

Research Article

Cypselas Diversity as Novel Taxonomic Marker in the Tribe Astereae (Family Asteraceae)

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Abstract

Despite the second largest tribe of the family Asteraceae and immense importance of morphology of cypselas for taxonomic delimitation at tribe level, no work has been done in this direction in the tribe Astereae. In order to evaluate taxonomic implication of cypselas features, diversity of morpho-anatomical features has been carried out in the tribe Astereae. Detail macro as well as micro-morphology and anatomy of cypselas of nine taxa including two species of *Aster*, two species of *Conyza* and one species each of *Erigeron*, *Grindelia*, *Kalimeris*, *Solidago* and *Vittadinia* of the tribe Astereae (Family Asteraceae) have been examined using Light Microscope. Experiments showed that surface characteristics like distribution arrangement, hair type, ribs and their number, pappus diversity and presence and absence of wing were taxonomically more important features in comparison to colour, size and shape of cypselas. Among anatomical features, mesocarpic characters like type of tissue (whether parenchymatous or sclerenchymatous), their distribution pattern, and nature of testal layer were found to be significant for the tribe Astereae. Finally, involving all these cypselas features an artificial key to the studied species is constructed. The key based on morpho-anatomical features could be used as reference key to identify taxa of the tribe Astereae solely based on its cypselas in absence of their flowering stage.

Keywords Astereae, cypselas, mesocarp, pappus, surface hair, testa

Introduction

Fruits of angiosperm dicot family Asteraceae are usually termed as cypselas or achenes. The term 'Cypselas' was first coined by C.de Mirbel (1815) and has often been confused with achenes (Bremer 1994). Though, cypselas varies from the achene by extra coat (perianth) over the pericarp due to the lower location of the ovary (Judd et al. 2002). The term cypselas was adopted as a complex, dry, indehiscent, unilocular fruit, with a single seed not adnate to the pericarp (connected only by the funicle) and initiating from an inferior ovary (Marzinek et al. 2008). Cypselas and pappus are two morphological features which are aiding in taxonomic classifications at tribal levels of Asteraceae (Talukdar 2008, Frangiote-Pallone and Antonio de Souza 2014, Talukdar and Mukherjee 2014). Cypselas and pappus morphology together with growth form, capitula size, florets, involucre bracts and leaf shapes were successfully used in separating daisy tribe Anthemideae into 12 sub-tribes (Bremer and Humphries 1993). For the taxonomic delimitation at tribe level, morphological diversity of cypselas has been used to distinguish the tribe Heliantheae and Eupatorieae of family Asteraceae from rest of the tribes by investigating carbonized cypselas (Bremer 1994). Another tribe Inuleae was usually characterized by the presence of calcium oxalate crystals in the cypselar epidermis (Merxmüller and Grau 1977, Breitwieser and Ward 2005).

The tribe Astereae is the second largest tribe of the family Asteraceae, with about 222 genera and ca. 3100 species distributed under 18 sub-tribes with high number of medicinal plants (Nesom and Robinson 2006, Talukdar and Talukdar 2013). The

tribe is primarily characterized by anther (tailless anther; ecaudate and ecalcarate anther base) and style characters (deltate to triangular or lanceolate style appendages). The tribe is also chemically distinct from other tribes by the absence of pentayne and sesquiterpene lactones (Bremer 1987, Brouillet et al. 2009). In any larger tribe like Astereae of family Asteraceae, tribe phylogeny required a thorough study of each and every organ of all the species without exception. Cladistic analysis based on broad morphological features was done in the tribe (Xiaoping and Bremer 1993). However, it is really a fact that morphology of cypselas in most of the tribes of Asteraceae has not been received much attention as it should be. Besides morphological features, poor knowledge regarding the anatomical characteristics of cypselas has hindered progress of taxonomic delimitations and tribe interrelationship in Asteraceae (Pandey 2003). Structure and anatomical characteristics of cypselas have been deeply observed to cover only few groups like Anthemideae and Cardueae and established to be taxonomically significant (Heywood et al. 1977). In this context, present investigation aims to deal with detailed study on morphological and anatomical features of nine taxa belonging to the tribe Astereae. Traditional features like size and shape of cypselas, structure of stylopodium, nature of surface pubescence, carpodium, pappus, nature and distribution of ribs and furrows etc. have been given distinctive preference. Anatomically, forms and comparative distribution of several tissues in pericarp wall, nature of embryo, composition of testa and endosperm, orientation of cotyledon and number of resin duct in each cotyledon etc. were observed in different ways. Finally, a sincere attempt has been made to construct an artificial key to studied taxa along with phylogenetic key involving all the observed characters.

