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RESEARCH ARTICLE

New Report of Vessel Elements in *Aleuritopteris* and *Cheilanthes*

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ABSTRACT: Investigations in the tracheary elements of rhizomes, roots and stipes of four cheilanthoid ferns from two genera *viz. Aleuritopteris albomarginata*, *A. bicolor*, *A. rufa* and *Cheilanthes tenuifolia* have been made both through maceration followed by light microscopic study and longitudinal sections through SEM study. Presence of vessel elements has been detected in the rhizomes of these species and roots of all species except *A. albomarginata.* No vessel elements were found in the stipes of any member. The vessel elements were having distinct obliquely elongated endplates mostly provided with compound perforation plate without any pit membrane. Detection of vessel elements in these plants which usually grow in dry habitats justifies their ecological success in rapid uptake of water with limited period of water availability.

KEY WORDS: *Aleuritopteris, Cheilanthes,* ecological success, vessel element.

INTRODUCTION

Cheilanthoid ferns represent the largest group of xerophytic ferns of the world with obscure systematics and hence need redefinition (Smith et al., 2006). Their primary centre of origin is in the Mexico province of tropical America (Tryon and Tryon, 1982). Cheilanthoid ferns are morphologically circumscribed very well by various workers from time to time (Ching, 1940; Nayar, 1962, 1970; Tryon and Tryon, 1982; Khullar, 1994; Fraser-Jenkins, 1997, 2008). Later on, a group of workers (Gastony and Rollo, 1992, 1995; Zhang et al., 2007; Prado et al., 2007; Schuettpelz et al., 2008; Kirkpatric, 2007; Rothfels et al., 2008; Windham et al., 2009) have studied this weird group of ferns cladistically on regional basis which supported the placement of these ferns in the subfamily cheilanthoideae of family Pteridaceae (Smith et al., 2006). The traditional basis for recognition of vessels as opposed to tracheids has been the absence of pit membranes in the perforations of end walls, whereas pit membranes are present in pits of lateral walls (Schneider and Carlquist, 1998). The cells representing intermediate condition between tracheids and true vessels were designated as "presumptive vessels" in a number of advanced ferns (White, 1963; Loyal and Bir, 1991). This was also designated as incipient vessel elements by Schneider and Carlquist (2000) and Carlquist and Schneider (2001) where different grades of porosity of the pit membranes are present in the obliquely horizontal end plates and or lateral perforation plates of the tracheary elements.

Bierhorst (1960) made a general survey of tracheary elements in the pteridophytes through light microscopic study. Previously, maceration of tracheary elements was followed by light microscopic study as the usual method of study of xylem elements. Following this method vessel elements have been detected in a scattered way by various workers in the roots and or rhizomes of some unrelated members like *Pteridium aquilinum* (Russow, 1872; Bliss, 1939) isophyllous members of *Selaginella* (Duerden, 1940; Mukhopadhyay and Sen, 1986), some species of *Marsilea* (White, 1961; Mehra and Soni, 1971; Bhardwaja and Baijal, 1977; Loyal and Singh, 1978), *Regnellidium diphyllum* (Tewari, 1975), *Actiniopteris radiata* (Singh et al., 1978), *Adiantum* spp. (Bhattacharya and Mukhopadhyay, 1989), *Pteris* spp., *Christella dentata* and *Ampelopteris prolifera* (Sen et al., 1989) etc. and helped developing the idea that occurrence of true vessels and or incipient vessel elements are not so frequent in pteridophytes. Among the cheilanthoid members, *Notholaena sinuata* has been reported for the presence of incipient vessel elements by White (1963) and later on confirmed through SEM study by Schneider and Carlquist (2000). Through SEM study, following maceration of tracheary elements, Carlquist and Schneider (1997a, b, 1998 a, b, 2000 a, b, c, d) published series of papers and reported vessel members in a number of ferns of different habits. But in a recent paper, Carlquist and Schneider (2007) devised a new technique of studying xylem elements through SEM of hand- razor cut longitudinal sections which were passed through aqueous alcohol grades and air dried. They stated that their earlier findings of tracheary elements of pteridophytes which were prepared through maceration techniques followed by SEM were incorrect except in cases of *Woodsia, Marsilea, Astrolepis* and *Pteridium.* They were of opinion that the macerating fluid might have damaged the pit membranes of tracheids in their earlier preparations, whose presence or absence plays a decisive role in designating tracheary element a tracheid or vessel.

