SURFACE MICROSTRUCTURE OF LEAF OF *CAPPARIS CARTILAGINEA* DECAISNE (FAMILY CAPPARACEAE)

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ABSTRACT

Surface microstructure of *Capparis cartilaginea* Decaisne leaves collected from coastal hills of Paradise Point, Karachi (Pakistan) was investigated. The leaves had unicellular strap-like trichomes of around 300 µm. The leaves were amphistomatic and as per Prabhakar's (2004) scheme of stomatal classification, there were four types of stomata identified in hexane treated leaves – anisocytic (70.79%), tetracytic (20.65%), anomocytic (7.74%) and staurocytic (0.65%). The density of stomata was relatively higher on dorsal surface (80.60 ± 1.45 per mm2) than on the ventral surface (61.9 ± 1.41 per mm^2). Cuticular striation, in mature leaves untreated with hexane, was in form of long parallel ridges radiating from the peristomatal rim and running over periclinal surface of the subsidiaries and continuing over adjacent epidermal cells. This Cuticular feature was strikingly different from the pattern reported for intraspecific taxa of *Capparis spinosa* described by S. Fici (2004), particularly the taxon viz. *C. spinosa* var. *galeata* Hook. F. & Thompson, an African collection, which has been considered synonymous to *C. cartilaginea* Decaisne by Jafri (1973).

Key words: *Capparis cartilaginea* Decaisne, Foliar surface microstructure, trichomes, stomata

INTRODUCTION

The genus *Capparis* includes about 250 species (Fici and Gianguzzi, 1997). Six species of *Capparis* are reported from Pakistan (Jafri, 1973) of which 3 species belong to *C. spinosa* complex [e.g., *C. spinosa* L., *C. himalayensis* Jafri (syn. C. leucophylla Collett; C. spinosa var. himalayensis (Jafri) Jacobs) *and C. cartilaginea* Decaisne. (Syn. *C. galeata Fresen*. *C. uncinata* Edgew. and *C. spinosa* var. *galeata (*Fresen) Hook. F. & Thoms. in Hook. F.)]. Two of the six species, *C. zeylanica* L. and *C. sapiaria* L., are not seen in wild and perhaps their specimens were collected as cultivated plants. The remaining one species, *C. decidua* (Forssk.) Edgew. (Syn. *Sodaba decidua* Forssk., *C. aphylla* Roth and *C. sodaba* R.Br. in Denh.) grow wild widely in Sindh and Baluchistan provinces of Pakistan.

Capparis cartilaginea Decaisne is a scrambling shrubby plant of coastal or sub-coastal to inland rocky habitats of Pakistan (Jafri, 1973; Jilani *et al.,* 2014) growing by sending their roots in rock crevices. It is distributed in several countries – North and tropical East Africa, Arabia, Israel, Iraq, South Iran and Pakistan (Jafri, 1973). The plants of genus *Capparis* L. are medicinal and source of nutrients (Misra *et al.,* 2007; Upadhyay, 2011; Lansky *et al.,* 2014). Lansky et al. (2014) have provided detailed pharmacognostic and ethanopharmacological exploitation of the genus *Capparis*.

C. cartilaginea Decaisne, *C. galeata* Fresen. and *C. spinosa* L. subsp. *cartilaginea* have treated as synonymous species for *C. sinaica* Veill. When Kamel *et al.* (2009) revisited the Family Capparaceae from Egypt. They reported the distribution range of *C. sinaica* to be East and South-East Africa and tropical Africa (Sudan, Ethiopia and Somalia), South Iran to Pakistan, South-West Asia to India. Moreover, *C. sinaica* Veill. has been considered as synonymous to *Capparis aegyptica* Lam.

In a study of nomenclature and typification in the genus *Capparis*, Dr. Diego Rivera and his colleagues (2003) discovered that proper name for the Israel's caper (*C. cartilaginea* Decne.) is *Capparis inermis* Forssk. However, since the name *C. cartilaginea* Decne was in wide use in floras of the area for a long time, the request to save its name was granted by the International Committee (Prof. Avinoam Danim – Flora of Israel on line).

Khan *et al*. (2015) have described the leaf architecture of *C. cartilaginea* from Paradise point, coastal hilly area of Karachi, Pakistan. Fici (2004) has described micromorphology of *Capparis* L. sect. *Capparis*, the section represented by a single species, *C. spinosa* L. divided into several intraspecific taxa. One of those taxa is *C. spinosa* L. var. *galeata* Hook. F. & Thomson distributed in Eastern Africa, South Western Asia to India. According to Fici (2004), the leaves of *C. spinosa* L. var. *galeata*, are hairy (567 um long), denser in young leaves, cuticular striations densely with crest and buttresses reticulate, stomata deeply sunken with peristomatal rim and sometimes obscured due to thick cuticularization. Stomata are reported by Fici (2004) to be of anomocytic type throughout the six investigated intraspecific taxa of *C. spinosa* L. Earlier publications (Metcalfe and Chalk (1950; Wilkinson; 1979;

Bokhari and Hedge, 1975; Singh *et al.,* 1987) have also reported anomocytic stomata in Capparaceae (previously Capparidaceae).

This paper attempts to investigate surface micro-structure in *C. cartilaginea* (syn. *C. spinosa* L. var. *galeata* Hook. F. & Thomson) collected from a coastal locality of Paradise point, Karachi and compares it with that of *C. spinosa* L. var. *galeata* Hook. F. & Thomson studied by Fici (2004) from African collection. Such studies are pertinent in view of their significant taxonomic value in characterizing several congeneric species (Haron *et al.,* 2015).

Fig.1. Habit of *C. cartilaginea* growing in crevice of a rock at Paradise point, Karachi. Its leaves are nearly round, thick, brittle and sun-reflecting. The leaf is provided with a spiny hook (directed inwardly) near apex on the ventral side. The leaf is somewhat notched at the point the petiole attaches on the ventral side. Photo March, 2012.

MATERIALS AND METHODS

The leaves of *Capparis cartilaginea* were collected from its plant growing in a rock fissure in coastal vicinity of Paradise point, Karachi in April, 2012 (Fig. 1). The surface ornamentation of the leaf was studied by preparing epidermal imprints (Wang *et al.,* 2006) of dorsal and ventral surfaces of leaf with and without hexane treatment (immersion of leaves in hexane for 10 days). Imprints were obtained by using transparent nail polish of fine quality. Hexane treatment was employed to remove wax and clearing of the leaf surface. Stomata were described following Prabhakar (2004). The leaf surface was also studied by Scanning Electron Micrography (SEM) of unimbibed (air-dried) leaves mounted on brass discs and coated with 300nm gold layer on to them by sputter coater. SEMs were made at 15 kV with JEOL JSM – 6380LV Scanning Electron Microscope at various magnifications. The images were saved digitally on computer. The statistical analysis of the data was performed following Zar (2010).

