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Research article

Comparative investigations on fruit microcharacters of four species of *Hieracium* L. (Asteraceae) and their taxonomic significance

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Abstract: In order to evaluate taxonomic implications of fruit or cypsela, detail macro as well as micro-morphology and anatomy in four species belonging to the genus *Hieracium* L. of the tribe Cichorieae, family Asteraceae have been investigated. Analysis revealed that in comparison to shape, size and colour of cypsela, surface features like markings, presence or absence of rib and their number along with carpopodium diversity were taxonomically more significant characters. Among anatomical features, mesocarpic characters, distribution of vascular trace and vallecular canal, and nature of testal layer were found to be diacritical for the studied species. These morphological and anatomical features of cypsela can be used as species determining characters in the genus *Hieracium* L. Finally, involving all these features of cypsela an artificial diagnostic key to the four studied species namely *Hieracium neopinnatifidum* Pugsley, *H. pilosella* L., *H. semigothiciforme* Zahn and *H. umbellatum* L. is constructed. This can be used as reference key to identify taxa solely based on its fruit.

Keywords: Hawkweed - Carpopodium - Cypsela - Endosperm - Morphology.

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INTRODUCTION

The genus *Hieracium* L., commonly known as hawkweed is a member of the tribe Cichorieae of the sunflower family (Asteraceae). *Hieracium* L. species are native to Asia, Africa, Europe and America (Strother 2007) and cytological analysis was carried out on *Hieracium* spp. from Indian Western Himalayas (Gupta *et al*. 2014). Being one of the largest genera with more than 10,000 species and subspecies *Hieracium* performs a putative role in making Asteraceae the second largest family of flowering plants (Coşkunçelebi 2003). Reproductive biology of the genus is very unique. They may reproduce exclusively by seeds, of either normal sexual or asexual apomictic type, and alternatively by both seeds and runner like stolon. The apomictic reproduction produces lots of minor geographic variations which in turn creates notorious difficulty in classifying the genus *Hieracium* L. up to species level.

Generic and sub-generic circumscription of *Hieracium* L. remained controversial (Szelag 2014). Recent treatments split *Pilosella* as a separate genus from *Hieracium* based on morphological, biochemical, cytological and genetical features (Bremer & Anderberg 1996). Sennikov (2012) revised the Himalayan (India and Pakistan) species of *Hieracium*. *H. korshinskyi*, *H. kuusamoense* and *H. subramosum* were found in place of *H. vulgatum*, while *H. robustum* were observed in place of *H. crocatum*. He distinguished *H. korshinskyi* from *H. vulgatum* and confirmed differences among rest of the taxa on the basis of morphological features such as leaf texture, habit, nature of hairs, geographical distributions etc. The presence of *H. umbellatum* and *H. virosum* is confirmed in South Asia (Sennikov 2012). In few taxonomic studies achene or cypsela characters along with pollen grain and stolon are taken into consideration (Mráz *et al*. 2002, Sell & West 1975). Strategic demarcation for *Hieracium* sensu lato and sensu stricto is not clear so far and reformation by shifting of species is still in a flux (Coşkunçelebi & Beyazoğlu 2002, Coşkunçelebi 2003). Moreover, hybrid origin of many of the species as evidenced by irregular meiosis, ploidy level variations, and apomictic development of embryos (Mráz 2003) put some more pressure on the present taxonomic difficulty.

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Micromorphological and anatomical features of different plant parts have enough potentiality for taxonomic consideration in Angiosperms (Parveen *et al*. 2000, Ramayya 1972, Talukdar 2012, 2013). Cassini (1975), the true founder of detailed and systematic studies in Asteraceae, believed that to understand a natural group like Asteraceae it was necessary to study all the organs of a plant belonging to all the species in the family without exception.

The fruit and seed characteristics in Asteraceae show marked variation that provides significant taxonomic information (Kadereit & Jeffrey 2007, Nordenstam & Mukherjee 2010, Talukdar 2013). Perusal of literature cited that there is very little comprehensive study on cypsela features in the genus *Hieracium* L. The aim of the present investigation is to provide a detail account of fruit or achene microcharacters including broad range of morphological and anatomical features of four species belonging to *Hieracium* s. lato. namely *H. neopinnatifidum Pugsley*, *H. pilosella* L., *H. semigothiciforme* Zahn, and *H. umbellatum* L. For assessing their taxonomic usefulness an artificial key to the studied species has also been constructed.

MATERIALS AND METHODS

Plant materials

Plant materials (cypselas) for the present investigation were obtained (courtesy Prof. Sobhan Kumar Mukerjee, FLS, Department of Botany, University of Kalyani, west Bengal, India) in the form of received herbarium specimens from the Hortus Botanicus Hauniensis herbarium (DK), Denmark, of the world which is mentioned in Index Herbarium (Holmgren *et al*. 1990). The list of the specimens with their collection number is presented in table 1. Voucher specimens (cypselas) were maintained at Departmental Herbaria, University of Kalyani, West Bengal, India.

Table 1. Source of materials with collection number.

S. No.	Name of Taxa	Locality	Collection Number
1.	*Hieracium neopinnatifidum* Pugsley	DK	Z S1973-1108
2.	*Hieracium pilosella* L.	DK	W DK: 44PG7976
3.	*Hieracium semigothiciforme* Zahn	DK	GE3026-0114
4.	*Hieracium umbellatum* L.	DK	GE 3026-0038

Note: DK-Hortus Botanicus Hauniensis, Denmark.

