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Research Article

MICROMORPHOLOGY OF FRUIT SURFACES IN SOME TAXA OF THE TRIBE ANTHEMIDEAE (ASTERACEAE) AND THEIR TAXONOMIC SIGNIFICANCE

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ABSTRACT
The detailed micromorphological features of mature cypselas of fifteen species, seven of *Artemisia*, three each of Achillea and Leucanthemum and two of Ligularia were studied using light compound and Scanning electron microscopes. Special emphasis was given to the surface structures particularly primary sculpture, cell shape, topography of anticlinal and periclinal walls, secondary sculpture, carpopodium and pappus bristles of cypselas. Six types of primary sculptures were observed, reticulate pattern with variations being the most common pattern. Surface ornamentations were seen to be significant for taxonomic delimitation for most of the taxa both at the generic and specific levels.

Key Words: *Anticlinal and Periclinal Walls, Carpopodium, Micromorphology, Primary Sculpture and Secondary Sculpture*

INTRODUCTION

A survey of micromorphological characters of cypsela in the family Asteraceae by Blake (1928), Kynclova (1970), Grau (1980), Mukherjee and Sarkar (1992, 1994, 1995, 1997, 2001), Abid and Qaiser (2002), Garg and Sharma (2005, 2007); and Abid and Qaiser (2009) reveals that these characters are very useful in delimiting various taxa. Bremer (1987) stated that "Testa structures are potentially interesting and although Grau (1980) described several types from the Mutisieae, we need further information from other tribes to make use of the data". Within the tribe Anthemideae, through various workers paid attention to the cypselar features in some taxa (Kynclova, 1970; Lovell *et al.*, 1986; Swelankomo *et al.*, 2007; and Abid and Qaiser, 2008), still there is a requirement of detailed information about the cypsela micromorphology for entire tribe of Anthemideae. The present investigation on micromorphology of cypselas was carried out to provide the strength to the systematic position of taxa in the tribe Anthemideae.

MATERIALS AND METHODS

Surface structures of cypselas were studied using SEM for which mature dried cypselas of 15 species belonging to the tribe Anthemideae (Asteraceae) were affixed on aluminium stubs with the help of transparent adhesine. The cypselas were coated with gold and examined at a range of magnifications in a Leo 435 VP Scanning Electron Microscope at AIIMS, New Delhi, India. The terminology of cypsela surface patterns adopted is mainly from Barthlott (1981), Hufford (1995), Mukherjee (2000), Johnson *et al.*, (2004), Garg and Sharma (2005, 2007) and partly improved by authors.
The following 15 species belonging to the tribe Anthemideae were studied:
Achillea ligustica All.
Achillea millefolium L.
Achillea ptarmica L.
Artemisia abrinthium L.
Artemisia abrotanum L.
Artemisia campestris L.
Artemisia camphorate Vill.
Artemisia dracumculus L.
Artemisia scoparia Waldst and Kit.

Artemisia vulgaris L.
Leucanthemum maximum DC.
Leucanthemum parthenium Gr.
Leucanthemum vulgare Lam.
Ligularia alpigena Pojark.
Ligularia clivorum Maximum.
Seeds were procured through Dr. A.S.R. Dathan (Retired Assoc. Prof. Department of Botany, University of Rajasthan, Jaipur) from Jardin Botanique de 1´ University Louis Pasteur de Strasbourg.

***Achillea ligustica* All. (Figure 1)**

Size - The size of cypsela varies from 1.0 to 1.1 mm in length and 0.4 to 0.5 mm in breadth
Shape - Oblong obovate
Colour- Dark brown
Pappus elements – Absent
Spermoderm patterns -

The spermoderm is irregular reticulate formed of more or less rectangular cells. Anticlinal walls appear straight and raised, periclinal walls are concave and secondary sculpture is smooth. Several periclinal walls also showed annular thickening giving a trachedial appearance. Transverse walls are not clearly visible. Some waxy depositions are also seen in randomly distributed over the surface. One sided thin carpopodium is present.

***Achillea millefolium* L. (Figure 2)**

Size - The size of cypsela varies from 2.0 to 2.1 mm in length and 0.9 to 1.0 mm in breadth
Shape - Oblong cylindrical
Colour - Light brown
Pappus elements – Absent
Spermoderm patterns -

The spermoderm shows reticulate pattern of more or less rectangular cells. Anticlinal walls appear straight and slightly raised, periclinal walls are flat to concave. Transverse walls are straight. Several anticlinal walls show series of perforations also. Some waxy depositions are seen randomly distributed. Secondary sculpture is smooth to granular. Carpopodium is not clear.

***Achillea ptarmica* L. (Figure 3)**

Size - The size of cypsela varies from 2.0 to 2.1 mm in length and 0.9 to 1.0 mm in breadth
Shape - Oblong obovate
Colour - Brown
Pappus elements – Absent
Spermoderm patterns -

The spermoderm pattern is striated reticulate. Anticlinal walls are depressed below the surface of outer periclinal walls which are highly convex. The oblique transverse walls are also depressed. The pattern near basal region of the fruit becomes compact. Some thread-like projections and waxy granules are also seen on the surface. Secondary sculpture is smooth. Carpopodium is not seen.

