Morphological, anatomical and histological studies on the genus *Icacina* (*Icacinaceae*) from Nigeria, West Africa

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Abstract. Morphological, anatomical and histological studies of three *Icacina* species (*I. mannii* Oliv., *I. oliviformis* (Poir.) J. Raynal, and *I. trichantha* A. (Juss)) were carried out by visual observation and microscopy. The species occur predominantly in Nigeria, West Africa. They exhibited anatomical variation in the midrib vascular bundle arrangement, trichome types and abundance, stomatal types, lamina thickness, stomatal index, and fruit morphology, all regarded as reliable diagnostic characters. Only *I. oliviformis* manifested druses and galls. The study revealed some morphological and anatomical similarities, such as leaf arrangement, non-glandular trichomes, anomocytic and tetracytic stomata, epidermis cell shape, and presence of tannin and starch grains in the corms of the species.

Key words: Anatomy, druses, histology, *Icacina*, stomata index, trichome.

Introduction

Icacinaceae is a pan-tropical family of trees, shrubs and lianas, with 50–55 genera and 300–400 species worldwide (Mabberley 1997; Kårehed 2001). Studies have shown that the family, as traditionally circumscribed, was polyphyletic (Savolainen & al. 2000; Soltis & al. 2000; Kårehed 2001). This resulted in a morphologically heterogeneous group, difficult to recognize in the field and in herbariums (van Balgooy 1998). Based on molecular phylogenetic work, the family has now been split into four families: *Icacinaceae* s. str. (Garryales), *Pennantiaceae* (a member of the order Apiales), *Cardiopteridaceae*, and *Stemonuraceae* (both members of the order Aquifoliales) (Kårehed 2001; Stevens 2001). The family *Icacinaceae* A. Juss comprises about 35 genera, including the genus *Icacina* with only three species, namely: *I. mannii* Oliv., *I. oliviformis* (Poir.) J.Raynal, and *I. trichantha* A. (Juss) in West Africa (Hutchinson & Dalziel 1954). These species are in abundance in some West African countries such as Nigeria, Chad, Benin Republic, Gambia, and Ghana. In West Africa, members of this genus have several common names: *False Yam* in the Anglophone countries, *Bakanas* in the Francophone countries, and in Nigeria they are called *Ibugo* or *Utu* by Igbos, *Gbegbe* by the Yorubas, *Pane* in Sudan, *Takwara* in Ghana, and *Kouraban* in Senegal (Burkill 1985; Fay 1987).

The *Icacina* species could be used as a food source because of the nutritional contents of their seeds: 13 % moisture, 72 % carbohydrates, 8–10 % protein, 0.1 % fat, and 0.5 % (Fay 1991). Fine flour domestically made from the tubers contains on the average 10–15 % of starch, which could either be spherical or elliptical (Fay 1973). The roots are toxic and are used in traditional medicine after detoxifying according to local means by macerating them in water or ethanol for about three days (Fay 1973). The paste or porridge made from the tubers of *Icacina* species contains 8–10 % protein, about five (5) times more than in the cassava flour and twice more than in potatoes (Fay 1991). The pharmacological uses and medical potential of the species are well documented (Sarr & al. 2011). *I. oliviformis* is used for treatment of *Plasmodium,*

due to its active antiplasmodial effect without host cell toxicity. It also contains dichloromethane, a very strong antimicrobial agent and thus is very promising in medical research against malaria (Sarr & al. 2011; Okoronkwo & al. 2014). *I. trichantha* contains different phytochemicals of economic importance (Ezeigbo 2010; Timothy & Idu 2011; Onakpa & Asuzu 2013; Shagal & al. 2014). In many communities, these species are misidentified, owing to their close resemblance based on morphological similarity. They are cultivated by tuber cutting (Mabberly 1997).

Leaf epidermal structures of some *Icacinaceae* species have been studied and described by different authors (Metcalfe & Chalk, 1979; Baas 1974; van Staveren & Baas 1973; Heintzelman & Howard 1948). Cuticular characters have been employed in the classification of sterile material down to the genus level in the Malesian *Icacinaceae*, including 28 genera outside Malesia (van Staveren & Baas 1973; Baas 1974). Contributions to leaf anatomy, other than epidermal structure, are scanty and relatively superficial (Solereder 1908; Scala 1917; Gerhard 1902), or have been neglected in systematical works on the *Icacinaceae* (Potgieter & van Wyk 1999). Lens & al. (2008), consequently, reported that the wood structure and anatomical characters of the stem of this family (including the genus *Icacina*) could be regarded as key features for intrafamily classification.

Among the African *Icacinaceae*, Potgieter & van Wyk (1999) have studied and described the leaf anatomy of eight species from the genera *Apodytes*, *Cassinopsis* and *Pyrenacantha*, which are trees and shrubs. Though Potgieter & van Wyk (1999) worked on these genera, the genus *Icacina* was not part of their study. Earlier studies have focused only on the wood structure and anatomical characters of *I. mannii* and *I. classeni* from West Africa (Lens & al. (2008). It has been difficult to distinguish the other members of the genus because of their close morphological characters (Akobudu & Agyakwa 1998; Akobudu & al. 2016). The epidermal structure, leaves and anatomy of the West African species of the genus *Icacina* are yet to be described. Therefore, this research work is aimed at describing the morphological features, epidermal characteristics, leaf anatomy, and histological characters of *I. mannii, I. oliviformis,* and *I. trichantha,* in order to aid the identification of these three species in West Africa.

Material and methods

Sample collection and study area

Samples of the *Icacina* species were collected from the environs of the University of Port Harcourt, Nigeria (University of Port Harcourt Biodiversity Conservation Centre and Faculty of the Agriculture Farm). The plants were properly identified, processed and deposited in the University of Port Harcourt Herbarium (Table 1). The analysis was carried out in the Plant Taxonomy and Biosystematics Research Laboratory, Department of Plant and Biotechnology, Faculty of Science, University of Port Harcourt, between February 2015 and April 2020.

Table 1. Voucher specimens of the studied *Icacina* species.

Species name	Locality	Collection date	Name(s) of collector	Herbarium number
I. oliviformis	University of Port Harcourt Biodiversity Conservation Centre	10/05/2019	Ekeke, C. & Joseph, T. O.	UPH/1123
I. mannii	University of Port Harcourt Biodiversity Conservation Centre	20/05/2019	Ekeke, C. & Joseph, T. O.	UPH/1402
I. trichantha	Faculty of Agriculture Farm, University of Port Harcourt	04/07/2019	Ekeke, C. & Nichodemus, C. O.	UPH/1056

Morphological study

The quantitative characters of the vegetative and reproductive parts were measured and recorded. Such traits like habit, habitat, leaves arrangement and type, morphology (leaf length, leaf width, petiole length, and degree of hairiness), inflorescence and flower, scent, hairiness, friut (size and hairiness), and size of tubers were recorded from 100 plant specimens. The range and average values were calculated using Microsoft Excel 2010. The terminology for the morphological description followed Hutchinson & Dalziel (1954), Davis & Heywood (1973), and Priti & Shital (1979). Photographs of the plants were taken and documented by a Canon digital camera.

Epidermal studies

Fresh foliar material for epidermal studies was collected from plants growing in the wild. The adaxial and abaxial epidermises were peeled, stained with 1%

Safranin or Alcian Blue, rinsed with distilled water to remove excess stain, mounted in a drop of pure glycerine on clean glass slides, placed under coverslips and sealed with nail varnish to prevent dehydration (Okoli & Ndukwu 1992). Twenty good slides were observed using a trinocular research microscope (T340B) fitted with Amcope digital camera. The epidermal features followed Metcalfe and Chalk (1979) determinations and the stomatal types are according to Malvey (2004) and Dilcher (1974). The length and width of 200 stomata and their complex were measured with graticule, and recorded. The mean and standard deviation was calculated by Microsoft Excel 2010.

Anatomical studies

One hundred (100) slides from twenty petioles, midribs, roots, and corms from mature plants and young stems were prepared and observed. The samples were fixed in FAA (formaldehyde: glacial acetic acid: ethanol in the ratio of 1:1:18 parts of 70 % ethanol v/v) for at least 48 hours. The samples were washed in several changes of distilled water, dehydrated in alcohol series (30 %, 50 %, 70 %, and 100 %) solution for two hours per series and embedded in wax. Sections were cut with a Leitz 1512 rotary microtome, with thickness between 15–20 µm. The selected sections were de-waxed and stained with 1 % Safranin O and counterstained with Alcian Blue, mounted on slides and micro-photographed with a trinocular research microscope (T340B) fitted with Amcope digital camera.

