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A COMPARATIVE STUDY OF CYPSELAR FEATURES IN TWO SPECIES OF THE TRIBE ARCTOTIDEAE, ASTERACEAE

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ABSTRACT

The family Asteraceae is categorized by the presence of a distinct type of fruit known as cypsela. The present study describes the detailed external and internal features of cypselae from two species within the *Berkheya* clade, *Berkheya multijuga* (DC.) Roessler and *Berkheya purpurea* (DC.) Benth. & Hook.f. ex Mast. The tribe Arctotideae was studied earlier in morphological perspective but the information regarding the cypselae is inadequate. The cypselae were studied with the help of light microscope and scanning electron microscope (SEM). Morphologically, the shape and size of cypselae, colour, pappus structure, hair, surface structure, stylopodia and its structure, carpopodium etc. were found to be important features for distinguishing the species. Anatomically, cellular nature of pericarpic region, number and shapes of ribs and elevations, cuticle features, epicarpic characters, mesocarpic differentiation, vascular trace, crystals, testa, endosperm, embryological details were studied and they are vital for differentiation of taxa. A diagnostic key was constructed for identification of the species. The present work showed a valuable taxonomical significance, on the basis of both morpho-anatomical characters of cypselae the identification and classification of various taxa can be easily performed.

1. INTRODUCTION

This group consists of flowering plants with special types of fruits called "cypsela". The tribe Arctotideae was first mentioned in the work of Cassini [1]. Later it was combined with the tribe Cynareae (now Cardueae) by Lessing [2,3] but again reestablished by Bentham [4], who illustrated three sub-tribes under Arctotideae as – Arctotidinae, Gorteriinae and Gundeliinae. This was supported by Hoffmann [5] with little deviation in generic circumscriptions. Leins [6] proposed forth monotypic sub-tribe Eremothamninae, based on palynological data, which was retained by Norlindh [7] along with those three sub-tribes.

Bremer's [8] cladistic analysis of Asteraceae based on morphology remained in concurrence with earlier placement of Arctotideae with the tribe Cardueae by Lessing [2,3]. Arctotideae was combined with Cardueae due to shared features including dissected, spiny leaves and style with a disc of sweeping hairs below the bifurcation. According to molecular analysis initiated by Kim and Jansen [9], Arctotideae appears to be nested in a monophyletic sub-family Cichorioideae *s.str*.

Among the four sub-tribes, the two sub- tribes Arctotidinae and Gorteriinae are supported by both morphological as well as molecular data while in the remaining two sub-tribes molecular data are not supportive proximity of the third sub-tribe Gundeliinae with that of the tribe Cichorieae (Karis et al., 2001; Funk et al., 2004, while the morphological data of the fourth sub-tribe Eremothamninae showed similarities with the tribe Arctotideae but molecular data do not supported Funk et al., 2004. After Norlindh's analysis [7] different morpho-anatomical characters like ray limb epidermis [10], cypselar anatomy [11], embryology [12], external cypselar morphology [13] were published. The cladistic analysis based on morphology of Gorteriinae [14] was also published. Molecular analyses regarding Arctotidinae [15]-[17] and Gorteriinae [18] were well documented and Gorteriinae was subdivided into the *Gorteria* clade and the *Berkheya* clade based on nuclear and chloroplast DNA sequence data [18].

Mckenzie et al. [13] estimated that the tribe Arctotideae consists of 220 species, generally South African in distribution, except only one *Cymbonotus*, which is native to South Australia. According to Karis [19] the tribe Arctotideae comprises of 17 genera and nearly 215 species.

Many species of Arctotideae are economically important as garden plants. They are cultivated as hybrids from the plants like *Gazania krebsiana*, *Gazania linearis* and *Gazania rigens* [20].

