1. LM AND TEM INVESTIGATIONS ON THE UPPER CRETACEOUS AJKAITE, OF HUNGARY I.

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Abstract

The fossil resin, (amber) occurring in the brown coal basin of Ajka in Hungary was denominated as Ajkaite. Multidisciplinary researches were started in our Laboratory on this kind of amber with the following purposes: 1. LM investigations on the plant microfossils (spores, pollen grains, tissue remnants) of the amber containing brown coal samples and other sedimentary types of the brown coal basin of Ajka. 2. Transmission electron microscopy of the Ajkaite to investigate the ultrastructure of the embedded organic material. 3. The aim of the first rank is to discover spores and pollen grains with well preserved intine and protoplasm in the Ajkaite and investigate the ultrastructure of the fossil protoplasm. In this first paper some previous palynological data of the investigated amber embedding coal sample, and the first TEM results on the organic material of some Ajkaite samples are presented.

Key words: Ajkaite, LM and TEM study, Upper Cretaceous, Hungary.

Introduction

The investigation of the chemofossils of amber to establish the "amber tree" was the subject of several papers. *Gymnosperm* origin: Possibly *Taxodioxylon* sp., HEY (1962), *Cryptomeria (Taxodiaceae), Cupressaceae,* HEGNAUER (1962), *Agathis,* THOMAS (1969), *Araucariaceae*, GOUGH and MILLS (1972). *Angiosperm* origin: *Hymenaea, Fabales* LANGENHEIM (1966), *Pistacia*, LANGENHEIM (1969), *Burseraceae*, VÁVRA (1991), KOSMOWSKA-CERANOWICZ, KRUMBIEGEL and VÁVRA (1993).

The well preserved animal remnants in the amber were well known a long time ago. WALTHER (1911) summarized that about 20.000 fly, and 13.500 further different kinds of arthropodous specimens were previously described. GYŐRFFY (1929) summarized the fossil moss taxa which were determined from amber samples of different ages. Among recent monographs those of POINAR Jr. (1992), was reviewed by WHALLEY (1992). First TEM results on the soft tissue of fossil fly (*Mycetophilidae: Diptera*) were published by POINAR Jr. and HESS (1982). Nucleus, cytoplasmatic vacuoles, cytomembranes, mitochondria, endoplasmic reticulum, plasma membrane were discovered.

From palynological point of view extremely important results were presented by DEJAX (DE FRANCESCHI, DEJAX and PLOËG, 1999); at the XVIth A.P.L.F. Symposium. Pollen grains were investigated with the LM and SEM method isolated from the amber

of Sparnatian sediments of the Paris Basin. Based on the presented LM data the intine and the protoplasm were in excellent preservation. This called our attention for the combined investigations on the fossil resin of Ajka.

The aim of our investigation is the following:

1. To investigate the full plant microfossil assemblages (spores, pollen grains, plant tissue remnants) of the Ajkaite containing brown coal in taxonomical, taphonomical and in paleoecological point of view.

2. To start to elaborate methods for the transmission electronmicroscopical investigations of the Ajkaite together with the embedded organic material.

3. Combined investigation of the ultrastructure of several Ajkaite samples, in the hope that well preserved protoplasm will also be found in the fossil spores and pollen grains. In this respect the early *Brevaxonate* pollen grains (*Normapolles*) seem to be the most importants for our researches.

Previous results on the amber containing brown coal layers of Ajka

HANTKEN (1867) published first that the brown coal of Ajka contains amber. SZABÓ (1871) established that the resinous remnants increase the caloric value of the coal. HLASIWETZ (1871) analyzed first the chemical compounds of the Ajkaite.

ZECHMEISTER (1926) investigated the origin of Ajkaite and he classed into the succinite and retinite. ROZLOZSNIK (1940) believed that the Ajkaite based on the chemical composition may be classed between the resinite and trinerite. SZÁDECZKY-KARDOSS (1953) observed a process of the liptobiolith and he concluded that the coal of Ajka originates of karstic swamp wood. The acidic water of the karstic swamps was neutralized or alkalined by the limestone and the microorganisms became very numerous. The sulphur content of the proteins of the microorganisms accumulate in the brown coal. PAÁL (1961) pointed that in the V. layer of the Kossuth pit the amber is common, but the microspores are sporadic. As regards the origin of the Ajkaite PAÁL (1961) based on an unpublished paper of TASNÁDI-KUBACSKA and GÓCZÁN emphasized that these may be the product of tropical decidous woods similar to kopal. In the same year, 1961 GÓCZÁN published the following; p. 796: "Das Ajkait ist wahrscheinlich ein fossiles Harz einer Kopal-Art."

