

Ultrastructure of the leaf cuticle of *Pachypteris indica* and its comparison with that of *Komlopteris indica*

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ABSTRACT. The ultrastructure of the leaf cuticles of *Pachypteris (Palissyia) indica* and *Komlopteris (Thinnfeldia) indica* was investigated in order to work out the taxonomic relationship between the two genera. Appreciable differences were observed in the cuticle ultrastructures of the two species which suggest that a detailed investigation involving more species of the two genera should be undertaken, in order to determine the exact relationship between them.

KEY WORDS: leaf cuticle, ultrastructure, *Pachypteris*, *Komlopteris*, *Thinnfeldia*, India

INTRODUCTION

The taxonomic status of the genus *Pachypteris* Brongniart 1828 against *Thinnfeldia* Ettingshausen 1852 has been long debated. Andrae (1855) was of the opinion that the leaves of the genus *Pachypteris* were indistinguishable from those of *Thinnfeldia*. Nathorst (1880) agreed with this view. Harris (1964) observed that the subsidiary cells of the cuticle in *Thinnfeldia* were arranged in the form of a collar, while in *Pachypteris* the arrangement was less regular and their number comparatively fewer. But at the same time he considered the difference between *Pachypteris lanceolata* and certain finely divided forms of *Thinnfeldia rhomboidalis* Ettingshausen to be slight, and believed that the two species might be very similar. Doludenko (1969, 1971) firmly believed that the leaves of *Pachypteris* and *Thinnfeldia* did not differ either in external morphology or in epidermal features. She treated *Thinnfeldia* as a younger synonym of *Pachypteris*. Bose and Roy (1968), however, noticed differences in the stomatal apparatuses of the two genera.

Doludenko (1974) observed that the epidermal features of a specimen from the type locality of *Thinnfeldia rhomboidalis* were quite

similar to those of *Pachypteris*, particularly *P. lanceolata* from Yorkshire. She opined that even the genus *Cycadopteris* Zigno 1853 might be a synonym of *Pachypteris*.

Barbacka (1993) argued that even if the specimens of *Thinnfeldia rhomboidalis* from the type locality were similar to those of *Pachypteris lanceolata*, other specimens referred to the former species did not necessarily belong to *Pachypteris*. She therefore instituted a new genus *Komlopteris* to accommodate all these "leaves which, according to their morphology and cuticular structure cannot be assigned either to *Pachypteris* or any other known genus". She also transferred *Thinnfeldia indica* Feistmantel, reported from the Early Cretaceous of the Rajmahal Basin, Bihar (Feistmantel 1877, Bano et al. 1979) and the Cauvery Basin, Tamil Nadu Maheshwari 1986) to the genus *Komlopteris*.

MATERIAL AND METHOD

For ultrastructural studies cuticular membranes (CM) were obtained from compression fossils. Specimens of *Komlopteris (Thinnfeldia) indica* (Feistman-

tel) Barbacka and *Pachypteris (Palissya) indica* (Oldham & Morris) Bose & Roy were collected by one of us (HKM) from the Sivaganaga (Naicolam, Cauvery Basin, Tamil Nadu) and Jabalpur (Sehora-on-Sher, Satpura Basin, Madhya Pradesh) Formations, respectively. For transmission electron microscopy the un-macerated phytollemma were treated in hydrofluoric acid for the complete removal of silica particles. After careful washing in distilled water, very small pieces of the phytollemma were fixed in 4 per cent glutaraldehyde in 0.2M cacodylate buffer (pH 7.2) for 6 hours at room temperature. After thoroughly washing in buffer, post-fixation was done in 2 per cent OsO₄ at room temperature. After washing in double distilled water, the pieces were passed through an ethanol series and acetone; embedding was done in Spurr's low viscosity medium. Thick sections were cut to check the orientation of the CM under the light microscope. Thin sections (up to 600 Å) were picked up on copper grids and stained in uranyl acetate and lead citrate.

On the basis of available published data, the current taxonomic status of the two genera can be summarized as follows.

DESCRIPTIONS

Genus *Pachypteris* Brongniart 1828 emend. Harris 1964

Pl. 1, figs 1–2

Type species. *Pachypteris lanceolata* Brongniart 1828 (designated by Harris, 1964, p. 122).

Horizon. Oolitic of Yorkshire, UK.

