

## Taxonomic relationships in *Lagenaria* Seringe (Cucurbitaceae) based on foliar epidermal morphology

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**Abstract:** The genus possesses a suite of leaf epidermal characteristics which reflect the affinities among the species. The most generic constant features are anomocytic stomatal type and undulate anticlinal wall pattern on the abaxial surface. Whereas the species distinguishing features include paracytic stomata which were recorded only in *L. breviflora*, hypostomatic leaf distinguishes *L. abyssinica* and *L. rufa* from other species that are amphistomatic, uniform epidermal cell shape on both surfaces of the leaf differentiates *L. guineensis* and *L. siceraria* from other species that have dissimilar patterns on the surfaces. Other features are possession of globular head trichome in *L. siceraria*, imperfect conical trichome in *L. abyssinica* and bicellular glandular conical trichome in *L. sphaerica*. Quantitatively, epidermal cells number generally varies from 28/mm<sup>2</sup> in *L. vulgaris* to 112/mm<sup>2</sup> in *L. breviflora* on the adaxial and abaxial surfaces respectively while stomatal number varies from 2-20/mm<sup>2</sup> and stomatal index is between 2.2 – 34.9 % on the adaxial surface and 8.2 – 20.8 % on the abaxial surface. Based on these features, two main groups and subgroups are proposed based on neighbour joining cluster derived from Euclidean similarity measure. Also, an indented dichotomous key is presented for species delimitation.

**Keywords:** leaf epidermis, light microscopy, taxonomy, *Lagenaria*, UPGMA dendrogram.

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## Introduction

*Lagenaria* Ser. is a genus in the family Cucurbitaceae, sub family Cucurbitoideae and tribe Benincaseae (BENTHAM & HOOKER 1963; HEYWOOD 1978; HUTCHINSON & DALZIEL 1958; JEFFERY 1967, 1980, 1990, 2005). It is pantropical in distribution and comprises eight species out of which one is poorly known. The genus was initially called *Cucurbita* (TEPPNER 2004). THORNEFORT (1700) first separated all specimens regarded as *Cucurbita* L. into two groups based on a combination of floral, fruit and seed characters, later LINNE (1753) supported the grouping and SERINGE (1825) recognized *Lagenaria* as being distinct from *Cucurbita*. The two genera are now placed in different tribe based on molecular, morphological and anatomical data (SCHAEFER & RENNER 2011). Other documented synonyms of the genus are *Adenopus* and *Sphaerosicyos* (JEFFERY 1967, 1980, 1990). Groups have been identified in *Cucurbita* based on seed coat anatomy (TEPPNER, 2004), but there is no report on infra specific grouping in *Lagenaria*. For the genus, the literature on exomorphological description are many (HUTCHINSON & DALZIEL 1958; JEFFERY 1967, 1980, 1990; ZHENGYI et al. 2004 and others); but documented information on leaf anatomy is scarce. The importance of leaf epidermal morphology for plant classification has been recognized and studied by many authors (BARANOVA 1992; METCALFE & CHALK 1950, 1979; YANG & LIN 2005). Therefore, a study of the foliar epidermis of the species of *Lagenaria* was undertaken with a tripartite view to discuss species identity, present inter specific relationships and to propose possible infra specific groups based on affinities in the studied epidermal features. BURKHILL (1985); ELUJOBA et al. (1985); TEPPNER (2004) have reported the long history uses of the species.

## Material and methods

The representative specimens deposited in the herbaria of Forestry Research Institute of Nigeria (FRI), University of Lagos Herbarium (LUH) and the Natural History Museum, London (BM) were collected with permission and investigated in the Department of Botany, University of Lagos, Nigeria. The provenances of the specimens are presented in Tab. 1. Herbarium abbreviations follow HOLMGREN et al. (1981) and the adopted method follows KADIRI (2003) and OLOWOKUDEJO (1991). For the study, 2-3 cm<sup>2</sup> portions were cut from the standard median portion of the leaf lamina near the mid-rib. They were revived by boiling in water for thirty minutes and they were either soaked in concentrated trioxonitrate (v) acid (HNO<sub>3</sub>) in capped specimen bottles for about 3-4 hours to macerate the mesophyll or irrigated in Sodium hypochlorite solution (commercial bleach) for 30–120 minutes to bleach the leaf portions. Tissue disintegration was indicated by bubbles and the epidermis were transferred into Petri dishes containing water for cleansing and then separated with forceps and mounting needle. 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 minutes before mounting in