RESULTS AND DISCUSSION

The leaves of this species, as described by Khan *et al.* (2015), are simple, cartilaginous, succulent, entire, smooth broadly ovate to circular, shiny green, alternate (one leaf at a node), petiole bent, petiolar attachment peltate eccentric, stipulate (two reddish brown stipules which are spinous, woody retrose), mucro curved yellowish brown and hook like inserted below the apex on the ventral side, lamina embayed in sinus in umbo, leaf base extension near zero to 6.5 mm, lamina basally cordate and venation brachidodromous type.

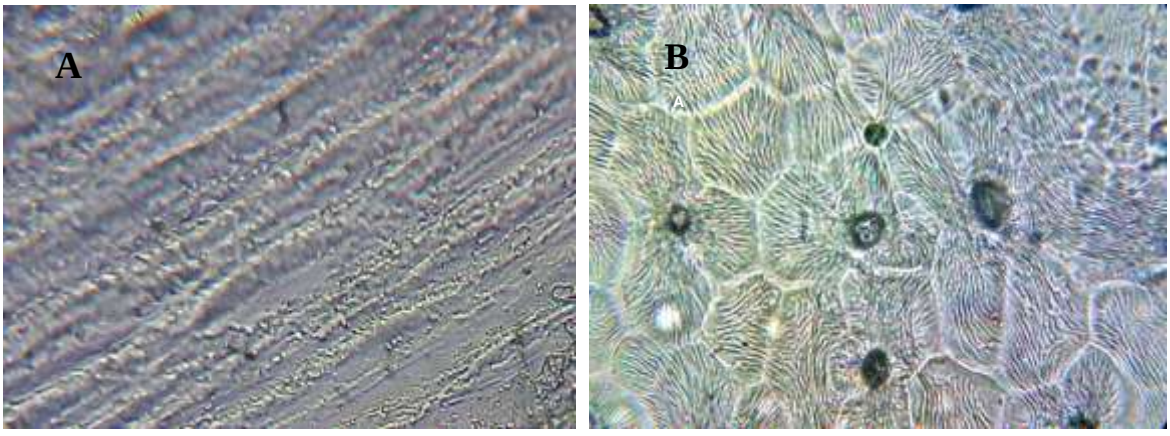

Fig 2. A view of dorsal surface of leaf of *C. cartilaginea* under optical microscope showing A) thick encrustation of wax and cuticle particularly in the vascular region of mature leaf (no hexane treatment) and B) the hexane

treated adolescent leaf showing the pattern of the cuticular striation. Cuticular striae may seldom be seen to traverse over adjacent cell. Magnification: 45 x 10 X.

Leaf surface is thickly encrusted with wax and cuticle material which is in the form of sheet with parallel running ridges (Fig. 2A). When leaves were immersed in hexane for 10 days, the surface structure became clear. The foliar epidermis cells appeared to be polygonal and isodiameteric in shape (Fig. 2B and 5). The anticlinal walls of the cells were straight. Density of epidermal cells on dorsal and ventral surfaces is reported to vary but slightly in this species (206 and 225 cells per mm^2, respectively) of Indian origin (Shodhganga.Inflibnet.ac.inbitstream /106031/58339/11/11_chapter6).

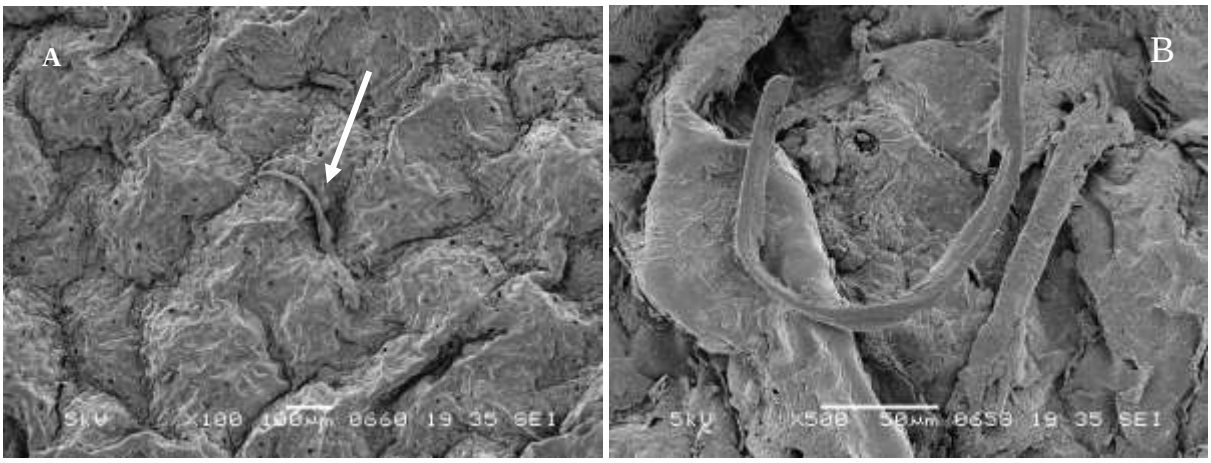

Fig. 3. Scanning electron micrographs of dorsal surface of dry leaf of *C. cartilaginea*. Note the strap like leaf hair at magnification 100X (A) and 500 X. (B).