***Artemisia abrinthium* L. (Figure 4)**

Size - The size of cypsela varies from 1.0 to 1.1 mm in length and 0.6 to 0.7 mm in breadth
Shape - The body of cypsela is curved near the basal region forming an inverted coma-like structure
Colour - Brown
Pappus elements – Absent
Spermoderm patterns -

The spermoderm shows reticulate ladder-like pattern. The anticlinal walls are thickened and raised and more or less straight, interconnected by mostly pericinal convex walls. This pattern becomes more compact in the basal region. The periclinal walls showed a annular thickening pattern with rugose structures here and there in the secondary sculpture. At higher magnification, some hairy projections and

waxy depositions are also seen randomly. Secondary sculpture is rugose type. Carpopodium is seen as a complete ring.

***Artemisia abrotanum* L. (Figure 5)**

Size - The size of cypsela varies from 1.2 to 1.4 mm in length and 0.4 to 0.5 mm in breadth
Shape - Oblong
Colour - Brown
Pappus elements - Absent
Spermoderm patterns-

The spermoderm shows reticulate annular ring-like pattern. These rings- like structures are seen arranged in longitudinal lines with straight and distinctly raised anticlinal and transverse walls and both these are interconnected by periclinal convex walls. Secondary sculpture is smooth. This pattern becomes a condensed structure near the basal region. Waxy granules are seen randomly distributed. They sometimes form clusters at certain placed. A lobed carpopodium is present.

***Artemisia campestris* L. (Figure 6A-C)**

Size - The size of cypsela varies from 1.2 to 1.4 mm in length and 0.8 to 0.9 mm in breadth
Shape - Oval shaped
Colour - Dark brown
Pappus elements – Absent
Spermoderm patterns -

The primary sculpture of spermoderm is revealed at higher magnifications as a reticulate pattern. The cells are rectangular to quadrangular shaped with anticlinal walls slightly raised and periclinal walls flat to concave. Longitudinal slits are also seen. Granular waxy depositions are present in random. Secondary sculpture is smooth to annular. Carpopodium is present in the form of a complete ring.

***Artemisia camphorata* Vill. (Figure 6D-F)**

Size - The size of cypsela varies from 1.8 to 2.0 mm in length and 0.6 to 0.8 mm in breadth
Shape - Oblong cylindrical with wrinkled surface
Colour - Dark brown
Pappus elements – Absent
Spermoderm patterns -

The primary sculpture of spermoderm is showed ladder-like reticulate pattern. The anticlinal and transverse walls are prominently raised forming small rectangules or quadrangles. The periclinal walls are concave. Longitudinal slits are prominently seen. Secondary sculpture is wrinkled to rugose. Granular waxy depositions are also present. Cap-like carpopodium is present.

***Artemisia dracumculus* L. (Figure 7)**

Size - The size of cypsela varies from 1.0 to 1.1 mm in length and 0.5 to 0.6 mm in breadth
Shape - Cylindrical with uniform width throughout
Colour - Light brown
Pappus elements – Absent
Spermoderm patterns -

The primary sculpture of spermoderm showed irregular reticulate pattern. The anticlinal walls are undulated and distinctly raised with flat to concave periclinal walls, which are giving a wrinkled appearance to the surface. Heavy waxy depositions in the form of irregular flaps are also seen on the whole fruit surface. Secondary sculpture is smooth. Cap-like carpopodium is present.

***Artemisia scoparia* Waldst and Kit. (Figure 8)**

Size - The size of cypsela varies from 0.6 to 0.7 mm in length and 0.5 to 0.6 mm in breadth
Shape - Oblong obovate
Colour - Light brown
Pappus elements – Absent
Spermoderm patterns -

The primary sculpture of spermoderm is showing reticulate pattern with annular ring-like transverse undulations on the periclinal walls. Anticlinal walls are straight and distinctly raised. These are interconnected by flat to convex periclinal walls. At higher magnifications irregular striations are seen on the periclinal walls which give a wrinkled appearance to it. Secondary sculpture is also wrinkled type. Some waxy depositions are seen randomly distributed. Cap-like carpopodium is present.

***Artemisia vulgaris* L. (Figure 9)**

Size - The size of cypsela varies from 1.8 to 1.9 mm in length and 0.5 to 0.6 mm in breadth
Shape - Oblong cylindrical
Colour - Dark brown
Pappus elements – Absent
Spermoderm patterns -

The Spermoderm shows reticulate ladder-like pattern. In primary sculpture, anticlinal walls are not continuous but distinctly raised flat to irregularly convex periclinal walls are clear. The transverse walls are also distinctly raised forming quadrangular to transversely rectangular shapes. Some waxy granular structure are seen here and there on the cypsela surface. Beyond this reticulate pattern, wrinkled type of secondary sculptures is also seen. Crown-like carpopodium is present.

***Leucanthemum maximum* DC. (Figure 10)**

Size - The size of cypsela varies from 3.0 to 3.1 mm in length and 0.9 to 1.0 mm in breadth
Shape - Oblong cylindrical with wrinkled longitudinal ridges; an apical beak is present near the hilum
Colour - Brown
Pappus elements – Absent
Spermoderm patterns -

Spermoderm exhibits irregular reticulate pattern with pronounced waxy undulations. The outline shows thick ridges and alternating furrows. The ridges also show undulations which are characterised by regularly disposed crests and troughs. Under high magnifications, undulated distinctly raised anticlinal walls and concave periclinal walls are seen. Some waxy deposits as outgrowths are also seen on the cypsela surface. Secondary sculpture is rugose type. Segmented cap-like carpopodium is present.

