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ON APPLICATION OF SCANNING ELECTRON MICROSCOPE TO THE STUDY OF ORGANIC INCLUSIONS FROM THE BALTIC AMBER

(Pl. I—IV)

Próby zastosowania scanningowego mikroskopu elektronowego do badań organicznych inkluzji z bursztynu bałtyckiego

(tabl. I—IV)

A b s t r a c t. This paper shows the usefulness of scanning electron microscope for the study of arthropods and plant fossils found in the Baltic amber. The value of this method for solving some fossilization and taxonomic problems has been discussed here.

INTRODUCTION

The Baltic amber, regarded to be of the Upper Eocene and Lower Oligocene age, frequently contains abundant plant and animal fossils. The state of preservation of those particular fossils was discussed many a time in the past. N. K o r n i l o v i c h (1903) reported chitinous exoskeletons, as well as remains of organs and muscles of insects preserved in succinite. H. C o n w e n t z (1890), A. J a c o b i (1937) and others maintained that due to intense carbonization, only empty space and slight amount of coal dust remained of organic fossils in succinite. This view, repeated in textbooks of palaeozoology, is still prevalent among palaeontologists.

Recently, the author has made an attempt to apply scanning electron microscope (SEM) to the investigations of plant and animal fossils preserved in the Baltic amber.

EXPERIMENTAL PROCEDURE

The investigations were carried out on the material that, due to substantial turbidity of the amber, was of little use for typical taxonomic studies. The following procedure was adopted while preparing the expe-

rimental material: 1) the thickness of a layer of succinite above the inclusion was reduced down to about 0,5 mm by polishing, 2) the thin layer of succinite that was left over was removed by pricking with a preparation needle and by levering, 3) the exposed inclusion or its fragment was transferred with a badger hair to the SEM stage. All these operations were controlled by means of the light microscope. Considering the character of the material (dehydration during fossilization, fragility), it was not treated with ethanol or acetone. The specimens were coated with carbon and gold with palladium by means of standard methods. The samples thus prepared were examined by the Jeol JSM-S1 scanning electron microscope, using an accelerating voltage of 10 kV and magnifications in a range of 100—12 000 ×. The investigations were carried out in the Electron Microscopy Laboratory of the N. Nencki Institute of Experimental Biology of the Polish Academy of Sciences in Warsaw.

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EXPERIMENTAL DATA AND DISCUSSION

The application of electron microscopy permitted the most thorough observations of the inclusions. Such results as obtained using this technique were definitely unattainable by means of the light microscope.

While preparing the samples, the author could observe a very good condition of preservation of the insect cuticles even with the naked eye. Upon being removed from the amber, the cuticle of Diptera from the family Dolichopodidae instantly recovered its characteristic green metallic lustre. This colour is never perceptible, even through a perfectly transparent succinite. In this situation, the widely accepted opinion that the cuticle of insects becomes discoloured in succinite due to fossilization changes must be revised. It seems probable that only pigment colours disappear or become less vivid. The structural colours, on the other hand, are preserved, and their apparent absence can be attributed to the disturbance of the light interference and diffraction in the cuticle effected by succinite. The electron micrographs (Pl. III) confirm the perfect state of preservation of the cuticle sculpture, as well as the lack of any indications of fossilization changes in its structure. The stability of the cuticle structure is also evidenced by the fact that, as for organic fossils in succinite, it shows a considerable elasticity.

On the inner surface of the cuticle, no vestiges of the underlying epithelium have been found. On the other hand, unidentified fragments, presumably scraps of indigested food and remains of entrails, could be observed. Occasionally, larger fragments of fossilized internal organs have

been noted among them (Pl. IV, fig. 2). It is interesting to observe that the entrails of Araneae are much better preserved in succinite than those of insects. The author succeeded in indentifying a bundle of muscle fibres almost completely preserved book-lungs, spinning glands and other organs.