We were interested to know the nature of tracheary elements present in the cheilanthoid members, which grow usually in drier habitats and the ecological role played by these elements in water stressed conditions. As such, we studied four species of cheilanthoid ferns belonged to two genera *Aleuritopteris* and *Cheilanthes*.

MATERIALS AND METHODS

Study site

Four species of cheilanthoid ferns, which fall under 2 genera, were studied for tracheary elements. The members are *Aleuritopteris* (3 spp.) viz. *A. albomarginata* (Clarke) Ching, *A. bicolor* (Roxb.) Fraser-Jenkins, *A. rufa* (D. Don) Ching, and *Cheilanthes tenuifolia* (Burm.) Sw. from different localities (places of eastern and western Himalayas, and local places of the province West Bengal) of India. Voucher specimens were prepared, labeled and have been deposited in the repository of Dept. of Botany, University of Burdwan (BURD).

Provenances of the specimens are mentioned in the Appendix-1 along with the geographical locations and habitat information of collection sites with mean annual rainfall and mean annual minimum and maximum temperatures in Table 1.

Two populations from each species were studied, one from open field (i.e. dry - exposed) and the other from moist shaded (i.e. in more or less close swamp woods) habitat. Mainly the open fileds are Ajodhya Hills, Purulia; and the exposed slopes of Karseong, West Bengal. Due to much more temperature fluctuations and low rainfall dry environment is created in Ajodhya Hills. Whereas Karseong area receives more precipitation but due to high wind (prevailing in) the exposed slopes of hills become dry. Moist shady habitats of Darjeeling, Almora (possess low rainfall) and Durgapur are maintained as these plants grow below the shaded canopy of woody trees where sunlight is low.

Methodologies

Study of the tracheary elements was done following two methods. In the first case standard method of maceration (Johansen, 1940) followed by light microscopic study was done and in the second method samples were not macerated. Instead, only hand-razor cut longitudinal sections were passed through aqueous alcohol grades and air-dried samples were placed on stubs, sputter coated with gold and examined with a Hitachi SEM (model no.530) (Carlquist and Schneider, 2007).

Following maceration procedure, three slides were prepared from each organ (root, rhizome and stipe) of each species and measurements of tracheary elements were taken at random from those prepared slides. The tissue somewhat distal in position from the apical parts and the portion of roots adjacent to the rhizome were taken for study. Mean and standard deviations of tracheids and incipient vessels length given here are the means of 25 readings taken.

RESULT

In the present survey, members of the cheilanthoid fern groups were mainly focused to search the divergence of tracheary elements present in the xylem of root, rhizome and stipe. All these four species studied, possessed incipient vessel elements in their rhizomes. Roots of all the species except *A. albomarginata* were found to have incipient vessel elements and reported for the first time in this paper. No vessel elements were noted in the stipes in any of these species.

The species containing the incipient vessel members are described below with a comparison with the tracheids. The mean length of incipient vessel members and tracheid; number of scalariform bars per endplate; length and width of endplates were measured. The average longest tracheid was found in the stipe of *A. rufa* (5.01±2.53mm) and average smallest tracheid was noted in the rhizomes of *A. bicolor* (0.73±0.27mm). The average longest endplate was noted also in the rhizome of *Aleuritopteris bicolor* (112.48±11.34 µm). Number of scalariform bars per endplate was variable, minimum was found in *C. tenuifolia* root (9) and maximum in *C. tenuifolia* rhizome (52). Species-wise descriptions of tracheary elements of studied taxa are given below.

Aleuritopteris albomarginata

In case of *A. albomarginata,* protoxylem tracheids of root and stipe were provided with single or double spiral secondary thickenings. Metaxylem tracheids were with scalariform alternate and opposite pitting (Fig. 2D). Scalariform bars were dichotomously branched (Fig. 2B). No incipient vessel elements were found associated with tracheary elements of roots and stipes.

In rhizome, protoxylem and metaxylem tracheids were found in association with incipient vessel elements. The protoxylem tracheids were mainly with

Table 1. Geographical position and climatological data of specimens collected from different places of India.