Histological localization of tannin

Thin microtome sections were fixed in a mixture of iron II sulphate and formalin ($FeSO_4$ + formalin). The darkly stained areas indicated the presence and occurrence of tannin (Okoli 1988).

Histological localization of starch

The test for starch was limited to only the corm of each species of *Icacina* species. Cut sections of the corms were stained with a mixture of potassium iodide and iodine (KI + iodine). The presence, distribution, and localization of starch grains in the different areas of the corm were noted and recorded, and photomicrographs were taken using a trinocular research microscope (T340B) fitted with Amscope digital camera.

Results

Macro-morphological description

I. mannii **(Fig. 1).** A scandent forest and forest re-growth, and swamp-forest shrub, 0.8–3.4 m high, leaves alternate and leaf stalks 0.4–1.2 cm long (Fig. 1a). Leaves broadly obovate-elliptic, abruptly acuminate at apex, more or less cuneate or acute at base, 11.3–22.5 cm long and 5.4–12.0 cm wide, glabrescent or sparsely hairy beneath. Flowers sessile, 5-merous, creamy, with scent, occurring in axillary/terminal clusters or short dense cymes towards the apex of the stem (Fig. 1b). Calyx much shorter than the petals; petals appressed-pubescent outside. Fruits

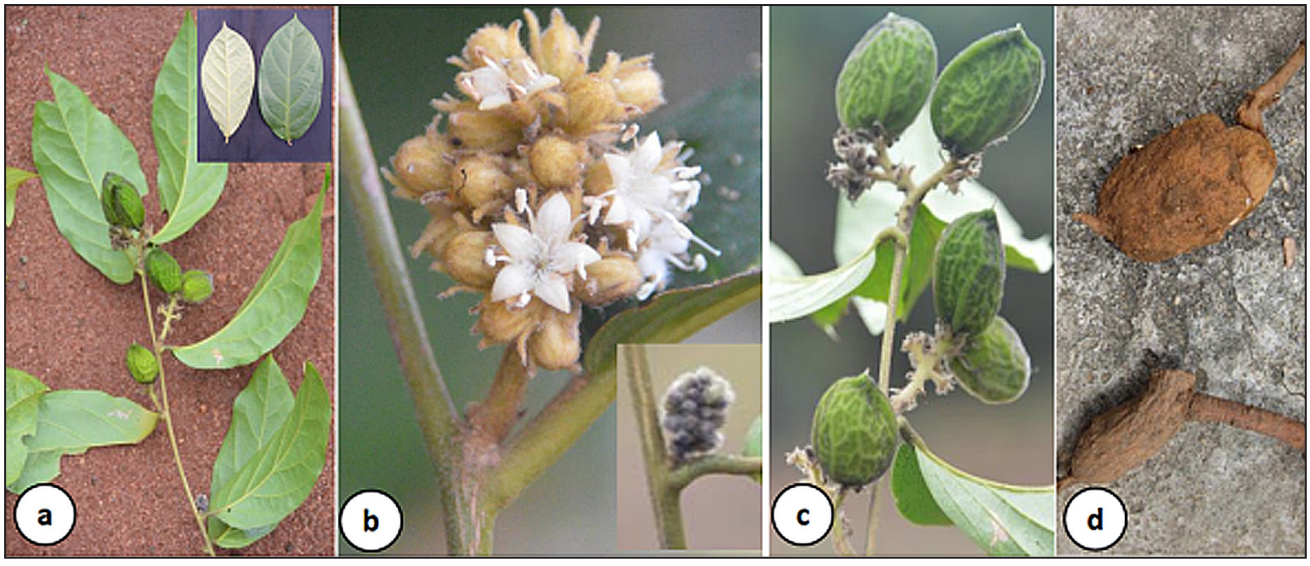


Fig. 1. Morphological features of *I. mannii* (**a**) habit, (**b**) inflorescence, (**c**) unripe fruits, (**d**) corm.

very hairy, rough, 2.0–2.5 cm long, and 1.4–2.0 cm wide (Fig. 1c). The tubers large and cylindrical, and weigh 0.2–0.4 kg (Fig. 1d).

***I. oliviformis* (Fig. 2).** A forest, forest regrowth and swamp-forest scandent shrub, with glabrous to partly pubescent stem, 0.3–0.9 m tall or more, leaves alternate and leaf stalks 0.6–2.0 cm long (Fig. 2a). Leaves lanceolate to obovate, base acute, apex acuminate to cuspidate and emarginate, 13.4–22.2 cm long, 7–114.2 cm wide, conspicuously reticulate and glabrous or nearly so, with galls (Fig. 2b). Flower 5-merous, creamw, on a short stalk, occurring in axillary clusters or short dense cymes; calyx short; petals shortly hairy outside. Fruits bright-red, obovoid to ovoid berries measuring about 2.5–3cm in length and 2–2.5cm in width, tomentellous, slightly wrinkled (Fig. 2c). The large underground fleshy tuber (corm) oval and weighs about 1.5–2.5 kg (Fig. 2d)

***I. trichantha* (Fig. 3).** A forest and forest regrowth scandent shrub, leaves alternate, leaf stalk 0.7–1.7 cm long (Fig. 3a). Leaves broadly elliptic or oblong, abruptly acute or cuspidate at the apex, rounded or cordate at base, 13.6–25.0 cm long, 5.7–13.3 cm wide, lower surface covered with long, non-glandular, fascicled-soft hairs. Flowers densely crowded on a stalk 2–3.5 cm long, subsessile and occurring on the older stem (Fig. 3b), creamy (Fig. 3c), calyx 0.2–0.4 mm long, nearly as long as the petals (Fig. 3c). Ripe fruits red and unripe fruits green; the underground tuber (corm) very large, oval and weighs 0.3–0.5 kg (Fig. 3d).

