



## COMPARATIVE MORPHOLOGICAL AND ANATOMICAL STUDIES OF CYPSELAS OF SOME MEMBERS OF THE TRIBE MILLERIEAE (ASTERACEAE)

BIDYUT KR. JANA, SOBHAN KR. MUKHERJEE

Taxonomy and Biosystematics Laboratory, Department of Botany, University of Kalyani,  
Kalyani, Nadia, West Bengal.

### Abstract

Accepted Date:

28/09/2012

Publish Date:

27/10/2012

Keywords

Cypselar features

Millerieae

Asteraceae

Corresponding Author

Mr. Bidyut Kr. Jana

Taxonomy and

Biosystematics

Laboratory, Department

of Botany, University of

Kalyani, Kalyani, Nadia,

The fruits of Asteraceae are technically termed as achene or cypsela which is developed from bicarpellary unilocular ovary. The fruit is basically non endospermous but it has one to two layers of endosperm at mature state. The tribe Millerieae is a new tribe (Panero et al, 2001) consisting of 34 genera and 400 species which are mainly distributed in Mexico and northern Andes and also in Old World tropics. Morphological and anatomical variations of cypselas are very important to distinguish the species of this tribe. For this purpose 3 species of the tribe Millerieae such as *Melampodium perfoliatum* (Cav.) Kunth, *Tridax procumbens* L., *Acanthospermum hispidum* DC. have been studied to show the diversity of cypselar features. All of them exhibit morphological as well as anatomical variations. Among the studied species, *Melampodium perfoliatum*, *Tridax procumbens* have different from it cypselas whereas *Acanthospermum hispidum* shows similar type of cypselas.

Some of the cypselar features like as morpho configuration of cypselas, surface hairs, pappus structure, cell layers in carpodium, nature and distribution of phytomelan layer, distribution of fibre cells, sclerenchyma layers in hypodermal region as well as mode of orientation of epidermal cells of cypselas are important for characterization of taxa. Hypodermal sclerenchyma cells are found as continuous ring in *Tridax procumbens* whereas other two (*Acanthospermum* and *Melampodium*) these are found in discrete bundle. Similarly phytomelanin layer exist as discrete bundle in *Melampodium* whereas in others it is seen in continuous ring. On the basis of observed morphological and anatomical features, an artificial key is presented.

### INTRODUCTION

The tribe Millerieae consists of 34 genera and approximately 400 species found mostly in Central Mexico and the northern Andes with a few species in tropical regions of the Old World, especially Africa. Some members of the Espeletiinae are the defining element of the paramos of northern South America. Millerieae have recently been resurrected (Panero et al. 2001; Panero and Funk, 2002) to accommodate the genera variously placed by Robinson (1981) and other authors (Stuessy 1977; Karis and Ryding 1994) in subtribes Desmanthodiinae, Espeletiinae, Galinsogiinae, Guardioliinae, Melampodiinae and Milleriinae. Based on historical considerations, Guardioliinae are tentatively placed in Millerieae, as there is

no strong support for their inclusion here or in any of the sister tribes. Most of them are annual or perennial herbs, shrubs or trees, sometimes rosulate or caulirosulate; herbage sometimes conspicuously and densely glandular, sometimes viscous. The present circumscription of the tribe is based primarily on results of chloroplast DNA sequence analyses (Panero et al. 2001). The tribe does not have any single character which defines it but most of its species tend to have opposite leaves, glandular herbage, scarios paleae, subterete cypselae, a pappus, when present, of scales or bristles arranged radially on the neck of the cypselae, and trilobed ray corollas. Delimitation of monophyletic groups at the subtribal level in Millerieae is still tentative. Subtribe

Dyscritothamninae was recently described (Panero 2005), based on results from molecular studies of chloroplast DNA. Molecular data support the sister-group relationship of Dyscritothamninae and Melampodiinae. Melampodiinae have also been recognized as a distinctive group within the Heliantheae alliance (Stuessy 1977; Robinson 1981; Karis and Ryding 1994) because of their involucre with dimorphic phyllaries, the outer series being herbaceous and the inner fused to the ray cypsela. Perusal of available literature (Mukherjee SK., 1991; John A, 1921; Bhar I, Mukherjee SK.,2004 ) indicated that there is no work on the cypselar features of the tribe Millerieae as a whole, but instead a few isolated works have been done on some genera as a part of other tribe. Therefore, the present work has been undertaken.