Sample collection site	Geographical position	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperature (minimum) (ºC)	Mean annual temperature (maximum) (ºC)	Site description
Ajodhya hills, Purulia	23°12'55.63"N 86°06'50.77"E	855	1100-1500	8	43	Open habitat
Durgapur,West Bengal	23.55 ºN 87.32ºE	34.2	1320	11	39	Moist shady habitat
Karseong, West Bengal	26.53ºN 88.17ºE	1458	3500	10.1	20.5	Open habitat
Darjeeling, West Bengal	27°01'04.39"N 88°18'35.97"E	2050	2624	8.9	14.9	Moist shady habitat
Almora, Uttarakhand	29.62°N 79.67ºE	1638	270	-3	28	Moist shady habitat

helical thickenings, and the metaxylem tracheids and incipient vessel elements were predominantly scalariformly thickened with opposite pitting. The incipient vessel members of rhizome possess oblique end plate (Fig. 1A) in LM. When the SEM images were taken from the longitudinal sections of rhizome the end plate of vessel members showed absence of scalariform bars and pit membrane in the end plate i.e. simple perforation plate was present here. Only remnants of pit membrane present in the plate (Fig. 3A). But the plate was very inclined. Mean endplate length (L): 39.85±6.55 µm, Width (W): 24.40±1.62 µm.

Aleuritopteris bicolor

In *A. bicolor* root, incipient vessel members were present with other tracheary elements. Protoxylem tracheids with helical thickenings and metaxylem tracheids with scalariform opposite pittings were found. Endplate was a compound perforation plate. No. of bars per endplate 12–38. Mean endplate L: 88.45± 35.87 µm, W:18.60±1.72 µm.

In rhizome, forked tracheids were present, metaxylem tracheids were with scalariform thickenings. Incipient vessel members were with compound perforation plates, scalariform bars were branched (Fig. 1B, Figs. 3B-D) at the end. SEM images revealed that no pit membranes exist at the endplate of incipient vessel members. No. of bar per endplate 21–40. Mean endplate L: 112.48± 11.34 µm, W: 26.05 ±9.25 µm.

Petiole tracheid possesses same type of thickening (Fig. 2F) but no vessel elements were found here. Scalariform bars were branched.

Aleuritopteris rufa

In rhizome, protoxylem tracheids were with helical thickenings. Metaxylem tracheids and vessel elements of rhizome were provided with scalariform pittings in their lateral walls (Fig. 1C, Figs. 3E, F). Compound perforation plate was present. The lateral walls of vessel members contained pit membranes in the pits (Fig. 3E), whereas the endplate was devoid of pit membranes (Fig. 3F). No. of bar per endplate 36–40. Mean endplate L: 93.96± 9.23 µm, W: 21.76 ±1.56 µm.

Root tracheary elements possess the incipient vessel elements with compound perforation plate (no. of bars per endplate 13–42. Mean endplate L: 93.50±35.33 µm, W: 20.63 ±3.73 µm.) and rest of the tracheary element with the helical or scalariform thickening. The vessel elements were absent in the stipe.

Cheilanthes tenuifolia

In case of *C. tenuifolia* root, all the tracheary members with scalariform opposite pittings were provided with spur like projections at the end of the tracheids. The vessel members from root were found to bear very long compound perforation plate (Fig. 1D, Figs. 4 A, B). No. of bars per endplate 9–32. Mean endplate L: 54.42±24.60 µm, W: 14.45±4.95 µm. The lateral walls of the vessel members were found to have residual pit membranes in their pits but the end plate showed no remnants of pit membranes. Vessel elements from rhizome were also found to bear very long compound perforation plate which was devoid of pit membrane. No. of bar per endplate 25–52. Mean endplate L: 84.08±40.29 µm, W: 14.96±4.76 µm.. The scalariform bars of the compound perforation plate present in the roots and rhizomes were branched (Figs. 1D–F). The vessel elements were absent in the stipe.

