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Comparative Leaf Epidermis of *Monodora* Dunal. (Annonaceae) From Central and Western African and its Taxonomic Value

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Abstract

A comparative assessment of the leaf epidermis of seven species of *Monodora* from Central and Western African was carried out with the aid of light microscope and species' affinities were shown with relationship polygraphs. The studied stomata and non-stomata characters yielded some outstanding taxonomic values leading to recognition of three groups that may equate infra-generic sections in the genus: *Monodora angolensis* and *M. brevipes* forming one group, *M. myristica* and *M. tenuifolia* forming the second group and, *M. crispata, M.undulata* and *M. zenkeri* as the third group. The quantitative characters overlap significantly but the discontinuity found within the values marks the species' limit. On the average, epidermal cell number varies from 34±2 in *M. myristica* to 92±2 in *M. zenkeri* on the abaxial surface whereas on the adaxial surface, it varies from 18±1 in *M. brevipes* to 126±3 in *M. undulata* and, usually the cell size is slightly more than twice greater than the stomatal size in all the species except in *M. tenuifolia*. The only feature that is constant across the genus is hypostomatic leaves, while brachyparacytic stomatal type is a new record in the genus. With these epidermal features, the species can be distinguished and their identification can be based on the documented data even when the leaves are fragmentary.

Keywords: Annonaceae, classification, epidermis, *Monodoreae*, statistics, taxonomy.

Introduction

Monodora Dunal.a genus in the family Annonaceae has 14 species in Africa (Bremer *et al.*, 2009; Smith, *et al.*, 2010; Zeng, *et al.*, 2014; Mols and Kessler, 2003) and seven (7) of them present in Central and Western Africa were used for the study. The species are generally called the nutmeg trees. Exo-morphologically, the species are recognizable by distichous phyllotaxis; presence of simple hairs; bracts, and

rhipidiate inflorescences which may be terminal or axillary, and bisexual flowers (Hutchinson and Dalziel, 1958). The species may be sometimes dioecious with free or fused carpels having parietal placentation. Other distinguishing morphological features of the species are presence or absence of stipe and aril and inaperturate pollen. The aromatic seeds of the plants are used as food, medicines and cosmetics. However, these features are common to all the species thus sometimes leading to confusion in identification. Leaf as an important source of taxonomic characters has not been particularly investigated in the genus for decision on species' affinity, delimitation, identification and in distinguishing the genus from its often confused other closely related genus *Isolona* which is sister to it based on syncarpous ovary (Couvreur*et al.*, 2008) and molecular data sets (Doyle and Le Thomas, 1994, 1996 and Richardson *et al.*, 2004). Therefore, it is aimed that the result of this investigation on leaf epidermis characteristics will assist in species distinction and distinguishing the species from other ones belonging to the sister's genus *Isolona*. Moreover, the problem of adulteration often encountered with medicinal plants in the regional herbal markets will be solved especially when samples of the species are mixed up or fragmentary.

Materials and Methods

Dried herbarium samples of the seven species of *Monodora* from Western and Central African obtained from the National Herbarium Yaounde, Cameroon (YA (IH) and Forestry Herbarium Ibadan, Nigeria (FHI) were used for the study. The herbarium abbreviations follow Holmgren and Holmgren (2003) while the specific samples of the studied species and their herbarium information were *Monodora angolensis*: R. Letouvzey, 23/10/54; *M. crispata*: J.J. Bos, 2/02/70; *M. myristica*: R. Letouvzey, 21/4/61; *M. tenuifoli*: MpomDenoit, 21/11/55: *M. brevipes*: R. Letouvzey, 8/3/84; *M. undulata*: D. Thomas, 25/1/84 and *M. zenkeri*: A.J.M Leeuwenberg, 31/1/84; showing species name, collector and date of collection respectively.

One to five square centimeters portions were obtained from the standard median portion of the leaf lamina near the mid-rib and leaf epidermal preparartion follows the approaches of Olowokudejo and Pereira-Sheteolu (1988), Ogundipe and Olatunji (1991), Olowokudejo (1993), Ogundipe and Akinrinlade (1998), Ogundipe and Daramola (1998), Ayodele (2000), Kadiri (2003), Kadiri and Ayodele (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 30-120 minutes to bleach the leaf portions. Tissue disintegration was indicated by bubbles and the epidermis was transferred into Petri dishes containing water for cleansing and then, separated with forceps. Tissue debris was cleared off the epidermis with fine-hair brush and wash in several changes of water. Drops of

different grades of ethanol: 50%, 70%, 75% up to 100% were added in turn to harden the cells.