***Leucanthemum parthenium* Gr. (Figure 11)**

Size - The size of cypsela varies from 1.5 to 1.7 mm in length and 0.5 to 7.0 mm in breadth
Shape - Oblong folded cylindrical
Colour - Golden brown
Pappus elements – Absent
Spermoderm patterns -

Spermoderm has a striated reticulate pattern. At the upper portion of the cypsela, the reticulation is polymorphic with irregular patterned waxy and thickened walls. At higher magnifications, in the middle part of the seed, the straight and distinctly raised anticlinal walls are seen along with convex periclinal walls. Long striations are also seen on the periclinal walls. Secondary sculpture is of wrinkled type. Cap-like carpopodium is present.

***Leucanthemum vulgare* Lam. (Figure 12)**

Size - The size of cypsela varies from 2.0 to 2.1 mm in length and 0.9 to 1.0 mm in breadth
Shape - Oblong cylindrical with longitudinal ridges
Colour - Light brown
Pappus elements – Absent
Spermoderm patterns -

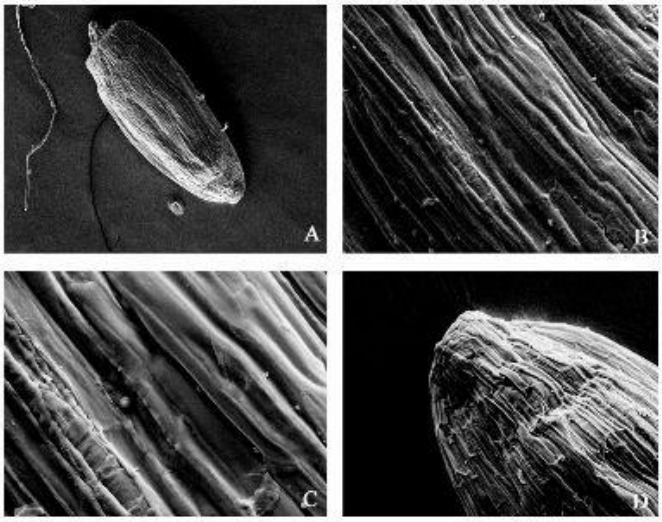
The Spermoderm exhibits a more or less reticulate pattern. Under higher magnification the anticlinal walls appear straight, distinctly raised with flat to concave periclinal walls. Transverse walls are oblique so that an irregular network of cells is formed. Some thread-like projections are also seen on the cypsela surface. Behind this primary sculpture, smooth to granular secondary sculptures are seen. Cap-like

carpopodium is present.

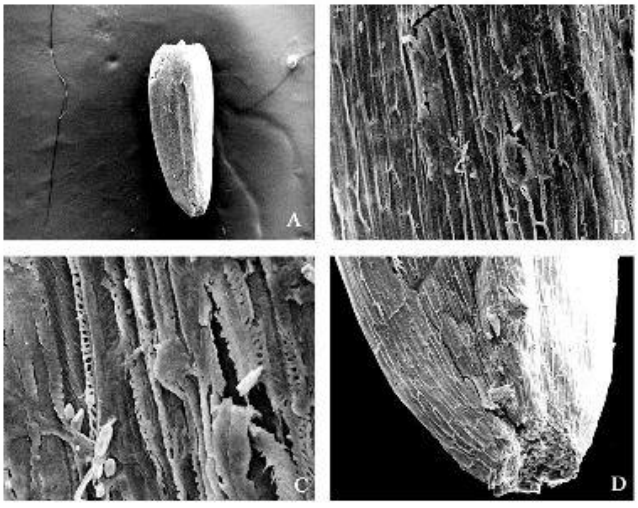
***Ligularia alpigena* Pojark. (Figure 13)**

Size - The size of cypsela varies from 5.0 to 4.1 mm in length and 0.9 to 1.0 mm in breadth
Shape - Oblong cylindrical with hairy surface
Colour - Brown
Pappus elements – Scabrous barbellate bristles
Spermoderm patterns -

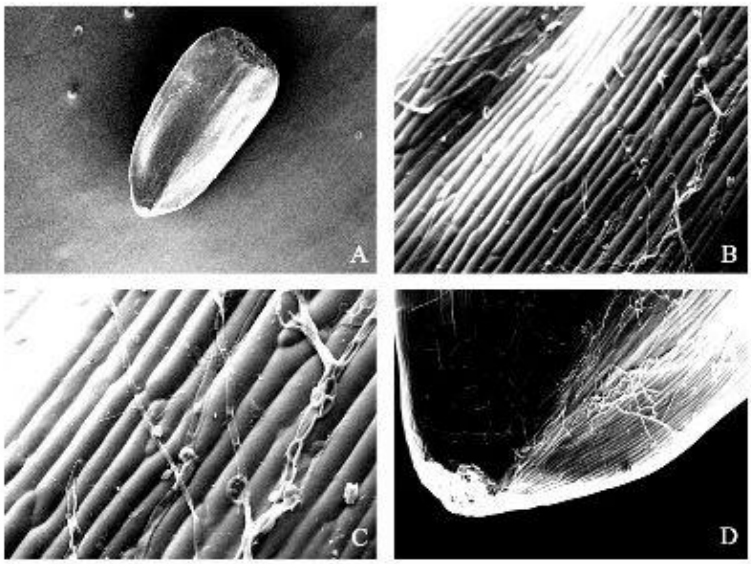
The Spermoderm pattern is striated reticulate with hairy surface. In primary sculpture, anticlinal walls are irregular straight and rose with flat periclinal walls. The short thick sac-like hairs with blunt tips and broad bases are present on the cypsela surface in random manner. Surface of the hairs is prominently wrinkled. Secondary sculpture is smooth. Carpopodium is present in the form of a complete ring.



