COMPLEX STUDIES ON THE POLLEN GRAINS OF ELAEAGNUS ANGUSTIFOLIA L.

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(Received December 1, 1980)

Abstract

Pollen grains of different maturity as well as acetolyzed and non-acetolyzed ones of *Elaeagnus angustifolia* L. were investigated under light, transmission resp. scanning electron microscope. During the investigation of the diameters of pollen grains the Cushing effect was also taken into consideration. Qualitative results revealed tetraexitus and plicate forms, too. This and the brevicolporate character with vestibulum are the most reminiscent of the ancien Normapolles, the *Complexiopollis* W. KR. 1959 em. TSCHUDY 1973. Further identities are the lamellar foot layer in the germinal region and the rugulate-corrugate surface.

Introduction

There is abundant literature on the pollen grains of the genus *Elaeagnus*. These were reviewed by THANIKAIMONI (1972). Earlier reports (AMBRUSTER and JACOBS, 1934; ERDTMAN, 1954, 1966, WANG FU-HSIUNG, CHIEN NAN-FEN, YANG HUI-QU and ZHANG YU-LONG, 1960; ERDTMAN, BERGLUND and PRAGLOWSKI, 1961; GUINET, 1962; HUANG, 1972; KUPRIANOVA and ALYOSHINA, 1972; MC ANDREWS, BERTI and NORRIS, 1973) have shown that the pollen grains of the *Elaeagnus* possess such ancient properties, which surpass the primitive character of the recent pollen grains of Amentiflorae. Fossil data (e.g. KRUTZSCH, 1962; GRAY, 1964; GRUAS-CAVAGNETTO; 1978) are also supportive of the significance of the pollen grains of Elaeagnaceae.

Pollen grains of *Boehlensipollis* W. KR. 1962 and the form-genus *Slowakipollis* W. KR. were related to Elaeagnaceae by several authors. (POKROVSKAYA and STELMAK, 1960; TIMOSINA 1965; POLUMISKOVA et al. 1966; BOITSOVA and POKROVSKAYA 1966; BLYAKHOVA, 1971; HOCHULI, 1978; CHATEAUNEUF, 1980; OLLIVIERRE-PIERRE, 1980). Further fossil form-genuses are: *Elaeagnacites* KE et SHI 1978 (in SUNG TZE CHEN et TSAO LIU), *Elaeagnuspollenites* HUANG 1980. Concerning recent taxons of the family only LEINS' (1967) TEM data are known. Detailed literature, particularly on the TEM structure of the germinal aperture is not available yet. In view of the phylogenetical value of pollen grains, it was thought justified to investigate them thoroughly. The studies on the pollen grains of Elaeagnus angustifolia L. were performed to

1. investigate quantitatively and qualitatively the pollen grains at two different levels of ontogenesis,
2. investigate the Cushing effect on pollen grain preparations by various methods,

3. establish the general ultrastructural and ultrasculptural properties,

4. evaluate TEM and SEM features in the function of the maturity of pollen grains and the methods applied.

Materials and Methods

The material investigated was collected by Z. SZABÓ in the Botanical Garden of Attila József University, Szeged during spring, 1979. Pollen sacs of mature resp. closed buds were removed and used in acetolyzed resp. non-acetolyzed condition for light, transmission and scanning electron microscopic investigations. For light microscopy 39.6% hydrated glycerin-jelly was used according to LOBREAU (1966). To investigate the variation of diameter, 200–250 pollen grains at both stages of maturity and prepared by each of the above methods were measured. For the investigation of Cushing effect, the measurements performed immediately after the preparations were repeated in December, 1979. For TEM studies, the material was fixed with OsO_4 (distilled water) and embedded into araldite. Ultrathin sections were cut with a glass knife. JEOL-100B electron microscope was used for examination and for taking micrographs. Its resolving power was 2Å. For SEM studies, the pollen grains were mounted on polyvinil-chloride adhesive-coated grids and evaporated with gold-palladium alloy. The fine sculpture was studied with the JEM-ASID scanning adapter of the aforementioned apparatus.

Results

1. Light microscopic results

The surface, contour and the nature of the germinal aperture of the pollen grain was not changed by the method used for the preparation of the material. The equatorial contour was triangular, with convex sides, the germinal region prominent. Surface finely rugulate-corrugate. Colpus narrow, 18–20 µm long (Plate I, 1, 2), vestibulum marked, endoaperture pore (Plate II, 1–7). Plicae, but principally pseudoplicae, as well as tetraexitus forms occasionally occurred (Plate I, 3–8). In the case of tetraexitus forms the contour was generally convex.