The preparations were later stained with safranin O in 50% alcohol for about 5 minutes before mounting in glycerine on the glass slide. The epidermis was 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 a compound light microscope at x100 and x400, and photomicrographs were taken using Toupview 3.2 Image Camera connected to a Pentium IV Compac Computer, USA.

For quantitative analysis of epidermal characters, 20 epidermal cells and 10 stomata were randomly selected for measurement. Stomata index was calculated using the formula reported by Stace (1965).

$$Stomata\ Index = 100\ (S/S + E)$$

Where S= No of stomata present (per unit area). E= No of Epidermal cell (per unit area)

Seven quantitative characters-number of cell per field, cell length, cell width, stomatal length, stomatal width, stomatal number per field and stomatal index were used to construct relationship polygraphs to reflect affinity.

Result and Discussion

The taxonomic relevance of leaf epidermis characters in the genus *Monodora* is undertaken with seven species occuring in Central and Western Africa. The species are clearly distinguishable and species grouping is possible with these taxonomically important leaf endomorphological characters.

All the studied *Monodora* species have straight anticlinal walls and polygonal cell shape on both surfaces except *M. crispata* which has irregular cell shape and undulate walls on the abaxial surface. This partially agrees with Metcalfe and Chalk, (1950, 1979) who reported isodiametric or irregular cell shapes. So far, only paracytic stomatal type was reported in *Monodora* and the family Annonaceae as a whole (Metcalfe and Chalk, 1950, 1979; Olowokudejo, 1990; Pereira-Sheteolu, 1992; Chatrou, 1998; Michael and James, 2012) but brachyparacytic type is now added to the existing information. Trichomes appear useful in distinguishing the species. Unicellular trichomes were encountered only in *M. crispate* on the adaxial surface (Fig. 1E, Table 1) and *M. myristica* on the abaxial surface (Fig. 2B, Table 1) while other species are glabrous. Trichomes have been used to show affinity and in defining taxa, especially in Proteaceae and Plantaceae and some other angiosperm plants (Olowokudejo 1990; Carpenter *et al*., 2005; Michael and James, 2012; Kadiri

and Olowokudejo, 2016). Quantitative characters significantly overlap among the species (Table 2). This supports the closeness of the studied species and their grouping as a genus. On the average, mean epidermal cell number varies from 34±2 in *M. myristica* to 92±2 in *M. zenkeri* on the abaxial surface whereas on the adaxial surface, it varies from 18±1 in *M. brevipes* to 126±3 in *M. undulata* (Table 2). On the abaxial surface, the cell size is slightly more than twice greater than the stomatal size in all the species except in *M. tenuifolia* whose values are 10.1±0.5 x 5.8±0.3 µm for cell size against the stomatal size of 5.2±0.1 x 1.1±0.1µm. In the genus, the stomatal index varies from 2.3% in *M. undulata* to 13.3% in *M. tenuifolia* (Table 2). Quantitative characters may overlap though; their value in defining taxa boundaries has been shown in many angiosperm taxa (Olowokudejo, 1990; Michael and James, 2012; Kadiri and Olowokudejo, 2016). The relationship polygraphs reveal the pattern of affinity that exist among the species. Based on the quantitative characters, a total three groups are recognizable; *Monodora angolensis* and *M. brevipes* forming one group, *M. myristica* and *M. tenuifolia* representing the second group*;* while *M. crispata, M. undulata* and *M. zenkeri* form the third group which are the possible infra-generic sections. It is therefore proposed that further investigation of this position should be verified with data from other taxonomic sources including molecular data set.