Methodology

Collection of plant material

Plant materials (cypselas) used in this study were received as herbarium specimens from the subsequent herbaria, which are mentioned in index Herbarium (Holmgren et al. 1981). The list of collected specimens has been shown in table 1.

DK: Hortus botanicus Hauniensis, Denmark.

Z: Botanischer Garten der Universität Zürich, Switzerland

Z: Zollikerstrasse 107, CH8008 Zürich, Switzerland.

For investigating stable and perfect stage of each character only fully matured and intact cypselas were observed. Investigations were carried out broadly in five categories as follows

Macro-morphological studies of cypselas

In situations with integrated cypselas, the initial and important process was to spot the posterior and anterior (abaxial) surface of the cypselas. Further, 10 dry and FAA (Formaldehyde, acetic acid, absolute alcohol) preserved mature cypselas were casually taken in glass and graphed slides and seen under Olympus stereo dissecting microscope (DM) and binocular microscope (Olympus Model 611062). Proper images were taken using Zeiss Stemi DV4 camera equipped microscope.

A careful noting has been done for the shape, color and direction of cypselas. Additionally, length and width of the cypselas were visually measured using graphed slides, and in few cases they were scored by ocular and stage micrometer. In this work, length of the cypselas was demarcated as the length of the body of cypselas from basal meristematic zone (carpodium) up to apical end excluding pappus. Moreover, the width of the cypselas was measured at the widest part of the cypselar body. In case of heteromorphic cypselas, all the features were examined for both the ray and disc cypselas and were studied separately. Summary drawings of complete cypselas and diverse cypselar portions were drawn by the Mirror type camera lucida.

Micro-morphological studies of cypselas

For micro-morphological experiments, mature cypselas were dipped in 1-5% sodium hydroxide (NaOH) solution for 2-7 d according to the stiffness. Further, they were moved to saturated chloral hydrate solution for some time, repetitively washed with water and appropriately stained in 0.2-0.5% aqueous Safranin solution.

Stained specimens were retained in 70% phenol glycerine solution and dissected cautiously for reviewing parts of cypselas. Proper photographs were taken using Olympus C-310 zoom digital camera (3.2 Megapixel) and Zeiss-stereo microscope.

Nature of ribs, types, distribution and orientation of hairs, nature of surface cells, other epidermal structures, carpopodial cells etc. all were judgmentally observed. Pappus characters such as

nature of pappus bristles, their number, arrangement, colour, length, apex organization etc. were also studied.

Table 1. Source of materials with collection number

Serial No.	Name of the Taxa	Locality	Collection Number
1.	<i>Aster radula</i> Aiton	Z	XXOZ- 20041675
2.	<i>Aster tripolium</i> L.	DK	W DK:06 NJ 0218
3.	<i>Conyza bonariensis</i> (L.) Cronquist	DK	276E2405-0007*AG
4.	<i>Conyza canadensis</i> (L.) Cronquist	DK	GE 2405-0008
5.	<i>Erigeron glabratus</i> Hook.	Z	CHOZ-20060915
6.	<i>Grindelia robusta</i> Nutt.	Z	XXOZ-20051345
7.	<i>Kalimeris mongolica</i> (Franch.) Kitam.	Z	XXOZ-20041851
8.	<i>Solidago virgaurea</i> L.	DK	W DK:06 NJ 0332
9.	<i>Vittadinia triloba</i> DC.	DK	488S1968-1357*AZ AU: Kimba