The present project was undertaken with a view to get some insight into the detailed external and internal features of cypselae (the fruit) of two species i.e., *Berkheya multijuga* (DC.) Roessler and *Berkheya purpurea* (DC.) Benth. & Hook.f. ex Mast. under one genus – *Berkheya* from the *Berkheya* clade of the tribe Arctotideae with the hope of getting some new information ultimately leading to better evaluation of the family. Cypsela features have potential value for characterization of taxa as these reproductive characters are less variable in different environmental conditions, they play a major role for identification of taxa when other floral features are inaccessible. As the characters of cypselae are the most important tool for the success of the family Asteraceae so, they can show the evolutionary diversity of the family as well.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Source of specimens:

The investigated plant materials (cypselae) were obtained **as a gift** in the form of dried herbarium specimens from Botanischer Garten der
Universitat Zurich, Zollikerstrasse 107, CH-8008 and Eidgenössische Technische Hochschule Zürich, Switzerland (United Herbaria- **Z and ZT**).

2.1.2. Other Materials required:

Scissors, scalpel, forceps, paper/ plastic packets, hand lens, desiccators, Formalin Aceto-Alcohol (FAA) solution, (1-5) % Sodium hydroxide
(NaOH) solution, saturated chloral hydrate solution, 0.2-0.5 % aqueous Safranin solution, 70% phenol glycerin solution, glycerin jelly,
lactophenol, ethanol, sodium hypochlorite, acetic acid, Canada balsam, wax, spirit lamp, blades, needles, slides, coverslips, water bath,
hot plate, stereo dissecting microscope, binocular microscope, microscopic camera, camera lucida, brush, notebook, pencil, eraser, graph
paper, scale, rotring isograph pen sets (0.1mm, 0.2mm, 0.3mm and 0.5mm), tracing paper, etc.

2.2. Methods:

2.2.1. Morphological study

a) For macro-morphological observation: 10 dried mature cypselae were randomly selected and observed in glass slides under the Olympus Stereo Dissecting microscope (DM) and Metzer binocular microscope (10 X). Diagram of different parts of cypselae were drawn with the help of drawing prism and pencil. Suitable images were taken by using microscopic camera.

b) For micro-morphological observation: 10 FAA solution preserved mature cypselae were taken, dipped in 1-5% NaOH soln. for 2-7 days. Then it was transferred into saturated chloral hydrate solution for few hours, repeatedly washed with water. Then, it was stained with 0.1% aqueous Safranin and observed under Stereo Dissecting microscope (DM) and Metzer binocular microscope (45X).

2.2.2. Anatomical study

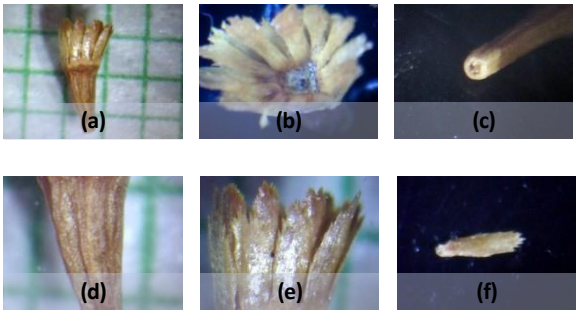
For anatomical observation, free hand thin transverse sections were made with a sharp razor blade. The sections were immersed in 0.2-0.5% aqueous Safranin solution for staining, mounted by 70% phenol glycerin solution on slides and placed under a compound light microscope (45 X) to observe the characters.

2.2.3. Scanning Electron Microscopic (SEM) study

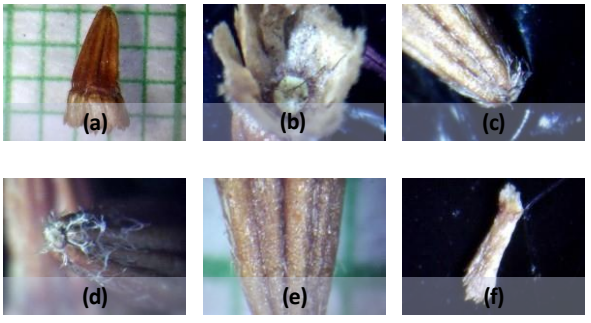
For Scanning Electron Microscopic (SEM) studies, a minimum of three cypselae were mounted in stubs and coated with a very thin layer of gold (200 A°). Scanning photomicrographs were taken in University Instrumentation Centre, Kalyani.

