STUDY OF ANATOMY OF THE GENUS *HURA* L. (EUPHORBIACEAE)

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ABSTRACT

Anatomy of leaf, wood and carpological structure of *Hura* L. was carried out with the aid of light microscopy. Useful taxonomic features for its delimitation and distinction are documented. Presence of scalariform perforation plates, narrow lumen and heterogeneous diameter are important features of the vessels. Laticiferous canals were found in the parenchyma tissue of petiole and midrib; where it occurs as a series of circular- to polyhedral-shaped cells. In the fruit peduncle, the ground tissue has some inclusions. Typical paracytic and anomocytic stomata of the Euphorbiaceae were recorded in the genus. The amphibrachyparacytic stomatal type found on the abaxial surface may be diagnostic. The range of interstomatal distance between any two paracytic and amphibrachyparacytic stomata is 35-45μm and 100-160 μm respectively. In between the cells, the former type has 2 cells whereas it varies from 5-6 cells in the latter. Glandular trichomes were restricted to the abaxial surface of the epidermis. Scalariform perforation plates in the wood link the genus to subfamilies like the recent Acalyphoideae, old Crotonoideae and Phyllanthoideae. Apart from this, other characters of the ground tissue and foliar epidermis offer strong support for its inclusion in the current subfamily Euphorbioideae. Since taxonomic description of this genus is lacking in the *Flora of West Tropical Africa,* published data on morphology from several sources and the present anatomical data are provided to fill this gap in knowledge.

Keywords: Anatomy, Euphorbiaceae, *Hura*, Huraea microscopy, taxonomy.

INTRODUCTION

Euphorbiaceae *s. l.* remains a large family despite its withering (Radcliffe-Smith, 1987 and APG III, 2009). It is found mostly in the tropics. The family consists of trees, shrubs, herbs and rarely woody climbers (Hutchinson and Dalziel, 1958 and Heywood, 1993). The family is estimated to consist of 300 genera and 7,500 species. *Hura* L. as a genus in the family is represented by two species worldwide. It is the sole genus in the monotypic and monophyletic tribe- Huraea (Park and Backlund, 2002). *Hura crepitans* L. originates from South America but naturalized and occupying all the moist parts of Africa, including Nigeria while *H. polyandra* Baill. is restricted to South America (Martínez Gordillo *et al.*, 2002).

Hura crepitans can grow up to 60 metres (Swain and Tom, 1977) and usually cultivated for canopy in many communities in West Africa (Burkill, 1994). Wood of *Hura* is priceless, used for constructing many durable household items (Chudnoff, 1984; Wangaard and Muschler, 1952); this has led to its drastic loss in the wild.

Taxonomic description of the genus is unavailable in our regional flora (see Hutchinson and Dalziel, 1958). Aworinde *et al.,* (2009) documented few exo-morphological features of the leaf (Table 2) and there is limited account on its anatomy (Metcalfe and Chalk, 1950). Only vessel type, presence of crystals in the petiole and cauline parenchyma cell arrangement which are sparsely described are accounted for by Metcalfe and Chalk, (1950). Given the rate at which the plant is lost, it is very important to conserve all its taxonomic information given the fact that it has naturalized in Africa.

Systematically, synonyms of *H. crepitans* include *Hura brasiliensis* Willd, *Hura senegalensis* Baill. and *Hura strepens* Willd. The genus occupies a position between tribe- Stomatocalyceae (represented by *Hamilcoa* and *Pimelodendron*) and tribe- Hippomaneae (represented by *Omalanthus* and *Sapium* Jacq.), following a cladistics analysis of the family based on cyathium character (Park and Backlund, 2002). With Hippomaneae, the genus shares presence of glands at the leaf blade base (Webster, 1994); this was confirmed during field work. Based on this character, Pax (1924) combined them into a single tribe Hippomaninae. The genus is currently placed in its own tribe –Huraea (Park and Backlund, 2002); and supported with evidence from molecular data (Wurdack, *et al.*, 2004; Wurdack and Davis, 2009).

Therefore, it is important to document all taxonomically useful features of the plant. There is paucity of anatomical information and there is no record of its exo-morphological description in the *Flora of West Tropical Africa* (see Hutchinson and Dalziel, 1958). We addressed these problems in the study.

Anatomical features are useful for taxa recognition in the family (Webster, 1994). The utility of this taxonomic tool for description of the family began since the 17th century (see Radlkofer, 1870; Pax 1884) and since then, many vegetative anatomical structures have been reported in several genera (Dehay, 1935, Breckon, 1975; Hickey and Wolfe, 1975; Dehgan, 1982; Miller and Webster, 1962; Thurston and Lersten, 1969; Uhlarz, 1978). An updated account on anatomical data of Euphorbiaceae *sensu stricto* is needed to improve our understanding of this new family segregate of the Euphorbiaceae *sensu lato*. Hence, it is expected that species-poor genera would play a role in this regard, and *Hura* is an excellent example.

MATERIALS AND METHODS

Leaf and Seed Anatomy

Both dry and fresh materials of leaf, seed and wood were used for the study. Nomenclatural authentication was carried out in the Lagos Herbarium (LUH) and a voucher specimen was deposited for future use. Herbarium abbreviation follows Holmgren *et al.*, 1990. All existing data sources (e. g. Metcalfe and Chalk, 1950; Olowokudejo, 1993; Kadiri *et al.*, 2009) were also consulted for further information.