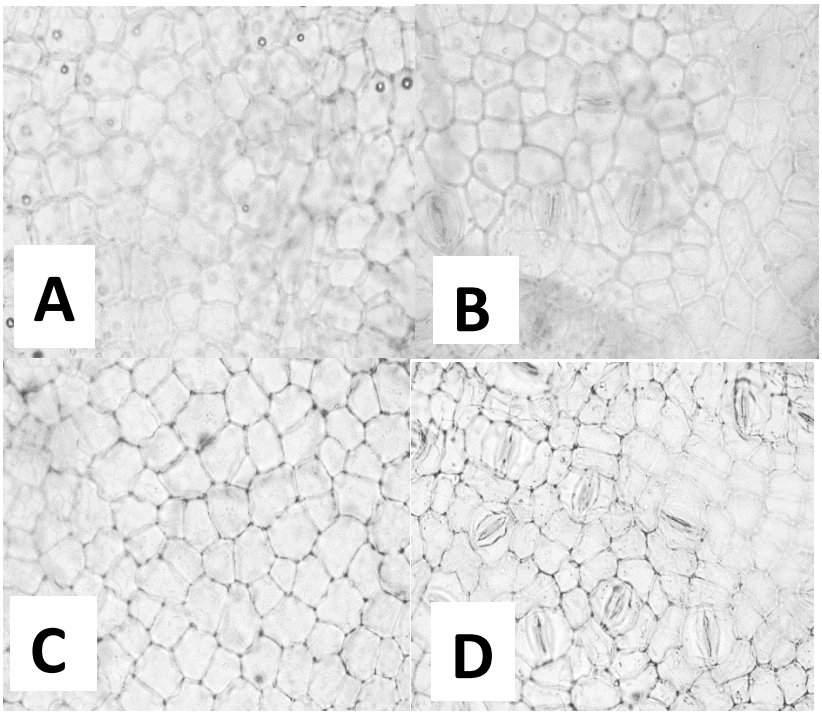
In conclusion, this study has furthered provided the distinctiveness between the genus *Monodora* and *Isolona* (its sister's genus) with respect to the shape of epidemal cell, type of stomatal, absence or presence of subsidiary cell amog other features and has ultimately confirmed the leaf epidermal morphology as taxonomic tool.

Table 1: Qualitative stomatal and non-stomatal cells of the leaf epidermis of some African *Monodora* species

Species	Surface	Epidermal cell shape	Anticlinal wall pattern	Stomata type	Trichomes	Crystal
Monodora angolensis	Abaxial	Polygonal	Straight/Curved	Paracytic, Hemiparacytic	Absent	Present
	Adaxial	Polygonal	Straight	Absent	Absent	-
M. brevipes	Abaxial	Polygonal	Straight/Curved	Paracytic	Absent	Present
	Adaxial	Polygonal	Straight	Absent	Absent	-
M. crispata	Abaxial	Undulate	Straight/Curved	Paracytic	Absent	Present
	Adaxial	Polygonal	Straight	Absent	Present	-
M. myristica	Abaxial	Polygonal	Straight/Curved	Paracytic, Brachyparacytic	Present	Present
	Adaxial	Polygonal	Straight	Absent	Absent	-
M. tenuifolia	Abaxial	Polygonal	Straight/Curved	Paracytic, Brachyparacytic	Present	Present
	Adaxial	Polygonal	Straight	Absent	Absent	-
M. undulata	Abaxial	Polygonal	Straight/Curved	Paracytic, Brachyparacytic	Absent	-
	Adaxial			Absent	Absent	-
M. zenkeri	Abaxial	Polygonal	Straight/Curved	Paracytic	Absent	-
	Adaxial	Polygonal	Straight	Absent	Absent	-

Table 2: Quantitative stomatal and non-stomatal cells of the leaf epidermis of some African *Monodora* species