Micromorphological analysis

For Micromorphological studies**,** mature cypselas were dipped in 1–5% NaOH solution for 2–7 days depending upon the hardness and transferred into saturated chloral hydrate solution for few hours. After repeated washing with water, cypselas were transferred into in 0.2–0.5% aqueous Safranin solution for 5–10 minutes and were placed in 70% phenol glycerine solution for studying different parts. Microphotographs were taken using camera equipped Zeiss-stereo microscope.

Surface ultra-structural study

For scanning electron microscope (SEM) analysis, cypselas were mounted on labelled aluminium brass stubs using double-sided adhesive tapes. All the carrying stubs were quick-dried using vacuum evaporator and examined using FEI-QUANTA 200 Autoscanning Electron Microscope at Regional Sophisticated Instrumentation Centre, Bose Institute, Kolkata, India.

For anatomical studies, mainly hand sections of cypselas from middle part were utilized. The cypselas were softened by dipping in boiling water for 5–30 minutes, with a few drops of glycerol. After softening and sectioning, the sections were dehydrated and stained using conventional method (Johansen 1940) with alcohol grades. All the observed features of cross-section were documented with the aid of camera lucida drawings and camera equipped microscope.

Terminology for the macro- and micro-morphological features, anatomical structures and SEM observation primarily followed Ramayya (1972), Stearn (1973), Barthlott (1981) and partially improvised by the author herself.

Measurement and statistical analysis

All measurements were taken from at least 10 intact and mature cypselas for each specimen and data were obtained by Olympus stereo dissecting microscope. The length of the cypselas was taken as the length of the

body of cypsela from basal meristematic zone (carpopodium) up to apical end excluding pappus. The width of the cypsela was measured from the widest point. The mean (M) and standard error (SE) of measurement were calculated.

RESULTS

In the present investigation the cypselas of four species of *Hieracium* (*H. neopinnatifidum*, *H. pilosella*, *H. semigothiciforme*, and *H. umbellatum*) were examined. Morphological features of cypsela like shape, size, colour, surface markings, carpopodium, pappus etc. are represented by figures 1–3. All anatomical observations are shown in figure 4–5.

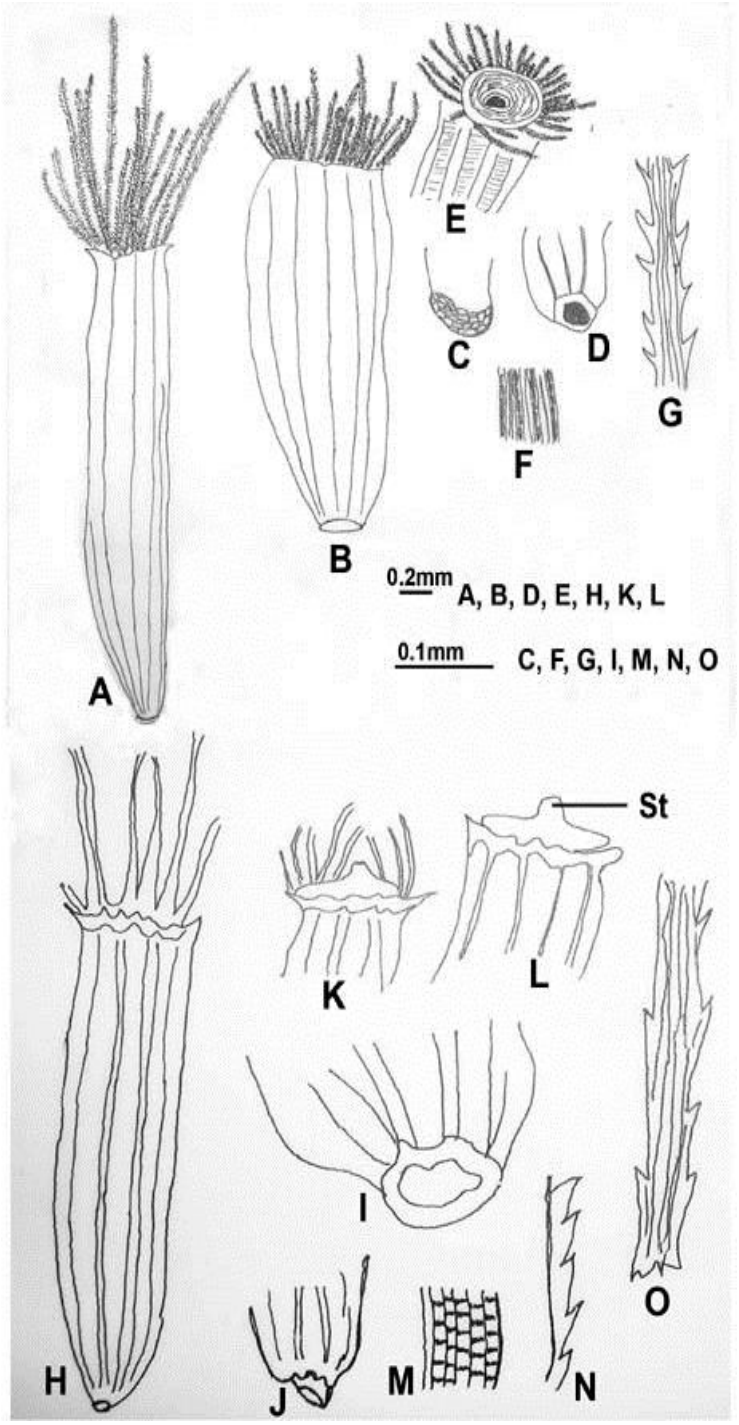

Figure 1. Cypselas morphology: **A-G**, *Hieracium neopinnatifidum*; **H-O**, *Hieracium pilosella*. (**A**, ray cypsela; **B**, disc cypsela; **C**, carpopodium of ray cypsela; **D**, base of disc cypsela; **E**, **K**, **L**, apex; **F**, **M**, surface; **G**, **O**, part of pappus bristle; **H**, cypsela; **I**, **J**, base; **N**, margin)