On the basis of the variation of measurements the following table was composed:

	October			December		
	measurements in					
	l	u	m	l	u	m
Mature flower, non-acetolyzed	30.0–60.4		44.1	39.0–60.4		44.0
Bud, non-acetolyzed	35.2–50.0		44.0	38.5–50.0		44.0
Mature flower, acetolyzed	34.7–56.4		49.0	38.0–62.0		49.0
Bud, acetolyzed	30.2–52.4		49.0	40.1–55.4		49.0

(l = lower, u = upper, m = maximum; the greatest and the smallest pollen grain, and the measurements occurring in maximal amount, in µm).

From the data the following conclusions were drown:

1. The measurements of pollen grains prepared by the same method were approx identical in the same time point, meaning that the pollen grains of the closed bud and those of the open flower do not differ from each other in this regard.

Plate I. Light microscopic picture of the pollen grains of *Elaeagnus angustifolia* L. x1000.
1, 2 typical specimen.
3–6. plicate forms.
7, 8 tetraexitus form.

Plate I

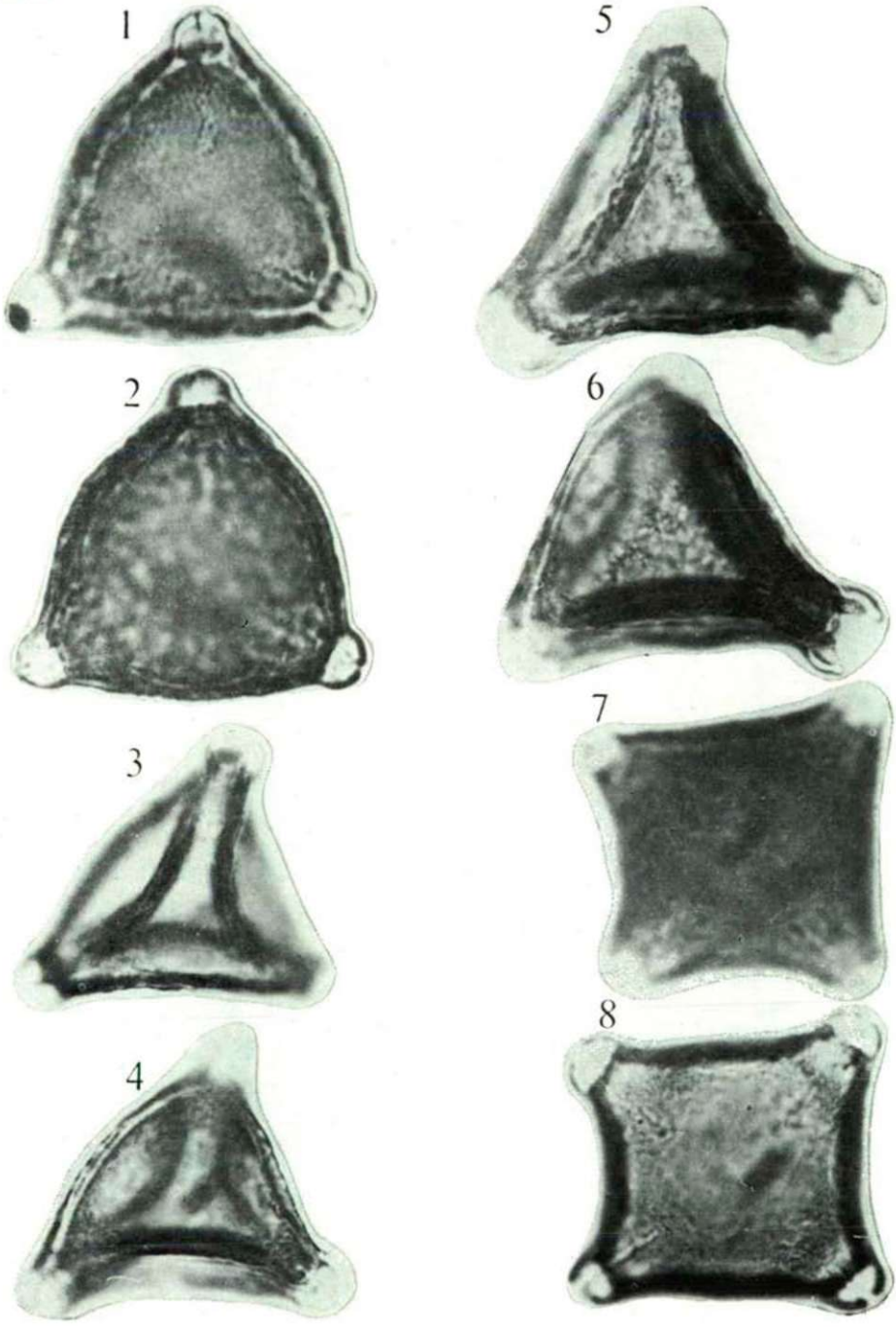


Plate II

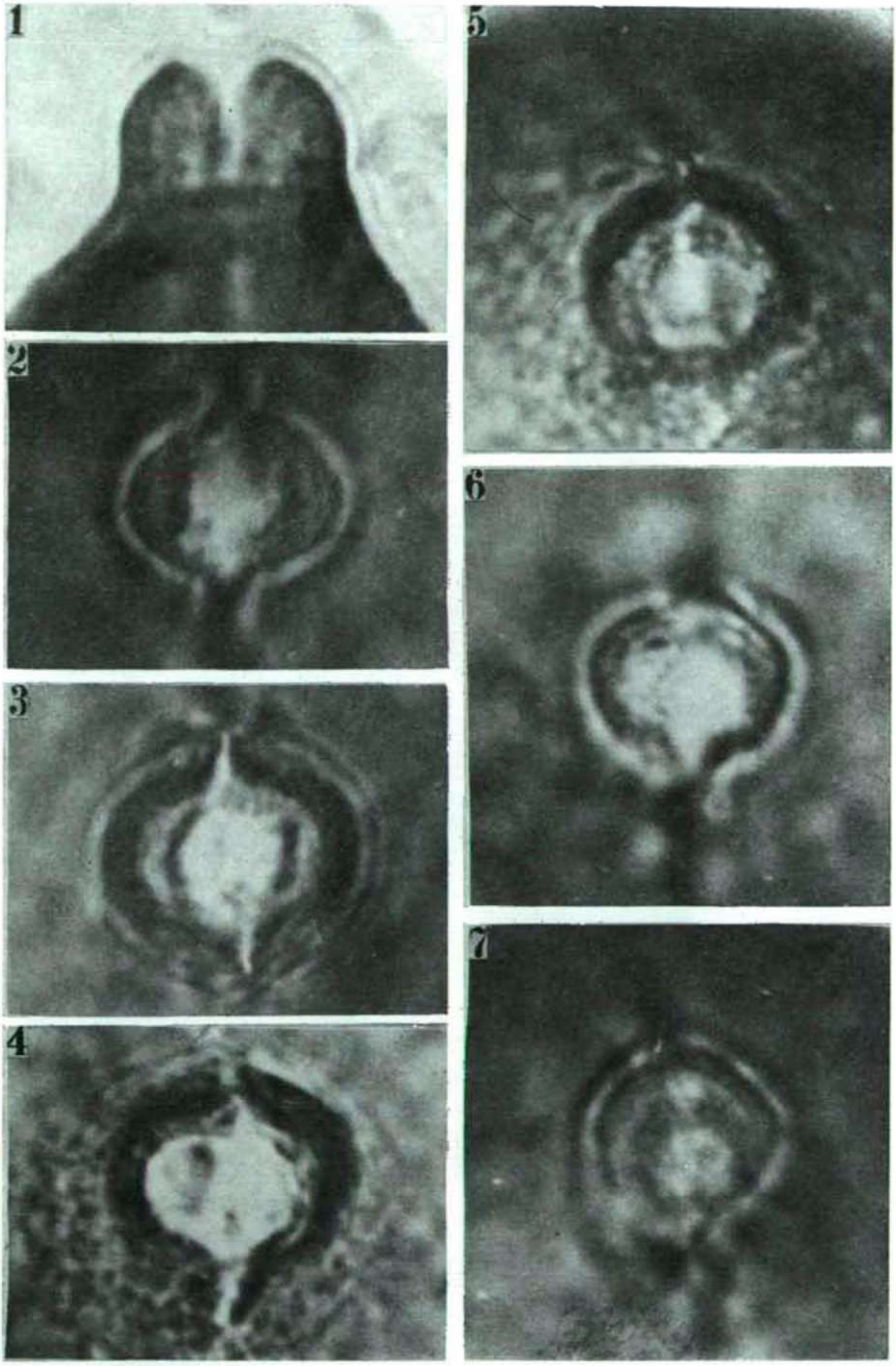
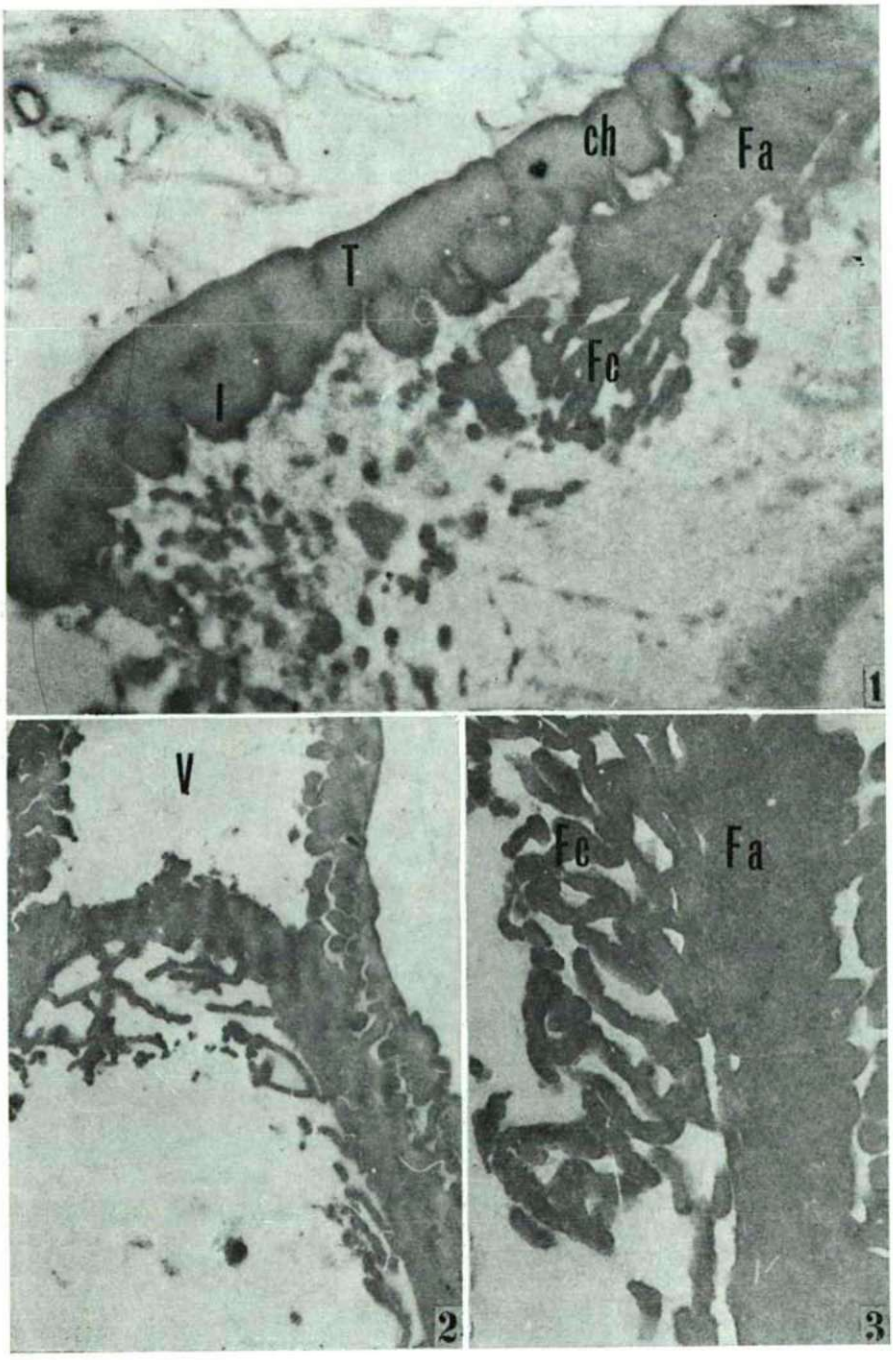


Plate II. Finer light microscopic structure of the germinal aperture of pollen of *Elaeagnus angustifolia* L. in optical section resp. top-view. x5000.