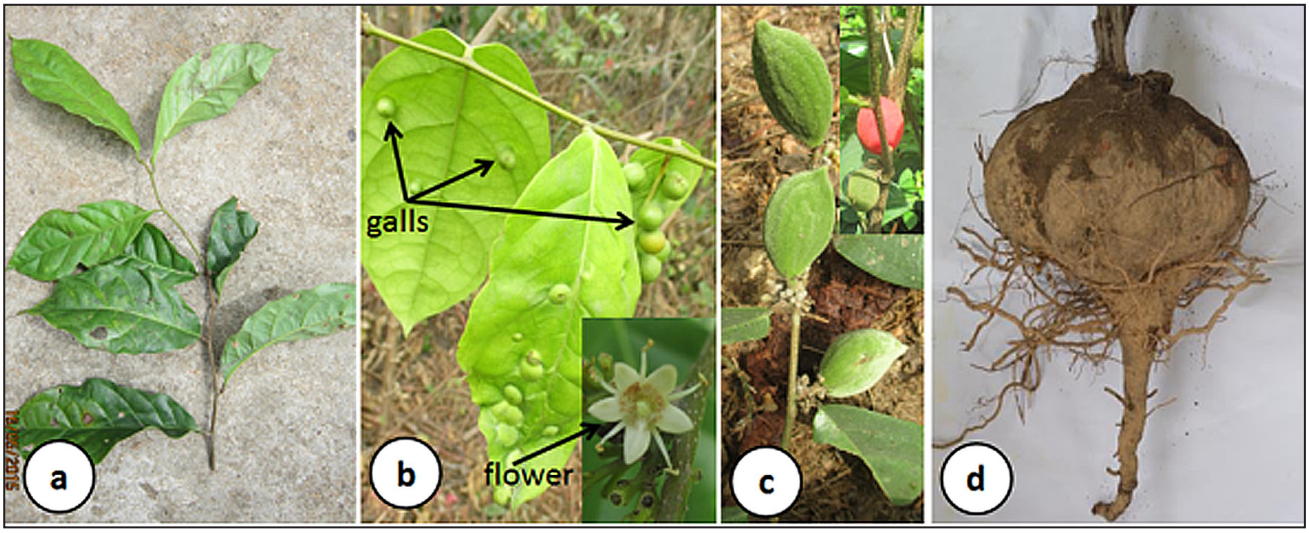
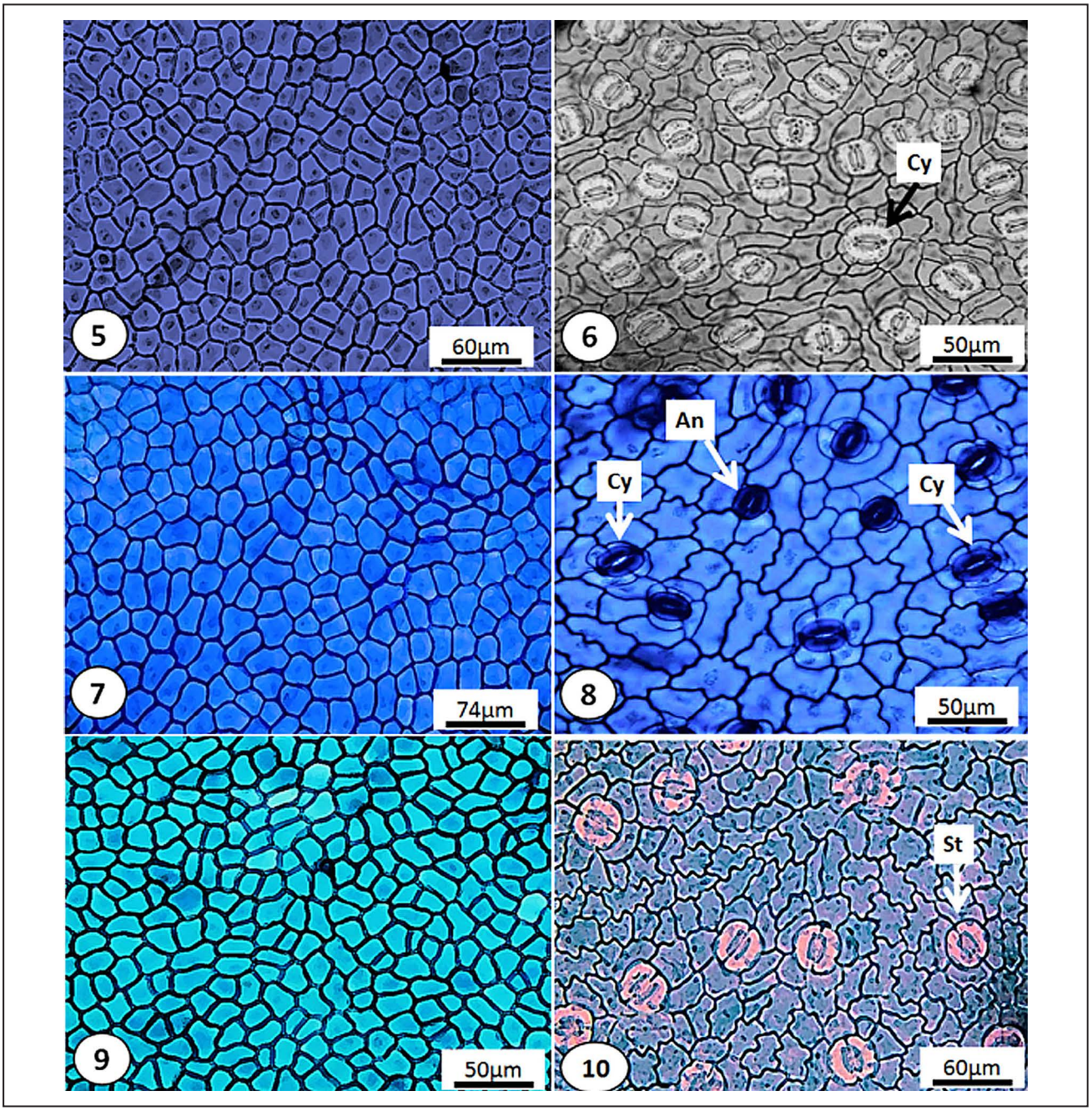


Fig. 2. Morphological features of *I. oliviformis* (**a**) habit, (**b**) leaves showing galls and flower, (**c**) ripe and unripe fruits and (**d**) corm.

Fig. 3. Morphological features of *I. trichantha* (**a**) habit, (**b** and **c**) inflorescence, (**c**) and (**d**) corm.

Leaf epidermal characteristics

All studied *Icacina* species have dorsiventral leaves and are hypostomatic (Figs 5-10 and Table 2). Stomatal index varied among the species: *I. manni* (28.33), *I. oliviformis* (15.29), and *I. trichantha* (8.77). Stomatal complex (guard cells and subsidiary cells) of the species ranged from 25.70–29.29 µm in length to 22.34–30.07 µm in width (Table 2). The adaxial epidermis of the leaves of all three species had cyclocytic and anomocytic stomata. Furthermore, *I. oliviformis* and *I. mannii* have tetracytic stomata, while *I. trichantha* has staurocytic stomata. The abaxial epidermal cells of the three *Icacina* species are pentagonal to polygonal, with straight to curved anticlinal walls. The abaxial epidermal cells have polygonal to irregular in shape, with wavy anticlinal walls (Figs 5-10 and Table 3).



Figs 5-10. Epidermal characteristics of *Icacina* species: (**5**) Upper epidermis of *I. mannii* with polygonal epidermal cells, (**6**) Lower epidermis of *I. mannii* with irregular epidermal cells, (**7**) Upper epidermis of *I. oliviformis,* (**8**) Lower epidermis of *I. oliviformis,* (**9**) Upper epidermis of *I. trichantha*, (**10**) Lower epidermis of *I. trichantha* with irregular epidermal cells and undulating anticlinal walls. **Cy** – cyclocytic stomata, **St** – staurocytic stomata, An – anomocytic stomata.

Table 2. Stomatal size, index and types in the studied *Icacina* species.

Species name	Leaf surface	Stomata type	Stomatal index	Stomatal size (µm)		Stomatal complex (µm)	
				Length	Width	Length	Width
I. mannii	Abaxial	Tetracytic, cyclocytic, and anomocytic.	28.33	21.17–31.75 (25.70±2.99)	26.46–31.75 (27.71±2.28)	40.24–85.56 (64.39±9.99)	40.92–84.40 (58.48±9.29)
	Adaxial	–	–	–	–	–	–
I. oliviformis	Abaxial	Tetracytic, cyclocytic, and anomocytic.	15.29	22.49–28.63 (25.78±1.87)	19.95–24.77 (22.34±1.93)	36.41–102.77 (56.50±19.83)	29.16–61.38 (42.29±10.62)
	Adaxial	–	–	–	–	–	–
I. trichantha	Abaxial	Tetracytic, anomocytic, and staurocytic.	8.77	24.67–35.00 (29.29±2.89)	20.99–36.47 (30.07±2.94)	48.08–77.82 (61.99±8.28)	47.27–68.55 (57.99±5.92)
	Adaxial	–	–	–	–	–	–

Table 3. Description of epidermal cells and trichomes in the studied *Icacina* species.

Species name	Leaf surface	Epidermal cells	Trichome Type	Abundance	Epidermal size (µm)		
					Size (µm)	Length	Width
I. mannii	Adaxial	Irregularly shaped anticlinal walls	Simple non-glandular unicellular hairs and sessile stellate hairs	++	8.1–76.0 (32.4±26.2)	15.88–42.33 (29.48±6.93)	5.29–21.17 (12.09±3.56)
	Abaxial	Pentagonal to hexagonal, with straight to curved anticlinal walls		++		10.58–31.75 (22.42±5.70)	10.58–21.17 (13.73±3.32)
I. oliviformis	Adaxial	Polygonal to irregular in shape, with wavy anticlinal walls	Simple non-glandular unicellular hairs	+	51–146 (66.3±27.1)	22.75–61.33 (36.73±8.51)	9.68–28.15 (20.39±4.76)
	Abaxial	Pentagonal to hexagonal, with straight to curved anticlinal walls		+		13.90–40.90 (25.39±5.82)	8.10–19.39 (13.48±3.75)
I. trichantha	Adaxial	Irregularly shaped anticlinal walls	Simple non-glandular unicellular hairs and short-stalked stellate hairs	+++	25.6–106 (67.7±28.5)	17.10–36.00 (25.31±5.01)	8.30–21.20 (13.93±2.87)
	Abaxial	Pentagonal to polygonal, with straight to curved anticlinal walls		+++		12.37–29.38 (21.59±4.17)	7.19–18.66 (13.11±2.18)

Keys: + = present, ++ = abundant and +++ = more abundant.

