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LEAF EPIDERMAL STUDIES OF THE SPECIES OF *EMILIA* CASS. (*SENECIONEAE, ASTERACEAE*) IN NIGERIA

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Abstract

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A taxonomic study of *Emilia* Cass. in Nigeria was conducted in search of useful and stable anatomical characters for the identification of the species. Stomatal type and index indicates that *Emilia praetermissa* is a hybrid between *E. coccinea* and *E. sonchifolia*. Larger cell size in all studied size attributes supports *E. praetermissa* as an allotetraploid. Trichome type, stomata type, stomata shape and size attributes are all characters of *E. sonchifolia* and *E. praetermissa* that were consistently close in resemblance and values to suggest *E. sonchifolia* as the closer parent of *E. praetermissa* exerting gene dominance. This would also suggest introgression between *E. sonchifolia* and *E. praetermissa*.

Keywords: anatomical characters, stomatal type, allotetraploid, trichome, gene dominance.

INTRODUCTION

The genus *Emilia* Cass. is distributed in different parts of the world, for example in Ghana, Western Polynesia, Hawaii and Thailand (Abbiw, 1990; Whistler, 1988; Wagner et al., 1999; Nakahara et al., 2002). According to Hutchinson & Dalziel (1954–1972), three species occur in West Africa and in Nigeria. They are *E. coccinea* (Sims) G. Don, *E. sonchifolia* (L.) DC. and *E. praetermissa* Milne-Redhead. The species of the genus are regarded as weeds (Wagner et al., 1999; Whistler, 1988). They are also edible and can be used for medicinal purposes (Abbiw, 1990; Azuine, 1998).

Olorode & Olorunfemi (1973) reported a chromosome number of 2n = 10 for *E. coccinea* and *E. sonchifolia*, 2n = 20 for *E. praetermissa* from the study of the chromosome dynamics in the genus. They concluded that *E. praetermissa* is an allotetraploid hybrid between *E. sonchifolia* and *E. coccinea*. Allopolyploids are species of hybrid origin combining the karyotypes of two or more ancestral species (White, 1973).

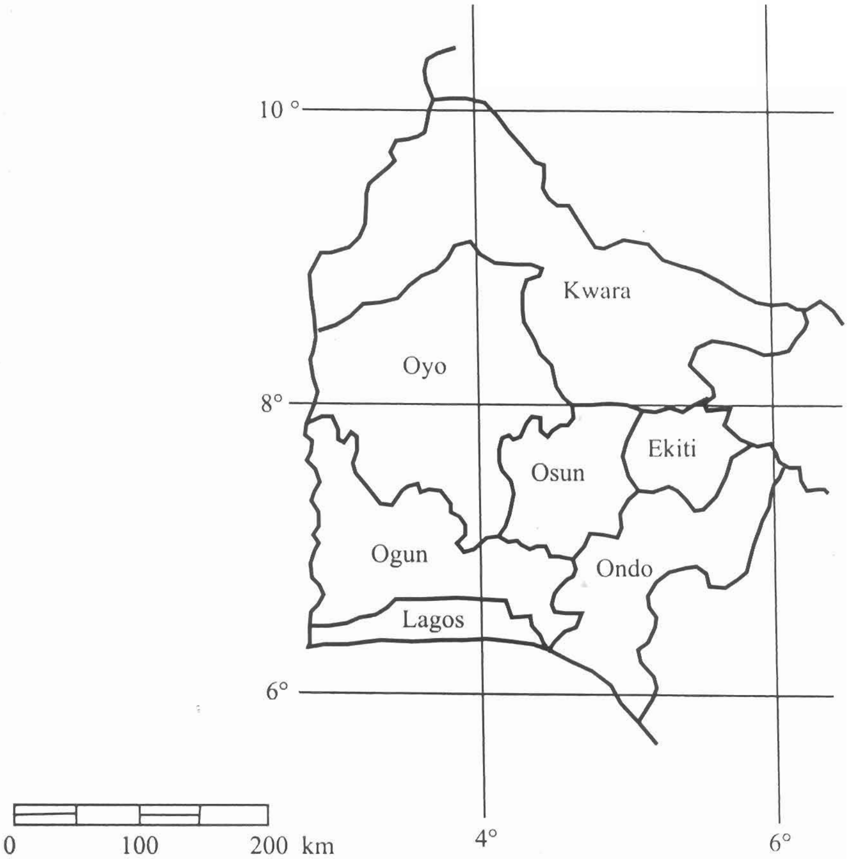

Fig. 1. Areas of collection of *Emilia* species used for the study in southwestern Nigeria

There is no published anatomical information on the genus. BASS (1978) pointed out that leaf anatomical characters deserve more attention in the analysis of naturally occurring or artificially produced hybrids. This study therefore aims to analyse the epidermal anatomy of the genus in order to establish the taxonomic and evolutionary relationships among the three species of the genus in Nigeria.

