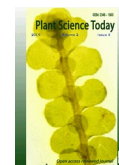




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Research Article

Taxonomic significance of Cypsela in *Vernonia anthelmintica* Willd. and *V. cinerea* Less. (Asteraceae; tribe – Vernonieae): Structural manifestations

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Abstract

An investigation was carried out to reveal the taxonomic importance of cypselar features of *Vernonia anthelmintica* Willd. and *Vernonia cinerea* Less. through morpho-anatomical manifestations in the plesiomorphic tribe Vernonieae of the dicot family Asteraceae. A light microscopic (LM) and Scanning Electron Microscopic (SEM) study unraveled various morphological features of the cypsela. Among them, apical part, wall surface, surface hairs, nature, structure and arrangement of carpopodium, and stylopodium are taxonomically important traits. Furthermore, presence or absence of glands in wall surface, pappus bristles, testal nature, distribution of crystal and in pericarp and/or testa and endosperms of cypselas can also be considered as taxonomically significant. Distinct differences were observed between two taxa for many characteristics. Notable among these were occurrence of stylopodium, size of cypsela (without pappus), nature of pappus, thickness of pericarp, presence or absence of ribs, layer of radially elongated cells in mesocarpic sclerenchyma and parenchyma, presence of additional uniseriate palisade like sclerenchymatous layer in *V. anthelmintica*, but not in *V. cinerea*, testal epidermal layer, nature of endosperm layer, and crystal formation. Similarities between two taxa were also noted. Based on structural manifestations of cypsela at morpho-anatomical levels, an indented dichotomous key is provided for identification of studied taxa.

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Introduction

Family Asteraceae is regarded as the largest family of Angiosperms comprising of more than 1,600 genera and 23,000 species of the flowering plants (Funk *et al.*, 2009) with large number of herbal medicinal plants (Manjunatha *et al.*, 2005; Chethan *et al.*, 2012; Talukdar and Talukdar, 2013a; Marwat *et al.*, 2015; Danalakshmi *et al.*, 2013). In India, this family is represented by approximately 900 species under 167 genera (Mukherjee and Sarkar, 2001). The tribe Vernonieae was one of the original tribes, and consistently recognized in subsequent treatments within the family Asteraceae (Cronquist, 1977; Jones 1977; Bremer, 1987). In more restricted sense, Vernonieae are part of sub-family Cichorioideae. Occurrence of narrow styles and long sweeping hairs differ the tribe from Arctotideae and Moquinieae, whereas usual lack of milky sap and the common actinomorphic corollas has differentiated it from Cichorieae. The tribe contains 126 currently recognized genera and about 1500 species (Keeley and Robinson, 2009), mostly

distributed in tropical parts of the world. Most of the species are assembled in the huge genus *Vernonia*, known as ironweeds in North America (Mukherjee and Nordenstam, 2004). It has two centers of distribution, one in Africa and other one in South America (Gleason, 1906). Old world species of *Vernonia* have a basic chromosome number of $X = 9$ or 10 , and New world species have a basic number of $X = 17$ (Jones, 1977). Root tip mitosis of *V. anthelmintica* exhibited $2n = 2x = 20$ chromosomes, while *V. cinerea* showed $2n = 2x = 18$ chromosomes composing both metacentric and submetacentric chromosomes (Mathew and Mathew, 1982). Vernonieae are notable for the frequent extreme cymose forms involving scorpioid cymes and a unique chemical vernolic acid and the tribe is probably most primitive among the tribes of the family Asteraceae (Jones, 1977; Robinson *et al.*, 1980). A good introduction to the detailed characters of the Vernonieae with a limited scanning electron micrograph (SEM) survey of Vernonieae pollen types has been given by Robinson (2009).

Table 1. Sources along with collection number of studied materials in tribe Vernonieae of Asteraceae

Taxa investigated	Locality	Collection Number
Genus – <i>Vernonia</i> Schreb.	Denmark (DK)	GE 2204-0001
Species – <i>V. anthelmintica</i> Willd. <i>V. cinerea</i> Less.	West Bengal, India	TT -01

Regarding the cypselar morphology in the tribe Robinson (2009) described that achenes were usually prismatic, rarely angled, 3-20 costate, and rarely with phytomelanin; carpodium was stopper-shaped to turbinate but was rarely obsolete. Pappus possessed long capillary bristles. *V. anthelmintica* and *V. cinerea*, two members of tribe Vernonieae, are commonly distributed in India (Talukdar, 2013a,b; Biswas *et al.*, 2014). Flower morpho-variants are available in *V. cinerea* (Manjunatha *et al.*, 2005; Bala and Gupta, 2013).

The fruits of Asteraceae are very distinct from fruits of other families. The cypselar, fruit of Asteraceae, differed from the achene by an additional layer (perianth) over the pericarp due to the inferior position of the ovary; however, many plant scientists have continuously used the wrong term achene (Judd *et al.*, 2002). Marzinek *et al.* (2008) adopt the term cypselar as a complex fruit, dry, indehiscent, unilocular, with a single seed not adnate to the pericarp (linked only by the funicle) and originating from an inferior ovary. Cypselar 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). It is off-course Bipontinus (1844), who was the first to draw the attention of taxonomists toward the potentiality of cypselar anatomical features and used them in the classification of taxa in the Asteraceae. Morpho-anatomical study of cypselar in different species of *Vernonia* has been conducted (Bar *et al.*, 2012; Jana *et al.*, 2013) but considering the complexity and variations of the features even in same species of *Vernonia* collected from different geographical regions (temperate and tropical), more study need to be carried out. Present endeavor was therefore carried out to explore the taxonomic significance of cypselar through morpho-anatomical manifestations of *V. anthelmintica* and *V. cinerea* of the family Asteraceae.

