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SYSTEMATIC IMPORTANCE OF PERIGYNIUM MORPHOLOGY AND ACHENE EPIDERMAL SILICA BODY IN SIXTEEN SPECIES OF *CAREX* L. (CYPERACEAE) FROM THE DARJEELING AND SIKKIM HIMALAYAS, INDIA.

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ABSTRACT

The cosmopolitan genus *Carex* L. represented about 1,800-2,000 species mainly in temperate and cold regions of the world. 49 species of *Carex* found in North-eastern India and 33 species in the rest part of India. Systematics of different taxa (subgenera, sections species pairs *etc*.) of the genus is quite complicated. In *Carex*, utricle morphology and fruit epidermal silicabodies have been used to delimit species and sections. The present study was undertaken to evaluate the taxonomic utility of these characters within sixteen species of *Carex* from three subgenera and thirteen sections. The following sixteen species (*viz. Carex baccans* Nees, *C. myosurus* Nees, *C. myosurus* subsp. *spiculata* Boott, *C. composita* Boott, *C. cruciata* Wahlenberg, *C. setigera* D. Don, *C. breviculmis* R. Brown, *C. speciosa* Kunth., *C. insignis* Boott, *C. polycephala* Boott**,** *C. fusiformis* Nees subsp. *finitima* (Boott) Noltie, *C. alopecuroides* D. Don ex Tilloch and Taylor, *C. teres* Boott, *C. longipes* D. Don ex Tilloch and Taylor, *C. nubigena* D. Don ex Tilloch and Taylor and *C. remota* L. subsp. *rochebrunii* (Franchet and Savatier) Kükenthal) are evaluated in the present study. Perigynia of all the studied species are more or less similar in their surface morphology. Surface appears smooth when examined with a dissecting microscope. However, the epidermal cells have a distinctive morphology when viewed under higher magnification; differ significantly in micromorphology (SEM-analysis). Perigynia have epidermal cells with thin convex/concave outer wall that are collapsed in dried specimen. Variation in size, shape, color and number of nerve/costa and texure of utricle are noticeable. Costal cells are narrower and more elongated, but also have thin, collapsed cell wall. Several species have conspicuously nerved, stipitate perigynia others were not. Epidermal cells of achene were polygonal to rhomboidal with little variation in size and shape. Epidermal cells of fifteen out of sixteen species (except *Carex nubigena* D. Don ex Tilloch and Taylor) each have a single, rounded or nodulose silica body with a basal platform. Although many unique characters were lacking, but interspecific variation in silica bodies was consistent with the high level of morphological variation of utricle and previously detected reproductive variation. The inclusion of micromorphological characters substantially enhances the resolving power of macromorphologic characters in taxon analysis. These data indicated that utricle morphology and micromorphological achene epidermal features (SEM features) are useful in identification of thirteen sections and sixteen species of *Carex*.

KEYWORDS: ***Carex, Utricle, Achene, Micromorphology, Systematics***

INTRODUCTION

The sedge family (Cyperaceae) numbers *ca*. 5000 species under 104 genera worldwide, making it the 3rd largest family of monocots (Goetghebeur P. 1998). *Carex* L. is the genus of Cyperaceae with the largest number of species, *ca*. 2000 according to Goetghebeur (1998). Although its main centers of diversity are in North America and East Africa (Starr J R et al. 1999), it is well represented in South East Asia and India, especially in high lands. *Carex* is also one of the most important genera in the Cyperaceae. It includes *ca*. 1800 species

