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Importance of Leaf, Stem and Flower Stalk Anatomical Characters in the Identification of *Emilia* Cass

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Authors' contributions

This work was carried out in collaboration between all authors. Author EC designed the study, performed the laboratory, statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OCA performed the laboratory, literature search and managed the literature searches. Author EC managed the analyses of the study. Author MSI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Three species of *Emilia* Cass. namely *E. cocinea* (Sims.) G. Don, *E. sonchifolia* (Linn.) DC and *E. preatamissa* Milne-Redhead were investigated using their petiole, stem, flower stalk, midrib and epidermal features. The specimens were pretreated, sectioned, stained in 1% Safranin O for two minutes, counter-stained with Alcian blue, mounted on a slide, observed under microscope and micro-photographed using Leica WILD MPS 52 microscope camera on Leitz Diaplan microscope. The results revealed that the variation in number of vascular bundle in the petiole, stem, flower stalk and midrib, stomata type, occurrence (on the adaxial and abaxial leaf surfaces) and orientation (contiguous or in groups) constituted dependable diagnostic characters among the species studied. In this study for instance, the contiguous stomata is restricted *E. cocinea.* The dipolar contiguous stomata are found on the adaxial leaf surface while mono-polar, lateral and di-polar contiguous stomata were found on the abaxial leaf surface. Also, stomata in groups of 2, 3 or more were found on the leaf surfaces of the *Emilia* species. Among the species, *E. sonchfolia* and

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E. cocinea have stomata in groups of 2, 3 or more on both the adaxial and abaxial leaf surfaces while *E. preatamissa* was distinct form these species by having stomata only in groups of 2 on the upper surface of the leaf. This also revealed that there are intraspecific and interspecific relationships among species.

Keywords: Flower stalk; midrib; petiole; contiguous stomata; stomata in group; vascular bundle.

1 INTRODUCTION

Asteraceae is among the cosmopolitan plant family [1] and is of the third largest plant family with 1,100 genera and over 20,000 known species [2]. Most of the species are herbaceous while others are shrubs or even trees. Many members of Asteraceae are economically important as weeds, ornamentals, medicinal and green vegetables [3]. Angiosperms are endowed with external morphological characters of significant taxonomic value which can be easily observed with the naked eye or with simple hand lens. Morphological attributes of vegetative organs have often constituted the mainstay of taxonomic studies in plants [4-10] and are very important in classification. The genus *Emilia* has three species in West Africa namely; *E. cocinea, E. preatamissa and E. sonchifolia* [11]. These species are basically identified base on vegetative morphological features and floral differences. Though the flowers are readily available on the plant all year round, when present it is difficult to actually distinguish between *E. preatamissa and E. sonchifolia* due to their close morphological similarities [12]. There are assertions that these species are hybrids of each other and this was based on the chromosome number of the species. Also it have been noted that the polyploids are always more in size when compared to the diploid species and that this genus has chromosome number 2n = 10, 20 or 30 i.e. n = 5 [13].

The use of anatomical methods in taxonomic investigations cannot be over emphasized. Although no character is absolutely immutable, some are more fixed than the others and it is on those that are less plastic that the systematic anatomist rely because they are not really affected by environmental conditions [14]; comparative plant epidermal, petiole, midrib and stem anatomy have been found to be reliable in taxonomy and systematics of many angiosperm species [15-23]. Metcalfe & Chalk [15,16], Naik & Nigrude [24], Palmer & Tucker [25], Adedeji [26] and Adedeji & Illoh [27] have all stressed the taxonomic importance of anatomical features which along with other characters are useful for identification and classification of plants. This article therefore uses micro-morphological characters from the petiole, midrib, floral and stem to enhance the delimitation of *Emilia* species. This study is necessitated by the fact that we need an additional ways to identify these species especially due to the morphological similarity between *E. sonchifolia* and *E. preatamissa* [12].

2. MATERIALS AND METHODS

2.1 Source of Materials

The materials used for this are fresh plant species of *Emilia* growing in the wild. These plant species were collected, properly identified and deposited in University of Port Harcourt Herbarium (UPH), Nigeria.

2.2 Anatomical Studies

One centimeter below the flower head (flower stalk), middle portion of the midrib, stem and petiole were cut off. These specimens were fixed in FAA (formalin, acetic acid and alcohol) for 12hrs, dehydrated in series of different percentages of ethanol (30% and 50%) and stored in 70% ethanol till when needed. The petiole (apex and basal portions), stem, flower stalk and the middle portion of the midrib were hand-sectioned [28,21]. The sections were stained in 1% Safranin O for two minutes, counter-stained with Alcian blue, mounted on a slide, observed under microscope and micro-photographed using Leica WILD MPS 52 microscope camera on Leitz Diaplan microscope. This method is the modified [29].