The greatest surprise in the course of investigations was the disclosure of perfectly mummified compound eyes of Diptera Dolichopodidae (Pl. I—II). It appeared that under the ideally preserved corneal lenses, the remaining elements of an eye could be easily distinguished. According to the method of preparation of the experimental material, preserved optic cells, pigment cells or crystalline cones have been observed. Moreover, the system of tracheae and tracheoles supplying oxygen to the eye has been also preserved. The perfect preservation of the basic stroma of the eye ultrastructure after about 45 million years is, indeed, an astounding phenomenon.

Some information has been also obtained on the character of milky rims frequently coating the animal inclusions. They used to be regarded as moulds overgrowing the decaying organisms or an emulsion of water and gases produced in resin due to putrefactive processes occurring in organic remains. N. J. Kuznetzov (1941) maintained that neither view was confirmed by the light microscope even at highest magnifications. The electron miscroscope studies have shown that it is right to associate the milky rims with the decomposition of organic substances. Furthermore, the electron micrographs yield certain data on the activity of saprogenic bacteria in the inclusions and on the intensity of the decompostion process (P. Mierzejewski, in press).

The author has also made an attempt to use SEM for the investigations of plant inclusions, particularly of the wood of *Pinus succinifera* (Conwentz) em. Schubert. The resultant electron micrographs (Pl. IV, fig. 1) are superior in quality to the best microphotographs (vide K. Schubert 1961). It seems feasible that in the future the electron microscope may play a significant role in the studies of flora of succiniferous forests, which still awaits an up-to-date comprehensive description. M. C. Boulter (1971) proved that the electron microscope images of the fossil Coniferae cuticles afford several pertinent taxonomic and evolutionary data. It is still more important as far as the flora of succiniferous forests is concerned, since R. Potonié (1924) pointed out to the possibility of separating the Coniferae cuticles from succinite, noting its perfect state of preservation. Thus, the new method gives a practical chance that plant fossils from succinite so far regarded as „indeterminable" will, partly at least, represent a greater value for a taxonomist.

On the basis of the analysis of the obtained data, the author has made certain observations concerning physical properties of the fresh resin of *P. succinifera* (Conv.) em. Schubert. Several authors have advoca-

ted a hypothesis that this resin was markedly thinner than that of the contemporary species of *Pinus* L. It should be expected then that such resin may have thouroughly infiltrated the organic remains stuck in it, filling the smallest fissures and penetrating inwards. Under such circumstances there should be a strong bond between the inclusion surface and the amber. If that were the case, it would be impossible to separate the delicate Dolichopodidae cuticle with bent chaetae (Pl. III, fig. 2); at the same time the trachae of the Coniferae wood (Pl. IV, fig. 1), derived from a small splinter only about 2—3 mm thick, would be filled with succinite. The presented electron micrographs show clearly that the contact beween the succinite and the fossils is very loose. They are also the case against attributing a different consistency to the resin of *Pinus succinifera* than to the resins of the contemporary Pinaceae.

The above preliminary results of the electron microscope studies encourage a presumption that the new method will permit elucidation of several problems connected with organic fossils in amber.

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STRESZCZENIE

Artykuł przedstawia wstępne wyniki badań prowadzonych przy pomocy SEM nad roślinnymi i zwierzęcymi szczątkami zawartymi w bursztynie bałtyckim. Opisano bardzo dobrze zachowane okrywy ciała owadów i przedyskutowano problem trwałości naturalnych barw kutikuli.

Zasygnalizowano odkrycie doskonałej mumifikacji niektórych narządów wewnętrznych stawonogów. Stwierdzono istnienie pod rogówkami oczu złożonych resztek stożków krystalicznych, komórek wzrokowych i komórek pigmentowych oraz systemu tchawek i tracheoli doprowadzających tlen do narządu wzroku.