extending throughout the world, while most occurring in Northern temperate zones (Jermy and Tutin, 1968). Different species are found in wide range of habitats (Schutz, 2000). Most of the species are able to spread laterally by rhizomatous growth and as a result, large clonal patches / tufts may be formed (Bouzille, 1992). Though Bernard (1990) estimated >2000 species worldwide. The genus *Carex* under tribe *Cariceae* comprises roughly 40% of the family by species, making it one of the largest genera of angiosperms (Reznicek, 1990; Mabberley, 1997). *Carex* species are economically important members of flood plain forests, dry prairies, alpine meadows, peat lands, swamp forests, sedge meadows and a wide range of other communities (Reznicek, 1990). The genus having diverse bio-geographical patches (Gondwanaland, Arcto-Tertiary, Bipolar; Nelmes, 1949; Ball, 1990). It is also remarkable within Cyperaceae because it is easy to recognize; all species of *Cariceae* are distinguished by the presence of consistently unisexual flower where a naked gynoecium is surrounded by a flask-like prophyll known as a perigynium or utricles (Blaser, 1944; Gehrke *et al.*, 2012).In *Carex* L., the largest genus (ca. 2000 species), micromorphological characters were successfully used to circumscribe species (Toivonen and Timonen, 1976; Wujek and Menapace, 1986; Ford and Ball, 1993) and even sections (Walter, 1975; Menapace and Wujek, 1987; Toivonen and Timonen, 1976; Menapace *et al*., 1986; Standley, 1987; Crins and Ball, 1988; Starr and Ford, 2001). However, it soon became apparent that many groups circumscribed on the basis of anatomical and (or) silica body characters conflicted significantly with taxa delimited on the basis of morphology and other features (Shepherd, 1976; Standley, 1987, 1990; Waterway, 1990; Ford *et al*., 1991; Ford and Ball, 1993). This led some authors to conclude that homoplasy in anatomical and silica body characters was high (Rettig, 1986; Standley, 1990; Waterway, 1990) and consequently, that they were unreliable indicators of evolutionary relationship (Rettig, 1986; Waterway, 1990; Ford and Ball, 1993). It is important to note, however, that these conclusions were drawn from groups that had not been phylogenetically analyzed. When anatomical and silica body characters were eventually used with morphology in the cladistic analysis of *Carex* section *Griseae* (18 species; Naczi, 1992), they provided characters that were essential to the definition of several small clades. Naczi (2009) stated that for phylogenetic analysis in sedges valuable sources of data were from the anatomy, and perigynia, as well as the micromorphology of achene/nut epidermal cells and were so vital to constracting phylogenetic trees. Ford *et al*. (2008) however stated that anatomic, micromorphologic, and phytogeographic characters shows a number of discontinuous supporting the recognition of these taxa at the species level. The perigynia surface appears smooth when examined with a dissecting microscope. However, the epidermal cells have a distinctive morphology when viewed under higher magnification. Consequently, the question is raised as to whether anatomical and silica body characters were inherently unreliable or whether previous negative conclusions regarding their phylogenetic utility in *Carex* can be explained by the factors such as personal bias, poor circumscription, or limited sampling (Starr and Ford, 2001). In India, study on morphology, micromorphology (SEM characters) on the family Cyperaceae in general and the genus *Carex* in particular is very scanty and discrete. Recently, Ghosh, A. (2013), Ghosh A. and Maiti (2014) and Ghosh et al. (2014) have studied different aspect of morphology, micromorphology and mychorrhizal status of the genus *Carex* from North-eastern India. Yarrayya K et al. (2015) have very recently published a paper on taxonomic significance of nutlet morphology in 15 species of Abildgaardieae Cyperaceae) from Tamil Nadu and provided a number of characters such as shape, size, colour and surface ornamentation to distinguish the species coming under the genera *Fimbristylis* and *Bulbostylis.* But systematic importance of Morphology, micromorphology (SEM) characters of the genus *Carex* still remain unresolved. This paper presents comparative study micromorphology of achene epidermal silica bodies in sixteen species of *Carex*. The purpose of this study was, first, to evaluate the taxonomic and potential of silica body characters within the genus *Carex* in general, and secondly, to determine whether silica bodies could help /supports or refutes the previous works like Starr and Ford (2001).

MATERIALS AND METHODS

Plant materials for this investigation were wholy obtained from *in situ* population (detail of the collection information is given in Table 1) Mature perigynia were removed from the upper part, middle part and upper part of the spikes/ inflorescences from 3-5 specimens' representatives of geographical range of each species. Perigynia, surrounding the achenes of half of the samples (fruits) was dissected away. For perigynia of members of *Carex*, we excised the bases of fruits,

removed the achenes, splitted the perigynia in half lengthwise at the margins for lenticular fruits (*Carex teres* and *C. longipes* of the subgenus Carex and *C. nubigena*, and *C. remota* of the subgenus Vignea) and along the angle for trigonous fruits (*Carex baccans, C. myosurus, C. spiculata, C. composita* and *C. cruciata* of the subgenus Vigneastra and *Carex setigera, C. breviculmis, C. speciosa, C. insignis, C. polycephala, C. finitima* and *C. alopecuroides* of the Subgenus Carex), slit the remaining two angles to allow the perigynia to lie flat. Both fruits and achenes were then air dried first and were then dried over night at 50^{o}c, mounted on to aluminum stabs with conductive carbon points (both side gummed cello tape) and then coated with 100-200nm of a gold/ palladium in a Edward Sputter Coater. Micrographs of whole utricle, base and apex and naked nutlets (whole, base and apex) were taken using Hitachi S-530 Scanning electron microscope (in 15 KV accelerating voltage). This study was conducted and photomicrographs were taken at different magnification at USIC, University of Burdwan, Golabag, Burdwan, W.B., India. The terminologies were described according to the explanation of Schuyler (1971), Strong (2006), Menapace and Wujek (1987), Starr and Ford (2001), Smith and Ashton (2006) and Ford *et al*. (2008).