glycerine on the glass slide. The epidermis were mounted on glass slide with the uppermost surfaces facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration. Slides were examined under light microscope at x100, x400 and photomicrographs were taken digitally using Toupview (x86) photomicroscopic camera attached to a Pentium IV computer. Quantitative and qualitative characters of the leaf epidermis were assessed and stomatal index was calculated using the formula as reported by STACE (1965):

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

Trichome description follows RADFORD et al. (1974). Twenty five epidermal cells and stomata were randomly selected for measurement. Fifteen character states from the leaf epidermis were selected for multivariate analysis using Euclidean similarity measure (HAMMER et al. 2001) and a UPGMA dendrogram generated reflect the species relationships.

## Results

The results are summarized in Figs. 1-3, Tabs. 2 and 3. The species have many foliar epidermal characters in common; though, there are some features that are distinguishing and useful for species delimitation. Anomocytic stomatal type is common in the genus but paracytic type was recorded only in *L. breviflora*. All the species are amphistomatic except *L. abyssinica* and *L. rufa* that are hypostomatic (Fig. 1A, B; 2K, L; Tab. 2). The epidermal cell shape is polygonal on the adaxial surface and irregular on the abaxial surface in all species except *L. guineensis* and *L. siceraria* that have irregular shape on both surfaces (Figs. 1 and 2, Tabs. 2 and 3). Anticlinal wall pattern is undulate in all species on the abaxial surface except *L. sphaerica* that has curved pattern. Conversely, the adaxial surface is varied from straight or curved to straight-curved among the species (Figs. 1 and 2; Tabs. 2 and 3). The leaf may be glabrous or pubescent. Four different types were encountered namely: multicellular conical trichomes in *L. abyssinica*, *L. siceraria* and *L. vulgaris* (Fig. 1A-C, F, I, and 2C, Tab. 2), globular head trichome in *L. siceraria* (Fig. 1F); imperfect conical trichome in *L. abyssinica* (Fig. 1I) and bicellular glandular conical trichome found only in *L. sphaerica* (Fig. 2C). These trichomes are either restricted to one surface or both surfaces of the leaf (Figs. 1 and 2). Scars left behind as a result of fallen trichomes have been described as trichome bases; which are either glandular as in *L. breviflora*, *L. sphaerica* and *L. vulgaris* while non-glandular forms were recorded in *L. sp.* *L. rufa* *L. guineensis* and *L. siceraria* (Figs. 1H, 2A, B, D-F). Highest number of epidermal cells, 112 was recorded on the abaxial surface of *L. breviflora* while lowest number, 28 was recorded on the adaxial surface of *L. vulgaris* (Tab. 3). Generally, cell size varies from 15.0(28.3)34.0µm x 13.0(21.0)28.0µm to 26.0(29.6)38.0µm x 15.5(20.3)25.0µm on the adaxial surface in *L. breviflora* and *L. rufa* respectively. But on the abaxial surface, stomatal size ranges from 12.8(15.2)18.5µm x 11.4(12.0)14.0 µm in *L. abyssinica* to 18.0(19.1)22.0µm x 12.0(14.0)15.5µm in *L. sp. A* (Tab. 3). Mean