3. OBSERVATIONS

3.1. Figures



CAM PP 1 –*Berkheya multijuga* (DC.) Roessler (a) Cypsela whole body (b) Stylopodium (c) Carpopodium (d) Surface (e) Pappus arrangement at apical part (f) Scale



CAM PP 2 –*Berkheya purpurea* (DC.) Benth. & Hook.f. ex Mast. (a) Cypsela whole body (b) Stylopodium (c) Carpopodium (d) Hairs at carpopodial region (e) Surface (f) Scale

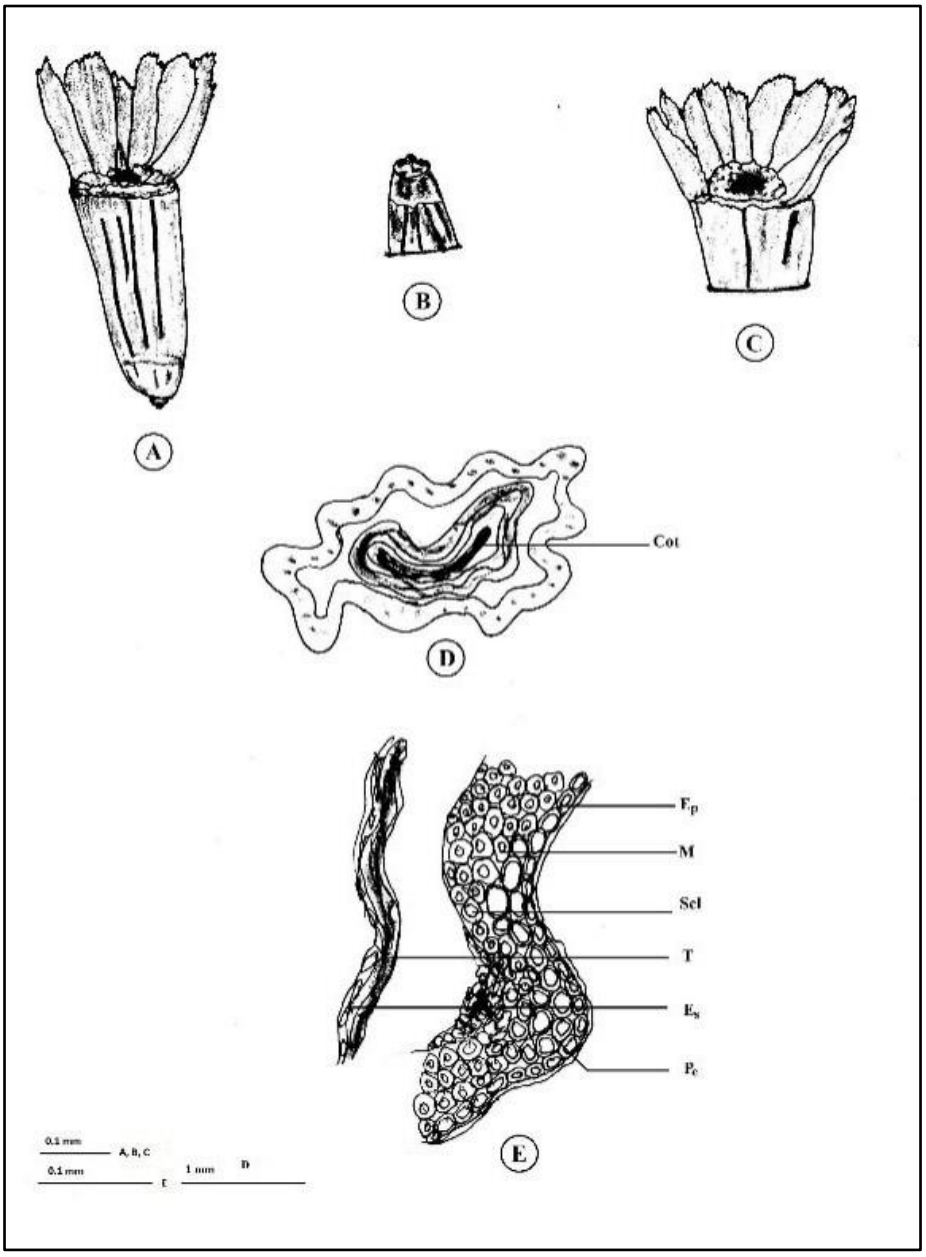


Figure 1 –*Berkheya multijuga* (DC.) Roessler (A) Cypsela whole body (B) Stylopodium (C) Carpopodium (D) T.S. of cypsela (diagrammatic view of entire section) (E) T.S. of cypsela (cellular view)