2.3 Epidermal Studies

Foliar materials for epidermal studies were collected fresh from the plants in the field. 0.5 cm – 1 cm square leaf cuttings were obtained from identical regions of each fresh leaf, generally from mid-way between the leaf base and apex of lamina including the mid-rib. The adaxial and abaxial epidermal peels were obtained using

forceps. Peels were stained with 1% safranin or alcian blue rinsed with distilled water to remove excess stain and mounted in a drop of pure glycerol on clean glass slides. A cover glass was placed over the drop and sealed with nail vanish to prevent dehydration [30]. The epidermal features like arrangement of the epidermal cells, shape and nature of the anticlinal cell wall of the leaf epidermis, stomata types, density and index were determined. The stomatal index (SI) was estimated based on [15] while the terminology for the stomata type is taken after [31,32] with modifications).

3. RESULTS AND DISCUSSION

3.1 Leaf Anatomical Characteristics

3.1.1 Petiole anatomy

Among the three species studied, the number of vascular bundles and layers of parenchymatous cell at the basal and apical sections of the petiole were observed to be diagnostic but the shape of the petioles were fairly diagnostic (Table 1; Figs. 1 and 2). For instance, at the basal portion of the petioles, *E. preatamissa* and *E. cocinea* have 7 vascular bundles each while *E. sonchifolia* has 7-11 vascular bundles. Subsequently, the layers of parenchymatous cells are as follows: *E. preatamissa* (5-11 layers), *E. sonchifolia* (6-13 layers) and *E. cocinea* (5-15 layers) (Fig. 1). In the apical region, the number of vascular bundles and parenchymatous layers showed variations such as *E. preatamissa* (7 and 6-17 layers), *E. sonchifolia* (15 and 10-18 layers) and *E. cocinea* (5-7 and 5-17 layers) respectively (Fig. 2). The shape of the basal and apical portion of the petiole are oval, curved, V-shaped or U-shaped (Figs. 1 and 2). These variations are diagnostic and could be used to differentiate the species. Among these characters, the parenchymatous layers and number of vascular bundles at the apical portion of the petiole was found to be contrasting. Metcalfe & Chalk [15] and Abbas [33] have noted that the number of petiolar apical vascular bundles (vascular bundles at the base of the leaf) is a reliable delimitating character among dicotelydonous plants. This assertion has been based on the fact that at the point of entering the leaf blade, the number of vascular bundles varies to represent the number of veins on the leaf. This character has been used in the taxonomy of many plant families. For instance, Nurul-Aini [34] described 11 types of petiole vascular bundles arrangement in *Microcos* L. (Malvaceae: Grewioideae). Also, Akinnubi [35] described the petiole anatomy of 12 species of Asteraceae around Ile-Ife in South-Western Nigeria and noted the distinguishing characteristics of these species as the shapes of the petioles, variation in the number, arrangement and shapes of vascular bundles, Noraini [36] found that the petiole vascular bundle as taxonomic tool in the identification of some species of the tribe Dipterocarpeae. Similarly, Sarala & Vijay [37], Taha [38] and Abbas [33] have brought the importance of the variation in the distribution of sclerenchyma, collenchyma and vascular patterns and shapes as observed in the transverse section of the petiole as taxonomic character in Euphorbiaceae, *Callistemon viminalis* and *Vitex* L. (Lamiaceae) repectively.

3.1.2 Midrib anatomy

The number of vascular bundles and the layers of parenchymatous cells in upper and lower epidermises of the midrib were found to be diagnostic in the species studied. For example, *E. sonchifolia* has one (1) vascular bundle trace (Fig. 3a), *E. preatamissa* has 3 vascular bundle traces (Fig. 3b) while *E. cocinea* has 4 vascular bundle traces (Fig. 3c and Table 2). Also, there are slight differences in the layer of parenchymatous cells in both upper and lower surfaces of the midrib. The parenchymatous layers in upper surface include; *E. preatamissa* 5-6 layers, *E. sonchifolia* 5-7 layers and *E. cocinea* 8-11 layers while in the lower surface they have 6-8 layers, 6-7 layers and 8-10 layers respectively. *E. cocinea* is more distinct based on this character and this showed intraspecific and interspecific variations among these species. Number of vascular bundles, shape of the midrib and other leaf epidermal structures have been employed in delimitation of many plant species [19,20,39,18]. André et al. [40] in their study, they found that the midrib outline is a diagnostic character in *Anthurium* section *Urospadix* subsection *Flavescentiviridia* (Araceae). They noted five distinct types of outlines and used them to distinguish *Anthurium.* Similarly, we have found three different number of vascular bundle in the midrib of the *Emilia* which could be used to distinguish members of this genus.