GREGUSS (1949) described as *Podocarpoxylon ajkaense* n. sp. a fusit remnant from the coal basin of Ajka. The fossil material was compared with the recent *Podocarpus neriifolius*, and an oceanic clima was reconstructed. Palynological researches were carried out by GÓCZÁN (1964), GÓCZÁN, GROOT, KRUTZSCH and PACLTOVÁ (1967), KOHN (1969), etc.

Materials and Methods

Two kinds of material are the subject of our first investigations: 1. Collection of Ass. Prof. M. SZÓNOKY (Department of Geology and Paleontology of the J.A. University, Szeged), 2. Samples collected by G. KOVÁCS PhD Student from the spoil-bank of the Ajka mine. The brown coal samples were treated with the HNO_3, KOH, and HF method, the slides were mounted in glycerine-jelly hydrated of 39.6 %. For transmission electronmicroscopical investigations small pieces of Ajkaite were placed into the gelatine capsules and some drops of propylenoxyde were added to dissolve the surface of the Ajkaite. We used partial and complete dissolution of the Ajkaite, some samples were

embedded in Araldite (Durcupan, Fluka) without previous propylenoxyde treatment. The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome in the Electron Microscope Laboratory of the Department of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences. The TEM pictures were taken on a Tesla BS-540 instrument (resolution 6-7 Å).

Results

Spores and pollen grains isolated from the Ajkaite containing brown coal

Spores

Appendicisporites cf. *tricuspidatus* WEYLAND et GREIFELD 1953 (Plate 1.1., fig. 1), *Punctatisporites aquisgranensis* WEYLAND et KRIEGER 1953 (Plate 1.1., figs. 2,3).

Pollen grains

Gymnospermatophyta

Araucariacites australis COOKSON 1947, *Araucariaceae* (Plate 1.1., fig. 4)

Angiospermatophyta

Brevaxones

Normapolles

Hungaropollis ajkanus GÓCZÁN 1964 (Plate 1.1., figs. 5-8), *H. krutzschi* GÓCZÁN 1964 (Plate 1.1., figs. 9-10), *Hofkeripollenites hemimechanicus* (PFLUG 1953a) KEDVES et HERNGREEN 1980 (Plate 1.1., figs. 11,12), *Trudopollis articulus* WEYLAND et KRIEGER 1953 (Plate 1.1., figs. 13,14), *Minorpollis gallicus* KEDVES 1969 (Plate 1.1., figs. 15,16), *Semioculopollis* cf. *maestrichtiensis* KEDVES et HERNGREEN 1980 subfsp. *maestrichtiensis* (Plate 1.1., figs. 17,18), *S. granulosus* KEDVES et HERNGREEN 1980 (Plate 1.1., figs. 19,20), *S. croxtonae* KEDVES 1980 (Plate 1.1., figs. 21,22), *Oculopollis minoris* KRUTZSCH 1973 (Plate 1.1., figs. 23,24), *O. orbicularis* GÓCZÁN 1964 (Plate 1.1., figs. 25-28).

In the slides some *gymnosperm* secondary woody remnant was also observed (Plate 1.1., figs. 29,30). The areolate pitting is of modern type, which also occur in the tropical *Podocarpaceae* family. The *Oculopollis* fsp. containing amber piece (Plate 1.1., fig. 31) is important in the forthcoming researches.

Transmission electron microscopy of some Ajkaite samples

During our TEM investigations we have observed different kinds of organic remnants. The MAT system (Matière Amorphe en Transmission) which was introduced by LUGARDON, RAYNAUD and HUSSON (1991) was followed. But further TEM data from kerogene samples were also used.

Sample 99-KG-1 (Plate 1.2., figs. 1,2)

The organic material consists of two kinds of substances of different electron density. There are holes of different size in the amber embedded organic matter. The relatively small electron dense particles are mostly globular occasionally elongated or amorphous. At the globular and elongated form bacterial origin may be presumed.