Tab. 1. Provenances of *Lagenaria* species investigated

SN	Species	Description	Accession number	Collectors	Locality	Region	Date of Collection
1.	<i>L. siceraria</i> (Molina) Standley	Growing among weeds Flowers white	BM 3120	O. Polunin, W. R. Sykes, & L.H. J. Williams	Junmla, Nepal	Asia, India	2. ix. 1952
2	„	Growing over shrubs, flowers white	„ 5831	O. Polunin, W. R. Sykes, & L.H. J. Williams	Jitachati, Jorda, JChola	Asia, India	21. x. 1952
3.	„	Trailer, flowers white	„ 1153	L.H. J. Williams	D. Lamburta	Asia, India	6. vii. 1969
4.	„	-	FHI 91039	T. K. Odewo	Nigeria	Africa	18. viii. 1977
5	„	Trailing vine, male flower white, female flower whitish	BM 14969	R. A. Howard, G. R. Proctor	Up to 25 km south west Kendal, in pasture	Central America, West Indies.	10. i. 1958
5.	„	Flor. Blanca, frutos lageniformes. Cultivada	BM 1024	D. Shutton, R. Hampshire & A. Reyes. G.	Approx. 8km al SO de Jaltenango, camino, Prusia	Central America, Mexico	13. xi. 1989
6.	„	Bejuco vigoroso con flor blanca, fruto discoidal de 30cm de diametro verde palido, Cultivada.	BM 938	D. Shutton, R. Hampshire J. C. Soto & A. Reyes. G.	Desviaccio a lagunas da Montebello, 12km. al SW de Comitán	Central America, Mexico	26. x. 1989
7.	„	Plantae herbacea, trepadora, flora blanca, escasa	BM 788	Ventura E Lopez	Matorra, terreno plano	Central America, Mexico	28. xi. 1984
8.	„	A trailer with broad leaves and coiled tendrils	BM 86784	R. Dale Thomas	Sandbar along West bank of Mississippi River	North America	8. xi. 1983
9	<i>L. abyssinica</i> (Hk. F.) Jeffrey	A trailer with coiled tendrils	„ 1843	G. Taylor	Virunga, Ntns. Kanaba Gap.	Africa, Uganda	19. xi. 1934
10.	„	Trailing herb, flowers with white petals, fruit globular, up to 10cm diameter, green with yellowish green spots	„ 887	J. K. Jackson	Gilo, near Ngairigi River	Africa, Anglo Egyptian Sudan	9. xi. 1949

Tab. 1. – cont.

SN	Species	Description	Accession number	Collectors	Locality	Region	Date of Collection
11	<i>L. vulgaris</i>	-	LUH	J.O. Opayemi	Nigeria	Africa	2. vi. 1982
12	„	General everywhere, handsome creeper, cream flowers, opening in evening turning brown in sun	BM -	M. T. Traill	Kipkabus	Africa, Kenya	-
13	„	Climber, up to 300cmhigh, flowers white with green veins	BM 1181	F. White	Native Reserve Embu district, near D. C.'s Camp, Keruogia	Africa, Kenya	18. viii. 1949
14	<i>L. brevipflora</i> (Benth.) G. Roberty	A climber of about 5m long, flower white, fruit green ovoid with many white patches	LUH 1439	A. B. Kadiri	Adeleye Town, Iwo-Ibadan Road, Oyo State, Nigeria	Africa, Nigeria	10. viii. 2007
15	„	Male flower white	BM 19	T. C. Wrigley	Pathside, Annobon, Anbo	Africa, Annonbon Island	12. vii. 1959
16	„	Climber, fruit green with yellow marks, flower white.	BM 254	T. C. Wrigley	Common in village roofs, hedges, etc., Annobon, Anbo	Africa, Annonbon Island	9. viii. 1959
17	<i>L. sphaerica</i> (Sond.) Naud.	Climber over grasses, trees and shrubs, flowers white.	BM 416	I. H. Patel & E. A. K. Banda	Monkey Bay road, Malawi	Africa, Malawi	31. iii. 1979
18	„	Large climber, stems with white lenticles, leaves deep green scabrid at base, flowers white like dog nose	BM 8141	N. C. Chase	South Eastern, Slope of Murabwa's Hill, Commmonage	Africa, Umtali, Rhodesia	18. iii. 1964
19	„	Climbing shrub, flowers large and white	BM 1	D. Gourtauld	District Gorongoge	Africa, Mozambique	26. viii. 1953
20	„	Climbing shrub, flowers larger, white, scented.	BM 5209	N. C. Chase	Hilltop, North of Morning side	Africa, Rhodesia	26 iii. 1954
21	<i>L. guineensis</i> (D.D.M.)	A forest climber	BM 464	P.A. Talbot	Not cited	Oban , Forest, Nigeria Africa	1961
22	<i>L. rufa</i> (Gilg) C. Jeffrey	A climber	BM 602	Kamerun	Not cited	Not cited	Not cited
23	<i>L. sp</i>	Nil	FHI 73353	Ekwuno, Macauley	Nigeria	Africa	14. viii. 1974

