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ULTRASTRUCTURAL STUDY OF THE CUTICLE OF *HIRMERIELLA MUENSTERI*

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ABSTRACT. A study of well preserved fossil cuticle material of *Hirmeriella muensteri* from the Liassic of Franconia (Germany) was carried out using transmission electron microscopy (TEM), as well as scanning electron microscopy (SEM) and light microscopy (LM). This paper is a summary of part of a complete study of this taxon (Guignard *et al*. in press), illustrating briefly by means of reconstruction and photomicrographs the different structures observed, stressing some of the structural/functional relationships revealed by the TEM, eg the ability of the guard cells to change in shape and volume for opening and closing.

KEY WORDS: Cuticular ultrastructure, *Hirmeriella muensteri*, Cheirolepidiaceae, Liassic, Germany

MATERIAL AND METHODS

The excellently preserved material came from the lower Liassic of the Franconia locality at Großbellhofen near Bayreuth (Germany), from the type species of the fossil conifer family Cheirolepidiaceae *Hirmeriella muensteri* (Schenk) Jung.

The cuticles were cleared by steeping in HF (40%, 12 hours) and HCl (10N, 6 hours) and subsequently macerated in Schulze's reagent (nitric acid + potassium chlorate, 1 hour), rinsed with water and neutralized in ammonia (3%, half an hour). These times can be changed if the chemical composition of the stone makes this necessary.

Samples for SEM were coated with 100% gold and observed with a JEOL JSM-35CF. The preparation of the material for TEM was carried out according to the 'one week technique' of Lugardon. After a few weeks of fixation with 4% paraformaldehyde solution in a phosphate-sodium buffer, the specimens were washed and postfixed in a 1% osmium tetroxide solution mixed in a phosphate-sodium buffer. Dehydrated in a graded ethanol series, the samples were dropped in propylene oxide with an increasing percentage of Epon resin before being embedded in fresh Epon resin. The preparations were subsequently treated at 60°C, then stored in a closed box containing silica gel.

The preparations were sectioned with a diamond knife and the sections deposited mainly on 300 Mesh (occasionally 200 Mesh) Formvar-coated and uncoated grids. They were stained manually both with a methanol solution of 7% uranyl acetate and an aqueous lead-citrate solution. The grids were observed with a Philips CM120. All the sections presented here are transverse, the negatives being kept in the authors' collection at Lyon.

DESCRIPTION AND DISCUSSION

The similarities between our fossil material and extant plants, as well as with other fossil material, observed using different techniques and data, demonstrate that the three problems generally discussed by authors about fossil cuticles (procedures, fossilization and diagenetic processes) have to be minimized, as has been suggested before (Archangelsky *et al.* 1986).

As is shown in Fig. 1, which is a reconstruction showing the cuticle of the three cell types observed, both the epidermal and subsidiary cell cuticles have the same basic ultrastructural pattern consisting of 4 layers, making up a cuticle proper (the two A layers) and a cuticular layer (the two B layers). The epidermal and subsidiary cells have a cuticle proper (Pl. 1, figs 3–5) composed of an external A1 layer (regular in thickness with 38% and 26% size variation, respectively; non-lamellate to polylamellate with 7–12 and 10 lamellae, respectively) and an A2 layer (variable in thickness with 67% and 43% size variation, respectively; granular). The cuticular layer (Pl. 1, figs 6, 7, 9) consists of a middle B1 layer (regular in thickness with 35% and 35% size variation, respectively; fibrillous and reticulate, respectively) and an innermost B2 layer (variable in thickness with 61 % and 100% size variation, respectively; granular). However, an interesting point is that the guard cell cuticle is different in that some layers are missing at various levels. The guard cell cuticle is made up of two different parts. The free part of the cuticle (Fig. 1), surrounded by the upper and lower stomatal chamber, has two layers but only the cuticle proper is present (Pl. 1, fig. 10), consisting of an external A1 layer (rather variable in thick-

