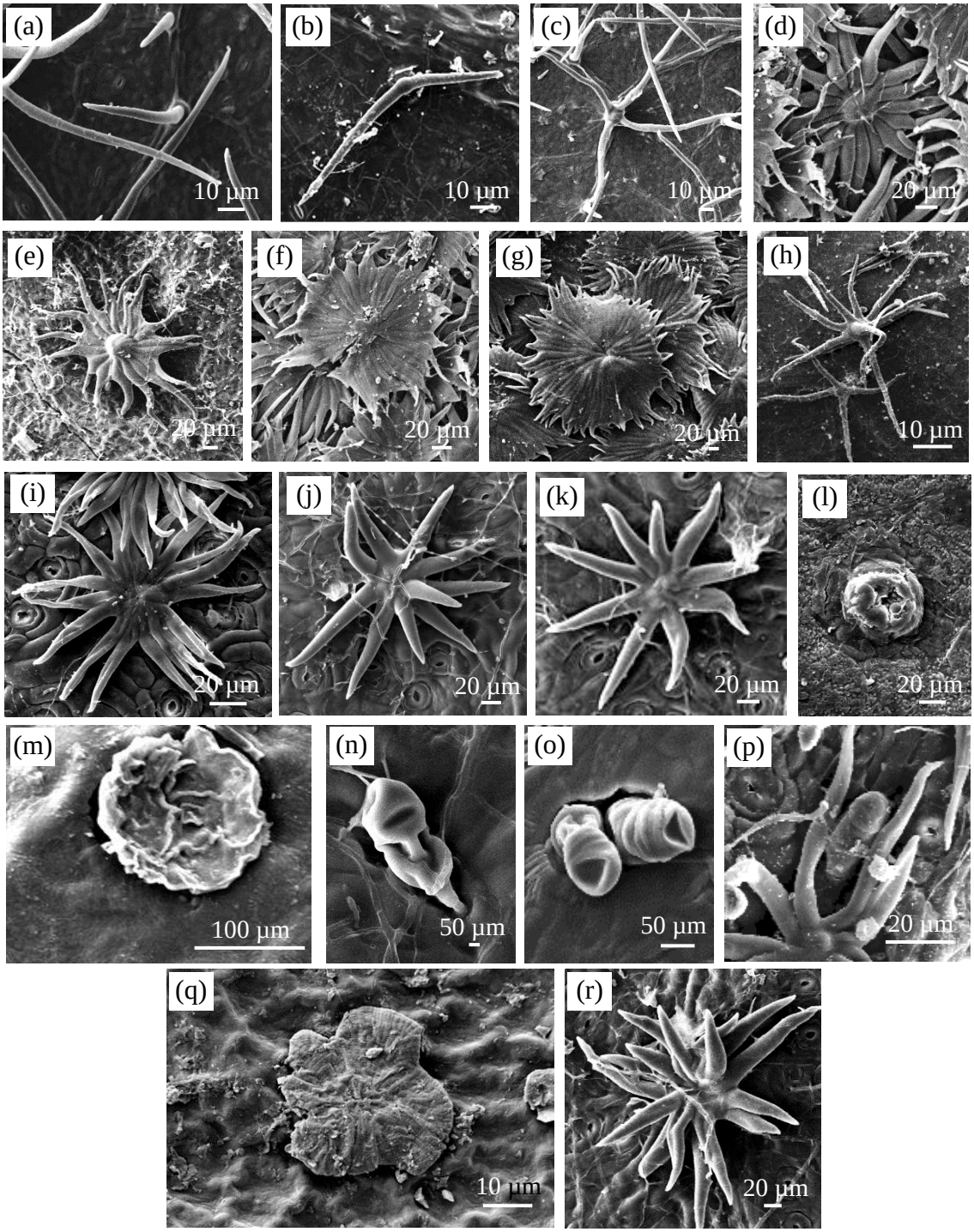

Figure 4. Trichomes present in the study (a) simple unicellular, (b) 2-armed cushion, (c) 4-armed cushion, (d) stellate-rotate (radii webbed 15-30%), (e) stellate-lepidote (radii webbed 30-50 %), (f) dentate-lepidote (radii webbed 50-80 %), (g) lepidote (radii webbed 80-100 %), (h) stellate-porate, (i) cushioned stellate (groove armed, thin walled), (j) cushioned stellate (long armed, thick-walled), (k) cushion stellate (short armed, thick-walled), (l) peltate glandular (multicellular terminal, thick-walled), (m) peltate glandular (multicellular terminal, thin-walled), (n) capitate glandular (multicellular terminal, unicellular short stalk), (o) clustered capitate glandular (multicellular terminal, unicellular short stalk), (p) digitiform, (q) scale and (r) tufted (multiangular).

In 1950 [20] had categorised four types of stomata based on the arrangement of subsidiary cells or cells that are directly beside the guard cell. These are anomocytic (*Ranunculaceous*), anisocytic (*Cruciferous*), paracytic (*Rubiaceous*) dan diacytic (*Caryophyllaceous*). In the order Malvales, more than one stomata can be present on the leaf lamina and this has taxonomic value. Anisocytic stomata were dominantly present in his study on the leaves of the species in Malvales order [24]. Findings in this study show similar trend as four out of six species was observed to have anisocytic stomata. Meanwhile the presence of anomocytic stomata in *H. littoralis* and *H. sumatrana* have been observed in other similar studies [23, 25-26].