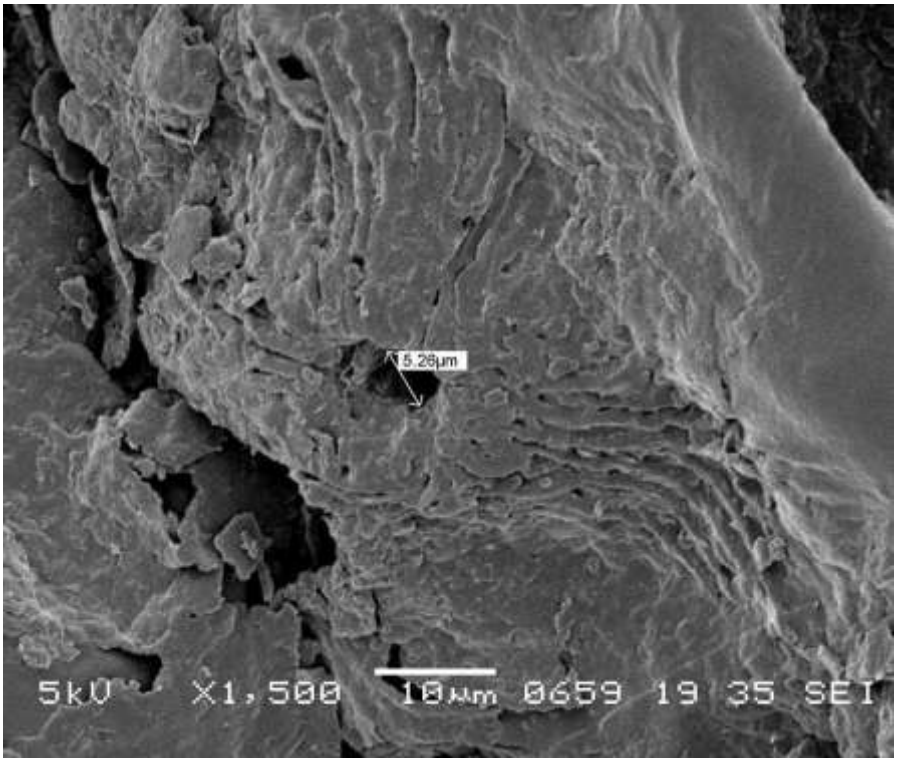

Fig. 4. Dorsal surface of dry leaf - SEM at 1500 X showing discrete cuticular striae radiating from the peristomatal rim.

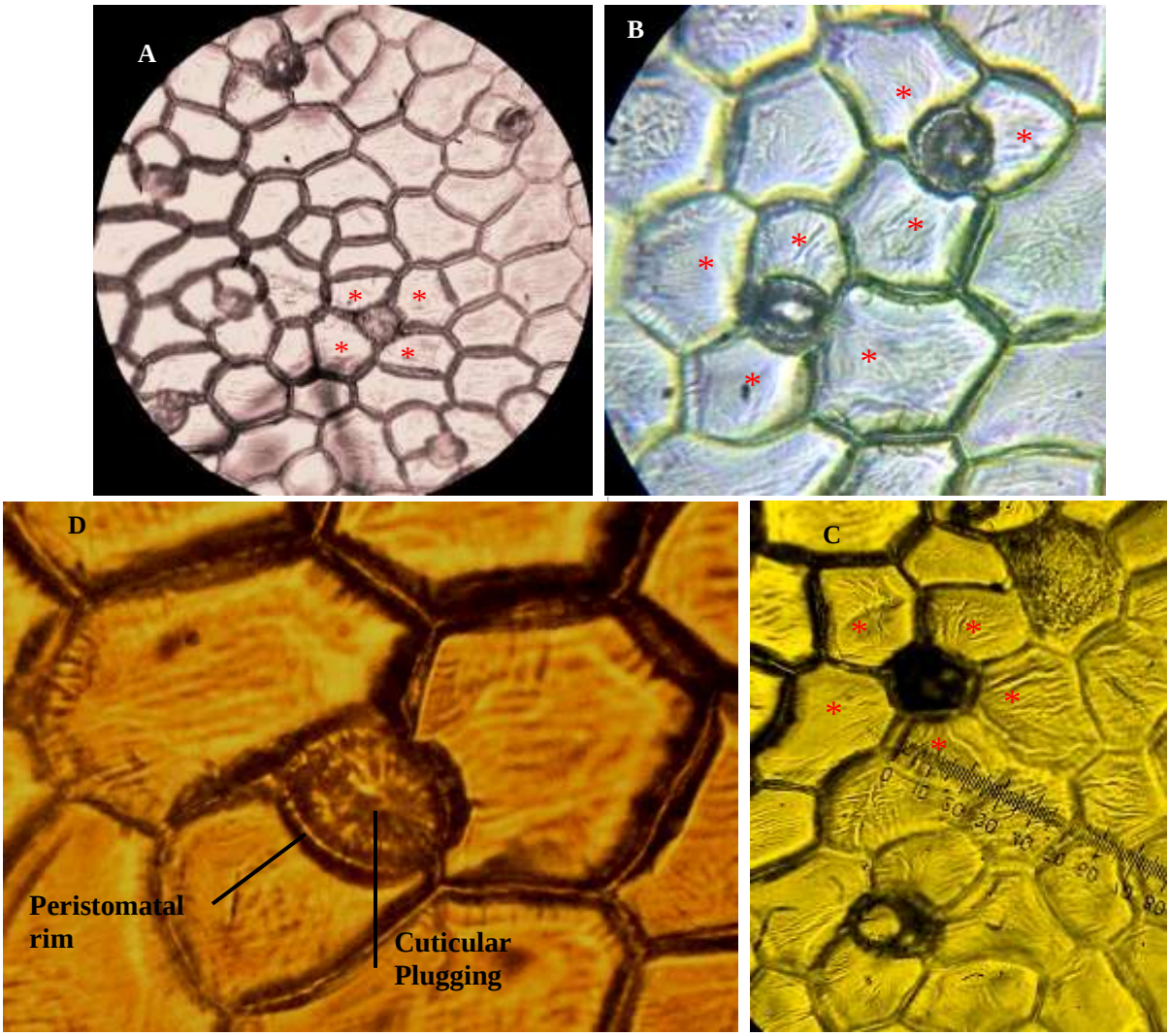

Fig. 5. Ventral surface of a hexane treated leaf. A) General view (45 x 10 X) showing polygonal cells with simple straight anticlinal walls and stomata; B) An anisocytic (three subsidiaries) and a tetracytic stoma (4 subsidiaries) stomata with common subsidiary; C), anomocytic stomata and D) Anisocytic stoma clogged with cuticle and other substances.

Trichomes: There were epidermal hairs distributed infrequently over the foliar surface. These epitrichomes were long (c 300 um) flat and strap-like appressed with the surface on the both surfaces of leaf (Fig. 3A and B). Fici (2004) has reported epitrichomes in younger *C. spinosa* var. *galeata* leaves to be quite denser.

Stomata: *C. cartilaginea* leaves are amphistomatic as have also been reported by Rhizopoulou and Psaras (2003). As per scheme of classification proposed by Prabhakar (2004), there were four types of foliar stomata in *C. cartilaginea* – anisocytic, tetracytic, anomocytic and staurocytic (Fig. 5 and 6). On ventral surface of hexane treated leaf, as studied in 30 microscopic fields of vision, anisocytic arrangement of subsidiaries in stomata was a common feature (70.97%) followed by tetracytic (20.65%), and anomocytic arrangement (7.74%). Staurocytic arrangement of subsidiaries was rare, 0.65% (Fig. 11). Several publications (Metcalfe and Chalk (1950; Wilkinson; 1979; Bokhari and Hedge, 1975; Singh *et al.*, 1987) have reported only anomocytic stomata in Capparaceae (previously Capparidaceae). This may perhaps be attributed to the indistinct nature of subsidiaries abutting stoma and the scheme of stomatal classification adopted by these workers. Aleykutti and Inamdar (1978) reported that the development of stomata in Capparidaceae follows various patterns, even in the same individual. Mature stomata are either without distinct subsidiaries ((anomocytic) or have one or several subsidiary cells. Various types such as cyclocytic, tricytic, staurocytic, and paracytic have been described by them in Capparidaceae. Such abnormalities as single guard cell, aborted guard cell, incompletely or completely divided guard cell, contiguous stomata, giant stomata and cytoplasmic connections may do occur.