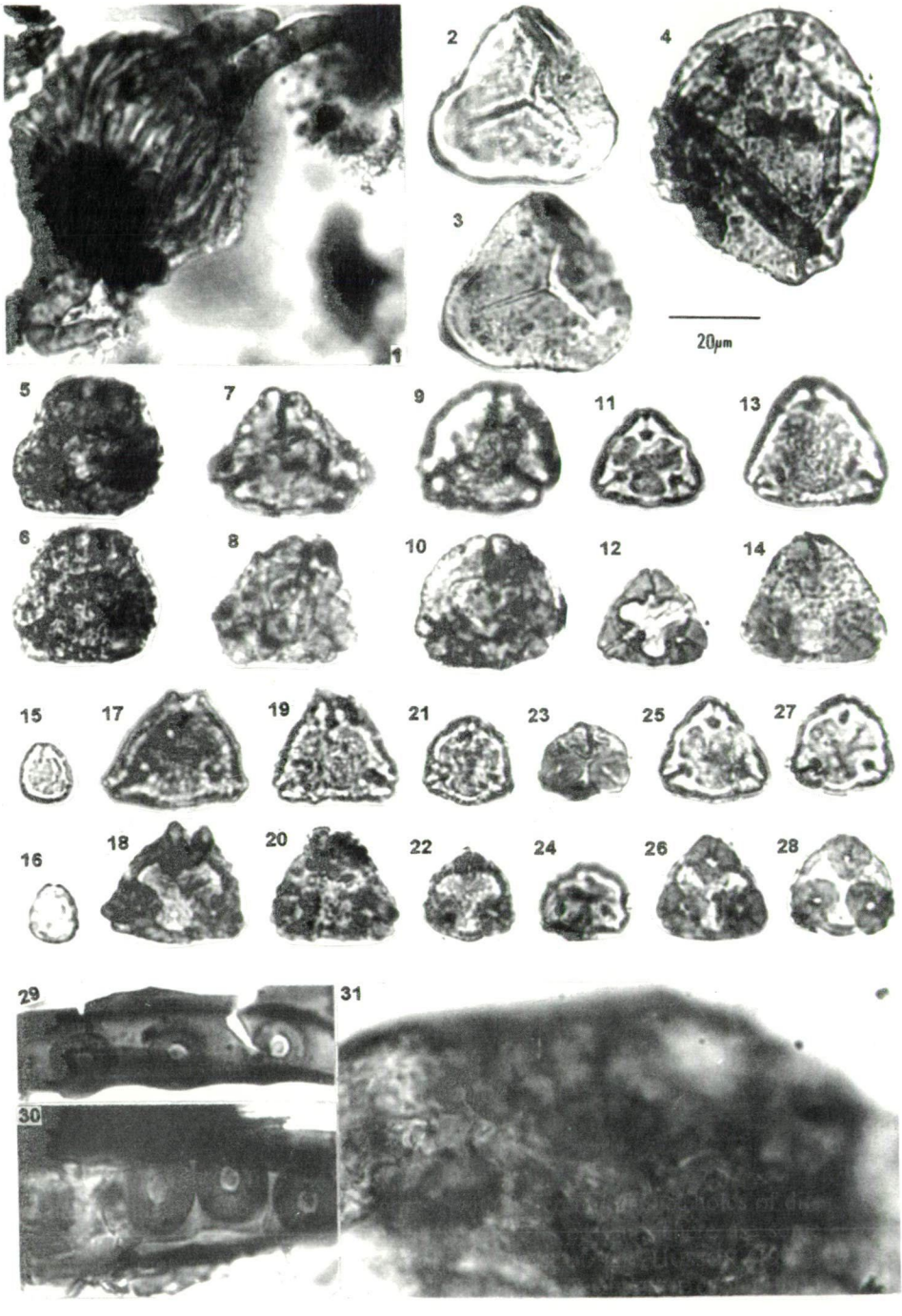
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Plate 1.1.