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EXPLANATION OF PLATES
OBJAŚNIENIE TABLIC

Plate — Tablica I

Fig. 1. Gen. et sp. indet. I (Diptera, Dolichopodidae).
Fragment of the compound eye with distinct hexagonal corneal lenses
Fragment oka złożonego. Widoczne sześciokątne rogówki.
1200 ×

Fig. 2. Gen. et sp. indet. II (Diptera, Dolichopodidae).
Ornamentation of corneal lenses
Ornamentacja rogówek. 12000 ×

Plate — Tablica II

Fig. 1. Gen. et sp. indet. II (Diptera, Dolichopodidae).
Corneal lenses of the compound eye seen from the inside
Rogówki oka złożonego od strony wnętrza. 1200 ×

Fig. 2. Gen. et sp. indet. II (Diptera, Dolichopodidae).
The compound eye with removed corneal lenses and crystalline cones
Oko złożone po usunięciu rogówek i stożków krystalicznych. 1200 ×

Plate — Tablica III

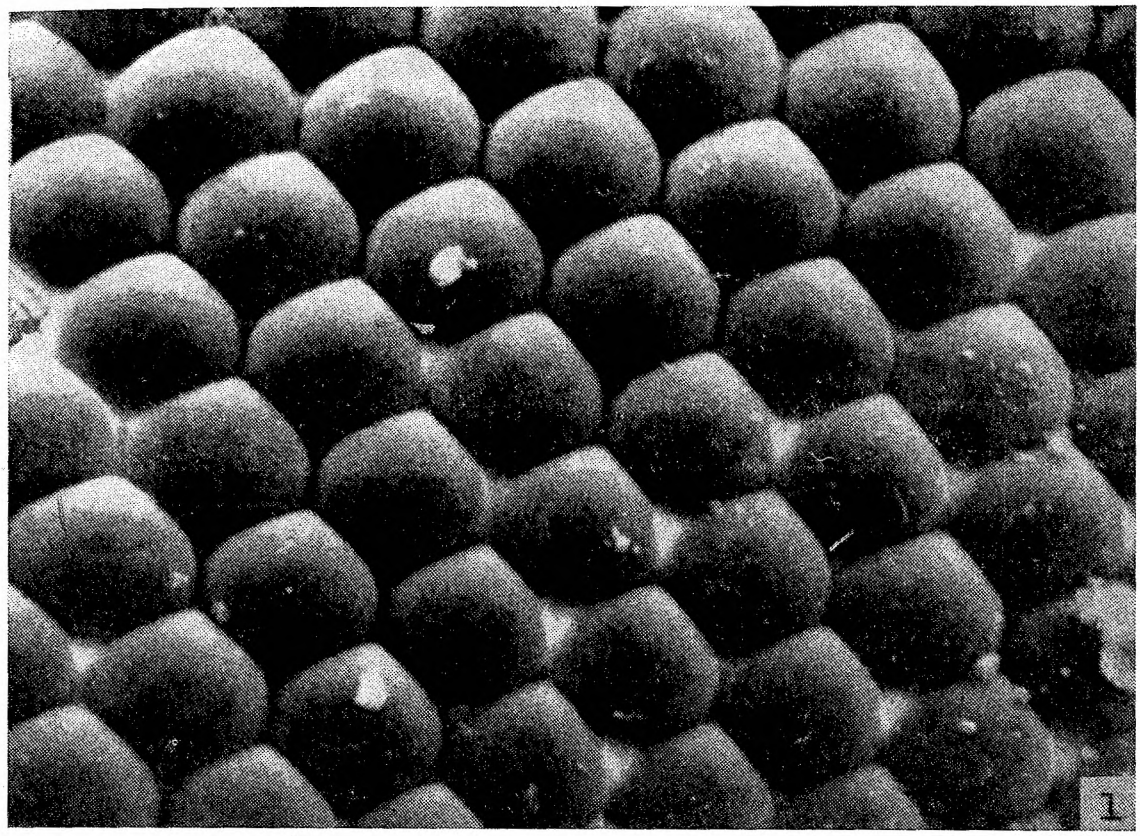
Fig. 1. Gen. et sp. indet. III (Coleoptera).
Fragment of the top surface of elytra
Fragment górnej powierzchni pokrywy.
2160 ×

Fig. 2. Gen. et sp. indet. I (Diptera, Dolichopodidae).
Fixed chaetae on the thorax
Nieruchome włoski porastające tułów.
3600 ×

Plate — Tablica IV

Fig. 1. *Pinus succinifera* (Conwentz) emend. Schubert, 1961.
Damaged tracheae with distinet pits
Uszkodzenie cewki — widoczne jamki.
2160 ×

