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# INDIAN CHEILANTHOID FERN - A NUMERICAL TAXONOMIC APPROACH

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**Abstract**

Twenty one species belonging to five genera (*viz. Aleuritopteris* Fēe, *Cheilanthes* Sw., *Doryopteris* J. Sm., *Notholaena* R. Brown, *Pellaea* Link.) of the Indian cheilanthoid ferns were studied to develop the new data set of micromorphological details *viz.* epidermal cells, stomatal morphotypes, venation pattern and spore ultrastructre. Cluster analysis was performed by using the two- state of multiple characters that separate the genus *Aleuritopteris* from *Cheilanthes* at the Eucladian distance of 5.1, though completely linked with other closely related genera, *viz. Doryopteris, Notholaena* and *Pellaea.* The taxonomic conundrum lies within these genera was resolved with numerical taxonomic study.

## Introduction

Cheilanthoid ferns form an evolutionary group with the strong tendency to be confined in the three large continental/archipelago land areas of America, Africa and Asia-Malaysia. The centre of diversity of the genera is in America and especially in Mexico, where about 100 species form the richest xeric fern flora in the world (Tryon and Tryon, 1982). In India, the distribution range is very wide from the altitudinal variations of plains (100m) to the slopes and small pockets of Himalaya (3000 m.), Nilgiri and Palni hills of South (Nayar, 1962; Dixit, 1984; Pande and Pande, 2003; Sen and Mukhopadhyay, 2011). The group is characterized by the sporangia on the abaxial side of the lamina, covered or not by a marginal pseudoindusium without veins, sporangia approximate in sori or soral lines, stipes at the base with one vascular bundle, sometimes with two, lamina farinose or efarinose, stems with scales, rarely with hairs, base chromosome no. n=29 or 30 (Nayar, 1962; Tryon and Tryon, 1990).

*Cheilanthes* and *Aleuritopteris* are old and phylogenetically problematical genera (genus 'arduum' of Fee) generally included among the gymnogrammeoid or placed in the Cheilanthaceae (family *nov*.) by Nayar (1962) or Pteridaceae (Tryon and Tryon, 1982; Smith *et al.*, 2006). Difficulties in identifying discrete generic boundaries among the cheilanthoids have long been attributed to convergent evolution driven by adaptation to arid environment (Tryon and Tryon, 1973). The workers frequently echo the comment on that "there is an obvious need for the development of new data which will give a better insight into the evolutionary lines within the group" (Tryon and Tryon, 1973). And the workers of molecular systematics also inferred for the genus *Cheilanthes* that "it needs redefinition" (Smith *et al*., 2006). Among cheilanthoid ferns the depositions of farina are used widely to delineate section *Aleuritopteris* (presence of farina) from section-*Cheilanthes* (absence of farina) though it breaks at the wider geographical scales (Nayar, 1962; Khullar, 1994). But the potential adaptive significance of the farina has made it a trait of evolutionary interest.

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To redefine *Aleuritopteris* and *Cheilanthes* and also to regenerate new character sets to resolve the ambiguity of their generic status our present study is attempted to focus mainly the micromorphological characters. On the basis of the multiple dataset of two character- state, taxa were clustered to establish the interrelationships that exist among them.

**Materials and Methods**

Detail list of specimens studied are mentioned in the Table 1. For cluster analysis of the 21 taxa studied as many as 9-two state characters (i.e. characters which exist in two alternative forms or states i.e. either present or absent for generic segregation (Table 2); for 12 spp. of *Aleuritopteris*, 43-two state characters (Table 3) and for 6 spp. of *Cheilanthes* 42-two state characters (Table 4), were taken into account to prepare the data matrix. Responses of each taxon to each of these characters were coded in a data matrix as '1' and '0' respectively for two alternative states i.e. presence or absence. The data thus recorded were further utilized in finding the overall similarities or rather the distance between taxa and putting them in clusters using the concept of 'Euclidean Distance' for measuring distance and 'Complete Linkage' for amalgamation or linkage. Statistical analysis was performed using Statistica-6 (Sneath and Sokal, 1973).

Images of the standard character-sets used for the analysis were taken in Leica QWIN 80 microscope and Scanning Electron Microscope, Model No. Japan Hitachi 530.