Anatomical studies of cypsela

For anatomical studies, fine hand sections of cypselas were utilized for examining the internal structures. Generally sections were made from the middle part of mature cypsela. The cypselas were dipped in different chemicals for different duration of times depending upon the hardness of wall, such as –

- (i) Cypselas were softened by dipping in boiling water for 5-30 min, with a few drops of glycerol.
- (ii) They were softened sometimes by putting in 2N NaOH solution for 1-10 h, and
- (iii) Sometimes placed in picric acid solution for few hours or inserted within lactophenol solution or 70% phenol-glycerine solution and boiled in water bath for 10-60 min.

After softening and sectioning, the sections were dehydrated and stained by conventional way (Johansen 1940) with diverse gradations of alcohol. A thorough study were undertaken to examine the following characters such as – nature of cells, their orientation, arrangement, wall thickness, and shape of different cells comprising the different pericarpic layers. Other structures such as secretory ducts, cavity, vascular trace, resin ducts etc. were also marked. All the experimental features of cross-section were documented with the help of camera lucida diagrams.

Terminology for the macro as well as micro-morphological features and anatomical structures were performed following earlier description

(Kynclova 1970, Barthlott 1981) and partially improvised by the author herself.

Results and Discussion

On the basis of morphological observations of cypselas, the tribe was characterized by the following features – cypselas are mostly homomorphic, dorsiventrally compressed, ribbed and/or winged; pubescent with twin, sometimes glandular hairs, less often glabrous; pappus of barbellate or scabrous, biseriate sometimes uniseriate bristles or absent.

On the basis of presence of rib and/or wing of cypsela, all the studied taxa, could be grouped into three distinct categories, such as; Category I- both winged and ribbed (cypselas of *Aster radula*, *Grindelia robusta*, *Kalimeris mongolica* and *Vittadinia triloba*; Category II- only winged, but ribs absent (cypselas of *Aster tripolium*, *Conyza bonariensis*, *Conyza canadensis* and *Erigeron glabratus*, and Category III- only ribbed, but wing absent as in *Solidago virgaurea*. In ribbed cypselas number of rib ranges from 2-15 (Fig. 1-4).

Length of cypselas ranged from 1.2 mm in *Conyza* to 10 mm in *Aster tripolium*. Usually, cypselas were brown, oblong to obovate to oblanceolate in shape and straight, though little to moderate curvature have been noted in cypselas of *Grindelia robusta* and *Conyza bonariensis* (Fig. 2, 3, 10, 12). Among the studied species, heteromorphic cypselas have been noted in *Grindelia robusta* (Fig. 3, 12).

Surface was generally pubescent with sparsely distributed biseriate twin hairs. Cypselas of *Vittadinia triloba* were markedly distinct having

densely distributed twin hairs along with bicelled, capitate glandular hairs (Fig. 4,13). Sessile glandular hairs were also reported on the ovary wall in *Brachycome iberidifolia* (Sharma and Murty 1977). The presence of twin hairs and few to many celled glandular hairs from the cypselar surface of the Astereae has also been reported (Grau 1977). Stylopodium was found to be either absent or ill-developed in majority of the species, however a well-developed, tubular stylopodium has been noted in *Aster radula*, *Conyza bonariensis* and *Solidago virgaurea* (Fig. 1, 2, 4, 9, 10, 13). Carpopodium is invariably symmetric, complete circular ring like among the studied species. Similar ring-like carpopodial structures have earlier been observed in other tribes of Asteraceae (Haque and Godward 1984, Sundberg 1985). Only differences in the present Astereae have been noted in row-thickness (height) and cell arrangement pattern of carpopodium. Tangentially oriented carpopodial cells have been noted in *Aster*, *Grindelia* and *Solidago*; whereas in others they are vertical. Row-thickness varied considerably at the generic level but at infra-generic level it became apparently stable. As for example, in both the studied species of *Aster*, carpopodia are 3-4 cells thick; in both the studied species of *Conyza*, it is 7-9 cells thick; in *Erigeron*, 8-12 rows; in *Kalimeris* and *Solidago*, 5-6 rows (Fig. 1-4). So, the character could be utilized efficiently at generic level and sometimes at species level too.