Morphology of cypsela

Hieracium neopinnatifidum Pugsley (Fig. 1A–G)

Cypsela was heteromorphic. Length of disc cypsela was ranged from 2.8–3.2 mm (excluding pappus) and width varied between 0.2 and 0.5 mm. Cypsela was black, oblong cylindrical, straight in direction, truncate at the apex and gradually tapered towards the base. Surface of cypselas was pubescent without any rib. After clearing, surface showed striate markings (*i.e.* marked with a series of fine narrow parallel bands wider than the lines of a lineate surface). A well developed, wide, tubular, hollow and broad based stylopodium with gradually tapering ring like apical part was noted. Carpopodium was asymmetrical, smooth, ring like structure with visible cells' outline. Carpopodial cells were rectangular, vertically oriented, and present in two to five rows. Diameter of carpopodium was lesser than the base of the body. Cypsela was basally inserted. Pappus was persistent but fragile, represented by two rows of many multicellular terete and scabrous bristles. Bristles were free from one another, unbranched, 1.2–2.3 mm in length, ivory white and somewhat coarser and rough due to projection tips of the lateral cells.

Ray cypselas were more or less similar to disc cypselas, except the following characters,

1. Ray cypsela was larger than disc cypsela with 3.6–4.0 mm (excluding pappus) in length and 0.10–0.15 mm in width,
2. lorate in shape,
3. with symmetrical, smooth triangular ring like carpopodium and
4. diameter of carpopodium was same as the base of the body.

Hieracium pilosella L. (Fig. 1H–O)

Cypsela was homomorphic. Length of cypsela was ranged from 2.0–2.2 mm (excluding pappus) and width varied between 0.3–0.5 mm. Cypsela was black, narrow oblong, cylindrical and straight in direction. Apex of cypsela was truncated wavy with two spiny projections and base was slightly narrower. Glabrous and ribbed surface with muricated margin was noted; ribs were ten in number, prominent and straight. After clearing, surface of cypsela showed lineate markings with transverse bands. A broad based stylopodium with upper tubular solid part was present on the apex of cypsela. At the base an asymmetric, ring like carpopodium was noted with one marked interruption. Carpopodial cells were visible and distinguishable from other cells of the cypsela. They were elongated, thin walled, tangentially oriented, and were arranged in five to six layers. Diameter of carpopodium was narrower than the base of the body. Cypsela was basally inserted and pappose. Pappus was represented by many, persistent but fragile, multicellular, unbranched and scabrid-barbellate bristles. Bristles were 0.23–1.00 mm. long, with conspicuous projection tips of the lateral cells. Base of pappus was free from one another.

Hieracium semigothiciforme Zahn (Fig. 2A–F)

Cypsela was homomorphic. Length was ranged from 2.0–2.4 mm (excluding pappus) and width varied between 0.2 and 0.5 mm. Cypsela was black, narrow oblong, dorsiventrally compressed and straight. At the apex cypsela was truncate and gradually narrower towards the base, with near about 10 lobes. Surface of cypsela was glabrous, and after clearing it showed foveate (pitted) markings, arranged in vertical rows. A well-developed stylopodium was noted at the apex. At the base carpopodium was symmetric, complete, smooth, circular ring like with visible and distinguishable cells. Carpopodial cells were rectangular to elliptic, thick-walled, tangentially oriented and arranged in five to six rows. Diameter of carpopodium was narrower than the base of the body. Cypsela was inserted basally. Pappus was represented by persistent but fragile, multicellular, terete and heteromorphic bristles. Bristles were many, free from one another, unbranched, 0.55–1.66 mm long. Two types of bristles were noted,

a) Narrower, barbellate bristles with the tips of the lateral cells elongated and approximately as long as width of the rachis of the bristles.

b) Broader scabrous bristles, with lateral projection that were narrower than the width of pappus bristles.

Hieracium umbellatum L. (Fig. 2G–N)

Cypsela was heteromorphic. Disc cypsela was 2.3–2.7 mm (excluding pappus) long and 0.5–0.8 mm wide. Cypsela was blackish brown, narrow oblong, dorsiventrally compressed and straight. At the apex cypsela was truncate and towards the base was gradually tapered. Surface of the cypsela was glabrous with ten lobes and showed lineate markings after clearing. A well-developed, narrow, tubular stylopodium was present at the apex.

At the base carpopodium was symmetric, ring like with one marked interruption. Carpopodial cells were visible and distinguishable. They were thick-walled, parenchymatous, square to rectangular, oriented vertically and arranged in seven to eight layers. Diameter of carpopodium was narrower than the base of the body. Cypsela was inserted basally and pappose. Pappus was represented by persistent but fragile, multicellular, terete and barbellate bristles. Bristles were few, free from one another, unbranched, 0.1–0.2 mm. long with the tips of the lateral cells as long as width of the rachis.

Ray cypsela was more or less similar with disc cypsela except in the following characters,

1. Lorate in shape with 3.0–3.2 mm (excluding pappus) in length and 0.2–0.4 mm in width.
2. Truncate at the apex and sharply tapered towards the base.
3. Carpopodium was symmetric, complete circular smooth ring like. Diameter of carpopodium was same as the base of the body.