Species	Surface	Number per field	Cell length (µm)	Cell width (µm)	Stomatal length (µm)	Stomatal width (µm)	Stomatal number per field	Stomatal Index %
Monodora angolensis	Abaxial	56(57±3)64	5.0(8.2±0.5)11.0	4.0(5.5±0.2)7.0	5.0(5.7±0.1)6.0	3.0(3.0±0.1)3.0	15(16±1)16	12.3
	Adaxial	66(78±2)95	5.0(7.0±0.2)8.0	3.0(5.5±0.3)8.0	-	-	-	-
M. crispata	Abaxial	39(44±1)47	8.0(10.1±0.2)11.0	4.0(5.4±0.2)7.0	5.0(5.8±0.1)6.0	4.0(4.4±0.1)4.8	13(13±1)13	12.9
	Adaxial	74(79±2)84	7.0(8.3±0.3)10.0	5.0(7.4±0.3)10.0	-	-	-	
	Abaxial	37(34±2)40	8.0(10.1±0.3)12.0	5.0(7.6±0.4)9.0	7.0(7.1±0.1)8.0	4.1(4.2±0.1)4.5	7(10±1)12	12.6
M. myristica	Adaxial	57(59±1)62	5.0(9.0±0.4)12.0	4.0(5.8±0.2)8.0	-	-	-	
M. tenuifolia	Abaxial	54(60±3)68	6.0(10.1±0.5)12.0	4.0(5.8±0.3)8.0	5.0(5.2±0.1)5.0	1.0(1.1±0.1)1.2	16(19±1)21	13.3
	Adaxial	64(67±2)75	6.0(8.8±0.4)11.0	4.0(6.2±0.4)9.0	-	-	-	
M. brevipes	Abaxial	70(79±2)85	5.0(7.5±0.4)10.0	4.0(5.9±0.3)8.0	7.0(9.2±0.2)7.4	1.0(1.1±0.1)1.2	16(19±1)21	7.7
	Adaxial	17(18±1)20	16.0(34.8±2.9)57.0	16.0(18.7±0.4)22.0	-	-	-	
M.undulata	Abaxial	80(89±2)100	5.0(6.7±0.4)10.0	5.0(5.1±0.1)6.0	4.0(4.7±0.1)5.0	1.0(1.1±0.1)1.2	6(8±1)10	2.3
	Adaxial	120(126±3)132	6.0(7.3±0.2)9.0	5.0(6.1±0.2)7.0	-	-	-	
M. zenkeri	Abaxial	86(92±2)100	9.0(7.8±0.2)7.0	4.0(4.4±0.1)5.0	4.0(4.9±0.1)5.2	1.0(1.1±0.1)1.2	13(14±1)18	8.1
	Adaxial	63(72±2)85	7.0(9.4±0.19)10.0	4.0(5.8±0.3)7.0	-	-	-	



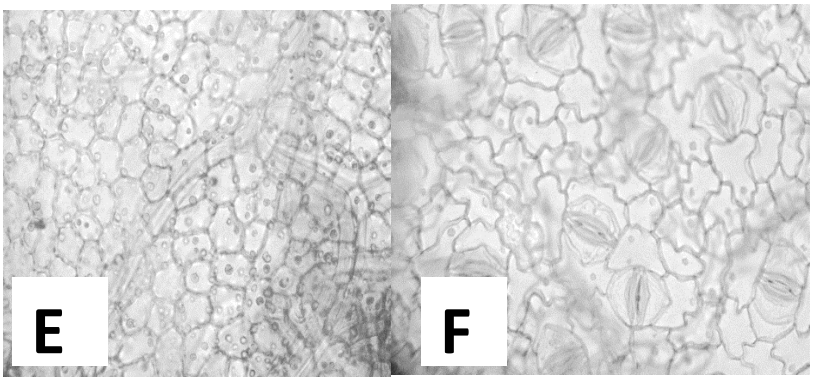
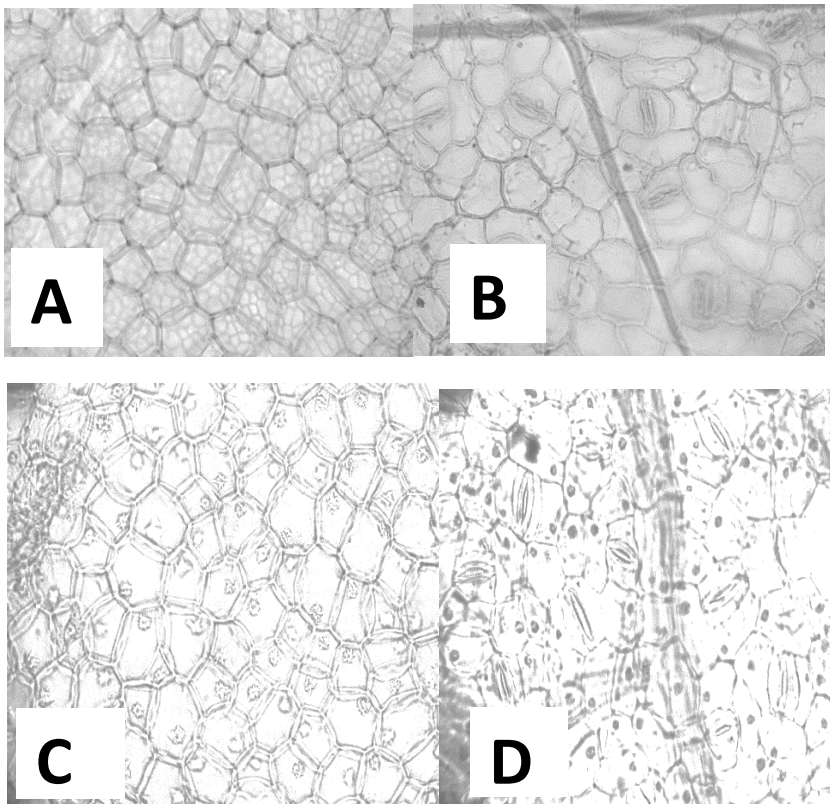