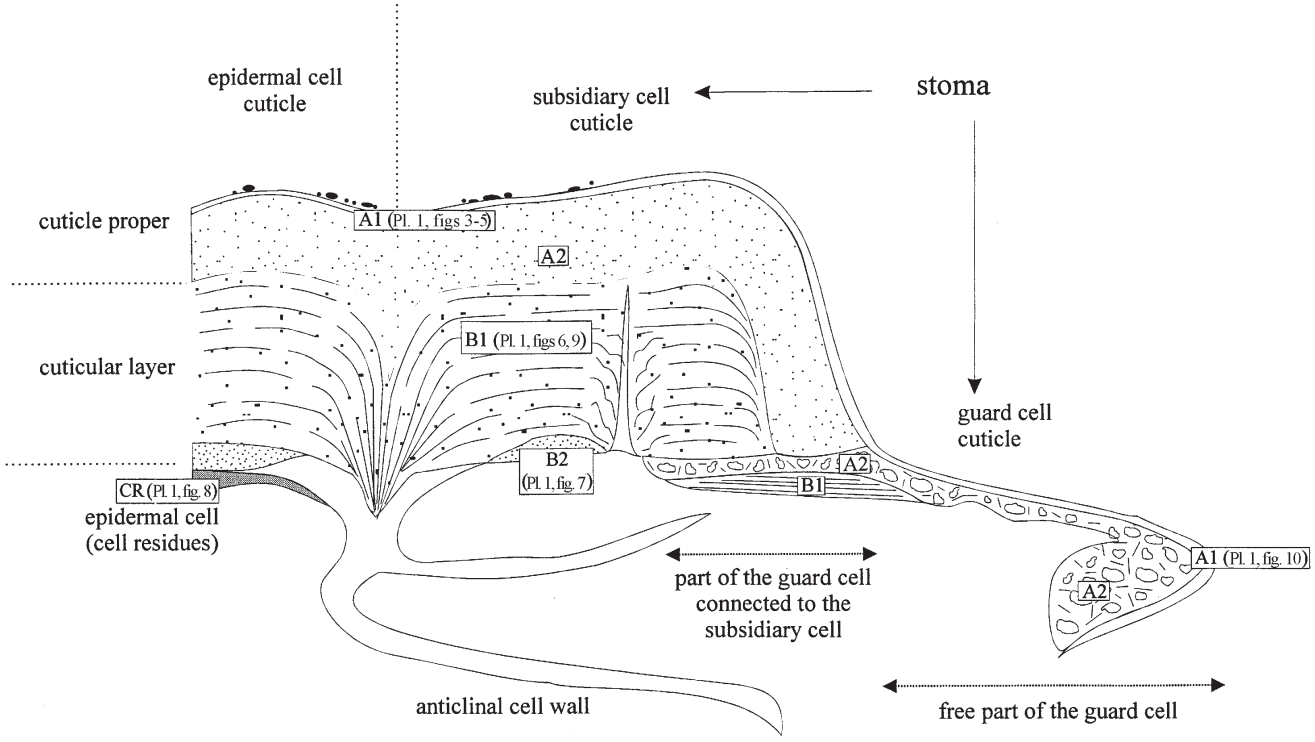

Fig. 1. Reconstruction of the ultrastructure of the cuticle of the three types of cell observed in *Hirmeriella muensteri*

ness with 58% size variation, non-lamellate to polylamellate) and an inner A2 layer (regular in thickness with 12% size variation, reticulate). That part of the cuticle connected to the subsidiary cell above (Fig. 1) consists of two layers only, the A2 layer being part of the cuticle proper (variable in thickness with 33% size variation, reticulate) and the B1 layer being part of the cuticular layer (regular in thickness with 12% size variation, fibrillous). It is remarkable that no A1 layer has been observed in this part of the guard cell so far. One suggestion is that the A1 layer is just an area of contact with the atmosphere, for it is present in the free part of the guard cell, as well as in the two kinds of cell already described.