**Results and Discussion**

The morphometric study was performed to delineate the taxa of the cheilanthoid fern at the generic and infrageneric level. For numerical taxonomic study the suitable characters used at the generic level are mentioned in the Table 2. For clustering at the infrageneric level used characters are mentioned in Table 3 (Genus *Aleuritopteris*) and Table 4 (Genus *Cheilanthes*) respectively. Figure 4(A-I) and Fig. 5(A-K) shows the contrasting character states as stated in Tables 2-4. Previously, all the works performed by various workers (Nayar, 1962; Tryon and Tryon, 1982; Khullar, 1994) have given much importance to the farina character, which is a potential synapomorphy and have some evolutionary interest (Sigel *et al*., 2011). But, the wholesome approach of character is giving a better clue of separation at both the generic and infrageneric level (Sen, 2014). The works at the molecular phylogeny also found some dispute for this group when the regional basis of works was performed (Gastony and Rollo, 1995, 1998; Zhang, 2007).

On the basis of phenetic study of the 5 genera, close relation or affinity with each other was noticed. The genus *Aleuritopteris* Fee and *Notholaena* R. Brown form a group and *Doryopteris* J. Sm and *Pellaea* Link. form another group; these two groups are allied with each other and form a broad group with *Cheilanthes* Sw. One important ambiguity which persisted so long regarding the generic segregation of *Aleuritopteris* from *Cheilanthes* gets some clear clue from this phenetic study in that they are quite apart from each other in phenogram but are linked. The characters as mentioned by Fraser-Jenkins and Dulawat (2009) to distinguish the genus *Cheilanthes* from *Aleuritopteris*, are narrow stipe scale and narrow leaf segment, which are very vague as is evident from our numerical data enlisted (Table 2).

To categorise the infrageneric taxa, phenetic study resolves some ambiguity. Placement of *C. subvillosa* Hook. under the genus *Cheilanthes* is also corroborated by our study as the taxon possesses similarity in generic characters with *Cheilanthes*. The placement of its synonym *Aleuritopteris subvillosa* (Hook.) Ching under the genus *Aleuritopteris* by Fraser-Jenkins and Dulawat (2009) is however not in conformity with the present study.

**Table 1. List of taxa studied is mentioned here. For each taxon only one specimen is enlisted.**

| Sl.No. | Name of the taxa | Herbarium details |
|---|---|---|
| 1. | *Aleuritopteris albomarginata* (Clarke) Ching | 52761, 02.05.1975,R.D.Dixit,Takdah -Athmal Reserve, Darjeeling, West Bengal, 9125(CAL); |
| 2. | *A. anceps* (Blanford) Panigrahi | 59343,27.03.1985,B. Ghosh and S.R. Ghosh, K.T. Road,950 m. Manipur, CAL |
| 3. | *A. argentea* (Gmel.)Fee | Zwa-Kabru, 6958 (CAL). |
| 4. | *A. bicolor* (Roxb.) Fraser-Jenkins | KS – 183, 12.10.2012; Kakali Sen, Almora, |
| 5. | *A. bullosa* (Kunze) Ching | 1878, Zy. King, Nilgiri Hills,(CAL); |
| 6. | *A. chrysophylla* (Hook.) Ching | 08.08.1892, G.A. Gammie,Lachung, Sikkim , 7136 (CAL) |
| 7. | *A. doniana* S.K.Wu | KS -146, 06.10.2010, Kakali Sen Dello Kalimpong(BURD) |
| 8. | *A. formosana* (Hayata)Tagawa | KS -149,06.10.2010,Kakali Sen,Dello, Kalimpong(BURD) |
| 9. | *A.grisea* (Blanf) Panigr. | 15205, Feb. 1972, Panigrahi,Bilaspur, M.P. (CAL). |
| 10. | *A. rufa* (Don) Ching | KS -204,09.10.2012, Kakali Sen, Samla Tal, Tanakpur, Uttarakhand(BURD) |
| 11. | *A. subargentea* Ching *ex* Sk. Wu | July, 1904, J. Walton, Sangpo valley, 6952 (CAL) |
| 12. | *A. subdimorpha* (C.B.Clarke and Baker) Fraser-Jenk. | KS -157,08.10.2010, Kakali Sen, Bhusuk, Gangtok(BURD) |
| 13. | *Cheilanthes acrostica* (Balbis) Tod | 02.01.1986, B.P.Uniyal Archi, Jammu and Kashmir, 80379, (CAL); |
| 14. | *C. belangeri* (Bory) C.Chr. | 21.10.1952, Rev. B. Godfrey, 7088, N.Lushai Hills, Assam (CAL); |
| 15. | *C. keralansis* Nair and Ghosh. | 49442, 29.07.1977, A.N. Henry, Kanyakumari, Keeriparai(CAL) |
| 16. | *C. mysorensis* Wall. ex. Hook. | 09.11.2001, P. Amrutalakshmi, Nellore, Andhrapradesh, 25119 (CAL); |
| 17. | *C. subvillosa* Hook. | 466, Tamilnadu, 9048 (CAL) |
| 18. | *C. tenuifolia* (Burm.) Sw. | 8614, 24.09.81, M.K. Mama and U.P. Samaddar, Netarhat, Palamau Dist., Bihar, 936 (CAL); |
| 19. | *Doryopteris concolor* (Langsd. and Fisch.) Kuhn | 04.11.1996, Sanchita Gangopadhyay, Kodaikanal (BURD); |
| 20. | *Notholaena marantae* (L.)Desv. | 3673, 18.09.1984, J.F.Duthie (BURD); |
| 21. | *Pellaea bovinii* Hook. | 7671, December, 1910, A. Meeblod, $6000^{ft,}$ Devicolani, S. India, 13487 (CAL). |