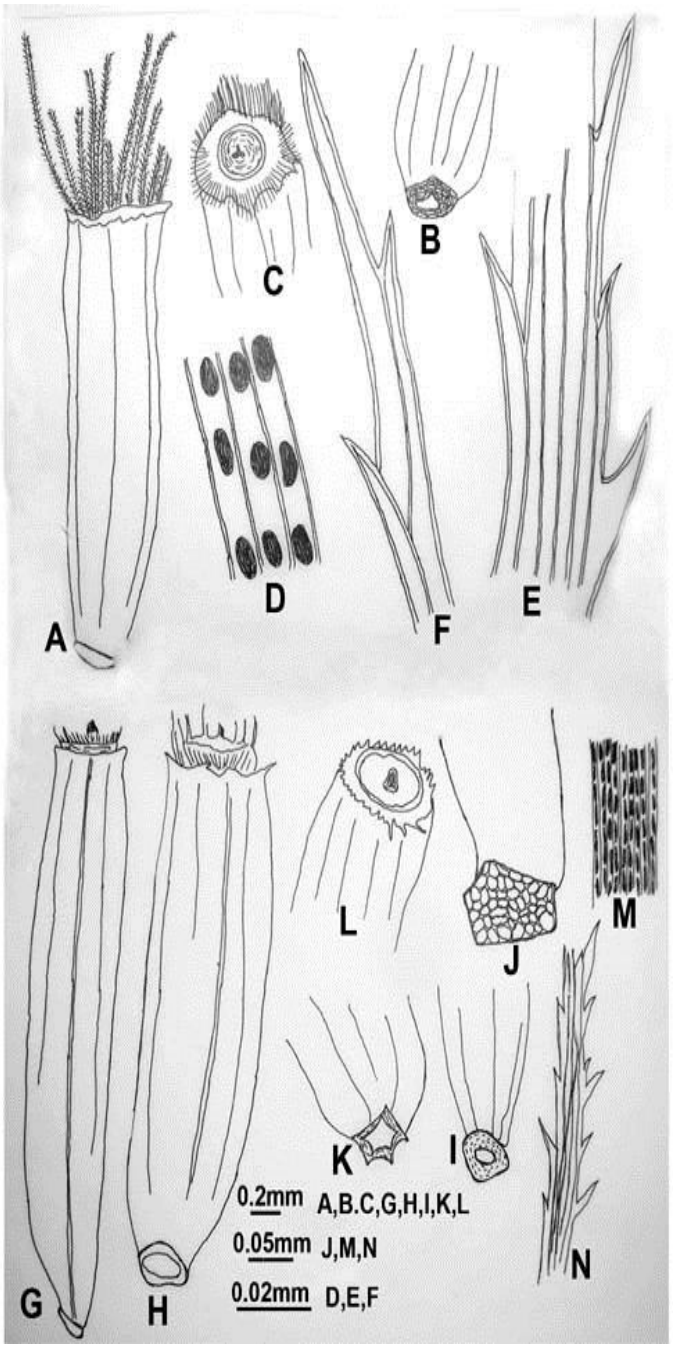

Figure 2. Cypselas morphology: **A-F**, *Hieracium semigothiciforme*; **G-N**, *Hieracium umbellatum*. (**A**, cypsela; **B**, base; **C**, **L**, apex; **D**, **M**, surface (after cleaning); **E**, part of outer bristle; **F**, part of inner bristle; **G**, ray cypsela; **H**, disc cypsela; **I**, base (ray cypsela); **J**, carpopodium (ray cypsela); **K**, base (disc cypsela); **N**, part of pappus bristle)

SEM survey of cypsela

Hieracium neopinnatifidum Pugsley (Fig. 3A–C):

Surface cells of cypsela were visible, rectangular, vertically oriented with straight anticlinal wall and shallow periclinal wall. Surface was pubescent with sparsely distributed, triangular, and upwardly directed hairs. Pappus was biseriate.

Hieracium pilosella L. (Fig. 3 D–F)

Surface cells of cypsela were visible, rectangular, vertically oriented, with straight anticlinal and periclinal wall. Surface was ribbed, muricate with short hard protuberances. Pappus was uniseriate.

Hieracium semigothiciforme Zahn (Fig. 3 G–I)

Surface cells of cypsela were visible, rectangular, vertically oriented, with wavy anticlinal wall and straight periclinal wall. Pappus was uniseriate.

Hieracium umbellatum L. (Fig. 3 J–L)

Surface cells of cypsela were not visible. Very small triangular protuberances were noted on surface. Pappus was uniseriate.