MATERIALS AND METHODS

Fresh leaf material was collected from 15–20 accessions per species from wild populations in the southwestern part of Nigeria, encompassing Osun, Oyo, Ogun, Ondo and Kwara States (Fig. 1). The leaf material was preserved in formalin-acetic acid-alcohol (FAA). Voucher specimens are deposited in the Herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Portions of the leaves were taken from the median part (midway between the tip and the base) from fifteen accessions of each species. For the purpose of studying the venation patterns, the portions were decolourised by boiling in 90 % ethyl alcohol for 15 minutes, then washed in 4–5 changes of water to remove traces of alcohol. The washed portions were transferred to 5 % sodium hydroxide and boiled for 20 minutes for further decolourisation after which they were washed thoroughly to remove the alkaline solution. The partially cleared leaves were further cleared in 5 % domestic bleach (Parozone) for about 2 hours. The leaf portions were again washed in 4–5 changes of water and stained in 1 % safranin O, rinsed thoroughly with water and temporary mounts made in 25 % glycerol.

For the purpose of studying the epidermal structures, hair types and stomata, portions of the leaves were again taken from the standard median part from fifteen accessions of each species. These were put into Jeffrey's maceration mixture (10 % chromic acid and concentrated hydrochloric acid) and kept in oven at 60 °C for about 15 minutes. Each sample was then washed thoroughly in 5 changes of water.

The adaxial and abaxial epidermes were separated by means of dissecting needle and forceps. The epidermal surfaces were stained in 1 % safranin O for about five minutes, washed with 4 changes of water to remove excess stain and then temporary mounts were made in 25 % glycerol. Stomatal index was calculated according to Dilcher (1974).

All processed materials were preserved in 50 % ethyl alcohol until when required. Illustrations were made with camera lucida fitted to M20 Wild microscope. All measurements were made with the aid of ocular micrometer and final figure obtained with ocular constant.

RESULTS

***Emilia coccinea* (Sims) G. Don.** (Fig. 2, Table 1)

Epidermal cells polygonal to irregular in shape with wavy anticlinal walls 61.60–95.20 μm long and 28.00–42.00 μm wide on the adaxial surface (Fig. 2 a), irregular with sinuous anticlinal walls 58.90–92.67 μm long and 25.20–42.00 μm wide on the abaxial surface (Fig. 2 b). Costal cells of adaxial and abaxial surfaces elongate and polygonal, occasionally rectangular, 95.20–173.60 μm long and 28.00–44.80 μm wide on the adaxial surface, 131.60–254.80 μm long and 22.40–36.40 μm wide on the abaxial surface, end walls often oblique on both surfaces (Fig. 2 c). Stomata amphistomatic, largely anisocytic, often also anomocytic, no brachyparacytic type observed (Fig. 2 a, b), circular in shape, rarely elliptic, small protrusions at the polar ends in some of the stomata, parallel contiguous stomata occasionally present on the abaxial surface (Fig. 2 d), stomatal size – adaxial 627.20–940.80 μm^2, abaxial 470.40–776.16 μm^2; stomatal index – adaxial 3.37–13.68 %, abaxial 26.20–33.40 %.

Trichomes. Only the eglandular trichomes present (Fig. 2 f–k) on the abaxial surface only, sparse in distribution, normal (Fig. 2 f–i) or shrivelled on some of the cells segments (Fig. 2 j–k) 98.00–176.40 μm long and 11.20–22.40 μm wide.

Venation. Major veins pinnate camptodromous cladodromous. Areoles polygonal to rectangular in shape, sizes variable, veinlet endings vary from 0 to 2 appearing singly to occasionally bifurcated or forked (Fig. 2 e).