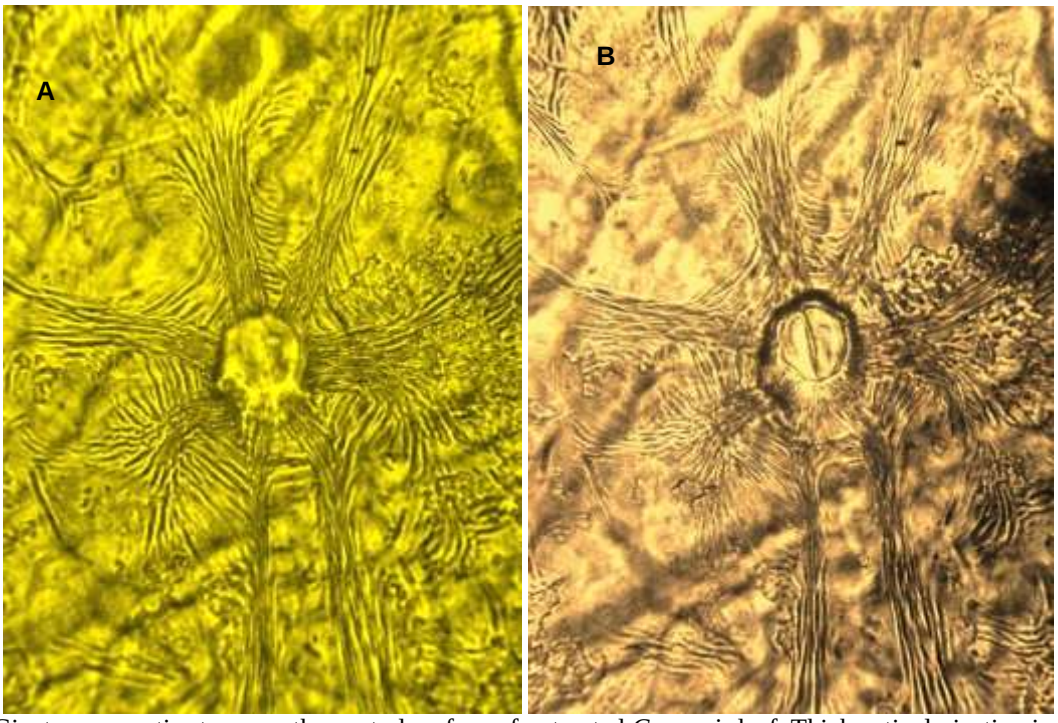

Fig. 6. Giant anomocytic stoma on the ventral surface of untreated *Capparis* leaf. Thick cuticularization is obvious as seen in this nail polish imprint. A) Focus on cuticular striations and B) the focus on the stomatal pore. (*Magnification: 45 x 15 X and zoomed). The stoma is almost round – admeasuring 28.8 µm in length and 27.2 µm in width. Note long parallel running striae.

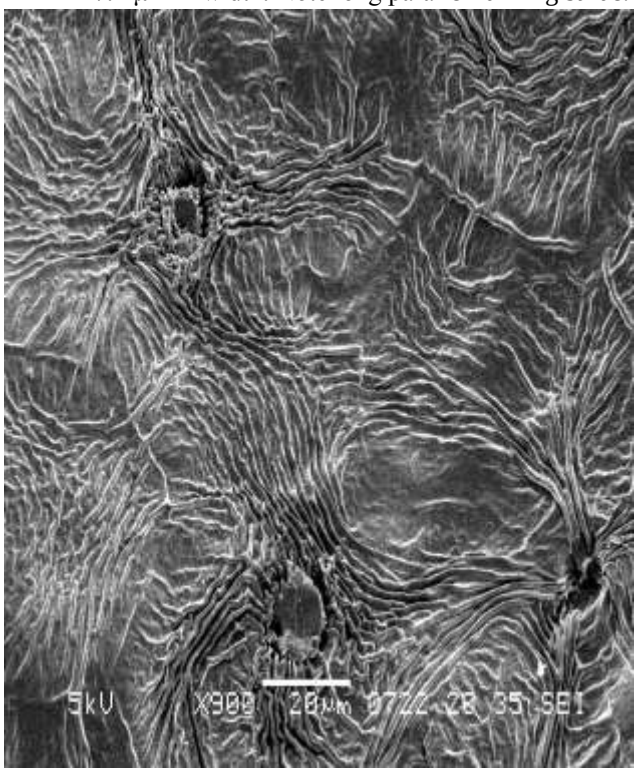

Fig. 7.SEM Surface of ventral surface of mature leaf of *C. cartilaginea* showing the pattern of cuticular striation as viewed at magnification of 900 X.

