Intraspecific variability assessment (Ecological and Morpho- anatomical) in *Conocephalum conicum* (L.) Dumort. in North- West Himalaya (Bhaderwah, J&K), India

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Abstract: The present paper includes information on intraspecific variability (ecological and morpho-anatomical) among 46 accessions of *Conocephalum conicum* (L.) Dumort. collected from diverse habitats in Bhaderwah (J & K state), India. The study revealed tremendous diversity in almost each studied character.

Keywords: Bryophyta, Liverwort, Conocephalum, Intraspecific, Ecology.

Introduction

Jammu and Kashmir, a part of North-West Himalaya is rich in hepatic diversity. One of the hilly areas of North-West Himalaya, which used to be least disturbed ecologically with unwavering habitats during the yesteryears but subjected to remarkable development and tourism related pressures for the last couple of years, Bhaderwah tehsil of Jammu region is under immense anthropogenic pressure.

Topography:

The area lying between 32°08´-32°52´ latitude and 75°48´ - 75°32´ E longitude has an altitudinal range of 821-4341msl. It receives an annual rainfall up to 249.6 mm with 84% maximum humidity (Chander, 2005). The climate of Bhaderwah is temperate; temperature varies from minimum mean 17.3°C to maximum mean 29.6°C in summers and minimum mean -0.9°C to maximum mean 2.8C° in winters. The area experiences severe cold during winters with snowy season between the months of December to March

with temperature remaining subzero. The snow melts off by April and weather becomes pleasant and remains so up to May. In June and July, temperature increases up to 29.6ºC. During August and September, the temperature subsides and it again becomes cold afterwards.

During the last decade, the anthropogenic activities on large scale have resulted in disappearance of various terrestrial and epiphytic taxa, particularly the endemic and the ones with specific habitat preferences. Studies on the assessment of intraspecific variability of *Conocephalum conicum* is therefore undertaken for this area.

Conocephalum conicum (L.) Dumort. is worldwide in distribution, as it is known to be distributed in America, Algeria, Alaska, Azores, Bhutan, Caucasus, Canary, China, Europe, Florida, India, Ireland, Italy, Japan, Korea, Kuriles, Liukium Maderia, Nepal, Pakistan, Rubki-Neylong, Seghalim and Siberia (Sato and Yamada, 1980).

From India, it is has been reported from Eastern Himalaya (Mitten, 1861; Chopra, 1938; Pande and Bhardwaj, 1949) and Western Himalaya (Stephani, 1898; Kashyap, 1929; Parihar, 1961-62; Parihar et al., 1994; Pande, 1958; Pant 1983).

In Jammu and Kashmir (North West Himalaya), it has so far been collected from Poonch (Tanwir, 2005), Udhampur (Tanwir et al., 2008) and Kathua districts of Jammu region and Ladakh (Tanwir and Langer, 2006)

Materials and Methods

Conocephalum conicum belongs to order Marchantiales; its thalli could be identified in the field based on (a) tiny hexagonal aerolae on dorsal surface (b) faint sweet scent, which it produces when rubbed between the fingers (Glime and Saxena, 1991) and (c) conical archegoniophores. Due to these three peculiarities, the plant is commonly called “Snake liverwort”or “great scented liverwort” or “cone-headed liverwort”. Another characteristic, which makes its identification easier, is the zigzag branching pattern, exhibited by thallus. It reported to grow generally at an altitude above 1000m in Jammu region (Tanwir, 2005; Tanwir et al., 2008).

During present investigations, 46 accessions of *C. conicum* have been collected growing at different sites ranging in altitude from 1230m-2600m. Populations were collected growing on substrate with pH ranging between 6.5 - 6.9. Plants of *C. conicum* have been collected from diverse habitats, such as eplithic (under snow cover) under dripping chilled water, caves, rock crevices, sides of stream, water channels, submerged under water, non-epilithic (cool and moist shady soil, loamy soil, litter/debris of humid forest floor) on bark of *Salix alba* and epiphytic (on decaying log of wood of *Cedrus deodar.*

Observation and Results:

Ecology of *Conocephalum conicum*

The species was observed to grow in association with a fungal associate (*Glomus* sp.), hepatic (*Dumortiera hirsuta, Marchantia palmata, Chiloscyphus kashyapii),* pteridophyte (*Athyrium attenatum, Dryopteris radactopinnata, Woodwardia unigemmata),* gymnosperm (*Cedrus deodara),* angiosperm *(Circium arvensis, Pilea umbrosa*, *Salix*

alba) and insect (*Componotus* sp.) taxa. Of these, *Dumortiera hirsuta* was the most commonly associated species as it was found associated with as many as 25 populations (Iqbal, 2009; Iqbal et al., 2011). Populations displayed wide tolerance to temperature from (-0.9 to 29°C).

