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Morpho-taxonomic description of cypsela features in two genera of tribe Mutisieae (Asteraceae)

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ABSTRACT

Micro-morphological, macro-morphological, and anatomical features of cypsela features of two genera *Leibnitzia nepalensis* (Kunze) Kitam. and *Onoseris sagittatus* Rusby of tribe Mutisieae of family Asteraceae have been investigated by light and scanning electron microscopy. Cypsela homomorphic and carpopodium symmetric in both genera. Cypsela narrows elliptic in cross-section, ribbed. However, in *Leibnitzia*, cypsela slightly curved with ill-developed ribs; hairs sparsely distributed; stylopodium ill-developed; diameter of carpopodium same as the base of the cypsela; insertion of cypsela oblique; pappus basally united; cypsela elliptic in T.S.; epicarpic cuticle absent; mesocarpic parenchyma absent; secretory ducts in each cotyledon equal in size. Contrastingly, in *Onoseris*, cypsela straight; ribs prominent; hairs densely distributed; stylopodium well-developed; diameter of carpopodium lesser than the base of the cypsela; insertion of cypsela straight; pappus basally free; cypsela angular in T.S.; epicarpic cuticle present; mesocarpic parenchyma present; secretory ducts in each cotyledon unequal in size.

KEY WORDS: Asteraceae, carpopodium, cypsela, Mutisieae, pappus, stylopodium

INTRODUCTION

The sunflower family Asteraceae is the largest family of flowering plants, consisting of over 1600 genera and 23,000 species excluding apomictic microspecies (Kadereit and Jeffrey, 2007) and is accounting about 10% of the total number of flowering plants. One of the principal centers of origin of the Compositae is in the dry high-land of central Mexico (Cronquist, 1981).The family is one of the most distinctive and isolated members of the class Magnoliopsida and placed in a monotypic order Asterales (Cronquist, 1981). It has many morphological specialties such as capitulum inflorescence, highly modified and reduced flowers, inferior bicarpellate ovary that develops into a cypsela, basal and erect ovule, and connate anthers, which support its isolated position. Besides that, the Asteraceae have a cosmopolitan distribution, highly diversified habitat, and evolutionary successful status which according to Cronquist (1981), may be due to a diversified chemical defense system with production of a combination of polyacetylenes and sesquiterpene lactones. Such extensive morphological, chemical, and biological diversification have occurred in the family very rapidly within a very short period of time as it have originated only 30 million years ago in the middle to upper Oligocene, supported by fossil evidence (Cronquist, 1981; Muller, 1981) and biogeographical considerations (Raven and Axelrod, 1974). Such explosive radiation and well-marked variability within the family in one hand created immense interest of taxonomists and, on the other hand, posed problems to resolve phylogenetic relationships and to understand character evolution (Jansen *et al*., 1991).

A history of research in Asteraceae reveals that as early as the mid 16th century Jean Ruel, a French botanist, is credited with many original observations on plants and coining of copious new morphological terminology. He described several Asteraceae and defined capitulum as being composed of several florets of different types (Ruel 1536, cited by Greene 1983). Vaillant (1719-1723) considered as the last serious contributor to Asteraceae before Cassini, first used the pappus character of fruit along with other characters derived from the phyllaries and receptacles, etc., in his classification system. Cassini was the true founder of detailed and systematic studies of Compositae, in his remarkable works, described characters from cypsela and pappus along with styles, stigmas, stamens, corollas and included all these characters

in the final classification scheme to recognize altogether 20 tribes of Asteraceae (Cassini 1816-1830). He 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.

Bremer (1994; 1996) classified Asteraceae into 3 sub-families- Barnadesioideae (monophyletic and contains less than 1% of the species in Compositae), Asteroideae (also monophyletic and contains ca 65% of the species in the family), and Cichorioideae (ca 35% of the species in the family) and 17 tribes. He acknowledged that sub-family Cichorioideae were most likely paraphyletic and showed variations in the morphological and molecular characters. With the use of chloroplast DNA data by Jansen and Palmer (1987; 1988), recently a new higher version of the classification system of Asteraceae had been proposed by Panero and Funk (2008). They have recognized 12 sub-families and 43 tribes altogether.

