Ultrastructure of the "cuticular membrane" in two Late Triassic corystospermaceous taxa from India

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Ultrastructure of the cuticular membrane in two types of corystospermaceous pinnae, referred to *Dicroidium gouldii* (=*D. coriaceum* sensu Pal 1984) and *Dicroidium* sp. (=*D. zuberi* sensu Pal 1984) has been studied. It is observed that while the epidermal pattern in the two species shows only minor variation, at the ultrastructure level the cuticular membranes of the two species show significant differences.

Key-words—*Dicroidium*, Cuticular membrane, Ultrastructure, Late Triassic, South Rewa Basin, India.

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सारांश

भारत से दो अनंतिम त्रिसंधी कोरिस्टोस्पर्मेसीय वर्गकों में "उपचर्मीय झिल्ली" की परासंरचना

हरिकृष्ण माहेश्वरी एवं ऊषा बाजपेयी

दो प्रकार के कोरिस्टोस्पर्मेसीय पत्रकों जो *डाइक्रोयडियम गोल्डाई (डा. कोरियेसियम सेन्सु* पाल 1984) एवं *डाइक्रोयडियम्* जा. (*डा. जुबेराई सेन्सु* पाल 1984) से नामाँकित हैं, में उपचर्मीय झिल्ली की परासंरचना का अध्ययन किया गया है और यह प्रेक्षित किया गया है कि उपचर्मीय लक्षण दोनों ही जातियों में कम विभिन्नता प्रदर्शित करते हैं जबकि परासंरचना स्तर पर दोनों ही जातियों की उपचर्मीय झिल्ली विशिष्ट विभिन्नता प्रदर्शित करती हैं।

THE epidermis of aerial parts of terrestrial plants is usually covered with an extra-cellular membrane which protects the plants from dessication, attack of phytopathogens and from its surrounding environment. This membrane, the 'cuticular membrane' or the cuticle, is attached to the outer surface of the epidermis through an intermediate pectinaceous layer (Martin & Juniper, 1970, p. 4). The main structural components of the cuticular membranes of plants are insoluble lipid polyesters - cutin (Holloway, 1982), or insoluble, non-hydrolyzable biomacromolecules - cutan (Tegelaar *et al.*, 1989). The cuticular membrane is made up of an inner cutinized layer (a layer of cellulose encrusted with cutin) and an outer cuticularized layer or cuticle (comprises cutin adcrusted on cell wall). The cuticular membrane or 'cuticle' has a very unique structure and chemical composition which vary considerably according to taxa, and also with developmental stage. The cuticle of extant plants has been investigated extensively at ultrastructural level (see, e.g., Martin & Juniper, 1970; Cutler *et al.*, 1982), but similar work has not been done to any great extent on the cuticle of fossil plants. There, thus, is a great potential for the study of ultrastructure of fossil cuticles. The study may provide data that may prove helpful in the identification, classification and correlation of compression fossils, particularly those of gymnosperms. Some work in this direction has already been done by Archangelsky *et al.*, (1986),

Archangelsky and Taylor (1986), Taylor *et al.*, (1989), Artabe *et al.*, (1991), Artabe and Archangelsky (1992) and Barale and Baldoni (1993).

So far no study has been done on the Indian compression material for the ultrastructure of the cuticle, though monographic studies have been carried out on the cuticular features of leaf fossils, particularly those of gymnosperms from the Gondwana Supergroup.

MATERIAL

The material for the present investigation comprises pinnules of two taxa, possibly belonging to the Corystospermaceae, recovered from bulk maceration of a Late Triassic grey argillaceous shale exposed on the east bank of the Janar Rivulet, about 1 kilometer south-south-west of Harai Village (23°40'53" N: 81°12'40" E, Text-figure 1) in the South Rewa Basin. This shale, placed in the Tiki Formation, is rich in palynofossils (Banerji *et al.*, 1978; Kumaran & Maheshwari, 1980), on the basis of which it is dated as Norian (Maheshwari *et al.*, 1978). Pal (1984) reported gymnospermous compression fossils from this shale, and identified *Lepidopteris madagascarensis, Dicroidium hughesii, D. zuberi, Xylopteris* sp., *Sphenobaiera janarensis* and *Baiera* sp. On bulk maceration we recovered several types of pinnae fragments, two types could be identified with *D. coriaceum* and *D. zuberi* as recorded by Pal (1984); the former species was not recorded by Pal (1984) from this locality.