Fig. 2. Gen. et sp. indet. II (Diptera, Dolichopodidae).
Fragment of an unidentified organ on the internal surface of the thorax cuticle. Distinct apertures in the cuticle, in which moving chaetae were set.
Fragment nie zindentyfikowanego narządu na wewnętrznej powierzchni kutikuli tułowia. Widoczne otworki w kutikuli, w których osadzone były włoski ruchome.
720 ×



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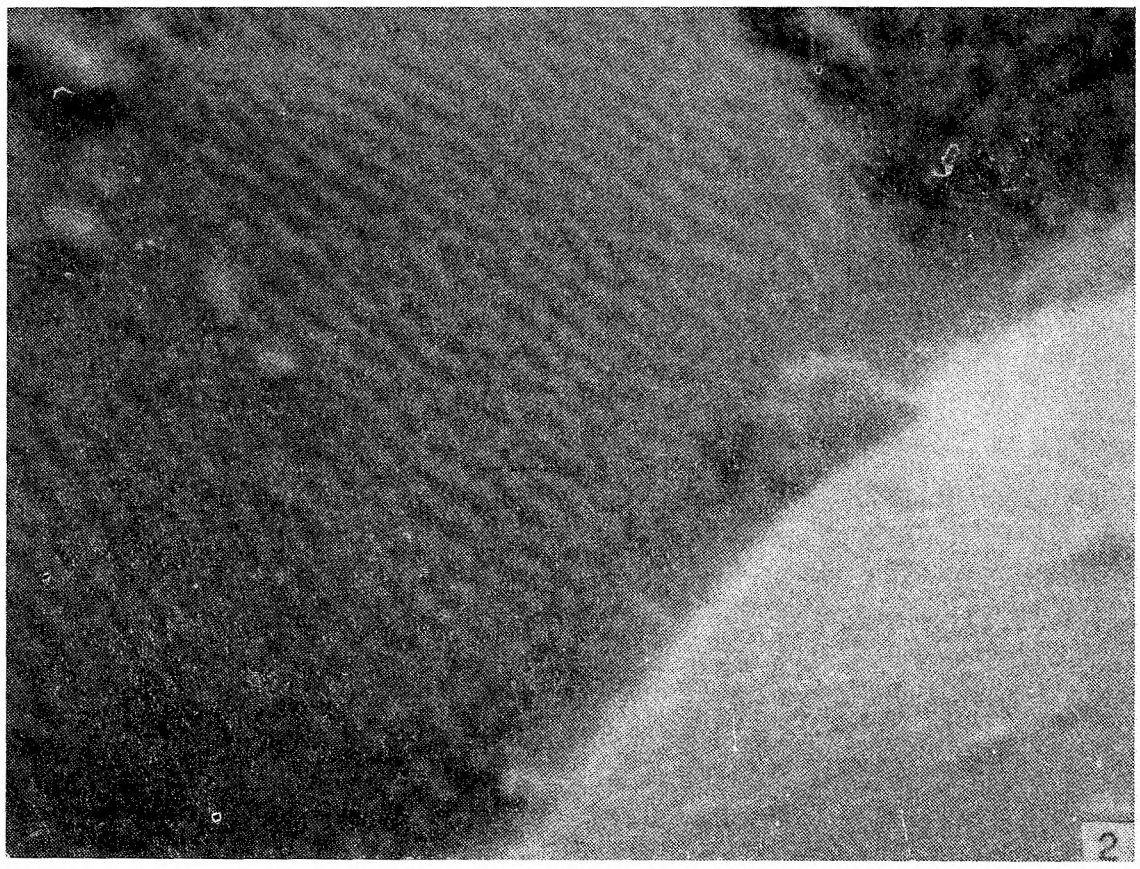