The phylogeny of Asteraceae even now is in a state of flux. Although DNA data provide the most reliable for estimating evolutionary relationships and distances between taxa, these data cannot explain how or why a particular plant evolved without phenotypic information, including a broad range of morphological and chemical characters (Calabria *et al.*, 2009). So, a valid need of morphometric analysis cannot be ruled out.

It is really a fact that cypselar morphology in the family has not been received as much attention as it should be. According to Heywood *et al*. (1977), cypsela structure and anatomical features have been studied in detail in only a few groups such as Anthemideae and Cardueae and found to be taxonomically valuable.

Perusal of literature cited that only very few instances of using cypsela characters as an aid in classification. Singh *et al*. (1972) have stressed the importance of the cypsela morphology in the Liguliflorae and studied 22 species using both fresh and herbarium material, and prepared a key to the seeds of Indian Liguliflorae mostly those occurring in the Himalayas.

In this context, our present investigation deals with detailed studies of cypselar morphological and anatomical features of two genera under the tribe Mutisieae distributed in temperate Europe of the family Asteraceae primarily using Light Microscope. Special emphasis has been given to traditional characters such as size and shape of cypselas, nature and distribution of ribs and furrows, nature of surface pubescence, the structure of stylopodium, carpopodium, and pappus. Anatomically, type and relative distribution of different tissues in pericarp wall, along with nature of phytomelanin layer and calcium oxalate crystals when present, the composition of testa and endosperm, nature of embryo, the orientation of cotyledon, and number of resin duct in each cotyledon, etc., all are studied from various aspects. Scanning electron microscopic (SEM) analysis has also been performed specially on surface characters, carpopodium, stylopodium, and pappus, with a purpose to create a reference set of micro-morphological features. Finally, a sincere attempt has been made to construct an artificial key to studied taxa along with phylogenetic key involving all the observed characters and to draw inter-relationships among the studied tribes.

MATERIALS AND METHODS

Plant materials (cypselas) for the present investigation were collected by the author and obtained in the form of received herbarium specimens (as gifted to guide) from the following herbaria of the world which are mentioned in Index Herbarium (Holmgren *et al*. 1981).

DK: Hortus botanicus Hauniensis, Denmark.

Z: Botanischer Garten der Universitat Zurich, Zollikerstrasse 107, CH8008 Zurich, Switzerland.

The present study includes 2 species belonging, to 2 genera under Mutisieae tribe of the family Asteraceae. The studied specimens are arranged according to tribe and genera after Kadereit and Jeffrey (2007). Under tribe, the species are alphabetically arranged with mentioning the locality and collection number of each species.

Source of Materials

Taxa investigated	Locality	Collection number
Tribe - Mutisieae		
Genus - *Leibnitzia* Cass.		
Species - *L. nepalensis* (Kunze) Kitam.	Z	XXO-BONN-8982
Genus - *Onoseris* Willd.		
Species - *O. sagittatus* Rusby	DK	405S1929 2425*AG

For investigating stable and perfect stage of each character only fully matured and intact cypselas were collected. The collected cypselas were properly air-dried and kept in desiccators for better maintenance. Few cypselas of each species were also fixed in formaldehyde:acetic acid:absolute alcohol (FAA) solution, to usual dry collection.

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."

Investigations were carried out broadly under five categories as follows:

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.

Color, shape, and 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 (carpopodium) up to apical end excluding pappus. The width of the cypselas was measured at the widest part of the cypselar body. Outline diagrams of complete cypsela 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 on 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 glycerin 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 stereomicroscope.

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, color, length, and apex organization, were also examined.

Anatomical Studies of Cypsela

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

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

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, the shape of different cells comprising the different pericarpic layers. Any other structures, for example, crystals, secretary 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 aluminum brass stubs with the help of double-stick cellophane tape. To obtain more analytical images, different angle views were taken by placing the cypsela obliquely. Along with normal surface features, such as surface cells, ornamentations, trichome, gland, and crystals, few localized observations were also made putting different parts of cypsela 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 Auto scanning Electron Microscope at Regional Sophisticated Instrumentation Centre, Bose Institute, Calcutta.