#### MATERIALS & METHODS

Mature cypselas of 3 species were collected from 3 herbaria from the world (Table.1)

A few randomly selected dry cypselas from the procured mass were immersed in 5% NaOH solution for 2-3 days. After that, the cypselas were softened. The softened

cypselas were stained in aqueous safranin solution (1%) and dissected the different parts of cypselas with the help of 2 sharp needles under dissecting microscope and stereo dissecting binocular microscope. Different parts of cypselas such as structure of epicarpic cells in cypselar surface, distribution of vascular trace through the ribs, relative thickness of ribs and furrows, structure of pappus bristle and scales were observed and were drawn in both compound research microscope as well as stereo dissecting binocular microscope. For anatomical study, free hand cross sections were done preferably from the middle part of cypselas with the aid of sharp razor blade. Selected sections were stained in safranin light green combination following standard method of staining. Properly stained good sections were observed under compound research microscope.

#### RESULTS AND DISCUSSION

*Acanthospermum hispidum* D C.

#### MORPHOLOGY (Figure 1A, 2 A-B)

Cypsela homomorphic, 7 mm x 3 mm with awns, 5 mm x 3 mm without awns,

yellowish brown, obviate, straight, upper part truncate, basal part tapered. Ellipsoidal in cross sectional configuration. Surface rough; remain covered with spine like outgrowth. Some of this outgrowth remains bifurcate at the terminal region. Ribs not observed clearly. Surface hair absent. At the upper portion of cypsela, stylopodium present, unenlarged, partially immersed in the nectar. Corona absent. Carpopodium not clearly observed. Pappus represented by 2 awns, yellow brown, approximately 2 mm x 3 mm.

#### **ANATOMY (Fig. 3 A)**

Cypsela narrows elliptic in cross section. Cypselar wall 0.03 mm and 0.05 mm wide at rib and furrow region respectively. Pericarp thin, on an average 0.04 mm wide, differentiated into two zones- epicarp and mesocarp. Epicarp uni-seriate, made up of thin-walled, cubical, parenchyma cells, compactly arranged, provided with cuticle. Just below the epicarpic region, phytomelanin layer present discontinuously arranged. Mesocarp consists of thick-walled, compactly arranged, hexagonal, sclerenchyma cells with small cell lumen. Mesocarpic region separated by crusted

tissue zone. Testa attached with pericarp, approximately 0.01 mm thick, uni-seriate, parenchymatous. Endosperm persists in mature cypsela, uniseriate, thick-walled, and parenchymatous. Mature embryo occupies a major part of the cypsela; cotyledons two in number, arranged at right angle to the axis of cypsela, containing 10 resin ducts( 5 ducts in each cotyledon)

#### ***Melampodium perfoliatum (Cav.) Kunth***

#### **MORPHOLOGY (Figure 1 B-C, 2 C)**

Cypsela heteromorphic. Disk cypsela 3.4 mm x 2.5 mm, whereas ray cypsela 5 mm x 3 mm. Disk cypselas blackish, obviate, upper part rounded, basal part truncate, whereas ray cypselas yellow-brown, ovate, curved, upper part truncate, basal part rounded. Ellipsoidal in cross sectional configuration. Surface rough and glabrous containing 10 ribs alternating with furrow; furrows wider than ribs. The distance between 2 ribs 0.1 mm. Surface hairs absent. At the upper part of cypsela, stylopodium present, inconspicuous, corona absent. At the basal region of cypsela, carpopodium present, narrower than the base, irregular ring like. Carpopodial cells thick-walled, rounded, not pitted, arranged

in 4-6 rows, distinguishable from other cells of the cypsela. At the upper portion of cypsela, pappus absent.

#### **ANATOMY (Figure 3 B)**

Cypsela elliptic in cross section. Ribs present; 8-10 in number, inconspicuous. Cypselar wall 0.02 mm and 0.04 mm wide at rib and furrow region respectively. Pericarp thin, on an average 0.02 mm wide, differentiated into two zones- epicarp and mesocarp. Epicarp uni-seriate, made up of thin-walled, rectangular-ovoid, compactly arranged, parenchyma cells, provided with cuticle. Just below the epidermal layer, sub epidermal layer present, made up of parenchyma cells. Mesocarp consists of thick-walled, compactly arranged, more or less hexagonal, sclerenchyma patches and elongated fibre cells, alternately arranged. Phytomelanin layer present as discrete manner in between sub epidermal layer and mesocarpic layer. Testa not observed. Endosperm not observed.