Pappus was represented by many, persistent, free, barbellate to scabrous, uni- to biseriate bristles, not above 5 mm in length long. However, epappose cypselas have also been noted in *Grindelia robusta*. Mostly bristle apex was composed of sharply pointed apical cells, but rounded apical cell has been noted in pappus of *Aster tripolium*. Number of apical cells also varied among the studied species, such as single apical cell in *Aster tripolium*, *Solidago* and *Vittadinia*; two apical cells in *Conyza*, *Erigeron* and *Kalimeris*; and three apical cells in *Aster radula* have been noted and thus could be utilized as significant taxonomic criteria in various treatments (Fig. 1-4, 9-13). However, much variation in size, shape, pappus elements and spermoderm features were reported in different other taxa of the tribe Astereae and five types of primary sculptures were observed in which reticulate pattern was the most common pattern (Kothari et al. 2012). These beside with surface

ornamentations were seen to be noteworthy for taxonomic delimitation for most of the taxa both at the generic and specific stages, as also opined in other taxa of the tribe Astereae (Kothari et al. 2012).

Cross section of cypselas showed its outline as mostly elliptic, but was sometimes rhomboid as observed in *Aster radula* (Fig. 5, 9). Epicarp was invariably uniseriate, with oval to rectangular, thin-walled parenchymatous cells. In *Solidago virgaurea*, they were distinctly different being columnar and filled with brown substances (Fig. 8, 13). In *Erigeron glabratus*, few epicarpic glandular structures have been marked and in *Vittadinia triloba* epicarpic cuticle is formed tubercle-like structures (Fig. 7, 8 and 11). Based on morphology of epicarpic cells in cypselas, the genus *Nolletia* of the tribe Astereae were divided into two groups: one group has oblong epicarpic cells organized in parallel rows, perceived in surface view, while the additional group has circular to elliptic epicarpic cells, seen in surface view (Herman 2013).

Regarding mesocarp, tissue differentiation has been observed only in *Grindelia robusta* and *Solidago virgaurea*, where mesocarp was composed of both sclerenchyma and parenchyma tissue (Fig. 7, 8, 12 and 13). In *Solidago virgaurea* both the tissue were present as discontinuous patches, whereas in *Grindelia robusta* both were continuous. Presence of radially elongated and pitted parenchymatous cells in lateral wings has been noted in *Grindelia robusta*. Such mesocarpic pitted parenchymatous cells also have been reported in the tribe Astereae (Mukherjee and Sarkar 2001). In all other studied taxa, mesocarp was found to be homogenous, made up of either parenchyma or sclerenchyma cells only. Parenchymatous mesocarp was observed mostly as discontinuous patches in *Aster tripolium*, *Conyza bonariensis* and *Erigeron glabratus* (Fig. 5-7, 10 and 11); whereas in all other studied species mesocarp is sclerotic, mostly as discrete braces or less frequently as continuous multiseriate zone as in *Kalimeris mongolica* (Fig. 8, 12). Both types of sclerenchymatous mesocarp (as discrete braces and as continuous multiseriate zone) have been noted earlier for the tribe Astereae (Pandey 1982, Mukherjee and Sarkar 2001). Usually single vascular trace was noted within each rib and wing. Mesocarpic secretory duct or cavity also has been observed in few studied taxa such as *Conyza bonariensis* and *Grindelia robusta* (Fig. 6,

7). Vallicular cavity was also reported in the mesocarpic region of *Aster albanicus* (Jana and Mukherjee 2014).