3.1.3 Nature of epidermal/subsidiary cells

These cells are predorminantly irregular in shape with undulating anticlinal walls (Table 3; Figs. 4 and 5). In *E. preatamissa* the epidermal cells are polygonal to irregular in shape and elongated

while the anticlinal cell walls are slightly straight, curved or undulating. In the adaxial leaf surface of *E. coccinea* they are rectangular-hexagonal or polygonal in shape with straight, curved or slightly undulating anticlinal walls. The characteristics of epidermal surface have been reported in members of angiosperms [18,19,20] and *Emilia* species [39].

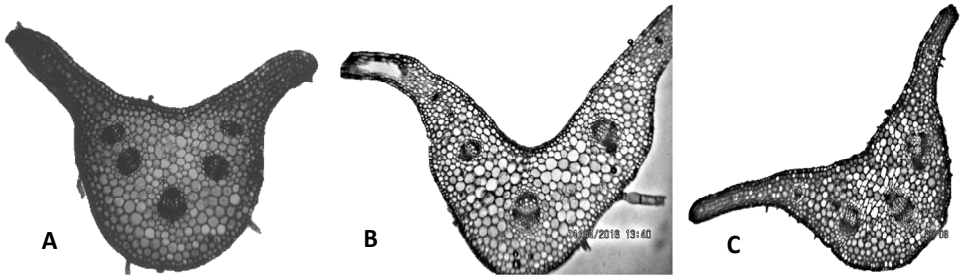

Fig. 1. Petiole basal portion (A) *E. preatamissa*; (B) *E. sonchifolia* and (C) *E. cocinea*

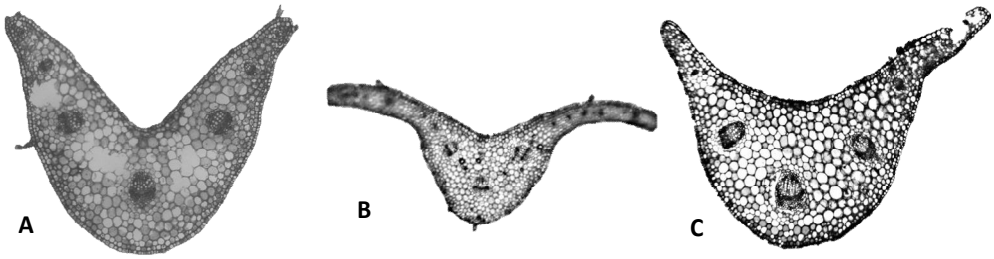

Fig. 2. Petiole apical portion (A) *E. preatamissa*; (B) *E. sonchifolia* and (C) *E. cocinea*

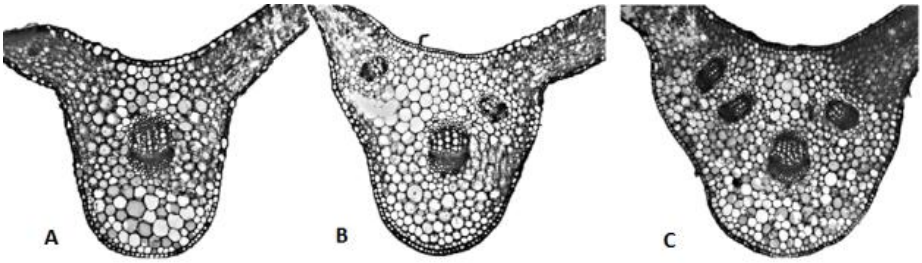

Fig. 3. Midrib (A) *E. preatamissa*; (B) *E. sonchifolia* and (C) *E. cocinea*

Table 1. Anatomical characteristics of the petiole

S/N	Species name	Basal portion			Apical portion			No. of epidermal layer
		No. of VB	Layer of PA	Shape	No. of VB	Layer of PA	Shape	
1	*E. preatamissa*	7	5 - 11	V-shaped	7	6 - 17	Arced	1
2	*E. sonchifolia*	7-11	6 - 13	U or V-shaped	15	10 - 18	V-shaped	1
3	*E. cocinea*	7	5 - 15	Arced	5 - 7	5 - 17	Arced	1

