

SOME MORPHOLOGICAL FEATURES OF THE LEAF EPIDERMIS IN FOSSIL SPECIES AND RELATED PRESENT-DAY VEGETAL SPECIES

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Abstract. Five fossil species (*Pseudocycas dunkeriana*, *Tsuga europaea*, *Taxodium dubium*, *Platanus neptuni* and *Juglans acuminata*) have been compared to several present-day related species (*Cycas revoluta*, *Tsuga canadensis*, *Taxodium distichum*, *Platanus hybrida* and *Juglans regia*) on the basis of some leaf epidermal features: the size of the epidermal cells of the leaves and stomata, as well as their other characteristics. We focused on similarities and some differences that exist between fossil and present-day related species.

Keywords: fossil and present-day species; Mesozoic and Cenozoic ages, leaf epidermal features.

INTRODUCTION

The analysis of the morphological features of leaves and other organs offers information about the climate conditions in different geological periods of our planet that are also of meaning in the taxonomical characterization of plants. Since the beginning of the XXth century, Bailey and Sinnott (1915, 1916) have been the first ones to observe a direct correlation between the proportions of dicotyledonous woody species with full leaves, and a moderate annual temperature. The importance of the leaf cuticle and stomata in Angiosperms was underlined by an exhaustive study (Upchurch-Jr, 1984) in order to establish the natural affinities between different plant groups.

Vickulin et al. (2003a, 2003b) used the anatomy of the epidermal leaf, especially the stomata architecture to distinguish fossil from living species and to separate different genotypes in the *Taxodium* genus. The stomata architecture in combination with the size and shape of epidermal cells is used in the characterization of different taxons.

Corneanu et al. (2004) analyzed quantitatively some leaf epidermal features in the fossil species *Buxus sempervirens*, *Ginkgo adiantoides*, *Magnolia liblarensis*, *Taxus inopinata*, and compared them with those of their present correspondent species. Based on this comparison, the authors ascertained that the speciation through polyploidy took place in the fossil species *Buxus sempervirens* Linne (Chiuzbaia, Maramureş) during the Miocene period.

Kovar-Eder (2004) analyzed Upper Miocene fossil flora from Styria (Austria) using the morphological parameters of the leaves, as well as some microscopic measurements for the characteristics of the cuticles.

Grote (2008) used leaf architecture to compare plant diversity of the Tertiary with their present-day correspondents in the flora of Thailand. He analyzed the cuticles of 253 fossil leaf specimens belonging to 157 species, 146 genera and 77 families (to which were added some other specimens with an uncertain classification), of the Early Miocene or Late Oligocene, from Thailand.

Kunzmann et al. (2009) studied Miocene specimens of *Taxodium dubium* species from Germany, former Czechoslovakia and Russia – based on taxonomical considerations. Their studies emphasized the evolutionary changes that occurred in this species during the Oligocene, expressed as changes in the ornamentation of the cone species. An analysis of interspecies variability has led to

the determination of two morph forms:

- a) *Taxodium dubium* form *heerii* (Dorofeev) Kunzmann, Kvaček, May and Walther stat. nov. et emend.;

- b) *Taxodium dubium* form *dubium*.

The authors ascertained the absence of interspecies variability when the morphology and anatomy of leaves were examined, underlining the possibility of their use as taxonomical features particularly in speciation analysis. Manchester and Kvaček (2010) have analyzed the inflorescences and compound leaves of the extent *Platanus neptuni* complex in the Oligocene of Oregon (U.S.A.) and highlighted several distinctions between the *Platanus bella* of the Early Paleocene, the *Platanus neptuni* of the Late Paleocene, and the Miocene species.

In the present paper, we have analyzed a few features of the epidermal cells (size of the epidermal cells and stomata, as well as their shape) in fossil species vs. in related present-day species.

The authors of this study are geneticists with a particular interest in species evolution. For achieving our goal, we have used some paleontological specimens kindly provided by Acad. Prof. Răzvan Givulescu, and compared the biometrics data of the epidermal cells and stomata in fossil species vs. present-day - correspondent or not - related species, with a notable exception: *Pseudocycas dunkeriana* fossil species of the Bennettiales (the Cycadeoides fossil), and an existing species, the *Cycas revoluta* from Cycadophyta (Gymnosperms). The Bennettiales present some similarities to cycads, as well as a few very unusual features apparently shared by the angiosperms and the Gnatales too (Rothwell et al., 2009). The Cycadeoides fossils are considered ancestors of modern flowering plants (Taylor et al., 2009). As far as Bennettiales are concerned, a leaf fragment of the *Pseudocycas dunkeriana* species was discovered in Anina (Givulescu, 1998), assigned to Lower Jurassic. The *Cycas revoluta* is the best-known species of the present-day genus *Cycas*, of the *Cycadaceae* family and it has almost 99 species (Wikipedia, 2010). The recorded data on Early Permian cycad fossils (280 million years ago) generated a controversy over older cycad fossils dated as Late Carboniferous (300-325 million years ago). The Cycads reached their maximum development stage, as far as their geographic distribution and numbers of taxa was

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concerned, during the Mesozoic.

