Bangladesh J. Plant Taxon. **27**(2): 283-291, 2020 (December)

COMPARATIVE MIDRIB ANATOMY OF *MONODORA* DUNAL. AND *ISOLONA* ENGL. (ANNONACEAE) FROM WEST-CENTRAL AFRICA

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Keywords: Comparative; *Monodoreae; Isolona; Monodora;* Midrib; Taxonomy.

Abstract

This article assessed midrib anatomical description of *Isolona* Dunal. And *Monodora* Engl. (Annonaceae) from West-Central Africa. Twelve species of tribe *Monodoreae* were investigated on the basis of micromorphology of midrib characters with the aid of light microscopy. The study provided important taxonomic characters which aid delineation of inter and infrageneric species within the duo genera. The generic features include centrally positioned, open collateral vascular bundle and furrow shaped midribs which are diagnostic to the genera.U shaped vascular bundles are present in most of the species with inviginating or expanded endings while marginal traces vary from 2 to 4. The presence of keel protrusion at abaxial surface established a closer affinity among *M. angolensis, M. crispata,* and *M undulata* with additional features species were delimited. Other variable useful features of midrib encountered are trichomes, parenchyma, collenchyma and sclerenchyma, and adaxial and abaxial shape. The midrib characters have been used to prepare an indented dichotomous key to delimit the species in the genera studied.

Introduction

Annonaceae Juss, the custard-apple, is the largest family of the Order *Magnoliales* (Bremer *et al.,* 2009; Smith *et al.,* 2010; Zeng*et al.,* 2014) and pantropical in distribution consisting of trees, shrubs and lianas (Mols and Kessler, 2003). The members play an important ecological role in terms of species diversity, especially in tropical rainforest ecosystems and the family is recognized as the most diverse plant family in the tropics (Phillips and Miller, 2002). There are about 42 genera (Couvreur *et al.,* 2012) and 500 species (Mols *et al.,* 2004) in Africa-Madagascar.

African genus *Monodora* and Afro-Malagasy genus *Isolona* with *ca.* 14 and 21 species respectively (Couvreur, 2009) are of the tribe *Monodoreae/Monodoroideae* (Hutchinson, 1923; Fries, 1959;Chatrou *et al.,* 2012) in the family Annonaceae. They occur in lowland or montane rainforest from West to East Africa with and comprise mainly of trees and shrubs up to 30-40m tall (Couvreur, 2009). In recent studies, morphological (Doyle and Le Thomas, 1996), palynological (Doyle and Le Thomas, 1994; 1997), and molecular phylogenies (Richardson *et al.,* 2004; Couvreur *et al*., 2008) indicated that both *Isolona* and *Monodora* form a well supported clade nested within the long branch clade, one of the two major clade recognized in Annonaceae (Richardson *et al.,* 2004; Couvreur *et al*., 2008). These genera are easily distinguishable with *Isolona* having one whorl of fused petals that are reflexed, spreading horizontally or recurved over the receptacle and the flower colour varying from bright yellow to bronzy red. In contrast, *Monodora* has two whorls each of three conspicuous petals slightly fused at their bases. The outer

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three petals areusually crisped or undulate and are white to yellow with purple-red streaks. The inner three petals are unguiculate and are sometimes connivent by a network of intricate trichomes or just pressed together over the receptacle to form a pollination chamber (Couvreur, 2008; Couvreur *et al.,* 2006).

African Annonaceae are largely understudied like most of the African flora, which partly explain the reported low diversity (Couvreur *et al.,* 2006). However, a number of publications have contributed to better understanding the African Annonaceae family (Couvreur *et al.,* 2006; 2008; Couvreur, 2009 ; Botermans *et al.,* 2011) but there is a sparse information on the anatomical character, hence the need for this study. This study therefore, will complement already reported taxonomic evidences, provides additional diagnostic features useful for understanding the family and specifically present midrib anatomical characters useful for the inter and intrageneric classification of the syncarpous genera of *Monodora* and *Isolona*.