Leaf lamina was held in fresh yam tissue and longitudinal sections were obtained with a razor blade by free-hand method. For foliar epidermis, leaf samples were examined using the standard method established for tropical plant taxa including Euphorbiaceae, e.g. Ogundipe and Kadiri (2012) on Amaranthaceae, Olowokudejo (1993) on *Jatropha*, Kadiri *et al.* (2009) on *Acalypha*, Yang and Lin (2005) on Schisandraceae. However, modifications were introduced where necessary for optimal result. The method involved cutting 1-5cm^2 portions from the standard median portion of the leaf lamina i.e. near the mid-rib and then boiled in water for 20-30 minutes. The leaf pieces were later soaked in concentrated trioxonitrate (v) acid (HNO_3) in capped specimen bottles for about 8-24 hours to macerate the mesophyll. Tissue disintegration was indicated by bubbles, and the epidermises were transferred into Petri dishes containing water for cleansing and then, epidermises were separated with forceps and mounting needles. Tissue debris was cleared off the epidermises with fine hair brush and washed in several changes of water.

For examination of the petiole, midrib and seed peduncle, this was done using freshly-collected materials. Petioles were examined from the distal, median and proximal regions. The materials were cut free-hand to make semi-permanent slides for microscopic studies. Drops of different grades of ethanol- 50%, 75% up to 100% were added in turn to harden the cells. Preparations were later stained with Safranin O in 50% alcohol before mounting in glycerine on a glass slide. The epidermises were mounted on a glass slide with upper surfaces facing up and then covered with cover-slips and ringed with nail varnish to prevent dehydration. Scraped epidermis of the seed was obtained and stained with Safranin O and then examined under the microscope. Slides were examined with light microscopes at x100 and x400.

Wood Anatomy

Cross sections of the wood were obtained after soaking in water using free-hand sectioning. Thin samples were stained with Safranin O and observed under the microscope. Some wood samples were macerated in concentrated tetra-oxo sulphate acid (H_2SO_4). The macerated samples were placed in water after which some portions were examined under the microscope.

Photomicrographs were obtained with ToupView 3.7 digital camera attached to an Olympus compound light microscope and viewed on a Pentium IV computer. Line diagrams were made with tracing paper mounted on photomicrographs.

For quantitative assessment of epidermal characters, 20 epidermal cells and stomata were randomly selected for measurement. Stomata index was calculated using the formula reported by Stace (1965).

$$\text{Stomatal index} = \frac{\text{Stomata number per mm}^2}{\text{Cell number per mm}^2 + \text{stomata number per mm}^2} \times 100$$

RESULTS

A summary of the results is presented in Tables 1- and Fig. 1-6.

LEAF IN LONGITUDINAL SECTION

The leaf is dorsi-ventral, having a layer of cylindrical/elongated palisade parenchyma cells which may be filled i.e. glandular or free of cell sap and another layer of spongy mesophyll parenchyma cells which have circular to round cells (Fig. 1). Cell length is *c.* 150 - 225μm in the palisade layer whereas cell diameter is *c.* 90 - 165μm in the spongy mesophyll layer. The epidermis consists of a single layer of cells on both adaxial and abaxial surfaces. The adaxial surface is covered by a layer of *c.*25-30 μm thick

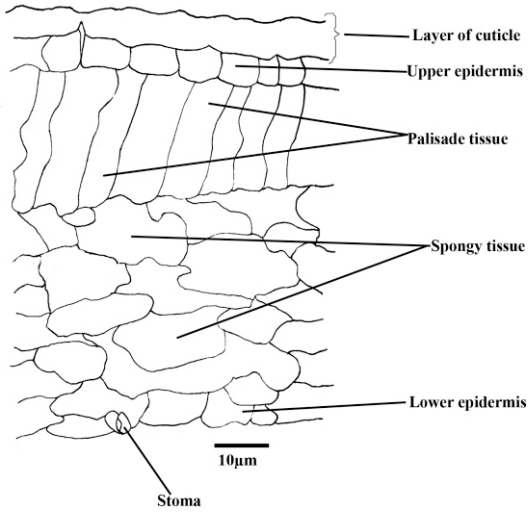

Fig. 1: Line drawing of the dorsiventral arrangement of tissues in the leaf of *Hura crepitans*.

LEAF: EPIDERMIS, MIDRIB AND PETIOLE

The leaf is amphistomatic. There are more stomatal cells on the lower surface than the upper surface of the leaf. On the latter surface, the anticlinal walls are straight (Fig. 2C), curved (Fig. 2B) to undulate (Fig. 2A). The cell lumen is sometimes filled with crystals of calcium oxalate (Fig. 2B) and striations were frequently encountered (Figs. 2A, C, 3D). Furthermore, the cell shape is polygonal and the stomatal types are paracytic and amphibrachyparacytic (Figs. 2 and 3, Table 1). Comparatively, on the lower surface, the anticlinal walls are straight (2A) to slightly curved (Fig. 3B, D). There are more crystals of calcium oxalate on lower surface than upper surface (Fig. 2A). In addition to this, there are unicellular, long acicular trichomes (Fig. 2C, D and Table 1). These trichomes are usually glandular at the base (Fig. 2D). Quantitatively, the epidermal cells are longer than wide on the surfaces (Table 1). The range of epidermal cell size is 34.0-62.0 μm x 25.0-48.5 μm on the upper surface and 35.0-85.5 μm x 20.5-35.0 μm on the lower surface (Table 1). Mean epidermal cell number per square millimeter is 600±5 and 574±5 on the upper and lower surface respectively. Stomatal cells are longer on the upper surface than lower surface. Stomata Index is higher on the lower surface (9.0%) than upper surface where it is 2.4%. The upper surface is glabrous whereas it is pubescent on the lower surface, the trichome length varies from 750-755μm (Table 1).