In the present investigation, testa of all the studied members of Astereae usually differentiated into

testa epidermis and an inner zone. Characteristic type of testal epidermal cells manifested as inverted U-shaped and radial cells have been observed in majority of the investigated taxa such as in *Aster*,

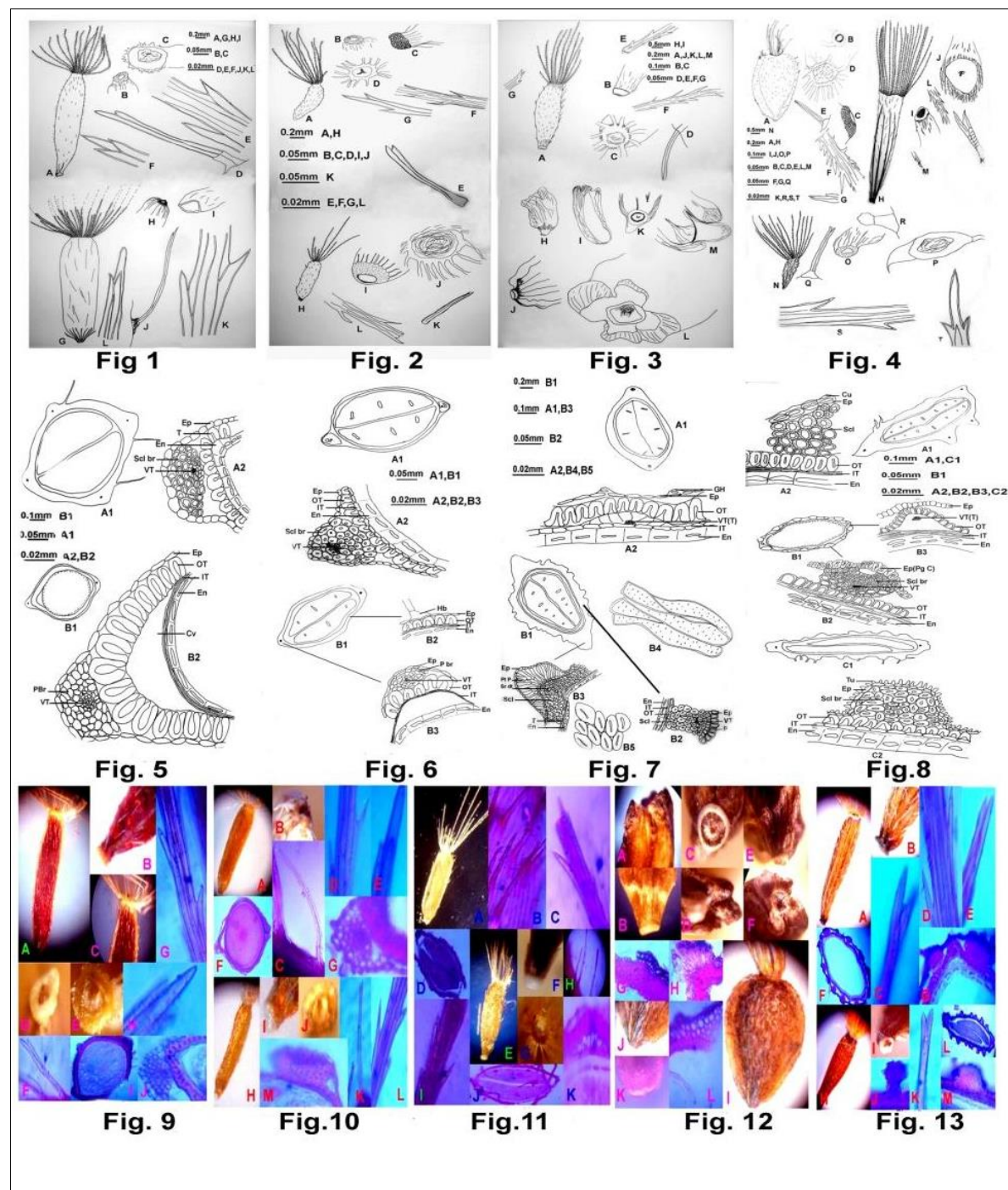


Figure legends

Fig. 1. A-F: *Aster radula*; A-cypsela, B-base, C-apex, D-surface hair, E-middle part of pappus bristle, F-apical part of pappus bristle, G-L: *Aster tripolium*; G-cypsela, H-base, I-apex, J-surface hair, K-middle part of pappus bristle, L-apical part of pappus bristle