Materials and Methods

Collection of plant materials

Plant materials (cypselas) for the present investigation were collected by the author and obtained in the form of received herbarium specimens (as gifted to Prof. Sobhan Kumar Mukherjee, Department of Botany, University of Kalyani, India) from the following herbarium of the world which are mentioned in Index Herbarium (Holmgren *et al.* 1981).

DK : Hortus botanicus Hauniensis, Denmark.

The present study includes two species of genus *Vernonia* i.e., *V. anthelmintica* Willd. and *V. cinerea*

Less. of the family Asteraceae. Under the tribe the species are alphabetically arranged with mentioning the locality and collection number of each species (Table 1).

For investigating stable and perfect stage of each character only fully matured and intact cypselas were collected. Fresh specimens were collected mainly from Nadia (23°24'N/88°30'E) and North 24-Parganas (22°08'N/88°30'E) of Gangetic West Bengal and few from Darjeeling (27°02'N/88°10'E) district of Sub-Himalayan West Bengal, India. During collection, field characters were carefully noted. In case of heterogamous capitula, both the ray and disc cypselas were placed in separate packets with proper labeling denoting date, locality and number of specimen. Collected cypselas were properly air-dried and kept in desiccators for better maintenance. Few cypselas of each species were also fixed in FAA (Formaldehyde, Acetic acid, Alcohol) solution, in addition to usual dry collection.

All the locally collected specimens were compared and verified in the Central National Herbarium, Calcutta (CNH, CAL) and all the voucher duplicate specimens were deposited in the Herbarium of the Department of Botany, University of Kalyani, Kalyani 741 235, Nadia, West Bengal, which was designated as "KAL".

Macro-morphological studies of cypselas

In cases, where intact cypselas were available, the first and foremost step was to mark the posterior and anterior (abaxial) surface of the cypselas. Then 10 dry and 10 FAA preserved mature cypselas were randomly taken in glass slides and graphed slides and observed under Olympus stereo dissecting microscope (DM) and Olympus binocular microscope (No.-611062). Suitable images were taken using Zeiss Stemi DV4 camera equipped microscope.

Colours, shape, direction of cypselas were noted carefully. Length and width of the cypselas were measured visually by graphed slides, in few cases they were counted by ocular and stage micrometer. The length of the cypselas in the present study is defined as the length of the body of cypselas from basal meristematic zone (carpodium) up to apical end excluding pappus. The width of the cypselas was measured at the widest part of the cypselar body. In case of heteromorphic cypselas, all the characters were studied for both the ray and disc cypselas and were noted separately. Outline diagrams of complete cypselar

and different cypselar part were drawn by the Mirror type camera lucida.

Micro-morphological studies of cypselas

Mature cypselas were dipped in 1-5% NaOH solution for 2-7 days depending upon the hardness. Then they were transferred into saturated chloral hydrate solution for few hours, repeatedly washed with water and properly stained in 0.2-0.5 % aqueous Safranin solution. After staining, specimens were placed in 70 % phenol glycerine solution and dissected carefully for studying different parts of cypselas. Suitable 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 critically observed. Pappus characters such as nature of pappus bristles, their number, arrangement, colour, length, apex organization etc. were also examined.

Anatomical studies of cypselas

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

1. Cypselas were softened by dipping in boiling water for 5-30 minutes, with a few drops of glycerol
2. They were softened sometimes, by putting in 2N NaOH solution for 1-10 hours.
3. Sometimes they were 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 minutes.

After softening and sectioning, the sections were dehydrated and stained using conventional method (Johansen, 1940) with alcohol grades. A thorough study were undertaken to examine the following characters such as – nature of cells, their orientation, arrangement, wall thickness, shape of different cells comprising the different pericarpic layers. Any other structures for example, crystals, secretory ducts, cavity, vascular trace, resin ducts etc. also marked. All the observed features of cross-section were documented with the aid of camera lucida drawings.

SEM studies of cypselas

For SEM analysis, 5 matured and air dried cypselas of each species were selected randomly. They were mounted on labeled aluminium brass stubs with the help of double-stick cellophane tape. To obtain more analytical images, different angle views were taken by placing the cypselas obliquely. Along with normal surface features such as surface cells, ornamentations, trichome, gland, crystals etc. few localized observations

were also made putting different parts of cypselas such as carpopodium, apical part or pappus separately on the stub with proper markings. All the carrying stubs were quick-dried using vacuum evaporator. During microscopic observations, all possible and suitable microphotographs of each specimen were taken using FEI – QUANTA 200 Autoscanning Electron Microscope at Regional Sophisticated Instrumentation Centre (RSIC), Bose Institute, Kolkata.

Terminology for the macro as well as micro-morphological features, anatomical structures and SEM observations primarily follows Ramayya (1962), Barthlott (1981) and partially improvised by the author himself.

Results

Vernonia anthelmintica Willd.