Morphological variations

Thalli dorso-ventrally differentiated, flat, notched, strap/ribbon shaped, with distinct and conspicuous penta/hexagonal aerolae with a prominent white pore in the centre, forming regular network on dorsal surface in all accessions, large, thick and opaque, thickest along the midline, repeatedly dichotomously branched with a characteristic zigzag pattern of branching, lateral and apical innovations present; margins undulate. Thalli exhibit variation in size, branching, colour and texture. Plants range between 0.4cm-11cm in length and 0.1cm-4.0cm in breadth; branching in majority of populations dichotomous and monopodial in few. As far as size variation among thalli of *Conocephalum conicum* is concerned, it is already on record. Thalli ranging from 1.5-25 cm in length have been reported by earlier workers, from time to time. Of these, largest thalli (12cm-25cm x 1.6cm-2 cm) were recorded by Schuster (1953), while Tanwir (2005) reported the smallest (1.5cm-5.2 cm x 0.5 cm-1.2 cm) ones. Other workers like Macvicar (1912) reported 10cm-20 cm x 0.8cm-1cm, Szeykowski et al., (2005) reported 5cm-11cm x 0.8cm-1.6cm, Kashyap (1929) reported 10cm-20cm x 0.8cm-1.0 cm and Bapna and Kachroo (2000) reported10cm x 1.cm-1.2 cm that is the thalli of intermediate size. Present observations, however, widened the range already reported for the species. Thus, while the minimum length reported earlier was 1.5cm by (Tanwir, 2005), present collection includes thalli only up to 0.4 cm long. Similarly, minimum breadth reported presently is 0.2cm against 0.5cm reported earlier by Tanwir (2005). It is interesting to note that the smaller plants collected earlier by Tanwir (2005) are also from an area (Poonch district) falling in Jammu region, which also includes the present study area. None of the earlier worker has attempted to correlate the thallus size of *Conocephalum conicum* with habitat. Such information is, however, available for *Reboulia hemispherica*. Plants of this taxon collected from stones and rocks were considerably larger (5mm-47mm x 3mm-7mm) as compared to those inhabiting soil (3mm-37mm x 2mm-9mm). Later ones, were, however wider than the former ones (Fardos, 2003). Plants growing on bark of *Cedrus deodara* were, medium sized (13mm-30mm x 5mm-9mm).

Similar correlation also exists, among the Populations of *Conocephalum conicum* studied presently. Accessions collected from moist shady areas were larger in size (0.4cm - 11 cm x 0.2cm-2.5cm) as compared to those growing as an epiphyte on rotten log of *Cedrus deodara* (0.3cm-3.5cm x 0.2-1.0cm) and *Salix alba* (0.4cm- 4.0cm x 0.2cm-1.0cm). Populations collected growing under dripping chilled water on soil capped stones (0.6cm-8.7cm x 0.2cm-1.7cm), growing exposed to sunlight (1.0cm-5.5cm x 0.3cm-1.5cm), submerged under water (1.0cm-5.5cm x 0.3cm-1.5cm), on the other hand bore thalli intermediate in size. Thalli of populations collected growing under snow were also intermediate in size (0.7cm-5.5cm x 0.2cm-1.0cm). Of all the Populations, the one collected from moist shady soil (Patnitop), had largest thalli (1.5cm-11cm x 0.5cm-2.5cm). While the smallest thalli (0.4 cm-2.5cm x 0.2 cm-0.9 cm) were recorded from Domail (moist shady soil).

As far as thallus colour, different workers have recorded concerned variations. Thus, while Macvicar (1926) and Kashyap (1929) have reported dark green thalli, Schuster (1953) observed yellowish- green plants of *Conocephalum conicum*. Bapna and Kachroo

(2000) used the term 'bright green' to designate the thalli collected by them. Tanwir (2005) reported thalli of various shades (light green, green and dark green). Macvicar (1926) and Szweykowski *et al.* (2005) also reported shining nature of thalli.