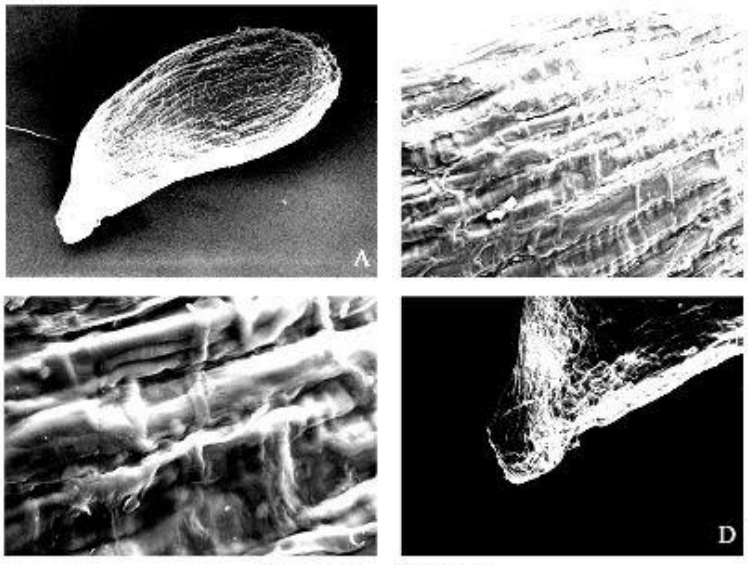
Figures-1 (A-D)
Cypsela and spermoderm patterns in *Achillea ligustica*
A - Cypsela, × 60; B - Surface, × 400; C - Surface, × 750
D - Carpopodium, ×250



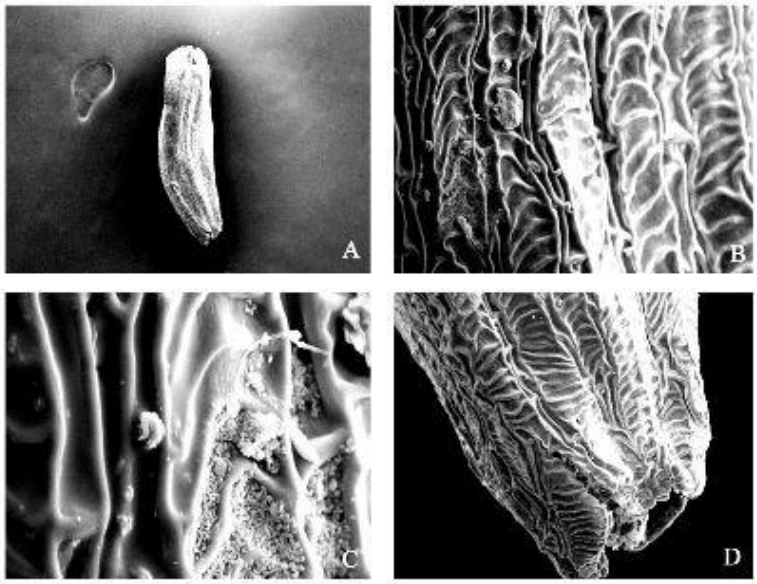
Figures-2 (A-D)
Cypsela and spermoderm patterns in *Achillea millefolium*
A - Cypsela, × 30; B - Surface, × 350; C - Surface, × 1000
D - Carpopodium, ×200



Figures- 3 (A-D)
Cypsela and spermoderm patterns in *Achillea ptarmica*
A - Cypsela, × 30; B - Surface, × 400; C - Surface, × 750
D - Carpopodium, ×160

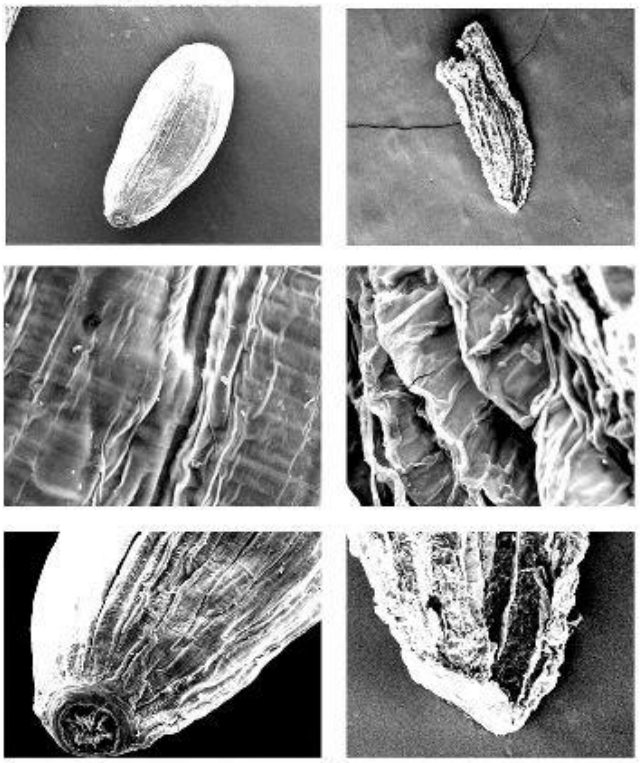


Figures - 4 (A-D)
Cypsela and spermoderm patterns in *Artemisia abrinthium*
A - Cypsela, × 100; B - Surface, × 500; C - Surface, × 1500
D - Carpopodium, × 215