Plate III. Transmission electron microscopic structure of the pollen grain from *Elaeagnus angustifolia* L.



1. Ultrastructure of pollen grain from non-acetolyzed bud. Germinal region. x25 000.
2. Ultrastructure of pollen grain from acetolyzed flower. Germinal region. x10 000.
3. Ultrastructure of the foot layer of pollen grain from acetolyzed bud. Germinal region. x25000.

T = tectum, I = infratectum, F = foot layer, Fa = compact foot layer, Fc = foot layer with lamellar ultrastructure, ch = channels, V = vestibulum.

2. Acetolyzation generally caused an approx 11.36% increase of measurements in the majority of pollen grains.

3. During measurements in October and December, the pollen grains were maximally equal in size, though the under and upper values of measurement for pollen grains increased somewhat.

2. Transmission electron microscopic (TEM) results

Since the ultrastructural features of pollen grains from buds resp. open flowers prepared with acetolyzation resp. without it were identical, they will be discussed here together. Extragerminal exine. — Tectate, perforated with channels. Of the layers of ectexine, infratectum is the most narrow one. It is made up of narrow columns. The tectum and that part of the foot layer which is near the infratectum are µneven. Of the layers of the ectexine the foot layer is the thickest, there is no endexine.

Germinal exine. — Tectum slightly perforated in the germinal region, as in the extragerminal part (Plate III, 1). Vestibulum marked (Plate III, 1, 2), before the endoaperture the foot layer is broken up, resp. becomes lamellar (Fc). In the vestibulum, the infratectum is not columellar, it is ellipsoid, globular or irregular in shape. There are tiny granules under the exoaperture (colpus) on the pollen grains from non-acetolyzed buds (Plate III, 1). In the germinal region, the lamellar ultrastructure of the inner part of the foot layer is noticeable (Plate III, 2, 3). Its endexine cannot be observed in the germinal region.

3. Scanning electron microscopic (SEM) results

Degree of maturity investigated and acetolysis did not affect essentially the superficial ultrastructure of pollen grains. There was only one difference that on the surfaces of non-acetolyzed pollen grains, spherical, most probably pollenkitt granules occurred (Plate IV, 1, 2, 4), which were particularly frequent on the pollen grains from closed buds. It is seen that the colpi are undulated at the margins, the about 5 µm wide zone near them is slightly sculptured. Ornamentation fine, measuring generally 1–3 µm. Its character is rugulate, occasionally corrugate.

Discussion

1. Complex investigation did not reveal noteworthy differences between pollen grains from closed buds and open flowers of *Elaeagnus angustifolia* L.