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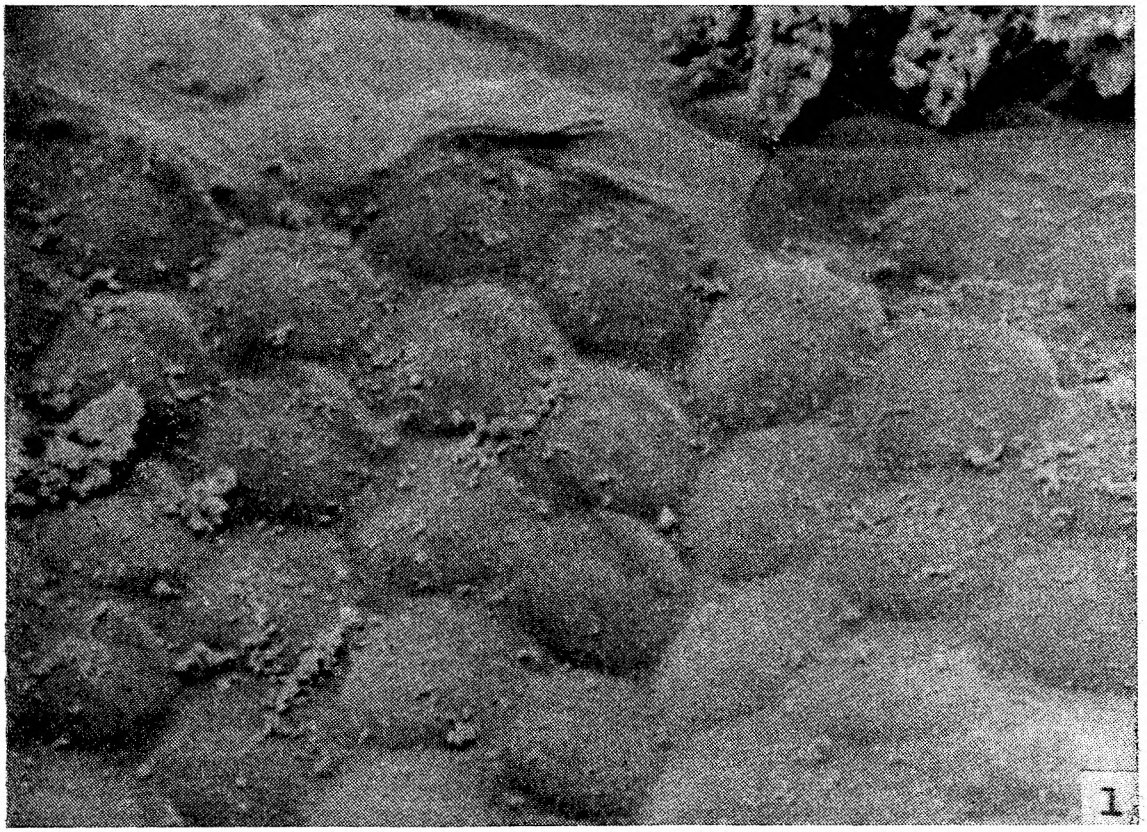
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