#### ***Tridax procumbens* L.**

#### **MORPHOLOGY (Figure 1 D-I, 2 D-G)**

Cypsela heteromorphic. Disk cypsela 2.5 mm x 0.05 mm, whereas ray cypsela 3 mm x

0.05 mm. Disk cypsela yellow brown, oblanceolate, straight, upper portion truncate, whereas basal portion slightly tapered. Ray cypsela light brown, obovate, straight, upper portion truncate, whereas basal portion tapered. Ellipsoidal in cross sectional configuration. Surface pubescent type containing 15-17 ribs, alternating with furrow; furrows wider than ribs. The distance between 2 ribs 0.02 mm. Surface after clearing shows phytomelanin layer. Surface hair ascending in orientation with the cypsela axis, containing body and basal cells. Pappus present; represented by plumose type of pappus bristles, arranged in 2 whorls, light brown, unequal. At the upper portion of cypsela, stylopodium present, inconspicuous. At the basal region of cypsela, carpopodium present, narrower than the base, irregular ring like, outline visible. Carpopodial cells thick-walled, elliptic, not pitted, arranged in 2 rows, distinguishable from other cells of the cypsela.

#### **ANATOMY (Figure 3C)**

Cypsela narrows elliptic in cross section. Cypselar wall 0.02 mm and 0.03 mm wide at rib and furrow region respectively. Pericarp

thick, on an average 0.02 mm wide, differentiated into two zones- epicarp and mesocarp. Epicarp Uni-seriate, made up of thick-walled, cubical, parenchyma cells, compactly arranged, provided with cuticle. Just below the epicarpic region, phytomelanin layer present continuously arranged. Mesocarp consists of thick-walled, compactly arranged, and more or less rounded, parenchyma cells with small cell lumen. Testa attached with pericarp, approximately 0.01 mm thick, uni-seriate, parenchymatous, horizontally arranged. Endosperm persists in mature cypsela, uniseriate, thick-walled, and parenchymatous. Mature embryo occupies a major part of the cypsela; cotyledons two in number, arranged at right angle to the axis of cypsela, containing 12 resin ducts( 6 ducts in each cotyledon).

## Discussion

### MORPHOLOGY

Three (3) species belonging to the tribe Millerieae have been studied. Cypselas are homomorphic (*Acanthospermum hispidum*) and heteromorphic (*Tridax procumbens*, *Melampodium perfoliatum*). The colour of cypselas is greatly variable, this is due to the

state of maturity of cypselas as well as inherent features but not have any important taxonomic value. . The size of cypsela is also variable. Among the studied species smallest cypsela has been reported in *Tridax procumbens* – 3 mm x 0.05 mm and largest cypsela has been reported in *Acanthospermum hispidum* -7 mm x 3 mm. Shape of cypsela is not an important taxonomic characters in the studied species. On the surface of cypsela number of ribs is also variable. In *Tridax procumbens*, cypsela has 15-17 ribs, *Acanthospermum hispidum*, ribs not clearly observed, *Melampodium perfoliatum*, containing 10 ribs. In *Melampodium perfoliatum* and *Acanthospermum hispidum*, surface rough and glabrous whereas in *Tridax procumbens*, surface pubescent. . Different forms of cypselar morphology have been noticed by Mukherjee & Sarkar, 1994; Jana & Mukherjee, 2012; Bhar & Mukherjee, 2004; Mukherjee & Sarkar, 1999; Mitra & Mukherjee, 2003. At the basal region of cypsela, carpodium present. Carpodium is the basal abscission zone of the cypsela, which is attached to the receptacle and composed of one or more