Fig. 2. A-G: *Conyza bonariensis*; A-cypsela, B-base, C-carpopodium, D-apex, E-surface hair, F-middle part of pappus bristle, G-apical part of pappus bristle, H-L: *Conyza canadensis*; H-cypsela, I-base, J-apex, K-surface hair, L-middle part of pappus bristle

Fig. 3. A-G: *Erigeron glabratus*; A-cypsela, B-base, C-apex, D-surface hair, E-base of pappus bristle, F-middle part of pappus bristle, G-apex of pappus bristle. H-M: *Grindella robusta*; H-disc cypsela, I-ray cypsela, J-base of disc cypsela, K-base of ray cypsela, L-apex of pappus bristle, M-apex of ray cypsela

Fig. 4. A-G: *Kalimeris mongolica*; A-cypsela, B-base, C-carpopodium, D-apex, E-surface twin hair, F-middle part of bristle, G-apex of bristle, H-M: *Solidago virgaurea*; H-cypsela, I-base, J-apex, K-surface twin hair, L-middle part of bristle, M-apex of bristle, N-T: N-cypsela, O-base, P-apex, Q-surface twin hair, R-surface glandular hair, S-middle part of bristle, T-apex of bristle

Fig 5. A1-A2: *Aster radula*; A1-T.S of cypsela (diagrammatic), A2- a part of cypsela in T.S. B1-B2: *Aster tripolium*; B1-T.S. of cypsela (diagrammatic), B2- a part of cypsela in T.S.

Fig. 6. A1-A2: *Conyza bonariensis*; A1-T.S. of cypsela (diagrammatic), A2-a part of cypsela in T.S. B1-B3: *Conyza canadensis*; B1-T.S. of cypsela (diagrammatic), B2 & B3-parts of cypsela in T.S.

Fig 7. A1-A2: *Erigeron glabratus*; A1-T.S of cypsela (diagrammatic), A2- a part of cypsela in T.S. B1-B5: *Grindelia robusta*; B1-T.S. of cypsela (diagrammatic), B2 & B3- parts of cypsela in T.S., B4-pitted parenchymatous cells, B5-mesocarpic sclerenchymatous cells

Fig 8. A1-A2: *Kalimeris mongolica*; A1-T.S of cypsela (diagrammatic), A2- a part of cypsela in T.S. B1-B3: *Solidago virgaurea*; B1-T.S. of cypsela (diagrammatic), B2 & B3- parts of cypsela in T.S., C1-C2: *Vittadinia triloba*; C1-T.S. of cypsela (diagrammatic), C2-a part of cypsela in T.S.

Fig. 9. A-F: *Aster radula*; A-cypsela, B-basal part, C-apical part, D-carpopodium, E-apex (after detachment of pappus), F-surface hair, G-middle part of pappus bristle, H-apical part of pappus bristle, I-T.S. of cypsela, J-part of cypsela in T.S. A-E $\times 75$; F, I $\times 110$; G, H, J $\times 725$

Fig. 10. A-F: *Aster tripolium*; A-cypsela, B-base, C-surface hair, D-middle part of bristle, E-apical part of bristle, F-T.S. of cypsela, G-part of cypsela in T.S. H-M: *Conyza bonariensis*; H-cypsela, I-base, J-apex (after detachment of pappus), K-surface hair, L-part of pappus bristle, M-part of cypsela in T.S. A, H $\times 30$; B, I, J $\times 75$; F, C, K $\times 170$; D, E, G, L, M $\times 725$