METHOD

For preparing ultrathin sections, individual pinnules were dissected from pinnae fragments and were treated again with 40 per cent hydrofluoric acid to remove last vestiges of silica. No further acid or alkali treatment was given to avoid any possible alteration in the structural pattern of the cuticles. After thorough washing in water the pinnule compressions were cut into small pieces suitable for processing.

The pieces of the compressions were washed in distilled water and fixed overnight in Gluteraldehyde (4%) prepared in 0.1M Cacodylate buffer (pH 7.2). After fixation the pieces were washed 2-3 times in buffer solution. After postfixation in Osmium tetraoxide (2%), the pieces were washed in distilled water and dehydrated in graded ethanol series, followed by two changes in 100 per cent ethanol and one change in 100 per cent acetone. After dehydration, the compression pieces were passed through various combinations of acetone and Spurr's low viscosity medium (3:1, 2:2, 1:3), each step being of 10-12 hours and included one mid-duration change of the solution. The pieces were then kept in pure plastic Spurr's medium for several days to allow proper infiltration of the plastic in the specimens. Each piece was then placed in the embedding medium in a mould and kept in a preheated oven for polymerization at controlled temperatures.

After polymerization, the blocks were removed from the moulds, trimmed under low power stereomicroscope. Thick sections were first cut for preliminary studies and proper orientation of the specimens. The blocks were accordingly trimmed precisely. 600-700 Å thick sections were cut and picked up on uncoated, 200 and 400 mesh copper grids. The sections were stained in aqueous uranyl acetate for 15 minutes and in lead citrate for 5 minutes.

DESCRIPTION

cf. *Dicroidium gouldii* Retallack 1977

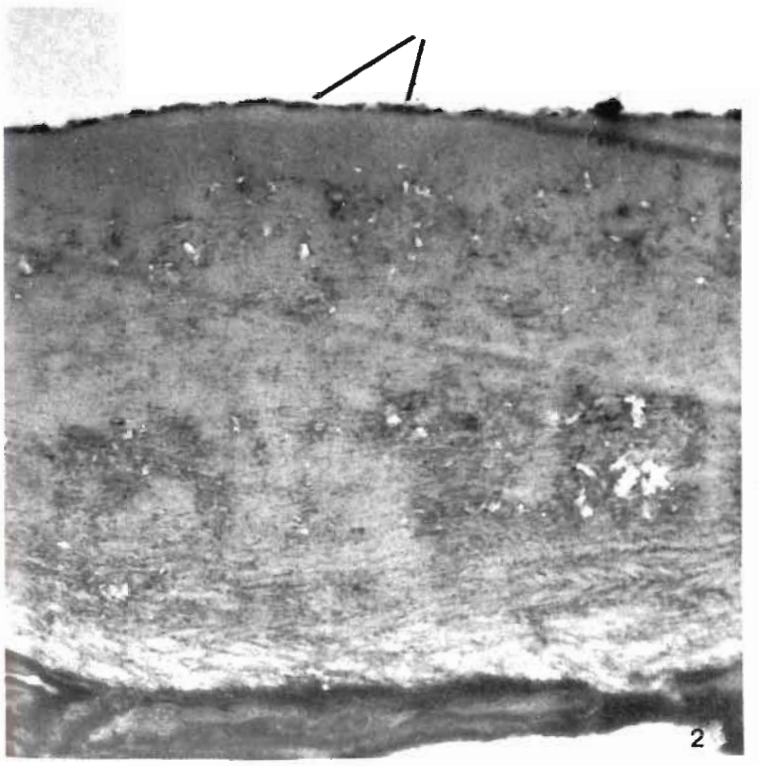
Pl. 1, figs 1-4; Pl. 2, figs 1-6; Text-figure 2B-D

1984 *Dicroidium coriaceum* (Johnston) Townrow: Pal, *Palaeobotanist* **32**:274, pl. 10, figs 82-89, text-fig. 16 A-E.