rows of cells, which are distinct morphologically from the rest of the cells of cypselar wall. In *Tridax procumbens*, carpopodial cells arranged in 2 rows; *Melampodium perfoliatum*, carpopodial cells arranged in 4-6 rows; *Acanthospermum hispidum*, carpopodium not clearly observed. Initial information about the different types of abscission zone of cypselar wall in Asteraceae was presented in the works of John, 1921; Mukherjee & Nordenstam, 2004. This zone has been recognized as 'callus or podocarp' by Robinson, 1913; 'separation tissue' by Roth, 1977. In *Tridax procumbens*, at the upper portion of cypselar wall, pappus present, represented by plumose type of pappus bristle, arranged in 2 circles. This pattern of the arrangement of pappus also present in some species of the tribe Vernonieae by Mukherjee & Sarkar, 2001. In *Melampodium perfoliatum* and *Acanthospermum hispidum*, pappus absent but in *Acanthospermum hispidum*, terminal awn present. The pappus is a classical source of taxonomic information at the generic and specific level but less at the higher levels. It may be persistent or caduceous and some time deciduous.

Cypselar wall without pappus has been reported in *Carpesium* of the tribe Inuleae by Mukherjee & Sarkar, 1994. At the upper portion of cypselar wall, stylopodium present. In *Tridax procumbens*, stylopodium inconspicuously arranged. In *Acanthospermum hispidum*, stylopodium present, unenlarged, partially immersed in the nectar. In *Melampodium perfoliatum*, stylopodium inconspicuously arranged. Diversity of stylopodial structure has been reported by Mukherjee, 2005.

#### ANATOMY

Cross sections of cypselar wall show pericarp, testa and endosperm. In all the studied species, epicarp is made up of uniseriately arranged parenchyma cells. Mesocarpic zone exhibits cellular variations in the studied species. In *Tridax procumbens*, mesocarpic region made-up of thick-walled parenchyma cells. In *Acanthospermum hispidum*, mesocarpic region made-up of thick-walled, hexagonal, sclerenchyma cells. In *Melampodium perfoliatum*, mesocarpic region made-up of compactly arranged, thick-walled, hexagonal, sclerenchyma cells and fibre cells. Variations in the anatomical features of cypselas have been reported by

many authors (Mukherjee, 2001; Basak & Mukherjee, 2001). Anatomically, phytomelanin layer is present in all the studied species. Phytomelanin is a unique type of resinous substance, which is usually present in the members of the tribe Heliantheae, Helenieae, Eupatorieae and *Arnica*, an unusual genus of the tribe Senecioneae by Mukherjee, 1991. But we observe the presence of phytomelanin layer in the tribe millerieae. In *Tridax procumbens* and *Acanthospermum hispidum*, phytomelanin layer present continuously whereas in *Melampodium perfoliatum*, phytomelanin layer arranged discontinuously. In *Tridax procumbens* and *Acanthospermum hispidum*, testal layer made-up of uniseriately arranged, parenchyma cells whereas in *Melampodium perfoliatum*, testal layer not clearly observed. In *Tridax procumbens* and *Acanthospermum hispidum*, endosperm layer made up of uniseriately arranged parenchyma cells but in *Melampodium perfoliatum*, endosperm layer not clearly observed. Number of resin ducts in the cotyledons also variable. In *Tridax procumbens* and *Acanthospermum hispidum*, cotyledons containing 12 and 10

resin ducts respectively. Presence of fixed number of resin ducts in cotyledon has been suggested by Pandey and Sing, 1982 and Cron et al, 1993.

#### Key to the studied species

1a. Cypselas homomorphic....

***Acanthospermum hispidum*.**

1b. Cypselas heteromorphic ..... (2)

2a. Phytomelanin layer present continuously... ***Tridax procumbens*.**

2b. Phytomelanin layer present discontinuously... ***Melampodium perfoliatum***

#### ACKNOWLEDGEMENTS

Authors are grateful to the curators of the 3 herbaria (Botanischer Garten und Institute fur Systematische Botanik, Zollikerstrasse 107, CH- 8008 Zurich, Switzerland, Swedish Museum of Natural History, P.O. Box 50007, SE-10405 Stockholm, Sweden, Colombia and Institute De Investigacao cientifica De Angola, Herbario (LUI)) for sending mature seeds for this study.