Figure 1: Characters of leaf epidermis of *Monodora* species under light microscope: A,B- *Monodora angolensis*, C,D- *M. brevipes*, E,F- *M. crispata*. A, C, E:Adaxial surface, B, D, F: Abaxial surface. Scale bars, 50 µm.



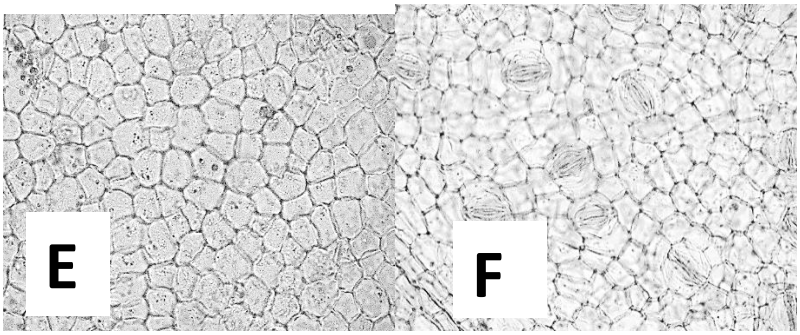


Figure 2: Characters of leaf epidermis of *Monodora* species under light microscope: A, B- *Monodora myristica.*C, D- *M. tenuifolia*, E, F- *M. undulata.*A, C, E: Adaxial surface, B, D, F: Abaxial surface. Scale bars, 50 µm.

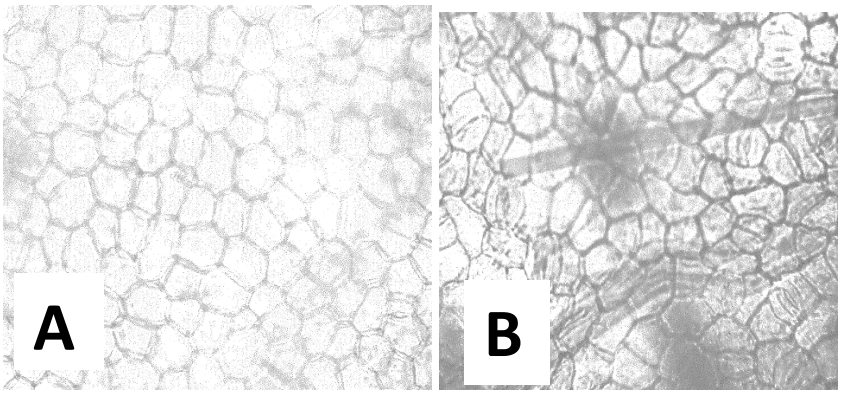


Figure 3: Characters of leaf epidermis of *Monodora* species under light microscope: A,B- *M. zenkeri*, Scale bars, 50 µm. A: Adaxial surface, B: Abaxial surface.