The pits on the overlap areas were somewhat larger than those of the lateral walls of incipient vessel members. Width of scalariform bars was found to reduce gradually in the end plate. A varied range of endplates or any intermediate forms are absent in our macerated material. Only one type of obliquely inclined end plate was present in all the cases. Study of three replications of each preparation from two habitats were made for confirmation of our observations and to

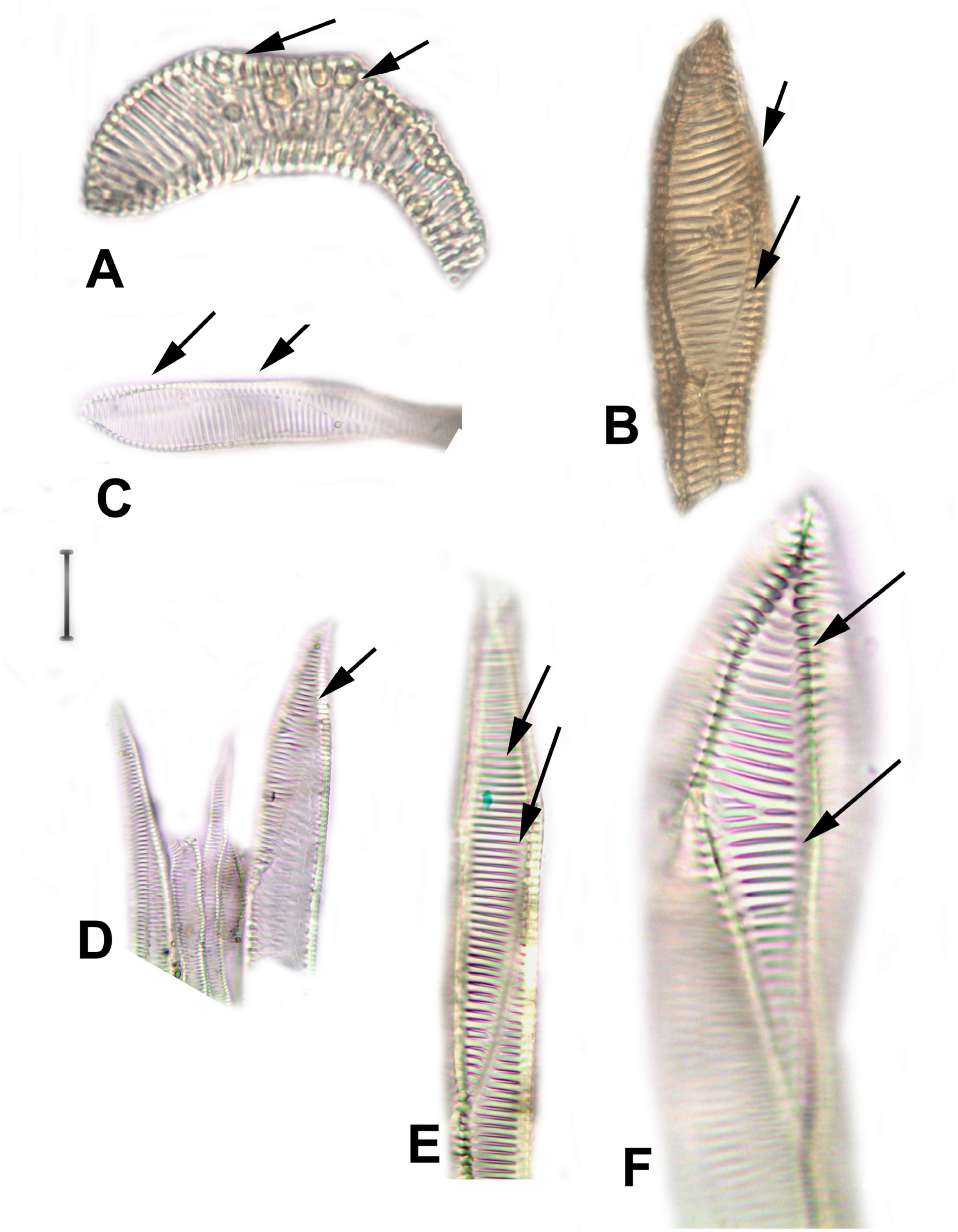

Fig. 1: LM images showing vessel elements from different organs of A: *Aleuritopteris albomarginata* – rhizome. B: *A. bicolor* – rhizome. C: *A. rufa* – rhizome. D: *Cheilanthes tenuifolia* – root. E & F: *C. tenuifolia* – rhizome. (arrows indicate perforation plate; scale bar = 20 μm).