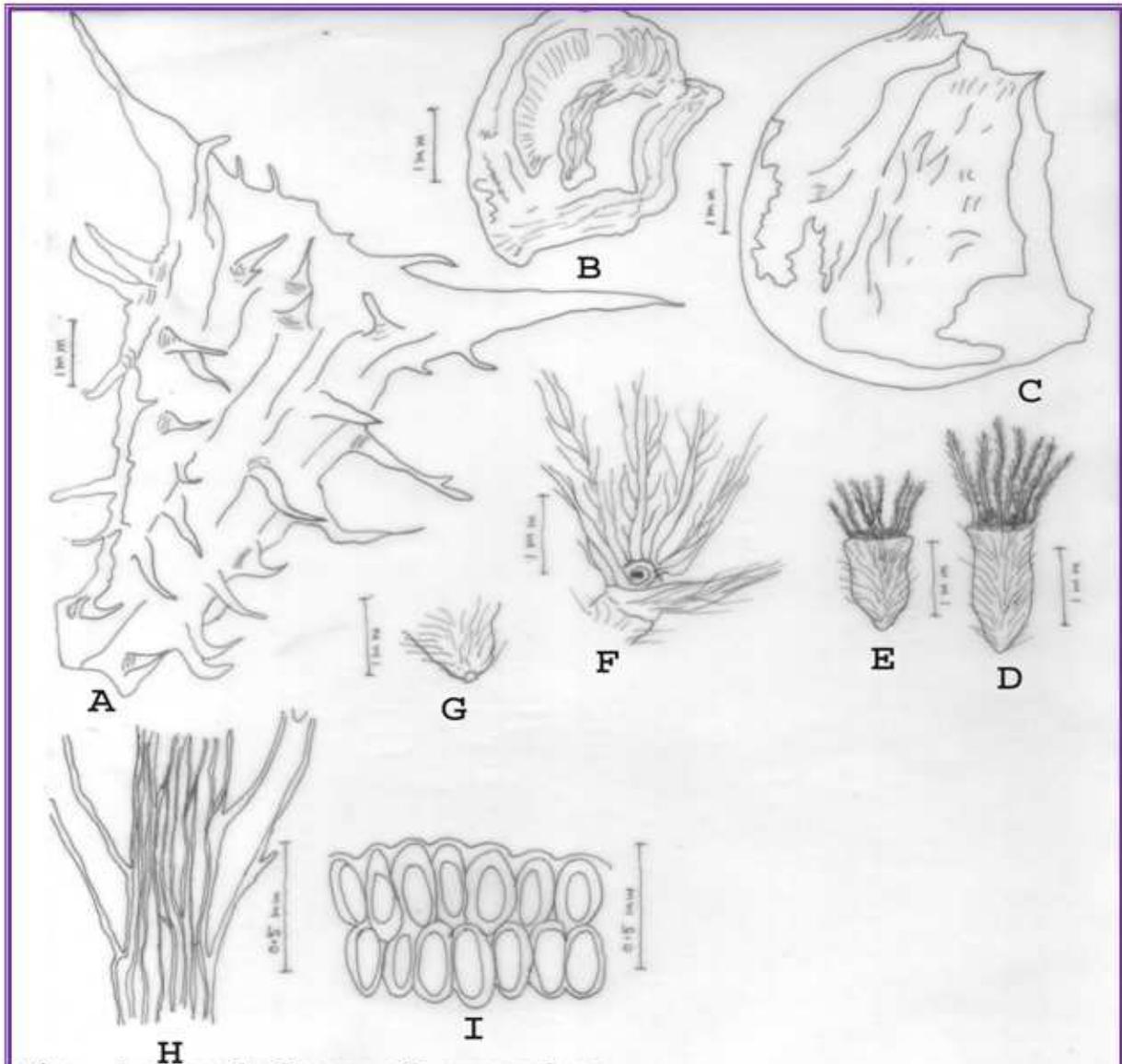