RESULTS

Leibnitzia nepalensis (Kunze) Kitam.

Cypselar morphology (DP 1A and B)

Cypsela homomorphic; 5.0-6.0 mm × 1.0-1.2 mm (excluding pappus), deep brown, oblanceolate, slightly curved, truncate at the apex, gradually tapered toward the base and apex, dorsiventrally compressed, ribbed; ribs seven to eight in number, inconspicuous. Surface

pubescent; hair covering villous, sparsely distributed on both surfaces, antrorse (bent or directed upword), non-glandular cypselar biseriate forked type; tips of the body cells of hair situated in same plane. Stylopodium absent. Carpopodium symmetric, complete, circular, ring-like, carpopodial cell outline visible and clearly distinguishable from other cells of the cypsela; carpopodial cells arranged in 2-3 rows, polygonal, vertical. Diameter of carpopodium same as the base of the cypsela. Insertion of cypsela oblique, basal. Pappus present; represented by many, persistent, multicellular, terete scabrous bristles. Bristles basally united, unbranched, 7-8 mm. long, with two, slightly unequal, pointed apical cells.

SEM survey of cypsela (SEM 1A-C)

Surface densely hairy. Surface cells visible; oval to rectangular, vertical, with straight anticlinal and periclinal wall. Pappus uni-seriate, 4-5 cells wide. Stylopodium absent. Carpopodium symmetric, complete, circular, ring-like, carpopodial cell outline visible and clearly distinguishable from other cells of the cypsela; carpopodial cells arranged in 2-3 rows, polygonal, vertical. The diameter of carpopodium same as the base of the cypsela.

Cypselar anatomy

Cypsela narrows elliptic in cross-section. Ribs present; seven to eight in number, inconspicuous. Cypselar wall 110 µ and 80 µ wide at rib and furrow region, respectively. Pericarp thin, on an average 30 µ wide, differentiated into two zones - epicarp and mesocarp.

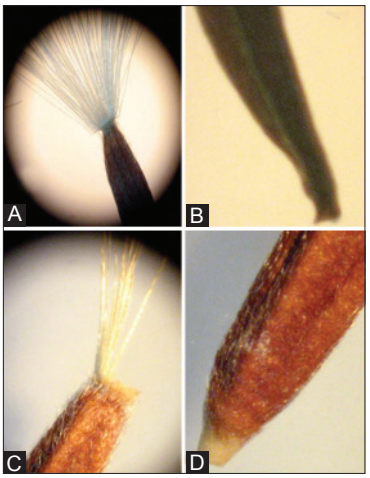
A. Epicarp - uni-seriate, made up of thin-walled rectangular to oval, compactly arranged, tangentially oriented, parenchymatous cells
B. Mesocarp consists of only sclerenchyma tissue and vascular tissue at mature state.
    1. Sclerenchyma tissue - present as discrete sclerotic braces of continuous cylinder of cells at each rib. Cells thick-walled, compactly arranged, rounded to oval, sclerenchymatous with large round lumen.

A single vascular trace present situated centrally within each sclerotic brace at each rib.