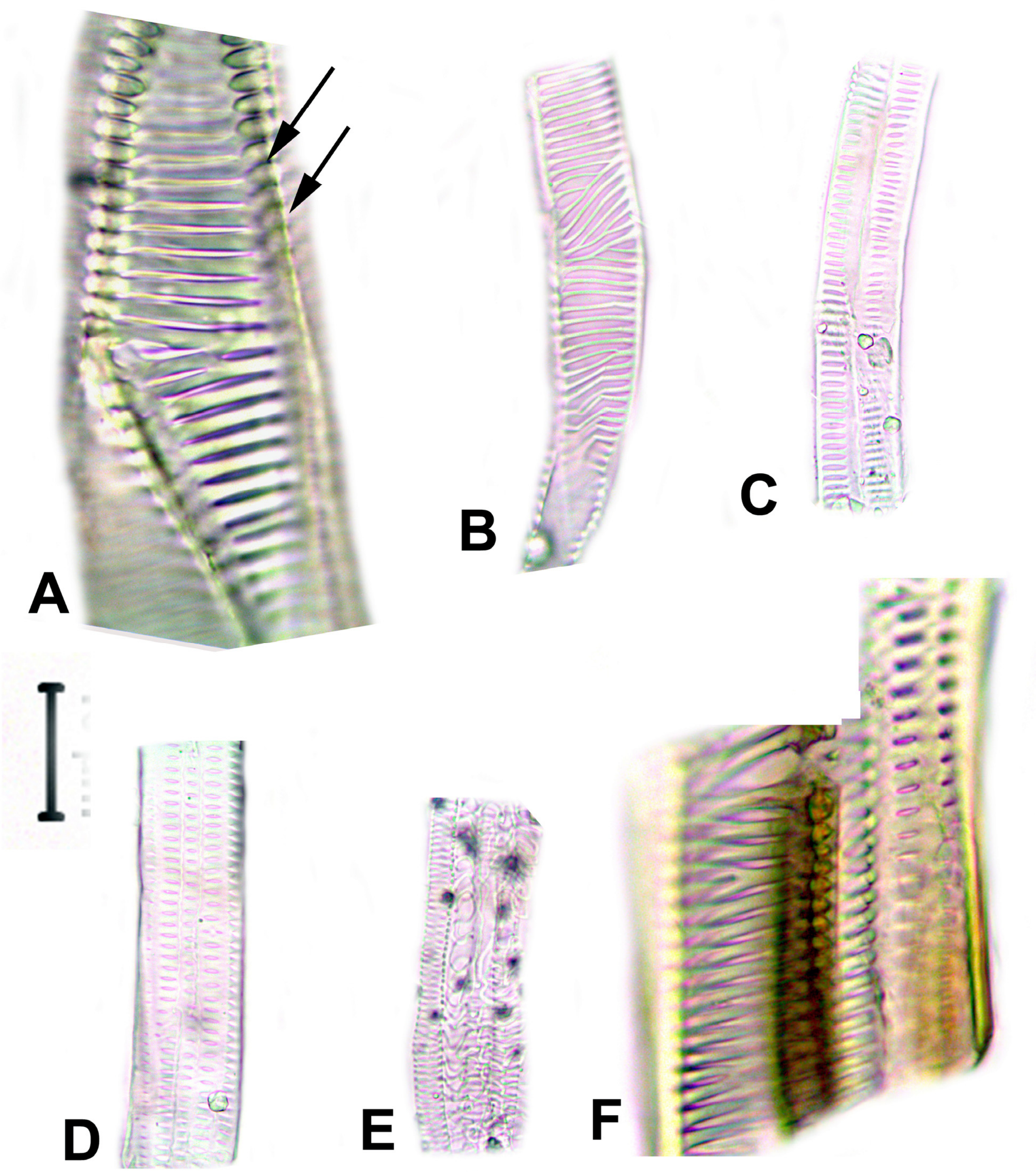

Fig. 2: LM images of A: *Cheilanthes tenuifolia* -vessel element with compound perforation plate from root. (B–E) different types of thickenings in the tracheid wall of *Aleuritopteris albomarginata* stipe. B: Helical to scalariform transition. C: scalariform thickenings. D: Scalariform opposite pitting. E: Protoxylem tracheid with loose and dense helical thickening. F: Tracheids from *A. bicolor* stipe with helical to scalariform transitional thickening and scalariform opposite pitting. (arrows indicate compound perforation plate; scale bar = 10 µm).