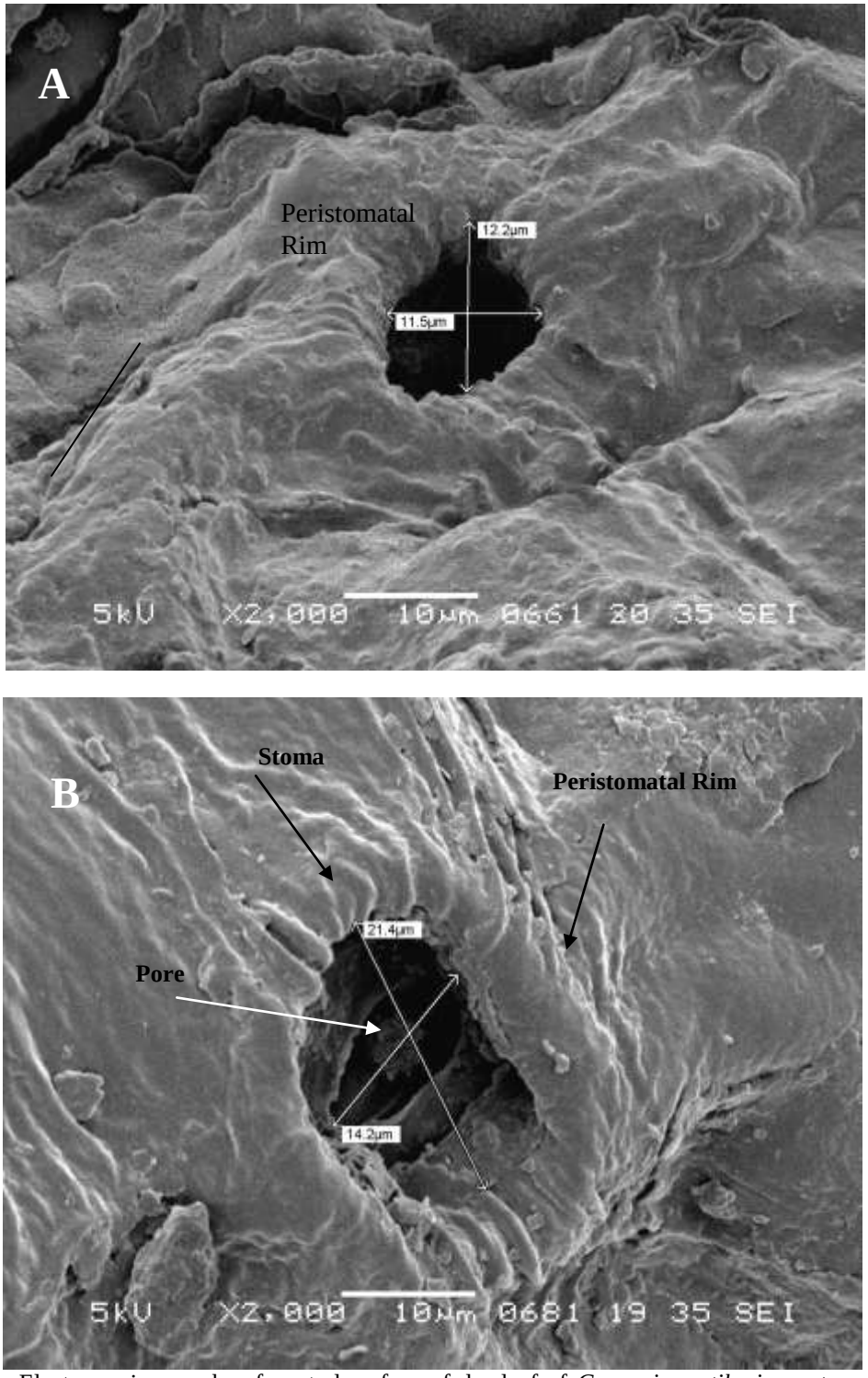

Fig.8. Scanning Electron micrographs of ventral surface of dry leaf of *Capparis cartilaginea at* magnification of 2000 X. Note the raised peristomatal rim and some cuticular striations. The peristomatal rim measures 12.2 x 11.5 µm (Fig. 8A) and 21.4 x 14.2µm in diameter (Fig. 8B). Stomatal pore measured 14.2 µm in length. Peristomatal rim is distinct. Stomatal pore is seated in depression. Pore axis is at right angle to the rim axis.

Stomata were arranged into various directions. Subsidiaries were indistinct. Stomata were sunken, provided with a peristomatal rim. The peristomatal rim varied in size – 12.2 x 11.5 µm (Fig. 8A), around 21.4 x 15 µm in

diameter (Fig. 8B) and even larger, 28.8 x 27.2 µm (Fig. 6). The Peristomatal rim varied in size and shape. They were nearly round to roughly rectangular in *C. cartilaginea*. In *C. spinosa* var. *galeata,* the peristomatal rim shaped like a tear drop (Fig. 12 b; Fici, 2004). As measured at magnification of 45 x10 X, peristomatal rim averaged to 28.85 ± 0.482 µm in length (N = 30, 22.4-35.2 µm, CV = 9.1 5%) and 24.13 ± 0.187 µm in width (N= 30, 19.2-28.8µm, CV =19.75 um). Axis of stomatal pore was generally seen at right angle to the axis of the peristomatal rim.

Stomata were occasionally found to be clogged presumably with cuticular and other substances (Fig. 4D). Fici (2004) has reported only one type of Stomata i.e. anomocytic type in this taxon. The clogging of stomata in *C. cartilaginea* may be related to its desert habitat. The stomata in some desert plants such as *Aristida ciliata, Sporobolus spicatus* and *Capparis spinosa* develop thickness on the wall of the guard cells or the pores and are blocked by the deposition of the resinous matter or wax which results in permanent closing of stomata (Singh *et al.,* 1987). Sometimes the stomata were in very close vicinity of each other (Fig. 9).

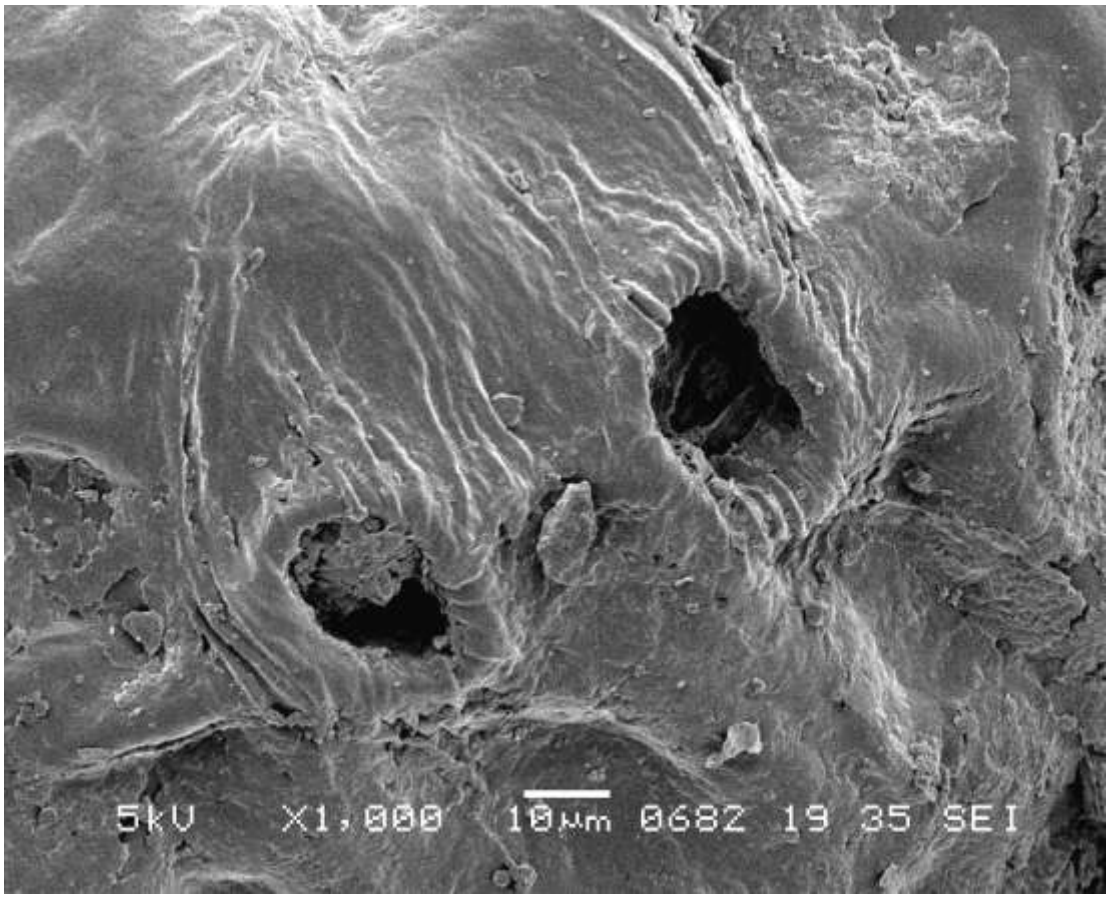

Fig. 9. Ventral surface of dry leaf of *C. cartilaginea* – SEM (1000X) showing three stomata (one small in the middle of two larger stomata) in close neighborhood. Occluding material may be seen in the vestibule (frontal cavity).