Cypselar morphology

Cypselas homomorphic, 4.2-4.3 × 1.0-1.5 mm. in size (excluding pappus), blackish brown in colour, narrow – oblong in shape, straight, truncate at the apex and gradually tapered towards the base, faintly dorsiventrally compressed, ribbed; ribs ten in number, prominent and straight. Surface was pubescent with pilose hairs covering, very closely appressed on both sides, antrorse in orientation, non-glandular, body undifferentiated, multicellular, biseriata, and flagellate, with sharply pointed apex. After clearing, cypselar wall surface shows glandular markings due to presence of rounded reddish and bilobed greenish glands (Fig. 1A-J). Stylopodium was ill-developed and narrow. Carpopodium symmetric, complete, circular, smooth, ring like; cells' outline visible and distinguishable from other cells of the cypselas, cells thin walled, round to oval, compactly arranged, tangentially oriented, parenchymatous, arranged in 14-15 rows; diameter of carpopodium same as the base of the body. Insertion of cypselas was straight, basal. Cypselas were pappose; outer pappus represented by many deciduous, capillary with squamellae (scale like), 0.25 mm. long, inner bristles not found (Fig. 1A-J).

SEM survey of cypselas

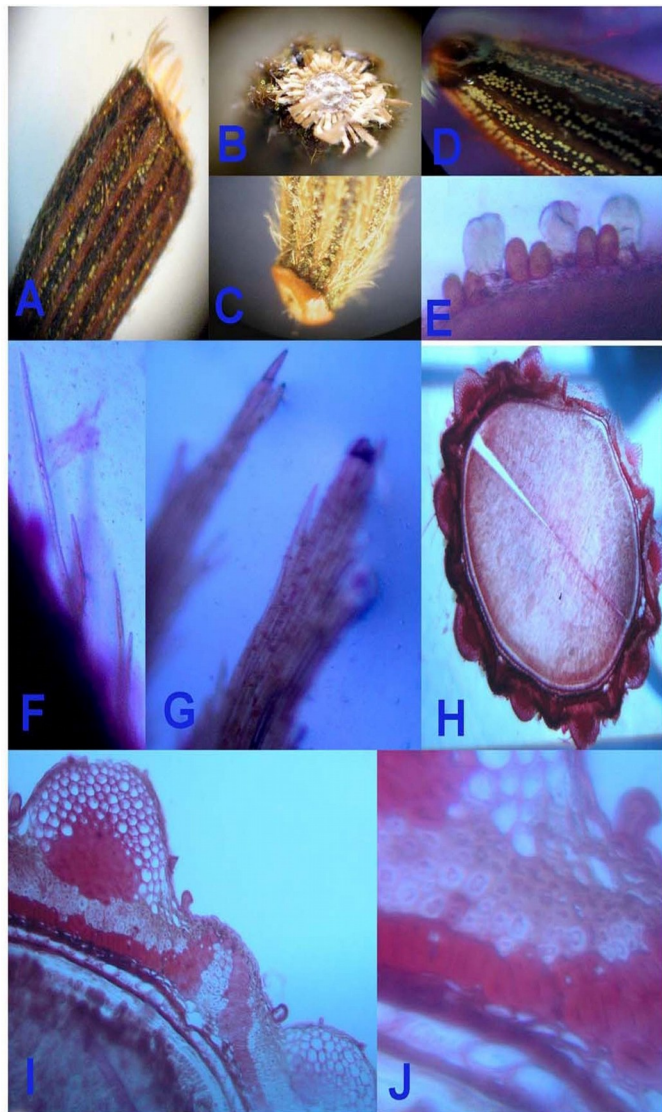
Surface cells visible; square to rectangular, vertical, anticlinal and periclinal wall straight. Surface strongly ribbed with simple hairs and vesicular bodies. Vesicular body glandular, equally bilobed, few with apical depressions (Fig. 1A-F). Stylopodium was ill-developed, narrow. Pappus was tri-seriate. Carpopodium symmetric, complete, circular, smooth, ring like; cells' outline visible and distinguishable from other cells of the cypselas, cells thin walled, round to oval, compactly arranged, tangentially oriented, parenchymatous, arranged in 14-15 rows; diameter of carpopodium same as the base of the body (Fig. 2 A-D).

Cypselar anatomy

Cypselas oblate in transverse section with ten prominent, triangular ribs. Cypselar wall 367 μ. and 233 μ. wide at rib and furrow region respectively. Pericarp thick, on

an average 229.0 μ . wide, differentiated into two zones, namely - epicarp and mesocarp (Fig. 3A-H).

A) Epicarp - uniseriate, made up of thin walled, rectangular, compactly arranged, tangentially oriented, parenchymatous cells. Cuticle was present.



A-D x 30; E-H x 170; I x 270; J x 725.

Fig. 1. A-J: *Vernonia anthelmintica*; A-cypsela, B-apex, C-base with carpodium, D-surface with glands, E-surface glands, F-surface hairs, G-parts of pappus, H-T.S. of cypsela, I & J-parts of cypsela in T.S. (A-D \times 30; E-H \times 170; I \times 270; J \times 725) (specimen number- GE 2204-0001).

B) Mesocarp - consists of different types of tissues and structures as follows (from outside to inner zone)-

1) Parenchyma tissue - uniseriate at furrow and six to seven seriate at rib. Cells thick walled, polygonal, compactly arranged, parenchymatous. Few cells contain prismatic crystals (Fig. 3I).