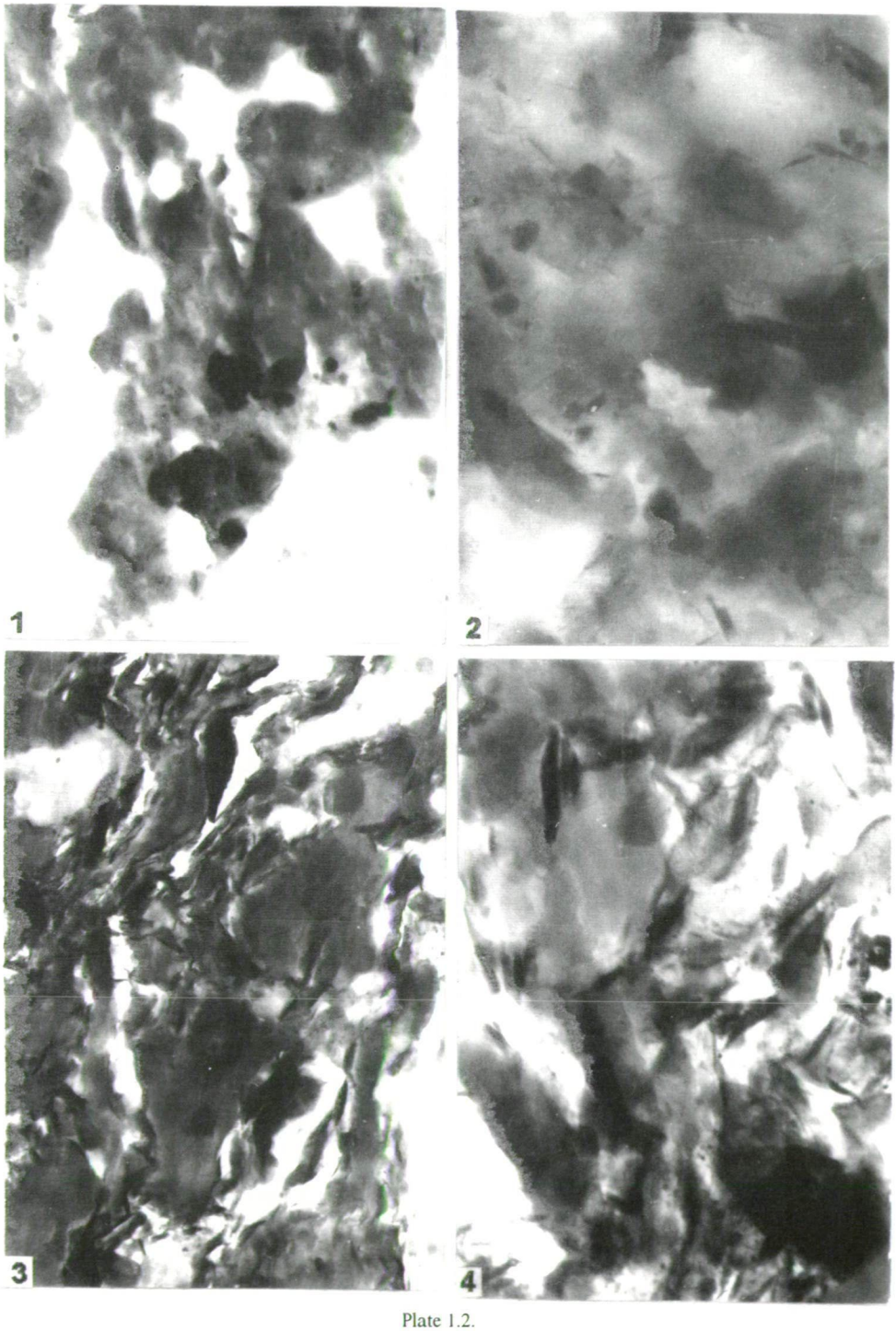

Plate 1.2.