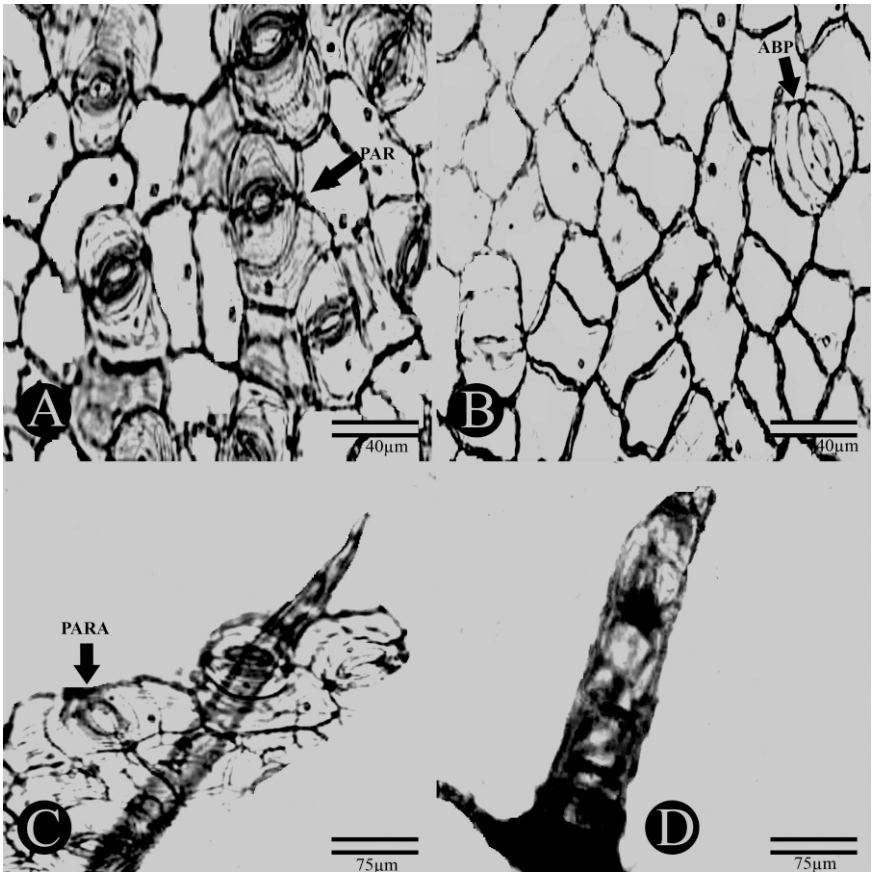

Fig. 2: Foliar epidermal features of *Hura crepitans*. A, C, D: Lower epidermis. Note presence of crystals of Calcium oxalate within the cell lumen. B: Upper epidermis. C, D; are two halves of an acicular type of trichomes that were observed (C= upper part; D= lower part). PAR = Paracytic, ABP= Amphibrachyparacytic. Stomatal terminology follows Stace (1989).

Table 1: Qualitative and Quantitative leaf anatomical features of *Hura crepitans*

S/N	Characters	Upper surface	Lower surface
	Epidermal cell shape	Polygonal	Polygonal
	Epidermal cell inclusion	Present	Present
	Striations	Absent	Present
	Anticlinal wall pattern	Straight, curved to undulate	Straight to slightly curved
	Stomatal type	Paracytic, amphibrachyparacytic	Paracytic, anomocytic
	Trichome type	Absent	Present, Unicellular, glandular , acicular
	Epidermal cell number/mm^2	595 (600±5) 610	567(574±5)584
	Epidermal cell length (µm)	34.0(52.0±0.2)62.0	35.0(60.0±0.2)85.5
	Epidermal cell width (µm)	25.0(32.0±0.2)48.5	20.5(29.0±0.2)35.0
	Epidermal thickness (µm)	2.0(2.5±0.01)3.0	1.6(2.0±0.01)2.8
	Stomata number/mm^2	12(15±1.0)18	55(57±1)60
	Stomata length (µm)	25.0(30.0±0.4)35	20.0(24.0±0.1)28.5
	Stomata width (µm)	10.0(12.2±0.23)15.3	15.8(20.5±0.6)25.3
	Stomata Index	2.4%	9.0%
	Trichome length (µm)	-	750-755µm
	Midrib: Shape Adaxial surface	Dome shape	
	Abaxial surface	Crescentiform	
	Surface	Glabrous	
	Vascular bundle position	Central	
	Vascular bundle type	Collateral	
	Collenchyma cell	Unevenly thickened	
	Sclerenchyma cell	Oval	
	Secretory cells	Present, Abundant in parenchyma	
	Parenchyma shape	Polyhedral, circular	
	Petiole: Outline	Circular to egg shape	
	Vascular bundle distribution	Discrete, continuous	
	Secretory cells	Present, Abundant in both perivascular region and within parenchyma cells	