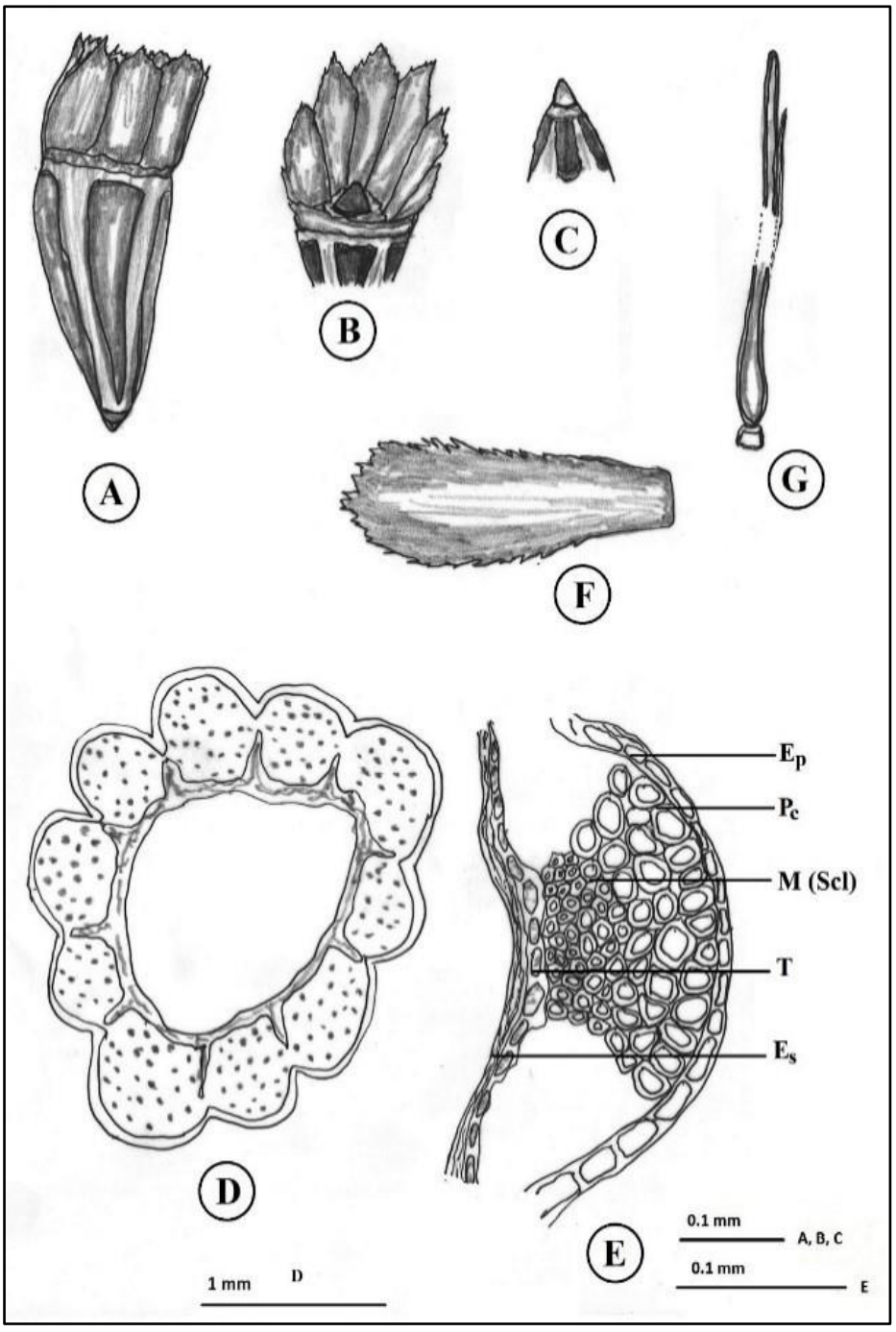
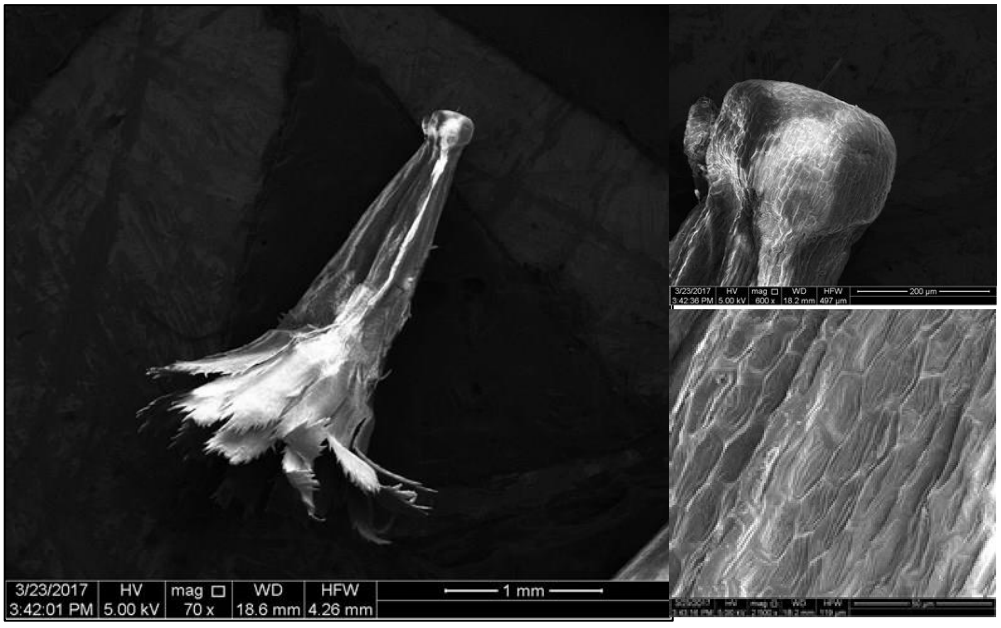