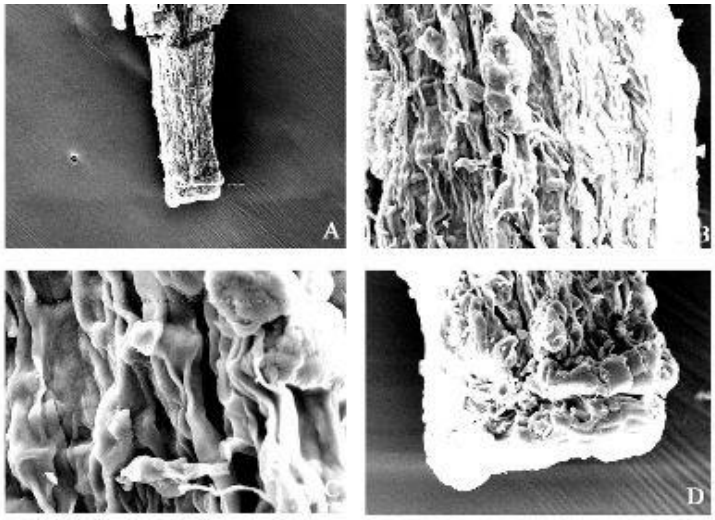
Figures - 5 (A-D)

Cypsela and spermoderm patterns in *Artemisia abrotanum*
A - Cypsela, × 40; B - Surface, × 500; C - Surface, × 1500
D - Carpopodium, × 300



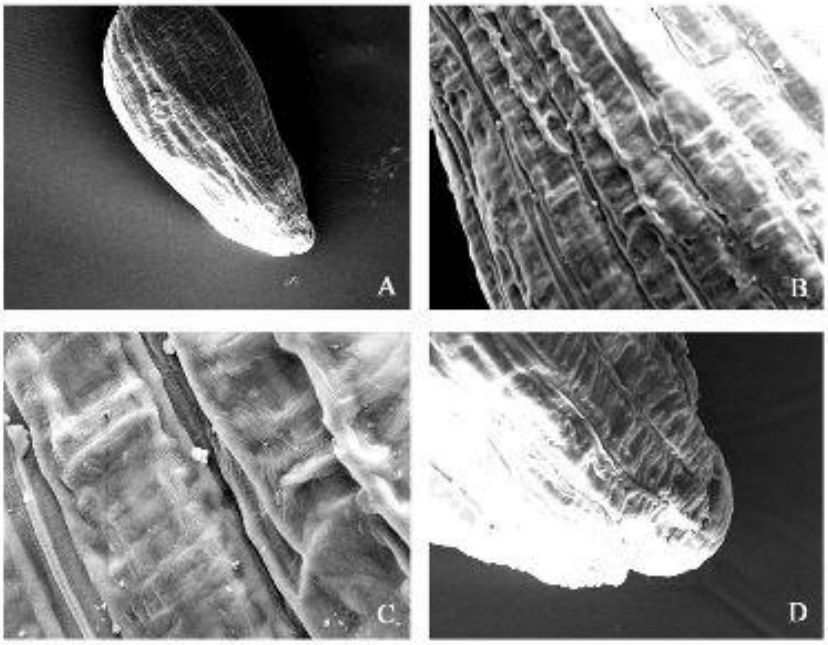
Figures - 6 (A-F)

Cypsela and spermoderm patterns in *Artemisia campestris* and *Artemisia camphorata*
A - Cypsela, × 70; B - Surface, × 500; C - Carpopodium, × 250
D - Cypsela, × 50; E - Surface, × 750; F - Carpopodium, × 250



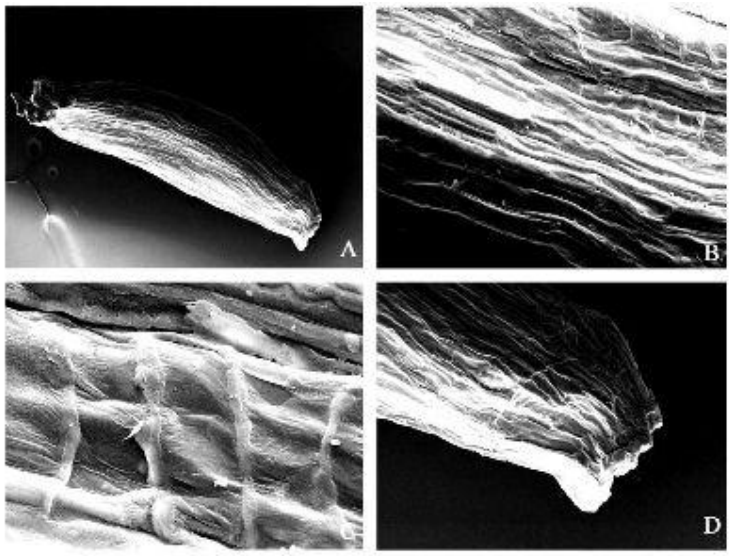
Figures - 7 (A-D)

Cypsela and spermoderm patterns in *Artemisia dracumculus*
A - Cypsela, × 100; B - Surface, × 500; C - Surface, × 1500
D - Carpopodium, × 400