Table 1
Collection information of the studied species and their systematic position (arranged after Dai et al., 2010)

Subgenus	sections	Species
1. Carex subg. Vigneastra (Tuckerman) Kükenthal	1. Carex sect. Polystachyae Tuckerman	1.Carex baccans Nees
		2.Carex myosurus Nees
		3. Carex myosurus subsp. spiculata Boott
		4.Carex composita Boott
	2. Carex sect. Indicae Tuckerman	5.Carex cruciata var. argocarpus Wahlenberg
2. Carex subg. Carex	3. Carex sect. Hirtae Tuckerman ex Kükenthal	6. Carex setigera D. Don
	4. Carex sect.Mitratae Kükenthal	7. Carex breviculmis R. Brown
	5. Carex sect. Radicales (Kükenthal) Nelmes	8. Carex speciosa Kunth
	6. Carex sect. Decorae (Kükenthal) Ohwi	9. Carex insignis Boott
	7. Carex sect. Hymenochlaenae (Drejer) L. H. Bailey	10. Carex polycephala Boott
	8. Carex sect. Debiles (J. Carey) Ohwi	11. Carex finitima var. finitima *Carex fusiformis* Nees subsp. *finitima* (Boott) Noltie
	9. Carex sect. Molliculae Ohwi	12. Carex alopecuroides D. Don ex Tilloch and Taylor *Carex japonica* Thunberg var. *alopecuroides* (D. Don exTilloch and Taylor) C. B. Clarke.
	10. Carex sect. Forficulae (Franchet ex Kükenthal) Raymond,	13. *Carex teres* Boott
	11. Carex sect. Graciles Kükenthal	14. Carex longipes D. Don ex Tilloch and Taylor
3. Carex subg. Vignea (P. Beauvois ex T. Lestiboudois) Petermann	12. Carex sect. Phleoideae Meinshausen	15. Carex nubigena D. Don ex Tilloch and Taylor
	13. Carex sect. Remotae C. B. Clarke	16. Carex rochebrunii subsp. rochebrunii *Carex remota* Linnaeus subsp. *rochebrunii* (Franchet andSavatier) Kükenthal

RESULTS AND DISCUSSION

Along with other morphological characters, perigynia morphological characters in the identification of different taxa of Cyperaceae were used by Kukenthal (1909); Kreczetovicz (1936); Nelmes (1951, 1952); Savile and Calder (1953); Faulkner (1972); Standley (1985, 1987); Reznicek (1990); Egorova (1999); Starr and Ford (2009). But most of the previous study was based on Herbarium specimens and non Indian specimens. The morphological characteristics of utricles and SEM characters of utricle and achene of sixteen species of *Carex* are represented in Table 2.Perigynia intercostal and costal cell differentiations are found in *Carex myosurus, C. spiculata, C. cruciata, C. insignis, C. alopecuroides, C. longipes* and *C. remota* and very typically in *C. nubigena*. Intercostal cells of distal and proximal end are rectangular or polygonal. Costal cells are narrower and more vertically elongated (*Carex nubigena*, Fig. 4., oii). Many studies have shown that silica body characters can be used to circumscribe species and sections in *Carex* (Walter, 1975; Toivonen and Timonen, 1976; Menapace *et al*., 1986; Menapace and Wujek, 1987) and species (Wujek and Menapace, 1986), others have found no distinguishing sectional characters (Waterway, 1990; Naczi, 1992; Starr and Ford, 2001; and this study), a lack of differences among seemingly distant species (Waterway, 1990) and marked intraspecific variation (Rettig, 1990; Salo *et al*., 1994). Several studies have now noted similarities in silica bodies between distantly related sections in *Carex* and even between *Carex* and other Cyperaceous genera (Rettig, 1986; Waterway, 1990; Salo *et al*., 1994). All members (except *Carex nubigena*, Fig. 4, oii) of the genus *Carex* possess achenes with an epidermal layer exhibited single primary silica body in each cell. Details of SEM studies on utricle and achene micromorphology (Fig. 1-4) are provided in Table 2. In addition the silica platforms are found to be either convex or concave without any peripheral satellite bodies. Table 2 is comprehensive listing of all the characters used in identification of species. The silicon foundation is referred to as a platform (Schuyler, 1971) and may and may not possess a number of peripheral protrusions known collectively as silicon bodies. Silica bodies in different sections of the genus *Carex* are very similar in morphology and not unlike those seen in other Cyperaceous genera. Members possessed a single, acute, conical central body that arose from the middle of either a convex or concave silica platform. Achene epidermal silica bodies that possess convex versus concave platforms and persistent outer periclinal walls are also diagnostic (Starr and Ford, 2001). The central body is also variable. The most notable differences among species are seen in the shape and relief of the silica platform. Based on these characteristic different sections can be divided into two principal groups distinguished by platform margins that are either concave (*Carex baccans, C. myosurus, C. composita, C. cruciata, C.setigera, C. breviculmis, C. speciosa, C. insignis, C. polycephala*, and *Carex remota*; Table 2; Fig.1-4) and others are with convex silica platform. Some of the species were recognized by distinctly raised silica platforms (*Carex baccans, C. spiculata, C. composita, C. cruciata, C. setigera, C. breviculmis, C. speciosa, c. insignis, C. polycephala, C. finitima, C. teres* and *C. longipes*) and other species are with slightly raised silica platform, Small conical central body (*Carex baccans, C. spiculata, C. composita, C. cruciata, C. insignis, C. polycephala,* and *C. fusiformis,* whereas, beaded central body found in *Carex teres, C. longipes* and *C. remota.* The ornamented silica platforms were found in *Carex baccans, C. Cruciata, C. setigera, C. breviculmis, C. speciosa, C. insignis,* and *C. finitima*. Combination of utricle morphology and SEM-micromorphological characters easily identified two related species i.e. *Carex myosurus* and *C. spiculata*. There is a serious debate regarding the systematic position of *Carex spiculata.* Sometime the species is considered as a subspecies of the species *Carex myosurus*, sometime considered as an individual species. Most of the previous treatments were on the basis vegetative and reproductive morphology. If we consider micromorphological characters, *Carex spiculata* can easily be identified by deeply raised silica body, conical central body of the silica and little costal and intercostal cell differentiation. But the second species (*Carex myosurus*) was with slightly raised silica body with distinct intercostal- costal cell differentiation in utricle epidermis (Fig.1; bi-biii and ci-ciii). On the other hand inclusion of *Carex composita* in the section Polystachyae of the Subgenus Vigneastra is justified on the basis of SEM-micromorphological characteristics like silica body, central body of the silica, basal platform, epidermal cell morphology of the achene, although the species vary significantly in vegetative anatomical characters. Two species *Carex setigera* and *C. breviculmis* of the sections Hirtae and Mitratae of the subgenus Carex of the genus *Carex* are very similar in vegetative and reproductive