Diagnosis (after Brongniart 1828). "Veg. Foliae pinnatae vel bipinnatae, pinnulis integris coriaceis enerviis vel uninerviis, basi con-

strictis nec rachi adnatis" (Leaves pinnate or bipinnate, pinnules entire, coriaceous, veinless or one-nerved, base compressed, adnate to the rachis below).

Emended diagnosis (after Harris 1964, pp. 121–122). Leaves petiolate, once or twice pinnate. Main rachis not forked, striate, pinna rachides or lamina segments borne laterally. Lamina segments ± lanceolate or lobed, apices blunt, bases contracted above, decurrent below. Veins inconspicuous, embedded in the lamina, consisting of a midrib and forked lateral veins. Cuticles thick, generally hypostomatic, stomata variably distributed and oriented between the veins. Stomata haplocheilic, subsidiary cells about five, guard cells sunken.

Remarks. Doludenko (1969) assigned a "topotype" for *Thinnfeldia* from the type locality Steierdorf-Anina, Romania, epidermal features of which she found to be similar to those of species of the genus *Pachypteris*. Barbacka (1993), on the basis of some specimens from the Liassic of Hungary, found recognizable differences in the stomatal apparatuses of the two genera.

Genus *Komlopteris* Barbacka 1994

Pl. 1, fig. 3

Type species. *Komlopteris nordenskiöldii* (Nathorst 1878) Barbacka 1994.

Horizon. Liassic, Palsjös, Sweden.

Diagnosis (after Barbacka 1994). Leaves

Table 1. A comparative chart of the salient features of the genera *Pachypteris* and *Komlopteris*

Characters	<i>Pachypteris</i>	<i>Komlopteris</i>
Leaf	pinnate/bipinnate	pinnate
Shape	lanceolate to obovate	elongate narrow
Apex	acute or obtuse	subacute
Margin	entire	entire, slightly undulate
Venation	veins concealed	alethopteroid, midvein
Stomatal distribution	hypostomatic, irregularly arranged, haplocheilic, guard cells sunken, thinly cutinized, subsidiary cells 5–8, unequal in size, inner part of subsidiary cells cutinized forming papillae	amphistomatic, stomata situated in intercostal bands, haplocheilic, subsidiary cells 5–7, forming a ring around the stomata pit
Ultrastructure of the cuticular membrane	exhibits two distinct zones: 1) outer electron-dense zone with homogeneous matrix and, 2) an inner electron-lucent zone, irregularly reticulate or fibrillate. Conforms to Holloway's Type-3	homogeneous, exhibits two distinct zones: 1) an electron-dense outer narrow zone and, 2) a relatively wider inner electron-lucent zone. Conforms to Holloway's Type-6

pinnate, pinnules alternate or opposite, elliptical-elongate in shape, apex acute, subacute or rounded, margins entire, occasionally slightly undulate; venation alethopteroid, lateral veins simple or once to twice forked. Cuticle amphistomatic, stomata on upper surface sparse, on lower surface distributed in the inter-costal areas only, subsidiary cells 5–7, mono- or di-cyclic; stomata sometimes surrounded by a more or less complete ring of encircling cells.

Comparison. The main difference between *Komlopteris* and *Pachypteris* is in the distribution of the stomata on the lower surface. In the former they are distributed in the inter-costal bands only, whereas in the latter the stomata are distributed all over the surface with the veins and the areas between them indistinguishable. Salient features of the two genera are compared in Table 1.

For the present report, ultrastructural studies were carried out on the cuticular membrane of a species each of the genera *Pachypteris* and *Komlopteris* from India in order to identify the difference between them at the level of ultrastructure of the cuticle.

OBSERVATIONS

ULTRASTRUCTURE OF THE CUTICULAR MEMBRANE OF *PACHYPTERIS INDICA*

Thin sections of the cuticular membrane (CM) revealed that osmiophilic bodies are randomly arranged at the leaf-air interface (Pl. 1 fig. 1). These probably represent epi-cuticular waxes. The outer surface of the CM had a wavy appearance in cross-section. There was an apparent variation in the thickness of the CM, which exhibited two distinct zones, an outer electron-dense amorphous zone with homogeneous matrix, and an inner electron-lucent, irregularly reticulate-fibrillate zone (Pl. 1 fig. 1). The fibrillae were very fine and showed distinct anastomoses, forming what appeared to be the basic framework at sub-cuticular level (Pl. 1 fig. 2). The fibrillae branched frequently and were most numerous towards the boundary between the cuticular membrane and the epidermal cell wall. Their orientation also varied. The uniform network of fibrillae in the structureless matrix probably was the innermost zone attached to the outer wall of the epidermal cells. In places abundant lipophilic

bodies or vesicles of various sizes appeared to permeate the sub-cuticular level (Pl. 1 fig. 2). These packages of vesicles subsequently added to the structural thickness of the cuticular membrane as also observed by us in the CM of a species of the extant *Cycas* (Pl. 1 fig. 4). The cuticular membrane seemed to be at the developing stage.