The summary of all the findings can be found in Tables 1 and 2, and Figures 1-4. The useful micro-features have been used to construct the graphical appearances of the species (Fig. 4). Both the non-stomatal and stomatal characters studied appear to yield information that can be relied upon for species discrimination. The uniform features such as

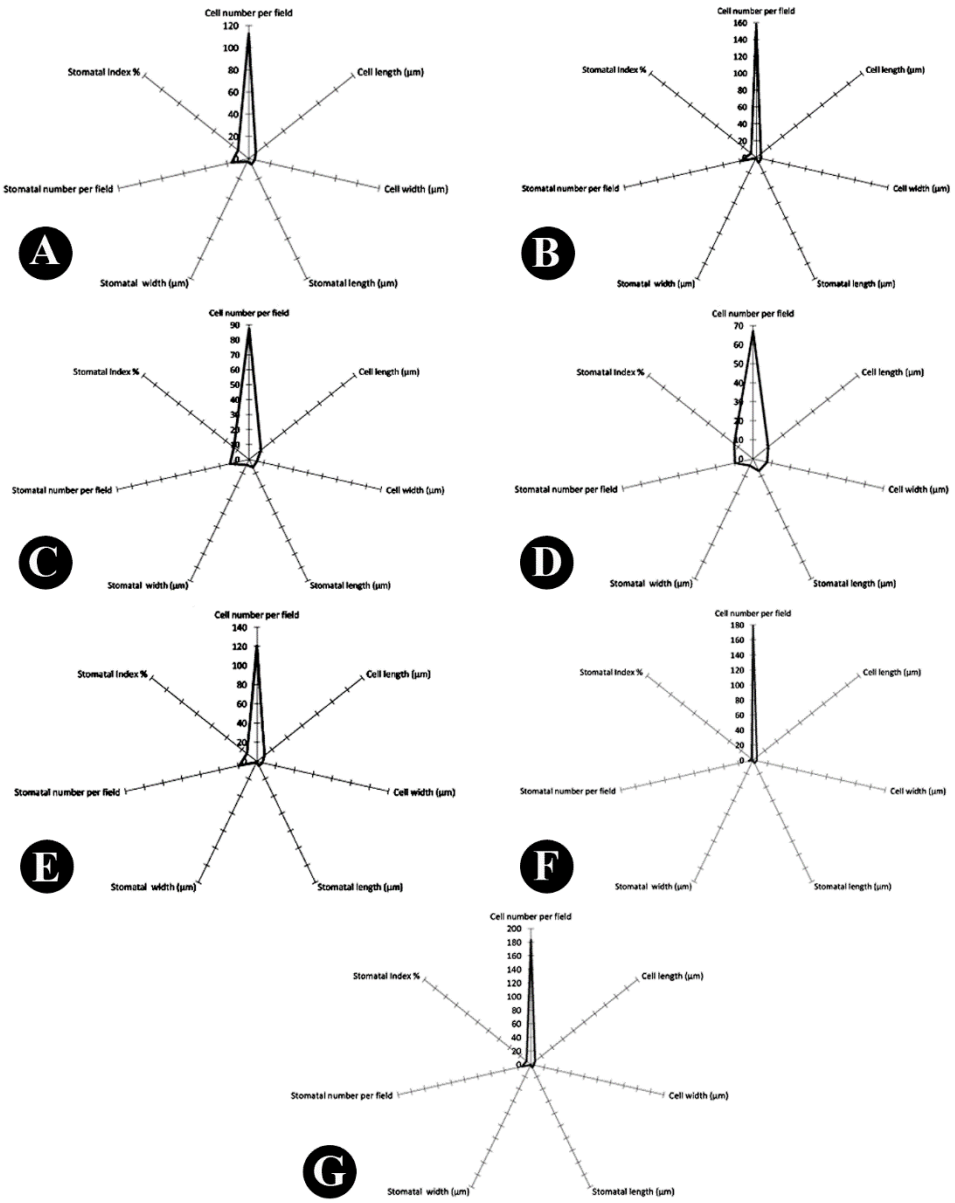


Figure 4: Relationship polygraphs showing character affinities and species closeness in some Central and West African species of *Monodora*. A: *Monodora angolensis*, B: *M. crispata*, C: *M. myristica*, D: *M. tenuifolia*, E: *M. brevipes*, F: *M.undulata* and G: *M. zenkeri*.

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