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Research Article

Comparative foliar epidermal morphology of *Isolona* Engl. (Annonaceae)

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Abstract

A qualitative micromorphological assessment of the seven species of *Isolona* occurring in Nigeria and the Cameroons was undertaken with the aid of light microscope. The stomatal cells are particularly useful, providing stable characters which can be reliably employed in distinguishing the species. Hypostomatic leaves and paracytic stomatal type are generic constant. Possession of only paracytic stomata is characteristic of *I. dewevrei* whereas other species may have in addition another type such as 1+2 laterocytic stomatal type found only in *I. zenkeri* and presence of brachyparacytic stomata which shows relatedness of *I. campanulata*, *I. congolana*, *I. hexaloba, I. pleurocarpus* and *I. thonneri*. The epidermal surfaces appeared glabrous but an indication of hairs is shown by the presence of a glandular trichome base only in *I. hexaloba*. The epidermal cell characters such as epidermal cell shapes and anticlinal wall patterns seem to intergrade and they are not as definite as the stomatal cells. However, a combination of these features will be helpful in defining the species better and their leaf fragments can be differentiated based on the various characters studied for effective utilization in herbal medicinal research.

Keywords: epidermis; leaf; *Isolona*; micromorphology; stomata

Introduction

Annonaceae Juss. are a pantropical family of trees, shrubs, and lianas belonging to the order Magnoliales (APGII, 2003). The family comprises cca. 42 genera and around 400 species (Maas *et al.*, 2003; Bremer *et al.*, 2009; Smith *et al.*, 2010; Couvreur *et al.*, 2012; Zeng *et al.*, 2014;). Compared to the Neotropical and South-East Asian Annonaceae, the African taxa are largely understudied (Maas *et al.*, 2003; Couvreur *et al.*, 2006; Couvreur *et al.*, 2008). One of the African genera in this category is *Isolona* Engl., a sizeable genus with *ca.*21 species distributed across the tropical zone. *Isolona* Engl. flowers are hermaphroditic and are unusual as all six petals are basally connate, forming a single whorl; although an enclosed pollination chamber does not form, the petals are inwardly curved to form a loose chamber (Couvreur, 2009). The petals are yellowish or reddish and are often darker towards the inside of the flower. Relatively little is known about the reproductive biology, although the flowers of *I. campanulata* Engl. and Diels have been shown to be protogynous, with the reproductive phases extending over a 2-day (or possibly 3-day) period, with diurnal receptivity (Gottsberger *et*

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al., 2011). The species are mainly trees and there are accounts on exomorphology (Maas *et al.*, 2003; Couvreur, 2009), but data on the anatomy are very sparse (Metcalfe and Chalk, 1950; 1979). Together with the genus *Monodora* Dunal, it nested in the same clade (Richardson *et al.*, 2004) based on morphological, palynological and molecular data, some of the species are often confused and, in the past, they have been traditionally placed in the same sub family *Monodoroideae* Kostel. based on true syncarpous ovary.

In search for additional identification criteria, the characteristics of the leaf epidermis of 7 African species occurring in Nigeria and Cameroons were investigated for this study with a view to describe the species better and update the existing data on the family at large.

Materials and Methods

Seven species of *Isolona* obtained at the Forestry Herbarium Ibadan, Nigeria (FHI) and National Herbarium Yaounde, Cameroon (YA, IH) were used for the study (Table 1). The herbarium abbreviations follow Holmgren and Holmgren (2003).

Table 1. List of species used for the study

S/N	Taxa	Collector	Date of Collection
1	*Isolona campanulata*	P.T. Francis	Dec. 1945
2	*I. congolana*	Westphal	15/5/78
3	*I. dewevrei*	R. Letouvzey	8/7/75
4	*I. hexaloba*	R. Letouvzey	16/5/63
5	*I. pleurocarpus*	R. Letouvzey	16/4/68
6	*I. thonneri*	R. Letouvzey	23/3/70
7	*I. zenkeri*	Endengle Elais	1955-1956