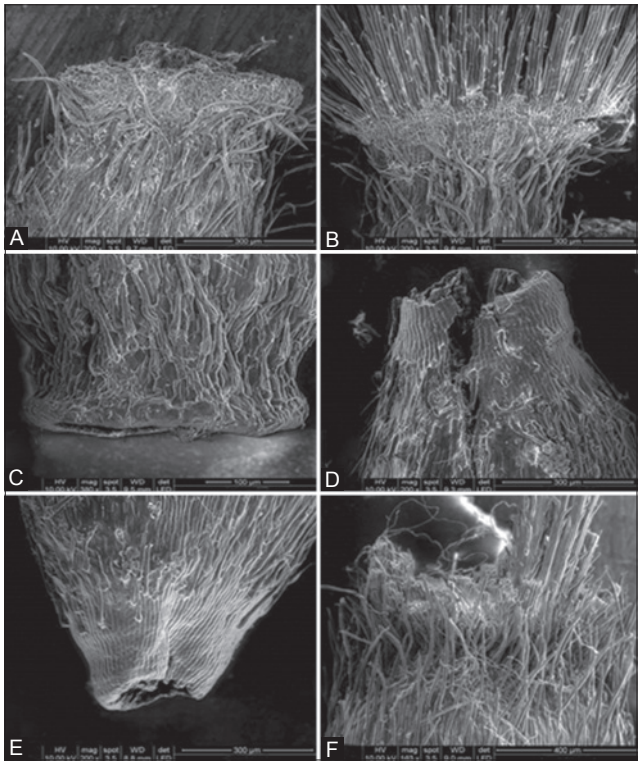
Testa/Seed coat - attached with pericarp, approximately 30-35 µ thick, differentiated into the outer and inner zone. Outer zone cellular, unlayered, organized; made up of radially elongated parenchymatous cells with the swollen radial wall. Inner zone disorganized, represented by a narrow layer of crusted parenchyma cells.

Endosperm - persists in mature cypsela, biseriate. Outer cells wide-barrel shaped and inner cells narrow-barrel shaped. Cells of both the layers thick-walled, parenchymatous, compactly arranged, tangentially oriented.

Embryo - mature embryo occupies a major part of the cypsela; cotyledons two in number, plano-convex, anterior-posteriorly oriented. Secretory duct in each cotyledon nine in number, equal.



DP No. 1: (A and B) *Leibnitzia nepalensis*; A - apical part of cypsela, B - basal part of cypsela. (C and D) *Onoseris sagittatus*; C - apical part of cypsela, D - basal part of cypsela



SEM No. 1: (A and C) *Leibnitzia nepalensis*; A-apex (after detachment of pappus), B - apex with pappus, C - base with carpopodium. D-F: *Onoseris sagittatus*; D and E - base with carpopodium, F - apex

Onoseris sagittatus Rusby

Cypselar morphology

Cypsela homomorphic; 6.0-7.0 mm × 1.0-1.5 mm (excluding pappus), brown, narrow-oblong, straight, truncate at the apex and gradually tapered toward the base and apex, faintly dorsiventrally compressed, ribbed; ribs five in number, promonent, straight. Surface pubescent; densely distributed all over the surfaces, antrorse (bent or directed upword), non-glandular achenial biseriate forked type; tips of the body cells of hair situated in more or less same plane. Stylopodium absent. Carpopodium symmetrical, complete, circular, ring-like. Carpopodial cell outline visible and clearly distinguishable from other cells of the cypsela; carpopodial cells arranged in 12-16 rows, thick-walled, square to rectangular, vertical. The diameter of carpopodium lesser than the base of the cypsela. Insertion of cypsela straight, basal. Pappus present; represented by many, persistent, multicellular, terete, scabrous bristles. Bristles basally free from one another, unbranched, 7-8 mm. long, with two, equal, pointed apical cells.

SEM survey of cypsela (SEM 1D-F)

Surface densely hairy. Surface cells visible; rectangular, tubular, dome-shaped, solid. Pappus 7-9 cells wide, without any midrib. Carpopodium symmetrical, complete, circular, ring-like. Carpopodial cell outline visible and clearly distinguishable from other cells of the cypsela; carpopodial cells arranged in 12-16 rows, thick-walled, square to rectangular, vertical. The diameter of carpopodium lesser than the base of the cypsela.

Cypselar anatomy

Cypsela pentangular in cross-section. Ribs present; five in numbers, prominent, semi-circular. Cypselar wall 260 µ and 110 µ wide at rib and furrow region, respectively. Pericarp thick, on an average 100 µ wide, differentiated into two zones - epicarp and mesocarp.

A. Epicarp - uni-seriate, made up of thin-walled rectangular to oval, compactly arranged and tangentially oriented, parenchymatous cells. Cuticle present.
B. Mesocarp consists of different types of tissues and structures as follows (from outside to inner):
    1. Parenchyma tissue - continuous, uni-seriate at furrow and five to six-seriate at rib region. Cells thick-walled, round
    2. Sclerenchyma tissue - present as discrete sclerotic braces of the continuous cylinder of cells at each rib. Cells thick-walled, compactly arranged, round, sclerenchymatous.