Tab. 2. Qualitative assessment of foliar epidermal characters *Lagenaria*

Taxa	Epidermal surface	Epidermal Cell shape	Anticlinal Wall pattern	Stomatal type	Trichome type	Other special feature
<i>L. abyssinica</i>	Adaxial	Polygonal	Straight	-	-	-
	Abaxial	Irregular	Undulate	Anomocytic	MCT, ICT	-
<i>L. breviflora</i>	Adaxial	Polygonal	Straight-curved	Anomocytic, Paracytic	-	Glandular trichome base
	Abaxial	Irregular	Undulate	Anomocytic	-	-
<i>L. siceraria</i>	Adaxial	Irregular	Curved	Anomocytic	MCT	Non-glandular trichome base
	Abaxial	Irregular	Undulate	Anomocytic	GHT	Multicellular scale
<i>L. sphaerica</i>	Adaxial	Polygonal	Straight	Anomocytic	BGCT	Glandular trichome base
	Abaxial	Irregular	Curved	Anomocytic	-	Glandular trichome base
<i>L. vulgaris</i>	Adaxial	Polygonal	Straight-curved	Anomocytic	MCT	Glandular trichome base
	Abaxial	Irregular	Undulate	Anomocytic	-	Glandular trichome base
<i>L. guineensis</i>	Adaxial	Irregular	Curved	Anomocytic	-	-
	Abaxial	Irregular	Undulate	Anomocytic	-	Non-glandular trichome base
<i>L. rufa</i>	Adaxial	Polygonal	Straight	-	-	-
	Abaxial	Irregular	Undulate	Anomocytic	-	Non-glandular trichome base
<i>L. sp.</i>	Adaxial	Polygonal	Straight	Anomocytic	-	Non-glandular trichome base
	Abaxial	Irregular	Undulate	Anomocytic	-	Non-glandular trichome base

MCT= Multicellular conical trichome, GHT=Globular head trichome, ICT= Imperfect conical trichome; BGCT= Bicellular glandular conical trichome