Leaf epidermal preparation

A total of 5 leaf samples each and 20 microscope fields were examined for constancy of characters. Dried herbarium samples were used. 1-5 cm^2 portions of the leaves were cut from the standard median part of the matured leaves, the midway between apex and base portion of the leaf lamina near the mid-rib following the approaches of Ogundipe and Olatunji (1991), Olowokudejo (1993), Ayodele (2000), Kadiri (2003). Leaves were hydrated by boiling in water and soaked in concentrated trioxonitrate(v) acid (HNO_3) in glass bottles for about 8-24 hours to macerate the mesophyll or irrigated in Sodium Hypochlorite solution (commercial bleach) for thirty minutes to two hours to bleach the leaf portions of recalcitrant species. In the former approach, tissue disintegration was indicated by bubbles and the epidermides were transferred into petri dishes containing water for cleansing and then, separated with forceps. Tissue debris was cleared off the epidermides with fine-hair brush and wash in several changes of water. Drops of different grades of Ethanol 75% up to 100% added in turn to harden the cells.

Preparations were later stained with safranin O in 50% alcohol for about five minutes before mounting in glycerin on the glass slide. The epidermides were mounted with the uppermost surface facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration. Then the slides were appropriately labeled. Slides were examined with light microscope at x100, and x400. Photomicrographs were taken using Topview 3.2 Image Camera attached to a microscope and computer.

Results

Summary of all findings is presented in Figures 1 and 2, and Table 2.

Table 2. Comparative qualitative characters of the foliar epidermis of *Isolona* species

Species	Surface	Epidermal cell shape	Anticlinal wall pattern	Stomata type	Trichome type	Crystals
I. campanulata	Adaxial	Irregular, beaded	Curved	-	-	-
	Abaxial	Irregular, beaded	Curved	Paracytic, brachyparacytic	-	-
I. congolana	Adaxial	Polygonal	Straight	-	-	-
	Abaxial	Polygonal	Straight-curved	Paracytic, brachyparacytic	-	-
I. dewevrei	Adaxial	Irregular	Straight-curved	-	-	Present
	Abaxial	Polygonal, beaded	Undulate	Paracytic	-	Present
I. hexaloba	Adaxial	Polygonal	Curved	-	Trichome base found	Present as sands
	Abaxial	Irregular, beaded	Undulate	Paracytic, brachyparacytic	-	-
I. pleurocarpus	Adaxial	Polygonal	Curved	-	-	Present
	Abaxial	Irregular	Undulate	Paracytic, brachyparacytic	-	-
I. thonneri	Adaxial	Irregular, beaded	Undulate	-	-	-
	Abaxial	Irregular	Undulate	Paracytic, brachyparacytic	-	-
I. zenkeri	Adaxial	Irregular	Curved	-	-	-
	Abaxial	Irregular, beaded	Undulate	Paracytic, 1+2 laterocytic*	-	-

*= stomatal type in the sense of Carpenter (2005).

Across the species, the interstomatal number is not constant; it varies from 1-5 (Figures 1 and 2) while stomatal aperture appeared usually narrower than wider in the genus. The guard cells usually lie parallel to the long axis of the subsidiary cells. The leaves are hypostomatic and paracytic stomatal type is typical. Other stomatal types such as brachyparacytic and 1+2 laterocytic type may accompany this basic type (Figure 3). The non-stomatal cells such as epidermal cells have closely varied wall thickness across the species. Cell shape may be irregular on both surfaces in *I. campanulata*, (Figure 1 A, B; Table 2), *I. thonneri* (Figure 2C, D; Table 2) and *I. zenkeri* (Figure 2E, F; Table 2) while polygonal cell shape was found on both surfaces of the epidermis in I. *congolana* (Figures 1C, D; Table 2) only. Beaded ornamentation was observed on the epidermal cells of *I. thonneri* on the adaxial surface (Figure 2C, Table 2) whereas on the abaxial surface, it was observed in *I. dewevrei* (Figure 1F, Table 2) but *I. campanulata* has beaded ornamentation on the epidermis on both surfaces (Figure 1A, B, Table 2). Other species have different cell shape which may be irregular or polygonal.

Under light microscope, the anticlinal walls of the epidermis in *Isolona* varied from curved, straight, undulate and straight-curved (Figures 1, 2, Table 2). Three of the species namely *I. hexaloba, I. pleurocarpus* and *I. zenkeri* are curved and undulate anticlinal wall in adaxial and abaxial respectively. On the upper and lower region *I. campanulata* has curved while *I. thonneri* has undulate wall pattern. Straight anticlinal wall is visible only in adaxial region of *I. congolana*.

