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Research Article

DIVERSITY OF CYPSELAR ANATOMY IN NINE SPECIES OF THE TRIBE ANTHEMIDEAE (ASTERACEAE)

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ABSTRACT
The paper deals with cypselar anatomical characters of 9 species belonging to 4 genera *(Achillea, Chamaemelum, Leucanthemopsis and Matricaria)* of the tribe Anthemideae. The anatomical features of the above said species have been investigated to establish their potential usefulness in taxonomy. The significant anatomical features of cypselas are cypselar shape in T.S., cypselar cuticle, ribs or elevations no., size of the ribs, thickness of cypselar wall (in µm) at ribs and furrow, pericarp thickness (in µm) at ribs and furrow, tissue differentiation of epicarp, epicarpic cell shape, epicarpic cell wall thickness, orientation of epicarpic cell, tissue differentiation of mesocarp, mesocarpic parenchymatous cell, mesocarpic sclerotic braces, mesocarpic vascular bundle, mesocarpic resin cavity, mesocarpic cavity, presence of endocarp, testa thickness (in µm), testal attachment with pericarp, tissue differentiation of testa and layers, cell content of testa, testal cell shape, thick walled cells of testa, crystals and their distribution, testal palisade cells, orientation of testal cells, endosperm in mature cypsela, non –cellular pellicle, nature of mature embryo, resin ducts/ secretory ducts in each cotyledon and relative size of the secretory ducts etc., have been examined. These characters of cypselas serve as reliable taxonomic marker in systematic study. The 'nature of testa' also has been used as significant taxonomic parameter for the identification of studied taxa. Based on these above features, an artificial key is prepared for easy identification.

Keywords: *Cypselar Anatomy, 4 Genera, 9 Species, Anthemideae, Compositae*

INTRODUCTION
The Compositae (Asteraceae) are considered as one of the largest, highly evolved, most successful and specialized family among the angiospermic plant families, approximately 24,000 species distributed in 1,600 – 1700 genera, 12 subfamilies and 43 tribes, found throughout the world except Antarctica. It is assumed that about 10% of total flowering plants are nested in this monophyletic family (Funk *et al*., 2009).

The tribe Anthemideae is included under sub family Asteroideae of the family Asteraceae. Anthemideae is one of the leading tribe, where anotomical features of cypselas provided important clues, which have been used to characterize the taxa. The tribe consists of 111 genera with 1,800 species, distributed throughout the world but central Asia, the Mediterranean region and Southern Africa are considered as mainland for the tribe (Kubitzki, 2007). Briquet (1916), Hegi (1918, 1929), Giroux (1933), Horvatic (1963), Kynclova (1970), Heywood and Humphries (1977), Kallersjo (1985, 1991), Weberling and Reese (1989), Mukherjee and Sarkar (1991), Bremer (1994), Mabberly (2008), Kadreit and Jeffrey (2007), Das and Mukherjee (2012) have been engaged and engrossed by the tribe Anthemideae and have blended the characters of cypsela either from anatomical or from morphological observation or both, using light microscope. Horvatic (1963) studied morphology as well as anatomy of cypsela of this tribe. Schermann (1967) had studied some species of achene of this tribe.

Cypselar external features have usually been included by different floristic workers during their preparation of floristic accounts in brief, but details morphological and/ or anatomical features of cypselas have not been stipulated. In this respect, Roth (1977) has pointed out that "Not only in the external morphology of the achenium very characteristic, but also its inner structure shows certain qualities which can be used taxnomically." Cypselar features both from morphological and anotomical studies or either

any one of them have been used for taxonomic evaluation in the tribe Anthemideae by some workers like Hegi (1918, 1929), Kynclova (1970), Heywood and Humphries (1977), Weberling and Reese (1988). Although these works have significant value, yet more and detail study in this respect are needed to fulfill the lacunae of the previous data.

The present investigation is executed to supplement the former works, reshaping the existing classification system and better understanding of taxa.

The present study deals with the detailed structure of cypsela in 9 species belonging to 4 genera of the tribe Anthemideae. An artificial key is presented here to recognize the studied taxa at the specific level.