2) Sclerenchyma tissue - present as continuous zone of uniseriate layer at furrow and three to four seriate layer at rib. Cells thick walled, rounded to oval, compactly arranged, sclerenchymatous, with very narrow elongated lumen. Outside of sclerenchyma

tissue discrete sclerotic braces made up of discontinuous cylinder of cells present at each rib.

3) Palisade tissue/layer - present as continuous, wavy zone of single layer of cells thick. Cells thick-walled, oval palisade like, sclerotic with narrow elliptic lumen, filled with ergastic matters, compactly arranged. This wavy layer forms a curvature at furrow, which filled with parenchymatous cells.

Testa/seed coat - attached with pericarp, approximately 38.2 μ thick, differentiated into outer and inner zones; outer zone made up of compressed and collapsed parenchyma cells. Inner zone cellular, unilayered, organized, made up of thin-walled, U-shaped, compactly arranged, tangentially oriented, parenchymatous cells (Fig. 3A-H).

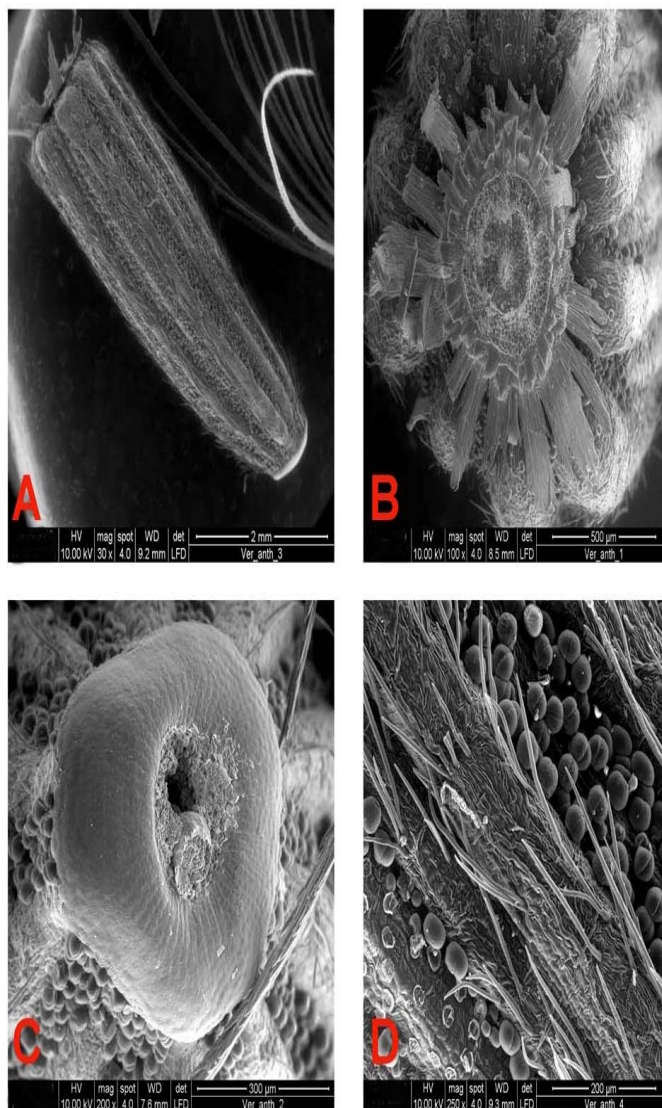


Fig. 2. A-D: *Vernonia anthelmintica*; A-cypsela, B-apex, C-carpodium, D-surface glands and hairs (specimen number- GE 2204-0001).

Endosperm - persists in mature cypsela, biseriate, cell layers slightly separated from each other. Cells of both the layer were thick-walled, barrel-shaped,

compactly arranged and tangentially oriented, parenchymatous cells. Embryo - mature embryo occupied a major portion of the cypselas; cotyledons two in number, plano-convex in shape, anterior-posteriorly oriented; secretory duct in each cotyledon three in number, of which central one larger than others (Fig. 3G, H).