Crystals of calcium oxalate may be found as flakes in *I. dewevrei* on both surfaces (Figure 1E, F, Table 2) or restricted to the adaxial surface in *I. pleurocarpus* (Figure 2B, Table 2). Crystals occurred as sands in *I. hexaloba* on the adaxial surface only (Figure 1G; Table 2). The species appear glabrous but trichome base was recorded in *I. hexaloba* (Figure 1H, Table 2).

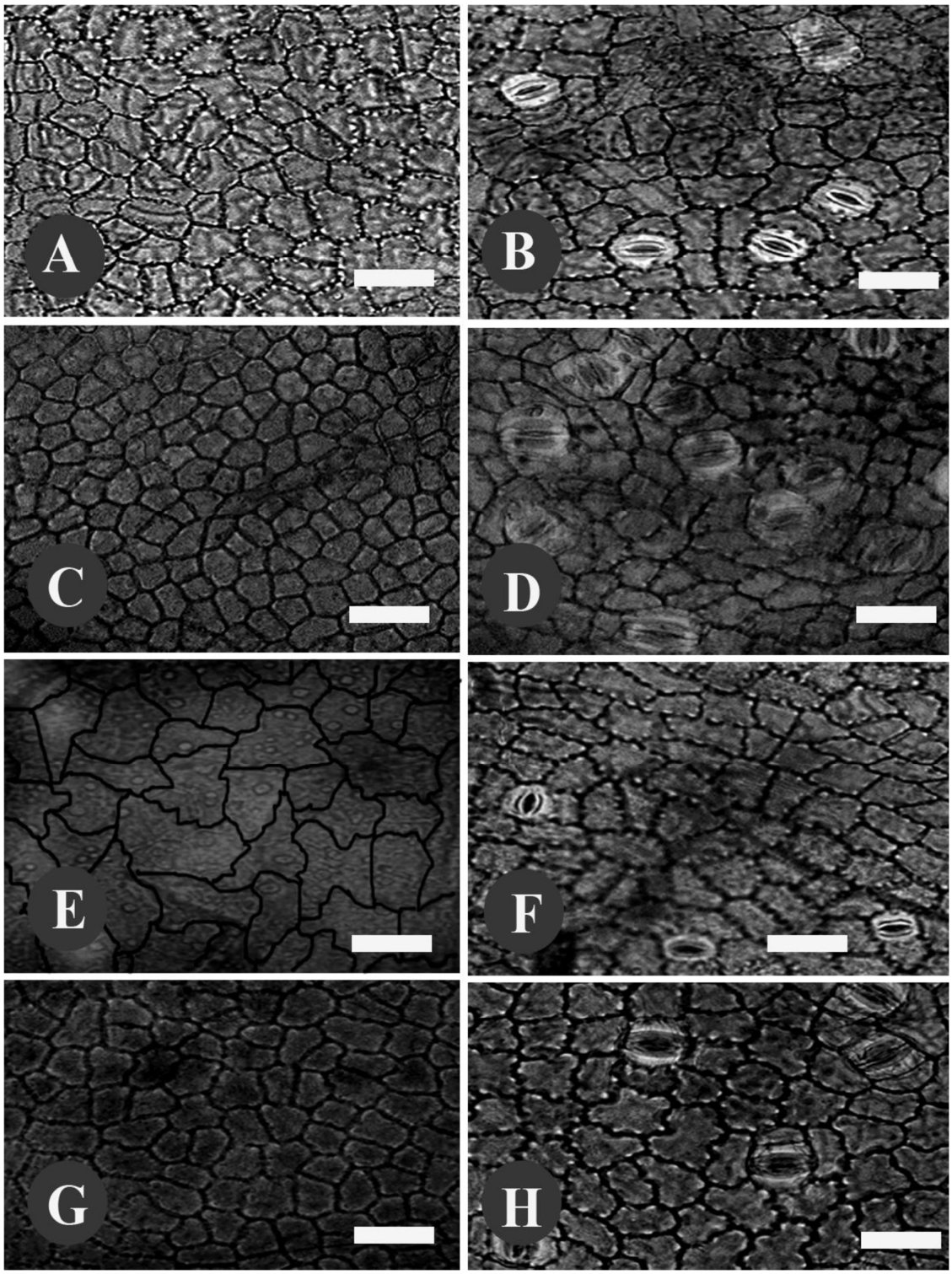

Figure 1. Comparative characters of leaf epidermis of the species of *Isolona*
A, C, E and G: Adaxial surfaces- B, D, F and H: Abaxial surfaces- A, B- *Isolona campanulata,* C, D- *I. congolana.* E, F- *I. dewevrei* G, H-*I. hexaloba*
Scale bar- 25 μm.