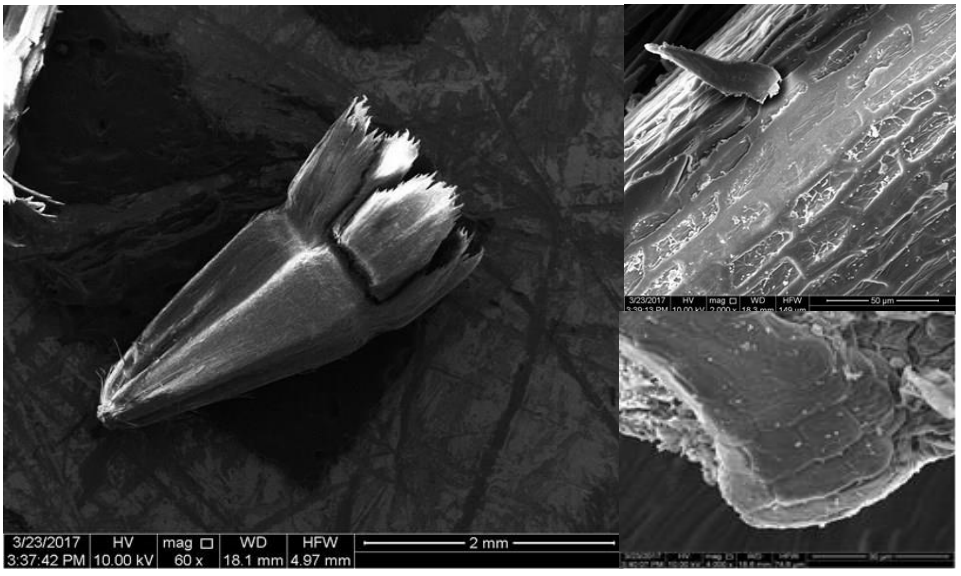


