

Systematic significance of foliar epidermal morphology in the West African species of *Ludwigia* (*Onagraceae*)

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Abstract. A light microscopic study of the comparative foliar epidermal characteristics of the West African species of *Ludwigia* was undertaken, with a view to elucidate the systematic significance of epidermal morphology in the genus. Species diagnostic features include amphistomata in *L. abyssinica*, pubescent leaf in *L. stolonifera* and possession of peltate scale in *L. octovalvis*. Other systematically useful characters include the stomatal index. The highest index of 36.7% was recorded in *L. brenanii* and stomatal size ranges from 12.3 µm to 41.1 µm in the genus. Trichomes are restricted to *A. stolonifera* only. Constant generic features are the paracytic stomatal type, amphi-, hypo-stomatic leaf and irregular to polygonal cell shape.

Key words: leaf epidermis, *Ludwigia*, morphology, taxonomy

Introduction

Ludwigia L. belongs to the family *Onagraceae*. It has species that may be submerged or growing at the banks of streams as annual, biennial or perennial herbs, shrubs, or trees. There are about 75 species across the world which are cosmopolitan and pantropical in distribution (Raven 1963; Mabberley 1987). Of these, 10 West African species deposited in the herbarium of the Forestry Research Institute of Nigeria are hereby investigated (Hutchinson & Dalziel 1958). The species are economically useful (Burkill 1994). The leaves of *L. abyssinica* A. Rich. are edible and used for dyeing of straw and fibres, and it is medicinally applied for enhancing memory. The leaves of *L. erecta* (L.) H. Hara are edible as vegetables and used for treating fevers. *Ludwigia hyssopifolia* (G. Don) Exell is valuable as green manure and the leaves can be used to treat wounds.

Ludwigia octovalvis (Jacq.) P.H. Raven is pulped and boiled as a vermifuge and laxative and its leaf infusion is used for treatment of dysentery (Oliver

1960). It also has analgesic properties, useful in the treatment of rheumatic pain (Dalziel 1937), *L. stenorraphe* (Brenan) H. Hara has unspecified veterinary use. Other species of unreported economic use but with certain ecological significance are *L. senegalensis* (DC.) Troch., *L. brenanii* H. Hara, *L. africana* (Brenan) H. Hara, *L. decurrens* Walter, and *L. stolonifera* (Guill. & Perr.) P.H. Raven.

There are good taxonomic accounts of the diagnostic features of the genus based on exomorphological features (Hutchinson & Dalziel 1958). Similarly, distinguishing features were obtained from the cuticle, genetics and stem anatomy and cellular inclusions in leaf. The report of Metcalfe & Chalk (1950, 1979) was based on fewer species of the genus and a general family account which provides scanty anatomical data. In view of this, obviously the endomorphological characteristics of the genus in West Africa are poorly known and the present investigation was carried out so as to document for the first time those characters of systematic importance and present comparative information on the species.

Material and methods

Preserved specimens obtained from the West African richest and largest herbarium of the Forestry Research Institute of Nigeria, Ibadan (FHI) and fresh specimens collected from field trips were used for the study. Parallel with that, specimens deposited in the herbaria of the universities of Lagos (LUH), Ibadan (UCI) and Obafemi Awolowo (IFE) were compared for the purpose of authentication. Herbarium abbreviations follow Holmgren & al. (1990). The attributions of the investigated specimens are given as follows: *L. abyssinica*: coll. J. Lowe, 14.05.1972 (FHI 82058); *L. brenanii*: coll. J.B. Hall, 01.08.1968 (FHI 32571); *L. africana*: coll. J. Lowe, 19.01.1968 (FHI 26127); *L. decurrens*: coll. T.P. Ramamorthy, 27.04.1977 (FHI 99356); *L. erecta*: coll. J. Lowe, 15.02.1978, (FHI 84993); *L. hyssopifolia*: coll. A. binuyo, 22.11.1961 (FHI 45422); *L. octovalvis* subsp. *brevisepala* (Brenan) P.H. Raven, coll. Daramola, Emwighon & Odewo, 01.07.1978 (FHI 103311); *L. senegalensis*: coll. Hall & Daodu, 17.01.1970 (FHI 32656); *L. stenorrhapha* subsp. *stenorrhapha*: coll. B.O. Daramola & M.G. Latilo, 17.11.1954 (FHI 28710); *L. stolonifera*: coll. Jean Lebrum, 12.1937 (FHI 32478).