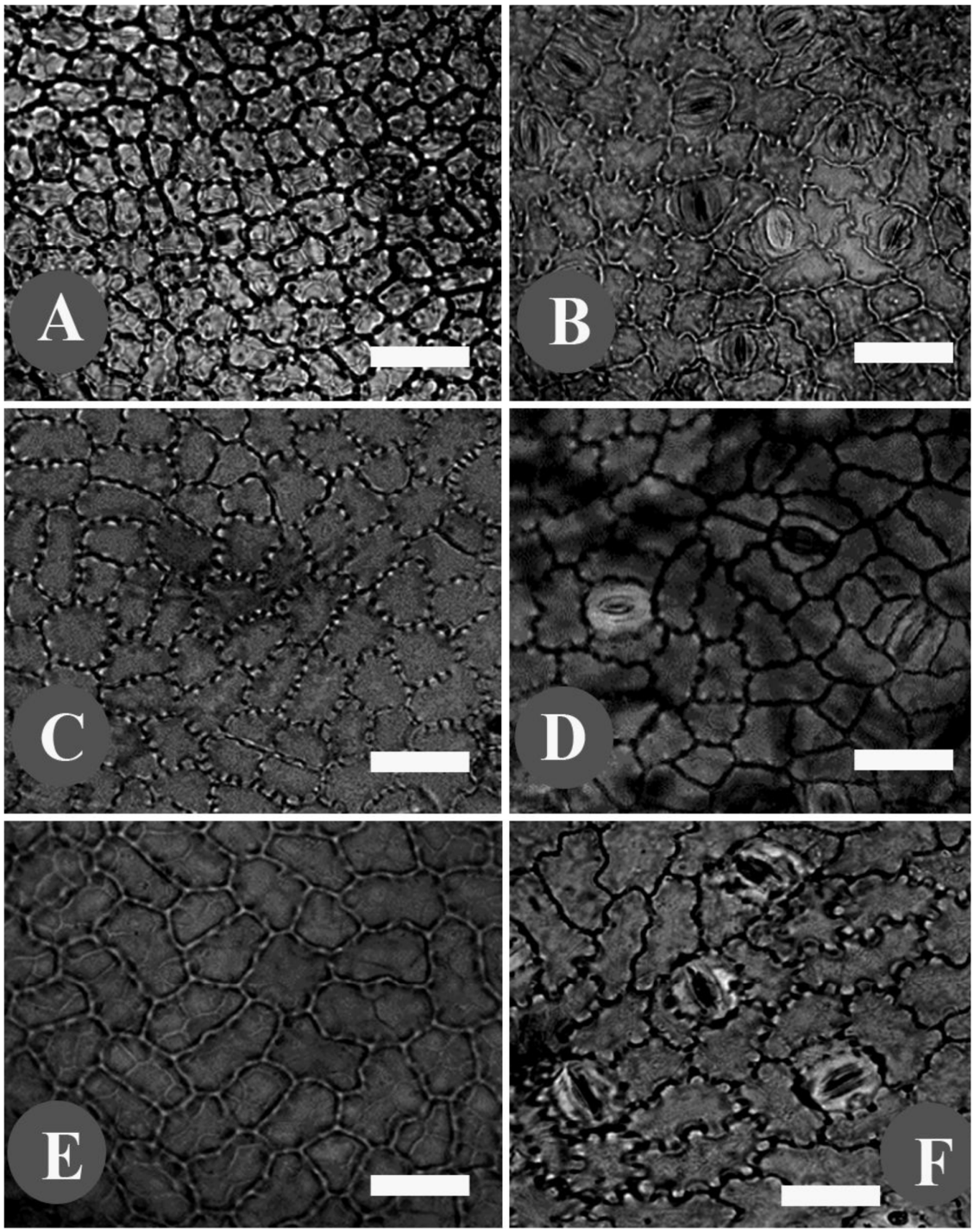

Figure 2. Comparative characters of leaf epidermis of the species of *Isolona*
A, C and E: Adaxial surfaces, B, D and F: Abaxial surfaces. A, B- *Isolona pleurocarpus,* C, D- *I. thonneri*, E, F- *I. zenkeri*
Scale bar- 25 μm.