morphology (earlier one is just robust form of the second one), but the two species are easily identifeable with the help of silica characters. The former one is with medianly raised silica boby with typical basal platform and later is with deeply raised, sharply pointed central body of the silica (Fig. 2, fiv and giv). Systematic position of *Carex polycephala* is also problematic and even left as untreated one (taxa *incertae sedis*) (Dai *et al*., 2010), but if we combine vegetative and reproductive morphology, anatomical characters along with SEM-micromorphological characters (like utricle epidermal characters, achene epidemal silibody characters), it can easily be treated near the section Decorae of the subgenus Carex. Sometimes artificial grouping may arise if we consider SEM-micromorphological characters alone. In our study, the grouping or placement of the species *Carex teres* of section Forficulae and *Carex longipes* of the section Graciles of the subgenus Carex and two other species (*C. nubigena* of section Phleoideae and *C. remota* of the section Remotae of the subgenus Vignaea) are very difficult only on the basis of SEM micromorphological characters. All of these four species of *Carex* are with biconvex (lenticular) achene morphology (Fig. 3, mi and 4, nii, oiii and pii) but achene epidermis of the species *Carex nubigena* devoid of any silica body (Fig. 3, oiii and oiv) supports the creation of separate section within subgenus Vigneae. On the other hand former two species of subgenus Carex are with more or less same type silica body, basal body and cells of achene epidermis (Fig. 4, miv and niii), again supports the retention of these species within the subgenus Carex. The present study indicate that utricle morphology and micromorphological achene epidermal features (SEM features) are useful in identification of different sections and species of *Carex*.

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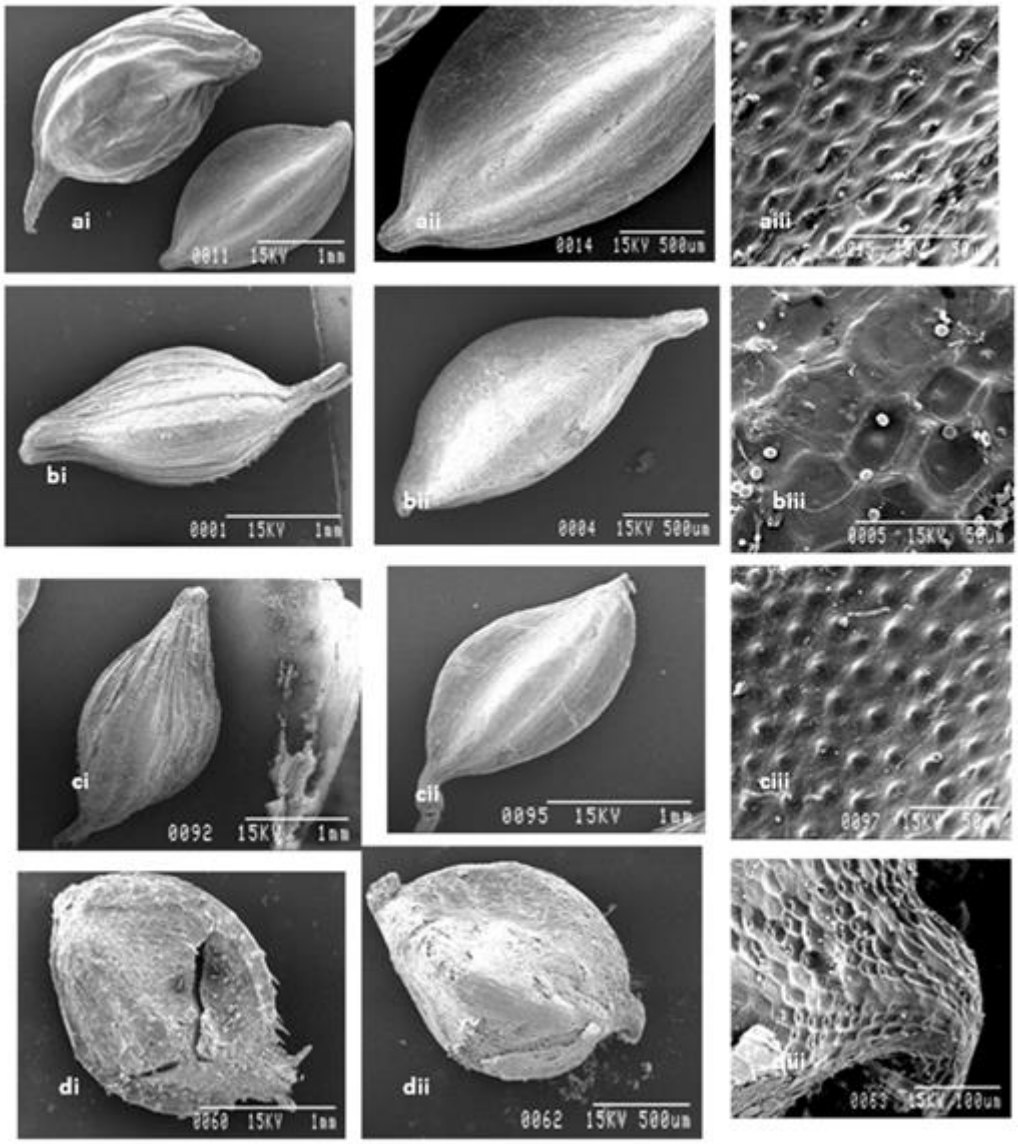