Anatomical structures of cypsela studied in detail using light microscope (LM). The main objective of this study is to specify the diacritical features of cypsela, which can be recruited as an ideal taxonomic marker for evaluation of taxa at or below the infra generic level and also to show the relationship among them.

MATERIALS AND METHODS

The present work is based on the 9-herbarium materials, which were obtained from the 5 herbaria of the world as a gift. Voucher specimens were stored in desiccators with fused Calcium Chloride, in the herbarium of the Department of Botany, University of Kalyani (KAL) and which are listed in details in table I.

Table I

Sl. No.	Name of the Studied Taxa	Source of the Specimens, Collector and/ or Collection No.
1	*Achillea nana* L.	Conservatoire et Jardin Botanique Geneve-Geneve (G), Valais, Gornorgrat, 3130m, Col. Lachaed, G. et.al., No. 608
2	*A. collina* J. Becker ex Rchb.	Flora of Austria, Lower Austeia, Thermenlinie, wineyards east of the Heberlberg 2km southwest of Gumpoldskirchen, 275 ms.s., 16°16'E ,No. 42
3	*A. millefolium*L.	BereichBotanik und Arboretum des Museums fur. Naturkunde der HumboldtUniversitatZuBerlin, DDR - 1195 BerlinSpathstr. 80/81 (BHU), No. 229/ 715
4	*A. ptarmica* L.	HortusUniversitatisHauniensis, Botanic Garden of Copenhagen (DK), No. 179
5	*Chamaemelum nobile* (L.) All.	BotanischerGarten der Universitat Zurich,Switzerland, Poschiavo GR, Bernnapasis2330 m asl, XX0Z19940181
6	*Leucanthemopsis alpina* (L.) Heywood.	BotanischerGarten der Universitat Zurich,Switzerland, Poschiavo GR, Bernnapasis2330 m asl , CHOZ-20110538
7	*Matricaria maritima* L.	BereichBotanik und Arboretum desMuseums fur. Naturkundeder Humboldt UniversitatZuBerlin, DDR - 1195 Berlin Spathstr. 80/81 (BHU), No. 229/758
8	*M. chamomilla*L.	Botanischer Garten der Universitat Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland., No. 301/ 284
9	*M. discoidea*DC.	Hortus Universitatis Hauniensis, Botanic Garden of Copenhagen (DK), No. 390

The herbarium specimens from various sources were given by respective Directors and Curators of the Herbaria.

Following steps have been successfully taken for the anatomical studies of the cypselas;

1. Depending upon the hardness of the cypselar wall, different solutions have been used for various time spans, such as -