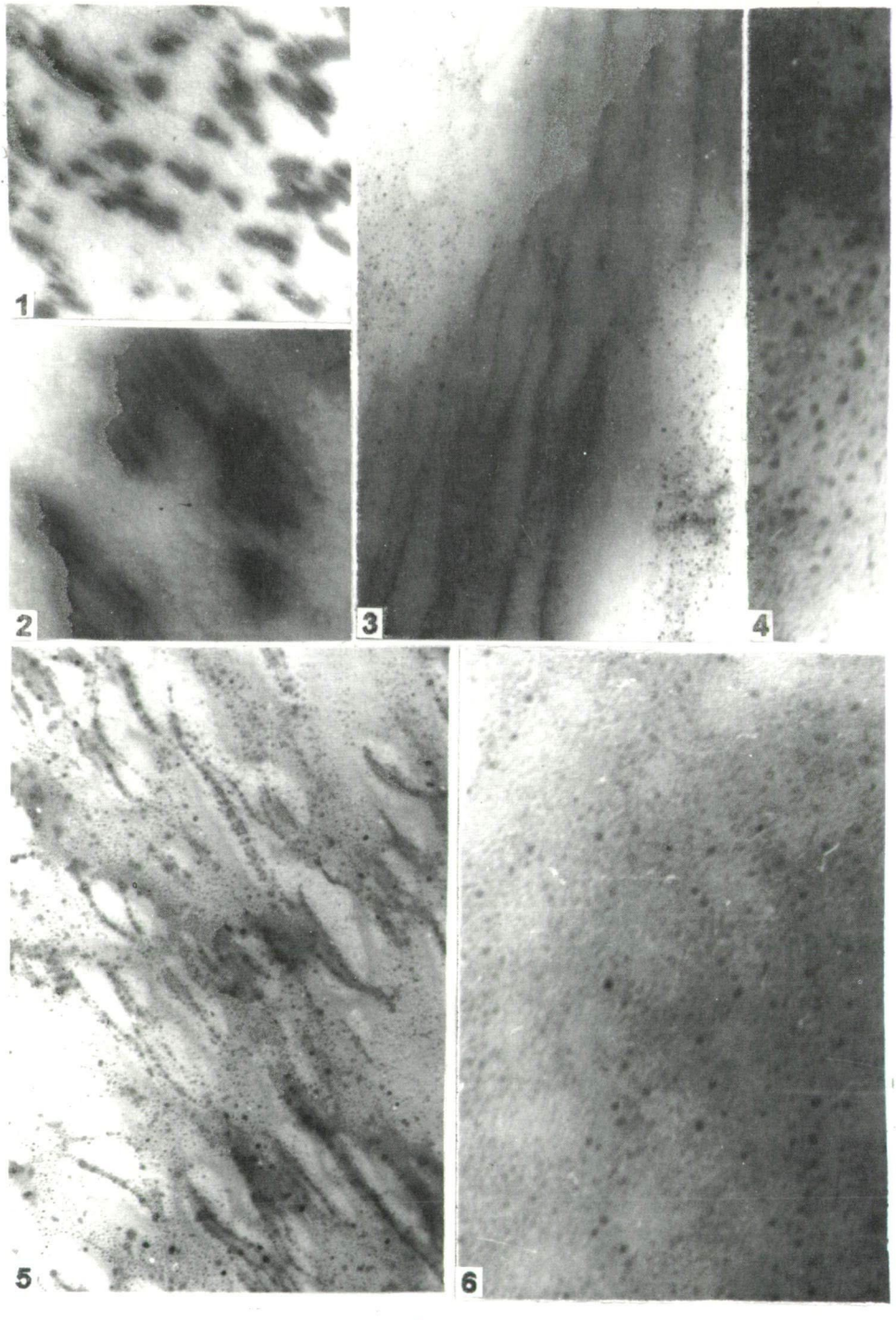

Plate 1.3.

Plate 1.1.

1. *Appendicisporites* cf. *tricuspidatus* WEYLAND et GREIFELD 1953, slide: KG-99-5; cross-table number: 19.6/126.8
2,3. *Punctatisporites aquisgranensis* WEYLAND et KRIEGER 1953, slide: KG-99-5; cross-table number: 18.2/128.4.
4. *Araucariacites australis* COOKSON 1947, *Araucariaceae*, slide: KG-99-2; cross-table number: 20.6/133.7.
5,6. *Hungaropollis ajkanus* GÓCZÁN 1964, slide: KG-99-3; cross-table number: 18.5/137.2.
7,8. *Hungaropollis ajkanus* GÓCZÁN 1964, slide: KG-99-3; cross-table number: 23.6/130.9.
9,10. *Hungaropollis krutzschi* GÓCZÁN 1964, slide: KG-99-2; cross-table number: 9.3/129.3.
11,12. *Hofkeripollenites hemimechanicus* (PFLUG 1953a) KEDVES et HERNGREEN 1980, slide: KG-99-4; cross-table number: 12.3/125.4.
13,14. *Trudopollis articulus* WEYLAND et KRIEGER 1953, slide: KG-99-4; cross-table number: 20.7/140.9.
15,16. *Minorpollis gallicus* KEDVES 1969, slide: KG-99-4; cross-table number: 19.2/128.1.
17,18. *Semioculopollis* cf. *maestrichtiensis* KEDVES et HERNGREEN 1980, slide: KG-99-5; cross-table number: 11.9/135.2.
19,20. *Semioculopollis granulosus* KEDVES et HERNGREEN 1980, slide: KG-99-5; cross-table number: 23.6/137.6.
21,22. *Semioculopollis croxtonae* KEDVES 1980, slide: KG-99-4; cross-table number: 15.2/137.2.
23,24. *Oculopollis minoris* KRUTZSCH 1973, slide: KG-99-4; cross-table number: 9.4/126.1.
25,26. *Oculopollis orbicularis* GÓCZÁN 1964, slide: KG-99-2; cross-table number: 11.6/128.4.
27,28. *Oculopollis orbicularis* GÓCZÁN 1964, slide: KG-99-3; cross-table number: 12.9/126.7.
29. *Gymnosperm* tracheid with areolate pits, slide: KG-99-4; cross-table number: 13.4/133.1.
30. *Gymnosperm* tracheid with areolate pits, slide: KG-99-2; cross-table number: 9.3/124.6.
31. *Oculopollis* fsp. pollen grains containing amber piece, slide: KG-99-2; cross-table number: 12.2/129.4.