Petiole anatomy

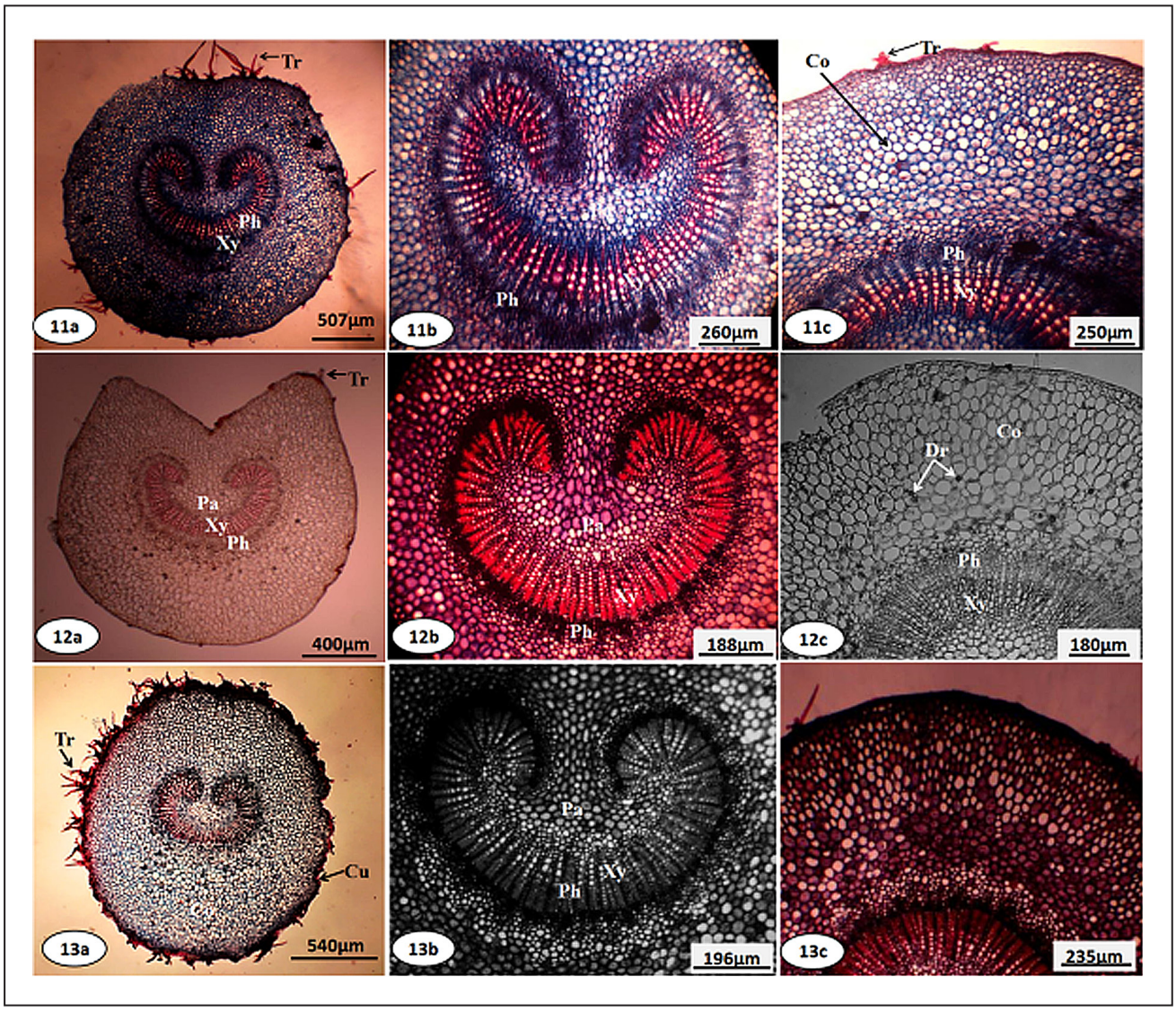
Generally, the petiolar vascular bundles of all three studied species have invaginated ends forming an island (Figs 11-13, Table 4). They are hairy in *I. mannii* (Fig. 11a), partly hairy or glabrous in *I. oliviformis* (Fig. 12a), and very hairy in *I. trichantha* (Fig. 13a). The shape of the petioles varied slightly among the studied species. For instance, the shape of the petiole is circular in *I. mannii* and *I. oliviformis,* but oval in *I. trichantha.* The adaxial cuticle outline in *I. trichantha* is convex, V-shaped in *I. oliviformis,* and flat/concave in *I. mannii*.

Midrib anatomy

The midrib of all three species contained deeply stained fibres, as well as xylem and phloem tissues. The outline of the adaxial midrib of *I. mannii* is flat (Fig. 14a), concave in *I. oliviformis* (Fig. 15a), and flat to convex in *I. trichantha* (Fig. 16a). The vascular bundles in midrib formed a closed semi-circle in *I. mannii* and *I. oliviformis*

Table 4. Characteristics of the lamina in the studied *Icacina* species.

Plant part	*I. oliviformis*	*I. mannii*	*I. trichantha*
Layers of spongy mesophyll	1	1–2	1–2
Layers of palisade mesophyll	6–8	7–8	8–9
Thickness of lamina (µm)	142–147	34–35	38–40
Percentage of palisade cells	13.53–17.02	24.68–25.70	15.85–16.45
Nature of palisade mesophyll	Loosely packed, 16–28 µm thick	Closely packed, 37.03–47.61 µm thick	Closely packed, 39.03–47.61 µm thick
Nature of spongy mesophyll	Loosely packed with air spaces, armed cells, 86–96 µm thick	Loosely packed with air spaces, armed cells, 111.09–121.67 µm thick	Loosely packed with air spaces, armed cells, 132.25–142.83 µm thick
Upper epidermis	1-layer, periclinally elongated, 13–24 µm long, 9–12 µm thick	1-layer, oval in shape, 5.29–15.87 µm long, 10.58 µm thick	1-layer, oval in shape, 5.29–15.87 µm long, 10.58 µm thick
Lower epidermis	1-layer, periclinally elongated, 13–24 µm long, 9–12 µm thick	1-layer, 10.58 µm long, 5.29–10.58 µm thick	1-layer, periclinally elongated, 10.58–15.87 µm long, 5.29–10.58 µm thick



Figs 11-13. Transverse section of petiole; (**11**) *I. mannii*, (**12**) *I. oliviformis*, (**13**) *I. trichantha,* **Pa** – parenchyma, **Xy** – xylem, **Ph** – phloem, **Dr** – druses, **Co** – cortex, **Tr** – trichome, **Cu** – cuticle.