Materials and Methods

Twelve *Isolona* and *Monodora* samples were obtained from specimens deposited at the National Herbarium Yaounde, Cameroon, YA (IH). Herbarium abbreviation follows Holmgren *et al*. (1990). Table 1 shows names of the specimens that were used for the study, Collector and Dates of Collection.

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. thonneri*	R. Letouvzey	23/3/70
6.	*I. zenkeri*	Endengle Elais	1955-1956
7.	*Monodora angolensis*	R. Letouvzey	23/10/54
8.	*M. crispata*	J.J. Bos	2/02/70
9.	*M. myristica*	R. Letouvzey	21/4/61
10.	*M. tenuifolia*	MpomDenoit	21/11/55
11.	*M. brevipes*	R. Letouvzey	8/3/84
12.	*M. undulata*	D. thomas	25/1/84

Six dried species each of both *Isolona* and *Monodora* were used for the study. Thin sections were made through free hand sectioning. The sections were obtained transversely and boil for about five minutes then drop of Sodium hypochlorite was added for atleast 20-30 minutes; washed in several changes of water and a few drops of concentrated ethyl alcohol were added in order to harden the tissue; then samples were stained in 1% aqueous Safranin O for 1 minute. Excess stain was washed off with a few drops of concentrated ethyl alcohol. The stained sections were mounted in glass slide and drops of glycerin added then covered with cover-slips and ringed with nail polish to prevent dehydration. Specimens were observed at X40 magnification and hand drawings were presented.

Results and Discussion

The results of the investigation are presented in Tables 2-3 and Fig. 1 and described for each species hereunder.

***Isolona campanulata* Engl. & Diels.**

The shape of midrib of *I. campanulata* is convex on the adaxial surface while it is flattened dorsoventrally on the abaxial surface. Parenchyma cells are polygonal of about4 to 5 cell layers. There are 2 cell layers of collenchymas cells while sclerenchymatous tissue is absent. Vascular bundle is positioned centrally with a U shape vascular bundle and open collateral bundles with 2 traces of vascular ring at the marginal axis. Trichomes are absent on both surfaces of the epidermis while the outline of the species is slightly uneven (Table 2, Fig. 1).

***Isolona congolana* (De Wild. & T.Durand) Engl. & Diels.**

Trichomes are absent on both surfaces of the epidermis. Midrib shape on the adaxial surface is convex while on the abaxial surface is crescentiform.A general view of midribis more like a furrow shape structure withslightly uneven outline. The vascular bundle is collateral bundle and polygonal parenchyma cells about 5 to 6 cell layers found around and in-between them. Within the medullary of the species towards the adaxial surface is found a bunch of sclerenchymous cell. Vascular bundle is more like a crescent and positioned at the center of the midribwith two traces vascular rings. (Table 2, Fig. 1). An overview of the shape of its midrib is furrow and uneven outlines.

***Isolona dewevrei* (De Wild.&T.Durand) Engl. & Diels.**

The midrib shape observed in *I. dewevrei* is convex at the adaxial surface but crescentiform on the abaxial surface of the epidermis and an uneven outline. Vascular bundle pattern is at central position on the cross section of the midrib. The vascular bundle is collateral and crescent shape without traces. Trichome is absent on both surfaces while polygonal parenchyma cells make up the pith in this species with about 4 to 5 layers at the perivascular region and up to 2 layers of collenchymas cell. The outline of the midrib of *I. dewevrei* is uneven with droplet of sclerenchyma cell at the parenchyma region.

***Isolona hexloba* (Pierre)Engl. & Diels.**

The shape of the vascular bundle of this species is U-shape and centrally located without marginal traces while the vascular type is collateral bundles. Trichome is absent on the abaxial and adaxial surfaces of the species. The perivascular tissues such as sclerenchyma, collenchymas and polygonal parenchyma cells were observed. Parenchyma cell has about 6 to 7 and collenchymas with about 1 or 2 cell layers.There are patches of sclerenchyma cells deposited within the perivascular tissue. On the adaxial surface, midrib shape is slightly convex while the on the abaxial surface, it is deeply crescentiform in shape with an uneven outline on the epidermal layer (Table 2, Fig. 1).