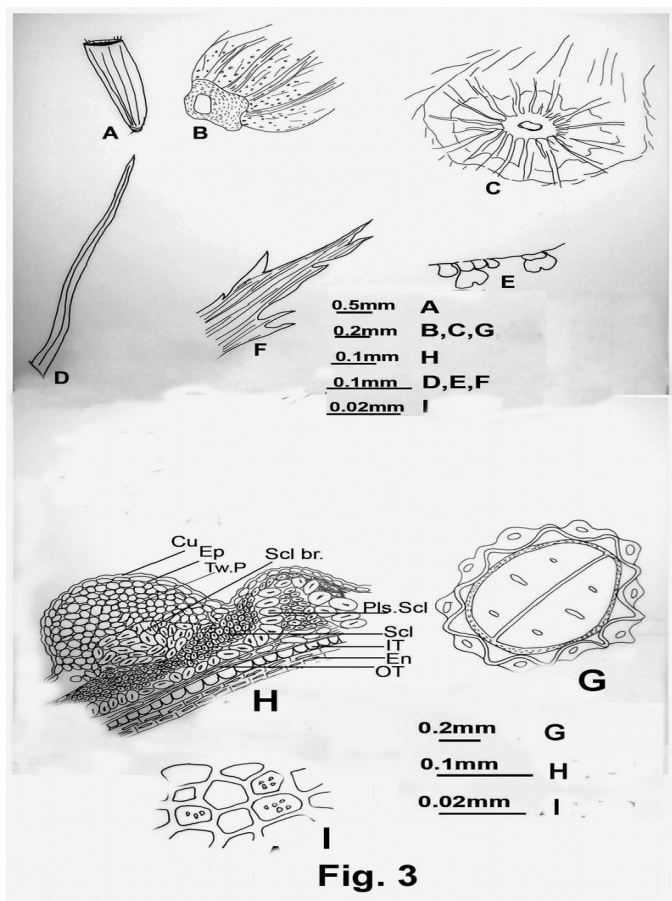


Fig. 3. *Vernonia anthelmintica*; A-cypselas, B-base, C-apex, D-surface hair, E-glandular surface hair, F-part of pappus, G-T.S. of cypselas, H-a part of cypselas in T.S, I-mesocarpic parenchymatous cells with crystals (specimen number- GE 2204-0001).

Vernonia cinerea Less.

Cypselar morphology

Cypselas homomorphic, 1.7-2.0mm x 0.52-0.60 mm. (excluding pappus), deep brown in colour, oblong in shape, straight, truncate at the apex and slightly tapered towards the base, sub-teret in transsection, slightly dorsiventrally compressed but not differentiated, without any rib. Surface is pubescent with pilose hair covering. Hairs sparsely distributed on both surfaces, antrorse, non-glandular, forked type, differentiated into foot and body; foot simple, more or less same in diameter as the base of the body cells; body un-differentiated, multicellular, biseriata, filiform, aseptate, with sharply pointed apex, tips of the body cells situated in slightly different planes. After clearing, the cypselar wall, surface shows numerous small, rounds, glossy vesicular structures, arranged in several vertical rows (foveate). Surface cells were thin-

walled, parenchymatous. Stylopodium was absent. Carpopodium present; symmetric, complete triangular ring like, cells' outline visible, six to seven rows thick; cells rectangular to elongated, thin-walled, radially oriented, distinguishable from other cells of the cypselas; diameter of carpopodium same as the base of the body (Fig. 4 A-H). Carpopodium bears a single vascular supply. Insertion of cypselas was straight, basal. Cypselas pappose; pappus represented by many, double, outer deciduous and inner persistent, terete, barbellate filiform bristles, free from one another, 4.2-4.8 mm long, white in colour. Tip of the lateral cells elongated and approximately as long as width of the rachis of the bristles, apex made up of two, more or less equal cells with pointed tips (Fig. 4 A-H).

Cypselar anatomy

Cypselas transversely elliptic in transsection. Cypselar wall 31.25 μ . wide. Pericarp thin, 25 μ . wide, differentiated into two zones, namely - epicarp and mesocarp (Fig. 5A-H).

A) Epicarp - uniseriate, made up of thin walled, oval to rounded, tangentially oriented, parenchymatous cells; persistent hair bases and vesicular cells present.

B) Mesocarp - consists of different types of tissues and structures as follows (from outside to inner zone)

1) Sclerenchyma tissue - present as continuous unilayered zone of cells; cells thick-walled, oval, radially elongated, sclerenchymatous with elongated large lumen.

2) Parenchyma tissue - uniseriate, continuous. Cells thin-walled, oval, tangentially oriented, compactly arranged. Two vallecular ducts present at 2 corners, just inside the parenchyma zone.

Testa/seed coat - adpressed with pericarp, approximately 6.9 μ thick, un-differentiated, disorganized represented by collapsed parenchyma.

Endosperm - persists in mature cypselas, uniseriate, cells thick-walled, barrel-shaped, tangentially oriented, parenchymatous. Embryo - mature embryo occupied more or less the entire portion of the cypselas; cotyledons two in number, plano-convex in shape, anterior-posteriorly oriented; secretory duct in each cotyledon-three in number, of which central one larger than others.

Key character to the *Vernonia*

Cypselas 5-10 costate; pappus almost always double; carpopodium symmetrical, ring-like; insertion of cypselas straight, basal; testa adpressed with pericarp; secretory duct in each cotyledon three in number.

Key to the species of *Vernonia*

1a. Hairs non-forked; stylopodium ill-developed, narrow; pericarp thick

(229 μ), with 3 mesocarpic layers, palisade sclerenchyma present, crystals

present; testa differentiated with U-shaped testal cells; endosperm

biseriate.

----- ***Vernonia anthelmintica***

1b. Hairs forked; stylopodium absent; pericarp thin (25 μ), with 2 mesocarpic

layers, palisade sclerenchyma absent, crystal absent; testa un-

differentiated without U-shaped testal cells; endosperm uniseriate.

----- ***Vernonia cinerea***

Vernonia anthelmintica VERN 25 19 11 26
19

Vernonia cinerea VERN 25 20 - 26 20

Discussion

Cypsela and pappus morphology undoubtedly provide immense taxonomic inputs for classification and phylogeny of Asteraceae (Talukdar, 2013a; Talukdar and Mukherjee, 2014). Cypsela and pappus morphology together with growth form, capitula size, florets, involucre bracts and leaf shapes were used in separating Anthemideae into 12 sub-tribes of Asteraceae (Bremer and Humphries, 1993). Present investigation revealed that the external and internal features of cypselas including pappus under LM and SEM study provide valuable information about the genus *Vernonia*.