Populations collected presently also comprise thalli of both light green and dark green colour. Colour seems to be apparently related with the age of plants. Thalli remain light green, thin and shining at early stages of development, but turn dark green thick and leathery as growth proceeds towards maturity. This has been indicated by our observations on five accessions collected growing under snow. In all these accessions dark green, thick, leathery, mature plants bore apical innovations that were invariably thin, shining and light green in colour. It has also been presently observed that thalli comprising populations collected from under dripping water and submerged under chilled water were shinier as compared to those growing at moist, shady places. Furthermore, thalli collected from rotten log of *Cedrus deodara* were distinctly dark green in colour.

Comparable observations have earlier been made by Fardos (2003) who undertook an exhaustive study on intraspecific variability among 71 populations of *Reboulia hemisphaeric*a growing in Jammu region (North-West Himalaya) and recorded light green thalli growing near water and dark green ones on bark *of Cedrus deodara*. Thalli bearing intermediate colour inhabited soil, stone or rock surface in dry or moist conditions, but never near water.

Ventral surface pale green in majority of the populations and violet in few. Midrib thick and conspicuous, extending from base to apex, bifurcating with the branching of thallus. From midrib on ventral surface, arise rhizoids and scales.
Rhizoids are dense, compactly arranged, hyaline, sinuous, generally unbranched, rarely branched; dimorphic; smooth walled; tuberculated rhizoids with peg like structures on inner wall, smooth walled rhizoids more in number and diameter 8µm-36µm (11.4µm – 26.6µm) in majority of populations as compared to tuberculated rhizoids 8µm - 24µm (10.4µm - 18.8µm). In few populations, tuberculated rhizoids were more in both number as well as diameter 8µm-32µm (12.5µm-28.5µm). Observations on intraspecific variability on these aspects of rhizoids have earlier been made for *Reboulia hemispherica* by Fardos (2003) and *Dumortiera hirsuta* by Butt (2008). Fardos (2003) recorded less abundant and larger (3.7µm-44.4µm) smooth walled rhizoids as compared to tuberculated rhizoids which were lesser in diameter (3.7µm-25.9µm) and more abundant in all populations except a single population inhabiting moist soil, where smooth walled rhizoids were lesser in diameter (3.7µm-7.4 µm) than tuberculated ones (10.1µm-14.8µm). Results obtained by Butt (2008) for *Dumortiera hirsuta* showed that smooth walled rhizoids were more in number and diameter (6µm-34µm) than tuberculated (3µm-32 µm) ones, except in one populations where the diameter of tuberculated rhizoids (16µm-32 µm) was more than that of smooth walled ones (12µm-30µm). Earlier studies on *C. conicum* by Tanwir (2005) also points towards a similar situation. He too recorded smooth walled and tuberculated rhizoids measuring 12µm–22.2µm and 9.25µm-18.5µm in diameter respectively. Not only in relative abundance and diameter, two types of rhizoids are also known to vary in their function and site of origin, (McConaha, 1939). Thus, while smooth walled rhizoids are believed to anchor thallus to the substratum, tuberculated ones perform the function of absorption of water and nutrients, from soil. As far as their origin is concerned, smooth walled rhizoids arise from the midrib region covered by free anterior portion of scale. They emerge laterally from behind the scale and extend downwards to the substratum. Tuberculated rhizoids, on the other hand, arise from the part of midrib covered by attached portion of scale and so are forced to lie nearly

parallel to the midrib and within the channels formed between the overlapping scales. Position (on ventral surface nearly parallel to midrib) and morphology (peg like structures on inner surface) of tuberculated rhizoids in all probability increases their efficiency in terms of absorption of more nutrients and water from soil. Mycorrhizae present; endomycorrhizae found mostly in smooth walled and rarely in tuberculated. Mycelium aseptate, spore wall differentiated into outer hyaline, middle dark brown and inner yellowish brown layers having single subtending hypha. Spores globose or ovoid; reddish brown to dark brown. Ligrone and Lopes (1989), reported that fungal hypha colonised the smooth walled rhizoids through which they pass into gametophyte parenchyma of the midrib. Vesicles were also found in rhizoids.