PLATE 1

Dicroidium gouldii Retallack 1977

1. Cross-section of the cuticular membranes of both the surfaces of a pinnule showing a darkish median tissue representing the palisade and other layers. x 14,200.
2, 4. Cuticular membrane of one of the surfaces showing irregularly distributed osmiophilic bodies (see arrow) at the leaf-air interface, and different zones; the outermost (a) electron dense, and the middle (b) and inner (c) zones fibrillar. 2. x 28,000; 4. x 62,000.
3. The middle (b) and inner (c) zones at higher magnification showing distinct fibrillar nature. In the inner zone the fibrillae acquire a "herring bone" pattern. x 62,000.



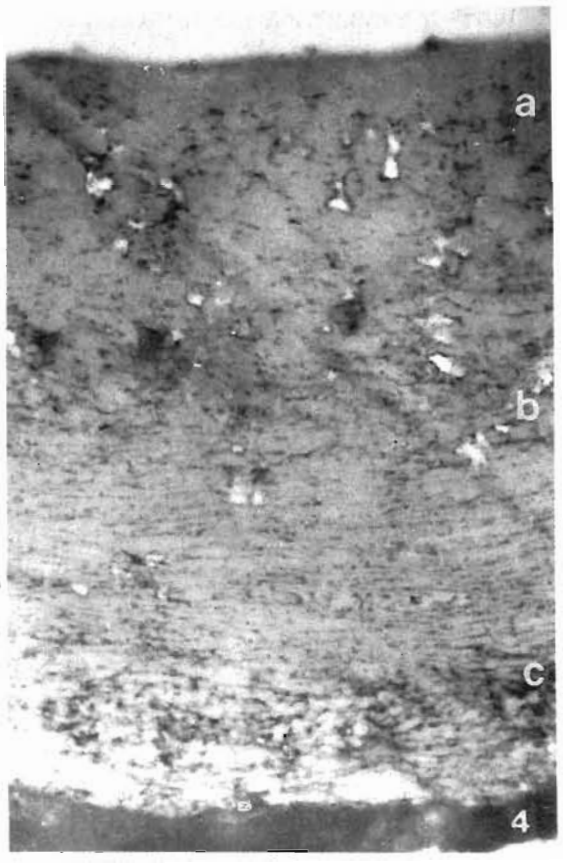
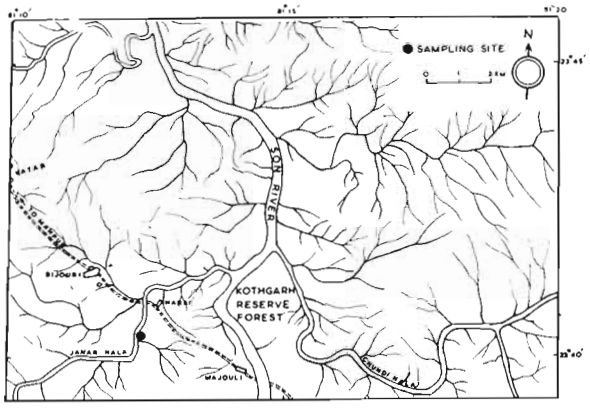