***Emilia sonchifolia* (L.) DC** (Fig. 3, Table 2)

Epidermal cells polygonal to irregular in shape with straight to slightly wavy anticlinal walls 72.80–168.00 μm long and 30.80–56.00 μm wide (Fig. 3 a), irregular with sinuous anticlinal walls 72.80–170.80 μm long and 16.80–58.80 μm wide on the abaxial

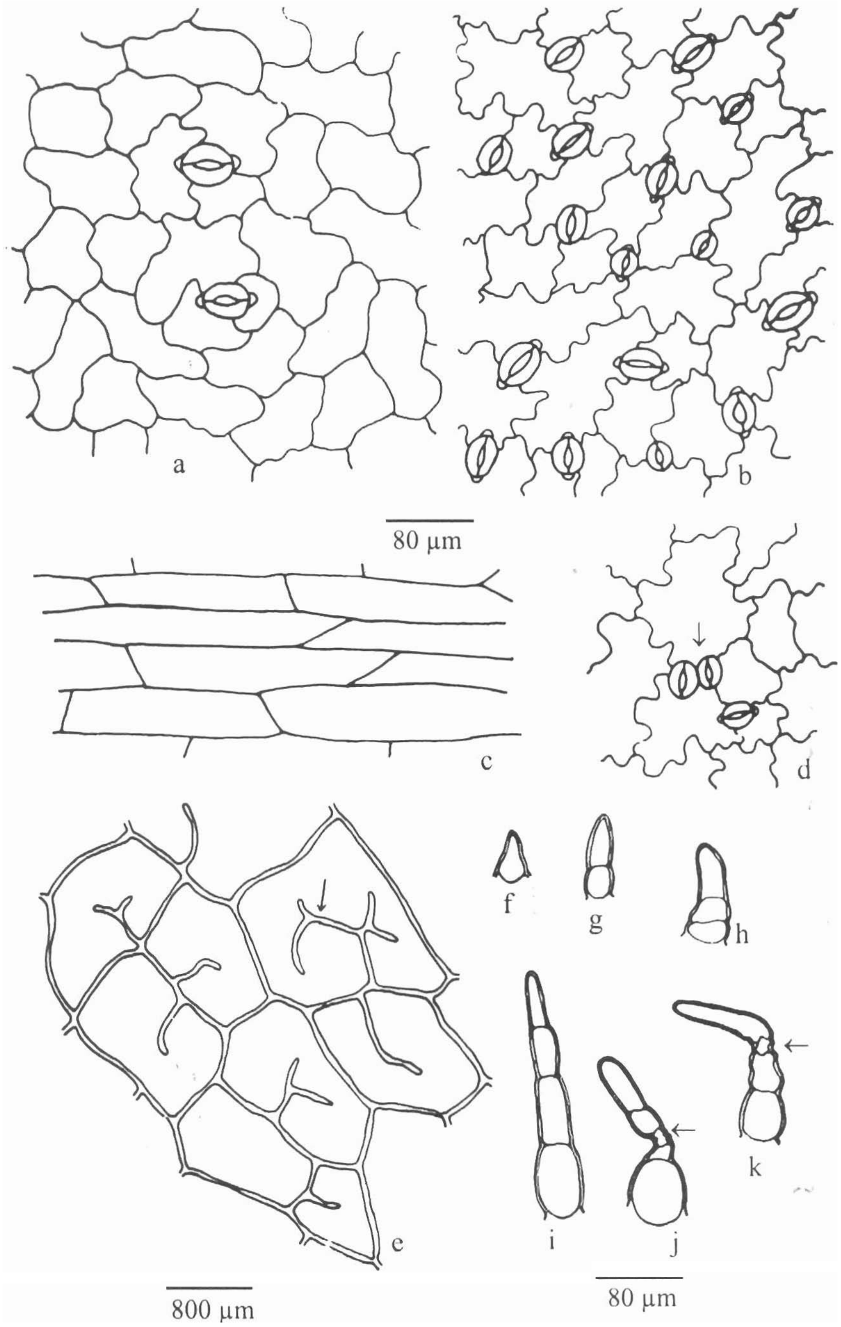

Fig. 2. *Emilia coccinea*: a – adaxial epidermis of lamina, b – abaxial epidermis of lamina, c – costal cells, d – parallel contiguous stomata (with arrow), e – venation pattern (arrow indicates veinlet ending), f–j – eglandular trichomes (f – unicellular, g – bicellular, h and i – multicellular with normal cells, j and k – multicellular with normal and shrivelled cells (arrows indicate shrivelled cells)