Tab. 3. Quantitative Assessment of foliar epidermal characters of *Lagenaria*

Species	Epidermal cell length ( $\mu\text{m}$ )	Epidermal cell width ( $\mu\text{m}$ )	Mean Epidermal cell No./ $\text{mm}^2$	Stomatal length ( $\mu\text{m}$ )	Stomatal width ( $\mu\text{m}$ )	Mean stomatal No./ $\text{mm}^2$	Stomatal index (%)
<i>L. abyssinica</i>	23.5 (26.2) 30.0	16.2 (18.5) 21.5	81	-	-	-	-
	21.6 (26.5) 34.5	17.0 (19.2) 22.0	62	12.8 (15.2) 18.5	11.4 (12.0) 14.0	8	11.4%
<i>L. breviflora</i>	15.0 (28.3) 34.0	13.0 (21.0) 28.0	38	10.0 (7.5) 12.0	11.0 (12.6) 13.0	2	5.0%
	18.0 (23.7) 30.0	14.0 (19.1) 24.0	112	13.0 (18.5) 21.0	11.2 (13.0) 14.0	10	8.2%
<i>L. siceraria</i>	21.0 (28.1) 32.0	18.0 (19.6) 22.0	90	14.0 (17.0) 20.0	13.0 (14.3) 16.0	2	2.2%
	21.0 (28.0) 34.0	17.0 (17.3) 21.0	79	15.0 (17.5) 21.0	12.0 (14.0) 16.0	9	10.2%
<i>L. sphaerica</i>	24.0 (27.8) 34.0	18.0 (20.5) 24.5	32	18.0 (19.1) 22.0	12.0 (14.0) 15.5	2	5.9%
	22.0 (27.3) 30.0	20.0 (20.8) 23.0	78	17.0 (20.1) 23.0	12.0 (14.0) 16.3	12	13.3%
<i>L. vulgaris</i>	20.0 (27.5) 35.2	17.0 (19.3) 23.0	28	15.0 (12.0) 18.0	9.0 (7.0) 11.0	15	34.9%
	25.0 (18.0) 31.4	16.0 (18.7) 21.0	73	16.0 (19.0) 24.0	15.0 (18.0) 21.0	18	19.8%
<i>L. guineensis</i>	17.0 (24.2) 31.0	15.0 (17.5) 23.0	35	12.8 (15.2) 18.5	11.4 (12.0) 14.0	2	5.4%
	17.0 (24.8) 32.0	16.0 (19.6) 24.0	50	12.0 (16.5) 21.0	11.0 (12.6) 13.0	8	13.8%
<i>L. rufa</i>	26.0 (29.6) 38.0	15.5 (20.3) 25.0	76	13.0 (18.5) 21.0	11.2 (13.0) 14.0	-	-
	23.5 (26.5) 20.0	16.2 (19.2) 22.0	44	14.0 (17.0) 20.0	13.0 (14.3) 16.0	10	18.5%
<i>L. sp.</i>	20.0 (26.4) 32.0	15.2 (17.7) 21.0	84	15.0 (17.5) 21.0	12.0 (14.0) 16.0	2	2.3%
	19.0 (26.5) 33.0	17.0 (18.3) 22.0	76	18.0 (19.1) 22.0	12.0 (14.0) 15.5	20	20.8%

Quantitative data are expressed as minimum (mean) maximum

stomatal number varies from 2-20 and stomatal index varies from 2.2 – 34.9 % on the adaxial surface and 8.2 – 20.8 % on the abaxial surface. A neighbour joining cluster derived from Euclidean similarity measure recognized two main groups in the UPGMA dendrogram. Group A comprises two sub-groups: sub group I has *L. guineensis*, *L. rufa* and *L. sp.* and sub group II consists of *L. sphaerica*, *L. vulgaris* and *L. breviflora*, while Group B is made up of *L. abyssinica* and *L. siceraria*.