PLATE 1

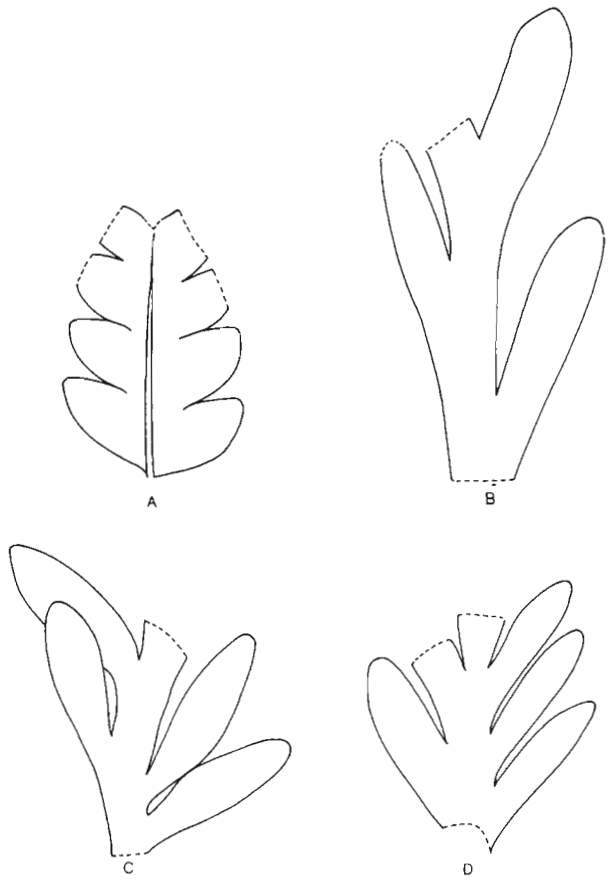


Text-figure 1—Map showing the approximate position of the site from where the fossiliferous sample was collected.

Pinnule morphology—Five fairly large pieces of pinnae were recovered. The pinnules are sub-opposite, closely spaced, oblanceolate in shape, and with odontopteroid venation. The pinnules are amphistomatic, the cuticle of both the surfaces being fairly thick. The stomata are sunken, usually with four subsidiary cells, each cell with a papilla that overhangs the stomatal pore. (for illustrations and detailed description *see* Pal, 1984, p. 274).

Ultrastructure of the cuticle—For reasons of expediency and for ease in processing of the specimens for ultrathin sections, cuticular membranes were not separated from each other and other tissues that are preserved, by nitric acid and/or alkali treatment. Study of thick sections under the light microscope shows that the cuticles of the two surfaces differ in thickness not only from each other, but the cuticle of the same surface shows lateral variation in thickness (Pl. 2, figs 5-6).

In fine structure, both the cuticular membranes are quite heterogeneous (Pl. 1, fig. 1). The cuticular membrane of one of the surfaces shows a polylamellate outer region delineated against an inner, mainly reticulate/fibrillate region (cf. Type 1, Holloway, 1982, p. 12). The polylamellate region is made up of parallel running, alternating, 4-6 electron dense, and 4-7 electron lucent lamellae, thickness of which is not uniform (Pl. 2, figs 1-3). Some of the lamellae are continuous while others run only for a short while (Pl. 2, fig. 4). This polylamellate region, "probably corresponding to the cuticle proper *sensu* von Mohl" (Holloway, 1982, p. 5), is also known as primary cuticle on the basis of TEM studies (Sargent, 1976).



Text-figure 2—A. Line drawing of a pinna fragment referred to *Dicroidium* sp., X 2; **B-D.** Line drawings of three pinnae fragments referred to *Dicroidium gouldii*, X 6.