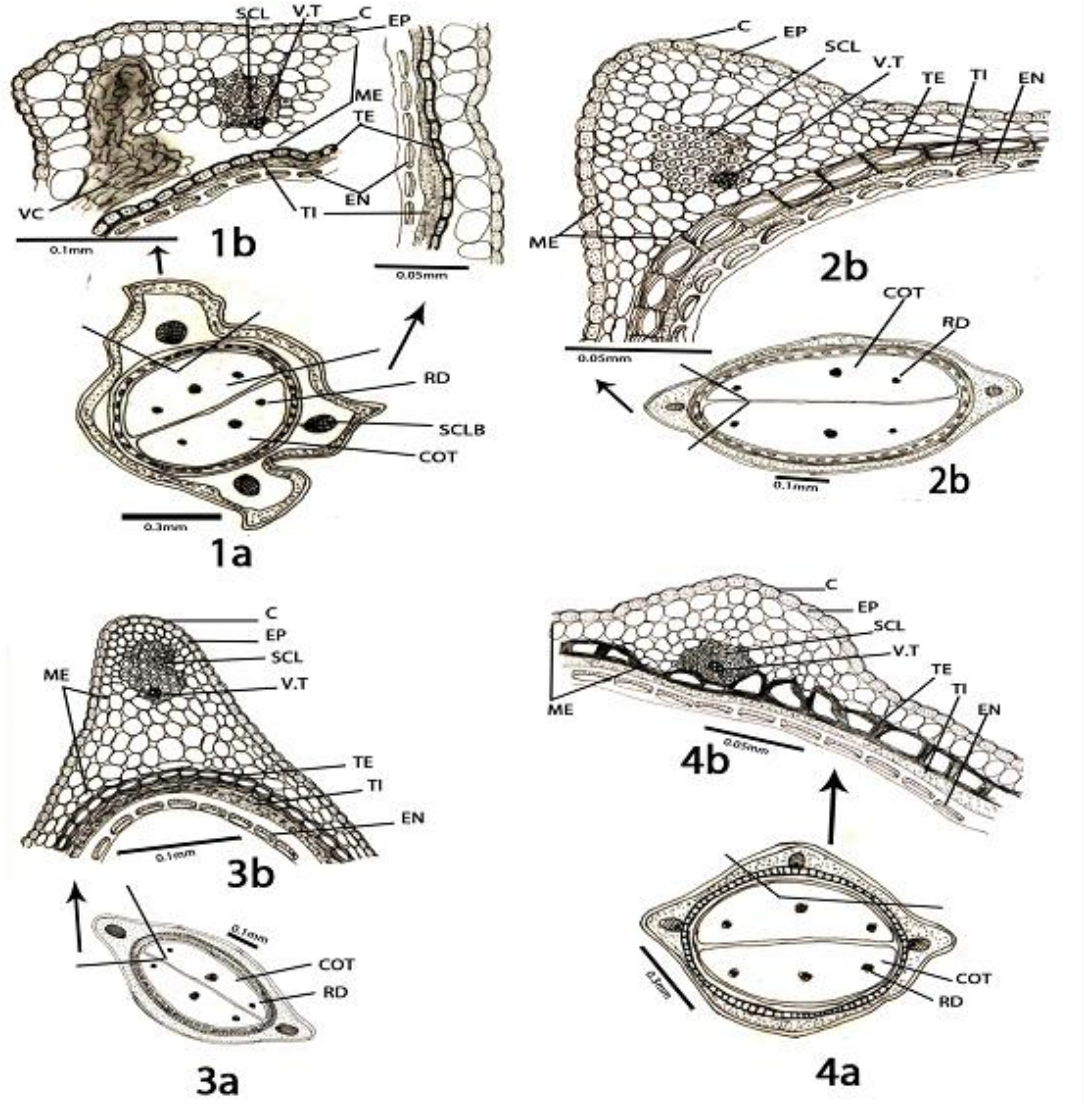
(i) Cypselas were treated by boiling in water bath for 5– 30 minutes, with a few drops of glycerol.

(ii) Occasionally cypselas are keeping in the 2N NaOH solution for 1 – 10 hours.

(iii) Sometimes used picric acid solution or 70% phenol-glycerine solution or lactophenol solution in boiling water bath for 10 – 60 minutes to treat cypselas.
2. To study the internal nature of the tissue of the cypsela, need very thin transverse section of the cypselas, which was done mainly by good handmade sections from the middle part of mature cypsela. The fine sections were dehydrated following the method of Johanson (1940) with various alcohol grades.
3. Various internal characters of cypselar -tissues such as- tissue type, arrangement, thickness, shape and orientation of cells, presence of cuticle etc. were observed under compound light microscope. Few other characters such as – presence of cavity, vascular trace, chemical deposition, crystals and no. of secretory ducts etc. were also observed.
4. Those observed above noted characters were documented with the help of camera lucida drawings.

RESULTS AND DISCUSSION

Comparative anatomical features of 9 studied species belonging to 4 genera *(Achillea, Chamaemelum, Leucanthemopsis, and Matricaria)* of the tribe are given in table II.