**Table 2. Characters taken for Generic segregation of cheilanthoid ferns by cluster analysis.**

| Characters taken | Character State | |
|---|---|---|
| | (0) | (1) |
| 1. Indusium | absent | present |
| 2. Farina | absent | present |
| 3. Leaf texture | coriaceous | membranous or herbaceous |
| 4. Pinna | sessile | stalked |
| 5. Vascular commissure | absent | present |
| 6. Indument on leaf surface | absent | present |
| 7. Pinna dissection | unipinnate leaf present | always more than 1-pinnate |
| 8. Pinna margin | dissected | entire |
| 9. Non-perinate spores | present | absent |

**Table 3. Characters taken for cluster analysis of *Aleuritopteris spp.*(infrageneric level).**

| Characters taken | Character State | |
|---|---|---|
| | (0) | (1) |
| 1. Stipe scale | base | throughout |
| 2. Rachis scale | absent | present |
| 3. Scales | present in costae & costule | absent |
| 4. Rhizome scale | non-clathrate | clathrate |
| 5. Rhizome scale | concolorous | bicolorous |
| 6. Rhizome scale | non-glandular | glandular |
| 7. Stipe scale | non-clathrate | clathrate |
| 8. Stipe scale | concolorous | bicolorous |
| 9. Farina | white | golden Yellow |
| 10. Indusial | margin entire | with fimbriated projections |
| 11. Stomatal type | polocytic | polo- and other type |
| 12. Position of stomata | hypostomatic | amphistomatic |
| 13. Pinnae | opposite | alternate |
| 14. Pinnae | sessile | stalked |
| 15. Lamina shape | lanceolate | boat shaped |
| 16. Lamina | glabrous | indument present (except farina gland) |
| 17. Petiole color | tan | black |
| 18. Venation | open dichotomous | not |
| 19. Vein ending | dilated | Not dilated |
| 20. Dichotomization pattern | ≤3 | >3 |
| 21. Vein goes | upto the margin | not |
| 22. Position of sorus | sorus at vein tip | some distance away from tip |
| 23. Epidermal cell surface | convex | concave |
| 24. Epidermal cellwall width | ≥5µm | <5µm |
| 25. Guard cell length | ≥30µm | <30µm |
| 26. Rhizome scale length | ≥4mm | <4mm |
| 27. Rhizome scale width | ≥0.5 | <0.5 |
| 28. Stipe scale length | ≥4 | <4 |
| 29. Stipe scale width | ≥0.5 | <0.5 |
| 30. Stipe/rachis length ratio | ≥1 | <1 |
| 31. Blade width | ≥5cm | <5cm |
| 32. Length/width ratio basalmost pinna of basal segments | ≥4 | <4 |
| 33. Length/width ratio median pinna of basal segments | ≥3 | <3 |
| 34. Length ratio of acroscopic/basiscopic segments of basal pinna | ≥0.5 | <0.5 |
| 35. Width ratio of acroscopic/basiscopic segments of basal pinna | ≥0.6 | <0.6 |
| 36. Spore Dia (P) | ≥30µm | <30µm |
| 37. Spore Dia (E) | ≥50µm | <50µm |
| 38. Exine thickness | ≥2µm | <2µm |
| 39. Laesural (L)longest arm | ≥25µm | <25µm |
| 40. Crassimarginate/tenuimarginate | Crassimarginate | tenuimarginate |
| 41. Perine | not cristate | cristate |
| 42. Tapetal depositions | absent | present |
| 43. Perisporic strands | present | absent |