Stomatal size

Stomatal length and width were measured in mature leaf after removal of wax by its immersion in hexane for about 10 days. At 45 x 10 X magnification, the stomatal length averaged to 28.85 ± 0.482 9 (N = 30) varying from 22.4 to 35.2 um (CV: 9.15%) and stomatal width averaged to 24.13 ± 0.87 (N = 30) varying from 19.2 to 28.8 um (CV: 19.75%). The stomata, therefore, tended to be nearly round in shape.

Stomatal density

Stomatal density was 80.60 ± 1.445 per mm^2 on dorsal surface and 61.90 ± 1.406 per mm^2 on the ventral surface (Fig. 10). On both surfaces parameter of stomatal density distributed asymmetrically (ranging from 48.14 – 117.95 and 19.66 – 108.12 per mm^2 on dorsal and ventral surfaces, respectively. Stomatal density was significantly higher on dorsal surface (80.60 ± 1.445 stomata per mm^2) as compared to that on ventral surface (61.50 ± 1.406 stomata per mm^2) ($t = 9.28$, $p < 0.0001$). Stomatal density in *C. spinosa* is reported to be quite high by Rhizopoulou and Psaras (2003) (225-600 stomata per mm^2). According to them stomata are around 10µm wide and around 28 um

in length distributed in both epidermises. The stomatal density was slightly higher on adaxial (upper) surface than that on the abaxial (lower surface). Moreover, stomatal density varied with the leaf position on the axis. Although stomatal density in our case was much lower but still higher on dorsal epidermis than that on the ventral epidermis. It is contrary to generally seen heterostomaty in amphistomtatous plants. However, higher stomatal density (132 stomata / mm^2) on upper surface than lower surface (112 stomata / mm^2) is also found in *Tribulus terrestris* by Aisha *et al*. (2010). The perfect amphistomaty is quite rare (Parkhurst, 1994). This may be due to the fact that adaxial surface is exposed to very high photon flux densities of solar radiation in desert environment while the abaxial one which is shaded by the mesophyll receive relatively lower photon flux densities (Lu *et al.*, 1993). Our estimate of stomatal density is, however, in agreement with that in *C. cartilaginea* from India (64 stomata per mm^2). (Shodhganga.Inflibnet.ac.inbitstream /106031/58339/11/11_chapter6).

Table 1. Surface characteristics of interspecific taxa of *C. spinosa* L. sect. Capparis (Fici, 2004) and *C. cartilaginea* (the present study). The stomatal size in *C. cartilaginea* was measured in nail polish imprint of mature leaf after wax removal by hexane treatment for 10 days.

Taxon (Distribution)	Trichome	Cuticle	Stomatal size		Reference
			Length (µm)	Width (µm)	
subsp. *spinosa* (Mediterranean region to Central Asia and Sahara)	Simple, unicellular, up to 517 µm	Randomly oriented reticulate striae	26.49 (24-32)	23.35 (20-26)	Fici (2004)
subsp. *rupestris* (Mediterranean)	Simple, unicellular, Up to 256 µm	Ridged to reticulate	25.80 (22-29)	21.25 (19-23)	
var. *marina* (Philippines, Oceania to Hawaii & Henderson Is.)	Simple, unicellular, Up to 200 µm	Reticulate	21.35 (15-26)	19.2 (12-25)	
var. *himalayensis* (N. India, W. Nepal, E. Pakistan)	Simple, unicellular, Up to 567 um	Undulate or rigid	24.45 (22-29)	21.79 (20-25)	
var. *galeata* (E. Africa, SW Asia to India)	Simple, unicellular, Up to 227 µm	Densely reticulate	32.98(25-49)	28.50 (23-36)	
subsp. *nummularia* (Western, Central and Eastern Australia)	Simple, unicellular, Up to 283 µm	Reticulate	32.12 (23-39)	26.87 (21-31)	
C. cartilaginea Decne.* (Karachi, Pakistan)	Simple, strap-like, unicellular, c. 350 µm	Long Parallel striate radiating from Peristomatal rim	28.85 ± 0.48 N = 30, (22.4- 35.2) CV= 9.15%	24.13 ± 0.87 N = 30, (19.2- 28.8) CV = 19.75%	Present study

*, Syn: *C. spinosa* var. *galeata* (Fresen) Hook.F. & Thom. in Hook. F.) OR *C. galeata* Fresen. OR *C. unicinata* Edgew.

Cuticular striations

The ground cells striations in adolescent leaf of *C. cartilaginea* treated with hexane, striations arising from the peristomatal rim were generally confined within the cell boundary, over periclinal surface (Fig. 2B) and only seldom traversed over an adjacent cell. In mature leaf (not treated with hexane) cuticular striations arising from the peristomatal rim were long, parallel and radiating (Fig. 4, 6, 7 and 12C & D). These folds or striae ran quite longer and continued to pass over adjacent epidermal cells. Similar striae have been reported in *Philippia capitata* Baker by Lavier-George (1936). Cuticle is known to vary in plants and in surface view may be smooth, granular or verrucose (Metcalfe and Chalk, 1979). This characteristic in *C. cartilaginea* may be due to its xerophytic nature. On the basis of 226 dicotyledonous species, Dunn *et al.* (1965) have reported that the most xerophytic species generally had the most wrinkled cuticle while the most mesophytic or water-loving species had the smoothest surface