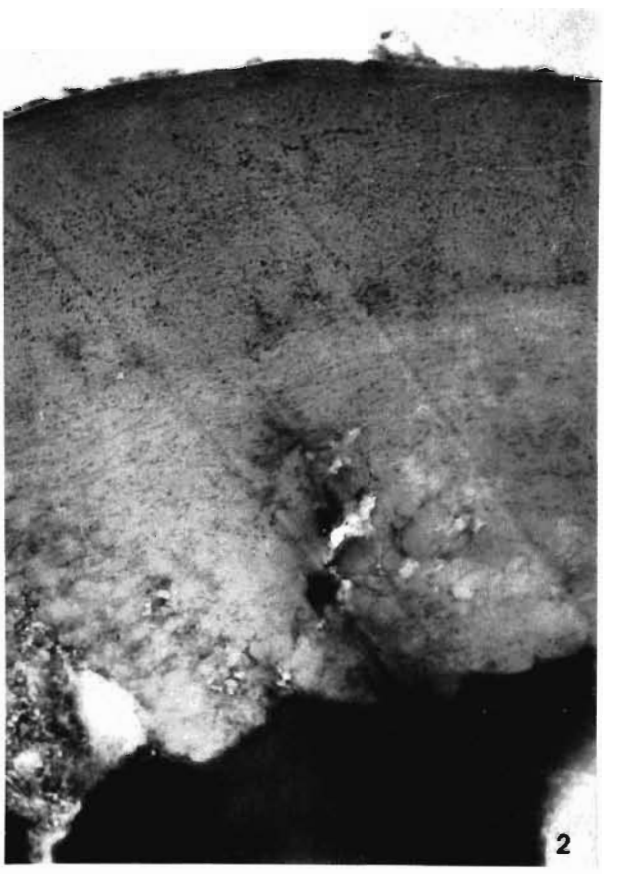
PLATE 2

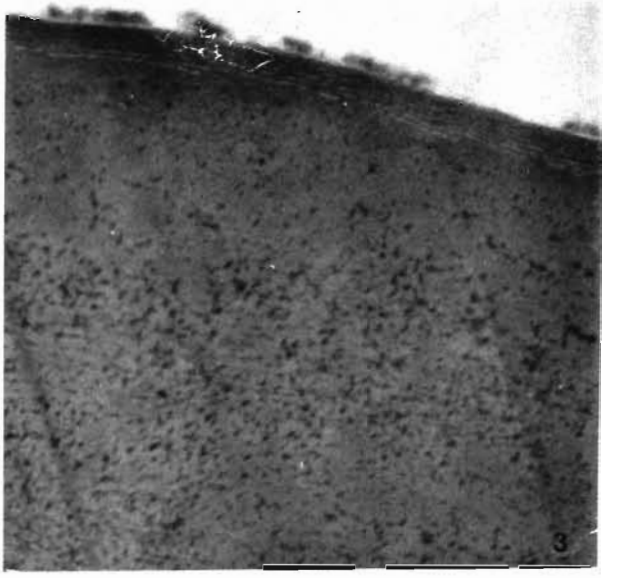
Dicroidium gouldii Retallack 1977

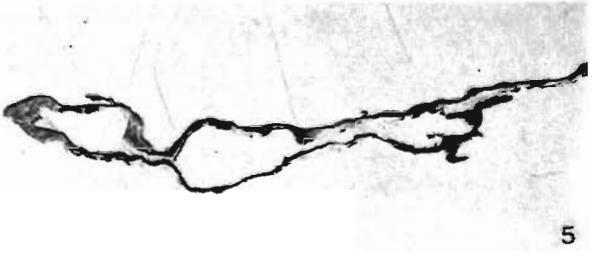
1-2. Fine structure of the other cuticular membrane. This membrane too shows three distinct zones (a, b and c), and an outer irregular deposition of osmiophyllic bodies.
Zones 'b' and 'c' are fibrillar, whereas zone 'a' is relatively homogeneous with 'canaliculate' appearance. Towards the leaf-air interface zone 'a' has a polylamellate layer. 1-2. x 28,000.

3-4. The outer zone (a) magnified to show the nature and number of the lamellae, and the remnants of wax deposit at the cuticle-air interface. 3. x 62,000; 4. x 2,24,000.

5-6. Cross-section of a pinnule as seen under a light microscope. Figure 6 taken under polarised light shows bireferigerence of the cuticle.







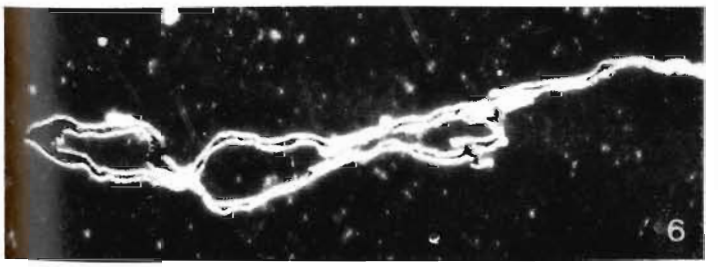


PLATE 2

On the outside of the cuticle proper, at the leaf-air interface, irregular osmiophilic deposits are present, which are possibly the remnants of the epicuticular wax, left after diagenesis.

Next to the polylamellate region is a comparatively thick amorphous region that shows incipient fibrillar components. The fibrillae in this region show osmiophilic deposits because of which it acquires a coarse canaliculate appearance (Pl. 2, figs 3-4).

The innermost zone is thickest of the three zones and is made up of distinct fibrillae. The fibrillae have a "herring bone" appearance and are oriented mainly parallel to the membrane surface (Pl. 2, fig. 1). This zone abuts on to a disformed, darkish tissue that probably is formed of palisade and other tissue of the leaf. At regular intervals the innermost zone forms wedge-shaped outgrowths which are taken to represent the cuticular pegs or anticlinal flanges that penetrate the interwall spaces between adjacent epidermal cells.