**Table 4. Characters taken for cluster analysis of *Cheilanthes spp.*(infrageneric level).**

| Characters taken | Character state | |
|---|---|---|
| | (0) | (1) |
| 1. Stipe scale | base | throughout |
| 2. Rachis scale | absent | present |
| 3. Scales | present in costae & costule | absent |
| 4. Rhizome scale | non-clathrate | clathrate |
| 5. Rhizome scale | non-glandular | glandular |
| 6. Stipe scale | non-clathrate | clathrate |
| 7. Stipe scale | concolorous | bicolorous |
| 8. Farina | white | golden Yellow |
| 9. Indusial margin | entire | with fimbriated projections |
| 10. Stomatal type | polocytic | polo and other type |
| 11. Position of stomata | hypostomatic | amphistomatic |
| 12. Pinnae | opposite | alternate |
| 13. Pinnae | sessile | stalked |
| 14. Lamina shape | lanceolate | boat shaped |
| 15. Lamina | glabrous | indument present (except farina gland) |
| 16. Petiole color | tan | black |
| 17. Venation | open dichotomous | not |
| 18. Vein ending | dilated | Not dilated |
| 19. Dichotomization pattern | ≤3 | >3 |
| 20. Vein goes | upto the margin | not |
| 21. Position of sorus | sorus at vein tip | some distance away from tip |
| 22. Epidermal cell surface | convex | concave |
| 23. Epidermal cell wall width | ≥1µm | <1µm |
| 24. Guard cell length | ≥40µm | <40µm |
| 25. Rhizome scale length | ≥4mm | <4mm |
| 26. Rhizome scale width | ≥0.5 | <0.5 |
| 27. Stipe scale length | ≥4 | <4 |
| 28. Stipe scale width | ≥0.5 | <0.5 |
| 29. Stipe/rachis length ratio | ≥1 | <1 |
| 30. Blade width | ≥5cm | <5cm |
| 31. Length/width ratio basalmost pinna of basal segments | ≥4 | <4 |
| 32. Length/width ratio median pinna of basal segments | ≥3 | <3 |
| 33. Length ratio of acroscopic/basiscopic segments of basal pinna | ≥0.5 | <0.5 |
| 34. Width ratio of acroscopic / basiscopic segments of basal pinna | ≥0.6 | <0.6 |
| 35. Spore Dia(P) | ≥30µm | <30µm |
| 36. Spore Dia(E) | ≥50µm | <50µm |
| 37. Exine thickness | ≥2µm | <2µm |
| 38. Laesural (L)longest arm | ≥25µm | <25µm |
| 39. Crassimarginate/tenuimarginate | crassimarginate | tenuimarginate |
| 40. Perine | absent | present |
| 41. Tapetal depositions | absent | Present |
| 42. Perisporic strands | present | absent |

The placement of the genera *Hemionitis* L., *Parahemionitis* Panigrahi and *Pityrograma* Link. with cheilanthoid group of ferns (Fraser-Jenkins and Dulawat, 2009) must not be supported as their taxonomic positions were clarified earlier by Smith *et al*. (2006) on the basis of morphology as well as molecular taxonomy in other subfamilies Hemionitidae and Taenitidae respectively.