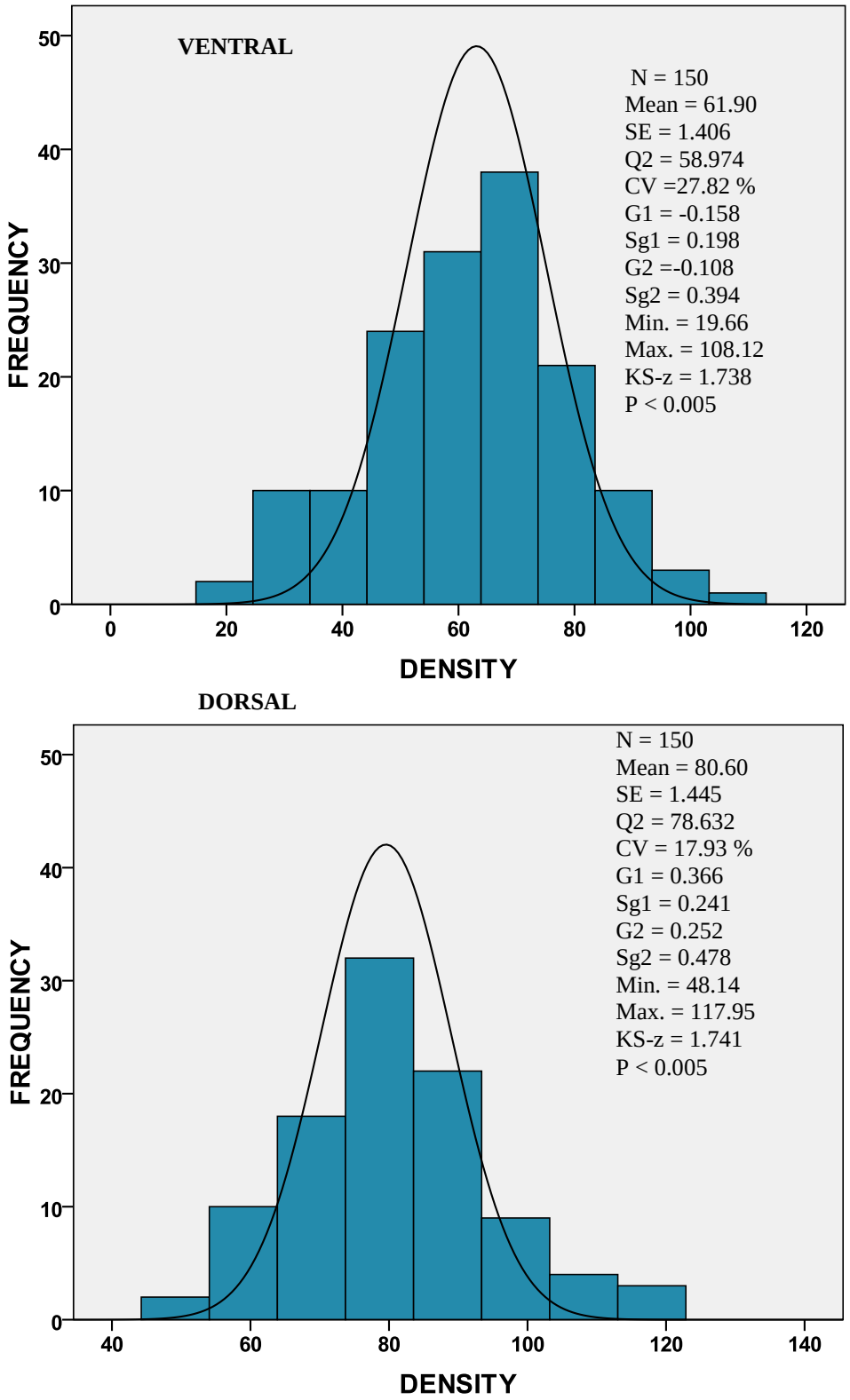

Fig. 10. Frequency distribution of stomatal density per mm^2 on ventral and dorsal surface of *C. cartilaginea* leaf.

Several workers have shown that many leaf surface patterns are under strong genetic control and therefore little affected by the environment (Mueller, 1966 a & b; Cutter, 1972; Cutter and Brandham, 1977). Metcalfe and Chalk (1979) referred to a number of papers of P. Martens published during 1931 to 1935 wherein he suspected relationship between protoplasmic streaming and cuticular markings. Time and place of the development of the cell may determine the exact pattern of cuticular striations and structural peculiarities of the cuticle layer could be a cause of the pattern of folds (Iterson, 1937).

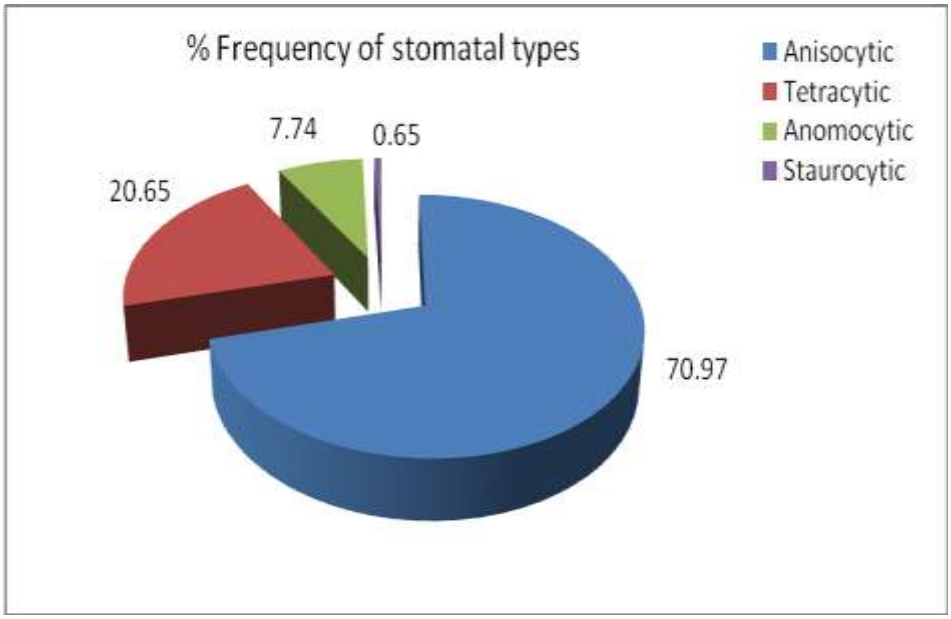

Fig. 11. *Per cent* frequency of occurrence of various stomatal types on ventral surface of *C. cartilaginea* leaf. The data is based on 155 stomata observed in 30 fields of vision at 45 x 10 X magnification.

The cuticle varies in plants and in surface view may be smooth, granular and verrucose. Kers (2013) reported that in Capparaceae, cuticle is generally smooth but sometimes minutely granular or striate with gently wavy or reticular lines. A differently more faintly marked patterns of striations are found to radiate from stomata and hairs e.g., in *Crateva adansonii, Boscia teitensis, Euadenia trifoliata* and *Maerua oblongifolia* (Pestalozzi, 1898; Aleykutty and Inamdar (1978).