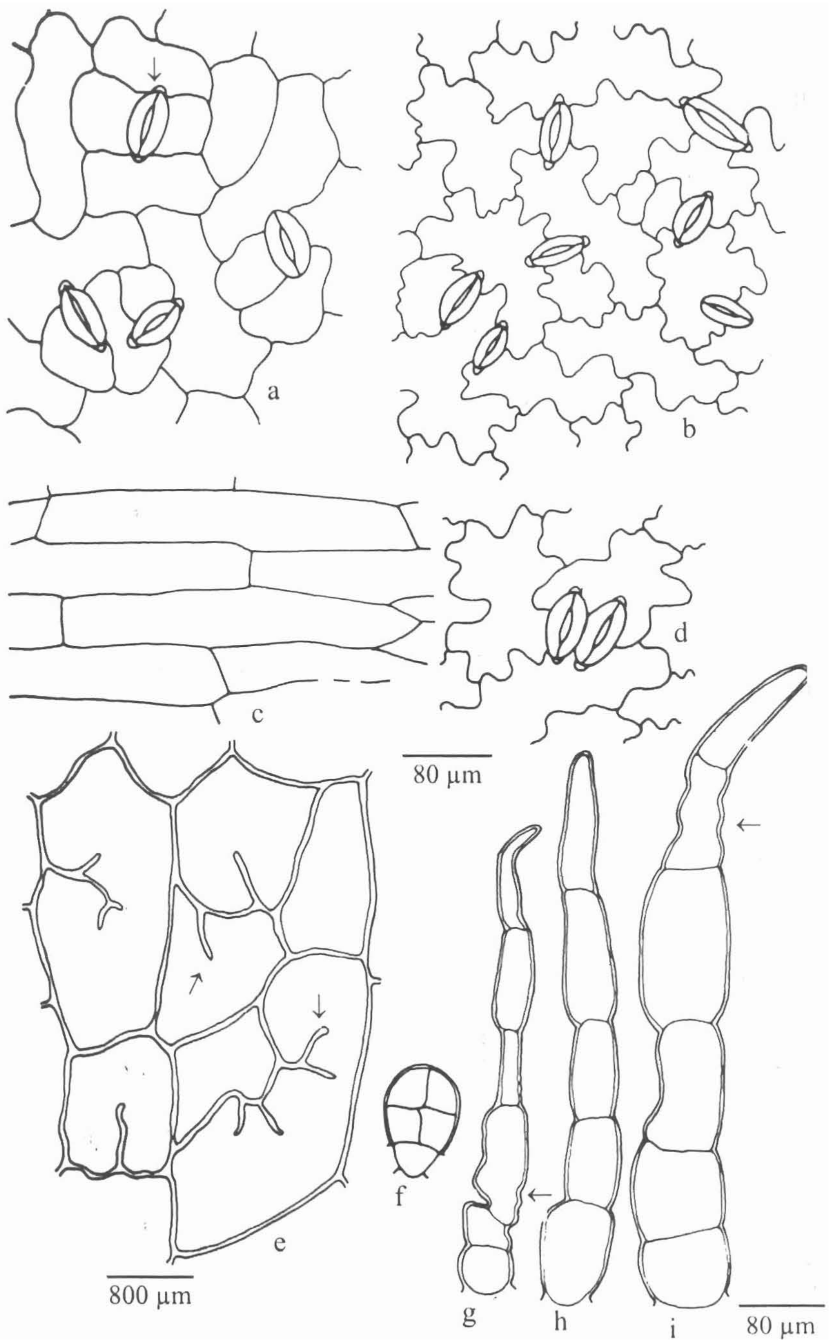

Fig. 3. *Emilia sonchifolia*: a – adaxial epidermis of lamina (arrow indicates brachyparacytic stomata), b – abaxial epidermis of lamina, c – costal cells, d – parallel contiguous stomata, e – venation pattern (arrows indicate veinlet endings), f – glandular trichome, g–i – eglandular trichomes, g and i – multicellular with normal and shrivelled cells (arrows indicate shrivelled cells), h – multicellular with normal cells

Table 1.