Portions of 2–5 cm² were cut from the standard median part of the leaf lamina near the mid-rib, or the whole leaf was used. Dried leaves were boiled in water for thirty minutes and subsequently soaked in either concentrated trioxonitrate (v) acid (HNO₃) [usually just called nitric acid] in capped specimen bottles for about 8–24 h to macerate the mesophyll, or irrigated in sodium hypochlorite solution (commercial bleach) for 30–120 min. to bleach the leaf portions. Tissue disintegration was indicated by bubbles and the epidermal layers were separated and transferred into Petri dishes containing water for cleansing. In case of fresh materials, the leaves were scraped with razor blade to separate epidermis. Tissue debris was cleared off the epidermis with fine-hair brush and washed in several changes of water. Drops of different grades of ethanol, 50% – 100%, were added in turn to dehydrate the cells. The preparations were later stained with Safranin O in 50% alcohol for about five min. before being mounted in glycerine on glass slides. The epidermal layers were mounted on glass slides with the uppermost surfaces facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration.

Line diagrams of epidermal structures were made with a camera lucida drawing apparatus attached to

a Zeiss Light microscope. For statistical analysis, the minimum and maximum values were calculated, while the stomatal index was determined by the formula reported by Stace (1965) as below:

$$\text{Stomatal index} = \frac{\text{Stomatal number per unit area}}{\text{Cell number} + \text{Stomatal number per unit area}} \times 100$$

Results

The following account confirms the systematic relevance of the foliar epidermal features in ten different species of the West African *Ludwigia*. Both qualitative and quantitative data presented in Tables 1 and 2 elucidate the generic characteristics and species' affinities. The leaf is hypostomatic in all species, except for in *A. abyssinica* (Fig. 1a, b), but paracytic stomata are generally present in all species. The cell geometry varies from irregular to polygonal. It may be similar on both surfaces – irregular in *L. abyssinica*, *L. africana*, *L. erecta*, *L. hyssopifolia*, *L. senegalensis*, and *L. stenorrhapha*, and polygonal in *L. brenanii*, or dissimilar on either surface; polygonal on the adaxial surface and irregular on the abaxial surface in *L. decurrens*, *L. octovalvis* and *L. stolonifera*. The anticlinal wall pattern varies from sinuous-undulate to straight and curved in the genus. It is straight in *L. brenanii* and undulate in *L. africana* and *L. senegalensis* on both surfaces. However, the pattern differs on either leaf surface in the other species (Figs 1–4, Table 1). Crystals of calcium oxalate exist as the only cell inclusion in three species, namely: *L. octovalvis*, *L. senegalensis* and *L. stolonifera*. Indumentum may exist as visible unicellular, tip-bent and glandular trichomes, as recorded in *L. stolonifera* on the abaxial surface (Fig. 4C - E), or as trichome scars occurring on the adaxial surface in *L. brenanii*, *L. decurrens* and *L. erecta* (Figs 1C, 2A, C). Peltate scales are present only in *L. octovalvis* (Fig. 3A). Although the quantitative values overlap, which confirms the affinities among the species, they have also proved promising for taxa delimitation in the genus. The cell wall is thickest in *L. decurrens* (1.8–6.1 μm) and thinnest (0.9–1.8 μm) in *L. senegalensis*, especially on both surfaces of the leaf (Table 2). The presence of stomata on the upper surface of *L. abyssinica* is interesting and diagnostic. The presence of the smallest stomatal index of 8.7% on the abaxial surface distinguishes *L. senegalensis* from the other species. The highest index (36.7%) was recorded in *L. brenanii*, while *L. decurrens* has the

highest stomatal size of $41.1 \mu\text{m} \times 39.4 \mu\text{m}$. The smallest stomatal size of $12.3 \mu\text{m} \times 12.3 \mu\text{m}$ was recorded in *L. stenoraphe* (Table 2). In *L. stenoraphe* and *L. stolonifera*, the stomatal shape is almost oval, because the

length and width are uniform (Table 2). On the abaxial surface, where stomata are copious and common, the maximum number varies from 10 in *L. senegalensis* to 77 in *L. brenanii*.