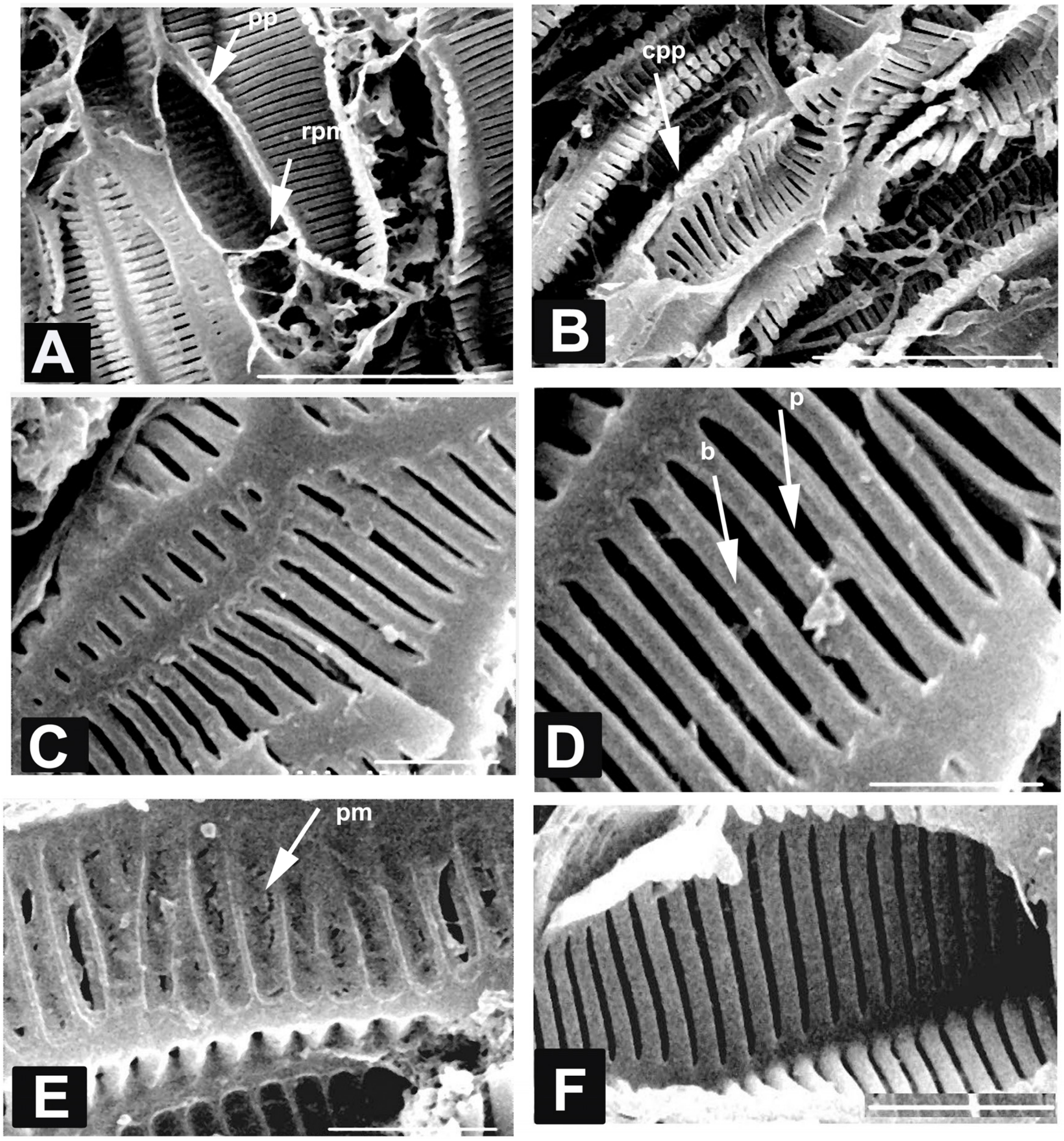

Fig. 3: SEM images showing A: *Aleuritopteris albomarginata* - open perforation plate from rhizome. B: *A. bicolor* - compound perforation plate from rhizome. C: *A. bicolor* - lateral wall of vessel element showing endplate with no pit membrane. D: Same - enlarged view showing scalariform bar and pit. E: *A. rufa* - lateral wall of vessel from rhizome with pit membrane. F: Same – end wall with no pit membrane. (arrows indicate pp – perforation plate, rpm – remnants of pit membrane, cpp - compound perforation plate, b – bar, p- pit, pm- pit membrane; scale bar: A, B = 50 µm, C, D, E =10 µm, F = 20 µm) .

see the length variations due to the ecological environment.

The effect of organographic location and ecological environment when considered on the length of tracheary elements of the studied specimens revealed the following results (see Table 2). Tracheary elements of different plant parts *viz.* root, rhizome and petiole were measured separately. The petiolar tracheids are longest in all the cases except in *A. bicolor*. In this plant, the longest tracheid was found in root (Table 2). In consideration with ecological environment, the taxa studied from moist shady habitat possess longer tracheids than the taxa of open habitat in all the four species studied (Table 2).