Ventral scales in two rows, one on either side of the midrib; one cell thick, inserted into the undersurface of thallus by a wedge shaped structure (Plate 1: Figures 1 and 2), each lateral series is formed by the overlapping of obliquely placed scale in such a manner that the posterior portion of one is overlapped by the anterior portion of next scale. Each scale consists of two parts (a) a free anterior portion with single apical reniform or orbicular appendage and (b) the lower elongated portion which is attached by its outer edge to the midrib. Scales distantly as well as compactly arranged. Scales and their appendages of same colour; violet in some accessions, brownish and purple in others (Plate 1: Figures 3 and 4). 18 populations possess both purple and violet scales and appendages; scales range between 0.62 mm-1.52 mm in length; 0.34mm-0.92mm in breadth at base, 0.21mm-0.77mm at neck and 0.12mm-0.42mm at apex. Earlier workers like Macvicar (1926) and Kashyap (1929) have also reported for *C. conicum* distinct, tender scales arranged in one row on either side of midrib, with single, violet, reniform or orbicular appendage. Observations of subsequent workers (Bapna and Kachroo, 2000 and Tanwir, 2005) are also similar regarding colour and arrangement of ventral scales and appendages in the species It is suprising that accessions collected from diverse habitats show constancy in number of appendages. This is in total contrast to the observations of Fardos (2003) for *Reboulia hemispherica* where she recorded variation between 0-4 appendages per scale. Variation in colour of scales does not seem to bear any correlation with habitat as both violet and purple coloured scales were present in accessions growing in diverse habitats (Plate 1: Figures 4 and 5). 0.65mm-1.52mm x 0.15mm-0.43mm scales were present in populations growing under dripping chilled water, against much smaller possessed by populations growing in moist shady places. Intermediate sized scales were present in populations growing under snow and those growing as epiphytes. As far as scale size in *C. conicum* is concerned, McConaha (1939) reported 5mm as average length. Tanwir (2005) also recorded 4.0mm-5.0mm long scales for the species. Similar variation in scale size (0.4mm-3.65mm x 0.41mm-2.45mm) has been reported by Fardos (2003) for *Reboulia hemisphaerica*. Large sized scales (0.58mm-3.65mm x 0.43mm-1.66mm) were present in populations growing in dry habitats (Plate 1: Figures 7 and 8), against much smaller (0.44mm-2.80mm x 0.43mm–1.66mm) possessed by those growing near water. Populations collected from bark of *Cedrus deodara* possessed extremely large (2.15mm-2.98mm x 1.49mm–2.45mm) scales. All scales have single appendage; constancy in appendage number notwithstanding, variability was exhibited by appendage shape. In some populations appendage was orbicular and in others it was reniform in shape. In few scales, appendage was slightly bifid and fish tail in shape. Reniform appendages range between 0.36mm-0.92mm x 0.12mm-0.91mm, and orbicular appendages range between 0.12mm-0.88mm in diameter. Variability was also exhibited by size of appendage in relation to the body of scale. Populations collected from moist shady places were having small sized appendage (0.12mm-0.39mm; Plate 1: Figures 3 and 4) as compared to the body (0.34mm-0.68mm) of scale, but those collected growing under chilled dripping

water were having large sized (0.36mm - 0.92mm) appendage (Plate 1: Figures 5 and 6) as compared to body of scale. Intermediate sized (0.30mm -0.78mm) appendages as compared to body (0.13mm-0.51mm) were present in populations growing in remaining habitats (Plate 1: Figures 7 and 8). Scales exhibited variation in size of constriction also. Small sized constriction (0.21mm-0.68mm) was present in populations growing in moist shady places, large sized (0.36mm-0.77mm) in those growing under dripping chilled water and intermediate sized (0.27mm-0.70mm) in remaining populations growing in other habitats (Plate 2: Figures 9-14).