***Phenetic study***

**Cluster analysis at generic level**

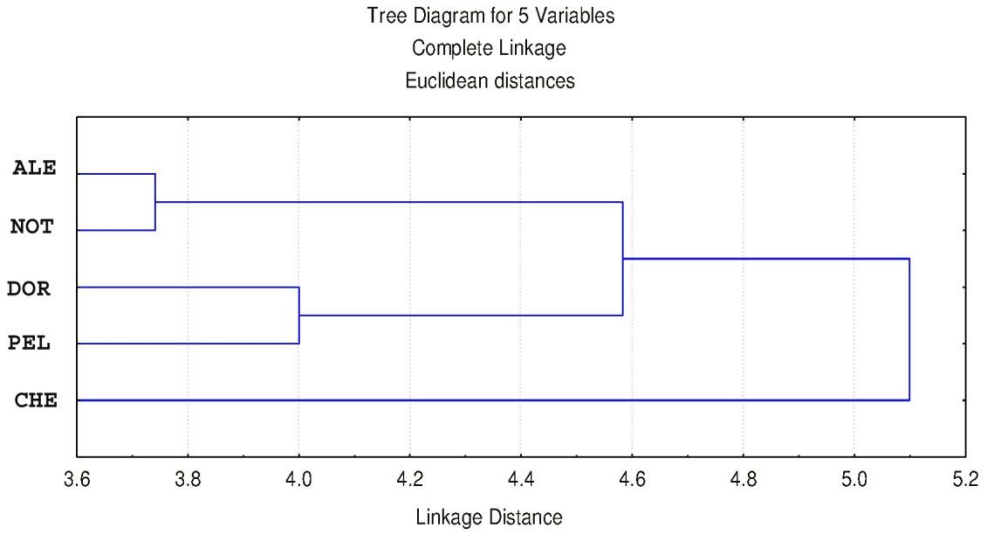


Fig. 1. Shows the relationship of cheilanthoid ferns at generic level (Abbrev.ALE-*Aleuritopteris*; CHE-*Cheilanthes*, DOR-*Doryopteris*, NOT-*Notholaena*, PEL-*Pellaea*).

*Aleuritopteris* (ALE) and *Notholaena* (NOT) have the nearest relation as is revealed from complete linkage at the Euclidean Distance of c.3.7. There is another closely related cluster formed by *Doryopteris* (DOR) and *Pellaea* (PEL) at the linkage distance of 4.0 which in its turn show a relationship with the first cluster at the distance of c.4.6 to form a larger cluster which shows a natural affinity with *Cheilanthes* (CHE) more or less at the Euclidean Distance of 5.1. All the OTUs under study, although individually distinct, are thus moderately related because of their overall similarity at the ED of 5.1

**Cluster analysis at infrageneric level**

Cluster analysis of *Aleuritopteris* spp.

*A. argentea* (AAR) and *A.subargentea* (ASR) have the nearest relation as is revealed from complete linkage at the Euclidean Distance of c.2.8. At the level of the linkage Distance of 3.5 as many as 8 clusters can be recognized of which 4 are with solitary OTUs (Operational taxonomic Unit), *viz. A. rufa* (ARU), *A. chrysophylla* (ACH), *A. formosana* (AFO), *A. subdimorpha* (ASD). However, at the Linkage Distance of 4.5 three large clusters are recognizable, *viz.* AAL, AAN, ARU; AAR, ASR, ACH, ABI, ADO, AFO; ABU, AGR, ASD. The relatedness of the last two clusters mentioned is greater than with the first cluster which is clearly expressed at the distance of 4.7. However all the OTUs under study, although individually distinct, are linked ultimately at the Ed of 4.8 because of their moderate overall similarity.