DISCUSSION

White (1961, 1963) reported vessel elements with

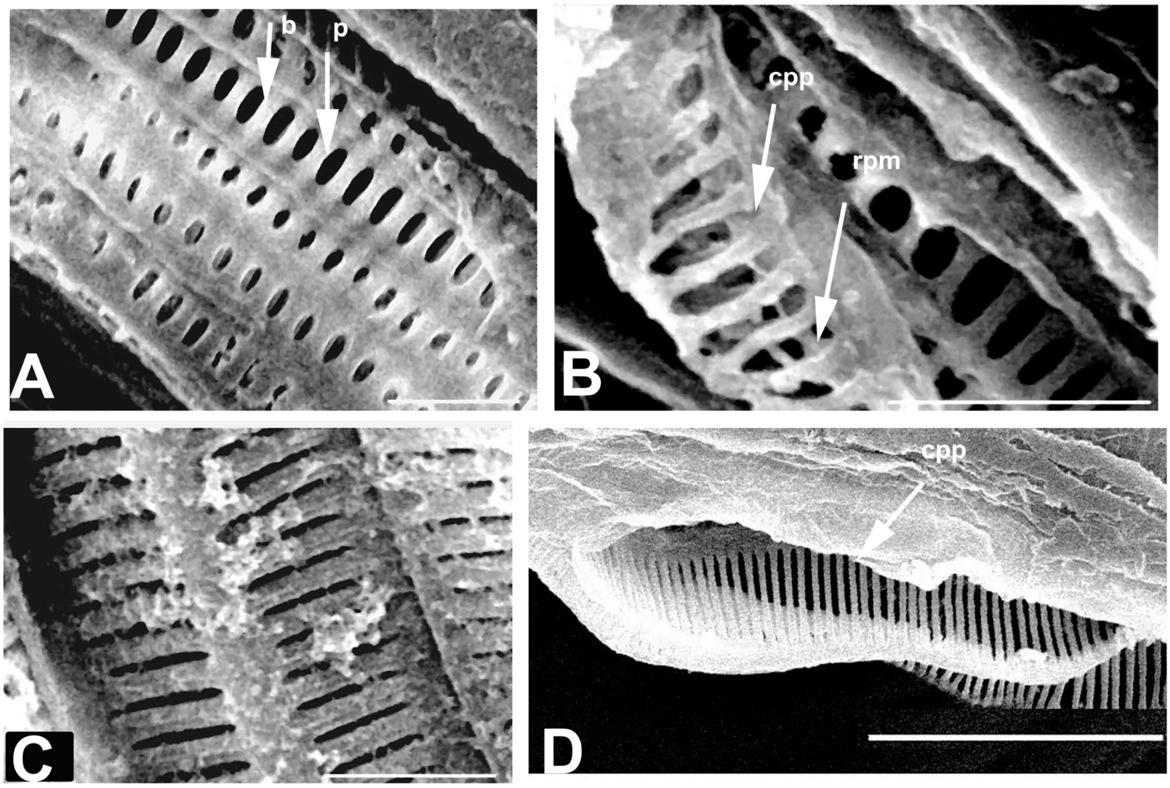

Fig. 4: SEM images of tracheary elements of *Cheilanthes tenuifolia* showing A: Lateral wall of tracheid from root with scalariform opposite pitting. B: Compound perforation plate on the vessel of root. C: Lateral wall of tracheid from rhizome. D: Vessel element with compound perforation plate (arrows indicate b-bar, p-pit, cpp- compound perforation plate, rpm-remnants of pit membrane; scale bar: A–C =10 µm, D = 50 µm).

transverse simple perforated or compound perforated end walls (true vessel members) in *Marsilea quadrifolia*, *M. drummondii* and *M. hirsuta* roots only. In *Pteridium* vessels were reported from rhizome, root and stipe (Schneider and Carlquist, 2000) and from various other genera (Duerden, 1940; Mehra and Soni, 1971; Mukhopadhyay and Sen, 1986; Tewari, 1975; Singh et al., 1978; Sen et al., 1989). As vessel elements were detected from diverse taxonomic groups of ferns and lycophytes, it may not be a reliable indicator of phylogenetic position among ferns (Schneider and Carlquist, 1998). This is the first report of vessel elements in some members of *Aleuritopteris* and *Cheilanthes*, which usually grow in drier habitats but sometimes in moist shady habitat. The selective value of vessel elements in these ferns is presumably facilitation of more rapid conduction during brief periods of water availability (Schneider and Carlquist, 1998). As tracheary elements occur in fascicles in fern steles, there are numerous lateral wall contacts on which perforation plates can develop and function as aggregated vessel elements (Carlquist and Schneider, 2007).