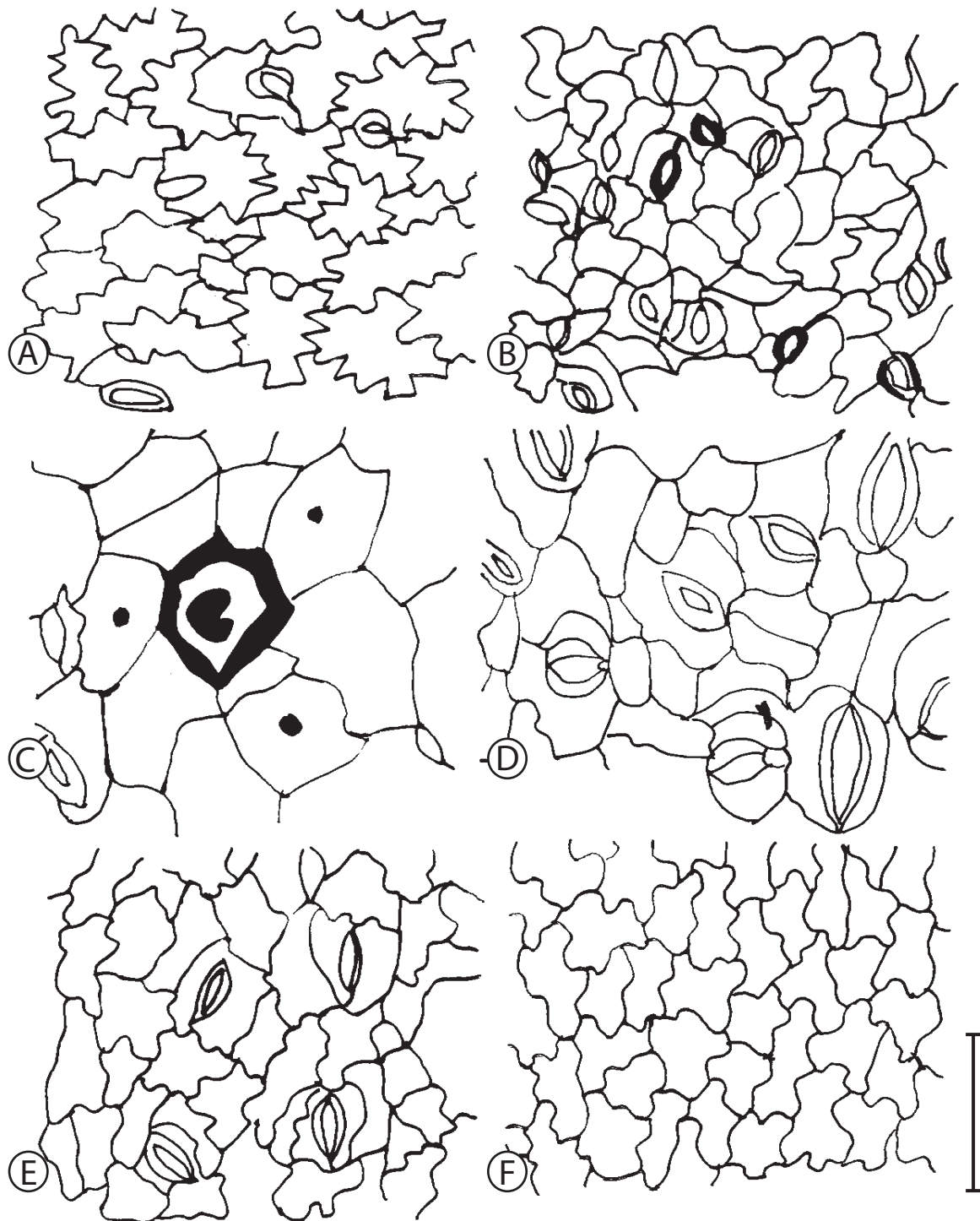


Fig. 1. Epidermal features of the species of *Ludwigia*: A, B – *L. abyssinica* (with trichome base on the adaxial surface); C, D – *L. brenanii*; E, F – *L. africana*; A, C, E – adaxial surface; B, D, F – abaxial surface. Scale bar = $50 \mu\text{m}$.