***Isolona thonneri* (De Wild.&T.Durand) Engl. & Diels**.

The perivascular tissue found in *I. thonneri* consists of both collenchymas and parenchyma cells littered with patches of sclerenchymatous cells. Parenchyma cell are polygonal and up to 7 cell layers while collenchyma cells is either a cell or 2. Midrib shape is furrowed and its surface is all round even. On the adaxial surface, the midrib shape is convex while on the abaxial the midrib shape is crescentiform. *I. thonneri* possesses collateral and centrally located vascular bundle which is arched crescent in shape. Trichome is not visible on the surface the epidermis (Table 2, Fig.1).

***Isolona zenkeri* (Engl.) Dyer.**

Adaxial surface shape of the midrib of this species is slightly flattenend but crescentiform on the abaxial surface of the epidermis. The vascular bundle is centrally positioned and collateral bundle found in *I. thonneri*. The vascular bundle is U-shaped with an invigorating end and uneven outline on the epidermis. Trichome is absent on the both abaxial and adaxial surface of the epidermis (Table 2, Fig. 1). Parenchyma cell are polygonal and 4 to 5 cell layers while collenchymas cells has 2 to 3 cell layers. Sclerenchyma cell is absent within the species.

***Monodora angolensis* Welw.**

Midrib shape of this species is convex on the adaxial surface while the abaxial surface is slightly crescentiform with lumpy keel pointing downward which makes its outline visibly uneven. The shape of the vascular bundles is crescent with 1 marginal trace. Trichome is sparingly present on the adaxial while it is absent on the abaxial of the epidermis. Pattern of arrangement of the vascular bundle is collateral and centrally located (Table 2, Fig. 1). Parenchyma cell are polygonal and 3 to 4 cell layers while collenchymas cells are up to 2 cell layers. Sclerenchyma cell is absent.

***Monodora crispate* Engl.& Diels.**

In *Monodora crispata* the vascular bundle is central having collateral type vascular bundle. On the adaxial surface, midrib shape is convex while on the abaxial surface, the midrib shape is V shape with protrusion of keel which makes the outline on the epidermal cell to be uneven. Vasculation pattern of this species is V-shaped bundles that form an unbroken vascular bundle and two traces of marginal bundles. Parenchymal cells are elongated with cell layers as much as 2 to 3 layers. Collenchyma cells are also present outside the pith below the parenchyma cells with about 2 layers. Trichome isabsent on both surfaces of the epidermis (Table 2, Fig. 1).

***Monodora myristica* (Gaertn.)Dunal.**

The transverse section of the species' midrib is slightly convex on the adaxial surface and crescentiform on the abaxial surface. Midrib outline is slightly uneven on the epidermal without trichomes. Vascular bundle is collateral and centrally positioned observed on the adaxial surface located. Vascular shape is U shape broadly open at the ends. Trichome is absent on the surface (Table 2, Fig. 1). Perivascular tissue like parenchyma is polygonal of about 8 to 9 layers of cell and collenchymas of two to three cell layers while sclerenchyma is absent in the species.

***Monodora tenuifolia* Benth.**

Midrib is convex on adaxial and crescentiform on the abaxial surfaces. Vascular bundles are situated at the center with 3 traces, present at the margins. Vasculation pattern of the midrib is collateral bundles that form a U shaped expanded endings and open free collateral type. Trichome is absent on both adaxial and abaxial surfaces. There are about 4 to 5 layers of polygonal parenchyma cell present in the perivascular tissue and about 2 layers of collenchymas cell. The surface is even at outwardly.