These vessel elements are characterized easily as their lengths are shorter than those of their tracheids and the presence of perforated oblique endplate with scalariform type of pitting (Table 2). Area of endplate and the number of scalariform bars varies in all the cases. Study of size of the tracheids also revealed that the longest tracheids were found in the stipe followed by the roots and the comparatively smallest tracheids were found in the rhizomes. In *A. bicolor* the longest tracheid was found in root (Table 2). It was also found that the taxa growing in open habitats have comparatively smaller tracheid elements than those of taxa growing in moist shady habitats (Table 2).

Among the four species studied, only *A. albomarginata* possesses simple perforated obliquely inclined endwalls and other species possess compound perforation plates in the end walls of vessel elements.

The homoplasy (i.e. the presence of similar features in unrelated groups of plants due to convergent evolution, Tryon and Tryon, 1982; Gastony and Rollo, 1995) and ecological success in the cheilanthoid fern group are also evidenced strongly with this report.

Table 2: The effect of environments on tracheary elements. Almost all the species show gradual decreasing of length of tracheary elements in dry open habitats than the spp. of moist shady habitats (measurement unit in mm). In each case Mean ± S.D. value of length is given.

Name of the taxa	Habitat	Root		Rhizome		Petiole	
		vessel element	tracheid	vessel element	tracheid	vessel element	tracheid
Aleuritopteris albomarginata	Open	-	2.08±0.14	0.21±0.07	0.80±0.03	-	2.62±1.49
	Moist shady	-	2.52±0.62	0.39±0.14	0.84±0.07	-	2.81±0.75
Aleuritopteris bicolor	Open	1.90±0.24	3.27±0.29	0.49±0.21	0.73±0.27	-	2.26±0.08
	Moist shady	2.16±0.24	3.57±1.14	0.53±0.20	0.87±0.14	-	2.43±0.16
Aleuritopteris rufa	Open	2.30±0.13	3.12±0.90	0.27±0.11	1.23±0.15	-	4.33±2.92
	Moist shady	2.43±0.27	3.23±0.89	0.32±0.14	1.24±0.14	-	5.01±2.53
Cheilanthestenuifolia	Open	1.22±0.07	1.50±0.33	0.59±0.13	1.33±0.15	-	2.80±0.55
	Moist shady	1.41±0.07	1.83±0.28	1.03±0.28	1.50±0.05	-	3.04±0.60

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