Simple descriptive statistics of leaf epidermal attributes of *Emilia coccinea*

Variable	Minimum		Maximum		Mean		Standard Deviation		Standard Error	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
X1	61.60	58.90	95.20	92.67	77.00	78.01	12.88	13.55	4.07	4.29
X2	28.00	25.20	42.00	42.00	35.56	33.88	6.61	6.11	2.09	1.93
X3	3.37	26.20	13.68	33.40	8.90	29.24	3.54	2.57	1.12	0.81
X4	627.20	470.40	940.80	776.16	752.64	631.90	97.64	107.19	30.88	33.90
X5	95.20	131.60	173.60	254.80	153.44	193.48	24.62	36.76	7.78	11.62
X6	28.00	22.40	44.80	36.40	33.88	28.84	5.97	4.18	1.89	1.32
X7										
X8										
X9										
X10										
X11		98.00		176.40		127.68		23.21		7.34
X12		11.20		22.40		16.80		3.49		1.10

- - absent

X1 Length of epidermal cells (µm)
X2 Width of epidermal cells (µm)
X3 Stomatal Index (%)
X4 Stomatal size (µm^2)
X5 Length of costal cells (µm)
X6 Width of costal cells (µm)
X7 Length of head, stalked glandular trichome (µm)
X8 Width of head, stalked glandular trichome (µm)
X9 Length of stalk, stalked glandular trichome (µm)
X10 Width of stalk, stalked glandular trichome (µm)
X11 Length of eglandular trichome (µm)
X12 Width of eglandular trichome (µm)

Table 2.

Simple descriptive statistics of leaf epidermal attributes of *Emilia sonchifolia*

Variable	Minimum		Maximum		Mean		Standard Deviation		Standard Error	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
X1	72.80	72.80	168.00	170.80	118.16	104.08	29.94	30.12	9.15	9.52
X2	30.80	16.80	56.00	58.80	42.56	35.28	9.22	13.01	2.92	4.12
X3	16.18	33.78	25.37	40.86	21.08	37.76	3.39	2.39	1.07	0.76
X4	820.80	658.56	1146.88	999.60	1002.82	740.10	90.49	178.85	28.61	56.56
X5	131.60	131.20	280.00	279.00	199.92	198.91	47.69	47.32	15.08	14.96
X6	19.60	19.60	36.40	30.80	31.36	25.60	4.90	3.55	1.55	1.12
X7	58.50	52.80	64.40	63.10	61.45	51.18	4.17	2.39	2.95	0.75
X8	39.20	36.90	42.00	42.05	40.60	38.61	1.98	1.75	1.40	0.55
X9	30.80	30.40	39.20	36.50	34.53	32.54	4.27	2.26	2.47	0.71
X10	28.00	24.20	36.40	35.50	33.60	29.68	4.85	3.83	2.80	1.21
X11	137.20	190.40	392.00	375.20	363.16	284.67	97.69	92.46	56.84	53.38
X12	19.60	12.60	67.20	28.80	44.24	20.41	16.10	5.64	5.09	1.77

- – absent

X1 Length of epidermal cells (μm)
X2 Width of epidermal cells (μm)
X3 Stomatal Index (%)
X4 Stomatal size (μm^2)
X5 Length of costal cells (μm)
X6 Width of costal cells (μm)
X7 Length of head, stalked glandular trichome (μm)
X8 Width of head, stalked glandular trichome (μm)
X9 Length of stalk, stalked glandular trichome (μm)
X10 Width of stalk, stalked glandular trichome (μm)
X11 Length of eglandular trichome (μm)
X12 Width of eglandular trichome (μm)

surface (Fig. 3 b). Costal cells of adaxial and abaxial surfaces elongate and polygonal, occasionally rectangular, 131.60–280.00 μm long and 19.60–36.40 μm wide on the adaxial surface, 131.20–279.00 μm long and 19.60–30.80 μm wide on the abaxial surface, end walls oblique to perpendicular on both surfaces (Fig. 3 c). Stomata amphistomatic, largely anisocytic, occasionally anomocytic and brachyparacytic (Fig. 3 a, b), often elliptic in shape, occasionally circular, small protrusions at the polar ends in some of the stomata, parallel contiguous stomata occasionally present on the abaxial surface (Fig. 3 d), stomatal size – adaxial 820.80–1146.88 μm^2, abaxial 658.56–999.60 μm^2; stomatal index – adaxial 16.18–25.37 %, abaxial 33.78–40.86 %.