***Monodora brevipes* Benth.**

Midrib vascular bundle is formed collateral bundle that form a V shape with invaginated ends. The vascular position is central with vasculation pattern that discontinuous. Adaxial surface of the midrib is V shape with 4 marginal vascular rings while the abaxial surface is crescentiform. Trichome is copiously absent on both surfaces of the epidermis. Parenchyma cell is polygonal of 6 to 7 layers of cell while sclerenchyma is absent.

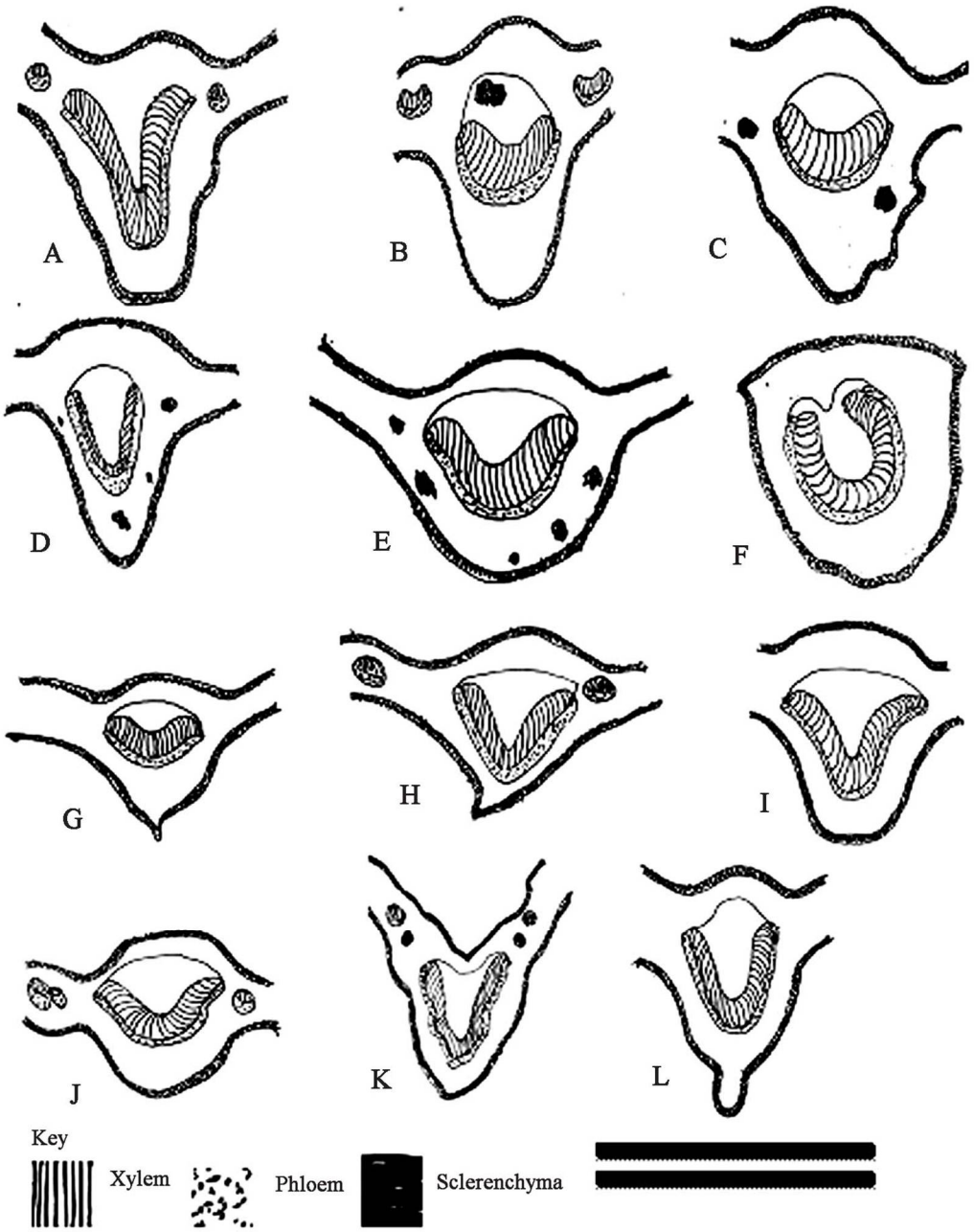

Fig.1.Midrib Anatomical Characters of 12 species of *Isolona* and *Monodora* from West-Central Africa - A. *Isolona campanulata,* B. *I. congolana,* C. *I. dewevrei,* D. *I. hexaloba,* E. *I. thonneri,* F. *I. zenkeri,* G. *Monodora angolensis,* H. *M. crispata,* I. *M. myristica,* J. *M. tenuifolia,* K. *M. brevipes,* L. *M. undulata,* Scale bar = 50 µm.

Table 2.Midrib characters of the *Isolona* and *Monodora* in West and Central Africa.

Species	Outline	Par.	Coll. layer	Scl	Trichome		Midrib shape	AD shape	AB shape	V. B position	V. B shape	V.B. type
					AD	AB						
Isolonacampanulata	Uneven	Poly 4-5	1-2	+	-	-	Furrow	Convex	flattened	Central	U shape with 2 traces	Col.
I. congolana	Uneven	Poly 5-6	3-4	+	-	-	Furrow	Convex	Crescentiform	Central	Crescent with 2 traces	Col.
I. dewevrei	Uneven	Poly 4-5	1-2	+	-	-	Furrow	Convex	Crescentiform	Central	Crescent	Col.
I. hexaloba	Uneven	Poly 6-7	1-2	+	-	-	Furrow	Convex	Deeply Crescentiform	Central	U shape	Col.
I. thonneri	Even	Poly 4-5	1-2	+	-	-	Furrow	Convex	Crescentiform	Central	Crescent	Col.