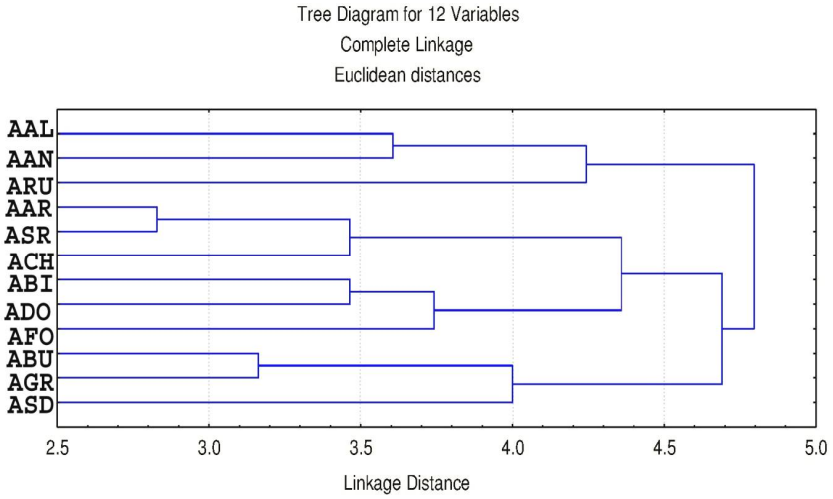


Fig. 2. Shows the infrageneric relationship of *Aleuritopteris* [Abbrev.: AAL- *Aleuritopteris albomarginata* (Clarke) Ching; AAN -*A. anceps* (Blanf.)Panigr. AAR- *A. argentea* (Gmel)Fee; ABI- *A. bicolor* (Roxb.) Fraser-Jenkins; ABU- *A. bullosa* (kze)Ching; ACH- *A. chrysophylla* (Hook) Ching; ADO- *A. doniana* S.K.Wu; AFO- *A. formosana* (Hay.) Tagawa; AGR- *A. grisea* (Blanf.)Panigr*;* ARU- *A. rufa* (D.Don)Ching; ASR- *A. subargentea* Ching ex Wu; ASD- *A. subdimorpha* (Clarke *et* Bak.)Fras.-Jenk.].

Cluster analysis of *Cheilanthes* spp.

*Cheilanthes acrostica* (Balbis)Tod (CAC) and *C. mysorensis* Wall ex.Hook. (CMY) have the nearest relation as is revealed from complete linkage at the Euclidean Distance of c.2.8. These OTUs in their turn show a relationship with *C. belangeri* (Bory) C.Chr. (CBE) at the distance of 4.0. At the same distance are linked *C.keralensis* Nair and Ghosh (CKE) and *C. subvillosa* Hook. (CSU). However, this cluster shows affinity with *C.tenuifolia* (CTE) more or less at the Euclidean Distance of 4.5. All the OTUs under study, although individually distinct, are related because of their overall similarity getting linked slightly above the Ed of 5.0.

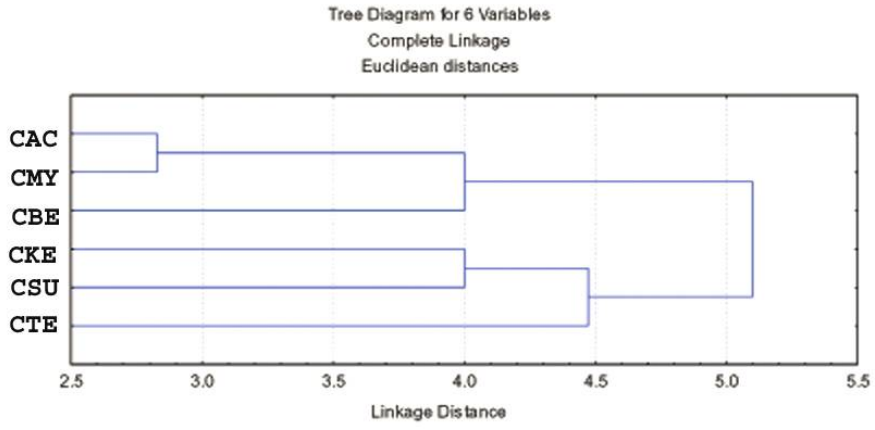


Fig. 3. Shows the infrageneric relationship of *Cheilanthes* (Abbrev.: CAC- *Cheilanthes acrostica,* CBE- *C. belangeri,* CKE- *C. keralensis,* CMY- *C. mysorensis,* CSU- *C. subvillosa,* CTE- *C. tenuifolia*).