Dorsal epidermis single layered, consisting of compactly arranged polygonal cells, angles 4-6 in 37 populations, 5-6 in 7 and 4-5 in 1 populations ranging between 0.005mm-0.006mm in length and 0.003mm-0.004mm in breadth, chlorophyllous with 8-22 chloroplasts per cell. Thalli with 4-6 angled cells were commonest and were found in accessions growing in different habitats and those with 5-6 angled cells inhabited moist shady places. Populations with 4-5 angles were collected growing under dripping chilled water at an altitude of 1820m on soil capped stone. Epidermal cells were thin walled and without trigones. Macvicar (1926) and Kashyap (1929) recorded thin walled epidermal cells in *Conocephalum conicum* with unthickened angles. Observations of Tanwir (2005) are, however, at variance, as he reported 4-7 angled, thick walled epidermal cells (10-17 chloroplasts/cell) without trigones in *Conocephalum conicum*. Number of areolae/mm² on dorsal surface of *Conocephalum conicum* varies between 1.2-4.1.

Air pores distributed regularly, conspicuous, elevate, circular or oval in shape, surrounded by 4-7 concentric rings, 4 rings being most frequent followed by 7, 6 and 5. Innermost ring composed of 6-7 cells; number of cells per ring varies from 6-11; 6-9 being most frequent (Plate 3: Figures 15-26). It has been observed that populations with 4-7 rings and 6-11 cells per ring grew under dripping chilled water on soil capped stones and those with 4-6 and 5-6 rings (with 6-9 cells/ring) were present in populations growing in remaining habitats. Earlier bryologists (Macvicar, 1926; Kashyap, 1929; Bapna and Kachroo, 2000) have also reported simple elevated air pores in *Conocephalum conicum*, with 5-6 concentric rings of cells; the innermost ring being composed of 6-7 cells. However, Tanwir (2005) noticed that in *C. conicum* dorsal air pores were simple, hexagonal, elevate, surrounded by 2-4 concentric rings, with unequal number (7-9) of cells in each ring.

Fardos (2003) observed three types of air pores in *Reboulia hemispherica* (circular, ovoid and oval) with 1-6 concentric rings, having 4-13 cells/ring. As far as size is concerned oval air pores range between 0.15mm-0.30mm x 0.10 mm-0.19 mm and circular between 0.13mm-0.31mm in diameter. Aperture oval (0.05mm-0.07mm x 0.02mm-0.06mm) or circular (0.02mm-0.07mm) in shape. It was observed that populations collected growing under dripping water were having comparatively larger aperture (0.04mm-0.07mm) than those collected from moist shady places (0.02mm-0.05mm). Intermediate sized (0.04mm-0.06mm) apertures were recorded in populations growing in other habitats (snow cover, submerged under water and epiphytic accessions).

Thallus bound by dorsal and ventral epidermis; internally differentiated into dorsal photosynthetic and ventral storage zones; photosynthetic zone being much narrower (0.06-0.09mm) than storage zone (0.34-0.68mm; Plate 4: Figures 27 and 28), populations vary with regard to various anatomical features such as outline of thallus in V.S, thallus thickness in midrib/wings, thickness of photosynthetic and storage zones, their relative proportion. Dorsal epidermis interrupted by hemispherical air pores (Plate 4: Figures 29-30), one cell thick, cells rectangular, varying between 0.07-0.15mm x 0.04m - 0.012mm; below dorsal epidermis is present 0.06-0.09mm thick photosynthetic zone, consisting of

green,1-3 cell high assimilatory filaments; photosynthetic filaments range between 0.004mm-0.005 in height; just below air pore apical cells of photosynthetic filaments are modified to form colourless pointed papillae having beak like structures (Plate 4: Figures 31 and 32). Distance between air pores in midrib ranges between 0.25-1.05mm. Tanwir (2005) also made similar observations related to photosynthetic zone except that he recorded 0.12-0.15mm thick photosynthetic zone with branched filaments.

Below photosynthetic zone, is present 10-18 cell high storage zone, 0.34-0.68mm thick in midrib region and 0.13-0.22mm in wing portion gradually decreasing into two cell thick margin in all accessions (Plate 4: Figure 33). Cells in storage zone 0.004-0.006mm x 0.002mm-0.005mm in size and 4-7 angled. Cells of storage zone compactly arranged, thin walled, polygonal and trigonous. Fungal infection observed in storage zone (Plate 4: Figure 34). Ratio between photosynthetic and storage zone varies between 0.10:1-0.23:1. Tanwir (2005) also observed comparable ratios.