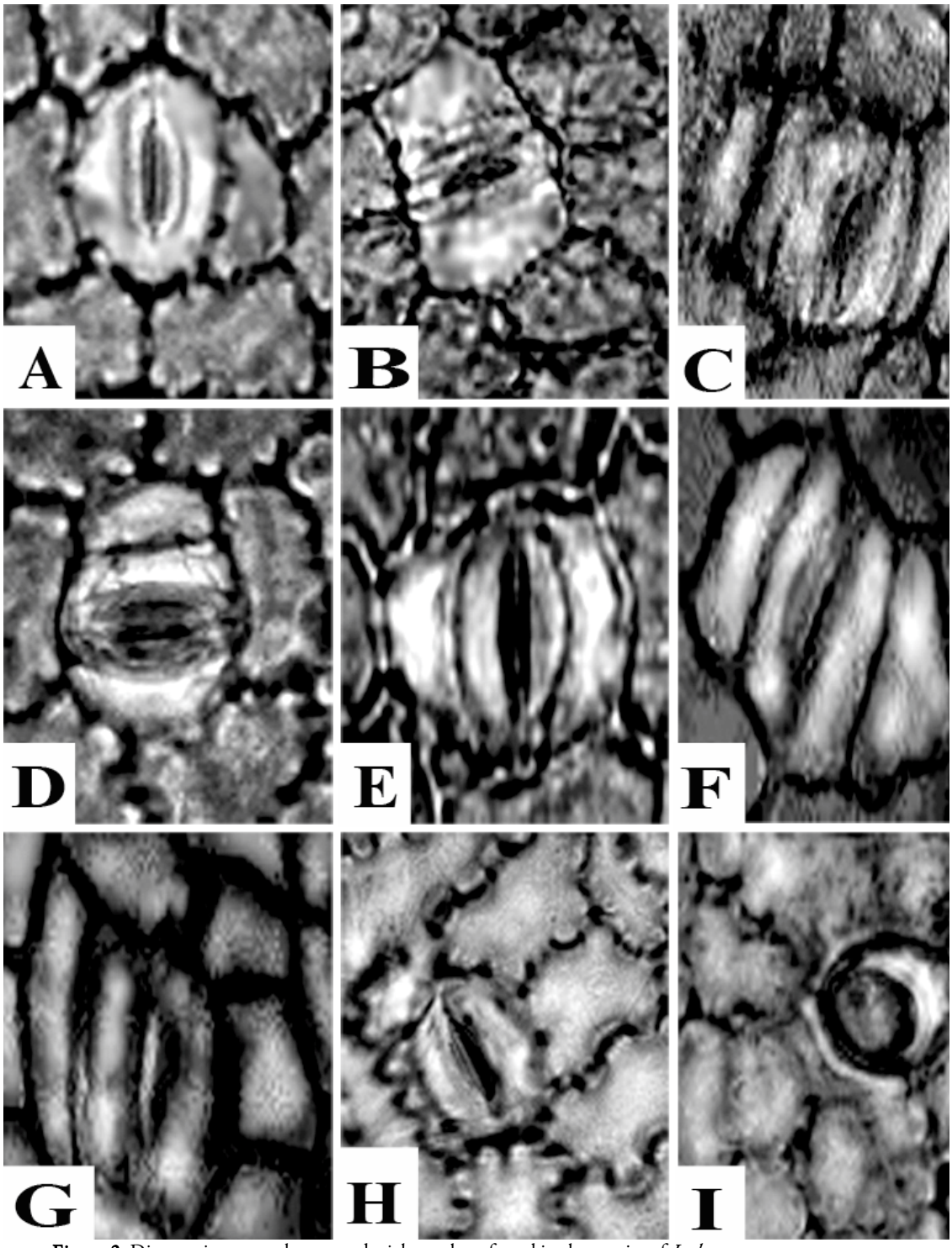

Figure 3. Diagnostic stomatal types and trichome base found in the species of *Isolona*
A= Paracytic, this common to all the species. B-G: Brachyparacytic type found in *I. campanulata, I. congolana*, *I. hexaloba*, *I. pleurocarpus* and *I. thonneri.* H: 1+2 laterocytic type, *sensu* Carpenter (2005). I: Trichome base found in *I. hexaloba*.