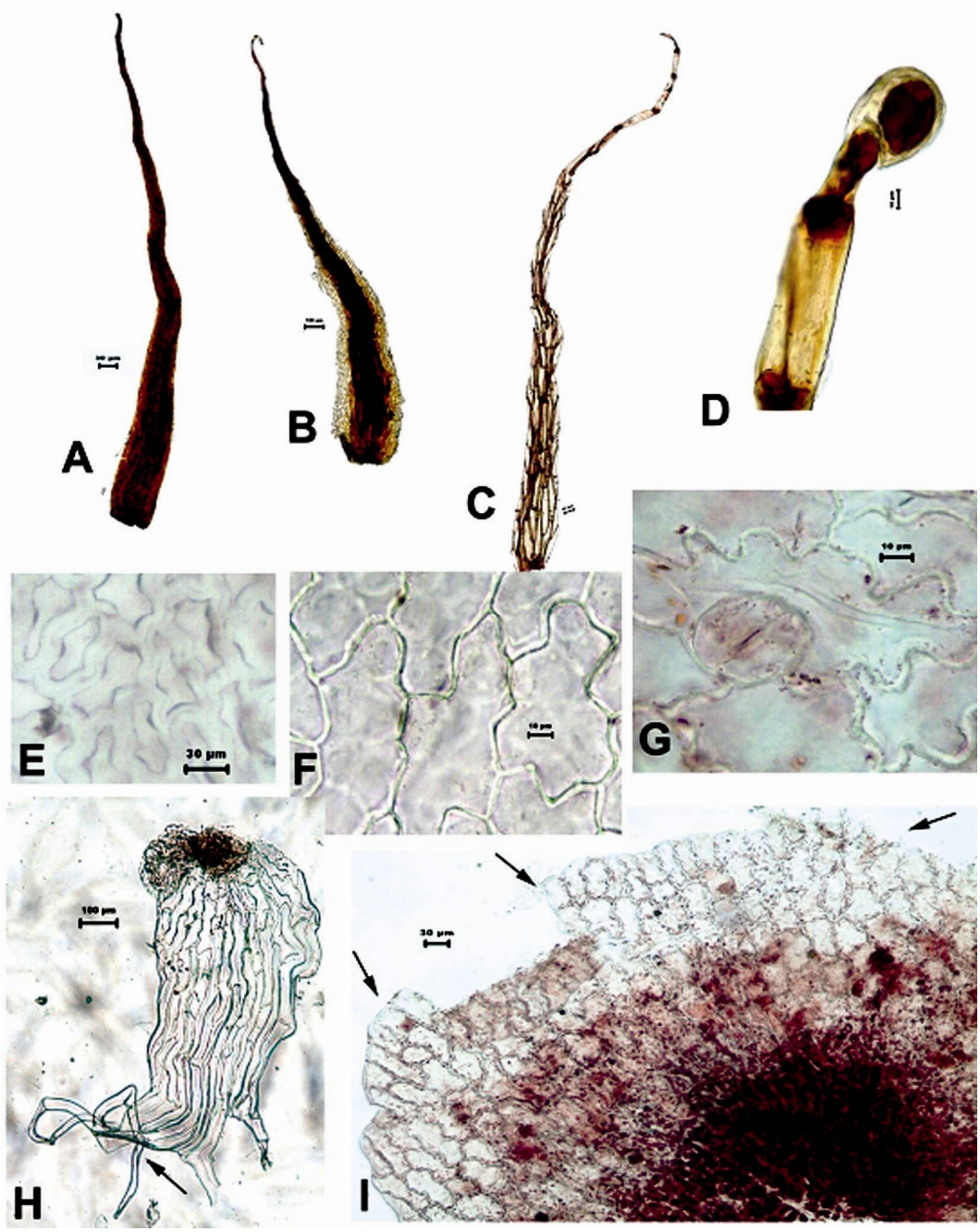


Fig. 4. LM images of different characters used in cluster analysis. A) concolorous scale of *Aleuritopteris subdimorpha* B)bicolorous scale of *A. formosana* C) clathrate non-glandular scale of *A. rufa* D) Glandular scale tip-*Cheilanthes mysorensis* E) epidermal cell-*A. chrysophylla* F) epidermal cell-*C. keralensis* G) polocytic stomata-*C.acrostica* H) fimbriated indusial margin-*A.rufa* (arrowhead shows the fimbriated margin) I) entire indusial margin-*A. chrysophylla* (arrowhead shows the entire margin).