C. conicum reproduces both, asexually as well as sexually; asexual reproduction occurs usually by regeneration from thallus fragments; thallus being light in weight, is easily transported by water; thallus produces ventral innovations, both apical and lateral. During present investigation, only 15 accessions were recorded in fertile form, of which 12 comprised both male and female thalli growing together, while remaining three had only female plants. Dioecious; male receptacles sessile, green, disciform, disc slightly raised above main thallus, surrounded by thin growth of thallus; terminal or subterminal; receptacles oval or circular having conspicuous papillae on their upper surface; borne on main thallus as well as on apical innovations; number of receptacles per thallus varied from 2-3; initiation of male receptacles occurred in April, and they disappeared in July.

Although female thalli were recorded presently in 15 accessions, mature receptacles could be observed only from 3; in the remaining 12 accessions, receptacles were observed at young stages only, as they degenerated before reaching maturity. Female receptacle terminal or slightly sub-terminal, 1 to 4 per thallus, sunken when young in a cup formed by dorsal growth of the thallus; receptacles appeared in first week of May; dense scales present below young female receptacles; one flask shaped archegonium present per lobe with broad venter and long neck; post fertilization changes recorded after mid June, when neck of archegonium degenerated; receptacles started emerging out of sunken cup, stalk elongated slowly till it reached maximum of 11 cm in March next year. Mature receptacles conical; raised on long stalk. T.S of stalk showed single rhizoidal furrow, undulating in outline, made up of thin walled cells, containing tuberculated rhizoids.

Mature spores observed only in Acc. MI 46; capsules of Acc. MI 37 and MI 38 possessed very few viable spores and possessed developing sporocytes and elaters; ratio between the two beings around 0.4:1. Capsules showed very low spore output as compared to that of elaters. Like the other two populations spore-elater ratio in population (Acc. no. MI 46) was around 0.4:1. Out of the spores formed in Acc. MI 46, another 10% were non-viable. These were small (16µm-20 µm x12 µm -16 µm), triangular and shriveled as compared to viable ones which were large (44.5 µm -102.3 µm x 25 µm -55.4 µm), green and healthy.

Elaters brown, healthy, bi- and tri spiral, unbranched and bluntly fusiform in Acc. MI 46. Elaters of abnormal morphology (branched and blackish) were also present in capsules of Acc. MI 37 and MI 38. Size of elaters varied between 50µm-150µm x 15µm-70µm.

Results and Discussion

It can be inferred from the observations and results obtained for various reproductive characters (female gametangia, sporocytes and spore-elater ratios) that there is minimal (if any) contribution of sexual reproduction to the variability recorded among various populations. Results thus obtained confirm the initial presumption that the variability recorded in the species is environmental and lacks any genetic basis. However, based on population status of this taxon, it can be concluded that due to increasing anthropogenic activities in the region the taxon is under extreme environmental stress which is evident by its changing morphological characteristics to adapt in accordance to the surroundings.

It comes under the criterion 5 proposed by Geissler *et al.* (1997) that the studied taxon exhibit unique reproductive biology as well a narrow ecological niche. Therefore, the studied species is under immense stress and can be consider as threatened in Bhaderwah region of Jammu and Kashmir.