Figures 1-4: Anatomy of Cypsela; a- Diagramatic View, b- Part of Cypsela; 1a- 1b: *Achillea Nana*; 2a- 2b: *A. Collina*; 3a- 3b: *A. Millefolium*; 4a- 4b: *A. Ptarmica*

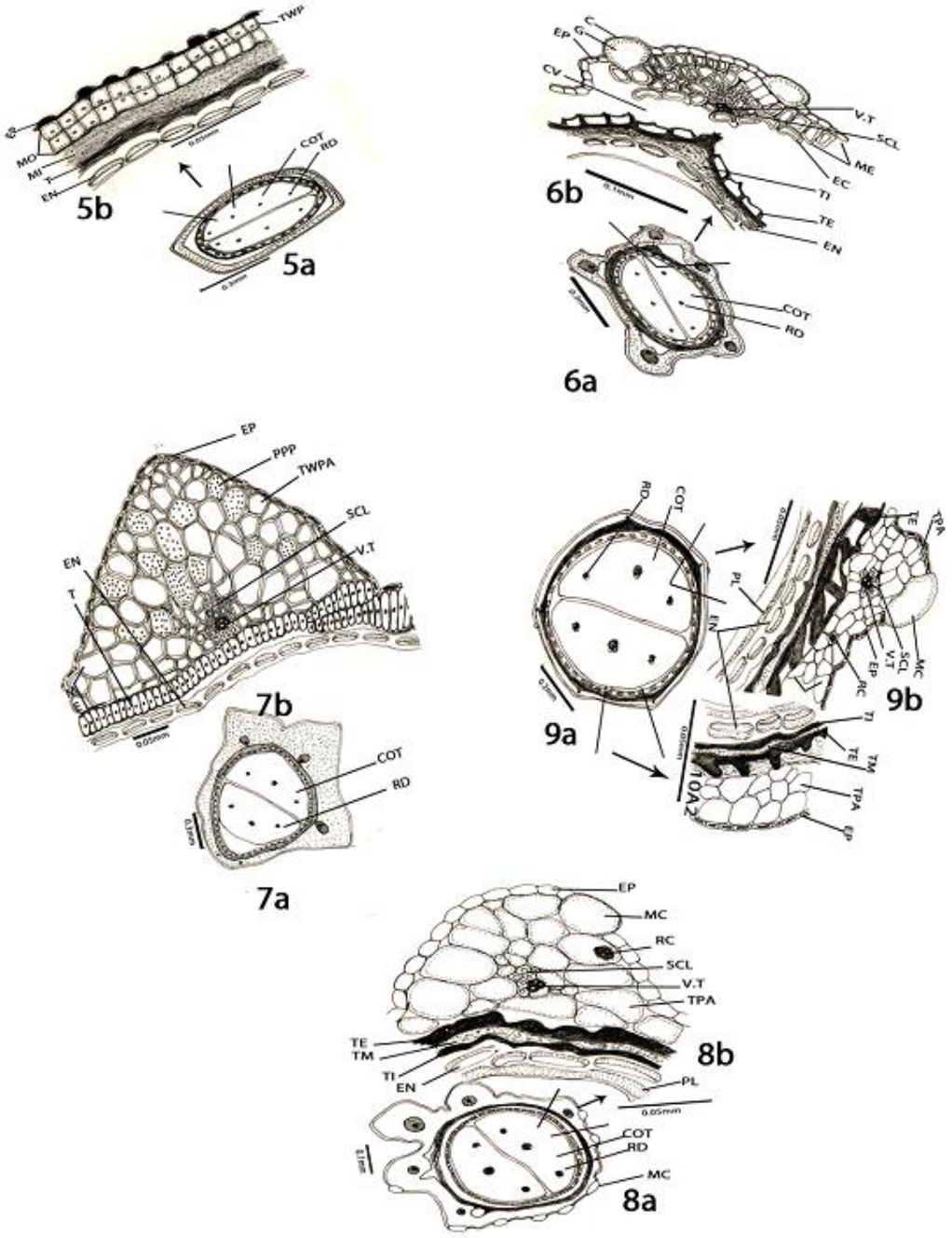
Table II

Sl. No.	Characteristic Features	*Achillea nana*	*A. Collina*	*A. Millefolium*	*A. Ptarmica*	*Chamaemelumnobile*	*Leucanthemopsis Alpina*	*Matricariamaritima*	*M. Chamomilla*	*M. Discoidea*
1	Cypselar shape in T.S.	Triangular	Ellipsoid	Ellipsoid	Round - ovoid	Four angular-Ellipsoid	Pent angular	Triangular	Pent angular	Round
2	Cypselar cuticle	Present	Present	Present	Present	Absent	Present	Absent	Absent	Absent
3	Ribs or Elevations	Present	Present	Present	Present	Present	Present	Present	Present	Present
4	No. of ribs	3	2	2	3	4	5	2	5	5
5	Size of the ribs	Cons-picuous	Cons-picuous	Cons-picuous	Cons-picuous	Incons-picuous	Cons-picuous	Cons-picuous	Cons-picuous	Incons-picuous
6	Thickness of cypselar wall (in µm) at ribs and furrows	130.4-163, 45.64-65.2	66.57-72.91, 9.17-12.34	130.4-146.7, 26.08-35.86	169.52-192.34, 42.38-48.9	74.98-81.5, 74.98	133.66-163, 48.9-55.42	365.12-423.8, 130.4-195.6	117.36-182.56, 39.12-58.68	58.68-94.54, 32.6-42.38
7	Pericarp thickness (in µm) at ribs and furrows	117.36-143.44, 35.86-42.38	50.72, 6.34- 9.51	114.1-130.4, 16.3- 26.08	149.96-176.04, 22.82- 32.6	65.2-71.72, 6.34- 9.51	114.1-136.92, 35.86-39.12	329.6-374.9, 81.5-163	91.28-153.22, 19.59-42.38	32.6- 65.2, 16.3-22.82
8	Tissue differentiation	Absent	Absent	Absent	Absent	Absent	Present	Absent	Present	Present

	of epicarp									
9	Epicarpic cell shape	Oval	Rectangular	Oval-Rectangular	Oval-Rectangular	Ellipsoid	Oval	Rectangular	Rectangular	Oval-Ellipsoid
10	Epicarpic cell wall thickness	Thin	Thin	Thin	Thin	Thick	Thin	Thin	Thin	Thin
11	Orientation of epicarpic cell	Tangential	Tangential	Tangential	Tangential	Radial	Tangential	Tangential	Tangential	Tangential