I. zenkeri	Uneven	Poly 4-5	2-3	-	-	-	Furrow	Slightly flattened	Crescentiform	Central	U shape	Col.
Monodoraangolensis	Uneven	Poly 3-4	1-2	-	+	-	Furrow	Convex	SligthlyCrescentiform with keel	Central	Crescent	Col.
M. crispata	Even	Elong 3-4	1-2	-	-	-	Furrow	Convex	SligthlyCrescentiform with keel	Central	V shape with 2 traces	Col.
M. myristica	Uneven	Poly 8-9	2-3	-	-	-	Furrow	Slightly convex	Crescentiform	Central	U shape	Col.
M. tenuifolia	Uneven	Poly 4-5	1-2	-	-	-	Furrow	Convex.	Crescentiform	Central	U shape with 3 traces	Col.
M. brevipes	Uneven	Poly 6-7	2-3	-	-	-	Furrow	V shape	Crescentiform	Central	V shape with 4 traces	Col.
M. undulata	Uneven	Poly 4-5	1-2	-	-	-	Furrow	Convex	Crescentiform with keel	Central	U shape	Col.

Par.-Parenchyma, Poly-Polygonal, Elong-Elongated, Coll.-Collenchyma, Scl.-Sclerenchyma,+-Present, --Absent, AD-Adaxial, AB-Abaxial, Col.-Collateral, V.B -Vascular Bundle

***Monodora undulata* (P. Beauv) Couvreur**

Midrib shape is convex adaxially and crescentiform with a lumpy keel protuberance on the abaxial which discontinue the evenness of the epidermal layers. It has a single trace of vascular bundle with U shape and collateral ring of bundles positioned at the center while the vascular pattern is a continuous. Trichome is unarguably absent on both surfaces of epidermis. Perivascular tissues are present with polygonal loosely packed parenchyma cells of 4 to 5 layers with about 2 collenchyma cell layers present near the epidermal layer.