Fig. 11. A-G: *Conyza canadensis*; A-cypsela, B-surface hairs, C-part of pappus bristle, D-T.S. of cypsela, E-K: *Erigeron glabratus*; E-cypsela, F-base, G-apex (after detachment of pappus), middle part of pappus bristle, H-surface hair, I-part of pappus bristle, J-T.S. of cypsela, K-part of cypsela in T.S. A, E $\times 30$; F, G $\times 75$; H $\times 110$; B, D, J $\times 170$; C, I, K $\times 725$

Fig. 12. A-H: *Grindelia robusta*; A & D-apical part of ray cypsela, B & C-basal part of ray cypsela, E-base of disc cypsela, F-apex of disc cypsela, G & H-parts of cypsela in T.S. I-L: *Kalimeris mongolica*; I-cypsela, J-basal part, K-carpopodium, L-part of cypsela in T.S.

A-F, I, J $\times 75$; K $\times 170$; G, H, L $\times 310$

Fig. 13. A-G: *Solidago virgaurea*; A-cypsela, B-base, C-surface hair, D-middle part of pappus bristle, E-apex of pappus bristle, F-T.S. of cypsela, G-part of cypsela in T.S. H-M: *Vittadinia triloba*; H-cypsela, I-base, J-glandular hair, K-twin hair, L-T.S. of cypsela, M-part of cypsela in T.S. A, H $\times 30$; B, I $\times 75$; F, L $\times 110$; G, M $\times 310$; C-E, J, K $\times 725$

Abbreviations used in figures

Cu = Cuticle, Cv = Cavity, En = Endosperm, Ep = Epidermis, GH = Glandular hair, Hb = Hair base, IT = Inner testa, OT = Outer testa, P = Parenchyma, P br. = Parenchymatous brace, Pg. C = Pigmented cell, Pt. P = Pitted parenchyma, Scl = Sclerenchyma, Scl. br. = Sclerotic brace, Sr. dt. = Secretory duct, T = Testa, Tu = Tubercle, VT = Vascular trace.

Conyza, *Erigeron glabratus*, *Solidago virgaurea* and *Vittadinia triloba* (Fig. 5-13). Testa epidermis is generally constituted by mono-layer of cells which are thickened on three sides (U-cells) in the tribe Astereae (Grau 1977). The epidermal cells were inverted U-shaped probably because the outer tangential walls and the radial walls of the cells were prominently thicker than the inner tangential walls (Mukherjee and Sarkar 2001). In *Grindelia robusta*, testal epidermal cells were oval to rectangular and tangential with medially depressed outer tangential walls. Contrastingly enough, they are elliptic or rectangular and radial in *Kalimeris mongolica*. Tangential, rectangular type of testal epidermal cells has also been reported from the tribe Astereae (Pandey et al. 1982). Inner testal zone was mostly disorganized and made up of collapsed parenchyma cells. However, well-organized, cellular inner testa was present in *Conyza bonariensis*, *Erigeron glabratus* and *Vittadinia triloba*. So, such dynamic testal features could be significant taxonomic differentiating marker at least for the tribe Astereae.

Endosperm persists in mature cypsela and is usually uniseriate, though biseriate endosperm is also noted in *Solidago virgaurea* (Fig. 8, 13G). Embryo of all the studied species occupied a major to entire part of the cypsela. It was made up of 2, plano-convex, parallelly oriented cotyledons with 3-7 secretory ducts, but sometimes secretory ducts were absent. Cotyledons of *Vittadinia triloba* was unique in the sense that they were obliquely oriented with one over arching end and another closely attached end with sub-terminal part of other cotyledon and could be treated as species delimiting factor (Fig. 8, 13L).

Micro-morphological and anatomical evaluation clearly indicates that members of the tribe Astereae are very rich source of diversity of cypsela. Presence of wing and rib, mesocarpic tissue differentiation and testal characteristics were found to be more substantial for the tribe Astereae and could be exploited as species delimiting aspects. All the observed cypselas features could also be utilized for improvement of existing tribal phylogeny in conjunction with other disciplines of taxonomy. Considering all these cypselas features, an attempt has been made to construct an artificial key to the species.