Trichomes stalked glandular (Fig. 3 f) and eglandular trichomes (Fig. 3 g–i) present on both surfaces. Stalked glandular head – 58.50–64.40 μm long and 39.20–42.00 μm wide on the adaxial; 52.80–63.10 μm long and 36.90–42.05 μm wide on the abaxial surface; stalk – 30.80–39.20 μm long and 28.00–36.40 μm wide on the adaxial surface; 30.40–36.50 μm long and 24.20–35.50 μm wide on the abaxial surface; eglandular – adaxial 137.20–392.00 μm long and 19.60–67.20 μm wide, abaxial 190.40–375.20 μm long and 12.60–28.80 μm wide; glandular trichomes less frequent than eglandular trichomes, both sparse in distribution, eglandular trichomes can be normal (Fig. 3 h) or shrivelled on some of the cells segments (Fig. 3 g, i).

Venation pinnate camptodromous cladodromous. Areoles variable in size, polygonal to rectangular in shape, veinlet endings 0 to 3 diverging singly, bifurcated or forked (Fig. 3 e).

***Emilia praetermissa* Milne-Redhead** (Fig. 4, Table 3)

Epidermal cells polygonal to irregular in shape with straight to slightly wavy anticlinal walls 117.60–254.80 μm long and 30.80–75.60 μm wide on the adaxial surface (Fig. 4 a), irregular with sinuous anticlinal walls 84.00–204.40 μm long and 22.40–44.80 μm wide on the abaxial surface (Fig. 4 b). Costal cells of adaxial and abaxial surfaces elongate and polygonal, occasionally rectangular, 196.00–397.60 μm long and 30.80–56.00 μm wide on the adaxial surface, 224.00–431.20 μm long and 22.40–28.00 μm wide on the abaxial surface, end walls oblique to perpendicular on both surfaces (Fig. 4 c). Stomata amphistomatic, largely anisocytic, occasionally anomocytic or brachyparacytic (Fig. 4 a, b, e), often elliptic in shape, occasionally irregularly shaped appearing more or less malformed; small protrusions at the polar ends in some of the stomata, parallel contiguous stomata occasionally present on the abaxial surface (Fig. 4 d), stomatal size – adaxial 846.72–1724.80 μm^2, abaxial 713.44–1066.24 μm^2, stomatal index – adaxial 13.16–22.95 %, abaxial 30.77–37.18 %.

Trichomes stalked glandular present on the abaxial surface only (Fig. 4 g), eglandular present on both surfaces (Fig. 4 h–j). Stalked glandular head – 70.20–81.50 μm long and 38.80–48.20 μm wide on the adaxial, 70.50–79.40 μm long and 36.50–42.00 μm wide on the abaxial; stalk – 53.20–56.80 μm long and 40.50–42.60 μm wide on the adaxial, 55.20–56.50 μm long and 38.50–43.40 μm wide on the abaxial; eglandular – adaxial 329.60–544.00 μm long and 30.80–58.80 μm wide, abaxial 310.00–524. 20 μm long and 28.40–56.50 μm wide. The eglandular can be normal (Fig. 4 h, i) or shrivelled on some cell segments (Fig. 4 j).

Venation pinnate camptodromous cladodromous. Areoles variable in sizes, more polygonal than rectangular in shape, veinlet endings 0 to 4, diverging singly, occasionally bifurcated or forked (Fig. 4 f).