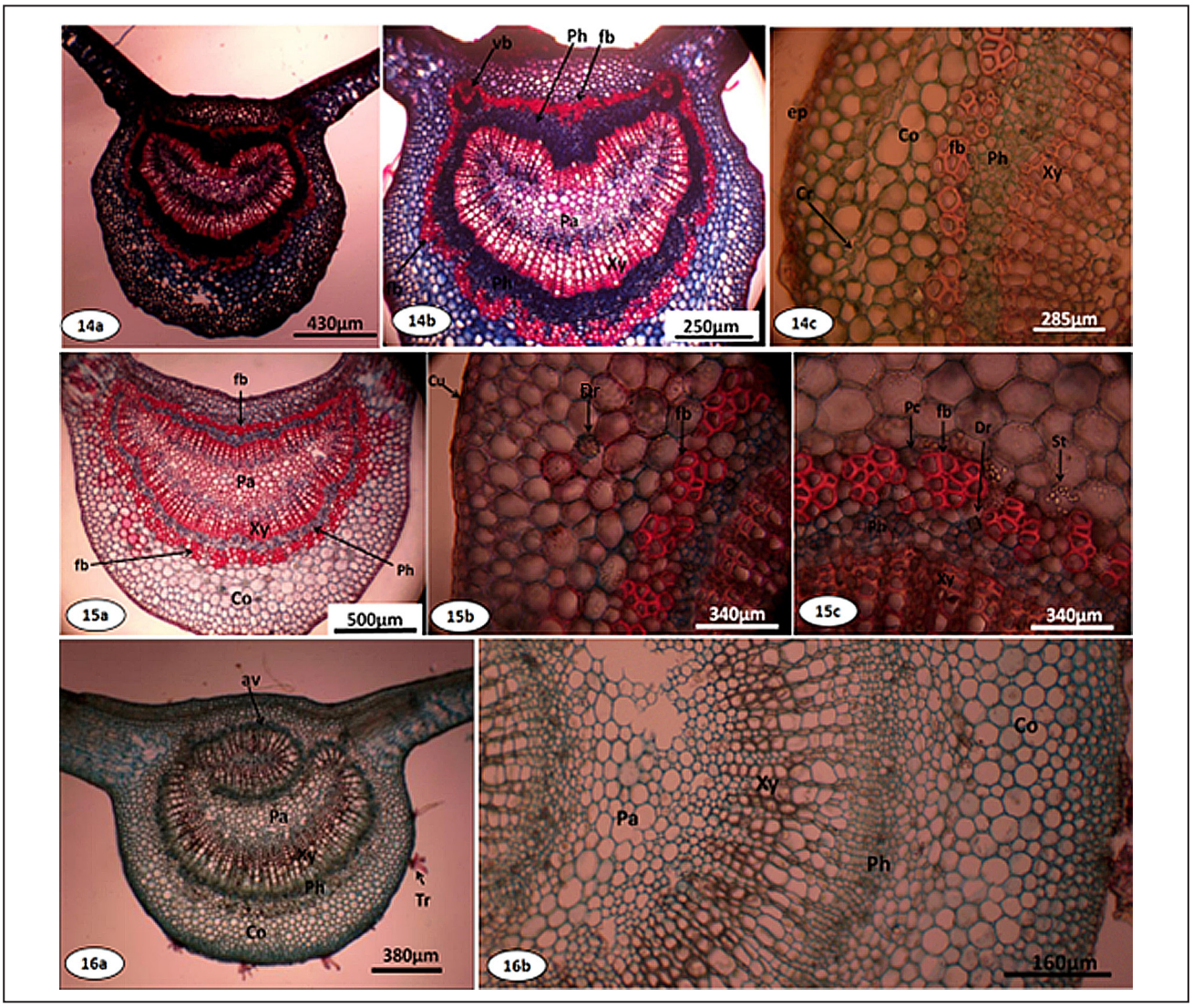
(Figs 14a and 15a), but with two adaxial rib traces in *I. mannii* (Fig. 14b). In *I. trichantha*, the vascular bundle formed a semi-circular arc with a central adaxial cylinder (Fig. 16a). The cortex parenchyma in *I. mannii* consisted of 7–8 layers, with crushed parenchyma (Fig. 14c), 6–8 layers in *I. oliviformis* (Fig. 15b), and 16–20 layers in *I. trichantha* (Fig. 16b).

Leaf lamina

The lamina of all studied species was dorsiventral and the spongy mesophyll was loosely packed with air spaces with epidermal mucilage (Fig. 17). The palisade mesophyll cells in *I. oliviformis* were loosely packed (Fig. 17b), while in *I. mannii* (Fig. 17a) and *I. trichantha* (Fig. 17c) the palisade mesophyll cells were closely packed. The bundle sheets were embedded in the spongy mesophyll in *I. oliviformis* and *I. trichantha*, but extended through the palisade mesophyll to the adaxial epidermal surface in *I. mannii*.

Stem anatomy

The stem anatomy of all three *Icacina* species showed persistent or patches of sclerenchymatous fibres in the outside portions of the vascular bundles. The vessels were pronounced, mainly solitary, with no visible rays (Fig. 18). They occurred seldom in radial pairs or multiples in *I. mannii* (Fig. 18a), in tangential and radial pairs in *I. oliviformis* (Fig. 18b), and in tangential pairs and radial multiples of 2–6 vessels in *I. trichantha* (Fig. 18c).



Figs 14-16. Midrib anatomy of the studied *Icacina* species: (**14**) *I. mannii,* (**15**) *I. oliviformis,* (**16**) *I. trichantha,* **Co** – cortex, **fb** – fibre, **av** – adaxial vascular bundle, **Tr** – trichome, **Xy** – xylem, **Pa** – parenchyma, **Ph** – phloem, **St** – crystal cluster, **Dr** – druses, **Cr** – crushed parenchyma, **vb** – vascular bundle, **ep** – epidermis, **Cu** – cuticle.

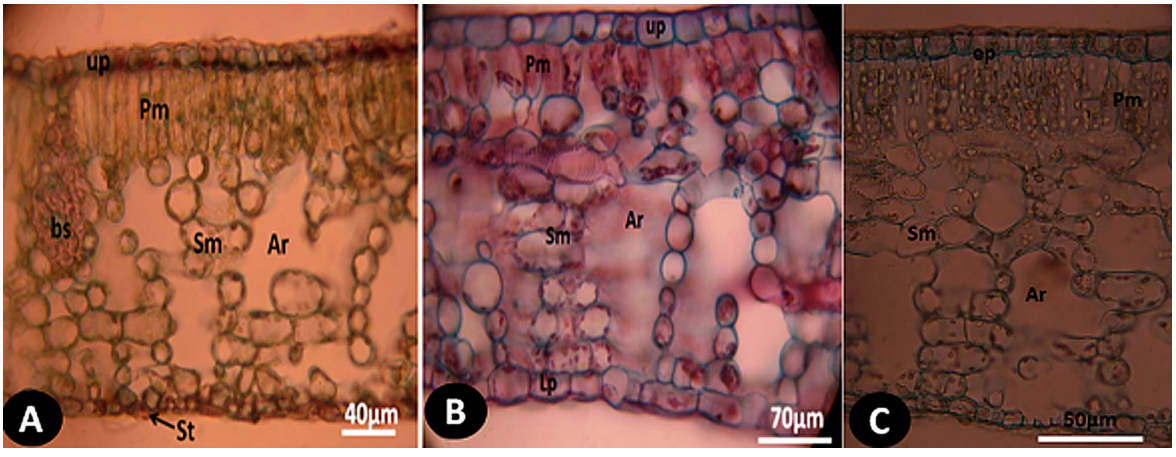


Fig. 17. Leaf lamina of the studied *Icacina* species: (**a**) *I. mannii,* (**b**) *I. oliviformis,* (**c**) *I. trichantha* (Note: **St**- stoma, **Ar**- air space, **Lp**- lower epidermis, **Sm**- spongy mesophyll, **bs**- bundle sheet, **up**- upper epidermis, **Pm**- palisade mesophyll).

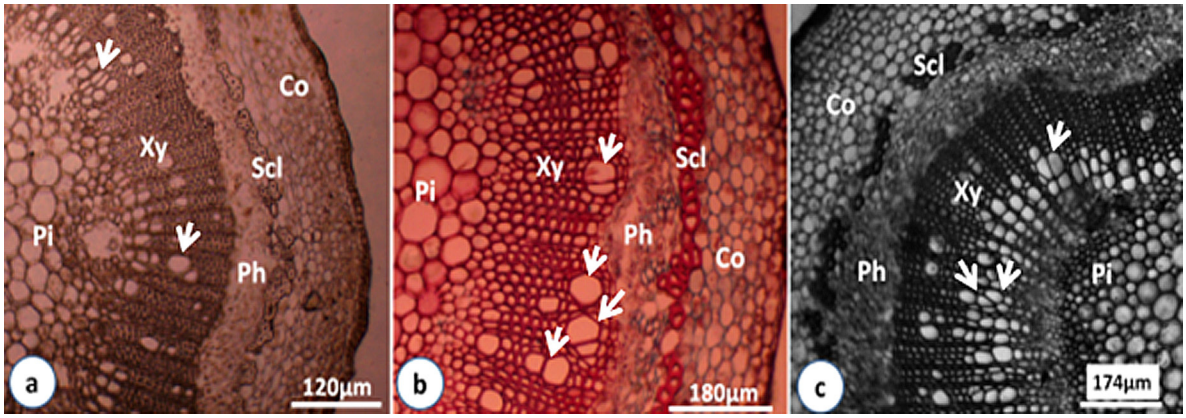


Fig. 18. Stem anatomy of the studied *Icacina* species: (**a**) *I. mannii,* (**b**) *I. oliviformis*, and (**c**) *I. trichantha*, **Pi** – pith, **Co** – cortex, **Scl** – fibre, **Ph** – phloem, **Xy** – xylem, and arrows show vessels.