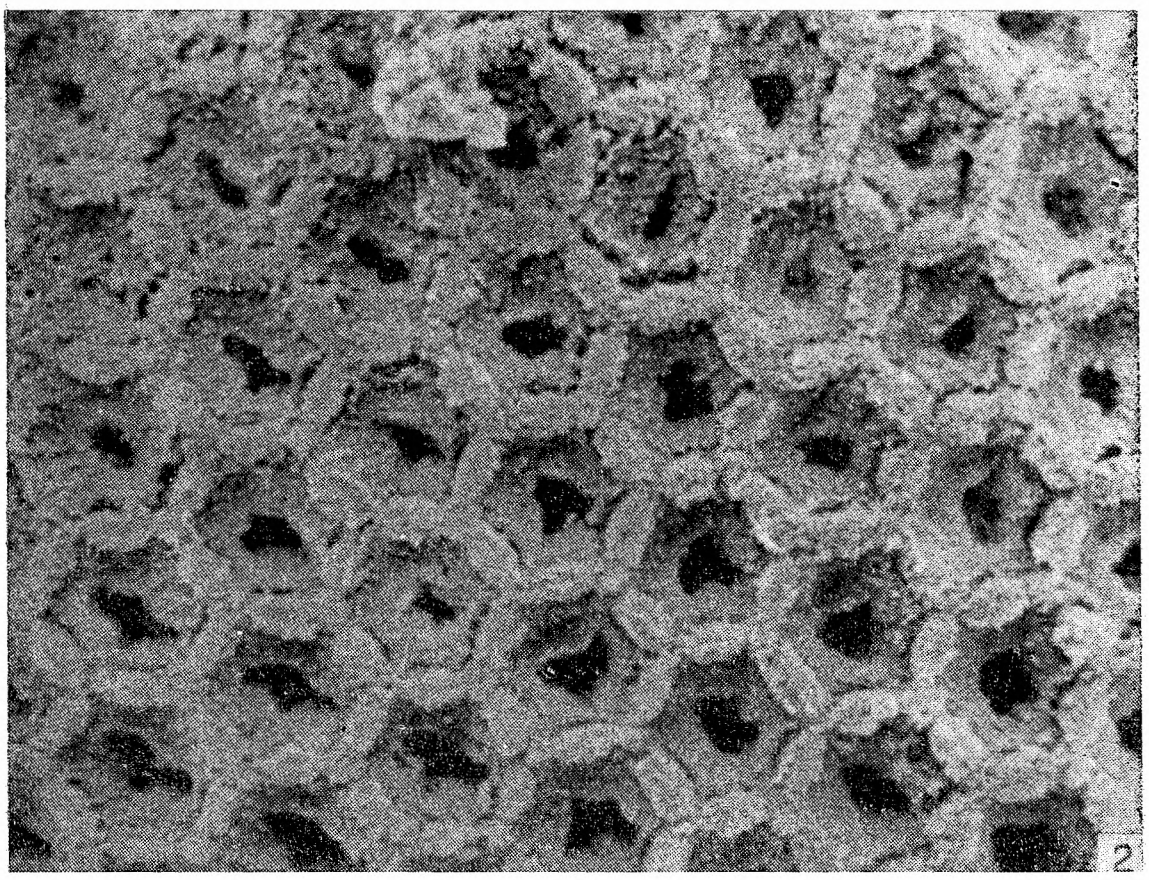
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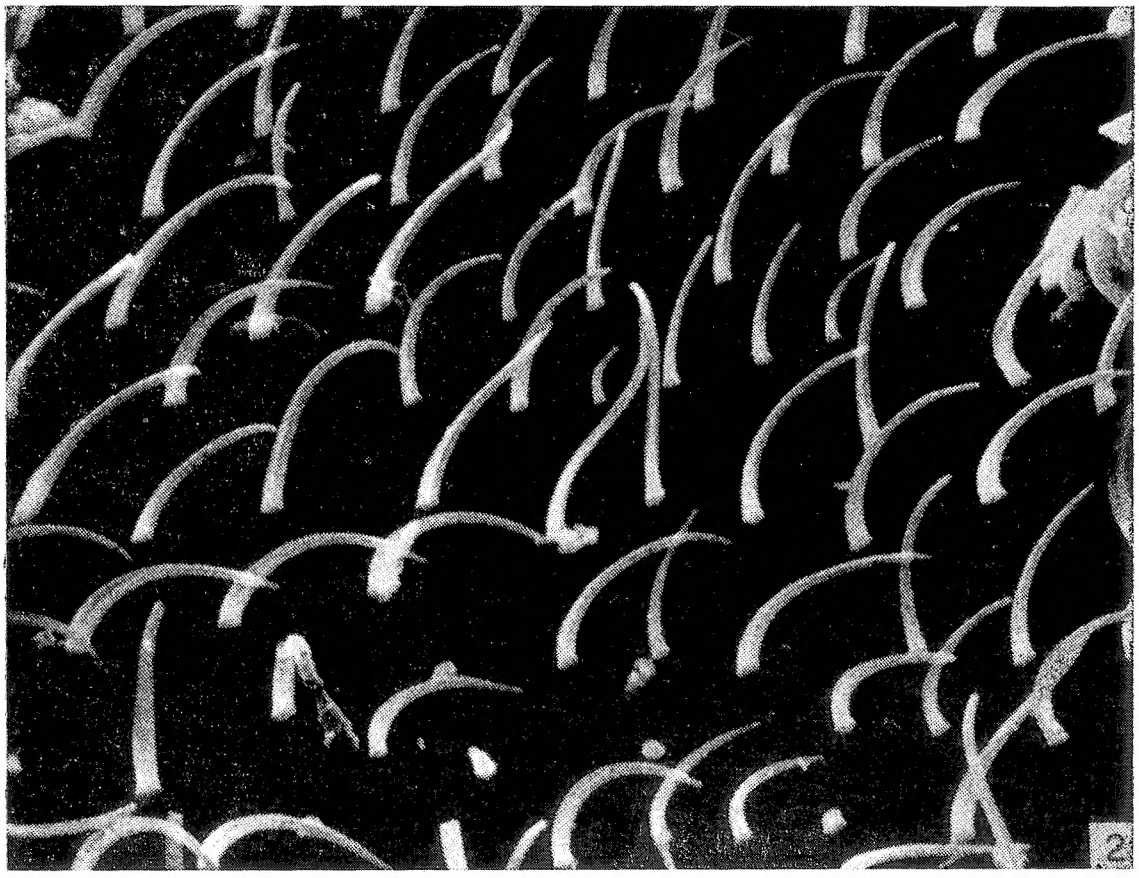