Apart from the A and B layers, i.e. the cuticle proper and the cuticular layer, three other different materials command attention. Firstly, extracutinized components are almost always present (Pl. 1, figs 3–5), which seem to be analogous to extracuticular waxes. Essentially different types of element have been observed above the A1 layer of both the subsidiary and guard cell cuticles (Pl. 1, fig. 10) that are much bigger and more heterogeneous in shape and size than the darkly stained bodies usually observed above the A1 layer in the cuticle of the epidermal cell. Although their chemical composition is unknown, these components may have functioned as a protective layer. The increased presence of these components in the stomata may relate to this region's clear need to be better protected. Secondly, an interesting point is that some very thick cuticles contain in their lower part thick fibres, different from the fibres of the B1 layer (Pl. 1, fig. 8). Whatever their location, at the base of the epidermal cell cuticle, in the anticlinal walls or in the embedded resinous parts of the leaf margin, these cell residues are more or less surrounded by darkly stained patches. Thirdly, anticlinal walls furnished with two horns can be seen. As in extant plants, so far as we know, this part never extends beyond the basal part of the epidermal cell, so this horned material shows that it cannot consist of anticlinal walls only.

It is well known that in extant plants the stomata cells (i.e. subsidiary and guard cells) are subject to variations in volume, mainly due to water exchanges (Willmer & Fricker 1996). Some interesting points arise for the fossil *Hirmeriella muensteri* studied. The guard cell cuticle, certainly the most prone to of movement due to its position adjacent to the opening and closing of the stoma, seems to be more malleable than that of the subsidiary cells, for several reasons (Fig. 1): it is about nine times thinner and it is composed of only two layers (the subsidiary cell cuticle can have up to four), A2 and B1 in the part connected to the subsidiary cell (B1 being fibrillous and probably quite strong, very helpful for anchoring to the subsidiary cell above), A1 and A2 in the free part of the guard cell in contact with the stomatal chamber (A2 being only reticulate and thus probably weaker and more malleable than B1's fibrillous structure; note for example the break at the level of the anchoring of the

guard cell (Fig. 1), which reveals a certain the lack of malleability of the B1 fibrillous layer).

In addition to the structural/functional relationships point of view, a comparison of these ultrastructural results with those which previously have been published of taxa related to the Conifers – *Ticoa harrisii* (Archangelsky *et al.* 1986, related to the Gymnosperms possibly to the Cycads or Pteridosperms), *Squamastrobus* (Archangelsky & del Fueyo 1989, Barale *et al.* 1992, belonging to the Podocarpaceae), *Tarphyderma* (Archangelsky & Taylor 1986, belonging probably to the Cheirolepidiaceae) and *Tomaxellia* (Villar de Seoane 1998, belonging also to the Cheirolepidiaceae) – show more similarities than differences, which is very interesting for the eventual use of cuticular ultrastructure as a taxonomic feature.

In comparison with the six ultrastructural types defined by Holloway (1982) for extant plants, the epidermal, as well as the subsidiary and guard cell cuticles studied here are attributable to his types 1 and 2, being lamellate in the outer region. But the photos in the present paper illustrate variations of these types. These approximate affinities, also observed in other fossil taxa (Barale & Baldoni 1993, Labe & Barale 1996) were discussed by Holloway himself in his conclusion in 1982: "... there is no typical plant CM (= cuticular membrane)... Thus the terms such as cuticle proper and cuticular layer are best reserved for those plant CM for which the nomenclature was originally intended ..." These observed variations have also been noticed more and more in research on extant plants, showing that the cuticle is a mixture of different components of inner fibrillous cellulose and pectin, amorphous and granular cutine and outer amorphous wax; these are more or less mixed and are thus responsible for many variations on a general scheme (Lyshede 1982, Willmer & Fricker 1996).

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PLATE

Plate 1

The photos illustrate details from different parts of the cuticle of *Hirmeriella muensteri* (Fig. 1)

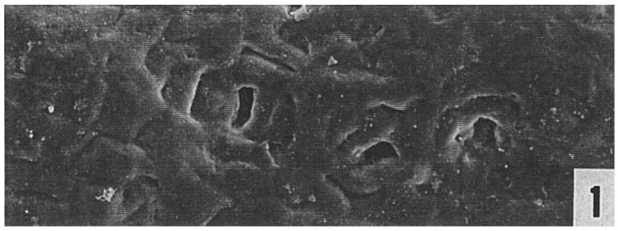
1. SEM. External view of a cuticle, showing three stomata among epidermal cells. No. FT0021, × 474
2. SEM. Inner view of a cuticle, showing three stomata among epidermal cells. No. FT0003, × 474
3. epidermal cell cuticle. TEM. Outermost A1 polylamellate layer, as typically observed, with the granular A2 layer beneath. Note the small and irregular extracutinized components over the A1 layer (arrow), corresponding probably to epicuticular waxes. No. GGG270, × 121 000