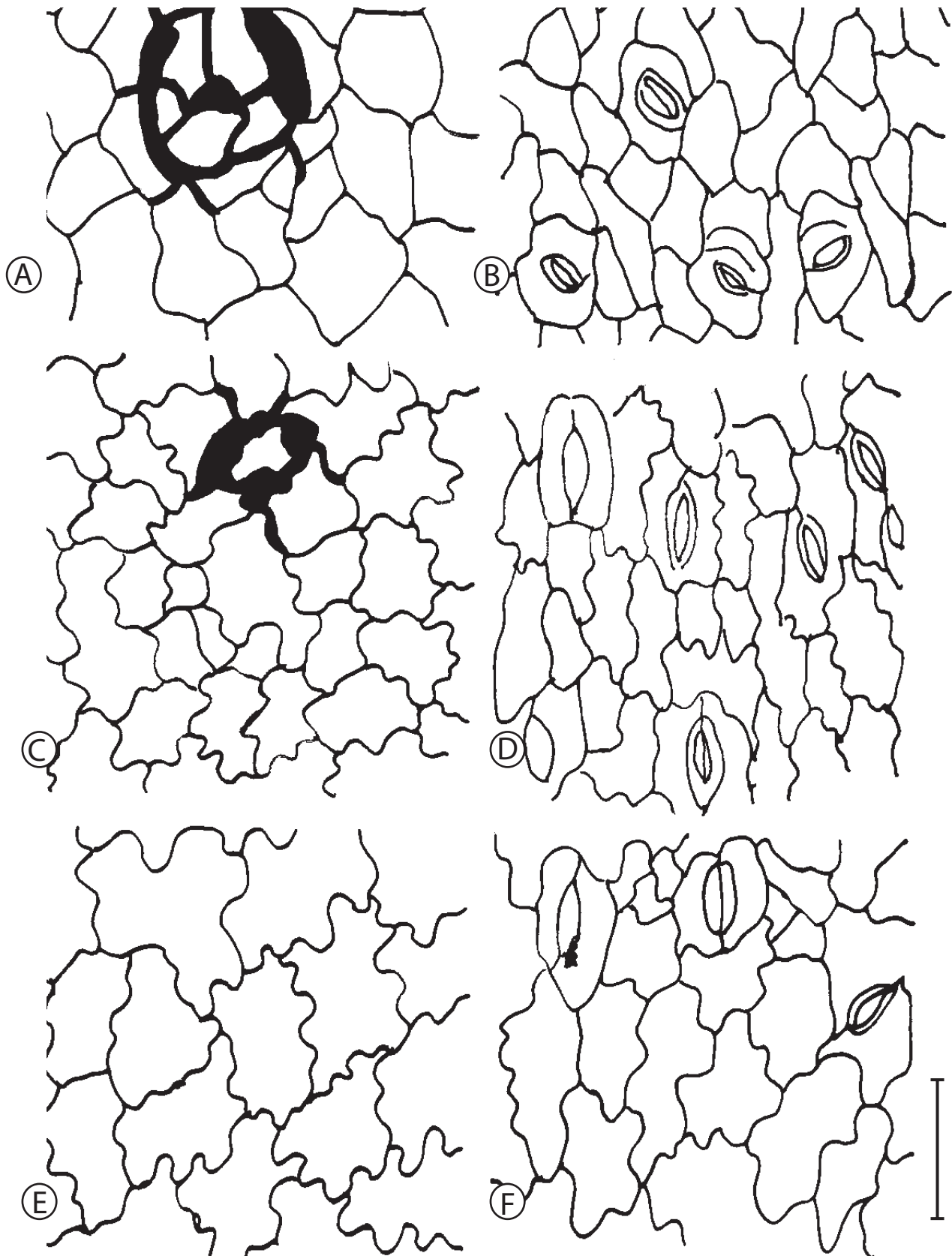


Fig. 2. Epidermal features of the species of *Ludwigia*: A, B - *L. decurrens* (with trichome base on the adaxial surface); C, D - *L. erecta* (with trichome base on the adaxial surface); E, F - *L. hyssopifolia*; A, C, E - adaxial surface; B, D, F - abaxial surface. Scale bar = 50 μ m.