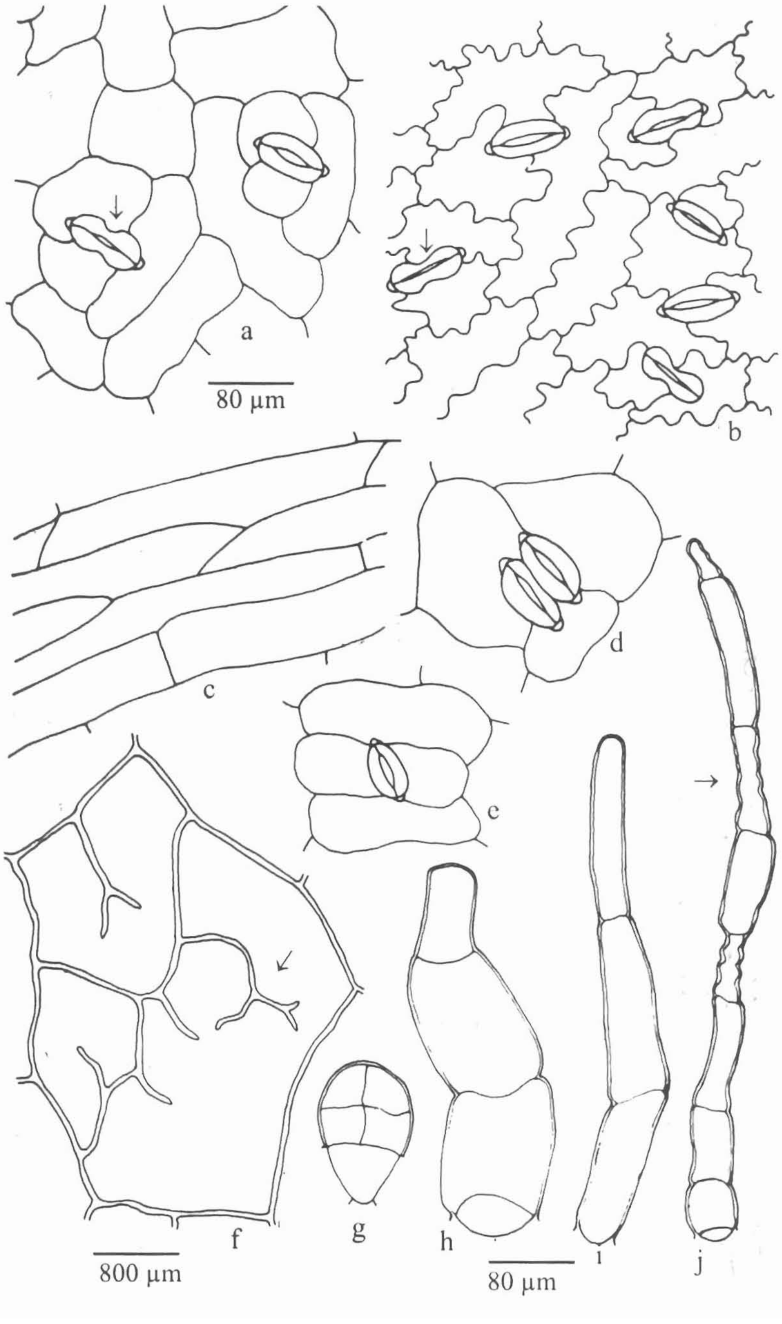

Fig. 4. *Emilia praetermissa*: a – adaxial epidermis of lamina (arrow indicates malformed stomata), b – abaxial epidermis of lamina (arrow indicates malformed stomata), c – costal cells, d – parallel contiguous stomata, e – brachyparacytic stomata, f – venation pattern (arrow indicates veinlet ending), g – glandular trichome, h–j – eglandular trichomes (h and i– multicellular with normal cells, j – multicellular with normal and shrivelled cells; arrow indicates shrivelled cell)

Table 3.

Simple descriptive statistics of leaf epidermal attributes of *Emilia praetermissa*

Variable	Minimum		Maximum		Mean		Standard Deviation		Standard Error	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
X1	117.60	84.00	254.80	204.40	206.08	132.72	54.06	38.49	17.09	12.17
X2	30.80	22.40	75.60	44.80	50.12	34.16	16.30	8.74	5.15	2.76
X3	13.16	30.77	22.95	37.18	16.82	33.72	3.07	1.87	0.97	0.59
X4	846.72	713.44	1724.80	1066.24	1401.79	854.08	243.16	155.20	76.89	49.08
X5	196.00	224.00	397.60	431.20	323.40	315.52	70.92	68.75	22.43	21.74
X6	30.80	22.40	56.00	28.00	41.72	25.76	7.40	2.57	2.34	0.81
X7	70.20	70.50	81.50	79.40	76.52	74.23	4.33	3.85	1.37	1.22
X8	38.80	36.50	48.20	42.00	41.74	39.91	3.90	2.57	1.23	0.81
X9	53.20	55.20	56.80	56.50	54.47	54.47	1.42	1.36	0.45	0.43
X10	40.50	38.50	42.60	43.40	41.13	39.83	0.90	3.69	0.28	1.17
X11	329.60	310.00	544.00	524.20	399.47	383.44	98.45	53.40	56.84	16.89
X12	30.80	28.40	58.80	56.50	47.60	39.03	14.82	7.87	8.55	2.49

-- absent

X1 Length of epidermal cells (μm)
X2 Width of epidermal cells (μm)
X3 Stomatal Index (%)
X4 Stomatal size (μm^2)
X5 Length of costal cells (μm)
X6 Width of costal cells (μm)
X7 Length of head, stalked glandular trichome (μm)
X8 Width of head, stalked glandular trichome (μm)
X9 Length of stalk, stalked glandular trichome (μm)
X10 Width of stalk, stalked glandular trichome (μm)
X11 Length of eglandular trichome (μm)
X12 Width of eglandular trichome (μm)

DISCUSSION

The data recorded in this study are sufficient for establishing the taxonomic and evolutionary relationships among the three *Emilia* species in Nigeria. Each species showed marked consistency for the anatomical characters examined.