Two different species of *Vernonia* are examined by LM and SEM, namely – (i) *V. anthelmintica* and (ii) *V. cinerea*. In both the species cypsela homomorphic, blackish brown to deep brown in colour, oblong, straight, truncate at the apex and tapered toward the base. Cypsela was weakly dorsiventrally compressed with ten prominent ribs, which was in accordance with the previous view of Misra (1972). However ribs totally absent in *V. cinerea*. Cypselas of both the species are hairy/trichomatous with pilose hair covering. Hair non-glandular, biseriate, non-forked in *V. anthelmintica* but forked (twin type) in *V. cinerea*. Presence of such biseriate forked hairs in the genus *Vernonia* is also reported by Narayana (1979) and Sahu (1984). The use of trichomes as one of the determining taxonomic criteria is well established not only in the family Asteraceae but for other family also. Hunter and Austin (1967), noted that evidence of hybridity of *V. guadalupensis* between *V. interior* and *V. tindheimeri* as obtained from morphological studies, field observations, herbarium studies as well as chromatographic data, were also supported from trichomes morphology. Narayana (1979) worked on the structure of vegetative trichomes of 15 species of *Vernonia* in South India and recognized 3 types of trichomes from the cypselas. He designated “twin hair” as ‘Biseriate-forked hair’, whereas Sahu (1984) reported six types of trichomes from the cypselas of 20 taxa of *Vernonia*. The twin hair has been designated by him as ‘Achenial hair’. Mukherjee and Sarkar (2001) carried out a detailed analysis of macro-morphological and anatomical features of mature cypselas of 18 species belonging to 6 genera of the tribe Vernonieae,

and found more or less uniform surface twin hairs with few exceptions. Recent study opined that although the trichomes may have taxonomic significance, the so-called twin hairs, which are very characteristic of the pericarp of many Compositae (Asteraceae) (Roth, 1977), are of no taxonomic value, as very distinct types occur within the same subfamily and even within the same genus (Frangiote-Pallone and Antonio de Souza, 2014). Hair character can be used successfully to delimit genera within the family Asteraceae, as for example the genus *Vernonia* can be delimited from other genera by the presence of T-shaped hair and *Chromolaena* has amoeboid shaped hair etc (Adedeji and Jewoola, 2008).

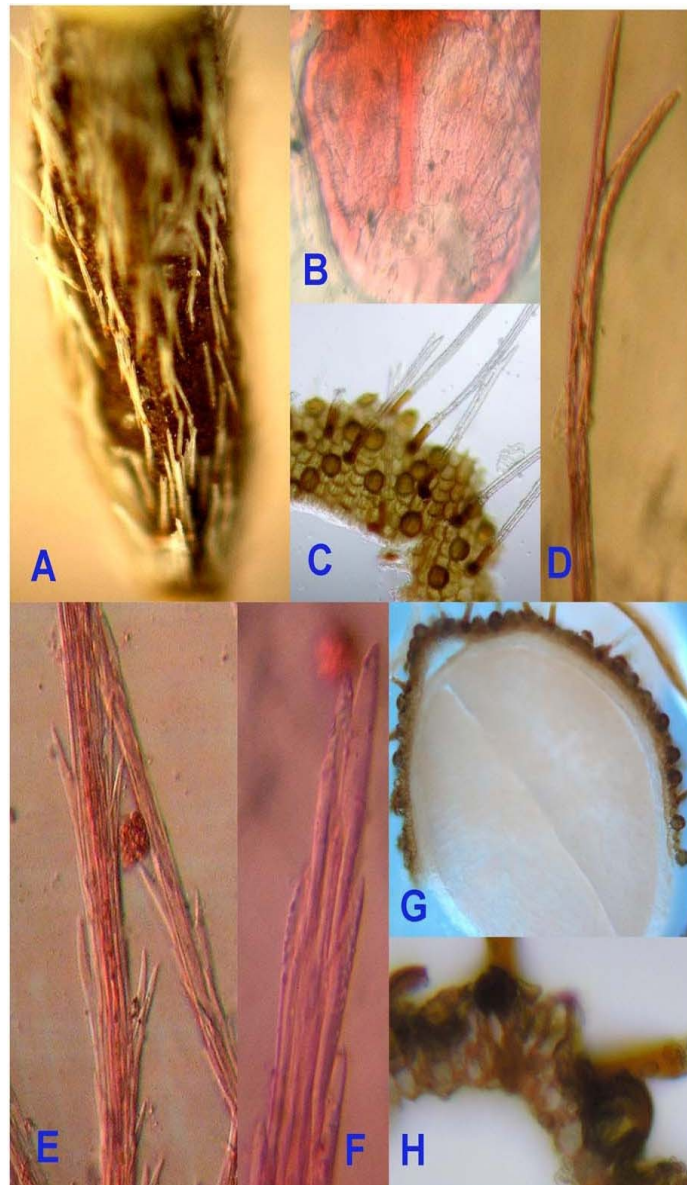


Fig. 4. *Vernonia cinerea*; A-cypselas, B-carpodium, C-surface with hairs and vesicular structures, D-twin hair, E-middle part of bristle, F-apical part of bristle, G-T.S. of cypselas, H-part of cypselas in T.S. (A \times 75; B, C, G \times 170; D-F, H \times 725) (specimen number- TT-01).