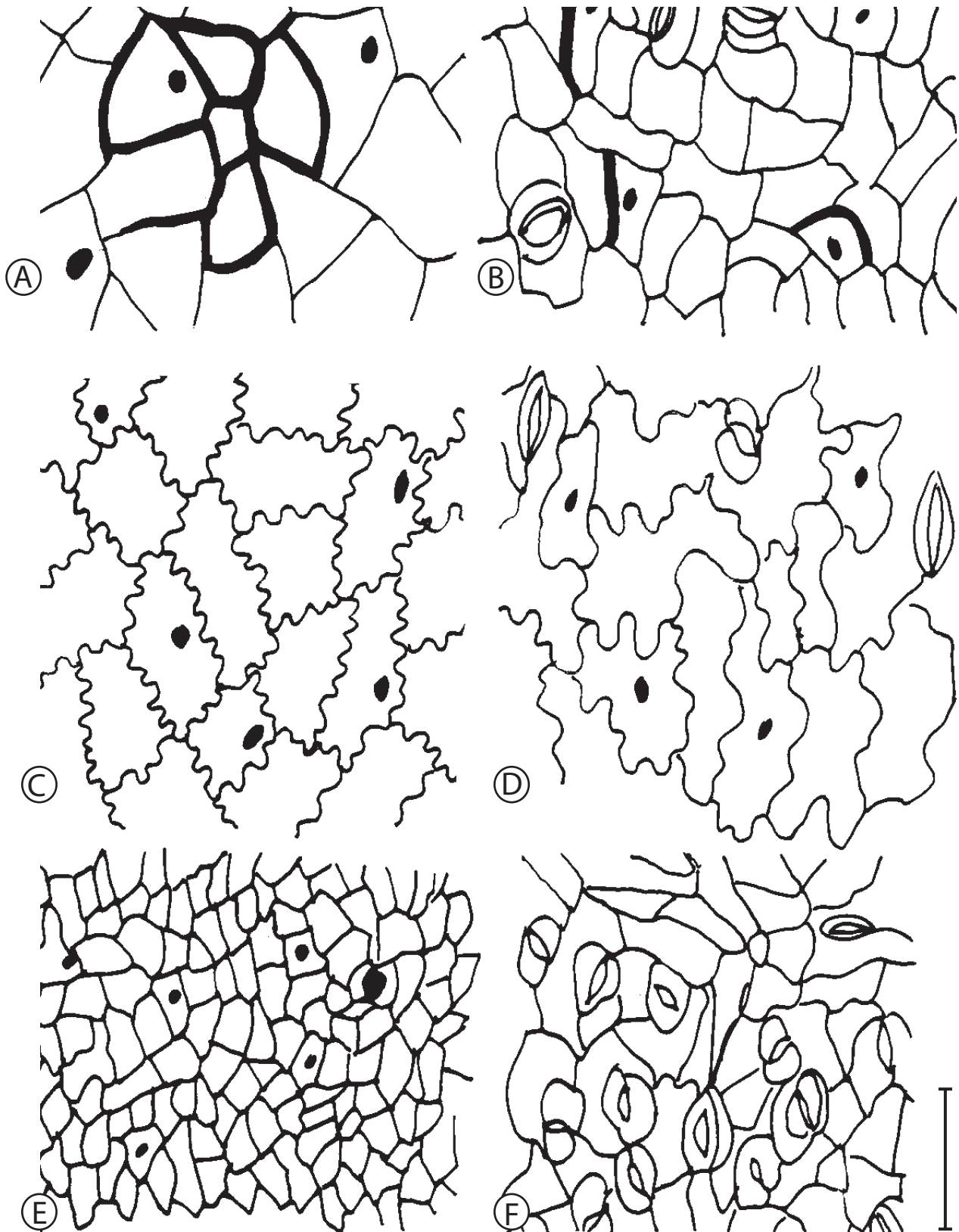


Fig. 3. Epidermal features of the species of *Ludwigia*: A, B – *L. octovalvis* (bearing scale on the surface on the adaxial surface); C, D – *L. senegalensis*; E, F – *L. stenorrhapha*; A, C, E – adaxial surface; B, D, F – abaxial surface. Scale bar = 50 μm .