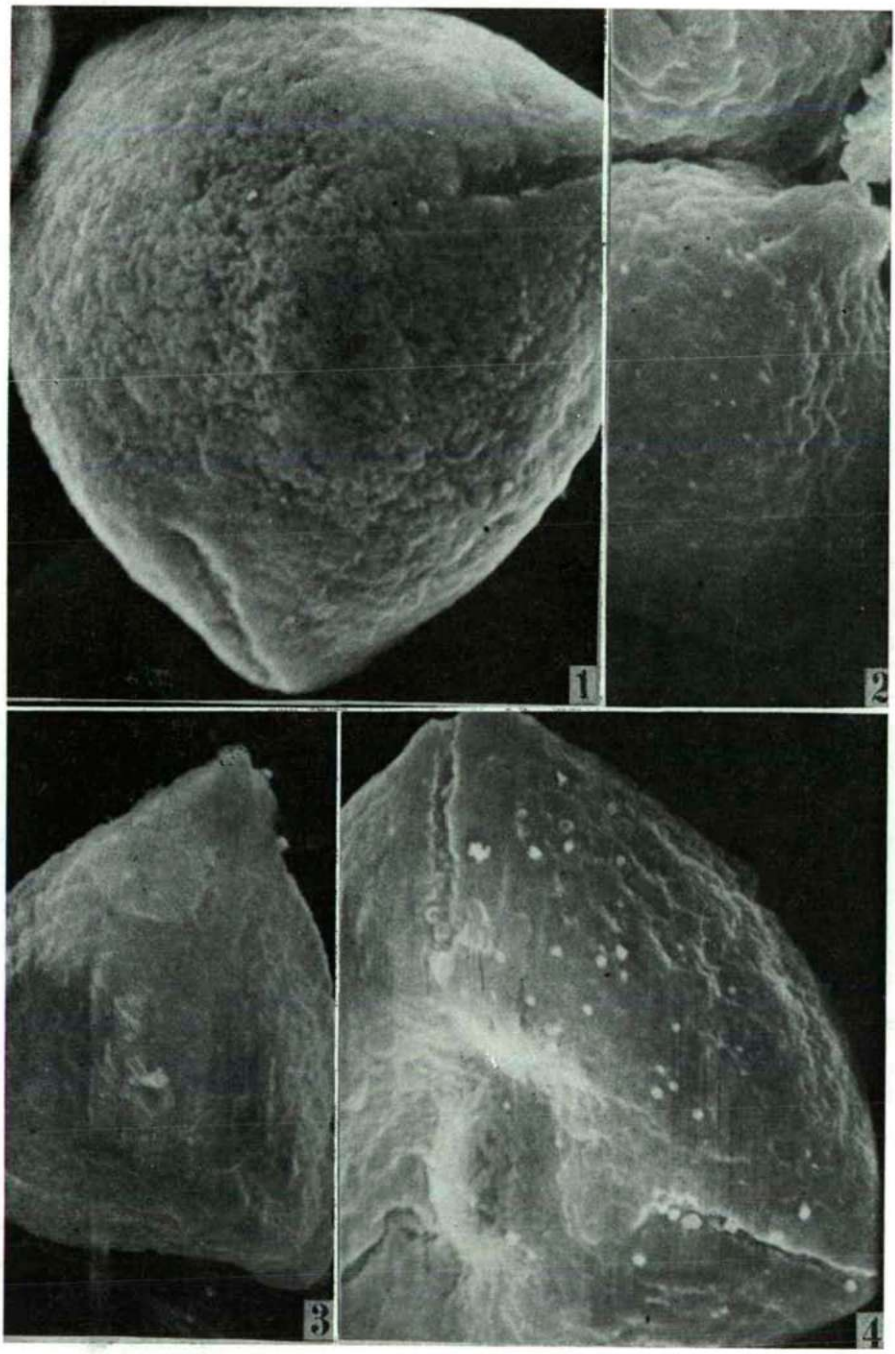
2. Only small time-dependent changes could be observed in pollen grains embedded into 39.6% hydrated glycerin-jelly. On the other hand, acetolysis considerably changed the measurements of pollen grains.

3. Of the qualitative results of light microscopic studies, the occasional occurrence of plicae, resp. pseudoplicae forms should be stressed. (The latter conception is used when two plicae occur on one surface and a third on the other one.) This is

Plate IV. Scanning electron microscopic (SEM) picture of pollen grains of *Elaeagnus angustifolia* L.

1. Non-acetolyzed pollen from flower. x2000.
2. Non-acetolyzed pollen from bud. x1000.
3. Acetolyzed pollen from flower. x1000.
4. Non-acetolyzed pollen from bud. x2000.

Plare IV



characteristic of one of the groups of Normapolles. A form-genus value has also been ascribed to this character. It should be emphasized that with fossil forms this question should be also considered with criticism, and that besides the tetraexitus forms, plicate specimens can also occur beside the "normal" ones. *Plicapollis pseudoexcelsus* is a good example for the opposite case. In a considerable proportion of its specimens plicae do not or only seldom occur.

4. Each of the complex methods, but principally the TEM one suggests that the pollen grains of *Elaeagnus angustifolia* L. may be regarded the morphological analogues of *Complexiopollis* W. Kr. 1959 em. Tschudy 1973, without supposing direct botanical relationship between the two.

Recent results gave grounds for the revision of the systematics of fossil pollen grains of Brevaxones (Kedves, 1981). At present it is the following:

1. Probrevaxones
2. Normapolles
2.1. Pronormapolles
2.2. Eunormapolles
2.3. Paranormapolles
3. Postnormapolles.

Recent analogies for Probrevaxones taxons are not known. There is only an analogue recent type for the classic form-genus of the ancient Normapolles (Pronormapolles). It is interesting that the light microscopic structure, but mainly the electron microscopic one of this group differs basically from Eunormapolles, Amentiflorae taxons, implying that there is a fairly sharp difference between the two stages of development. The establishing of transitional, connecting types is the task of further investigations.

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