Table 4. Types of trichomes in six *Heritiera* species studied

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
H. albiflora	-	-	-	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-
H. elata	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	-
H.javanica	+	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	-	+
H. littoralis	+	+	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	-
H. simplicifolia	-	-	-	+	+	-	-	-	+	-	-	-	-	+	-	+	-	+
H. sumatrana	+	-	+	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-

Legend: + = present, - = absent

1 = Simple unicellular
2 = 2-armed cushion
3 = 4-armed cushion
4 = Stellate-rotate (radii webbed 15-30%)
5 = Stellate-lepidote (radii webbed 30-50 %)
6 = Dentate-lepidote (radii webbed 50-80 %)
7 = Lepidote (radii webbed 80-100 %)
8 = Stellate-porate
9 = Cushioned stellate (groove armed, thin-walled)
10 = Cushioned stellate (long armed, thick-walled)
11 = Cushion stellate (short armed, thick-walled)
12 = Peltate glandular (multicellular terminal, thick-walled)
13 = Peltate glandular (multicellular terminal, thin-walled)
14 = Capitate glandular (multicellular terminal, unicellular short stalk)
15 = Clustered capitate glandular (multicellular terminal, unicellular short stalk)
16 = Digitiform
17 = Scale
18 = Tufted (multiangular)

Table 5. Types of stomata in six *Heritiera* species studied

Species	Types of Stomata
H. albiflora	Anisocytic, Tetracytic
H. elata	Anomocytic, Paracytic
H.javanica	Anisocytic
H. littoralis	Anomocytic
H. simplicifolia	Anisocytic, Paracytic, Tetracytic, Cyclotetracytic
H. sumatrana	Anisocytic, Anomocytic, Paracytic

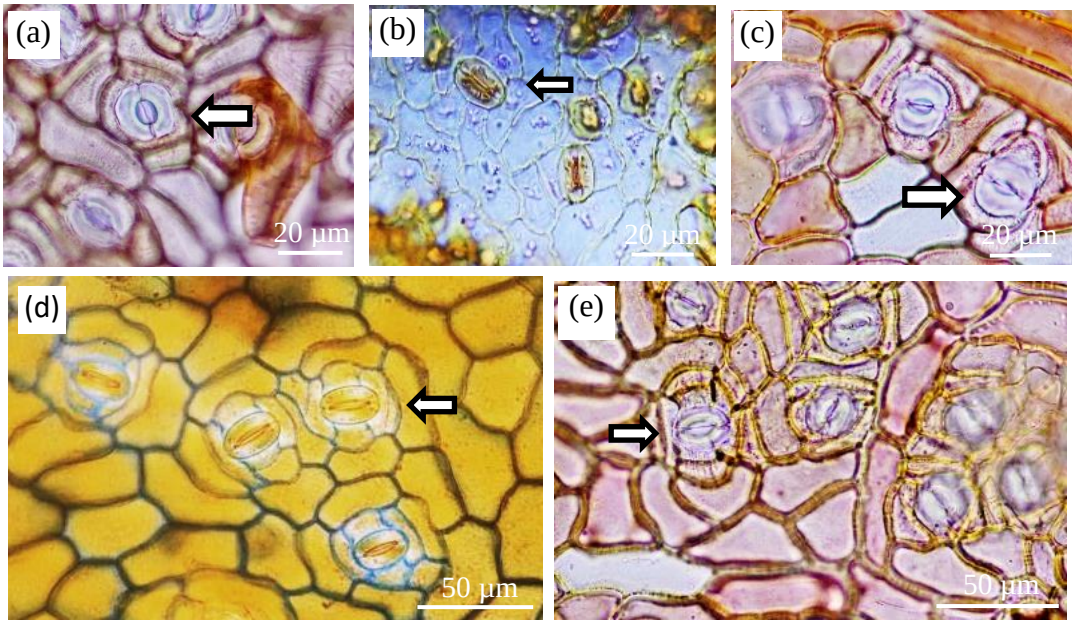

Figure 5. Stomatal types in *Heritiera* (a) Anisocytic, (b) Anomocytic, (c) Paracytic, (d) Tetracytic and (e) Cyclotetracytic in *H. simplicifolia.*

Conclusion

Results of the study revealed, a number of important features that have taxonomic value to assist and support species identification. This is true in species with diagnostic characters such as, stellate wax sheet, 2-armed cushion and lepidote (radii webbed 80-100%) trichome that was only observed in *H. littoralis*. Other species such as *H. simplicifolia* can be identified by having cushioned stellate (groove armed, thin-walled) trichome and cyclotetracytic stomata. *H. elata* was the only species with sinuous and curved to wavy anticlinal wall shape among the six species studied. This indicates that leaf micro morphological characteristics of *Heritiera* species studied, which was observed through light and scanning electron microscope showed significant taxonomic value which will be useful in species identification.

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Author Contributions

All authors contributed toward data analysis, drafting and revision of 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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