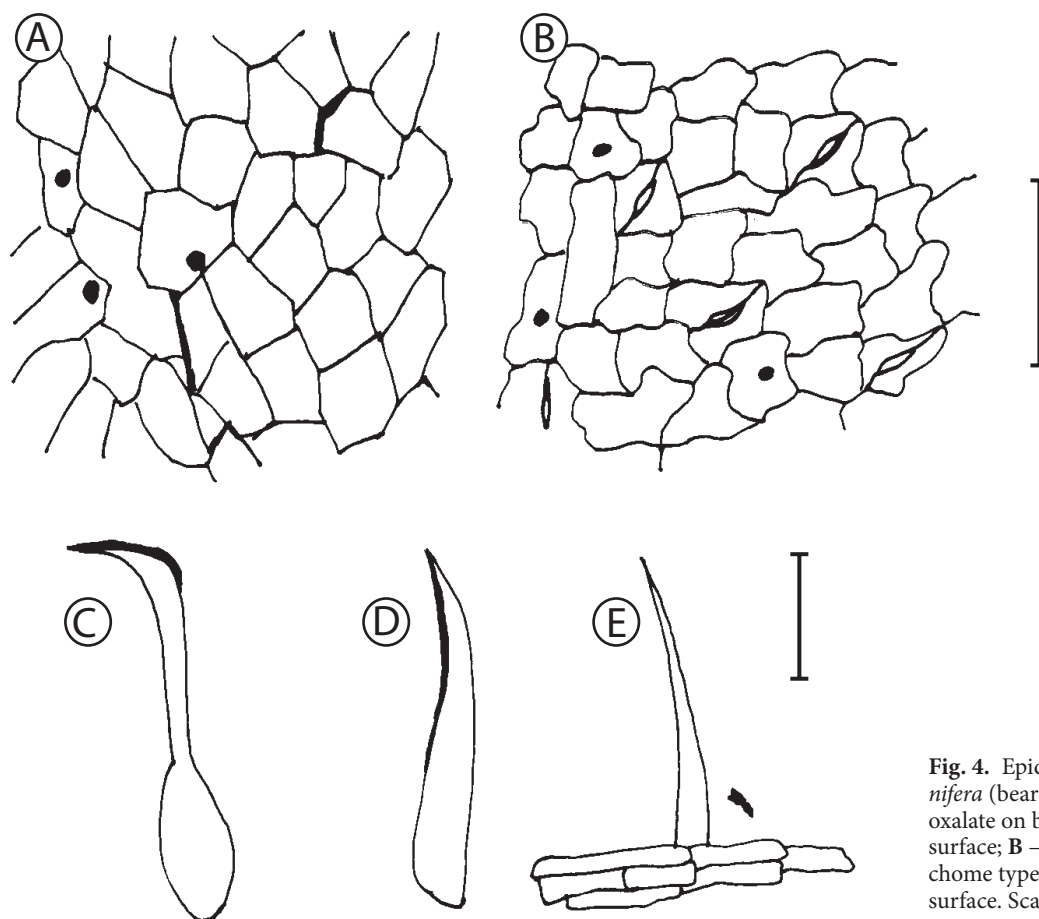


Fig. 4. Epidermal features of *L. stolonifera* (bearing crystals of calcium oxalate on both surfaces): **A** – adaxial surface; **B** – abaxial surface; **C-E** – trichome types recorded on the abaxial surface. Scale bar = 50 μ m.