After clearing the cypselar wall, the surface shows glandular or foveate marking. Among the studied taxa,

V. cinerea do not possess stylopodium but *V. anthelmintica* bears ill-developed, narrow stylopodium. However, partially immersed, well developed style base have been noted in other species of *Vernonia* like *V. gracilis* and *V. senegalensis* by Mukherjee and Sarkar (2001) and Mukherjee (2005). Consistent occurrence of non-interrupted and complete ring like carpopodium in the present two species is in agreement with earlier notes given by Mukherjee and Nordenstam (2004). Another study with 18 species belonging to 6 genera (*Bothriocline*, *Centratherum*, *Elephantopus*, *Rolandra*, *Vanillosmopsis* and *Vernonia*) of the tribe Vernonieae with the aid of LM and SEM revealed that the tribe Vernonieae has many primitive features from the cypselar point of view like – (i) beakless cypselas, (ii) presence of vesicular bodies in most taxa, (iii) presence of symmetrical carpopodium usually formed by many cell layers, (iv) pappus represented by scabrous bristles or scarios or setaceous scales. The results suggested that the tribe can be regarded as one of the most primitive tribe in the Asteraceae (Mukherjee and Sarkar, 2001). Present results with more insights in cypselar features further confirmed these findings.

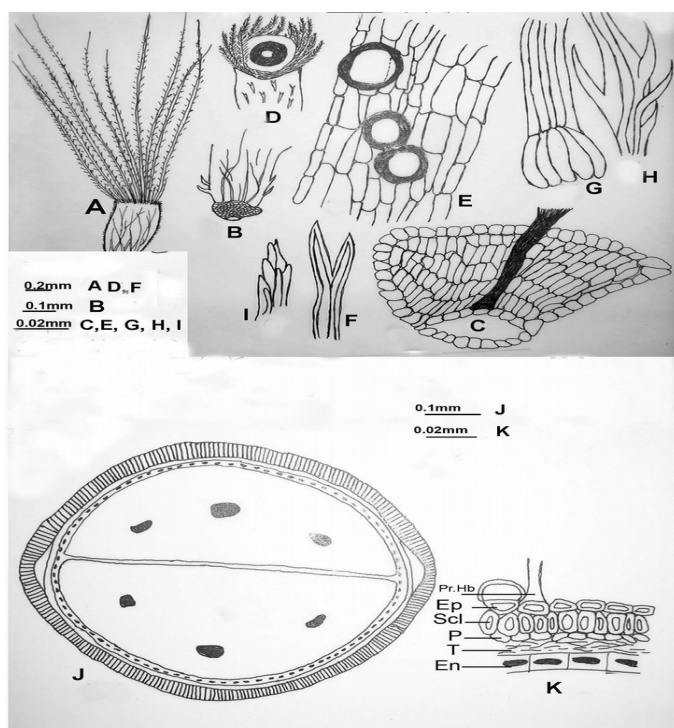


Fig. 5. *Vernonia cinerea*; A-cypselas, B-base, C-carpopodium, D-apex, E-cypselar wall after cleaning, F-surface hair, G-I-basal part, middle part and apical part of pappus bristle, respectively, J-T.S. of cypselas (diagrammatic), and K-a part of cypselas in T.S. (specimen number- TT-01).

Widespread variations within the genus *Vernonia* were noticed regarding the size of cypselas. Bar et al. (2013) reported smallest cypselas in *V. gracilis* (1.5 mm × 1.0 mm excluding pappus) and largest cypselas in *V. diffusa* (3.2 mm × 0.7 mm excluding pappus). In the present study, cypselas (excluding pappus) in *V. anthelmintica* exhibited nearly 1.3-2-fold higher size than these two species but size was comparable to *V.*

gracilis in case of *V. cinerea*. Cypselas shapes have not been particularly useful for grouping the species but can be used for species delimitation (Mukherjee and Nordenstam, 2010). In the present study, occurrence of narrow (*V. cinerea*) to narrow-oblong (*V. anthelmintica*) cypselas are suggestive of the above concept.

In Asteraceae, pappus plays an important role in the dispersal of seed which has been regarded as one of the powerful mechanisms of colonization of some of the weedy daisies such as *Parthenium hysterophorus*, *Eupatorium odoratum*, *Ageratum conyzoides* to spread as worst invasive and alien flora with high allelopathic effects on native plants in India (Marngar and Kharbuli, 2003; Belgeri and Adkins, 2015; Wang et al., 2015). Pappus nature is diversified in Asteraceae (Mukherjee and Nordenstam, 2008; Talukdar, 2013a). Under Light microscope, pappus characters provide valuable information about the genus *Vernonia*. In both the species pappus dimorphic, usually of long capillary bristles. Both barbelate and scaly type of pappus present in *V. cinerea*. However, occurrence of only barbelate type of pappus bristles was reported in *V. anthelmintica* (Jana and Mukherjee, 2012; Talukdar, 2013a, b). Despite biseriate arrangement of pappus in present two species, uniseriate arrangement was not uncommon in other species of *Vernonia* (Basak and Mukherjee, 2003).