Figure 1

Scanning electron microphotographs(SEM) of Carex spp. ai-aiii. C. baccans; ai. Utricle & nut; aii. Nut surface. aiii. Nut surface(magnified) bi-biii. C.myosurus; bi. Utricle ; bii. Nut surface; biii. Nut surface (magnifies) ci-ciii. C. spiculata; ci. Utricle; cii. Nut surface; ciii. Nut surface (magnified)di-diii. C. composita; di. Utricle; dii. Nut; diii. Nut surface(magnified).

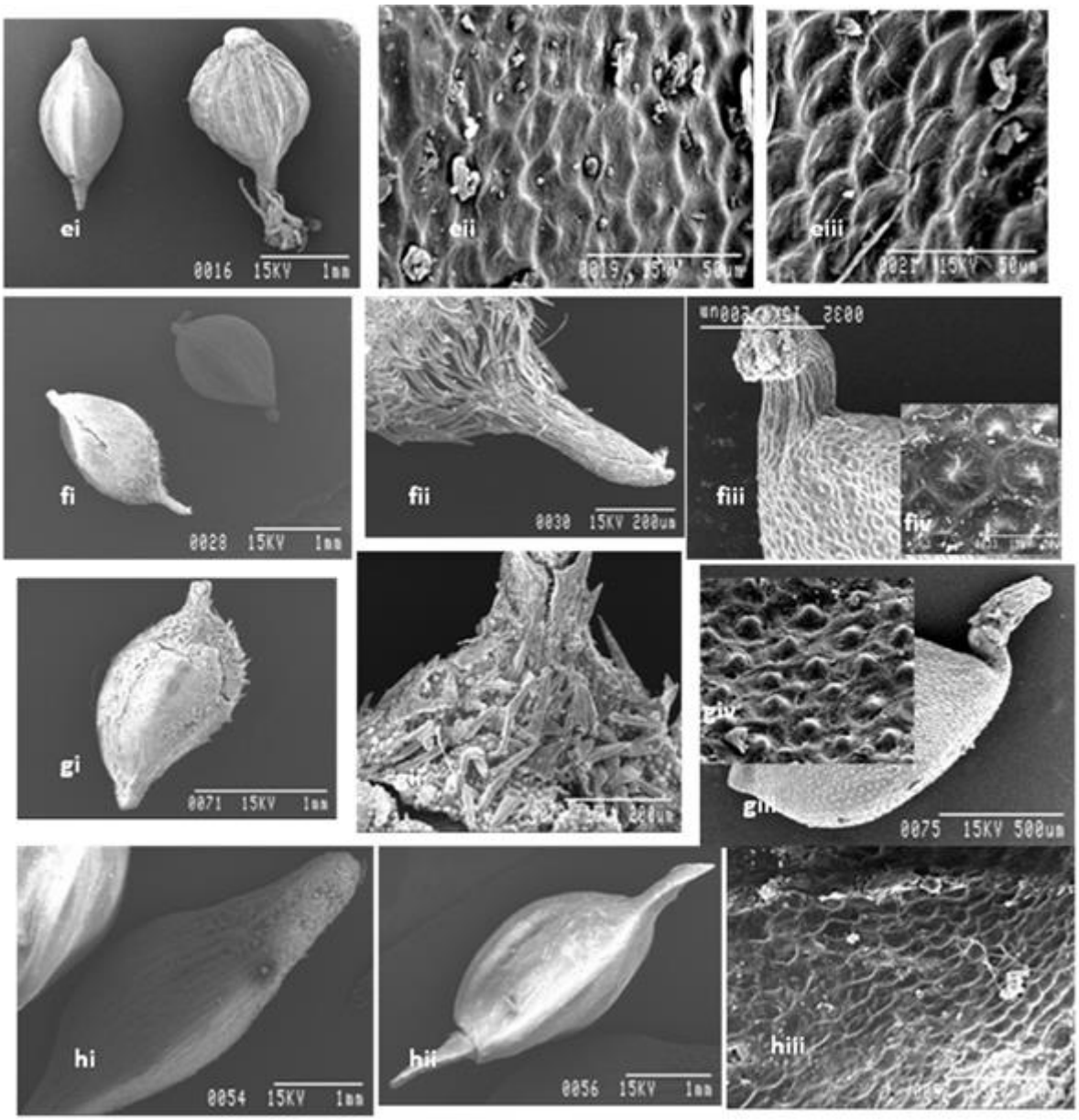

Figure 2

Scanning electron microphotographs(SEM) of Carex spp. ei-eiii. C. cruciata; ei. Utricle & nut; eii. Nut surface magnified(eii-middle & eiii- top of nut) fi-fiv. C. setigera; fi. Utricle & nut;fii. Top of utricle; fiii. Nut surface; fiv. Nut surface magnified. gi-giv. C. breviculmis; gi. Utricle; gii. Top of utricle ; giii. Nut surface; giv. Nut surface magnified. hi-hiii. C. speciosa; hi. Nut & a part of utricle; hii. Nut surface; hiii. Nut surface magnified.