Figure 2–*Berkheya purpurea* (DC.) Benth. & Hook.f. ex Mast. (A) Cypsela whole body (B) Stylopodium (C) Carpopodium (D) T.S. of cypsela (diagrammatic view of entire section) (E) T.S. of cypsela (cellular view) (F) Scale (G) Hair



SEM PP 1 – *Berkheya multijuga* (DC.) Roessler (a) Cypsela whole body (b) Carpopodium (c) Surface cells showing striations



SEM PP 2 – *Berkheya purpurea* (DC.) Benth. & Hook.f. ex Mast.(a) Cypsela whole body (b) Surface showing twin hairs (c) Carpopodium

3.2. Table

Table1: Comparative morphological and anatomical characters of cypselae of the studied species.

Character / Genus	*Berkheya multijuga*	*Berkheya purpurea*
Morphology		
Colour/ Shape	Bright brown	Bright brown
Apex	Dorsiventrally flattened	Slightly dorsiventrally flattened
Surface	Surface cells visible; thick walled; shape: rectangular; straight anticlinal and concave periclinal walls of surface cells; surface markings: furrowed or sulcatus and striated; surface texture: asperous (rough surface)	Surface cells visible; thick walled; shape: rectangular; straight anticlinal and concave periclinal walls of surface cells; surface markings: furrowed or sulcatus; surface texture: glaucous (covered with bloom or slight waxy coating)
Ribs	10 in number	10 in number
Stylopodium	Present	Present
Carpopodium	Symmetrical, complete, lobed ring like; morphologically and anatomically well-differentiated, multi-layered; outline visible, distinguishable from other cells of the cypsela, arranged in 11-12 rows, small; square to rectangular	Symmetrical, complete, circular complete ring-like; morphologically and anatomically well-differentiated; multi-layered; outline visible, distinguishable from other cells of the cypsela, arranged in 3-4 rows; large; square to rectangular
Anatomy		
Shape in cross section	Multicostal, ellipsoidal	Multicostal, ellipsoidal
Epicarp	Uniseriate, oval to rectangular, parenchymatous, thin-walled, tangentially oriented, compactly arranged cells; cuticularized and persistent hair bases present	Uniseriate, oval to rectangular, parenchymatous, thin-walled, tangentially oriented, compactly arranged cells; cuticularized and persistent hair bases present
Mesocarp	Made up of parenchyma, sclerenchyma and vascular tissues	Made up of parenchyma, sclerenchyma and vascular tissues
Testa	Testa adpressed to pericarp	Testa adpressed to pericarp
Endosperm	Persists in mature cypsela, biseriate, 2 layers separated, elongated oval, parenchymatous, thin-walled, tangentially oriented, compactly arranged, continuous	Persists in mature cypsela, biseriate, 2 layers separated, elongated oval, parenchymatous, thin-walled, tangentially oriented, compactly arranged, continuous
Embryo	Mature embryo occupied major part of the cypsela; cotyledons two in number, equal	Mature embryo occupied major part of the cypsela; cotyledons two in number, equal

3.3. Diagnostic key to the studied species of Arctotideae

1A. Carpopodium lobed ring like; carpopodial cells thin – walled, arranged in 11-12 rows, vertically or longitudinally oriented; diameter of carpopodium: wider than the base of the cypsela**…**……*Berkheya multijuga*

1B. Carpopodium circular complete ring-like; carpopodial cells thick – walled, arranged in 3-4 rows, vertically or longitudinally as well as tangentially oriented; diameter of carpopodium: narrower than the base of the cypsela**…**……*Berkheya purpurea*

4. DISCUSSION

The tribe Arctotideae was studied earlier in morphological point of view (Pandey, 1983) but the information regarding the cypselae is inadequate. Present investigation deals with two species i.e *Berkheya multijuga* (DC.) Roessler and *Berkheya purpurea* (DC.) Benth. & Hook.f. ex Mast. under one genus – *Berkheya* from the *Berkheya* clade.

4.1. Cypselar Morphology

The tribe Arctotideae was studied earlier in morphological point of view [21, 22] but the information regarding the cypselae is inadequate. Cypselae characters varies with in the tribe, generally ovoid rarely oblong-elliptic or sometimes prismatic to obconical, distinctly ribbed. Pappus of scale type or rarely bristles or absent [19].