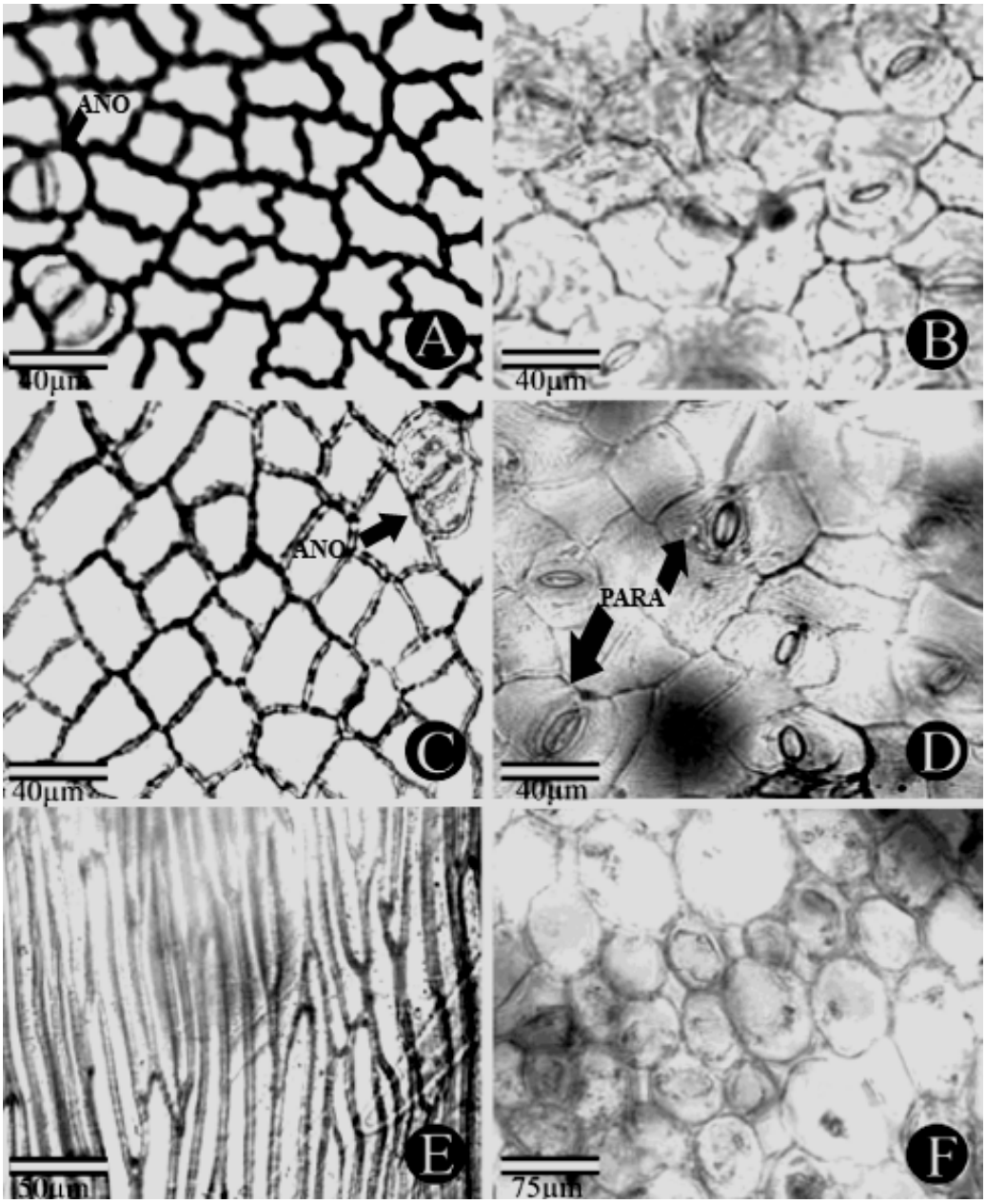

Fig. 3: Some vegetative and reproductive anatomical features of *Hura crepitans*. A, C: Upper epidermis. B, D: Lower epidermis. E: Seed surface showing thick walled and elongated epidermal cells. F: Parenchyma cells of seed peduncle with some inclusions. ANO= Anomocytic, PAR = Paracytic. Stomatal terminology follows Stace (1989).

Vasculature of the midrib is medulated. Laticiferous canals are on the wings and the ground tissue is mostly composed of parenchyma cells. The surfaces are glabrous. Parenchyma cells are ovoid to polyhedral and most abundant (Fig. 4A-D). The upper surface is dome-shaped whereas it is crescentiform on the lower surface (Fig 5A).

The petiole is structurally different at the distal, median and proximal regions; it is circular in shape at the proximal and median sections but it tends towards egg shape at the distal end. The cortical region accommodates the vascular bundles where there are laticiferous canals and parenchyma cells abundantly occupy the pith region (Fig. 4E, F). However, vascular bundles of different sizes were observed in these three regions (Fig. 5B-D); more different in proximal area and distal areas than median zones where they appear uniform.

Interfascicular cambium was observed only at the median and distal regions of the petiole (Fig. 5B-D). Similar to midrib, the parenchyma cells are most abundant and the shape varies from ovoid to polyhedral (Fig. 4F).