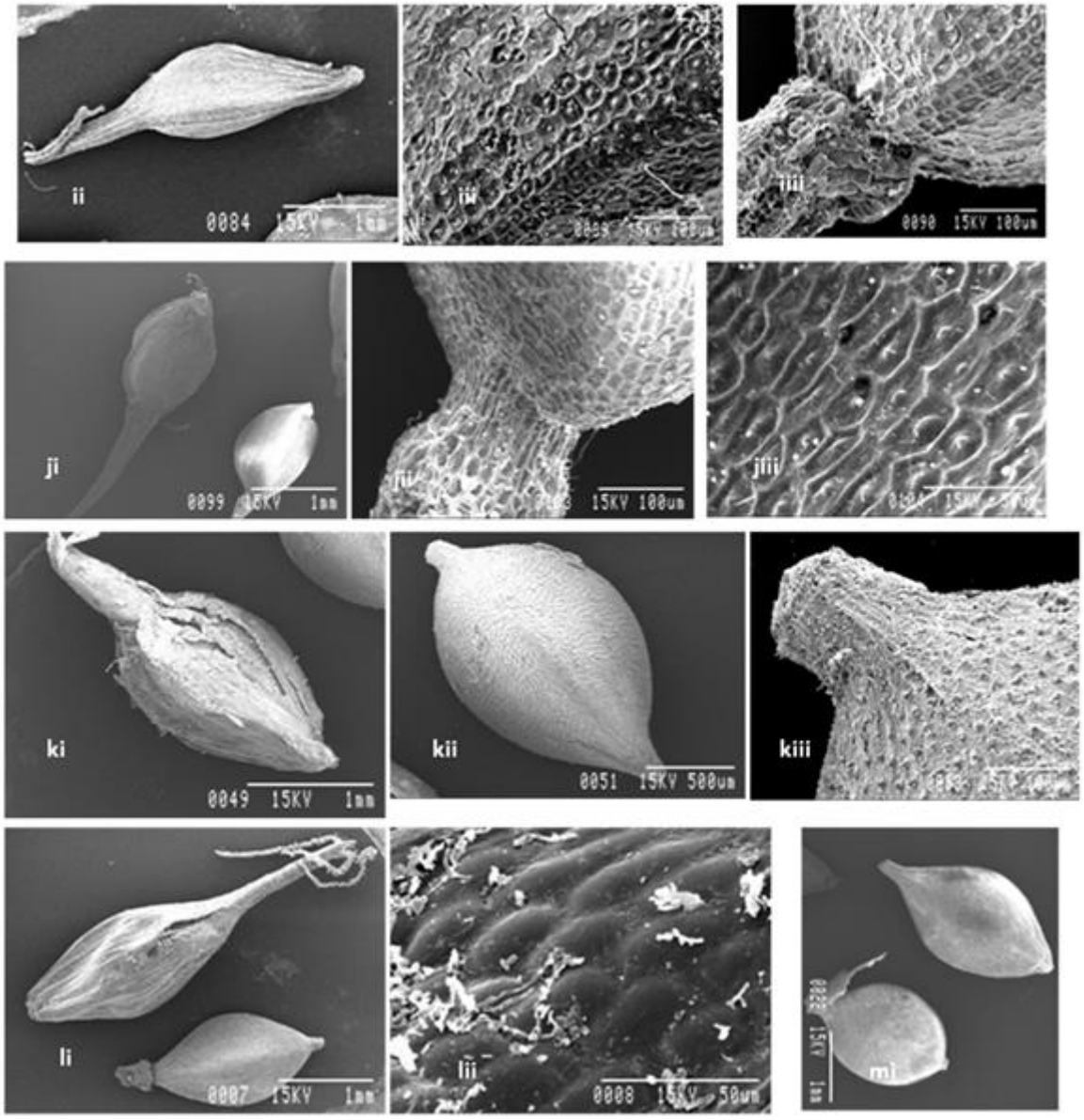

Figure 3

Scanning electron microphotographs(SEM) of Carex spp.ii-iiii. C. insign is, ii. Utricle; iii-iii. Nut surface(Middle & Top respectively).ji-jiii. C. polycephala; ji. Utricle; jii. Top of nut; jiii. Surface of nut(magnified). Ki-kiii. C. fusiformis, ki. Ruptered utricle; kii. Nut surface; Base of nut slightly magnified. li-lii. C. alopecuroides; li. Utricle and nut; lii. Nut surface magnified. mi. C. teres; mi. C. teres. Utricle & nut.

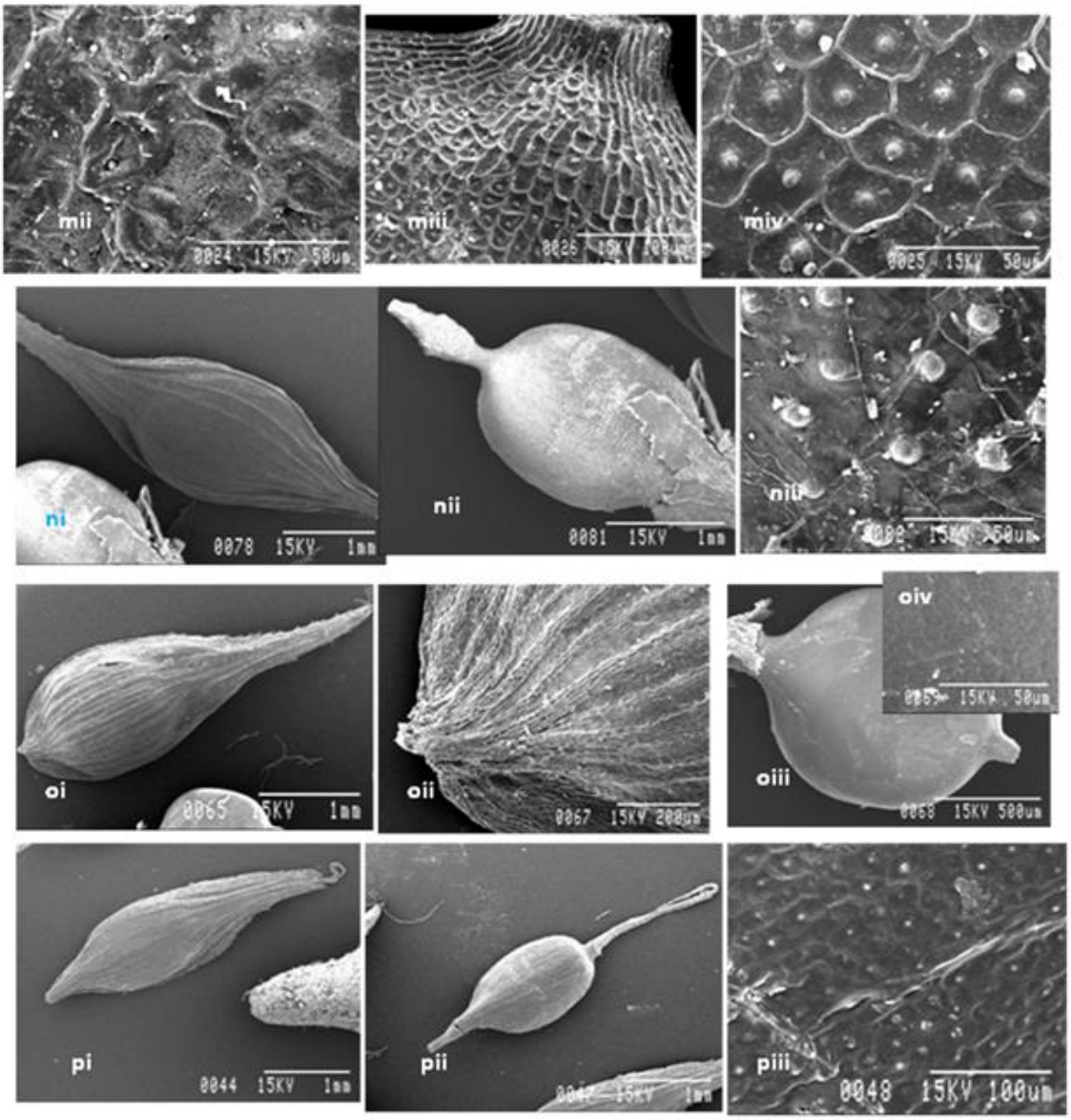

Figure 4

Scanning electron microphotographs(SEM) of Carex spp. Mii-miii. C. teres; mii. Utricle surface at middle; miii. Nut surface at the base; miv. Nut surface at the middle (magnified). ni-niii. C. longipes; ni. Utricle; nii nut surface; niii. Nut surface at the middle(magnified). oi-oiv. C. nubigena; oi. Utricle; oii. Base of utricle with multiple costae; oiii. Nut surface; oiv. Nut surface (magnified). pi-piii. C. remota; pi. Utricle; pii. Nut; piii. Nut surface at the middle(magnified).