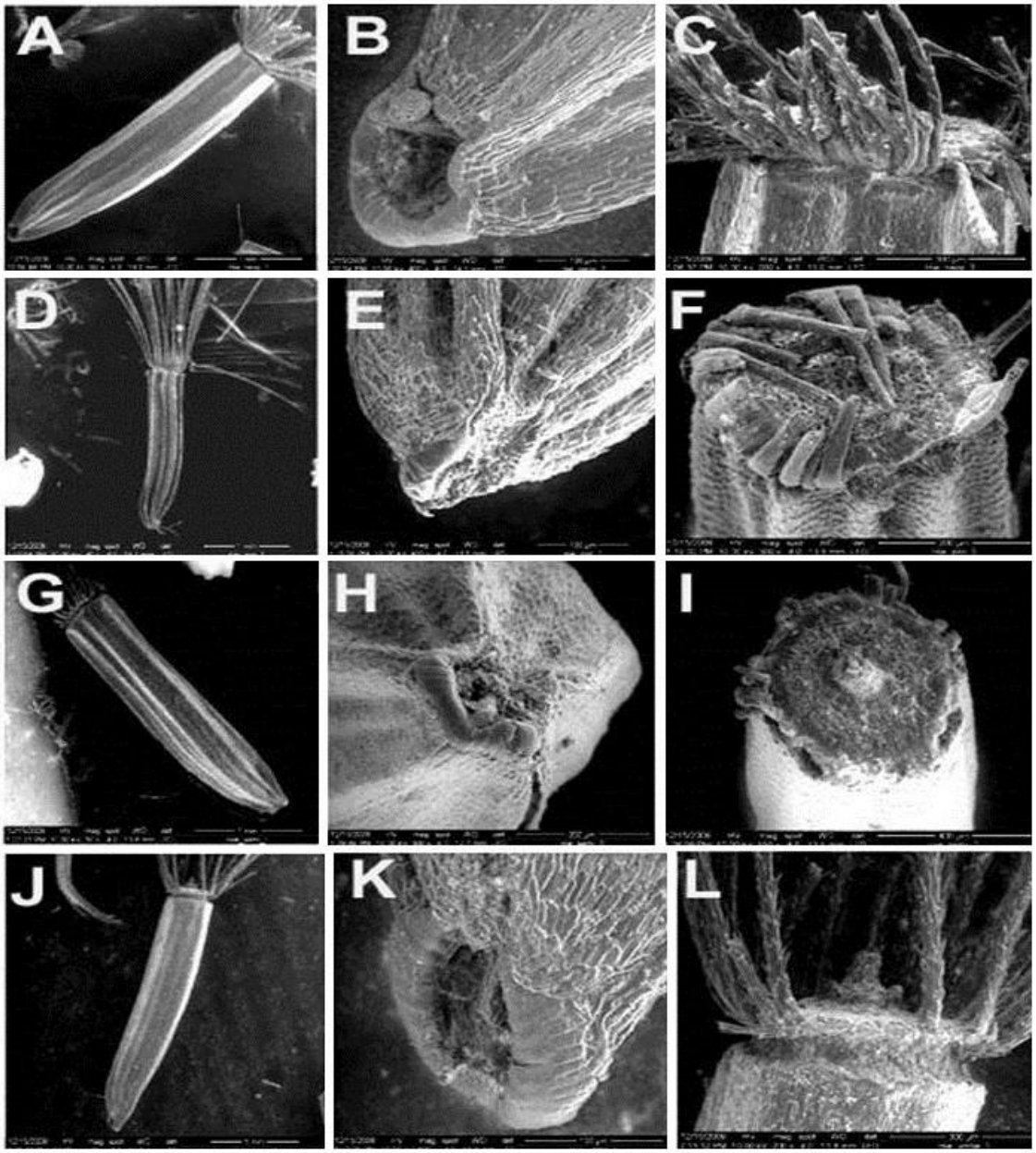

Figure 3. SEM analysis of cypselas: **A-C**, *Hieracium neopinnatifidum*; **D-F**, *Hieracium pilosella*; **G-I**, *Hieracium semigothiciforme*; **J-L**, *Hieracium umbellatum*. (**A**, **D**, **G**, **J**, cypsela; **B**, **E**, **G**, **K**, base; **C**, **F**, **I**, **L**, apex)

Anatomy of cypsela

Hieracium neopinnatifidum Pugsley (Fig. 4 A1–A2)

Both ray and disc cypselas were same in anatomical features. Cypsela was oblate in transection with convolute margin. Wall of cypsela was approximately 106.6 µm wide in T.S. with 66.6 µm thick pericarp. Pericarp was found to be differentiated into two zones-epicarp and mesocarp. Epicarp was uniseriate, made up of thin-walled, elliptic to rectangular, compactly arranged and tangentially oriented parenchymatous cells. Mesocarp made up of continuous four to five layers of scelerenchymatous cells; cells were thick-walled, compactly arranged, variously shaped, with large and round lumen. Three to four vascular traces and five to six vallecular cavities were noted inside of sclerenchyma tissue in the mesocarpic zone. Testa/seed coat was secondarily separated from pericarp, approximately 6.9 µm thick, unilayered, cellular and compressed. Testal cells were parenchymatous, thin-walled, elliptic, loosely arranged and tangentially oriented. Endosperm was persisted in mature cypsela and biseriate. The two endospermic cell layers were dissimilar, of which the outer cells were larger. Cells of both the layers were thin-walled, barrel shaped, tangentially oriented and parenchymatous. The mature embryo occupied a major part of the cypsela. Cotyledons were two in number, plano-convex in shape, anterior-posteriorly oriented (parallel to surface) with three secretory ducts in each cotyledon, of which central one was larger than others.

Hieracium pilosella L. (Fig. 4B1–B2)

Cypsela was circular in transection, with ten broadly semicircular ribs. Cypsela wall was 88 µm and 41 µm wide at rib and furrow respectively with on an average was 58.5 µm thick pericarp. Pericarp was differentiated into two zones-epicarp and mesocarp. Epicarp was uniseriate, made up of thin-walled, rectangular, wavy, compactly arranged and tangentially oriented parenchymatous cells. Epicarpic cuticle was noted which form tubercle like outgrowths throughout the entire surface of epidermis. Mesocarp was sclerenchymatous with variable thickness. At furrow mesocarp was uniseriate and was multiseriate at ridge. Mesocarpic cells were thick-walled, oval to angular, compactly arranged and radially oriented with large elongated lumen. At each rib, one central vascular trace and two lateral vallecular canals were noted just inside of sclerenchyma. Testa was found to be adpressed with pericarp, approximately 4.13 µm thick, unilayered, cellular and compressed. Testal cells were parenchymatous, thin-walled, transversely elliptic, compactly arranged and tangentially oriented. Endosperm was persisted in mature cypsela and biseriate. Cells of two endospermic layers were dissimilar with larger and wider outer cells. Cells of both the layers were barrel shaped, thick-walled, parenchymatous, compactly arranged and tangentially oriented. The mature embryo occupied more or less the entire part of the cypsela. Cotyledons were two in number, plano-convex in shape, anterio-posteriorly oriented (parallel to surface) with three secretory ducts in each cotyledon, of which central one was larger than others.