Note: VB = Vascular bundle; PA = parenchymatous cells

Table 2. Anatomical characteristics of the midrib

S/N	Species name	No. of VB	Layer of PA in upper surface	Layer of PA in lower surface	No. of epidermal layer
1	*E. preatamissa*	3	5 - 7	6 - 8	1
2	*E. sonchifolia*	1	5 - 6	6 - 7	1
3	*E. cocinea*	4	8 - 11	8 - 10	1

Note: VB = Vascular bundle; PA = parenchymatous cells

Table 3. Epidermal characteristics of the *Emilia* species studied

S/N	Species name	Upper (adaxial) epidermis				Lower (abaxial) epidermis			
		Stomata type	**Nature of subsidiary cells**	**Nature of epidermal cells**	**SI range (Maen±STD)**	**Stomata type**	**Nature of subsidiary cells**	**Nature of epidermal cells**	**SI range (Maen±STD)**
1	*E. preatamissa*	Diacytic, paracytic, tetracytic, anisocytic, and partly in group of 2.	Polygonal - irregular in shape, anticlinal walls slightly straight, curved or undulating.	Polygonal-irregular in shape, short or elongated, anticlinal walls slightly straight, curved or undulating.	16.67 - 55.56 (27.38±13.65)	Tetracytic, anisocytic and diacytic	Irregular in shape, anticlinal walls undulating.	Irregular in shape, elongated with undulating anticlinal walls.	74.58 - 85.71 (80.87±4.67)
2	*E. sonchifolia*	Paracytic, diacytic, anisocytic, isotricytic and in group of 2, 3 or more	Irregular in shape with slightly undulating anticlinal walls.	Irregular in shape, elongated or large with slightly undulating, curved or straight anticlinal walls	14.29 - 18.87 (17.00±2.03)	Tetracytic, anisocytic and in group of 2 or 3.	Irregular in shape, anticlinal walls undulating.	Irregular in shape, elongated with undulating anticlinal walls.	75.00 - 88.89 (82.04±5.75)
3	*E. cocinea*	Anisocytic, tetracytic, mono-polar contiguous and in group of 2, 3 or more	Polygonal in shape with straight-curved anticlinal walls.	Rectangular-hexagonal or polygonal in shape with straight, curved or slightly undulating anticlinal walls.	23.53 - 26.09 (24.71±1.09)	Anomocytic, tetracytic, anisocytic, dipolar and mono-polar contiguous, lateral contiguous and in group of 2 or 3.	Irregular in shape with undulating anticlinal walls.	Irregular in shape, elongated with undulating anticlinal walls.	47.37 - 90.01 (75.40±19.24)

Note: STD = Standard deviation; SI = Stomata index

3.1.4 Stomata types

We identified six (6) stomata types, stomata in groups and contiguous among the species. The stomata types include diacytic, paracytic, anisocytic, isotricytic, tetracytic and anomocytic stomata (Table 3; Figs. 4 and 5). These stomata occurred on the upper (adaxial) and lower (abaxial) surfaces of the leaves. On the abaxial surface of *E. preatamisssa* diacytic, anisocytic and tetracytic stomata (Fig. 4a-d), *E. sonchifolia* has anomocytic, anisocytic and isotricytic (Figs. 4e-g) while *E. cocinea* has anomocytic, anisocytic and tetracytic stomata (Figs. 4h-l). On the adaxial surface, *E. preatamisssa* has diacytic, paracytic, tetracytic and anisocytic stomata (Figs. 5a-d), *E. sonchifolia* has tetracytic, isotricytic, paracytic and anisocytic stomata types (Figs. 5e-i) while *E. cocinea* has tetracytic and anisocytic stomata (Figs. 5j-l). This result confirms the work of Ndukwu and Agbagwa [39] on the epidermal characteristics of *Emilia* species.