4–5. subsidiary cell cuticle

4. TEM. Outermost A1 layer, non – lamellate in this case (quite rare!), with the granular A2 layer beneath. Note again the small and irregular extracutinized components over the A1 layer (arrow), corresponding probably to epicuticular waxes. No. GGG450, × 58 100
5. TEM. Outermost A1 layer, undulate and very variable in size in this quite rare case, non – to very slightly lamellate, covering the granular A2 layer. No. GGG451, × 125 000

6–9. epidermal cell cuticle

6. TEM. Normal B1 fibrillous layer (compare with photo 9), containing granules of differing size. No. GGG0340, × 57 000
7. TEM. B2 innermost granular layer, very slightly lamellate (rare!). No. GGG063, × 125 000
8. TEM. Cell residues located at the bottom of the cuticle. Note the fibrillate material, differing from the B1 layer (photos 6 and 9). Note also the round particles attached to the cell residues (arrow), which seem to be a characteristic of this material. No. GG0350, × 78 000
9. TEM. B1 fibrillous layer observed in one of the rare cases where the fibrils vary in density and are very undulate (compared to photo 6), and contain granules of variable size No. GGH584, × 60 000
10. guard cell cuticle. TEM. Free part surrounded by stomatal chambers (upper and lower). The A1 non – lamellate layer is covered with quite thick epicuticular components (arrow). The A2 layer is alveolate, with a quite clear reticulum of granules of variable size. No. GGH609, × 120 000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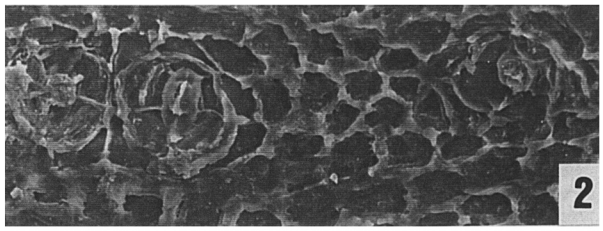
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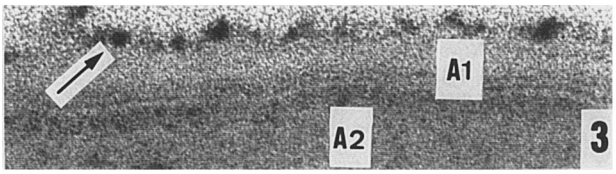
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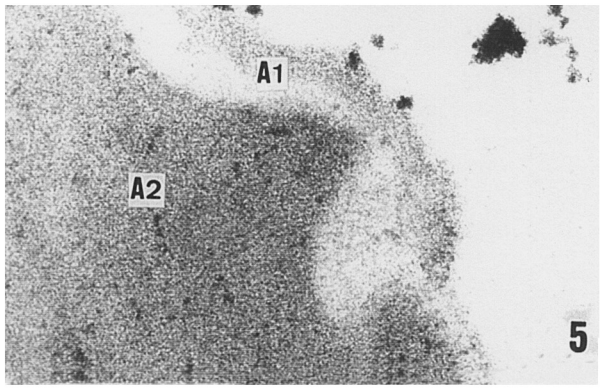
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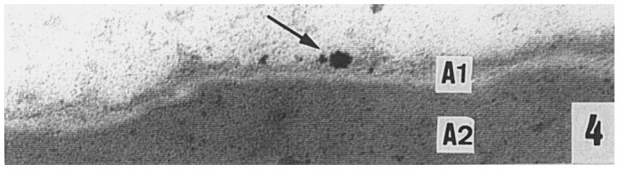
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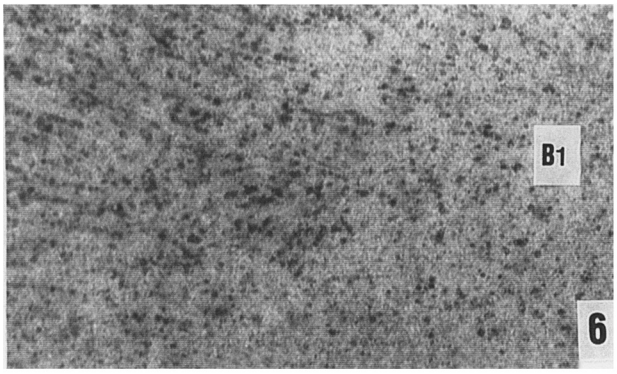
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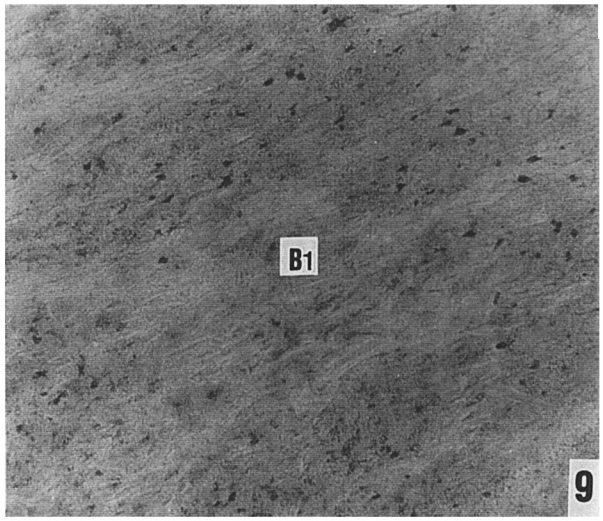
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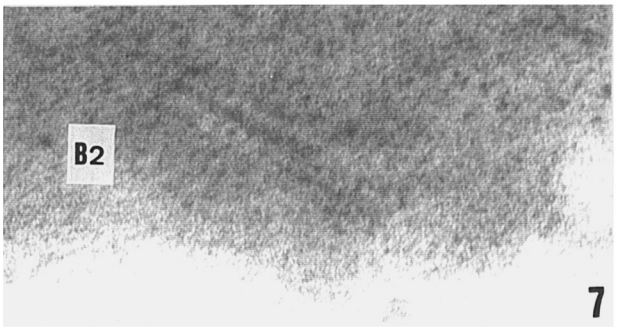
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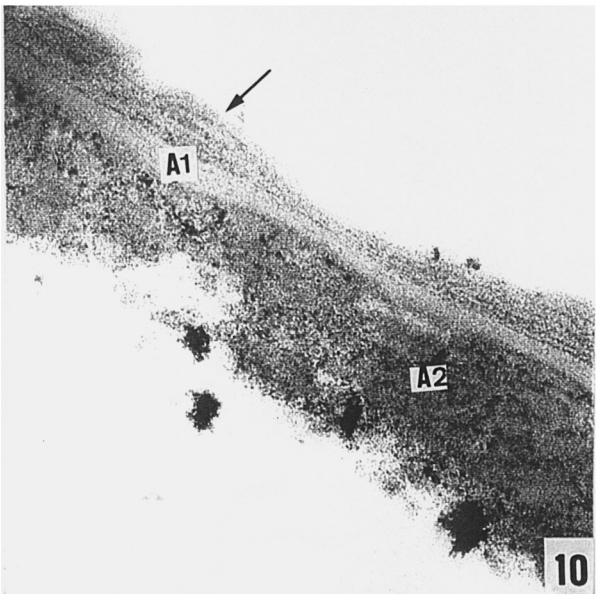
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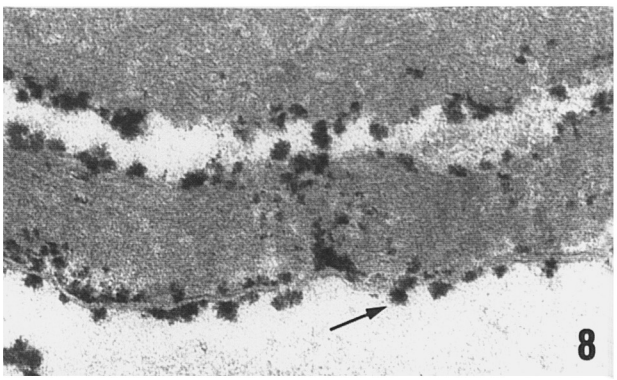
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