The cuticular membrane of the other surface too shows three identifiable zones (Pl. 1, fig. 2). The outermost zone is almost homogeneous and does not show even incipient lamellae. The middle and inner zones have basically the same composition as the other surface except that the fibrillae are seen much more distinctly and the inner zone has a very pronounced "herring bone" look (Pl. 1, fig. 3). Cuticular pegs or anticlinal flanges are formed by this zone, too.

Dicroidium sp.

Pl. 3, figs 1-5; Text-figure 2A

1984 *Dicroidium zuberi* (Sazajnocha) Archangelsky: Pal, *Palaeobotanist* **32:** 257, 275; pl. 3, figs 20-32; pl. 10, figs 90, 91; pl. 11, figs 92, 93.

Pinnae morphology—Only three small pieces of the pinnae were recovered in the bulk macerate. The pinnules are sub-opposite, closely placed, roughly rhomboidal in shape, amphistomatic and with relatively thick cuticle (for illustrations and detailed description, *see* Pal, 1984, p. 258, 275).

Ultrastructure of the cuticle—Cuticular membranes of the two leaf-air interfaces in this taxon differ from each other in thickness and composition. One of the cuticular membranes, which is thinner of the two, is heterogeneous and shows at least 3 recognisable zones (Pl. 3, fig. 1). This CM, which shows much variation in thickness laterally, has an electron dense outer homogeneous zone without any identifiable finer structure (Pl. 3, fig. 3). At places, on the leaf-air interface, this zone has osmiophilic bodies and possibly remnants of epicuticular waxes also. The inner zone is fine reticulate and has a median electron lucent sub-zone bounded on either side by an electron dense sub-zone each of variable thickness. The alveolae in the median sub-zone apparently form layers that are seen as strips in the transverse section. One anticlinal flange (f) is seen in micrograph 1 on Plate 3. Figure 5 shows the cuticular membrane of a small papilla. In this micrograph, the outer extremely narrow osmiophilic zone and the middle homogeneous zone are clearly discernible. The inner zone is not well-preserved; the holes in this zone are areas where the plastic medium has not impregnated. Another dome-shaped protuberance illustrated as figure 4 on Plate 3 has only homogeneous matrix.

The cuticular membrane of the other surface shows two distinct zones, an outer amorphous zone and an inner distinctly reticulate zone (cf. Type 3, Holloway, 1982, p. 15). The inner zone has two subzones, an electron dense and an electron lucent.

DISCUSSION

The two types of pinnae that have been investigated by us for ultrastructure of the cuticular membrane have been placed under the genus *Dicroidium* Gothan with great hesitation. The