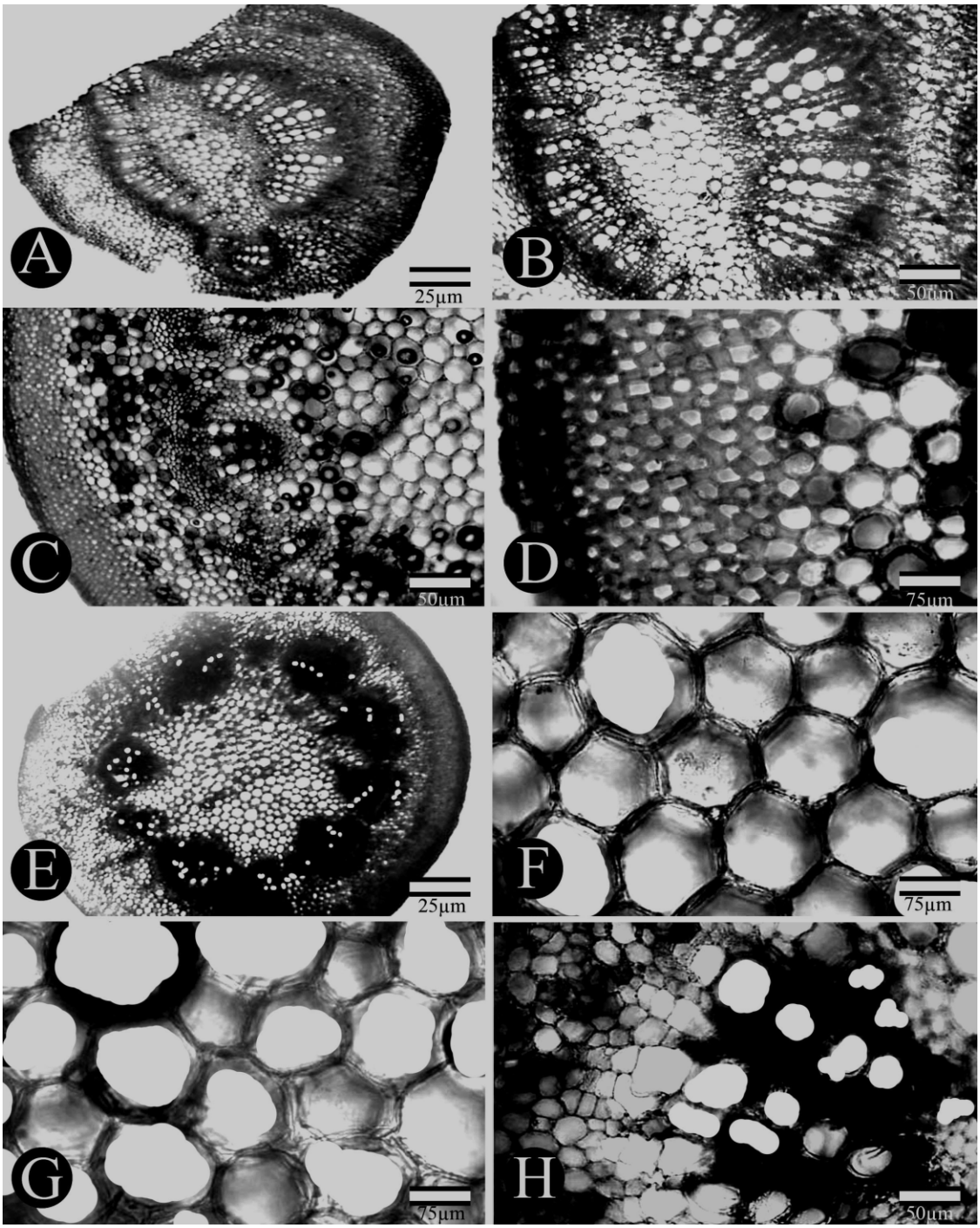

Fig. 4: Midrib and petiole anatomy of *Hura crepitans*. A-D: Midrib showing centrally located vascular bundle, some secretory cells observed under both low- and high- power microscope. E-F: Petiole revealing centrally positioned discrete but continuous vascular bundles. Parenchyma cells are filled with ergastic substances. Note thick walled sclerenchyma cells in G, recorded at the upper surface. H shows unevenly- shaped and sized parenchyma cells, and oval to oblong xylem vessel pores.