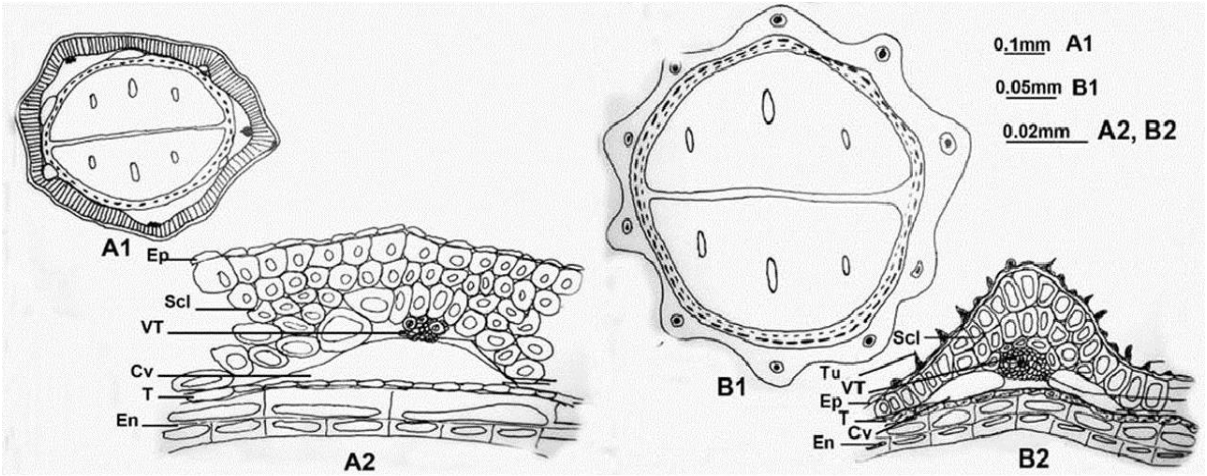

Figure 4. Cypselas anatomy: **A1-A2**, *Hieracium neopinnatifidum*; **B1-B2**, *Hieracium pilosella*. (**A1**, **B1**, T.S. of cypsela (diagrammatic); **A2**, **B2**, a part of cypsela in T.S.; **Cv**, cavity; **Ep**, epidermis; **En**-endosperm; **Scl**, scerenchyma; **T**, testa; **Tu**, tubercle; **VT**, vascular trace)

Hieracium semigothiciforme Zahn (Fig. 5A1–A2)

Cypsela was widely elliptic in transection, with near about 10 lobes. Cypselar wall was 75 µm wide with 68 µm thick pericarp. Pericarp was differentiated into two zones- epicarp and mesocarp. Epicarp was uniseriate,

made up of square to rectangular, compactly arranged and tangentially oriented parenchymatous cells. Papillate out growths were noted in the epicuticular layer. Mesocarp was heterogenous, consists of outer sclerenchymatous and inner parenchymatous tissues. Sclerenchymatous tissue was composed of continuous, four to five cell layers. Cells were thick-walled, angular with narrow elongated lumen. Inner parenchymatous tissue was uniseriate, made up of rectangular, thin-walled, compactly arranged and tangentially oriented cells. Inside of sclerenchyma tissue vascular trace was noted. Corresponding to each vascular trace, single vallecular cavity was found to exist inside of parenchyma tissue. Testa was attached with pericarp, approximately 6.9 µm thick, unilayered and cellular. Testal cells were parenchymatous, elliptic, thin-walled, compactly arranged and tangentially oriented. Inner cross walls of testal cells were much more thickened than outer wall. Endosperm was persisted in mature cypsela and biseriate. The two cell layers were dissimilar with large, barrel-shaped outer cells and small, narrow elliptic inner cells. Cells of both the endospermic layers were thick-walled, tangentially oriented and compactly arranged. The mature embryo occupied major part of the cypsela. Cotyledons were two in number, plano-convex in shape, anterior-posteriorly oriented (parallel to surface) with three secretory ducts in each cotyledon of which middle one was larger than others.

Hieracium umbellatum L. (Fig. 5B1–B2)

Both disc and ray cypselas were more or less anatomically similar. Cypsela was circular in transaction, with ten broadly triangular lobes including five more pronounced lobes. Wall of the cypsela was 160 µm and 93 µm wide at rib and furrow region respectively with on an average 119 µm thick pericarp. Pericarp was differentiated into epicarp and mesocarp. Epicarp was uniseriate, made up of thin-walled, elliptic, compactly arranged and tangentially oriented parenchymatous cells. Epicuticular layer was present forming papillate outgrowths. Mesocarp was homogenous, sclerenchymatous, made up of continuous three to four layers of cells. Mesocarpic cells were thick-walled, angular, with small and elliptic lumen. Single vascular trace and single vallecular canal were situated inside of sclerenchyma tissue at each lobe. Testa was represented by thin, approximately 4.13 µm wide, non-cellular pellicle layer. Endosperm was persisted in mature cypsela and biseriate. The two endospermic cell layers were dissimilar with outer larger cells. Cells of both the layers were thick-walled, parenchymatous, barrel-shaped, tangentially oriented and compactly arranged. The mature embryo occupied more or less the entire part of the cypsela. Cotyledons were two in number, plano-convex in shape, anterior-posteriorly oriented (parallel to the surface) with four more or less equally developed secretory ducts in each cotyledon.