12	Tissue differentiation of mesocarp	Present	Present	Present	Present	Present	Present	Present	Present	Present
13	Mesocarpic parenchymatous cell	Oval - round	Oval - round	Oval - round	Oval - round	Rectangular	Rectangular	Hexagonal(pitted cell)	Round	Round
14	Mesocarpic sclerotic braces	at centre of the rib	close to testa	close to epicarp	very close to testa	Absent	at centre of the rib	more closer to testa	near to testa	near to testa
15	Mesocarpic vascular bundle	Present	Present	Present	Present	Absent	Present	Present	Present	Present
16	Mesocarpic resin cavity	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Present
17	Mesocarpic cavity	Present	Present	Present	Absent	Absent	Present	Absent	Present	Absent
18	Endocarp	Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent	Absent

19	Testa thickness (in µm)	Thick 19.56-26.08	Thin 15.85	Thin 13.04- 16.3	Thin 16.3	Thin 6.52- 9.78	Thick 19.56-35.86	Thick 32.6-48.9	Thick 19.56-22.82	Thick 19.56-22.82
20	Testal attachment with pericarp	Absent	Present	Present	Present	Present	Absent	Present	Present	Present
21	Tissue differentiation of testa and layers	Present, 2	Present, 2	Present, 2	Present, 2	Absent	Present, 2	Absent, NA	Present, 3	Present, 3
22	Cell content of testa	Partially Cellular	Partially Cellular	Partially Cellular	Partially Cellular	Non-cellular	Partially Cellular	Totally cellular	Non-cellular	Partially Cellular
23	Testal cell shape	Rectangular	Rectangular	Rectangular	Rectangular	Disorganized	Rectangular	Rectangular	Disorganized	Rectangular
24	Thick walled cells of testa	Absent	Present	Present	Present	Absent	Present	Present	Absent	Present
25	Testal crystals	Absent	Absent	Absent	Absent	Absent	Absent	Present	Absent	Absent
26	Testal palisade cells	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
27	Orientation of testal cells	Tangential	Tangential	Tangential	Tangential	Tangential	Tangential	Radial	NA	Tangential