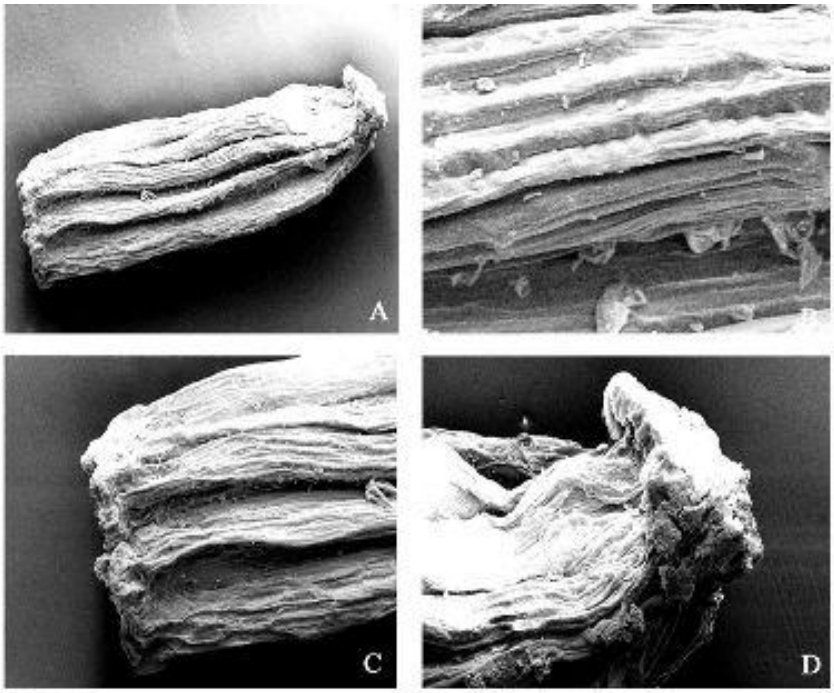
Figures - 8 (A-D)

Cypsela and spermoderm patterns in *Artemisia scoparia*
A - Cypsela, × 125; B - Surface, × 500; C - Surface, × 1500
D - Carpopodium, × 550



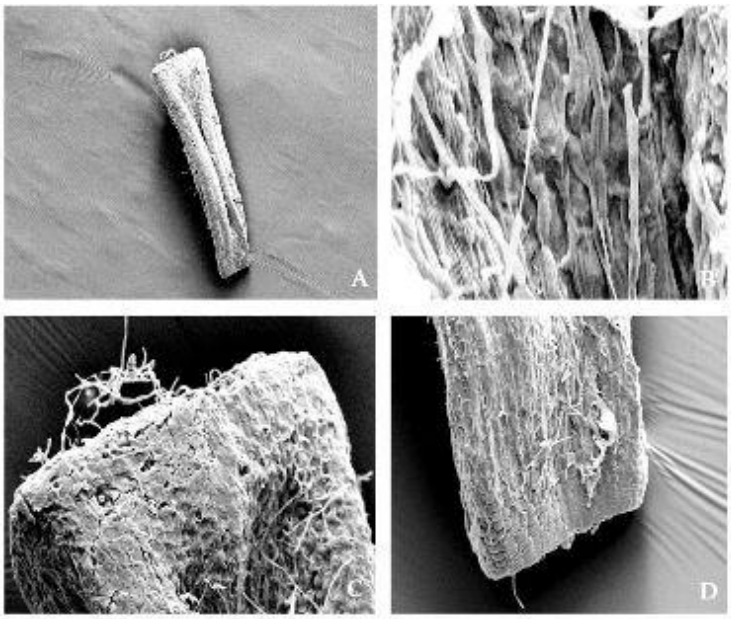
Figures - 9 (A-D)

Cypsela and spermoderm patterns in *Artemisia vulgaris*
A - Cypsela, × 50; B - Surface, × 400; C - Surface, × 1500
D - Carpopodium, × 200



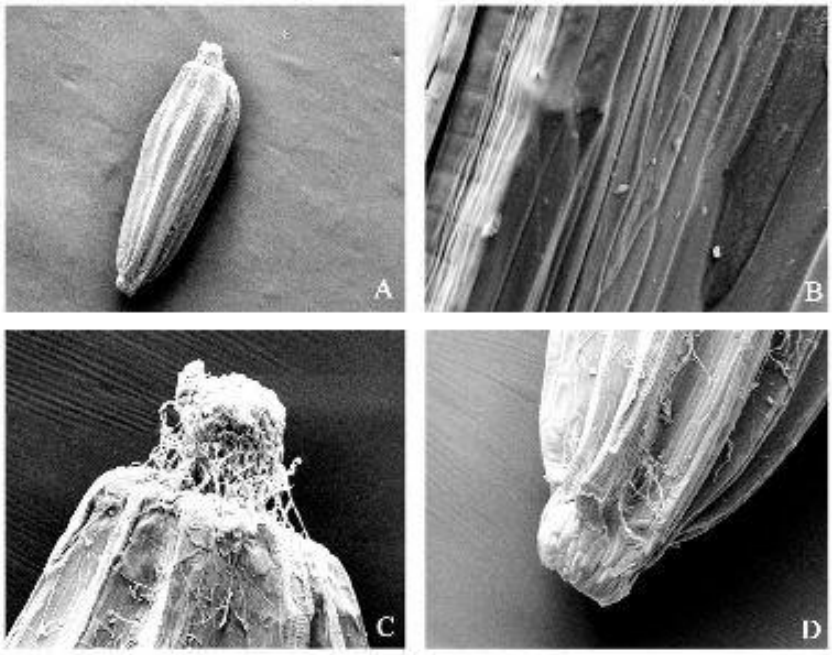
Figures - 10 (A-D)

Cypsela and spermoderm patterns in *Leucanthemum maximum*
A - Cypsela, × 50; B - Surface, × 500; C - Apical surface, × 100
D - Carpopodium, × 150