A single vascular trace situated within each sclerotic brace at each rib.

Testa/Seed coat - attached with pericarp, approximately 50µ thick, differentiated into the outer and inner zone. Outer zone cellular, bilayered, organized; made up of thick-walled, oval, radially oriented and compactly arranged parenchymatous cells. Inner zone disorganized, represented by a narrow layer of collapsed parenchyma.

Endosperm - persists in mature cypsela, biseriate. Outer cells transversely elongated, narrow and inner cells oval. Cells of both the layers thick-walled, parenchymatous, compactly arranged, and tangentially oriented.

Embryo - mature embryo occupies a major part of the cypsela; cotyledons two in number, plano-convex, anterior-posteriorly oriented. Secretory duct in each cotyledon seven in number of which central one larger and elongated than others.

Key to the Genera and Species

L. nepalensis

Cypsela slightly curved; ribs not prominent; hairs sparsely distributed; stylopodium ill-developed; diameter of carpopodium same as the base of the cypsela; insertion of cypsela oblique; pappus basally united; cypsela elliptic in T.S.; epicarpic cuticle absent; mesocarpic parenchyma absent; secretory ducts in each cotyledon equal in size.

O. sagittatus

Cypsela straight; ribs prominent; hairs densely distributed; stylopodium well-developed; diameter of carpopodium lesser than the base of the cypsela; insertion of cypsela straight; pappus basally free; cypsela angular in T.S.; epicarpic cuticle present; mesocarpic parenchyma present; secretory ducts in each cotyledon unequal in size.

DISCUSSION

Widely recognized placements of Mutisieae as the sister group to the remainder of the family, make their taxonomic resolution more fundamental than before. Probably, Lagasca (1811) first grouped the present day Mutisious genera in his *Chaenanthophorae*, followed by De Candolle (1812) in his Labiatiflorae. Cassini (1816-1830) proposed the tribe Mutisieae to include the genera with bilabiate corollas. Giving importance on style characteristics Cassini (1816-1830) established two related tribes: Mutisieae and Nassauvieae. The first modern systematic review of the whole tribe Mutisieae was presented by Cabrera (1977). Author defined Mutisieae by having bilabiate corollas,

caudate anthers, and characteristic style shape and divided the tribe into four sub-tribes on the basis of corolla and style features: Barnadesiinae, Gochnatiinae, Mutisiinae, and Nassauviinae. Cabrera (1977) also described the cypselas of this as "turbinate or obconic, truncate, attenuate or rostrate at the apex, glabrous, or hairy with two-armed hairs. Pappus usually of one or more series of bristles, rough or plumose, seldom formed of paleae or absent." Later, among the four sub-tribes, the Barnadesiinae was removed from the tribe Mutisieae due to the absence of chloroplast DNA inversion (Jansen and Palmer, 1987) and established as the sub-family Barnadesioideae (Bremer and Jansen, 1992). Bremer (1994) in his cladistic analysis also considered Barnadesioideae as basal, followed by Mutisieae and proposed Mutisieae as a non-monophyletic tribe. In contrast, Hansen (1991) considered Mutisieae as monophyletic on the basis of a petal epidermal pattern.

Presently, there are two views - one is the concept of the tribe Mutisieae *sensu lato*, which mainly based on morphological grounds and employed by Cabrera (1977), Bremer (1994), Jeffrey (2009), and Katinas *et al*. (2009). Other is the concept of the tribe Mutisieae *sensu stricto* (Mutisieae *s.str*.) based on molecular phylogenetic data involving DNA sequences of the chloroplast gene *ndhF* (Panero and Funk, 2008). Mutisieae *s.str*. or the Mutisieae clade contains three main clades - the *Onoseris* clade, the *Mutisia* clade, and the *Nassauvia* clade. A total number of genera and species of the tribe varied according to the different authors. Bremer (1994) included 76 genera and ca. 970 species within Mutisieae.