28	Endosperm in mature cypsela	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent
29	Endosperm layer	Uniseriate	Uniseriate	Uniseriate	Uniseriate	Uniseriate	Uniseriate	Uniseriate	Uniseriate	Uniseriate
30	Non –cellular pellicle	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present
31	Portion of cypsela occupied by mature embryo	Major part	Major part	Major part	Major part	Major part	Major part	Major part	Major part	Major part
32	Resin ducts/ secretory ducts in each cotyledon	3	3	3	3	4	3	3	3	3
33	Relative size of the secretory ducts	Unequal	Unequal	Unequal	Equal	Equal	Equal	Equal	Unequal	Unequal



Figures 5-9: Anatomy of Cypsela; a- Diagramatic View, b- Part of Cypsela; 5a- 5b: *Chamaemelum Nobile*; 6a- 6b: *Leucanthemopsis Alpina;* 7a- 7b: *Matricaria Maritima*; 8a- 8b: *M. Chamomilla;* 9a- 9b: *M. Discoidea*

Internal morphology or anatomy of cypselas slightly varies in different species of the tribe like ellipsoid with 2 lateral ribs (*A. collina, A. millefolium)*, ovoid to round with 3 ribs (*Achilleaptarmica*), round with 5 weakly developed lobes (*M. discoidea*), triangular with 3 lobs/ ribs (*A. nana, M. maritima*), four angular

to ellipsoid with 4 weakly developed lobes (*C. m nobile*), and pentangular with 5 unequal lobs (L.alpina, *M. chamomilla*). Heywood and Humphries (1977) reported that the number of the ribs in cypselas varies from 2- 10 with in the tribe Anthemideae. The opinion also confirmed by Bhar and Mukherjee (2004). Majority of the species with conspicuous ribs and few with inconspicuous ribs (*C. nobile*, *M. discoidea*). Pericarp usually differentiated into two zones in all the studied species except in *L. alpina*, where an extra layer- the endocarp is present. Epicarpic cells are usually thin walled, narrow and tangentially oriented but seldom thick walled, radially oriented (*Chamaemelumnobile*), thinly cuticularised except in *Matricaria*and *Chamaemelum*. In *Matricaria*, mucilage cells are found in the epicarpic zone as reported by Mukherjee and Sarkar (1990) but in *M. maritima*no such epicarpic mucilage cells found. Mesocarpic zone is huge and compose of mostly thin walled parenchymatous cells interrupted by sclerenchyma tissue (braces).

But thick walled parenchymatous cells observed in *M. maritima* and *L. alpina*. In *M. maritima*, pitted parenchyma also noticed. In *C. nobile*no such sclerenchyma braces observed. Sclerenchyma braces composed of multiseriatesclerenchymatous cells; cells polygonal, multilayered, present at centre at each ribs in *L. alpina*and *Achillea nana*, close to epicarp in *A. millefolium* and close or very close to testa in the remaining studied species. The location of sclerenchyma brace is very unique for the identification of the studied genera. Vascular trace is regularly found at the centre or at the base of the braces. In *Matricaria*, presence of mesocarpicresin cavity is very distinctive. Existence of resin glands in cypsela of *Matricaria* has also been reported by Kynclova (1970). The studied species can be separated into 4 following types on the basis of the nature of testa: (I) – Testa represents uni layer, non- cellular, collapsed thick-walled parenchymatous cells in *C. nobile*. (II) Testa represents uni or bilayers, cellular, thick-walled, radially oriented cells in *M. maritima*.(Ill) Testa represents 2 layers- outer cellular and inner disorganized, translucent thin walled parenchymatous cells in *A. nana***,** *A. collina***,** *A. millefolium, A. ptarmica***,** L. alpina. (IV) Testa represented by 3 layers- outer layer cellular or partially cellular, cells; sclerenchymatous thick-walled, middle layer shown disorganized, translucent thin walled parenchymatous cells and inner layer completely composed of collapsed thick wall cells in *M. chamomilla*, *M. discoidea*. Mukherjee and Sarkar (1990), Bar (2014) observed only 3 types of testa among their studied species of Anthemideae. Testa usually remains attached with pericarp except in *A. nana, L. alpina.*