Table 1. Comparative qualitative foliar epidermal features of West African species of *Ludwigia*.

Species	Stomatal type	Cell geometry	Anticlinal wall pattern	Cell inclusion	Trichome	Trichome base	Scale
<i>L. abyssinica</i>	Paracytic	Irregular	Sinuuous	-	-	-	-
	Paracytic	Irregular	Undulate	-	-	-	-
<i>L. brenanii</i>	-	Polygonal	Straight	-	-	+	-
	Paracytic	Polygonal	Straight	-	-	-	-
<i>L. africana</i>	-	Irregular	Undulate	-	-	-	-
	Paracytic	Irregular	Undulate	-	-	-	-
<i>L. decurrens</i>	-	Polygonal	Straight	-	-	+	-
	Paracytic	Irregular	Curved	-	-	-	-
<i>L. erecta.</i>	-	Irregular	Sinuuous	-	-	+	-
	Paracytic	Irregular	Undulate	-	-	-	-
<i>L. hyssopifolia</i>	-	Irregular	Sinuuous	-	-	-	-
	Paracytic	Irregular	Undulate	-	-	-	-
<i>L. octovalvis</i>	-	Polygonal	Straight	+	-	-	+
	Paracytic	Irregular	Curved	+	-	-	-
<i>L. senegalensis</i>	-	Irregular	Undulate	+	-	-	-
	Paracytic	Irregular	Undulate	+	-	-	-
<i>L. stenorrhapha</i>	-	Irregular	Curved	-	-	-	-
	Paracytic	Irregular	Curved	-	-	-	-
<i>L. stolonifera</i>	-	Polygonal	Straight	+	-	-	-
	Paracytic	Irregular	Curved	+	unicellular	-	-

Features are stated adaxial / abaxial. + = present; - = absent.

Table 2. Comparative quantitative foliar epidermal features of West African species of *Ludwigia*.

Species	Stomatal length	Stomatal width	Stomatal number	Stomatal index	Epidremal cell length	Epidermal cell width	Cell wall thickness
	Min – Max	Min – Max	Min – Max		Min – Max	Min – Max	Min – Max
<i>L. abyssinica</i>	11.4–19.3	9.6–20.1	0.0–5.0	1.1 %	11.4–32.4	17.5–38.5	0.9–2.0
	14.9–26.3	9.6–33.3	32.0–37.0	27.5 %	15.8–70.9	6.1–31.5	0.9–3.5
<i>L. brenanii</i>	–	–	–	–	10.5–35.9	12.5–38.5	0.8–4.4
	9.6–20.1	13.1–34.1	68.0–77.0	36.7 %	8.8–68.3	5.3–31.5	0.9–2.6
<i>L. africana</i>	–	–	–	–	18.4–41.1	21.0–38.5	1.8–5.3
	7.9–26.3	1.3–28.0	22.0–25.0	21.4 %	17.5–49.9	17.5–29.8	1.8–5.3
<i>L. decurrens</i>	–	–	–	–	14.0–37.6	9.6–31.5	0.9–6.1
	9.6–41.1	11.4–39.4	9.0–15.0	16.8 %	16.6–43.8	16.6–54.3	1.8–6.1
<i>L. erecta</i>	–	–	–	–	8.8–31.5	9.6–31.5	1.8–2.6
	16.6–33.3	16.6–31.5	24.0–29.0	19.7 %	8.8–43.8	7.0–26.3	0.9–2.6
<i>L. hyssopifolia</i>	–	–	–	–	10.5–40.3	11.4–31.5	0.9–2.6
	8.8–30.6	11.4–31.5	21.0–24.0	19 %	16.6–50.8	33.3–45.5	0.9–2.6
<i>L. octovalvis</i>	–	–	–	–	7.0–30.6	13.1–31.5	0.9–2.6
	12.3–22.8	8.8–21.0	23.0–31.0	19 %	13.1–35.0	13.1–37.6	0.9–1.8
<i>L. senegalensis</i>	–	–	–	–	8.8–30.6	12.3–28.9	0.9–1.8
	6.1–12.3	7.0–14.0	9.0–10.0	8.7 %	21.0–29.8	19.3–37.6	0.9–1.8
<i>L. stenorrhapha</i>	–	–	–	–	5.3–21.0	5.3–19.3	0.9–2.6
	5.3–12.3	5.3–12.3	39.0–44.0	22.6 %	8.8–26.3	5.3–23.6	0.9–1.8
<i>L. stolonifera</i>	–	–	–	–	14.9–56.9	13.1–58.6	1.8–3.5
	14.0–30.6	10.5–30.6	14.0–20.0	13.2 %	15.8–38.5	14.5–61.3	1.8–3.5