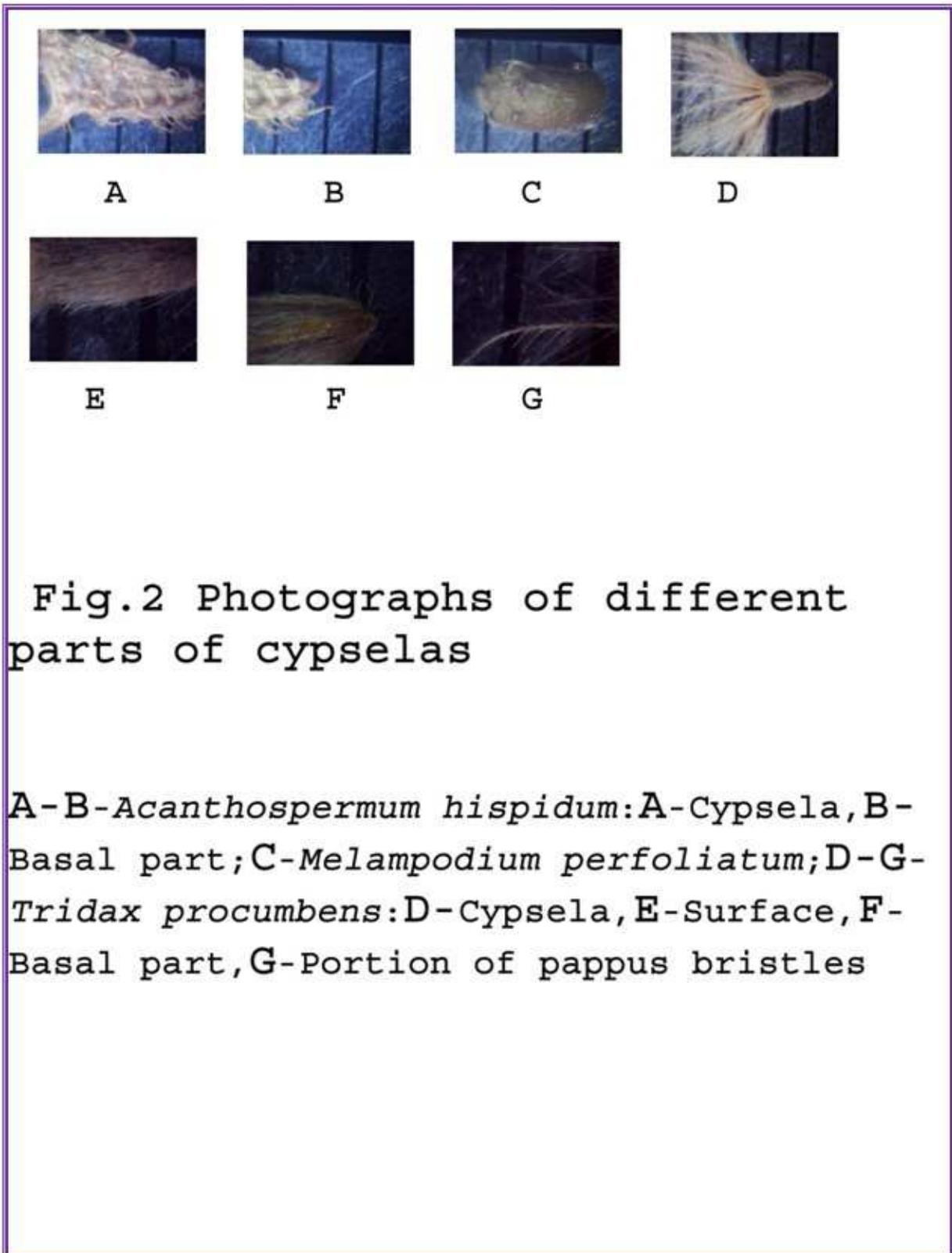