The distribution of calcium oxalate crystals within testa is taxonomically important. Crystals are found in the testa in *M. maritime*. Kynclova (1970) did not notice any crystal structure while studying the same species. Jana and Mukherjee (2014), Bar (2014) also informed about the crystal structure and their distribution.

Endosperm is uniseriate for the studied species. But Talukdar (2012) has reported that "Endosperm is generally uniseriate, but biseriate in *Nivellea*, *Ursinia* and *Tanacetum macrophyllum*" in this tribe. Most of the authors remain silent about the non– cellular pellicle, but it is observed in *M. chamomilla* and *M. discoidea*.

From the above discussion it is obvious that cypselar internal characters are important for isolation and characterization of taxa at the species and generic level along with other reproductive or floral features.

List of Abbreviation Used

C- Cuticle, COT- Cotyledon, CPA- Collapsed / Crusted Parenchyma, EN- Endosperm, EP- Epidermis, MC- Mucilage cell, ME- Mesocarp, MI- Inner mesocarp, MO- Outer mesocarp, NA- Not applicable, PL- Pellicle, PPA- Pitted Parenchyma, RC- Resin cavity/ resin containing cell, RD- Resin Duct, SCL- Sclerenchyma, SCLB- Sclerenchyma brace, SCV- Secretary duct / cavity, T- Testa, TE- Testa epidermis, TI- Testa inner zone/ Inner Testa, TM- Testa middle zone, TO- Outer Testa, TPA- Thin wall Parenchyma, TWPA- Thick wall Parenchyma, VC- Valecular Cavity, VD- Valecular duct, VT- Vascular Trace**).**

An Artificial Key to the Studied Species

1a. Testa thick (>20µm)……..(2)
1b. Testa thin (<20µm)……(6)
2a. Mesocarpic resin cavity present; sclerenchymatous brace very close or close to testa……..(3)

2b. Mesocarpic resin cavity absent; sclerenchymatous braces at the centre of the rib……(5)
3a. Ribs (elevations) conspicuous or inconspicuous, number of ribs 5; mesocarpicparenchyma cells round and without pits; sclerenchymatous brace close to testa……(4)
3b. Ribs (elevations) conspicuous, number of ribs 2; mesocarpicparenchyma cells hexagonal and pitted; sclerenchymatous brace more closer to testa……..***Matricariamaritima***
4a. Ribs equal and inconspicuous; epicarpic cells oval to ellipsoid……..***Matricaria discoidea***
4b**.** Ribs unequal and conspicuous; epicarpic cells rectangular……***Matricaria chamomilla***
5a. Testal cells thick- walled, cells without brown substance ……....***Leucanthemopsis alpina***
5b.Testal cells not thick walled, cells with brown substance ……..***Achillea nana***
6a. Ribs conspicuous, number of ribs 2……(7)
6b. Ribs conspicuous or inconspicuous, number of ribs 3 or 4……...(8)
7a. Mesocarpicsclerenchymatous brace near the testa; thickness of cypsealr wall (excluding endosperm) at furrow about 9.17- 12.34µm……***Achillea collina***
7b. Mesocarpicsclerenchymatous brace very closer to testa; thickness of cypsealr wall (excluding endosperm) at furrow about 26.08- 35.86µm……***Achillea millefolium***
8a. Ribs conspicuous; number of ribs 3; thickness of cypsealr wall (excluding endosperm) at furrow < 50µm……***Achillea ptarmica***
8b. Ribs inconspicuous;, number of ribs 4; thickness of cypsealr wall (excluding endosperm) at furrow >50µm……***Chamaemelum nobile***

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