Kurer (1917) used cuticular striae and other characters to distinguish adulterants in China tea. Paganelli Cappalletti (1975) indicated the usefulness and diagnostic value of cuticular characters. Stace (1965) also considered striations to be of great taxonomic value in some groups of plants. Cuticular ornamentation was, however, rated of no diagnostic value at family level (Hüller. 1907). Like other taxonomic evidence epidermal characters need to be interpreted with great circumspection. Lavier-George (1936), however, used cuticular ornamentation as one of the characters of diagnostic value to separate species of *Philippa* as follows:

Philippa capitata – long parallel striae passing over several cells
P. myriadena – Striae short and not overlapping
P. andringitensis– Striae fine and warty
P. danguana– Cuticle striate
P. ibytiensis - Cuticle smooth
P. ciliata - Striae branched
P. jumellei – Striae parallel
P. decomtei – Concentric striae confined to a cell

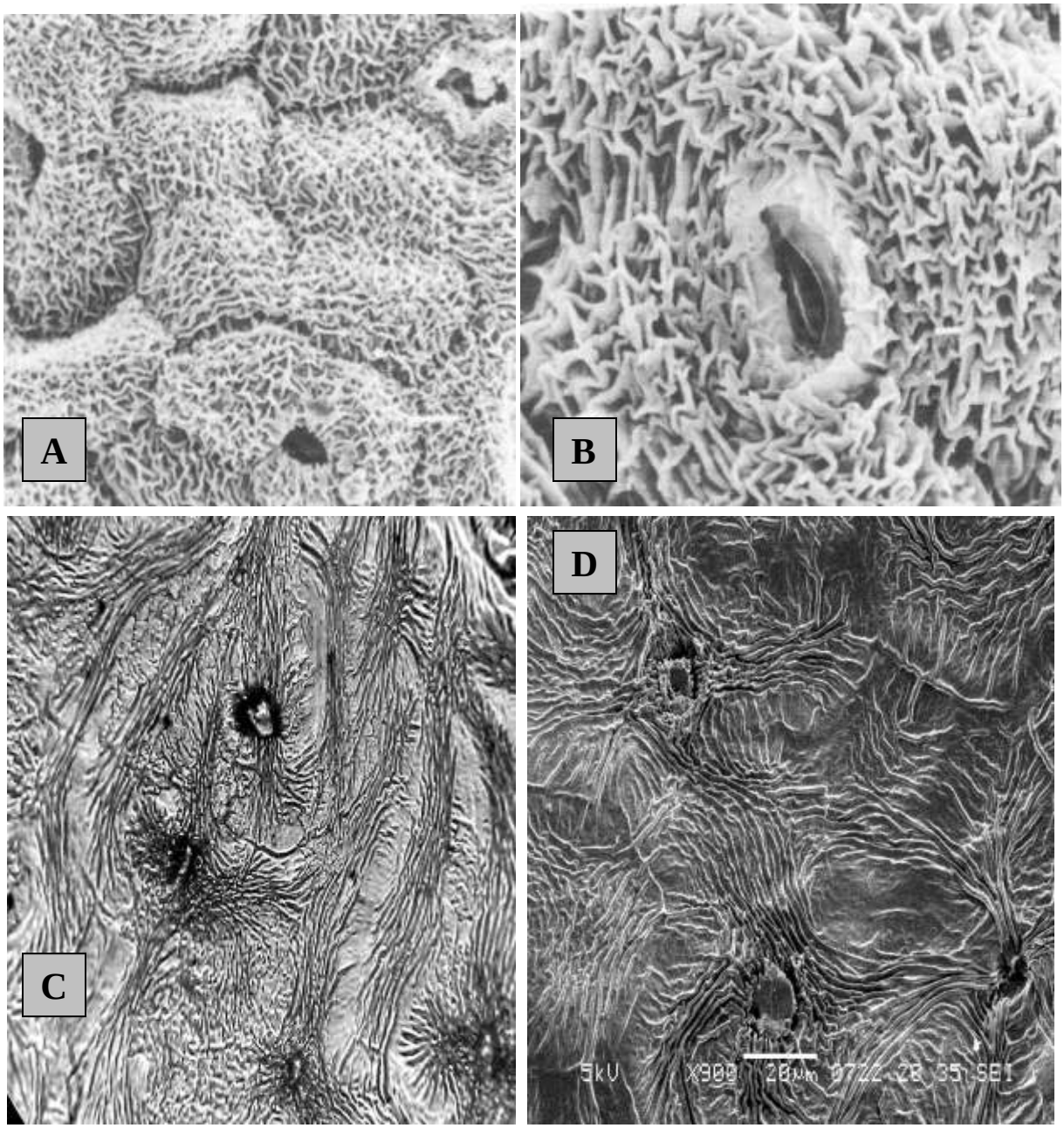

Fig. 12. SEM images of *Capparis spinosa* var. *galeata* - abaxial leaf surface A) and adaxial leaf surface B) as given by Fici, 2004). C) Cuticular striations of *C. cartilaginea* (present study) as viewed in nail polish imprint of hexane treated leaf (wax removed); D) Cuticular striation as seen in SEM at 900X. Prominently parallel striation is evident.

Capparis is a large polymorphic genus with a worldwide distribution in the tropics and subtropical zones (Kamel *et al.,* 2009). Species within genus *Capparis* are highly variable and interspecific hybridization has taken place frequently in its long evolutionary history. *C spinosa* is considered to be composed on multiple distinct species (Zohary, 1960). Others consider it to be a species with multiple varieties or subspecies. (Jacobs, 1965; Heywood, 1993). In Capparaceae, cuticular striation has been regarded as one of the characters of taxonomic value (Metcalfe and Chalk, 1950, 1979; Solereder, 1908; Fici, 2004) but there is a paucity of data on cuticular striation for many species of this family. Fici (2004) has, however, given micro-morphological characteristics of *Capparis* L. sect. *Capparis.* The comparative account of the trichome type, pattern of cuticular striation and stomatal size of various

intraspecific taxa of *Capparis spinosa* L. as given by Fici (2004) and *C. cartilaginea* collected from coastal locality of Karachi, Pakistan (present study) is presented in Table 1. Trichomes in all taxa were unicellular but varying in length. Cuticle was generally reticulate with little variation in rigidity and density. Stomata were somewhat larger in var. *galeata* and ssp. *nummularia*. In *C. cartilaginea*, stomata were on larger side but not as large as in var. *galeata* and ssp. *nummularia*, which may probably be attributed to their arid environment. *C. cartilaginea* was, however, strikingly different in having long parallel cuticle striations arising from the peristomatal rim and continuing on the adjacent cells (Table 1, Fig. 12 C & D). It should be interesting to explore the micro-morphological characteristics (cuticle patterning) in *C. cartilaginea* from Indian part of the sub-continent for comparison.

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