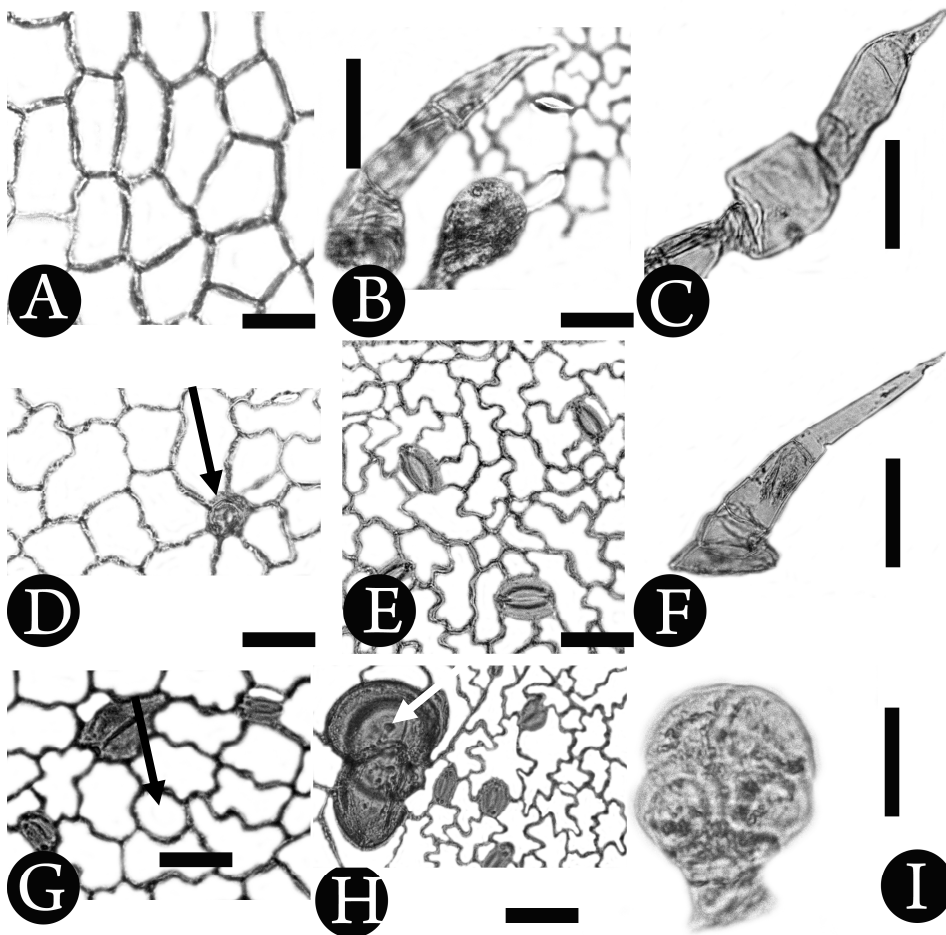


Fig. 1. Leaf epidermal characters of *Lagenaria*.

A-C: *L. abyssinica*; D, E: *L. breviflora*; F-I: *L. siceraria*. B, C, F & I are the various trichomes recorded in *L. abyssinica* and *L. siceraria*. H: a multicellular scale found in *L. siceraria*.

Scale of epidermal surfaces is 50µm and trichome is 70µm.