Plate 1.2.

1,2. Ultrastructure of the MAT of the Ajkaite sample No: 99-KG-1.
1. Negative number: 7792, 15.000x.
2. Negative number: 7793, 50.000x.
3,4. Ultrastructure of the MAT of the Ajkaite sample No: 99-KG-3.
3. Negative number: 7795, 15.000x.
4. Negative number: 7796, 50.000x.

Plate 1.3.

1-6. Ultrastructure of the MAT and the Ajkaite of the sample No: 99-KG-4.
1. Negative number: 7799, 5.000x.
2. Negative number: 7800, 15.000x.
3. Negative number: 7802, 50.000x.
4. Negative number: 7802, 15.000x.
5. Negative number: 7804, 15.000x.
6. Negative number: 7702, 15.000x.

Sample 99-KG-3 (Plate 1.2., figs. 3,4)

The substance of the organic material is spongy, with different kinds of holes of different size. Sometimes not characteristic lamellar structure is present. There are some similarities with the picture published by LUGARDON, RAYNAUD and HUSSON (1991), Pl. II., fig. 2, from the sample of Upper Cretaceous of Oman, and DERENNE et al. (1991) from the *Gloeocapsomorpha prisca* from Estonian Kukersite, K1 A and B. The electron dense particles are in general elongated.

The TEM results of this sample are the most interesting, because of the variety of the observed ultrastructures. Similar to the "small elongated bacteria-like bodies" of LUGARDON, RAYNAUD and HUSSON (1991), Pl. II., fig. 7, from the Upper Cretaceous, Oman were also observed (Plate 1.3., figs. 1,2). Characteristic strands or lamelles (Plate 1.3., fig. 3), with electron dense globular particles (Plate 1.3., fig. 4) were observed. Cf. LUGARDON, RAYNAUD and HUSSON (1991), DUBREUIL, et al. (1989) from the Darwin Coorongite (Australia) and TEMPLIER et al. (1992) from the A and B races of *Botryococcus braunii* KÜTZ., after lipid extraction. There are holes in the amber (Plate 1.3., figs. 5,6) with globular electron dense particles. Picture 5 in Plate 1.3., illustrate globular electron dense particles in linear arrangement, which may also be of bacterial origin.

Discussion and Conclusions

This paper as it was emphasized presents the first results of the new research program of our Laboratory.

We have to point that in the future large LM investigations will be carried out on the sporomorphs of the Ajkaite containing layers and the woody remnants. Based on our preliminary data the investigated material may be identical with the "Upper part of the palynological zone B" Santonian of GÓCZÁN (1964). Regarding the secondary woody remnants, till this time we have observed only fragments of *gymnosperm* tracheids. The areolate pitting of the wall is modern, similar to some of the *Podocarpaceae* (cf. *Podocarpoxylon ajkaense* GREGUSS 1949), or with some so-called modern coniferous taxa. In this way the genus of *Pinus* is also possible. But it is necessary to emphasize that till this time *Araucariaceae* secondary woody remnant was not observed. The areolate pitting of the radial wall of the tracheids is very characteristic (cf. GREGUSS, 1955, 1972) as well as the pits of the cross fields.

As regards the TEM investigations, in this moment it is the most important that the ultrathin sectioning of the amber was successful, and the organic material was suitable for TEM investigations together with the amber. A remarkable number blocks will be ultrathin sectioned and investigated with transmission electron microscope. Preparation of further material for TEM investigations is in progress. We planned to select the sporomorphs containing Ajkaite pieces under the light microscope.

Our preliminary data on the MAT of the Ajkaite are encouraging for further remarkable results.

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