Table 3. Identification key based on midrib anatomic

1. Vascular bundle U-shape----------	2
2. Adaxial shape flattened----------	*I. campanulata*
2. Adaxial shape crescentiform----------	3
3. Vascular bundle U shape with inviginating end----------	*I. zenkeri*
3. Vascular bundle U shape without inviginating ends-------	4
4. Marginal traces about 4 ----------	*I. tenuifolia*
4. Vascular bundle U shape, no marginal trace----------	5
5. Sclerenchyma present----------	*I. hexaloba*
5. Sclerenchyma absent----------	6
6. Vascular bundle U shaped, expanded endings---------	*M. myristica*
6. Vascular bundle U shaped without expanded endings	*M. undulata*
1. Vascular bundle Vascular V or Crescent----------	7
7. Vascular bundle V shape----------	8
8. Marginal traces up to 4 rings ----------	*M. brevipes*
8. Marginal traces up to 2 rings----------	*M. crispata*
7. Vascular bundle Crescent----------	9
9. Abaxial surface Keel protrusion present----------	*M. angolensis*
9. Abaxial surface Keel protrusion absent ----------	10
10. Marginal leaf traces present----------	*I. congolana*
10. Marginal leaf traces absent----------	11
11. Abaxial outline uneven----------	*I. dewevrei*
11. Abaxial outline even----------	*I. thonneri*

Taxonomic significance of anatomical characters as an aid to establishing relationships between taxa has been exploited (Davis and Heywood, 1963; Radford *et al.* 1976; Bacic *et al.* 1992 and Woltz *et al.* 1987). The midrib anatomy of the twelve species understudied showed marked differences and provided important taxonomic characters which aid delineation of species within *Monodora* and *Isolona* genera from West-Central Africa. The presence of centrally positioned and open collateral vascular bundle with furrow shaped midrib was shared by all the studied species and has established the interrelationship between duo genera.

The report of Mantovani *et al*. (2009) highlighted importance of keel in the identification of *Anthurium* species. Possession of keel protrusion in *M. angolensis, M. crispata* and *M undulata* on the abaxial surface established a closer affinity among the species and ultimately advance the understanding of the character in classifying the genus. As a result of the keel, the unevenness of the surface is copiously visible in the said species. The occurrence of scanty trichomes on the adaxial surfaces of *M. angolensis* stands out among other species. Metcalf and Chalk (1979) reported medullary bundle within a ring of collateral strand in *Cananga odoratus.* The number of

marginal traces varies from two *in I. congolana, I. campanulata* and *M. crispate* while three and four traces are seen in *M. tenuifolia* and *M. brevipes* respectively at the marginal axis of the adaxial surface. This feature is a unifying character and limited to the five species mention above. Many researchers have employed anatomical parameter to solve taxonomic intricacies (Olowokudejo, 1987; Mantovani, *et al.* 2009; Kadiri and Olowokudejo 2010; Anorue *et al.* 2020). U shaped vascular bundles are present in most of the species with inviginating or expanded endings. Crescentiform arched shaped vascular bundle clearly separate the trio species of *Isolona congolana, I. dewevrei* and *I. thonneri* from the other *Isolona* species studied. Other helpful characters in distinguishing the species are parenchyma, collenchymas, sclerenchyma; presence and absence of trichomes. The occurrence of perivascular tissues like parenchyma, collenchymas and sclerenchyma played a vital role in adding to the classification criterion. The number of parenchyma layers varies from 3-9 and the shape is more of polygonal except *M. crispata* that is elongated whereas sclerenchyma layers is between1-4 layers. Scanty scattered sclerencymatous cells were present at the parenchyma or collenchymas region of *I. campanulata, I. dewevrei, I thonneri,* and *I hexaloba* while a clog of sclerenchyma present in the pith of *I. congolana* separates it from other species. This could be useful in constructing phylogenetic tree and quantitative characters.

Conclusively, the study present and compare different anatomical characteristics of twelve species of duo genera: *Isolona* and *Monodora*. The results show several similarities which justify the grouping of the genera in the same family and also differences that distinguish the species from one another. The midrib anatomical features observed in the genera are of taxonomic importance to the classification of the family. A taxonomic key is constructed using the combined midrib characters for better understanding.

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(Manuscript received on 12 May 2020; revised on 7 November 2020)