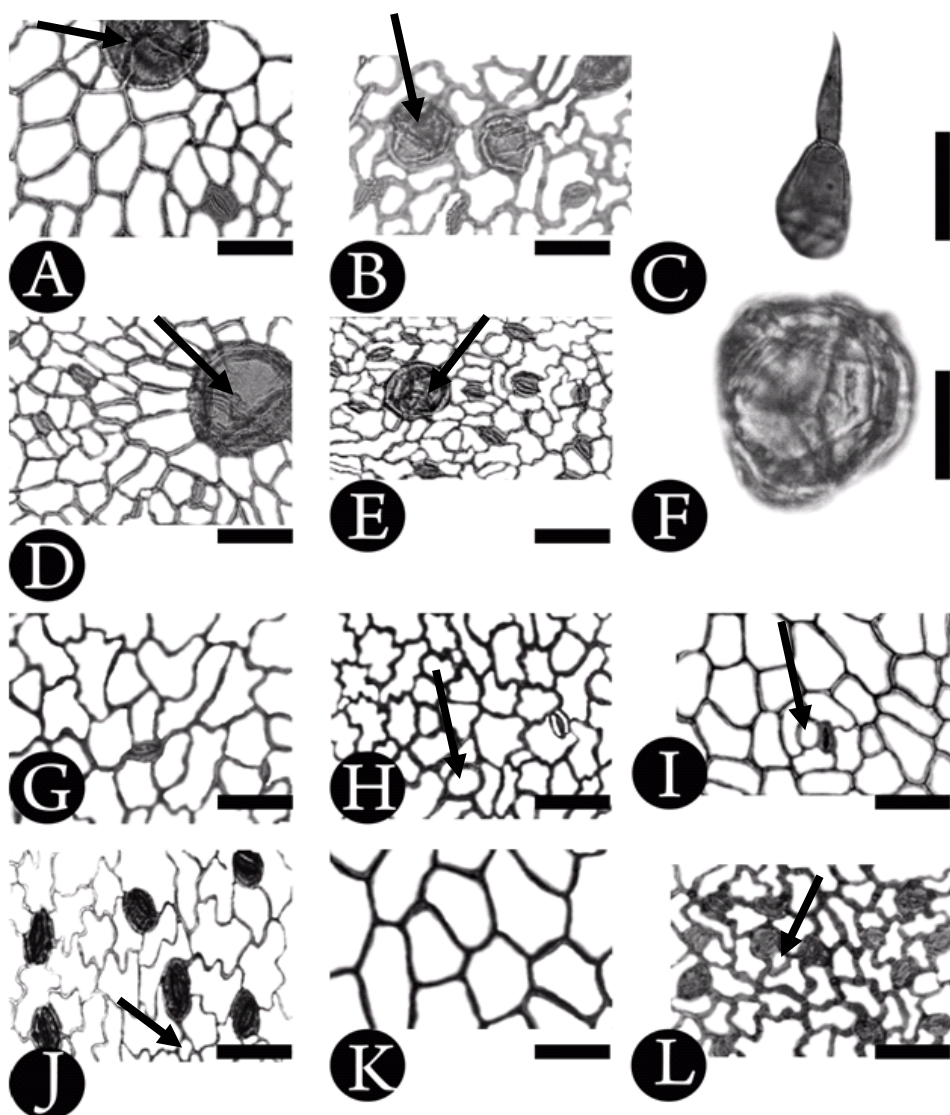


Fig. 2. Leaf epidermal characters of *Lagenaria*.

A-C, F: *L. sphaerica*; D, E: *L. vulgaris*; G, H: *L. guinensis*; I, J: *Lagenaria* sp.; K, L: *L. rufa*

Scale of epidermal surfaces and trichome is 50µm while trichome base is 25µm.

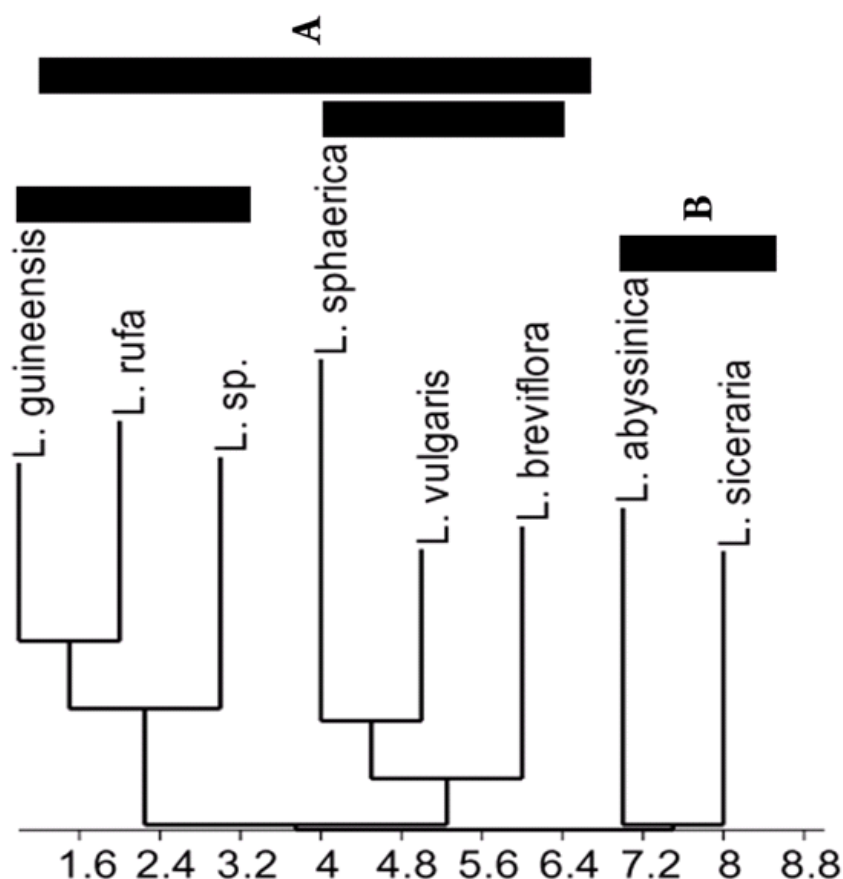


Fig. 3. Neighbour joining cluster using Euclidean similarity measure. A total of 15 character states of the leaf epidermis were used. Two groups: (A with 2 sub groups) and B were recognized by the UPGMA dendrogram.