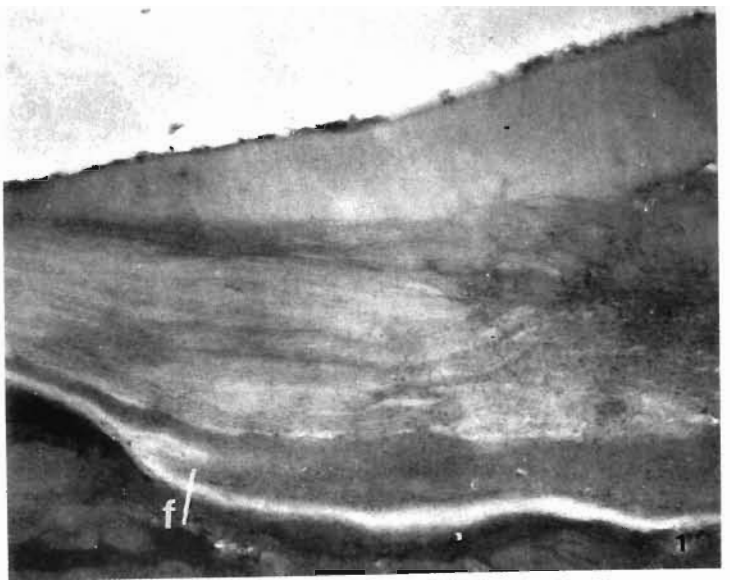
PLATE 3

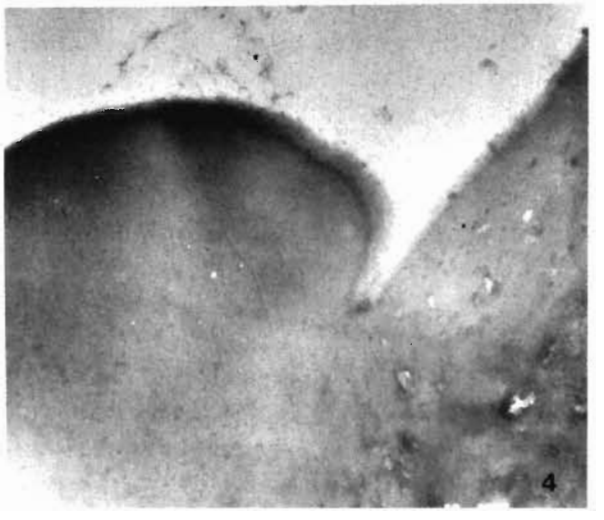
Dicroidium sp.

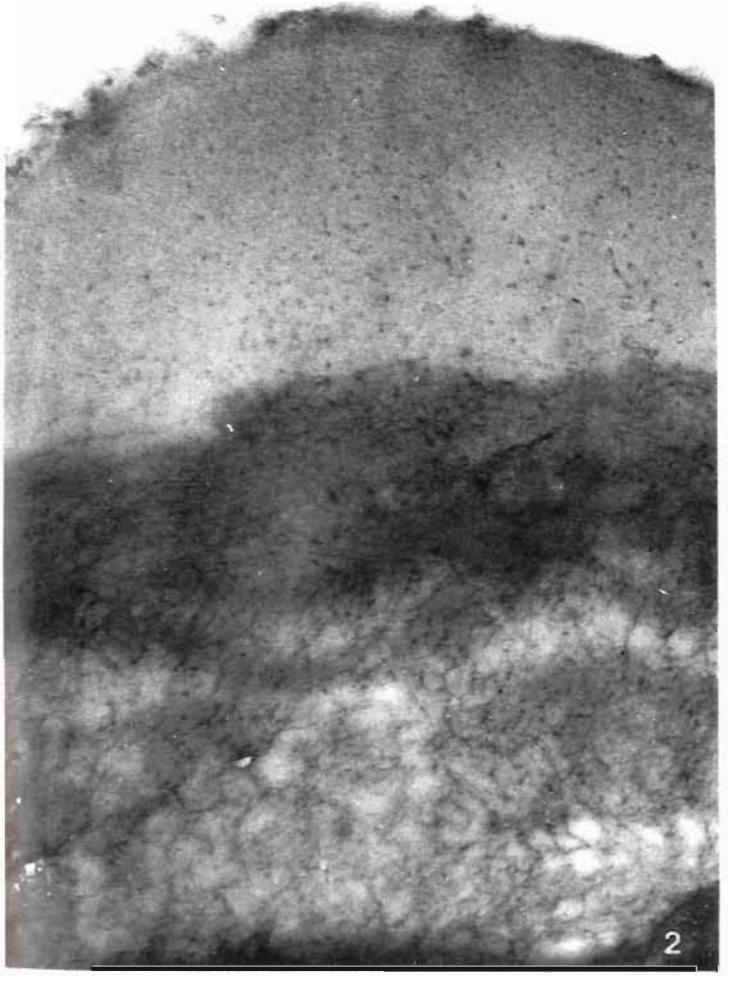
1, 2. Cross-section of the cuticular membrane of one surface showing an outer amorphous zone and inner reticulate zone. An anticlinal flange (f) is also seen in this micrograph. 1. x 33,800; 2. x 42,000.

3. Details of the cuticular membrane of the other side, showing an outer homogeneous zone, and an inner reticulate zone; the inner zone shows an electron dense and an electron lucent subzones, the latter with very clearly defined reticulations. x 42,000.

4,5. Cuticular membrane comprising a papilla and a protuberance, respectively. 4. x 14,000; 5. x 21,000.