The cypselae are homomorphic; 2.5 mm X 1.0 mm (excluding scales) and 3.5 mm X 1.0 mm (including scales); slightly asymmetrical in *Berkheya multijuga or* 3.5 mm X 1.0-1.25 mm (excluding scales) and 4.5 mm X 1.0-1.25 mm (including scales); symmetrical in *Berkheya purpurea*; both are narrow-obovate; straight; gradually tapered towards the base and with truncated apex; dorsiventrally flattened. Both are without wings, however, the wing like structure is reported by Mckenzie & al.2006 in *Arctotis*.

Based on colour, it is harder to distinguish the species as both *Berkheya multijuga* and *Berkheya purpurea* are bright brown in colour.

Berkheya multijuga and *Berkheya purpurea* both have persistent pappus scales, showing ear like structure, homomorphic; free; double whorled and represented by many structures, equal in length, unbranched; the colour in case of both the species are same i.e., pale brown; pappus apices are serrated, unequal, sharply pointed, base of pappi is free; however, length of scales slightly varies from species to species 0.9-1.0 mm (*Berkheya multijuga*) and 0.8-1.0 mm (*Berkheya purpurea*). Coronate based pappus was also observed in *Arctotheca populifolia*, *Haplocarpha hastana*, *Haplocarpha schimperi* etc. of this tribe [13]. The pappi are responsible for the dispersal of these one-seeded dry fruits, the cypselae [23]. An overall idea about the diversity in pappus structure was given by Mukherjee & Sarkar [24].

Surface hairs are visible in both the species when studied under SEM, both have pilose sharply pointed type hairs which are sparsely distributed on both the surfaces, an observation supported by Karis [14], who reported presence of twin hairs of different forms, length and colour [14, 19] ; hairs in both the cases are oriented antrorsely i.e. bent or directed upward; cypselar hairs are of simple type; tips of the body cells of hairs situated in different plane and types of hairs are either simple as in *Berkheya multijuga* or dimorphic as twin (biseriate forked) and simple types both in *Berkheya purpurea*. Herman [25], examined the surface in four species of *Artotheca* of this tribe and observed the presence of septate type of hairs. He also reported in the cypselae of *Arctotheca calendula*, the presence of multi-cellular, bi-seriate hairs, such hairs matched with the twin hairs of *Berkheya purpurea*, which were noticed in the present study.

The surface cells were visible and usually thick walled, rectangular with straight anticlinal and shallow periclinal walls, surface markings showing furrow and ridges and specific ornamentation (striated in *Berkheya multijuga*), although no such striation were seen in Berkheya *purpurea* during the SEM study of the specimens Surface texture varies in the two species: either asperous (*Berkheya multijuga*) or glaucous (*Berkheya purpurea*). Ribs present, 10 in number; conspicuous, (*Berkheya purpurea*) ribs wider than furrows; slightly incurved or recurved (converginervis) at base. Similar types of ribbed cypselae were also revealed in the study by Mukherjee & Sarkar [26] in *B. zeyheri* ssp. *zeyheri*.

Carpopodium as defined by Mukherjee & Nordenstam [27] is the basal, abscission zone of cypselae, and it helps in the detachment of cypselae from the thalamus. It is symmetrical, complete, lobed ring like (*Berkheya multijuga*) or circular complete ring-like (*Berkheya purpurea*); morphologically and anatomically well-differentiated; multi-layered; outline visible, distinguishable from other cells of the cypsela, thin-walled (*Berkheya multijuga*) or thick-walled (*Berkheya purpurea*), not pitted but striated or ornamented, arranged in 11-12 rows (*Berkheya multijuga*) or 3-4 rows (*Berkheya purpurea*); cell size comparatively small (*Berkheya multijuga*) or large (*Berkheya purpurea*); cell shape is square to rectangular; vertically or longitudinally oriented (*Berkheya multijuga*) or both way oriented as in *Berkheya purpurea*; compactly arranged; diameter either wider (*Berkheya multijuga*) or narrower than the base of the cypsela (*Berkheya purpurea*). Detachment area is straight basal. Haque & Godward [28], pointed out the absence of carpopodium in case of *Arctotis stoechadifolia* of this tribe.