Root and corm anatomy

The roots of all species have some similar anatomical features, namely, starch grains, tannins and secretory canals (Fig. 19). There is abundance of secretory canals in *I. mannii* (Fig. 19a and b) and *I. oliviformis* (Fig. 19c and d). *I. trichantha* has thick-walled sclereids, with obvious secretory canals (Fig. 19e and f). The corms have patches of vascular bundles containing tannins (Figs 33-35). *I. mannii* had scanty thick-walled sclereids in the parenchymatous cortex (Fig. 33), and in *I. oliviformis* (Fig. 34) and *I. trichantha* (Fig. 35), the thick-walled sclereids are in abundance in the parenchymatous cortex.

Calcium oxalate, tannins and starch distribution

In this study, calcium oxalate crystals (druses) are observed only in the petioles (Fig. 12c) and midrib (Fig. 15b and c) of *I. oliviformis.* Tannins are found mainly in stem, midrib, petiole, and root of the species (Figs 20-34), while starch grains mainly occurred in the corms (Figs 35-37). Tannins are concentrated in the xylem vessels, with patches in the endodermis, fiber cells, and parenchymatous cortex. The presence of these compounds (their abundance) varied slightly amongst the species. Variation in concentration in the different parts of the plants is presented in Table 5.

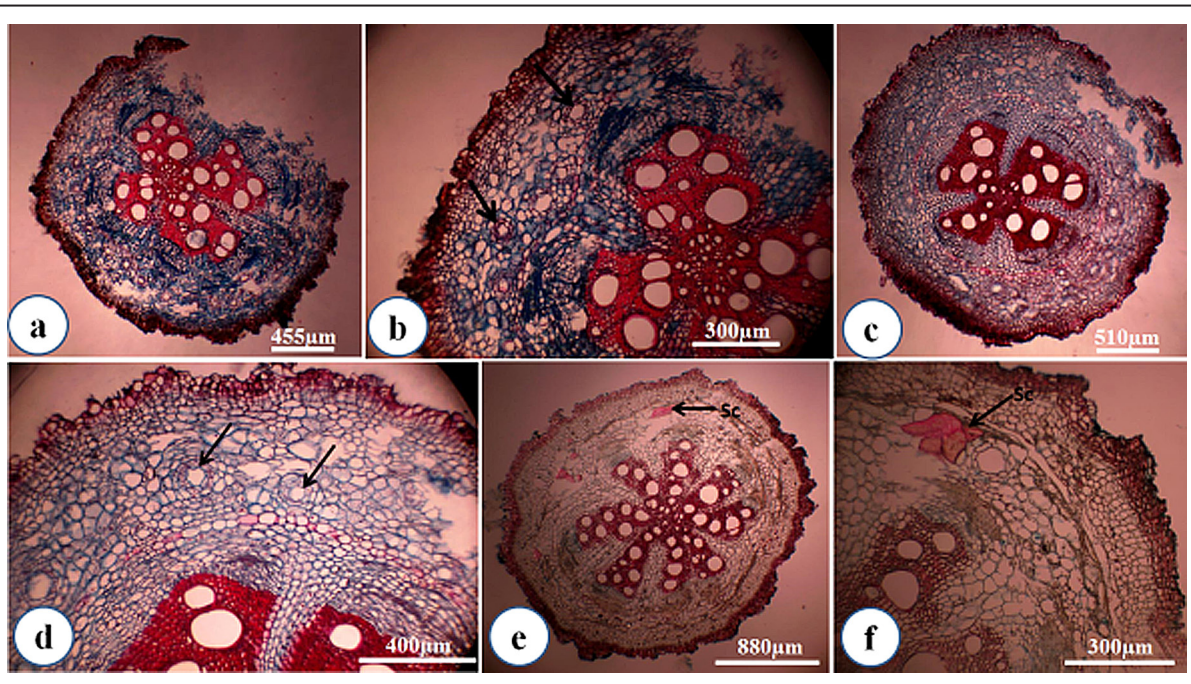
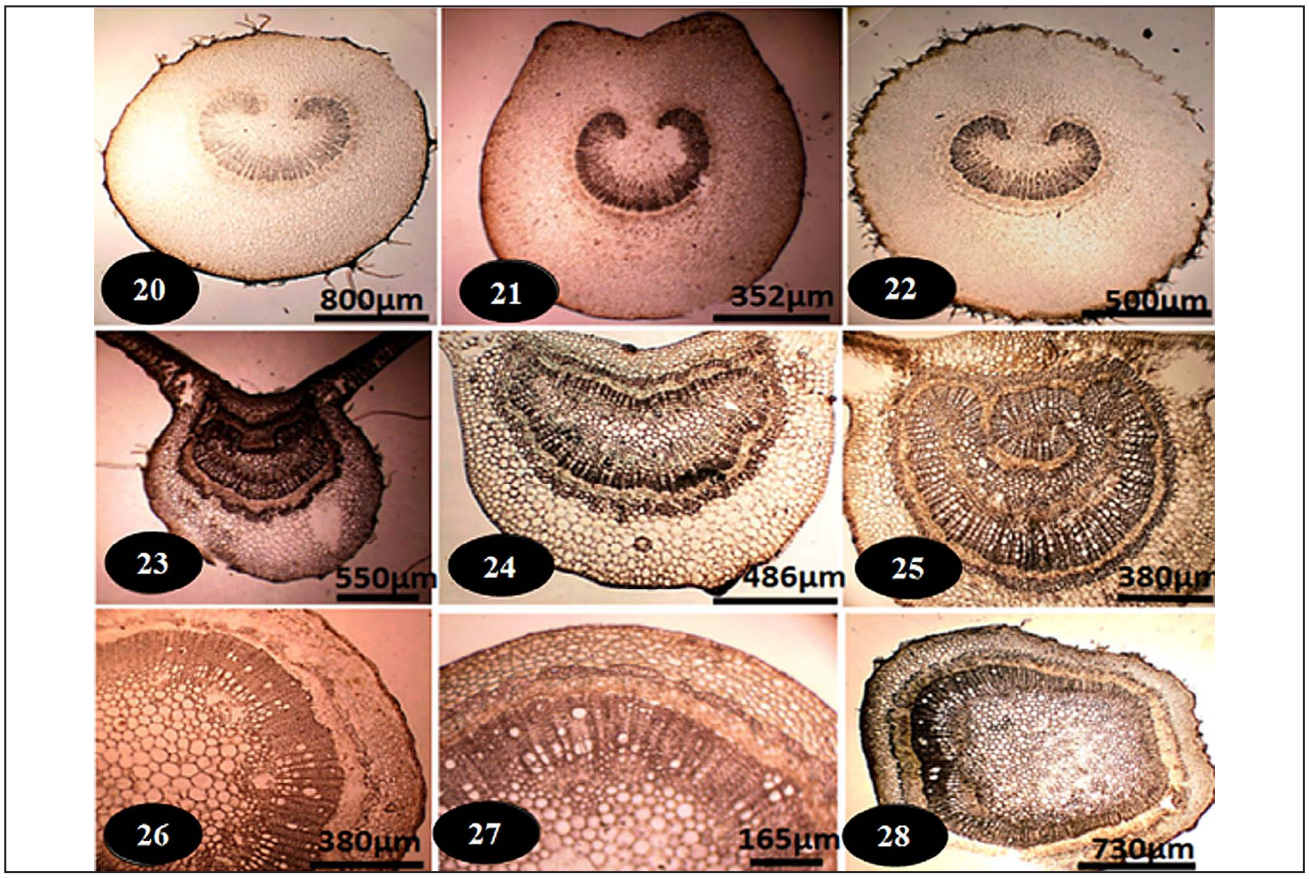
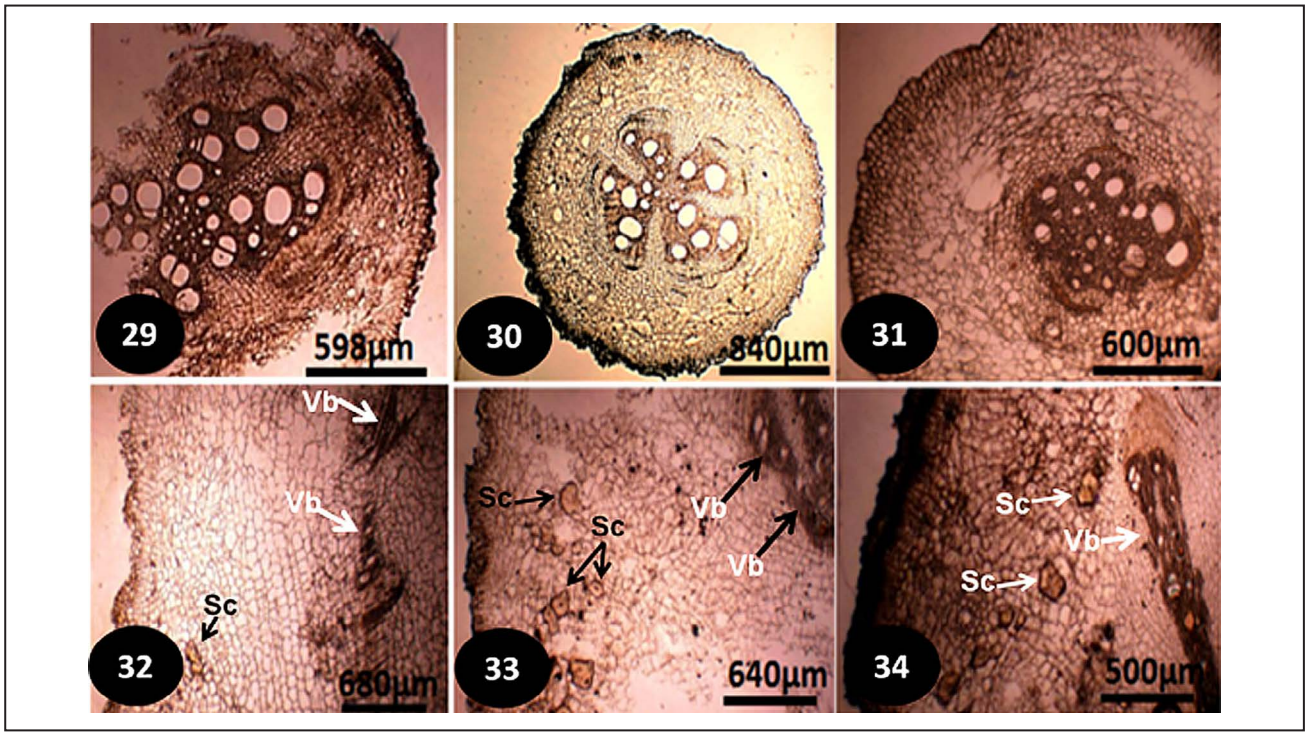