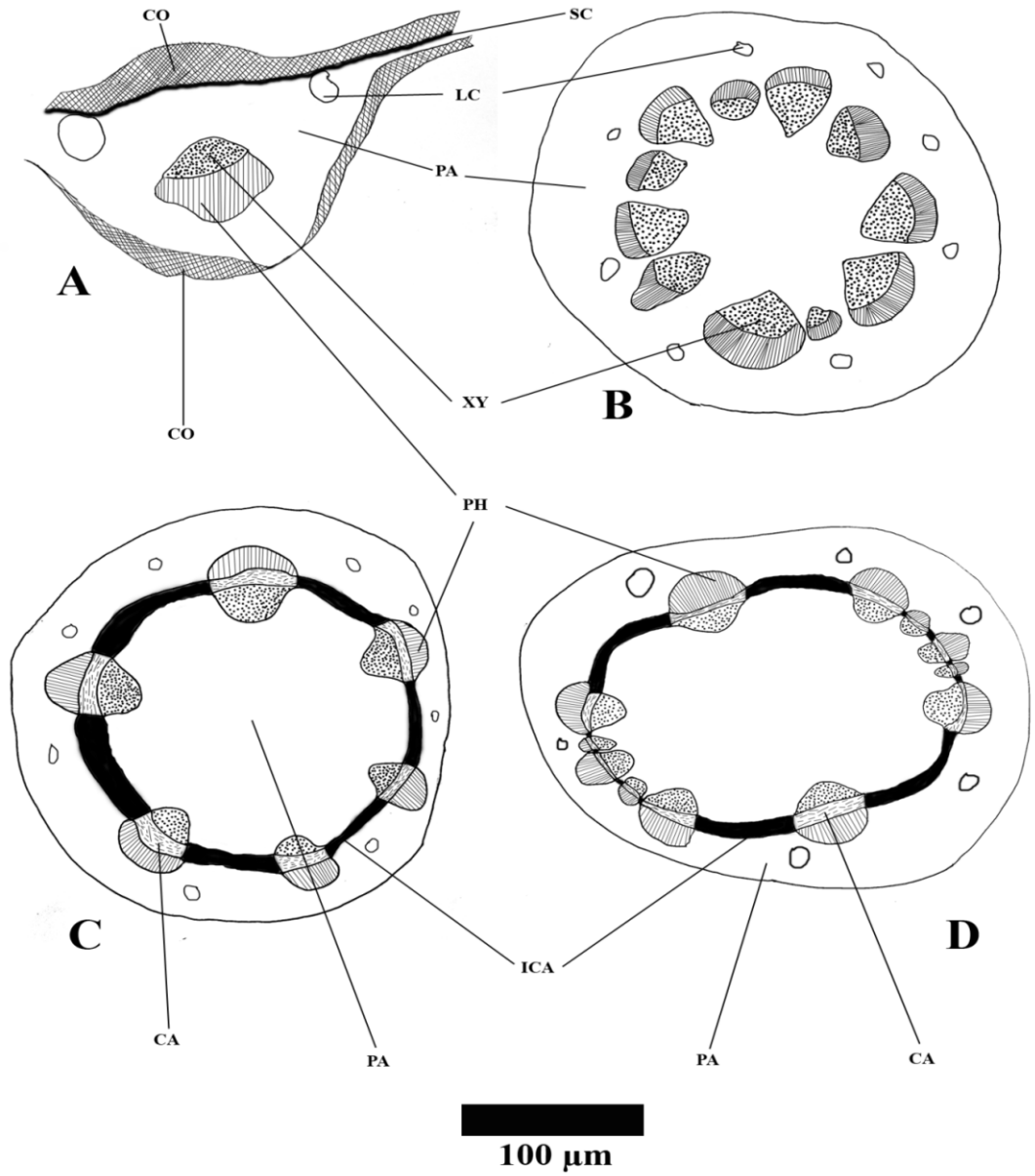

Fig. 5: Line drawings of midrib and petiole characteristics of *Hura crepitans*. A: Midrib showing central vasculature and laticiferous canals on the wings. B-D: Petiole characteristics recorded at different portions. B: Proximal section, C: Median zone of the petiole and D: Distal region of the petiole. Note laticiferous canals in the ground tissue. CA: cambium, CO: collenchyma, ICA: interfascicular cambium, XY: xylem, PH: phloem, SC: sclerenchyma, LC: laticiferous canal, PA: parenchyma.

CARPOLOGICAL FEATURES

The epidermal cells of the seed coat are double-walled and thick. They are very much longer than wider (Fig. 3E), and about 3-5 times longer than leaf epidermal cells. The parenchyma cells of the peduncle are commonly ovoid-shaped. Some deposits of ergastic materials were seen in the lumen of the thick walled parenchyma cells (Fig. 3F).

WOOD

Transverse sections of the wood revealed vessels having both scalariform and simple perforation plates (Fig. 6A, C). The pits are either bordered or non-bordered (Fig. 6A). The vessel size may be large or small (Fig. 6C, D) with their length varying from 60 μm to 120 μm (Fig. 6F-G). The fibres are longer than the vessels and they often have tiny or narrow cavities (Fig. 6E, H).

Table 2: Diagnostic characters of *Hura crepitans* based on morphology (According to Park and Backlund, 2002; Webster, 1994) and anatomy (Present study)

Morphology		**Anatomy**	
1. Succulent stems	Absent	Leaf in T/S	Layer of cuticle
2. Male inflorescences	Condensed		Cylindrical palisade cells
3. Staminate calyx	Present	Spongy mesophyll	Limited intercellular spaces
4. Stamen number	>8		
5. Pistilate calyx	Present	Thorn	Presence of conical cavity / free pith
6. Pistilate flower	Basal		Rich in parenchyma cells
7. Style	Undivided	Stomatal type	
8. Stigma	Thin (tapered)	Upper surface	Amphibrachyparacytic
9. Staminate flower articulation	Absent	Lower surface	Paracytic, very common
10. Pistilate flower pedicel	Present	Trichome	
11. Gland position	Base of male bracts	Upper surface	Absent
12. Glands	Present *sensu* Webster, 1994	Lower surface	Present / acicular
13. Gland appendages	Present *sensu* Webster, 1994	Trichome length	Up to 755 μm
14. Pollen colpus margins	Present with smooth margin	Stomatal Index	2.4% (Adaxial), 9.0% (Abaxial)
15. Pollen tectum	Reticulate or microreticulate	Upper surface	Less than 5%
16. Seed	Smooth	Lower surface	More than 5% but less than 10%
17. Latex color: colourless	Colourless	Cell inclusion	Present on both leaf surfaces
18. Sexuality	Monoecious	Anticlinal wall pattern	
19. Petiolar glands at the base of leaf blade	Present	Upper surface	Straight, curved to undulate
20. Ovary	> 3	Lower surface	Straight to slightly curved
21. Bracts	Free		
22. Petaloid bracts	Absent		
23. Capsule trichomes	Absent.		
24. Staminate petals	Absent		
**Leaf: Apex (acute), margin (undulate),	shape and base (cordate)	Plus some quantitative exo-morphological data of leaf	