ULTRASTRUCTURE OF THE CUTICULAR MEMBRANE OF *KOMLOPTERIS INDICA*

Under the transmission electron microscope the leaf-air interface was in some places seen to be covered with remnants of epicuticular waxes (Bajpai 1997, Pl. 1 figs 2, 5). The structural configuration of the cuticular membrane showed an almost homogeneous matrix. The thickness of the CM was not uniform throughout. The cuticle conformed to Type-6 of Holloway (1982). The CM showed two zones, a narrow electron-dense outer zone, and a comparatively much wider electron-lucent homogeneous inner zone (Pl. 1 fig. 3). At the innermost limit of the sub-cuticular level, where the cuticle came into contact with the epidermis, the sub-cuticular zone formed cuticular pegs (anticlinal flanges) into which extended a narrow strip of the electron-dense zone. The cuticular pegs were pointed at the tip and had wide bases (Bajpai 1997, Pl. 1 figs 6–7). These flanges were located between the walls of the adjacent epidermal cells.

DISCUSSION

Compilation of available information on the ultrastructural configuration of the CM in modern plants led Holloway (1982) to recognize six primary types of CM, based on the presence or absence of distinctive ultrastructural components, such as, lamellae and fibrillae.

The CM of *Pachypteris indica* shows close resemblance to CM Type-3 of Holloway (1982) in possessing an outer region that was mainly amorphous, and a reticulate/fibrillate inner region. The significant thickness of the outer homogeneous zone and the inner reticulate zone could be attributed to a number of factors, the most definite of which were the ecological conditions. The electron density of the homogeneous layer was greater than that of the underlying reticulate region. The CM of *Komlopteris*

indica, on the other hand, showed a homogeneous matrix (Holloway's CM Type-6); its significant thickness suggested a protective role in extremely dry climates.

From the above it is evident that, at the ultrastructural level, the CMs of *Pachypteris indica* and *Komlopteris indica* show appreciable differences. Ultrastructural studies in many more species of the two genera, however, would need to be undertaken before concluding that these differences are genus specific. The ultrastructure of the CM has also been investigated in a few other taxa of fossil plants, for example, *Ticoa harrisii* (Archangelsky et al. 1986), *Tarphyderma* (Archangelsky & Taylor 1986), *Dicroidium* spp. (Maheshwari & Bajpai 1996), *Ginkgoites tigrensis* (Taylor et al. 1989, de Seoane 1997), *Tomaxiella biforme* (de Seoane 1998), etc. The ultrastructure of the CM in specimens of *Ginkgoites tigrensis* and *Ginkgo biloba* studied by Taylor et al. (1989) was markedly different from that in other specimens of the same two species investigated by de Seoane (1997). The very important polylamellate layer observed by Taylor et al. (1989) was not found in the specimens investigated by de Seoane. Recently Dr. Gaetan Guignard and colleagues in the Palaeobotanical Laboratory of University of Claude-Bernard, Lyon investigated sun and shade leaves of *Komlopteris nordenskiöldii* from Hungary (communication from the reviewer). They observed that cuticles showed differences in ultrastructure. These differences are supposed to have been caused by adaptation of plant to varying intensities of solar radiation. This raises some doubt about the importance of the CM in taxonomy, but then very little information is available on the subject as yet.

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PLATE

Plate 1

1. Cross-section of the cuticular membrane of *Pachypteris indica* showing two zones, an outer electron-dense zone and an inner electron-lucent zone with fibrillae. Remnant of an anticlinal flange is seen as a small protuberance in the lower central region, $\times 4.800$
2. Part of the cross-section in fig. 1 enlarged to show the differently oriented branched fibrillae, and lipophylic bodies of various size, adding to the sub-cuticular level, $\times 14.200$
3. Cross-section of the cuticular membrane of *Komlopteris indica* showing the homogeneous matrix. A protuberance at the sub-cuticular level probably represents an anticlinal flange, $\times 8.800$
4. Cross-section of the cuticular membrane of *Cycas* sp. showing the reticulate-fibrillate inner region and the development of extensive cutinization, $\times 33.800$