Fig. 19. Cross-section of *Icacina* roots: (**a** and **b**) *I. mannii* (arrows show secretory canals), (**c** and **d**) *I. oliviformis* (arrows show secretory canals) and (**e** and **f**) *I. trichantha* (arrows show thick-walled sclereids), **Sc** – sclereids.



Figs 20-28. Tannin distribution is indicated by darkly stained areas in the petiole, midrib, and stem of the *Icacina* species: (**20**, **23** and **26**) *I. mannii,* (**21**, **24** and **27**) *I. oliviformis* and (**22**, **25** and **28**) *I. trichantha.*



Figs 29-34. Tannin distribution in the studied *Icacina* species: (**29–31**) root, (**32–34**) corm. Tannins are indicated by the darkly stained tissues: (**29** and **32**) *I. mannii,* (**30** and **33**) *I. oliviformis* and (**31** and **34**) *I. trichantha.* **Vb** – vascular bundle containing tannins, and **Sc** – sclereids.

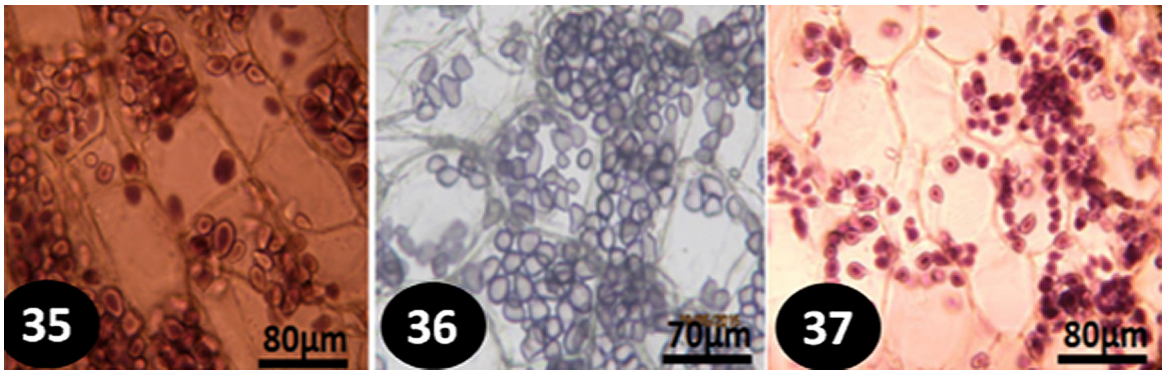
Table 5. Distribution of tannins and starch in the studied *Icacina* species.

Compound	Plant part	Species name		
		I. mannii	*I. oliviformis*	*I. trichantha*
Tannin	Petiole	+	++	+++
	Stem	++	+	+++
	Midrib	++	++	+++
	Root	++	+	+++
	Corm	+	++	+++
Starch	Corm	+	+++	++

Keys: + = present, ++ = abundant and +++ = more abundant.

Trichome types

The trichomes observed in this study varied in types and abundance among the species (Fig. 38, Table 3). They included simple non-glandular, uncinate, and multi-armed (sessile or short-stalked stellate) hairs. Trichomes are more abundant in *I. trichantha,* followed by *I. mannii. I. oliviformis* was the least hairy (Table 3). The length of trichomes slightly varied among the taxa. Simple non-glandular unicellular hairs (Figs 38a and b) occurred in all studied species,



Figs 35-37. Starch distribution in the corm of the studied *Icacina* species: (**35**) *I. mannii,* (**36**), *I. oliviformis* and (**37**) *I. trichantha*.

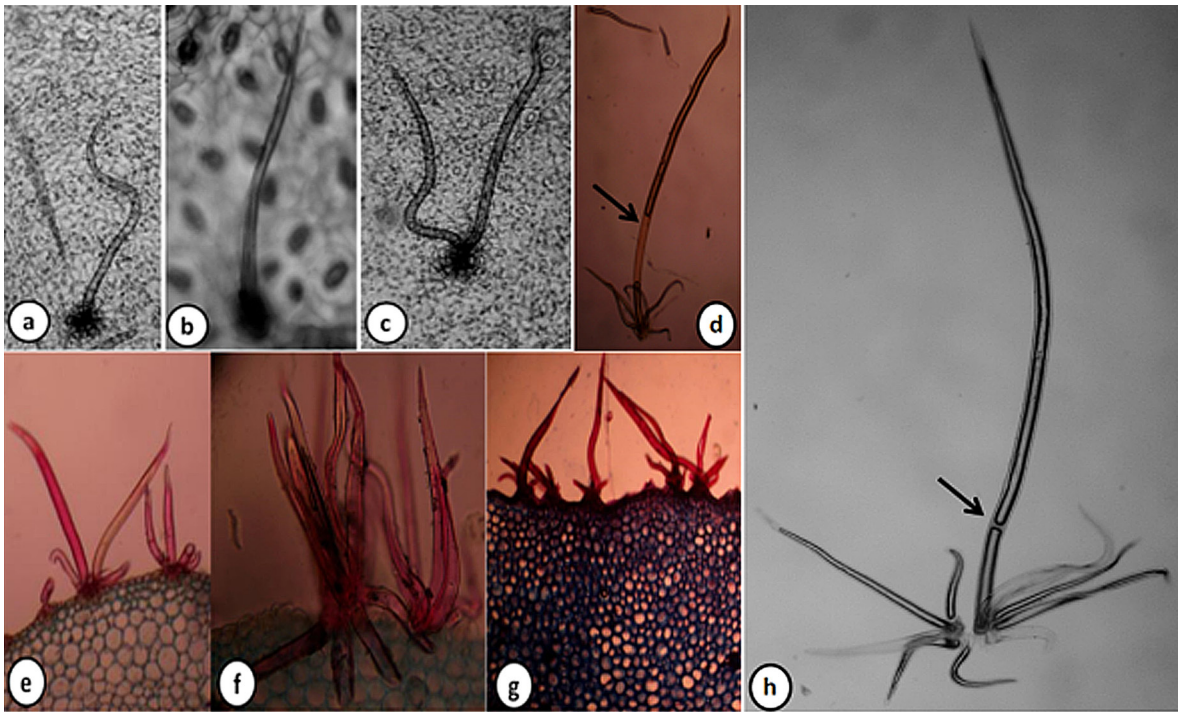


Fig. 38. Trichomes observed in the *Icacina* species: (**a**-**b**) simple non-glandular unicellular trichomes, (**c**-**h**) stellate non-glandular trichomes. Arrows show biseriate arms.