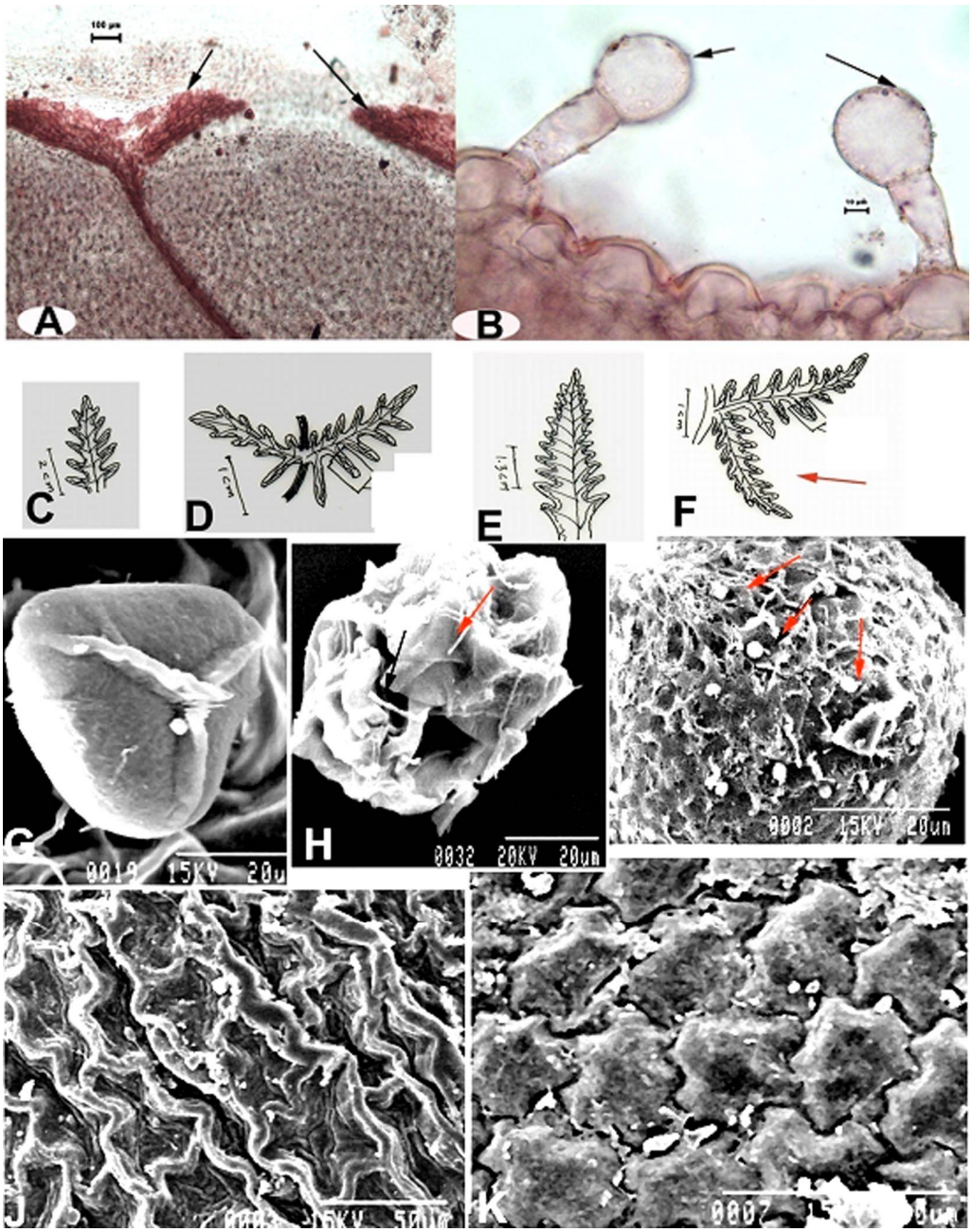


Fig. 5. LM (A-B) , Free hand drawing (C-F) & SEM (G-K) images of characters used in cluster analysis. A) Commissural vein of *Doryopteris concolor* (arrowhead shows the marginal joining of veins)B) leaf gland of *Cheilanthes keralensis* C-D)ultimate & basal segment of leaf – *Aleuritopteris albomarginata* E-F) ultimate & basal segment of leaf- *A.bicolor* G) non-perinous spore-*C. tenuifolia* H) perisporic strands-*Pellaea falcata* I)tapetal deposits-*A.chrysophylla*(arrowhead shows the globular deposits) J) convex epidermal surface(adaxial)-*A. bullosa* K) concave epidermal surface(adaxial)-*A.formosana.*

The dendrogram based on phenetic study clearly revealed the interrelationships of five Indian cheilanthoid genera of arid region. Despite of their homoplasy of characters (Tryon and Tryon, 1973; Sen and Mukhopadhyay, 2014; Sen, 2014) they can be separated, though linked, clearly by using a multiple sets of characters. Present study is the first report describing the correlation between the cheilanthoid ferns of India at generic and infrageneric level and also establishes the generic segregation of *Aleuritopteris* and *Cheilanthes* by doing numerical taxonomic study.

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