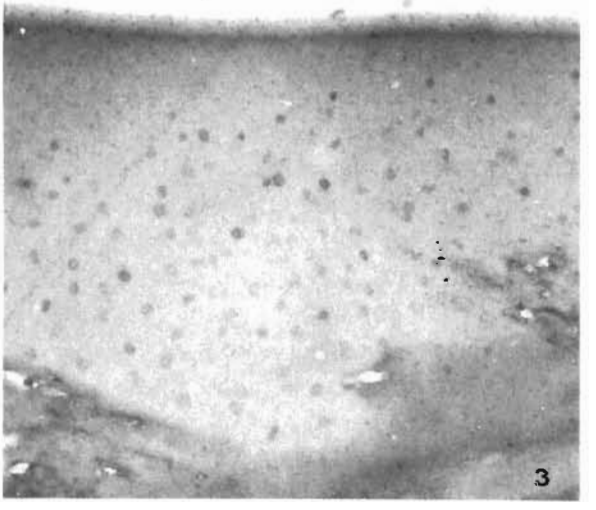


PLATE 3

material available to us, and even to Pal (1984), comprises only small portions of the pinnae, none of which shows dichotomy/forking of the main rachis. Our only excuse to identify the specimens with *Dicroidium* is their epidermal pattern which is on the lines of the genus *Hoegia* (Townrow, 1957), which the later authors merged in *Dicroidium*.

The two taxa reported in the present paper resemble specimens placed under *Dicroidium coriaceum* and *D. zuberi*, respectively by Pal (1984). However, to our consternation we noticed that Pal's identifications may altogether be wrong. The specimens he identified with *D. coriaceum* (Pal, 1984, pl. 10, figs 82-86; text-fig. 16A-C) do not show the distinguishing features of the species, that is, "frond simple, margin entire to slightly sinuate, leaf blade narrow" (Anderson & Anderson, 1983, p. 92, pl. 31, figs 1-6; pl. 76, figs 1-11). This species has also been reported by Jain and Delevoryas (1967, pl. 91, figs 1, 2), Archangelsky (1968, pl. 97, fig. 2), Delevoryas (1973, fig. 9), Retallack (1977), and others. None of the specimens illustrated by these authors show even vague resemblance with Pal's specimens.

On the other hand, the holotype of *D. gouldii* Retallack (='*Dicroidiopsis*' sp. *sensu* Gould, in Bourke *et al.*, 1977, fig. 12) has pinnules that in gross morphology are similar to our specimens. In the absence of data about the epidermal pattern in this named species, we have only tentatively placed our material and Pal's *D. coriaceum* under this species.

The specimens that Pal (1984) identified with *D. zuberi*, too, do not show distinguishing features, particularly of the epidermal pattern, of this species. Pal reports amphistomatic condition with almost similar stomatal frequency on both the surfaces and almost straight-walled epidermal cells, whereas in the authentic material studied by Anderson and Anderson (1983, p. 200, pls 101, 102; fig. 6.4), the stomatal frequency is relatively very high on the lower surface, and the cell walls, particularly those of the lower surface are distinctly sinuous. Evidently Pal's specimens (our's, too) do not fit in within the circumscription of *D. zuberi*. For the present study, we have named these as *Dicroidium* sp.

The epidermal pattern of the two species as reported by Pal (1984) shows only minor variation (table 1). However, observations on the ultrastructure of the cuticular membrane in two specimens assignable to the two species shows significant differences. The most significant difference is the presence of a polylamellate outermost zone of the cuticular membrane in *Dicroidium gouldii*, and the absence of the same in *Dicroidium* sp.

	D. gouldii (=*D. coriaceum* of Pal)		*D.* sp. (=*D. zuberi* of Pal)
1.	Lamina amphistomatic; stomatal frequency same on both the surfaces	1.	Lamina amphistomatic; stomatal frequency more on one surface
2.	Cells isodiametric-polygonal	2.	Cells isodiametric-polygonal
3.	Cell walls straight	3.	Cell walls minutely sinuous
4.	Each cell with a papilla	4.	Each cell with a papilla
5.	Subsidiary cells 4(5)6	5.	Subsidiary cells 4

Holloway (1982) illustrated six types of ultrastructure of the cuticular membrane. The ultrastructure in two species of the genus *Dicroidium* compares with that of Holloway's Types 1 and 3 and no new type of cuticular membrane has been found.

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