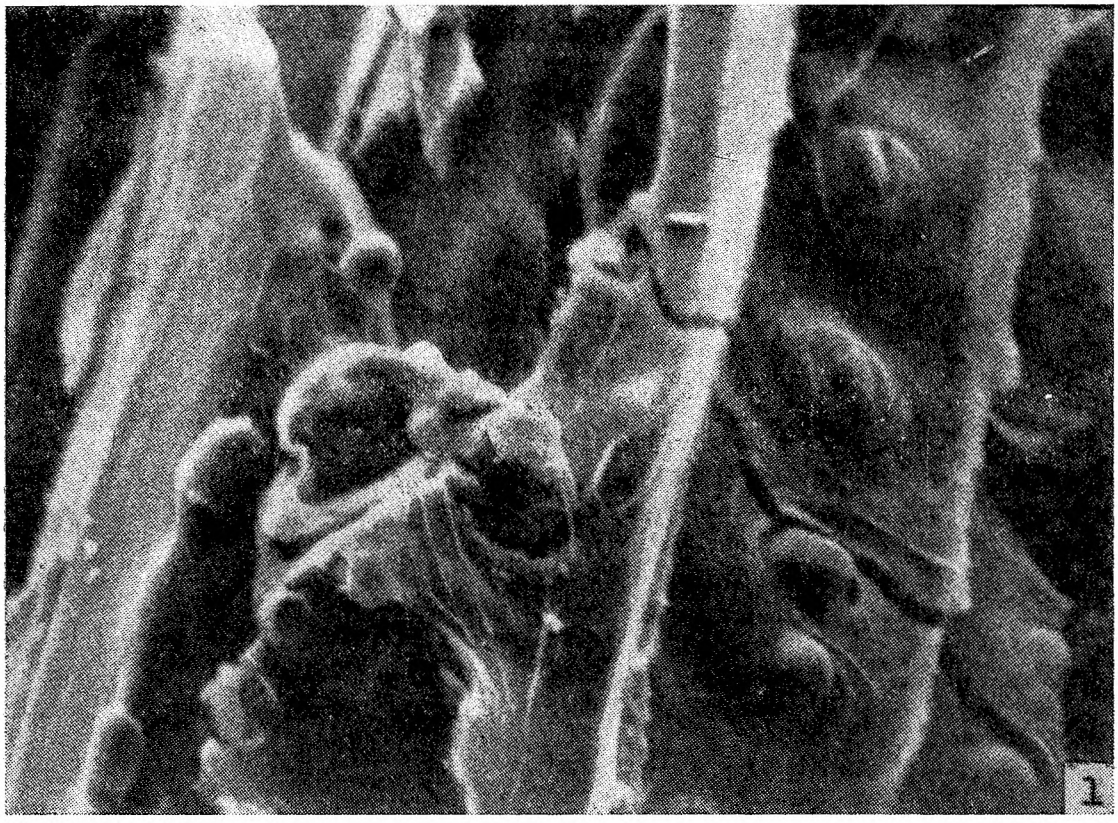
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