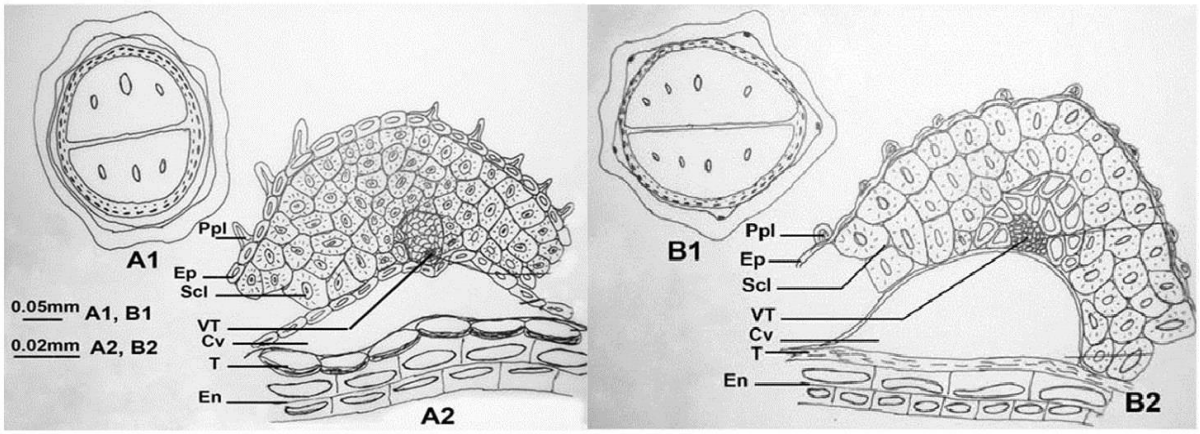

Figure 5. Cypselas anatomy: **A1-A2**, *Hieracium semigothiciforme*; **B1-B2**, *Hieracium umbellatum*. (**A1**, **B1**, T.S. of cypsela (diagrammatic); **A2**, **B2**, a part of cypsela in T.S.; **Cv**, cavity; **Ep**, epidermis; **En**, endosperm; **Ppl**, papillate structure; **Scl**, scerenchyma; **T**, testa; **VT**, vascular trace)

DISCUSSION

Cypselas of the studied species were categorised into heteromorphic type and homomorphic type. Heteromorphic cypselas were noted in *H. neopinnatifidum* and *H. umbellatum.* Cypselas were black to blackish brown in colour and oblong to narrow oblong in shape. However, ray cypsela of *H. neopinnatifidum* and *H. umbellatum* were lorate in shape. Length of cypselas was ranged from 2.0–4.0 mm (excluding pappus) and width varied between 0.1 and 0.8 mm. Cypselas were mostly cylindrical but dorsiventral differentiation was noted in cypsela of *H. semigothiciforme.* In contrast to other species, two apical spiny projections were observed

on cypsela of *H. pilosella*. Cypsela surface of all the studied taxa was glabrous and marked with varied striation. Mostly cypselas were ribbed or lobed, but rib was totally absent in heteromorphic cypselas of *H. neopinnatifidum*. In ribbed cypselas, number of ribs was generally 10. Similarly, 10-ribbed cypsela was also noted in *Lactuca pseudo-umbrella* of the tribe Cichorieae (Maity & Maity 2001). According to Babcock and Stebbins (1937)**,** the longitudinal main ribs of the cypsela are generally five in this tribe. However, the occurrence of other than five-ribbed (*i.e.* 10, 15 or 20 ribbed) cypsela as noted in the present observation was well explained by Kilian (1997). Generally, main ribs were frequently differentiated and sub-divided into less-defined, smaller secondary ribs on either side, resulting in 10, 15 or 20-ribbed cypsela (Kilian 1997).

In all of the presently studied species stylopodium and carpopodium were well developed. Carpopodium showed a wide range of variation between the studied taxa and could be species delimiting factor**.** Two basic types of carpopodium was noted in the present study, A. complete ring like as in *H. semigothiciforme,* in disc cypsela of *H. umbellatum* and in both the cypselas of heteromorphic *H. neopinnatifidu* and B. interrupted ring like as in *H. pilosella* and ray cypsela of *H. umbellatum*. Completely ring like carpopodium also varied in shapes of the ring, which may be either circular in *H. semigothiciforme* and in disc cypsela of *H. umbellatum* or triangular in ray cypsela of *H. neopinnatifidum*. A complete ring like carpopodium with marked interruption was observed in *H. jutlandicum* while carpopodium was complete ring like in *H. norvegicum* (Mukherjee & Das 2008). Marked variations also exist in carpopodium thickness among the different studied species. While highest magnitude of thickness (7–8 rowed) was exhibited in *H. umbellatum* carpopodium, it was moderately thick in *H. pilosella and H. semigothiciforme* (5–6 rowed). Contrastingly, lowest extent of carpopodium thickness (2–5 rowed) was noted in *H. neopinnatifidum*. Mostly, diameter of the carpopodium was found to be lesser than the body of the cypsela except in ray cypsela of *H. neopinnatifidum* and disc cypsela of *H. umbellatum*. Pappus features were found to be less variable among the studied taxa, suggesting this feature may not be a taxonomic delimiting factor in these four taxa. Pappus was mostly persistent, represented by unbranched, free, scabrous and uniseriate bristles of fragile nature. In *H. neopinnatifidum* biseriate bristles and in *H. semigothiciforme* heteromorphic bristles were noted.

Distinct differences were noticed between different species in many of the micro-morphological features of cypsela like surface characters, nature of carpopodium etc. Such type of inter-specific demarcation between *H. pilosella* and *H. umbellatum* was also evidenced by their chromosomal and ploidy level variations (Mráz 2003). This correlation between morphological markers and genomic variations in turn signifies the fact that visual morphology of any parts of plant is as competent as any molecular marker in predicting interrelationship of taxa.