3.1.5 Contiguous stomata

In this study we have observed and reclassified contiguous stomata into the following (a) mono-polar contiguous (Mp) stomata (when one pole of the stomatal guard cell is touching the lateral side/portion of the guard cell of an adjacent stoma) Fig. 4h (b) lateral contiguous (La) stomata (when the lateral portions of the guard cells of two adjacent stomata are touching each other) Fig. 4j and (c) di-polar contiguous (Di) stomata (when the two poles of the guard cells of two stomata are touching each other) Figs. 4k and 5j. The distribution of these stomata types could be used to delimit the species. In this study for instance, the contiguous stomata is restricted *E. cocinea.* The dipolar contiguous stomata are found on the adaxial leaf surface while mono-polar, lateral and di-polar contiguous stomata were found on the abaxial leaf surface (Table 3). These stomata types have been reported in some members of angiosperms. In their studies Chen and Li [41] recorded 2 or 3-contiguous stomata in *Ginkgo bilola* - Ginkgoaceae, Su [42] recorded 2 or 3-contiguous stomata in *Zamia* – Zamiaceae while 2-contiguous stomata were recorded in *Casuarina* - Casuarianaceae [43] *Ephedra* - Ephedraceae [44], *Camptotheca* – Nyssaceae [45], *Nelumbo* – Nymphaceae [46]. Also, Awasthi [47], Xi [45] and Kothari & Shah [48] have reported lateral and polar or diagonal contiguous stomata in Amaryllidaceae, Nyssaceae and Papilionaceae respectively. In a similar study Ndukwu & Agbagwa [39] have reported contiguous stomata in *E. cocinea* and *E. sonchifolia.* The findings of this work is consistent with the works of Ndukwu and Agbagwa [39], Sun [49], Stockey [50], Zhao [51], Chen [52] and Gopal & Shah [53] who reported lateral and polar contiguous stomata in angiosperms.

3.1.6 Stomata in groups

In this study, we observed stomata occurring in groups of 2, 3 or more on the leaf surfaces of the *Emilia* species. Among these species, *E. sonchfolia* and *E. cocinea* have stomata in groups of 2, 3 or more on both the adaxial and abaxial leaf surfaces (Figs. 4i and 4l, 5c, f, j and k) while *E. preatamissa* was distinct from these species by having stomata only in groups of 2 on the upper surface of the leaf (Fig. 5a). This character was found to be diagnostic among the species and could be used to distinguish them. Metchalfe and Chalk [17] found that members of the family Gesneriaceae, Ixonathaceae, Melastomataceae, Portulacaceae, Rubiaceae, Saxifragaceae, Simaroubaceae and Theaceae have non-contiguous stomata on their leaf surfaces. Also, stomata in groups of 4-6 were recorded by Payne [54], Dehnel [55] Tang [56] and [57] among members of the *Begonia* – Begoniaceae and Baranova [58] in *Himantandra* – Himantandraceae. Members of Crassulaceae have 2-6 stomata in groups (Payen, 1970). These authors showed occurrence of these non-contiguous stomata among these families as a character for delimiting them from other families, genera or species. In the same vein, we observed non-contiguous stomata in groups of 2 on the adaxial leaf surface of *E. preatamissa* while in *E. sonchifolia* and *E. cocinea* they occurred in groups 2-3 in the abaxial leaf surfaces and 2, 3 or more on their adaxial surfaces. This character confirms that these last two species are more closely related and affirms the works of Hutchinson and Daziel [12,16] that these three species are distinct.

3.2 Transverse Section of Stem and Flower Stalk

The stem and flower stalk are oval in shape (Figs. 5 and 6). The number of vascular bundles and collenchyma in the stem and flower stalk varied from one species to another and is diagnostic. In the stem, the variation in number of vascular bundles include: *E. preatamissa* 22 (Fig. 5a), *E. sonchifolia* 18 (Fig. 5b) and

E. cocinea 16 (Fig. 5c) while in the flower stalk we found *E. preatamissa* 11 (Fig. 6a), *E. sonchifolia* 8-10 (Fig. 6b) and *E. cocinea* 15 (Fig. 6c). This confirms that these species are distinct as reported by Metchalfe & Chalk [59]. We further observed that there is slight variation in the layers of collenchyma cells among the species studied. For instance, in the stem, we noted 3-6 layers, 4-7 layers and 4-8 layers for *E. preatamissa*, *E. sonchifolia* and *E. cocinea* respectively while in the flower stalk we recorded 5, 4-5 and 5-6 respectively. This result showed intraspecific relationship among these species and supports Ajuru & Okoli [60] who noted variation in the layers of collenchyma in *Mormodica* species as distinguishing character.