Many authors have used leaf anatomy as a taxonomic tool. These include CHANDRA & KHARE (1980), ILLOH & INYANG (1998), ADEDEJI & FALUYI (2001). A survey of literature on leaf anatomy shows that the data obtained can be used for the clarification of taxonomic and phylogenetic relationships. The commonly used characters like venation patterns and epidermal structures (including stomata and trichome types) are largely employed in this study.

The epidermal cells are generally polygonal, occasionally irregular on the adaxial surface and generally irregular cells on the abaxial surfaces in all the studied species. The anticlinal walls are wavy to slightly wavy on the adaxial surface and sinuous on the abaxial surfaces. Costal cells are generally polygonal, occasionally rectangular with oblique to perpendicular end walls. Sizes of epidermal cells and costal cells are largest in *E. praetermissa* followed by *E. sonchifolia* and smallest in *E. coccinea*. Major veins are pinnate camptodromous cladodromous, veinlet endings diverge singly and are bifurcated or forked.

OLORODE & OLORUNFEMI (1973) established that *E. praetermissa* is an allopolyploid or tetraploid hybrid of *E. sonchifolia* and *E. coccinea*. Allopolyploids will generally exhibit a mingling of the parental characteristics (SWANSON, 1960). Stomatal type is of taxonomic value. It is largely anisocytic, occasionally anomocytic and brachyparacytic in *E. sonchifolia* while it is only anisocytic and anomocytic in *E. coccinea*. *E. praetermissa*, as a hybrid, inherited the three stomatal types with *E. sonchifolia* exerting some dominance. According to OLATUNJI (1983), stomatal index is highly constant for a certain species and can be used for species delimitation. In this study, stomatal index is the lowest in *E. coccinea* and highest in *E. sonchifolia* with *E. praetermissa* having intermediate values on both adaxial and abaxial epidermal surfaces. This suggests the hybridisation of the stomatal indices of the putative parents in which this attribute is the lowest and highest. Stomata shape is largely elliptic in *E. sonchifolia* and *E. praetermissa*, but circular in *E. coccinea*. Some irregularly shaped stomata, appearing malformed, are found occurring in *E. praetermissa*. According to SWANSON (1968), in many instances though not always, polyploidy causes changes in shape of organs as morphological or anatomical characters are the products of gene action.

The presence of a particular type of trichome can frequently delimit species, genera or even whole families (METCALFE & CHALK, 1979). The glandular trichome is present in *E. praetermissa* and *E. sonchifolia* but absent in *E. coccinea*. Eglandular trichome is present in all the three species. Unicellular and bicellular eglandular types are present in *E. coccinea* only. *E. sonchifolia* and *E. praetermissa* have largely the multicellular type. Some of the eglandular trichomes in the three species have shrivelled cells. They are longest in *E. praetermissa* and *E. sonchifolia*, shortest in *E. coccinea*. The trichomes are sparse in all the studied species.

According to SWANSON (1968), the most immediate and universal effect of polyploidy is an increase in cell size. *E. praetermissa* exhibits an increase in cell size when all the size attributes are considered with *E. sonchifolia* having closer values to it than *E. coccinea*. A consistent closeness in an array of anatomical attributes would suggest introgression between *E. sonchifolia* and *E. praetermissa*, while retrogression could be the reason why *E. coccinea* and *E. praetermissa* are less close. Sneath (1968) suggests that the phenetic position of a hybrid in relation to its parents is largely determined by gene dominance; if most characters of one parent are dominant, then the hybrid will lie close to it. This opinion was supported by White (1973). The findings in the present study support the observation of Olorode & Olorunfemi (1973) that *E. praetermissa* is the tetraploid hybrid of *E. coccinea* and *E. sonchifolia*. Furthermore, the study has revealed that out of the two parents, *E. sonchifolia* is closer to *E. praetemissa* than *E. coccinea*.

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