SEM analysis clearly revealed rectangular surface cells of cypsela with visible anticlinal and periclinal wall, although surface cells were totally non-visible under SEM in *H. umbellatum*. Anticlinal and periclinal wall of the surface cells were mostly straight, but in *H. semigothiciforme* wavy anticlinal wall and in *H. neopinnatifidum* shallow periclinal wall was also noted.

Cypselas were circular to elliptic in cross section. Among the studied species, pericarp was the thinnest in *H. pilosella* (58.5 µm) but the thickest in *H. umbellatum* (119 µm), indicating high range of variation in shapes of cypsela. Formation of tubercle or papillate outgrowths was noted on the cuticle layer except in *H. neopinnatifidum*. Pericarp was clearly differentiated into epicarp and mesocarp. Epicarp was usually uniseriate; made up of thin walled, rectangular to elliptic, tangentially oriented, parenchymatous cells. Occurrence of dark brown tanniniferous substance in epicarpic cells of *Hieracium* L. was reported (Pandey *et al*. 1978), but in present observation this was totally absent. Absence of mesocarpic parenchyma as observed in the present case was also observed in *Sonchus asper* of Cichorieae (Uyar & Cirli 1982), and in *Mikania* and *Ageratum* of Eupatorieae (Talukdar & Mukherjee 2008). Only heterogenous mesocarp comprising of both sclerenchymatous and parenchymatous cells was noted in *H. semigothiciforme* among all the studied taxa. Mesocarpic sclerenchyma in all of the studied taxa was present as continuous and multiseriate zone of thick walled compactly arranged angular cells. Mesocarpic parenchymatous layer in cypselas of *H. semigothiciforme* was uniseriate.

On the basis of mesocarpic tissue distribution pattern, two types of fruit wall structures -winged type and ribbed type was earlier proposed in *Ixeris* of Cichorieae tribe of Asteraceae (Pak & Lawano 1990). In more or less similar way, Zhu *et al*. (2006) defined winged type cypsela, in which mesocarp was composed of parenchymatous cells and distinct fibrous strand (*i.e.* thick-walled sclerotic cells). In the contrary, cypselas of

the studied taxa are supposed to be of ribbed type due to bearing continuous thick-walled sclerotic cells in their mesocarp.

Usually few vascular traces were noted in the sclerotic layer. Notably, few larger vallecular canals were present in the same layer corresponding to each vascular trace side by side. They are generally considered as collapsed protoxylem and might be an indicator of reduced mechanical strength. On the other way these empty canals were also act as aerating passage in the cypsela and possibly favour their wind dispersal and dissemination over varied eco-geographical regions and thus, could be regarded as one of the adaptive strategies of *Hieracium* spp.

Testa or seed coat characters are found to be very diacritical and can be considered as potential marker in sub-genus level of *Hieracium* L. Usually testa was uniseriate and cellular as also reported earlier in the tribe Cichorieae (Borthwick & Robbins 1928; Lavialle 1912). Non-cellular pellicle like testa was noted only in *H. umbellatum* among the studied species. Occurrence of non-cellular pellicle like seed coat was also noticed in *Aposeris foetida* of Cichorieae by (Pandey *et al.* 1978). In *H. neopinnatifidum* testal layer was secondarily separated from pericarp, but testa was adpressed to pericarp in heteromorphic species like *H. pilosella* and *H. semigothiciforme*. Notably, in *H. semigothiciforme* differential thickening was noted on inner tangential wall of testal cells, as reported earlier in other members of Cichorieae (Borthwick & Robbins 1928, Pandey *et al*. 1978).

Uniformity in endosperm layer in all the studied species was manifested as biseriate with two dissimilar cell layers, of which outer cells were larger than inner. The embryo occupied major to entire portion of the cypsela. Cotyledons were 2 in number, equal, plano-convex, anterio-posteriorly oriented and possess secretory ducts. Cotyledon of *H. umbellatum* bears 4 equally developed secretory ducts. However, in rest of the studied species secretory duct was 3 in number, of which central one was well developed and larger than others.

Considering all these micro features of cypselas, an artificial key to the species was constructed by which studied species can be identified in their fruiting stage.

Key to the species

Genus - *Hieracium*

1a. Cypsela heteromorphic **2**

2a. Cypsela cylindrical; ribs absent; pappus biseriate; epicarp without any papillate outgrowths; surface cells visible in SEM study; testa cellular. . . . . ***H. neopinnatifidum***

2b. Cypsela dorsiventrally compressed; ribs present; pappus uniseriate; epicarp with papillate outgrowths; surface cells not visible in SEM study; testa disorganized . . . . . ***H. umbellatum***

1b. Cypsela homomorphic **3**

3a. Apex with spiny projections; cypselar margin muricated; carpopodium asymmetric, ring-like, with one marked interruption; mesocarp with sclerenchyma tissue only; testal cells without any wall thickenings . . . . . ***H. pilosella***

3b. Apex without spiny projections; cypselar margin not muricate; carpopodium symmetric, ring-like, without interruption; mesocarp with both sclerenchyma and parenchyma tissue; testal cells with inner cross-wall thickenings . . . . . ***H. semigothiciforme***

CONCLUSION

The present study on detailed macro and micro-morphological and anatomical features of cypselas of four different species of *Hieracium* L. is a strong attempt to assess the potentiality of cypsela as species delimiting factor. The analysis clearly indicates that in comparison to size, shape and colour of cypsela; surface characters, nature of carpopodium, mesocarpic differentiation, testal features etc. are much more reliable for inter-specific taxonomic grouping or separation of *Hieracium* genus and should have a positive impact on its present status.

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