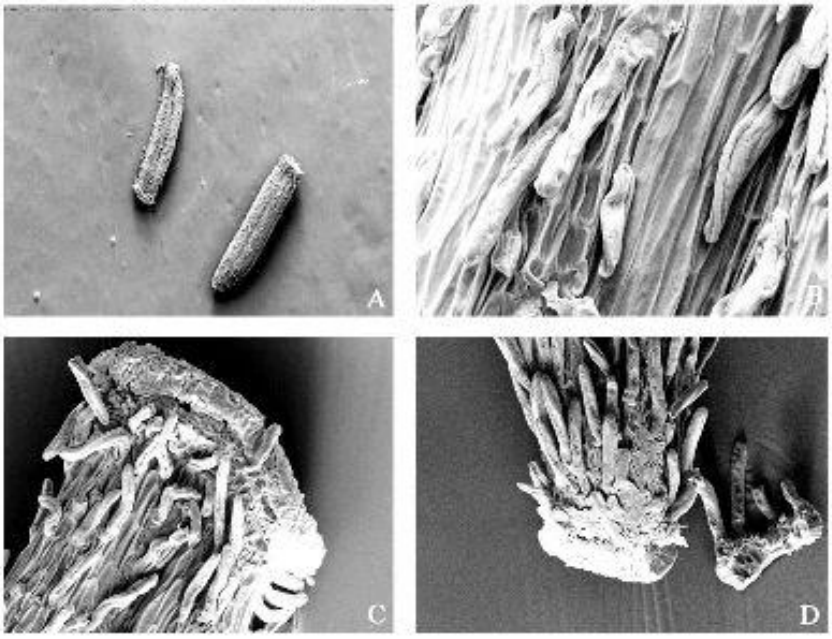
Figures - 11 (A-D)

Cypsela and spermoderm patterns in *Leucanthemum parthenium*
A - Cypsela, × 40; B - Surface, × 1000; C - Apical surface, × 250
D - Carpopodium, × 250



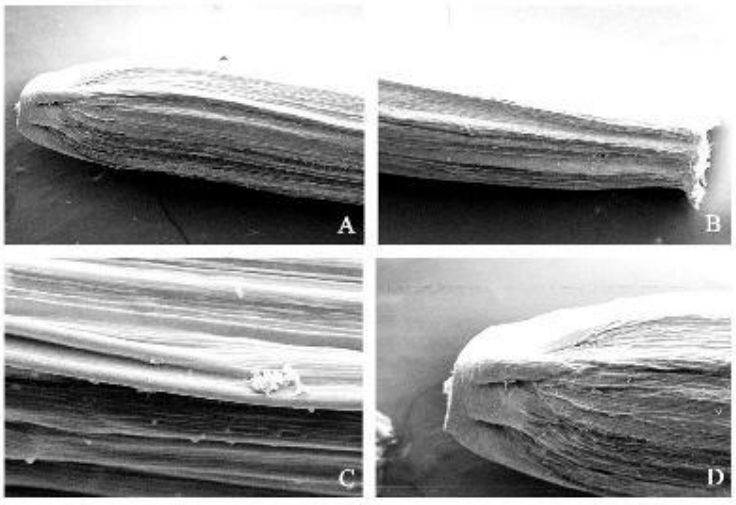
Figures - 12 (A-D)

Cypsela and spermoderm patterns in *Leucanthemum vulgare*
A - Cypsela, × 20; B - Surface, × 1000; C - Apical surface, × 150
D - Carpopodium, × 150



Figures - 13 (A-D)

Cypsela and spermoderm patterns in *Ligularia alpigena*
A - Cypsela, × 20; B - Surface, × 500; C - Apical surface, × 250
D - Carpopodium, × 250



Figures - 14 (A-D)

Cypsela and spermoderm patterns in *Ligularia clivorum*
A - Cypsela, × 30; B - Apical surface, × 30; C - Surface, × 1000
D - Carpopodium, × 60

Table 1: Morphological and Scanning Electron Microscopic characters in the 15 species of the tribe Anthemideae

Tribe – Anthemideae											
S. no.	**Name of cypsela**	**Size**	**Shape**	**Colour**	**Primary sculpture**	**Cell shape**	**Anticlinal wall**	**Periclinal wall**	**Secondary sculpture**	**Carpopodium**	**Pappus elements**
1.	*Achillea ligustica*	1.0×0.4	Oblong obovate	Dark brown	Irregular reticulate	Rectangular	Straight and raised	Concave	Smooth	One sided thin	Absent
2.	*Achillea millefolium*	2.0×0.9	Oblong cylindrical	Light brown	Reticulate	Rectangular	Straight and slightly raised	Flat to concave	Smooth to granular	Not seen	Absent
3.	*Achillea ptarmica*	2.0×0.9	Oblong obovate	Brown	Straight reticulate	Nearly rectangular	Depressed	Convex	Smooth	Not seen	Absent
4.	*Artemisia abrinthium*	1.0×0.6	Inverted coma-like	Brown	Reticulate ladder-like	Square to rectangular	Thick and raised	Convex	Rugose	A complete circular ring	Absent
5.	*Artemisia abrotanum*	1.2×0.4	Oblong	Brown	Reticulate annular ring	More or less square	Straight and distinctly raised	Convex	Smooth to granular	Lobed	Absent
6.	*Artemisia campestris*	1.2×0.8	Oval	Dark brown	Reticulate	Rectangular to quadrangular	Slightly raised	Flat to concave	Smooth to annular	In a complete ring	Absent
7.	*Artemisia camphorata*	1.8×0.6	Oblong cylindrical with wrinkled surface	Dark brown	Reticulate ladder-like	Rectangular	Raised	Concave	Wrinkled to rugose	Cap-like	Absent
8.	*Artemisia dracumculus*	1.0×0.5	Cylindrical with uniform width throughout	Light brown	Irregular reticulate	More or less rectangular	Undulated and distinctly raised	Flat to concave	Smooth	Cap-like	Absent