Presence of carpopodium is another important features of cypselar morphology in *Vernonia* spp. Situated at the base of cypselas, carpopodium in both species exhibited comparable features with symmetric, ring like structure, and are distinguishable from other cells of cypselas.

Cypselar anatomy

Regarding anatomical features studied taxa showed minor to moderate variations. Cross section of mature cypselas of *V. anthelmintica* usually shows ten prominent triangular ribs and furrows, whereas in *V. cinerea* ribs are either absent or very inconspicuous. Pericarp is very thin (ca. 25 μ in *V. cinerea*) to very thick (ca. 229 μ in *V. anthelmintica*) and clearly differentiated into two zones – epicarp and mesocarp. In both the species of *Vernonia*, epicarp is commonly uniseriate and homomorphic. A cuticle layer can be observed in *V. anthelmintica*. Mesocarpic zone shows considerable variations. In *V. cinerea* mesocarpic sclerenchyma tissue represented by a uniseriate layer of radially elongated cells, whereas in *V. anthelmintica* it is uni- to multiseriate with additional sclerotic braces at each rib. Mesocarpic parenchyma is uni- to multiseriate with thick-walled, polygonal cells in *V. anthelmintica*, whereas in *V. cinerea* this layer is uniseriate throughout with oval, thin-walled cells. Notably an additional uniseriate palisade like sclerenchymatous layer exists in *V. anthelmintica*, which is totally absent in *V. cinerea*. A unique mesocarpic zone was revealed in *V. hymenolepis*, where this zone shows an inverted 'T' shaped sclerenchymatous bundle near the ribs in the tribe Vernonieae (Mukherjee and Sarkar, 2001). The results suggested that the tribe Vernonieae has many

primitive cypselar features like thick pericarp, absence of pitted parenchyma cells etc. (Mukherjee and Sarkar, 2001).

Taxa having well organized testal epidermis have been considered as primitive one compared to disorganized testal epidermis exhibiting testal epidermal cells lacking sclerification and wall thickening as advanced features (Reese 1989). In the present study, testa is often addressed with the pericarp. It is differentiated into an outer non-cellular layer and an inner cellular layer in *V. anthelmintica*, whereas in *V. cinerea* it is totally un-differentiated and non-cellular, suggesting that testa is usually less specific and less distinct, as noted in different other members of Vernonieae (Jana and Mukherjee, 2014). However, occurrence of U-shaped cells in inner testal layer was found as unique in *V. anthelmintica* in the present study.

Endosperm also shows moderate variations among the studied taxa. It is uniseriate in *V. cinerea* but biseriate in *V. anthelmintica*. Embryos of both the species show rather uniformity. They occupied a major to entire portion of cypselar with two, parallelly oriented, plano-convex cotyledons having three secretory ducts in each.

Calcium oxalate crystals and druses, found to be present in different plant parts including cypselar wall in some members of the family Asteraceae. Its significant role to detoxify heavy metals (Nakata, 2003; Talukdar, 2013c), as well as to overcome water and salt stress (Hurkman and Tanaka, 1996) along with other functions and its restricted occurrence in few taxa probably enough to consider it as a evolutionary important taxonomic tool. The presence of calcium oxalate (CaOx) crystals in different plant parts such as leaves, stems, roots, floral parts, fruits and seeds specially as intracellular granular deposits have been recorded from over 215 angiospermic families including Asteraceae (Franceschi and Nakata, 2005). In the present observation, few mesocarpic parenchyma cells manifested calcium oxalate crystals in *V. anthelmintica*. Basak and Mukherjee (2003) observed prismatic calcium oxalate crystals in *V. blanda*, *V. hymenolepis* and *V. stenolepis* of the tribe Vernonieae. In *V. blanda* and *V. hymenolepis* crystal found to be present only in sclerenchymatous tissue whereas in *V. stenolepis* crystals found in both sclerenchymatous and thick-walled parenchymatous tissue. Presence of such type of prismatic crystals in few other species of *Vernonia* has also been noted (Mukherjee and Nordenstam, 2010). Present results pointed out that distribution of crystals and druses in pericarp and/or testa is very useful and important for the tribes Vernonieae, as also found much important in Astereae, Inuleae (*s.lato.*), Senecioneae, Anthemideae and Cynareae and is less important in Arctoteae, Calenduleae, Pertyeae, Dicomeae, Mutiseae and Lactuceae (Mukherjee and Nordenstam, 2010). Unique features of pappus, trichomes, endosperms and crystals have recently been elucidated as potential biomarkers of toxic metalloid stress in organs of medicinal daisy, *Wedelia chinensis* (Talukdar, 2013b). The outcome of

the present structural considerations of two members of Asteraceae assumes further significance in this regard.

Conclusion

The present investigation revealed taxonomic significance of cypselar morphological and anatomical features of two daisies namely *V. anthelmintica* and *V. cinerea*. LM and SEM study revealed sharp differences between two taxa as well as some uniformity in diverse aspects morpo-anatomical features of cypselar. Occurrence of lobed vesicular structure along with biseriate hairs all over the cypselar surface of *V. anthelmintica* is a unique feature and can be used in dual purposes such as potential taxonomic marker as well as easily observable stress responsive biomarker. Presence of well-differentiated distinct carpodium can be used as species delimiting factor under the tribe Verbineae, Asteraceae.

Competing interests

Author declares that she does not have any competing interests.

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