Apex truncated, without any beak. Stylopodium unenlarged, free, fully immersed in the nectary; coronate but weakly or ill developed; ebordered. Cypselar beak and wing both were not found in the investigated species *viz. Berkheya multijuga* and *Berkheya purpurea*. So, the present observation is similar with the observation of Mukherjee & Sarkar [26]. Mukherjee & Sarkar[26] mentioned about inconspicuous stylopodial development in other species (*Arctotheca calendula*, *Arctotis venusta* etc.) of this tribe.

4.2. Cypselar Anatomy

In cross sectional configuration, it is multicostal, ellipsoidal, distorted; dorsiventrally strongly compressed, ribbed; outlines of the section: both are rotundus.

In *Berkheya purpurea* and *Berkheya multijuga*, 10 conspicuous ribs were noticed, ribs wider than furrows and round.

Pericarp thick, tissue differentiated into 2 zones - epicarp and mesocarp. Cuticle present in both. In *Berkheya purpurea* epicarpic cells are uniseriate, oval to rectangular, parenchymatous, thin-walled, tangentially oriented, compactly arranged cells; cuticularized and persistent hair bases present. The orientation of epicarpic cells and related structures are vital for taxonomic study [29]. In the cypselae (*Arctotis* and *Arctotheca*), of this tribe, tangential orientation was reported by [29]

Mesocarp usually made up of parenchyma, sclerenchyma and vascular tissues: parenchyma 5-6 seriate, oval to polygonal, angular, thick-walled, with large lumen, tangentially oriented, compactly arranged, continuous; sclerenchyma, 3-4 seriate, hexa- to poly-gonal, angular, thick-walled, with small lumen, tangentially oriented, compactly arranged, discontinuous, present as sclerotic braces, vascular traces present. Reese [11], advocated that only a small number of Gorteriinae have oblong sclerified cells in one or two sub-epidermal layers in the pericarp, while remaining of the examined species have cells with equal length and width [11, 13]. Interestingly, in *Berkheya purpurea* few upper mesocarpic cells contain prismatic crystals. Sub-epidermal crystals were also reported in the testa of many Gorteriinae by other workers [11, 13].

In *Berkheya purpurea* single vascular trace present at each rib, situated inside of sclerenchyma tissue. Pericarpic phytomelanin layer, cavities like vallecular, secretory or resin and pellicle were not found. In few upper mesocarpic cells in *Berkheya purpurea*, crystals were seen.

Testa adpressed to pericarp, not differentiated into outer and inner layer; cellular, bilayered, organized, collapsed, thick-walled, rectangular, tangentially oriented, compactly arranged, parenchymatous cells in *Berkheya purpurea* [30]. Jana & Mukherjee [31] observed the presence of U-shaped testal layer in some species (*Solidago virgaurea*, *Solidago arborescens* etc.) of the tribe Astereae and in the tribe Lactuceae (*Cichorium endivia*), Gnaphaleae (*Leptorhynchos elongates*) etc. [32-35]. According to Reese [11], this tribe is somehow primitive than the other tribes. Endosperm persists in mature cypselae, biseriate in nature, 2 layers separated, elongated oval, parenchymatous, thin-walled, tangentially oriented, compactly arranged and continuous. According to Bartthlott [36], the endosperm layer is single layered in mature cypselae. Uniseriate endosperm layer was also observed in some other studied genera (*Arctotheca* and *Arctotis*) [29]. The studied taxa however, deviated from this observation.

In *Berkheya purpurea* mature embryo occupied a major part of the cypselae; cotyledons are two in number, equal, plano-convex, anterio-posteriorly oriented, secretory ducts in each cotyledon were not evident.

CONCLUSION

In the taxonomy and bio-systematics point of view, the morphology and anatomy of cypselae of this species are of great importance and these features may be employed for better understanding of the taxa.

Also, the analysis evidently indicates that in comparison to size and shape of cypselae; nature of carpopodium, presence or absence of ribs, pappus features like arrangement of pappus bristles, types of cells present in mesocarp region, number of resin ducts present in cotyledons etc. are much more consistent characters for grouping or separation of different species.

Apart from the taxonomists, as these species are economically important, these characters are significant for the agriculturist as well as pharmacologists for identification of weeds or detection of adulterants.

Acknowledgements

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