## Discussion

The leaf epidermal characteristics of eight species of *Lagenaria* were studied by the means of light microscopy and the features examined proved useful for species identification and discrimination. The few reported information on leaf epidermal morphology on the family holds promise for taxonomic understanding of the plants. ABDULRAHAMAN et al. (2011) and METCALFE & CHALK (1950, 1979) on Cucurbitaceae, ADEBOOYE et al. (2012) in *Trichosanthes* and KOLB & MULLER (2004) on *Cucurbita pepo*. Some of the features reported by these authors were found in *Lagenaria*, which suggest that taxa composition in the family is in order. In *Lagenaria*, anomocytic stomatal type and undulate anticlinal wall are particularly constant in all species except *L. sphaerica* which has somewhat curved pattern. PATIL & PATIL (1987) investigated stomatal distribution,

frequency, index and size in *Chlorophytum* L. and commented on their significance at sub generic level. CARLQUIST (1961) and RAJU & RAO (1977) also reported the contribution of stomatal size in species delimitation. Other studies on stomata revealed the application of the character at various taxonomic levels (ALBERT & SHARMA 2013 and KOTHARI & SHAH 1975). The species are either amphistomatic or hypostomatic; *L. abyssinica* and *L. rufa* are hypostomatic while other species are amphistomatic. Paracytic stomata were recorded only in *L. breviflora*. Stomatal distribution is a reliable feature of angiosperm leaf which can be employed for delimiting taxa (STACE 1965). Stomatal index varies from 8.2% to 20.8% in *L. breviflora* and *L. sp.* while other species occupy intermediate positions. The species are either pubescent or glabrous. Distribution of trichomes may be restricted to the adaxial or abaxial surface or present on both. Although trichome distribution has been documented to be influenced by environment and age of plant in Cucurbitaceae and other plants (ADEBOOYE, et al. 2012, DAVIS & HEYWOOD 1963 and STACE 1965) but its application in taxa delimitation has been presented (ADEBOOYE et al. 2012, KOLB & MULLER 2004 and INAMDAR & GANGADHARA 1977). The four different types identified appear useful for differentiating the species. Multicellular conical trichomes were found in *L. abyssinica*, *L. siceraria* and *L. vulgaris*, globular headed type in *L. siceraria*, imperfect conical form was recorded in *L. abyssinica* and bicellular glandular conical type was found only in *L. sphaerica*. In related studies on other members of Cucurbitaceae, unicellular and globular headed trichomes were found in *Trichosanthes cucumerina* (ADEBOOYE et al. 2012) and globular headed type with one or more cells have been reported in *Cucurbita pepo* by KOLB & MULLER 2004. Other sophisticated types such as stellate hairs have been documented absent in the core Cucurbitales; *Lagenaria*, is grouped here (NANDI et al. 1994). Functionally, trichomes prevent herbivory, excessive heat and sunlight; and they are offensive to animals when in contact (KOLB & MULLER 2004, STACE 1965). Based on a combination of these features using neighbour joining cluster and Euclidean similarity measure (HAMMER 2001), two major groups and subgroups are therefore proposed. However, whether the position in this study would initiate recognition of infra specific ranks among the species or not is a question to be answered with other combined findings from morphological and molecular data in future investigations.

Therefore, based on the numerous numerical variations and qualitative differences in the features of the species, an indented dichotomous key is thus presented for delimitating them.

1. Leaf hypostomatic.....2
  2. Trichome present, stomatal index less than 15%.....*L. abyssinica*
  2. Trichome present, stomatal index greater than 15%.....*L. rufa*
1. Leaf amphistomatic.....3
  3. Paracytic stomata present, glandular trichome base present .....  
.....*L. breviflora*
  3. Paracytic stomata absent, non-glandular trichome base present .....  
.....*Lagenaria* sp.

4. Cell shape uniform on both surfaces.....5
  5. Stomatal index 13.8% on abaxial surface.....*L. guineensis*
  5. Stomatal index 10.2% on abaxial surface.....*L. siceraria*
4. Cell shape dissimilar on either surface.....6
  6. Bicellular glandular trichome present, stomatal index 5.9 % on adaxial surface .....*L. sphaerica*
  6. Multicellular conical trichome present, stomatal index 34.9 % on adaxial surface.....*L. vulgaris*

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