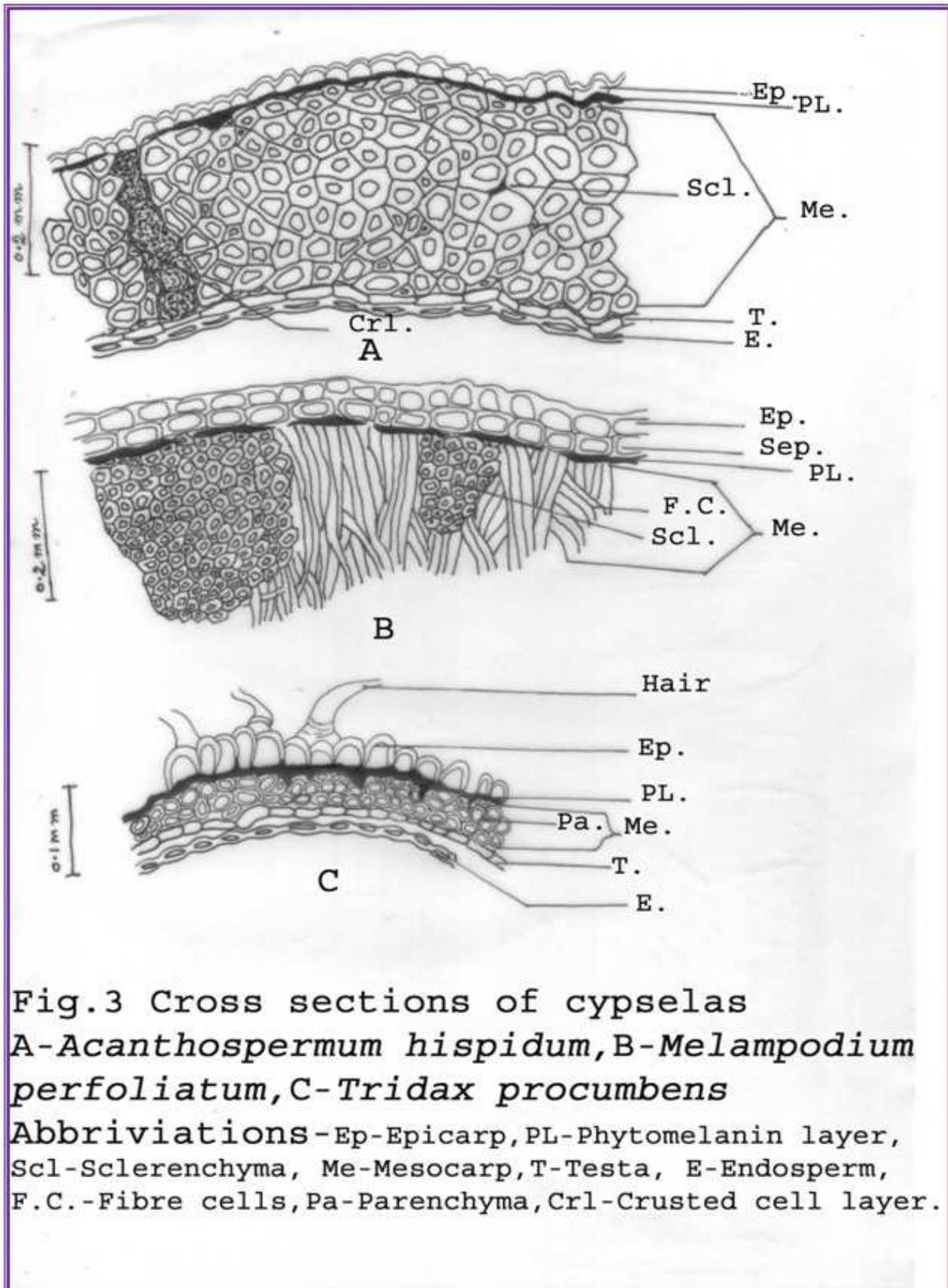