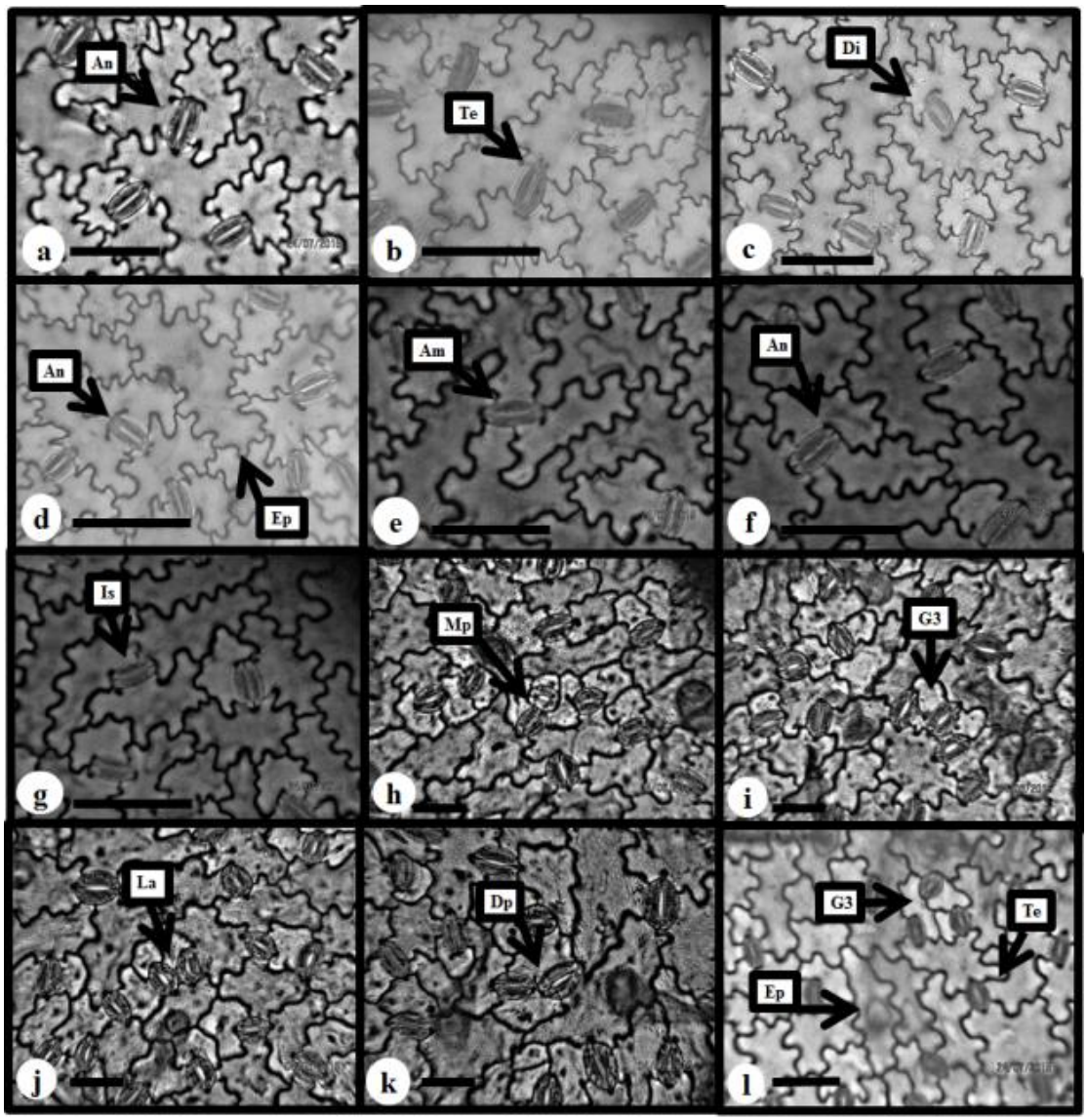

Fig. 4. Abaxial (lower) epidermal surfaces of *Emilia* species studied showing different stomata types (a-d) *E. preatamissa;* (e-g) *E. sonchifolia* and (h-l) *E. cocinea;* diacytic (Di), anisocytic (An), paracytic (Pr), isotricytic (Is), anomocytic (Am), tetracytic (Te), mono-polar contiguous (Mp), di-polar contiguous (Dp), lateral contiguous (La), group of 2 (G2), group of 3 (G3) and Epidermal cell (Ep) and bar = 130 µm