Key to the genera

1a. Cypsela heteromorphic, glabrous, surface with lineate markings; insertion of cypsela oblique, basal; pappus absent; secretory duct in each cotyledon 5 in number.

----- ***Grindelia* (*G. robusta*)**

1b. Cypsela homomorphic, pubescent, surface without markings; insertion of cypsela straight, basal or lateral; pappus present; secretory duct not as above.

----- **2**

2a. Cypsela cylindrical, not winged; epicarpic cells columnar with brown substances; mesocarp composed of both sclerenchyma and parenchyma tissues; testa secondarily separated from pericarp; endosperm biseriate.

----- ***Solidago* (*S. virgaurea*)**

2b. Cypsela dorsiventrally compressed, winged; epicarpic cells not as above; mesocarp composed of either sclerenchyma or parenchyma tissue; testa attached to pericarp; endosperm uniseriate.

----- **3**

3a. Epicarpic cells cuticularized; persistent hair bases exist at epicarp; embryo occupied major part of cypsela; carpopodium with radially arranged cells.

----- **4**

4a. Surface wrinkled and sparsely hairy with twin hairs; vesicular glandular hair absent; diameter of carpopodium lesser than the base of the cypsela; pappus uniseriate of scabrous bristles with 2 apical cells; epicarp without any tuberculate outgrowths; mesocarpic sclerenchyma present as continuous zone; inner testa disorganized of crusted parenchyma cells; cotyledon parallel, plano-convex, with 7 secretory ducts in each.

----- ***Kalimeris* (*K. mongolica*)**

4b. Surface smooth and densely hairy with both twin and vesicular glandular hairs; diameter of carpopodium same as the base of the body; pappus biseriate of barbellate bristles with single apical cell; epicarp with tuberculate outgrowths; mesocarpic sclerenchyma present as discrete sclerotic braces; inner testa organized by rectangular parenchyma cells; cotyledon oblique, with over-arching end; secretory duct absent.

----- ***Vittadinia* (*V. triloba*)**

3b. Epicarpic cells not cuticularized; persistent hair bases present or absent; embryo occupied major or entire part of cypsela; carpopodium with radially or

tangentially arranged cells.

----- 5

5a. Pappus uniseriate; epicarp with glandular structure; testal vascular trace present.

----- *Erigeron (E. glabratus)*

5b. Pappus biseriate; epicarp without any glandular structure; testal vascular trace absent.

----- 6

6a. Carpoidium 3-4 rows of cell high; tips of the body cells in twin hair situated in prominently distant planes; each cotyledon with 3 secretory ducts.

----- *Aster (2 spp)*

6b. Carpoidium 7-10 rows of cell high; tips of the body cells in twin hair situated in more or less same planes; cotyledon without any secretory duct.

----- *Conyza (2 spp)*

Key to the species

Genus – *Aster*

1a. Cypsela quadrangular, ribs present; stylopodium well-developed, tubular; pappus bristles with sharply pointed apical cells; persistent hair bases exist in epicarp; mesocarp sclerenchymatous; testa un-differentiated.

----- *A. radula*

1b. Cypsela dorsiventrally compressed, ribs absent; stylopodium absent, tubular; pappus bristles with rounded apical cells; persistent hair bases absent; mesocarp parenchymatous; testa differentiated.

----- *A. tripodium*

Genus – *Conyza*

1a. Cypsela curved; stylopodium well-developed; persistent hair bases absent; mesocarp sclerenchymatous with two lateral secretory ducts; inner testa organized.

----- *C. bonariensis*

1b. Cypsela straight; stylopodium ill-developed; persistent hair bases exist in epicarp; mesocarp parenchymatous without any secretory duct; inner testa disorganized.

----- *C. Canadensis*

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