Table 2

Characters description of utricle and achene microcharacters of the studied spp. of Carex L.

	Perigynia							Achene/nut/nutlet epidermal silica bodies				
Species	**Luster At maturity**	**Shape in cross section**	**Tightness of investiture of schene**	**Length**	**Texture**	**Beak**	**Number and position of nerves**	**Epidermis(Costal and intercostal cells)**	**Silica platform**	**Platform. Margins**	**Platform, distinctly raised (D) vs. raised(R).**	**platform**	**Satellite body**
1. *Carex baccans*	Glisering red	Triangular with rounded margins	Tightness ratio low	Long	Smooth, membranous	Median Slightly bidentate	No distinct nerves found	Not differentiated	Concave	Tough	Distinctly raised	Ornamented	-
2. *Carex myosurus*	Glossy brown	Triangular	Tightness ratio high	Long	Tough, slightly hispidulous at apex	Long bidentate	5-7 at base	differentiated	Concave	Not thickened	Raised	Not ornamented	-
3. *Carex myosurus* subsp. *spiculata*	Pale brown	Triangular	Tightness ratio high	Long	Tough, slightly hispidulous at apex	Long bidentate	7-12	differentiated	convex	Not thickened	Distinctly raised	Not ornamented	Absent
4. *Carex composita*	Reddish brown	Triangular with lobed margins	Tightness ratio high	Comparatively Short	Membranous, upper half hispid	Very short, aperture not clear	No distinct nerves	Not differentiated	Concave	Not thickened	Distinctly raised	Not ornamented	-
5. *Carex cruciata* var. *argocarpus*	Glisering white	Triangular	Tightness ratio high	Medium	Smooth with ridge and forrow	Long, bidentate	More than 15 nerves	differentiated	Concave	Tough	Distinctly raised	Ornamented	-

6. *Carex setigera*	Light brown	Triangular	Tightness ratio high	Short	Upper 2/3 hugely hairy	Median, bidentate	No distinct nerves	Not differentiated	Concave	Tough	Distinctly raised	Ornamented	Absent
7. *Carex breviculmis*	Light brown	Triangular	Tightness ratio high	Short	Upper 2/3 moderately hairy	Short, oblique-bidentate	No distinct nerves	Not differentiated	Concave	Not thickened	Distinctly raised	Ornamented	-
8. *Carex speciosa*	Light brown	Triangular	Tightness ratio high	Very Very Long	Tough	Long, bidentate	No distinct nerves	differentiated	Concave	-	Distinctly raised	Ornamented	-
9. *Carex insignis*	Pale-olive brown	Triangular	Tightness ratio low	Long	Tough, membranous	Long, bidentate	3-5	Differentiated	Concave	Not thickened	Distinctly raised	Ornamented	-
10. *Carex polycephala*	Creemish	Triangular	Tightness ratio low	Very Long	Smooth membranous	Very very long, apex bidentate	No distinct nerves	Not differentiated	Concave	Not thickened	Distinctly raised	Not ornamented	Present
11. *Carex finitima* var. *finitima*	Light brown	Triangular	Tightness ratio high	Long	Tough membranous	Long, bidentate	Not clear	Differentiated	Convex	-	Distinctly raised	Ornamented	-
12. *Carex alopecuroides*	Light brown	Triangular	Tightness ratio high	Medium	Membranous smooth	Very very long, bidentate	3-5	Not differentiated	Convex	Not thickened	Raised	Not ornamented	-
13. *Carex teres*	Olive greenish	Triangular	Tightness ratio high	Medium	Membranous smooth	Very short, round-oblique apertur	No distinct nerves	Not differentiated	Convex	Not thickened	Distinctly raised	Not ornamented	Present

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14. *Carex longipes*	Light brown	Lenticular	Tightness ratio low	Long	Membranous, smooth,beak margins often hispid	Very long, distinctly bidentate	9-12	Differentiated	Convex	Not thickened	Distinctly raised	Not ornamented	-
15. *Carex nubigena*	Light brown	Lenticular	Tightness ratio high	Medium	Ridge and furrowed, membranous, upper half hispid.	Very long, bidentate	15-25	Differentiated	No silica	Not thickened	-	-	-
16. *Carex rochebruni i* subsp. *rochebruni i*	Golden brown	Lenticular	Tightness ratio low	Medium	Tough, upper half winged hispid	Very long, bidentate	3-7	Differentiated	Concave	Not thickened	Raised	Not ornamented	-

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