9.	*Artemisia scoparia*	06.0×0.5	Oblong obovate	Light brown	Reticulate with annular ring	Square	Straight and distinctly raised	Flat to convex	Wrinkled	Cap-like	Absent
10.	*Artemisia vulgaris*	1.8×0.5	Oblong cylindrical	Dark brown	Reticulate with ladder-like	Rectangular	Irregular and distinctly raised	Flat to irregularly convex	Wrinkled	Crown-like	Absent
11.	*Leucanthemum maximum*	3.0×0.9	Oblong cylindrical	Brown	Irregular reticulate	Not clear	Undulated and distinctly raised	Concave	Rugose	Segmented cap-like	Absent
12.	*Leucanthemum parthenium*	1.5×0.7	Oblong folded cylindrical	Golden brown	Striated reticulate	More or less rectangular cells	Straight and distinctly raised	Convex	Wrinkled	Cap-like	Absent
13.	*Leucanthemum vulgare*	2.0×0.9	Oblong cylindrical with longitudinal ridges	Light brown	More or less reticulate	Not clear	Straight and distinctly raised	Flat to concave	Smooth to granular	Cap-like	Absent
14.	*Ligularia alpigena*	5.0×0.9	Oblong cylindrical with hairy surface	Brown	Striated reticulate	Rectangular	Irregular straight and raised	Flat	Smooth	A complete ring	Scabrous barbellet bristles
15.	*Ligularia clivorum*	10.0×2.0	Oblong elongated	Light brown	Ribbed	Unclear	Straight and distinctly raised	Flat to convex	Not clear	Lobed	Scabrous barbellet bristles

***Ligularia clivorum* Maximum (Figure 14)**

Size - The size of cypsela varies from 10.0 to 10.1 mm in length and 2.0 to 2.2 mm in breadth
Shape - Oblong elongated
Colour - Light brown
Pappus elements – Scabrous barbellate bristles

Spermoderm patterns

The Spermoderm pattern is ribbed. The anticlinal walls are parallel to the longitudinal axis of the cypsela. They are straight and distinctly raised and at some points are interconnected by unclear transverse walls.
On the surface of anticlinal walls waxy structures are observed at random. Also they are not in uniform thickness. Long striations are seen on the flat to convex periclinal walls giving a wrinkled appearance to it and reveal the secondary sculpture of the surface. Undulations are seen on the periclinal wall here and there. Some waxy depositions are also present. A complete ring of carpopodium having lobs towards proximal side is present.
Comparative analysis of different characters of cypsela morphology is given in Table -1.

DISCUSSION

The spermoderm pattern in 15 species of Anthemideae studied here showed 6 types of primary sculptures viz., reticulate, irregular reticulate, straited reticulate, reticulate ladder like, reticulate – annular, and ribbed. The data are in accord with some previous reports like Kynclova (1970), Mukherjee and Sarkar (1992), and Abid and Qaiser (2008, 2009). The reticulate – ladder like patterns found in 3 species of *Artemisia*, reticulate – annular ring- like in 2 species of *Artemisia* and ribbed in *Ligularia clivorum* are seen for the first time in the tribe Anthemideae.
The most common type of cell shapes are rectangular in most of the species studied here whereas square cell shape is seen in *Artemisia abrotanum* and *A. scoparia*. The other types of cell shapes are square to rectangular in *A. abrinthinum* and rectangular to quadrangular in *A. campestris*. Mukherjee and Sarkar (1998) and Garg (2008) also observed these types cell shapes in the cypsela surface structures in the family Asteraceae.
Gohary and Mohamed (2007) described anticlinal walls under three heads: (1) anticlinal wall's shape – undulated / wavy (ii) anticlinal wall's thickness – thin / thick (iii) anticlinal wall's level – raised / grooved. The species studied here also showed raised / depressed anticlinal walls which are thin or thick. Shape of the anticlinal walls reported here falls under undulate, wavy, straight or irregular types. Similarly the periclinal walls are also of different types viz., concave, flat to concave, convex and flat to convex. Such observations have also been made by Mukherjee and Sarkar (1995) and Garg and Sharma (2007) in the tribe Astereae and respectively.
In the present investigation 5 types of the cells secondary sculptures are recognized. The most common type of secondary sculptures are the smooth and smooth to granular types in the tribe – Anthemideae. The new terms wrinkled, wrinkled to rugose are used by the authors to describe the secondary sculptures.
Abid and Qaiser (2008) reported two types of caropopodia in 23 species of *Artemisia* (Except *A. annua* which is characterized by the absence of carpopodium). The carpopodia reported by them were broad circular disc-like structures found in 22 *Artemisia* sps. and broad angular disc-like structures found in *A. rutifolia*. The crown – like carpopodium in *A. vulgaris*, lobed carpopodium in *A. abrotanum* reported here resemble those of Abid and Qaiser's (2009) observations in 15 genera of the tribe – Anthemideae. The one sided carpopodium seen in *Achillea ligustica* is reported for the first time.
Abid and Qaiser (2008, 2009) studying 44 species belonging to 15 genera of Anthemideae categorised them into two main groups on the basis of pappose or epappose cypsela following Bremer (1994). They reported the genus *Achillea* as epappose. The present report agrees with this. The pappus are absent in all the three species of *Achillea* studied here. The data agree with those reported by Kynclova (1970), Lovell *et al.*, (1986) and Swelankomo *et al.*, (2007) in that cypselas are epappose in Anthemideae generally. However, scabrous barbellet bristles are also observed in *Ligularia* species.

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