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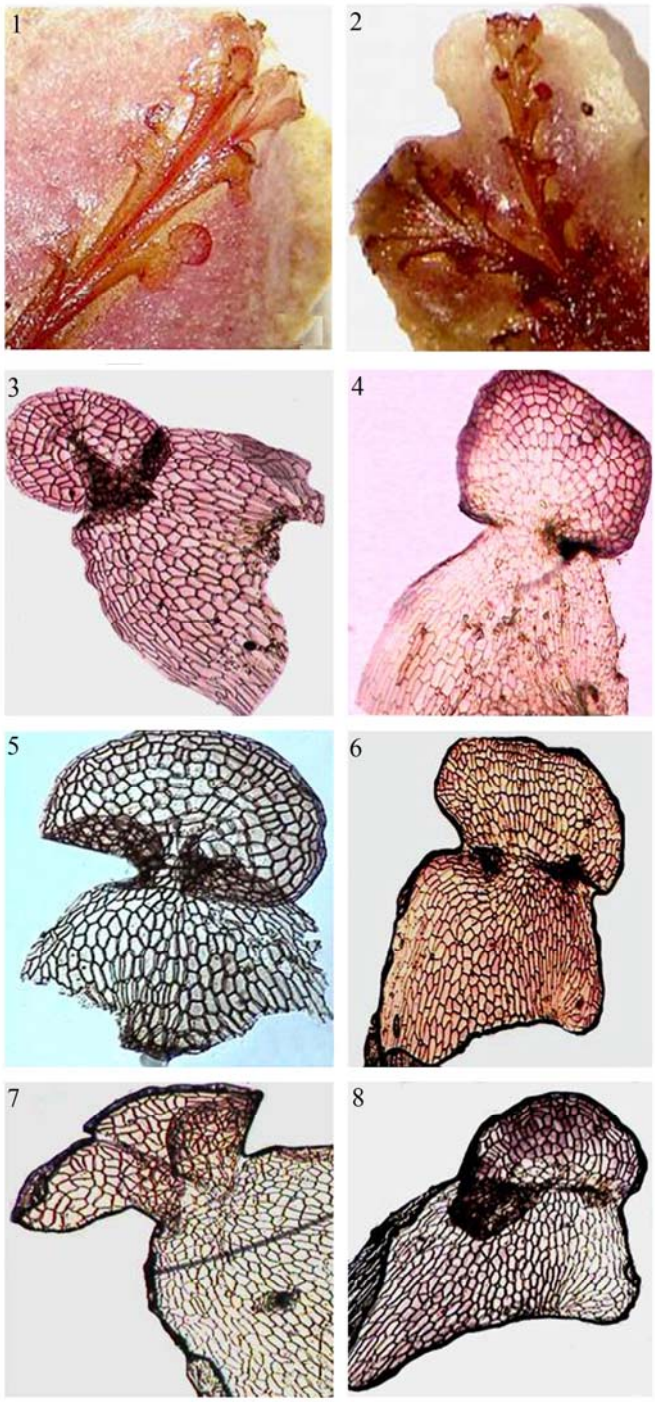

PLATE 1: FIGURES 1-8

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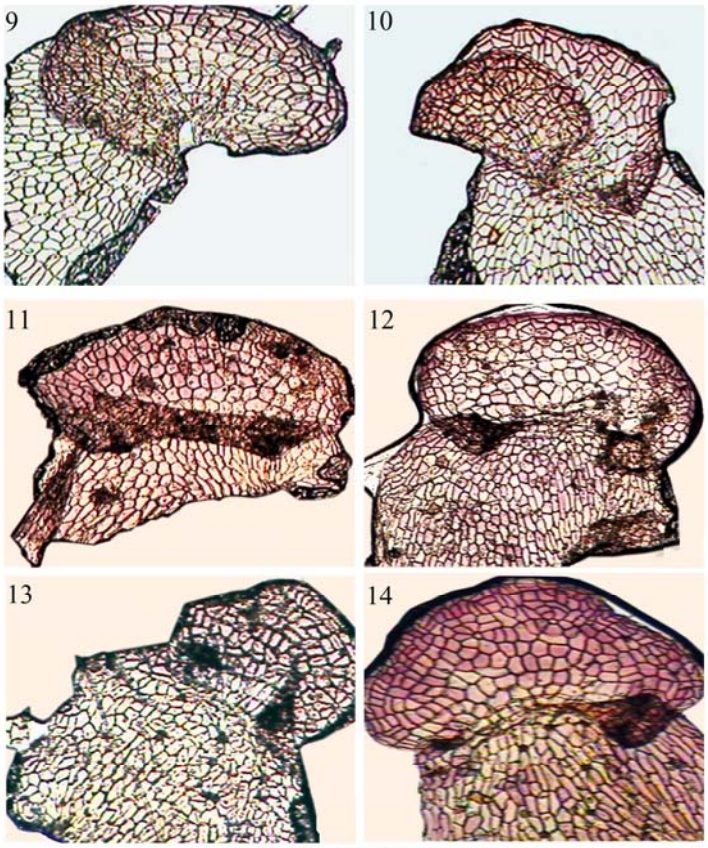