Discussion

The foliar epidermis is one of the most noteworthy taxonomic characters from the biosystematic point of view and the taxonomic studies of a number of families are made on the basis of leaf epidermis (Stace, 1984; Baranova, 1992). Contrasting and comparing the epidermal characters of *Isolona*, it revealed relationship among the species. Polygonal and irregular cell shape is seen in all on both surfaces. This study is in agreement with the report of Pereira-Sheteolu (1992) on genus *Monodora,* the sister genus to *Isolona*. The Annonaceae studies according to Metcalfe and Chalk (1950); Michael and James (2012); Olowokudejo (1990); indicated confinement of paracytic stomata to the abaxial surface in the family, but complexity in stomata apparatus of Annonaceae is first reported by Metcalfe and Chalk (1950) who for saw the possibilities of the stomata having more than one pair of subsidiary cells. The subsidiary cells surrounding the stomata in this study are obviously different from each other which aid to classify into different stomata types. This investigation revealed *I. campanulata, I. congolana, I. hexaloba, I. thonneri* and *I. pleurocarpus* recorded brachyparacytic accompanying paracytic stomata and a new record laterocytic stoma present in *I. zenkeri*.

The presence or absence of trichomes can frequently be used to delimit species, genera, or the whole family (Metcalfe and Chalk, 1979). Theobbald *et al.* (1979) established the importance of trichomes in comparative systematic of angiosperm. A number of reports on Annonaceae suggested the presence of unicellular unbranched trichomes (Metcalfe and Chalk, 1950; 1979; Olowokudejo, 1990; Michael and James, 2012). The presence of glandular base trichomes on the abaxial is diagnostic in *I. hexaloba*. Olowokudejo (1990) reported wax of various size, shapes and pattern in *Annona*. Base on the presented Table 2, the stomata type of *I. campanulata and I. congolana* differ from each other beyond that epidermal cell shape in *I. campanulata* is irregular on both surfaces while it is polygonal in *I. congolana.* Epidermal cells are larger in *I. congolana* compare to *I. campanulata.* The quantitative characters also indicated significantly difference from one another. *I. pleurocarpus* and *I. hexaloba* show distinct differences between themselves ranging from stomata type, present of druses and number of the cell per field.

An indented artificial dichotomous key for delimiting the seven species of Isolona occurring in Nigeria and the Cameroons

1a. Stomatal type paracytic only.....*I. dewevrei*
1b. Stomatal type paracytic and another type..... 2
2a. 1+2 laterocytic type present.....*I. zenkeri*
2b. Brachyparacytic stomata present..... 3
3a. Epidermal cell shape polygonal on both surfaces.....*I. congolana*
3b. Epidermal cell shape irregular on both surfaces.....4
4a. Anticlinal wall pattern straight-curved onadaxial surface..... *I. campanulata*
4b. Anticlinal wall pattern undulate on the adaxial surface.....*I. thonneri*
5a. Trichome base absent.....*I. pleurocarpus*
5b. Trichome base present.....*I. hexaloba*

Conclusions

The foliar epidermal assessment provided a significant source of information and evidence in determining the interrelationship among the seven *Isolona* species occurring in Nigeria and the Cameroons. The study revealed similar and different characters used for separating the species within the genus. Some of the diagnostic characters used for delineating the genus were stomatal type, presence or absence of hair, epidermal cell shape, and anticlinal wall pattern. Hypostomatic leaves and paracytic stomatal types that generic constant. Complex stomata types were present in the genus with the possession of 1+2 laterocytic type stomatal as a new record.

Authors' Contributions

ABK and JDO contributed to manuscript development and literary work as supervisors of ASA who did data acquisition, writing and laboratory work. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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