Fig. 1 Morphology of cypselas  
A:Cypselus-Acanthospermum hispidum; B-C-Melampodium perfoliatum-B: Cypselus ( Disk cypselus), C:Cypselus (Ray cypselus); D-I-Tridax procumbens: D-Ray cypselus, E-Disk cypselus, F-Upper part of cypselus, G-Lower part of cypselus, H-Parts of pappus bristles, I-Carpodial cells.





**Table 1**  
**Studied materials and their sources**

Taxa	Sources
1. <i>Acanthospermum hispidum</i> D.C.	Instituto De Investigacao Cientifica De Angola, Herbario (LUAI). Col: A. Menezes, No- 4223, Dated 28.6.1972
2. <i>Melampodium perfoliatum</i> (Cav.) Kunth	Botanischer Garten der Universitat Zurich, Zollikerstrasse 107, CH- 8008 Zurich, Switzerland. Specimen number: XXOMJG 19- 46810, 78, 2009 Drawer.
3. <i>Tridax procumbens</i> L.	B. Nordenstun, 2009.02.02. Swedish Museum of Natural History, P.O. Box 50007, SE-10405 Stockholm, Sweden. Ref No: Colombia, Cartagena, 8.11.1928, Herb. Erik Wall s.n.(S).

### REFERENCES

1. Basak N and Mukherjee SK: The morphology and anatomy of the Cypselas of some species of *Helianthus* L. (Asteraceae-Heliantheae) and its taxonomic significance. Nat. Bot. Soc., 2001; 55: 111-124.
2. Bhar I and Mukherjee SK: Macro morphological and Micro morphological study of cypselas in seven species of the tribe Anthemideae- (asteraceae). J. Econ. Taxon. Bot. 2004; 28(3): 788-794.
3. Corn GV, Robbertse PJ, Vincent, PLD: The anatomy of the cypselae of species of *Cineraria* L. (Asteraceae-Senecioneae) and its taxonomic significance. Bot. J. Linnean Soc., 1993; 112: 319-334.
4. Jana BK and Mukherjee SK: Diversity of cypselar features of seven species of the genus *Crepis* L. in Compositae. Indian Journal of Fundamental and Applied Sciences. 2012; 2(1): 51-58.

5. John A: Beitrage Zur Kenntnis der Ablosungsvorrichtungen der Kompositenfruchte, Beih, Bot. Zentralblatt, 1921; 38: 182-203.
6. Karis PO and Ryding O: Tribe Heliantheae. In: Bremer K. (ed.) Asteraceae. Cladistics & classification. Portland, OR: Timber Press, 1994: 559-624.
7. Mukherjee SK and Sarkar AK: Morpho-anatomical studies of Cypselas in some members of the *tribe Inuleae* (asteraceae) with the help of SEM. J. Natl. Bot. soc. 1994; 48 19-39
8. Mukherjee SK and Sarkar AK: Studies of Macro-morphological structures of Cypselas of two Taxa of the tribe Calenduleae (Asteraceae) by sem and Lm. J. Swamy Bot. Cl. 1999; 16: 21-24
9. Mitra S and Mukherjee SK. Morpho-Anatomical study of Cypselas of *Crassocephalum crepidioides* (Benth.) S. Moore- A rare plant of West Bengal (India). J. Swamy Bot-Cl. 2003; 20: 19-22
10. Mukherjee SK and Nordenstam B: Diversity of carpopodial structure in the Asteraceae and its taxonomic significance. Comp. Newsl. 2004; 41: 29 -50.
11. Mukherjee SK: comparative studies of Stylopodium in some Asteraceae. Plant Taxonomy. Advances and Relevance, Eds. A.K. Pandey, Jun & J.V.V. Dogra, 2005: 493-503.
12. Mukherjee SK and Sarkar AK: Study of macro-morphological and anatomical structures of Cypselas of eighteen taxa of the tribe Vernonieae (Asteraceae). Nat. Bot. Soc., 2001; 55: 85-104,
13. Mukherjee SK. (1991). Carpological studies in Compositae. Ph.D. thesis, Kalyani University, W.B. India. (Unpublished).
14. Panero JL., Baldwin BG., Schilling EE and Clevinger JA: Molecular phylogenetic studies of members of tribe Helenieae, Heliantheae and Eupatorieae (Asteraceae). 3. General systematic and proposed taxonomic changes in current classification. In: Osborn, J.M. (Prog.dir.) Botany, Abstracts, part 3. Systematics. St. Louis, Mo: Botanical society of America. 2001.
15. Panero JL and Funk VA: Toward a phylogenetic sub familial classification for the Compositae (Asteraceae). Proc. Biol. Soc. Wash. 2002; 115:909-922.

16. Pandey AK and Sing RP: Development and structure of seeds and fruits in Compositae: *Coreopsis* species. J. Indian Bot. Soc., 1982; 61: 417-425.

17. Panero JL: New combinations and infrafamilial taxa in the Asteraceae. Phytologia. 2005; 87: 1-14.

18. Robinson H: A revision of the tribal and sub tribal limits of the Heliantheae (Asteraceae). Smithsonian contr. Bot. 1981; 51: 1-102.

19. Robinson BL: Revisions of *Alomia*, *Ageratum* and *Oxylobus*. Proceedings of the American Academy of Arts and Sciences 1913; 49; 438-491.

20. Roth L: Handbuch der Pflanzenanatomie, Spezieller Teil, Band X, Teil I. Gebruder Borntraeger, Berlin. 1977.

21. Stuessy TF: Heliantheae- Systematic review. In: Heywood, V.H., Harborne, J.B., Turner, BL. (Eds) the biology and chemistry of the Compositae, Vol.2. London: Academic Press, 1977: 621-671.