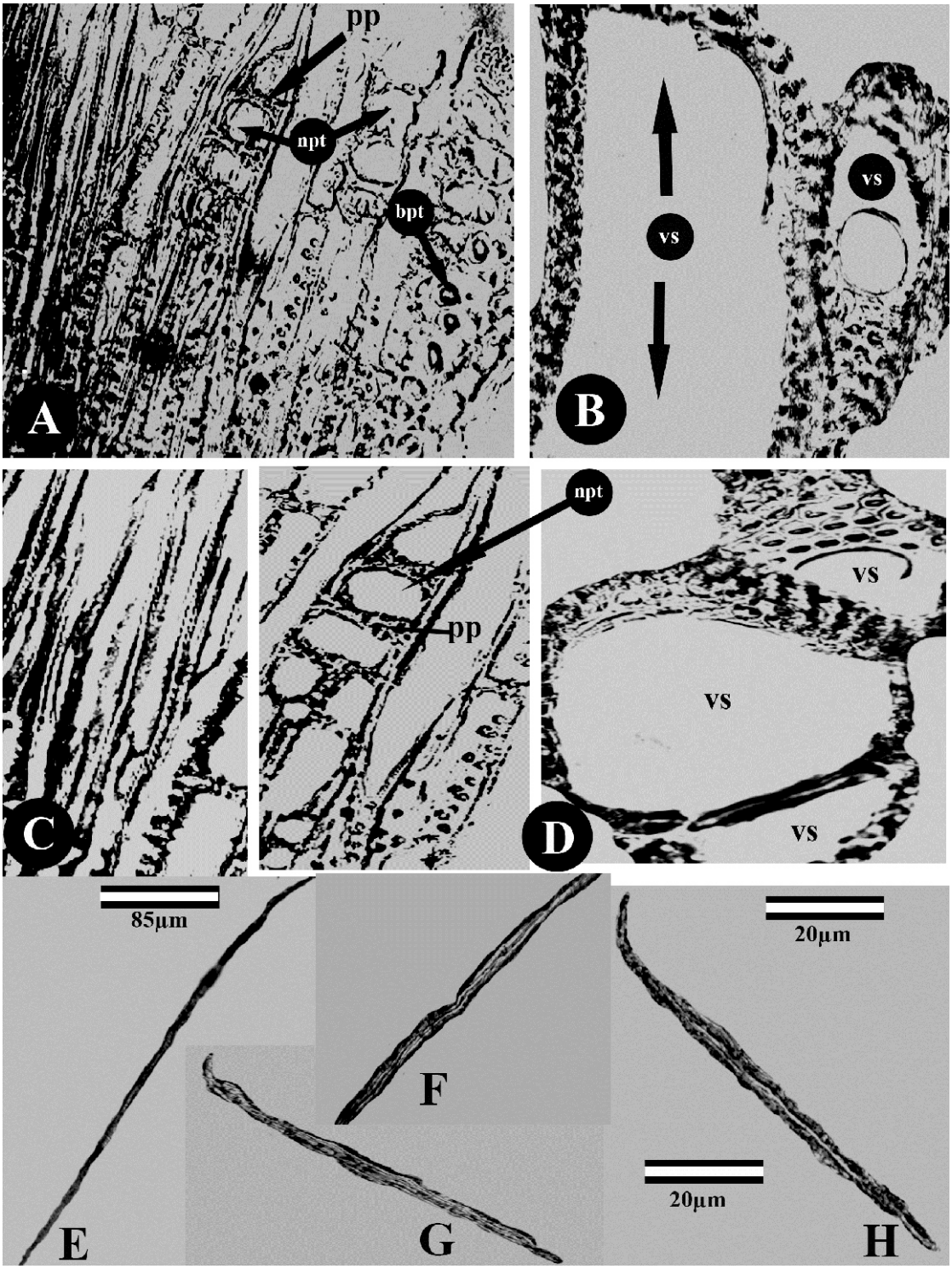

Fig. 6: Some wood anatomical characteristics of *Hura crepitans*. A, C: Wood in longitudinal section revealing scalariform perforation plates and both bordered and non-bordered pits. B, D: Wood seen from tangential view showing wide to narrow vessel pores. E, H: Long fibres. F-G: Vessels of different sizes showing narrow cavity and absence of it in the vessels. Note the thick walls of the vessels. bpt: bordered pit, npt: non-bordered pit, pp: perforation plate, vs: vessel.

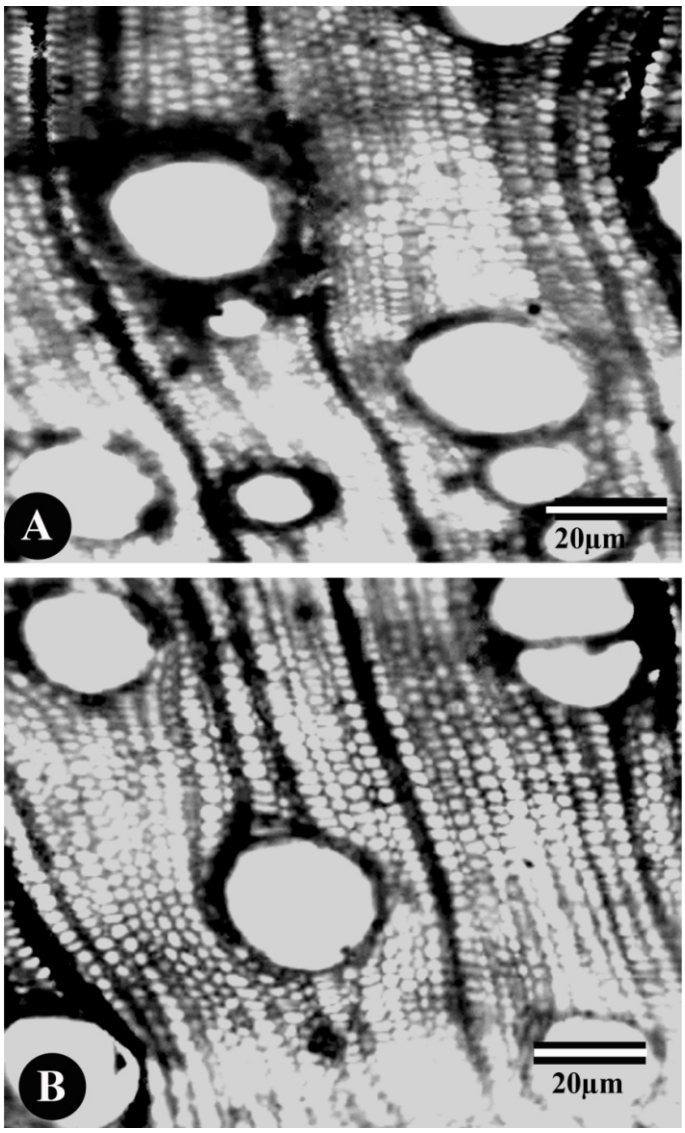

Fig. 7: Transverse section view of wood of *Hura crepitans* showing paratracheal parenchyma somewhat circumvascular, a situation whereby the cells surround the vessels. Ray cells are narrow and the vessels

DISCUSSION

The relevance of anatomical characters to the taxonomy of the Euphorbiaceae has been observed since antiquity (Pax 1884). Taxonomically useful features in the family are those of wood structure, trichomes and stomata (Mueller, 1866; Solereder, 1899; Solereder, 1908; Thurston and Lersten, 1969; Breckon, 1975; Inamdar and Gangadhara, 1977; Rao and Raju, 1985; Webster, 1994). Petiole anatomy has been particularly used for separating related genera in the family (Miller and Webster, 1962; Uhlarz, 1978; Dehgan, 1982). In addition, characters of the leaf epidermis have been used to explain affinities in infra and intra-generic groups in the family (Dehgan, 1980; Olowokudejo, 1993; Gilani *et al.*, 2002; Aworinde *et al.*, 2009; Kadiri *et al.*, 2009; Thakur and Patil, 2014). Presence of striations is a significant character of the epidermis in the genus and they can be a reliable diagnostic tool. This and other epidermal characters have proven to be important in plant classification (Stace, 1984), and they have been widely used in taxonomic treatments and systematic studies in many angiosperm families (Wang and Tao, 1993; Luo and Zhou,2001; Shi and Li, 2003; Yang and Lin, 2005; Ren, *et al.*, 2007).