Present investigation deals with two species - *L. nepalensis* and *O. sagittatus*. In both the studied taxa, cypsela homomorphic, brownish, oblanceolate to narrow-oblong, slightly curved to straight in direction, truncate at apex, dorsiventrally compressed and ribbed. Truncate cypselas also observed by Mukherjee (2001) in *Dicoma* of this tribe along with attenuate in *Ainsliaea*. In *L. nepalensis* ribs 7-8 in number and inconspicuous but in *O. sagittatus* ribs 5 in number, prominent and straight. Brownish tapering cypsela with about 5-11 ribs with pilose or villose hair covering have been also noted in other members of Mutisiinae by Hansen (1988). Both the studied taxa have sparsely or densely distributed villous hair covering of twin type or forked hair. Jeffrey (2009) noted that very long, silky-villose hairs are common in tribe Mutisieae, which is in accordance with the present observation. Such fine, slender, twin or forked cypsela hairs have been noted in *Leibnitzia* by Hansen (1990) also. According to Hansen, "the achene hairs are providing by far the most important diagnostic characters."

Stylopodium is absent in both the taxa. Carpopodium in both the species symmetrical, complete, ring like but varied in diameter, height, and cell-shape. In *L. nepalensis* diameter of carpopodium same as the base of the cypsela, carpopodium made up of polygonal cells, arranged in 2-3 rows. Whereas in *O. sagittatus* diameter of carpopodium is lesser than the base of the cypsela, cells are square to rectangular, arranged in 12-16 rows. In the contrary, the absence of carpopodium has been reported by Haque and Godward (1984) in the genus *Mutisia* of the tribe Mutisieae.

Pappus represented by many, uni-seriate, persistent, scabrous bristles. In *L. nepalensis* bristles basally united but in *O. sagittatus* they are free. All the pappus bristles are devoid of midrib. Hansen (1990) noted that "A midrib many cells wide is clearly primitive." SEM analysis shows variation in pappus width which can be utilized taxonomically (Jeffrey, 2009). In *L. nepalensis* bristles are 4-5 celled wide, whereas 7-9 celled wide in *O. sagittatus*. A similar type of observation is also noted in *Gerbera* (Hansen, 1990), *Ainsliaea*, *Dicoma* (Mukherjee, 2001) of the tribe Mutisieae.

Cypselar Anatomy

Cypselar cross-section is narrow-elliptic in *L. nepalensis* but angular in *O. sagittatus* with semicircular ribs. Pericarp thickness varies from 30 to 100 µ. In both the species epicarp is uniform. The presence of cuticle is noted in *O. sagittatus*. Mesocarpic zone shows little variations. In *L. nepalensis* mesocarp made up of only sclerenchymatic tissue represented as discrete sclerotic braces, whereas in *O. sagittatus*, in addition to mesocarpic sclerotic braces a continuous uni-seriate parenchymatous layer is also present. It is made up of thick-walled cells and multi-seriate at rib regions. Vascular traces are generally associated with sclerenchymatous tissue in both the species. Same feature is noted in *Gerbera* (Mukherjee, 2001).

Testal features in cypsela are quite unique (Talukdar, 2008; 2015a; 2015b; Talukdar and Mukherjee, 2014). In both the species, testa attached to pericarp, differentiated into an outer cellular zone and an inner disorganized zone of crusted parenchymatous cells. The outer testal zone or testal epidermis in *L. nepalensis* is unilayered, made up of radially elongated parenchymatous cells with notable thickening and swelling on their radial walls. In the contrary, testal epidermis in *O. sagittatus* is bilayered, with uniformly thickened parenchymatous cells. Such type of testal cells was reported in *Gerbera* and stated these cells as prosenchymaticin *Gerbera* (Grau and Hope, 1985).

Endosperm in both the studied taxa persists in mature cypsela and biseriate. Mature embryo occupied a major part of the cypsela with two, plano-convex and parallely oriented cotyledons. In *L. nepalensis* nine, equal secretory ducts present in each cotyledon, whereas in *O. sagittatus* they are seven and unequal, central one larger, and elongated than others. So anatomically, it can be said that testal features are much more unique than pericarpic features within the tribe Mutisieae. Such view was also supported by Mukherjee (2001).

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