PLATE 2: FIGURES 9-14

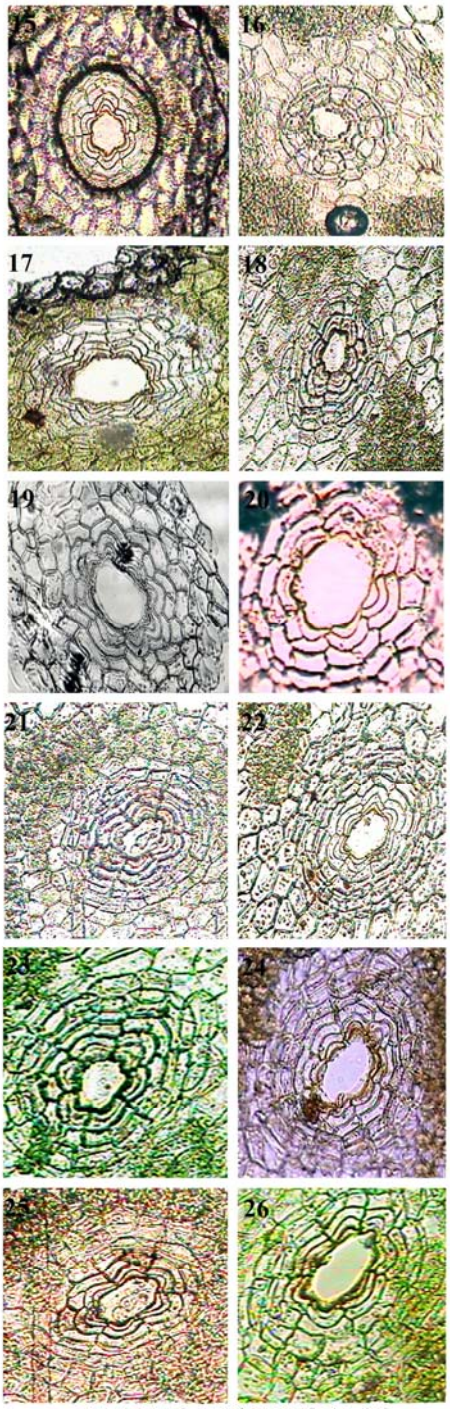

PLATE 3: FIGURES 15-26

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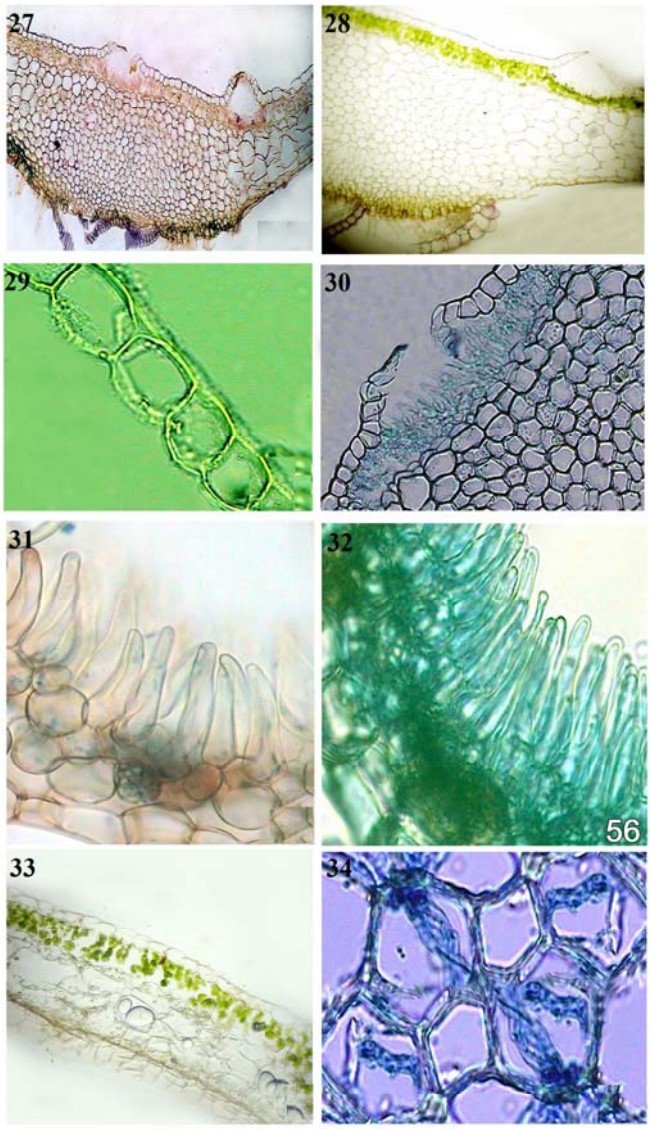

PLATE 4: FIGURES 27-34