All measurements are in microns and value ranges are presented except stomatal index.

Values are presented adaxial / abaxial. + = present; – = absent.

Discussion

The taxonomic value of epidermal features is well documented in literature, where they have been used to delimit a number of plant taxa (Kadiri & Ayodele 2003; Kotresha & Seetharam 2000; Ogundipe & Olatunji 1991; Olowokudejo & Nyananyo 1990; Rejdali 1991; Olowokudejo 1993; Singh & Dube 1993; Ogundipe & Wujek 2004). The anatomical characteristics of *Ludwigia* are sparingly mentioned in the general account of Metcalfe & Chalk (1950, 1979) and earlier reports on the foliar epidermal morphology of the genus elsewhere in West Africa are lacking. Paracytic stomata found in the present work have already been reported in the family (Metcalfe & Chalk 1950, 1979). Other taxonomically useful features recorded in the present account of the genus include stomatal distribution which is usually hypostomatic, or occasionally amphistomatic, as recorded in *L. abyssinica*; anticlinal wall which is either curved or straight; and epidermal cell shapes that are isodiametric, polygonal and irregular (Table 1; Figs 1–4). Stace (1965) has documented systematic relevance of the stomatal index. The

stomatal index has been found useful in diagnosis of some of the species; generally, it ranges from 8.7 % to 36.7 % in the genus. Cell wall thickness seems to reflect closeness and appears useful for distinguishing the species. The wall is either uniform in thickness on both surfaces of the leaf of *L. africana*, *L. hyssopifolia*, *L. senegalensis*, and *L. stolonifera*, or different in thickness on either surface of the leaves of other species. Inclusions of crystals of calcium oxalate (Figs 3A, C & 4A) have distinguished *L. octovalvis*, *L. senegalensis* and *L. stolonifera* from the other species. The role of epidermal appendages, such as trichomes, trichome bases, scales and cell inclusions in systematic work has been reported by Metcalfe & Chalk (1950, 1979) and other researchers. *L. stolonifera* is the only pubescent species in the genus and the presence of peltate scales also distinguishes *L. octovalvis* from the other species. The foregoing analysis and the presented illustrations make it apparent that some of the epidermal features are constant for the genus, while others are distinctive of the species. Based on these features, an artificial indented dichotomous key is presented hereunder to delimit the species.

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An artificial indented dichotomous key to ten West African species of *Ludwigia*

1. Trichomes absent, epidermal cell width under 60.0 µm
 2. Leaves amphistomatic, epidermal cell length up to 70.0 µm *L. abyssinica*
 - 2*. Leaves hypostomatic, epidermal cell length under 70.0 µm
 3. Stomatal index higher than 30 %, cell geometry polygonal on both surfaces *L. brenanii*
 - 3*. Stomatal index lower than 30 %, cell geometry irregular on both surfaces *L. africana*
 4. Crystals of calcium oxalate present on both surfaces *L. senegalensis*
 - 4*. Crystals of calcium oxalate absent on both surfaces *L. hyssopifolia*
 5. Peltate scales absent, stomatal index 22.6 *L. stenorrhaphae*
 - 5*. Peltate scales present, stomatal index 19 *L. octovalvis*
 6. Stomatal number greater than 20, anticlinal wall sinuous and undulate on adaxial and abaxial surfaces respectively *L. erecta*
 - 6*. Stomatal number under 20, anticlinal wall straight and curved on adaxial and abaxial surfaces respectively *L. decurrens*
- 1*. Trichomes present, epidermal cell width up to 60.0 µm *L. stolonifera*

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