Trichomes, stomata and structure of the seed coats have been reported useful for family classification and taxa description too (Metcalfe

and Chalk, 1950; Rao, 1963; Stace, 1965; Raju and Rao, 1975, 1977, 1987; Van, 1970; Levin, 1986; Inamdar and Gangadhara, 1978; Webster, 1994; Carpenter, 2005; Thakur and Patil, 2014). Only Metcalfe and Chalk (1950) sparingly mentioned the genus, therefore there is huge absence of useful taxonomic details about it. This is probably because it is not species-rich. Different types of trichomes such as simple unicellular to multicellular types, which may be branched or unbranched, tufted or stellate, stalked or sessile glandular and stinging hairs have been reported in the family (Metcalfe and Chalk, 1950). We found glandular simple unicellular conical / acicular were restricted to the abaxial surface in the genus. This supports its correct grouping in the family. Trichomes are useful for taxa distinction and also for inferring phylogeny (Ghahremaninejad *et al.,* 2012).

In the tribe genus, the leaf is amphistomatic, one of the two distribution types (amphistomatic and hypostomatic) reported by Metcalfe and Chalk (1950) for the family. This indicates closeness with the tribe *Crotoneae sensu* Metcalfe and Chalk (1950). Rubiaceous (i.e. paracytic) and Ranunculaceous (i.e. anomocytic) stomata were reported across the family by Metcalfe and Chalk (1950) without stating where they were found on the surfaces. In the study, we found the two stomatal types in addition to amphibrachyparacytic which is a derivative of the Rubiaceous stomata. Comparatively, amphibrachyparacytic stomata are distinguishable from paracytic by having the guard cells being flanked by more than one subsidiary cell on the sides. In the genus, the stomata are longer in amphibrachyparacytic but wider in the paracytic type. However, the amphibrachyparacytic type is quite uncommon like the paracytic form. Not only this, the former stomatal type is restricted to the upper surface of the epidermis. Paracytic and anomocytic stomatal types were found on the lower surface but paracytic and amphibrachyparacytic stomata were distributed on the upper surface of the epidermis. Furthermore, between any two paracytic stomata, the interstomatal distance is about 35-45μm occupying two epidermal cells whereas it is about 100-160 μm between any two amphibrachyparacytic stomata, occupying up to 5-6 epidermal cells (Fig 2A, B). Aworinde *et al.*, (2009) documented parallelocytic stomata which were not decipherable from the micrographs; however, this can be established in future studies. Nevertheless, taxonomic relevance of stomatal cells has been demonstrated in several plant taxa; they have been used to suggest affinities among taxa and also employed in taxa grouping (Stace, 1965; Carpenter *et al*, 2005; Ghahremaninejad *et al.*, 2012).

With limited taxa used for petiole anatomy, Metcalfe and Chalk (1950) reported that petiole anatomy is similar at any level of the structure. Contrary to this, in *Hura*, vasculature having interfascicular cambium was found at the median and proximal regions; this arrangement was similar to the interconnecting phloem recorded in the stem of *Mercurialis perrenis* by Metcalfe and Chalk (1950). Therefore, the existing account on petiole is hence updated; also, with the number of vascular bundles that varies from *ca.* 6-12. It has been pointed out that differently-sized vascular bundles may reflect the prevailing ecological situation of an area (Metcalfe and Chalk, 1950). On the other hand, the diagnostic features of the mid rib include centrally-positioned vascular bundle having a thin layer of sclerenchyma tissue that subtends copious collenchyma adaxially; and the shape which is domed on the upper surface and crescentiform on the abaxial surface.

Wood anatomy of the Euphorbiaceae has been shown to be taxonomically significant in resolving controversies among taxa (Hayden and Brandt, 1984; Hurusawa, 1954; Mennega (2005; Merev *et al.* (2005). From transverse view, the wood samples used revealed paratracheal parenchyma which is somewhat circumvascular, a situation whereby the cells surround the vessels. Ray cells are narrow and the vessels are ring porous. In *Hura*, the vessels may be long or short (*ca.* 60 to 120 μm; but it is 25-200μm in sub tribe Crotonoideae by Metcalfe and Chalk, 1950). The vessels have thinly narrow cavities and their diameter is heterogeneous; some possessing scalariform perforation plates and others having simple forms which are thick walled. Compared to other taxa in other subfamilies such as Acalyphoideaeae, perforation plates are simple with admixture of irregular scalariform plates (Hayden and Hayden, 2000); and in Crotonoideae, perforations are typically simple but scalariform

plates were observed in some genera such as *Bridelia* and *Antisdesma* (Metcalfe and Chalk, 1950). Moreover, in *Hura*, the wood features are similar to *Aporosa* wood type of subfamily Phyllanthoideae. Laticiferous canals are usually found in all parenchyma cells of the species. Mennega (2005) reported that laticfers are; generally not surrounded by special cells, except in some genera of certain subtribe where radiallaticifers are present. *Hura* possesses these features; thus its placement in the new family segregate (Radcliffe-Smith, 1987) and the Euphorbiaceae *sensu lato* is supported. With all the features described in this study, *Hura crepitans* can be differentiated from allied taxa even when the materials for study are either mixed up or fragmentary.

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