as well as sessile stellate hairs. Sessile stellate hairs with unicellular arms (Figs. 38c) and biseriate arms (Fig. 38d and h) occurred in *I. mannii,* while short-stalked stellate hairs with unicellular arms (Fig. 38 e-g) occurred in *I. trichantha.*

Discussion

In this research, such morphoanatomical characters as the dorsiventral leaf, collateral vascular bundles, presence of non-glandular trichomes (in the young stems, leaves and petioles), starch grains, shape of the epidermal cells, shape of the vascular bundles in the petioles, alternate leaf arrangement, etc. are similar in all *Icacina* taxa. However, the following characters could be emphasized as useful to distinguish the species: midrib vascular bundle arrangement, adaxial outline of the petioles, percentage of palisade cells, thickness of the lamina, presence or absence of druses in the petiole and midrib, size of the epidermal cells, stomatal type and size, stomatal index and complex.

Morphologically, the studied species could be distinguished by their leaf shape, size and shape of the corms, fruit morphology (shape, size, wrinkles, and hairiness), and position of the inflorescence.

In *Icacinaceae*, the differences found in the epidermal characteristics, stomatal types, leaf and wood anatomy have always been discussed at generic and family levels (Bailey & Howard 1941; van Staveren & Baas 1973; Baas 1974; Potgieter & van Wyk 1999; Lens & al. 2008). The size of the epidermal cells on both adaxial and abaxial leaf surfaces varied among the species. In this study, the authors observed tetracytic and anomocytic stomata in all species. However, cyclocytic stomata were only found in *I. mannii,* paracytic stomata in *I. oliviformis* and staurocytic stomata in *I. trichantha.* The existing reports by Potgieter & van Wyk (1999) on the leaf structure of three South African genera of *Icacinaceae* using light and scanning electron microscopy had shown that the stomatal and trichome types, and lamina characters (such as mucilage cells, pectic warts and 'unidentified cell inclusions') were diagnostic characters for these genera. According to their findings, both *Cassinopsis* Sond. and *Pyrenacantha* Hook. had cyclocytic stomata, as opposed to the anomocytic type in *Apodytes* E. Mey. Ex Arn. The presence of a stomatal ridge in *A. dimidiata* was a useful character in separating this species from the other two South African members of that genus. Also, in the epidermal characters of 109 species of the Malesian *Icacinaceae* and the genus *Pennantia* from Australia and New Zealand, great diversity was encountered in the stomatal types, including paracytic, anomocytic, cyclocytic, anisocytic, helicocytic, and several intermediate stomata types. The paracytic and anomocytic stomata were restricted to a few genera and were primitive for the family, while the cyclocytic stomata were most frequent in the different genera (van Staveren & Baas 1973; Baas 1974).

In another study based on a comparative anatomical investigation of the leaf lamina and petiole of six genera of *Icacinaceae* in West Malaysia, Teo & Haron (1998) reported that these genera can be classified into four closely-knit groups by virtue of their many shared characters, such as the shape of the vascular bundles in both midrib and petiole, presence of funnel-shaped palisade cells, foliar sclereids, tannin crystals, a hypodermis layer and accessory or wing bundles in the petiole. They also mentioned that there is variability in the petiole outline, midrib and stem anatomy, including vascular bundle arrangement in the midrib of these species.

Anatomical characters of the stem have been considered key features for the intrafamily classification of *Icacinaceae* (Lens & al. 2008). Lens & al. (2008) noted that wood structure of the four *Icacinoideae* subfamilies (Icacineae, Iodeae, Sarcostigmateae, and Phytocreneae) does not offer straightforward characters to define the family boundaries, because *Icacinaceae* s. str. are probably not monophyletic (Kårehed 2001). Nevertheless, some wood features merit special emphasis because of their predictive value to assign a species to one of the four subfamilies. Also, within *Icacinaceae* s. str., the Icacina-group (including the bulk of the climbing genera plus some non-climbing ones) is rather homogeneous and can not be easily distinguished from the other *Icacinaceae* lineages, due to a combination of anatomical characters. These include simple vessel perforations, solitary vessels plus tangential multiples, a tendency to alternate vessel pitting, and relatively short vessel elements and fibers (Kårehed 2001). In another member of *Icacinaceae*, Patel and Bowles (1978) reported more or less evenly distributed vessels, angular, and mostly solitary, with frequent false tangential pairs, owing to the overlapping oblique end walls, sometimes in radial multiples of 2–3, occasionally in tangential

pairs. In our study, we have observed mainly solitary vessels with no visible rays, seldom in radial pairs or multiples in *I. mannii,* tangential and radial pairs in *I. oliviformis*, and tangential pairs and radial multiples of 2–6 vessels in *I. trichantha*.

This study confirms non-occurrence of druses in *I. mannii* as reported by Lens & al. (2008), presence of druses (calcium oxalate crystal) in *I. oliviformis* only in the midrib and petiole of this species, and absence of druses in *I. trichantha.* Lens & al. (2008) have also observed the presence of crystals in clusters: raphides and prismatic crystals. In this study, the authors have recorded a cluster of crystals in the midrib of *I. oliviformis.* Their finding is in conformity with Metcalfe and Chalk (1950), who reported absence of druses in most members of *Icacina,* and with Lens & al. (2008), who reported that druses are absent in *I. mannii.*

Abundance of sclereids differed from species to species in the studied corm of the species, and thick-walled sclereids were observed in the roots of *I. trichantha*. The *Icacina* species contained tannins and starch in varying amounts. These compounds have different medicinal properties (Ezeigbo 2010; Sarr & al. 2011, Timothy & Idu 2011; Onakpa & Asuzu 2013; Okoronkwo & al. 2014; Shagal & al. 2014) and starch is a major food substance (Fay 1991). Starch in the corms and tannins in the leaves and stems of the *Icacina* species have been confirmed in the diet of different peoples in Africa (Fay 1991, 1973; Ezeigbo 2010; Sarr & al. 2011; Timothy & Idu 2011; Onakpa & Asuzu 2013; Shagal & al. 2014; Okoronkwo & al. 2014).

The existing reports have shown that differences in the trichome (hair) ontogeny, type, size, shape, abundance, and distribution could be useful in delimiting the plants of the same family and genus (Levin, 1973; Payne, 1978; Werker, 2000; Yang and Ye, 2013; Wagner et al., 2004; Ma & al. 2016). These trichomes are epidermal appendages, unicellular or multicellular, branched or unbranched, glandular or non-glandular (Levin, 1973; Werker, 2000; Yang and Ye, 2013). Ma & al. (2016) have used trichome morphology, structure and ontogeny, diversity, and distribution to delimit 34 species of *Vitis* (*Vitaceae*). The indumentum of *Pyrenacantha* consists of simple (unmodified), 'globular' and 'uncinate' trichomes, whereas that of *Apodytes* and *Cassinopsis* consists of simple hairs. Mucilage cells were found only in members of *Apodytes*. Intercellular, predominantly wart-like pectic protuberances are present in the mesophyll of mature leaf samples of *A. geldenhuysii* van Wyk & amp; *Potgieter* and *Cassinopsis* (Potgieter & van Wyk 1999). Simple non-glandular unicellular hairs and sessile or short-stalked stellate hairs were observed in the studied species, varying from 8.1 μm in *I. mannii* to 146 μm in *I. oliviformis*. *I. trichantha* could be easily distinguished from *I. mannii* because of the presence of short-stalked stellate hairs and the length of trichomes.

The study on the *Icacina* species has shown some morphoanatomical similarities. Midrib vascular bundle arrangement, outline of the petioles, abundance of hair, percentage of palisade cells, thickness of lamina, presence or absence of druses in petiole and midrib, stomatal types, stomatal index, and presence of galls on the leaves of *I. oliviformis* are reliable characters for distinguishing the studied species.

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