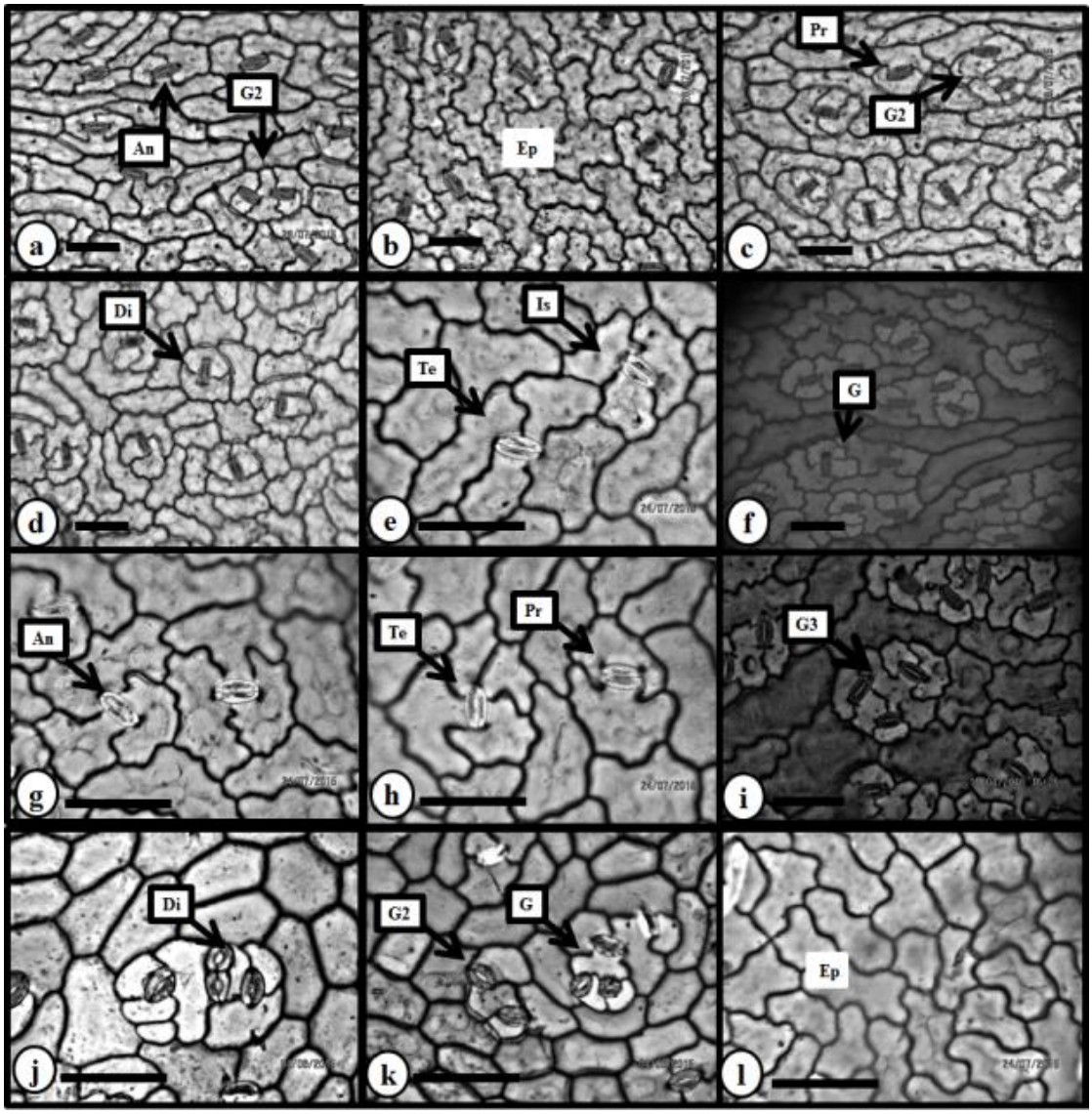

Fig. 5. Adaxial (upper) epidermal surfaces of *Emilia* species studied showing different stomata types (a-d) *E. preatamissa,* (e-i) *E. sonchifolia* and (j-l) *E. cocinea,* diacytic (Di), anisocytic (An), paracytic (Pr), isotricytic (Is), anomocytic (Am), tetracytic (Te), mono-polar contiguous (Mp), di-polar contiguous (Dp), lateral contiguous (La), group of more than 3 stomata (G), group of 2 stomata (G2), group of 3 stomata (G3), Epidermal cell (Ep) and bar = 130 µm

Table 4. Anatomical characteristics of the stem and flower stalk

S/N	Species name	Stem			Flower stalk		
		No. of VB	COL layers	Shape	No. of VB	COL layers	Shape
1	*E. preatamissa*	22	3 - 6	Oval	11	5	Oval
2	*E. sonchifolia*	18	4 - 7	Oval	8 - 10	4 -5	Oval
3	*E. cocinea*	16	4 - 8	Oval	15	5 - 6	Oval

Note: VB = Vascular bundle; COL = Collenchyma

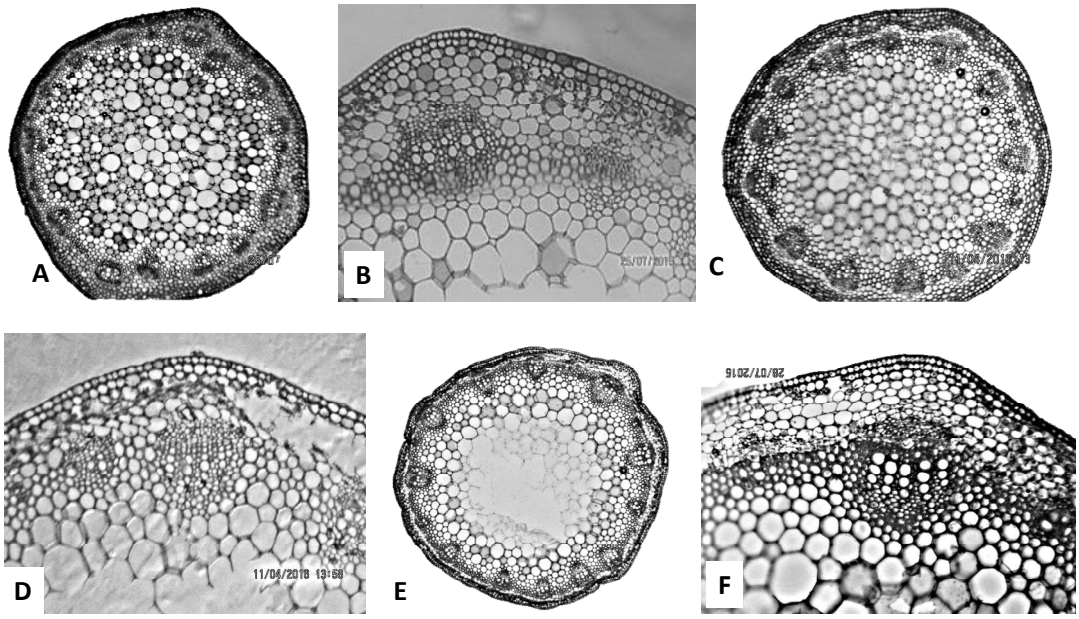

Fig. 6. Stem anatomy of *Emilia* species (A-B) *E. preatamissa*; (C-D) *E. sonchifolia* and (E-F) *E. cocinea*

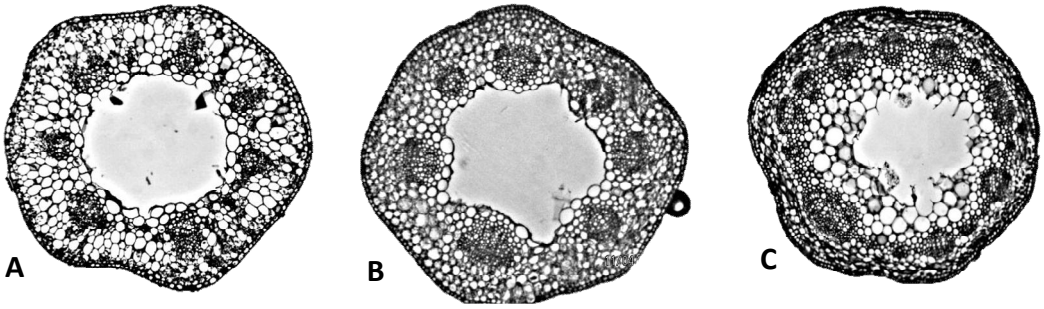

Fig. 7. Flower stalk anatomy of *Emilia* species (A) *E. preatamissa*; (B) *E. sonchifolia* and (C) *E. cocinea*

4. CONCLUSION

The study revealed that variation in number of vascular bundle in the petiole, stem, flower stalk and midrib; stomata type, occurrence (on the adaxial and abaxial leaf surfaces) and orientation (contiguous or in groups) constituted dependable diagnostic characters among the *Emilia* species and revealed intraspecific and interspecific relationships among these species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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