According to Crepet (2000), Cycads are most closely related to the extinct Bennettitales, and are also relatively closely related to the Ginkgoales. In some phylogeny schemes, the Bennettitales and Cycadales present a common ancestor (Miller, 2010), but so far a direct relationship based on a common ancestor has not been established. The foliage of the Bennettitales is morphologically very similar to that of the cycads both in macro-morphology and in their development (Taylor et al., 2009). That explains the great interest in comparing the size of the epidermal cells and the stomata size, as well as the shape of the fossil species *Pseudocycas dunkeriana*, with those characteristics of the present-day species *Cycas revoluta*, a living fossil.

MATERIAL AND METHODS

Biological material

Fossil species. We have measured some cytological indices (the size of the epidermal cells and stomata), as well as some morphological features of the leaf, on some fossil species from Mesozoic (Jurassic) and Cenozoic (Tertiary). Below are listed the fossil species, their origin and the appropriate bibliographical references:

Pseudocycas dunkeriana (GOEPPERT 1844) FLORIN 1933, Mesozoic, Lower Jurassic, Anina (Givulescu, 1998).

Tsuga europaea (MENZEL 1913) SZAFER 1947, Cenozoic, Tertiary, Neogene-Upper Miocene, Chiuzbaia (Givulescu, 1990).

Taxodium dubium (STERNBERG 1823) HEER 1853 emend., Cenozoic, Tertiary, Paleogen-Oligocen, Petroșani (Givulescu, 1996).

Platanus neptuni (ETTINGSH. 1877) BŮŽEK, HOLÝ & KVÁČEK 1967, Cenozoic, Tertiary, Late Miocene, Delureni (Givulescu, 2003).

Juglans acuminata A. BRAUN 1845 ex UNGER 1850, Cenozoic, Tertiary, Neogene-Upper Miocene, Borod (Givulescu, 2003).

Present-day species. In the case of present-day species, we have similarly analysed species related, or corresponding to the fossil ones (with one exception, *Cycas revoluta*). The specimens were collected from the Botanical Gardens in Craiova, located at 85 meters altitude. The basic systematic assignment of the present-day species (Tutin et al., 1964; Ciocârlan, 2009) is as follows:

Phylum Spermatophyta, Subphylum Pinophytina (Gymnospermae), Cycadopsida Class, Cycadales Order, *Cycadaceae* Family, *Cycas revoluta* Thunb. 1783;

Phylum Spermatophyta, Subphylum Pinophytina (Gymnospermae), Pinopsida Class (Coniferopsida), Pinales Order, *Pinaceae* Family (*Abietaceae*), Abietoideae subfamily, *Tsuga canadensis* (Linné 1753) Carrière 1855;

Phylum Spermatophyta, Subphylum Pinophytina (Gymnospermae), Pinopsida Class (Coniferopsida), Pinales Order, *Taxodiaceae* Family, *Taxodium distichum* (Linné 1753) L.C.M. Rich 1810;

Phylum Spermatophyta, Subphylum Magnoliophytina (Angiospermae), Magnoliopsida Class (Dicotyledonatae), Hamamelidae Subclass, Hamamelidales Order, *Platanaceae* Family, *Platanus hybrida* Brot. 1804;

Phylum Spermatophyta, Subphylum Magnoliophytina (Angiospermae), Magnoliopsida Class (Dicotyledonatae), Hamamelidae Subclass, Juglandales Order, *Juglandaceae* Family, *Juglans regia* Linné 1753.

Methodology

For fossil species, we have used biometric values as well as some morphological features of the epidermal cells and stomata, benefiting from the kind assistance provided by Acad. Prof. Răzvan Givulescu (Cluj-Napoca) and from scientific bibliographical sources. For present-day species, we have made 30 biometric observations on mature plants (during the flowering stage). By the same token we have also observed the size (length and width) and shape of the epidermal cells, the length of the stomata and other structural features of the leaves. The biometric values were statistically interpreted.

RESULTS AND COMMENTS

Pseudocycas dunkeriana, Lower Jurassic, Anina (Givulescu, 1998). The slightly cutinized upper epidermis (adaxial) is made up of polygonal cells with rounded corners disposed in more or less regular rows. The length of the epidermal cells varies from 30.76 μm to 73.89 μm , with an average of 50.82 μm ; their width varies from 18.53 μm to 36.99 μm with an average of 26.73 μm (Table 1). The lower epidermis (abaxial) displays a thicker cuticle, its cells being grouped in the same manner as the superior epidermis cells. The length of the inferior epidermal cells varies from 30.80 μm to 55.44 μm in its lower limits – with an inferior average value (41.97 μm), comparing favourably with the length of the upper epidermis cells; at the same time, the cells width registers values comparable to those of the superior epidermal cells (their limits ranging from 18.48 μm to 36.96 μm , and a slightly superior average value - 27.72 μm). Stomata disposed in irregular rows present a slightly inferior length with limits between 33.9 μm and 41.1 μm and an average of 37.5 μm , since they are epidermal cells adapted to gaseous exchange (Fig. 1).

The *Cycas revoluta* existing species is grown as an ornamental plant throughout the world. When we compared this species to the fossil species *Pseudocycas revoluta* we have noticed several structural and biometric differences of the leaves' epidermal cells. The upper epidermis (Fig. 3) is monolayered and it is covered by a very thick cuticle (ranging from 5.00 μm to 6.25 μm). The polygonal cells whose sides vary from 3 to 6 have rounded corners; length-wise, they are usually laid out in regular rows, by the longitudinal axle of the leaves. The desmosomes are quite obvious. Variability limits for upper epidermis cells length and their average value (Table 1) are ostensibly lower as compared to the same values of the fossil species (Fig. 3). The width of the cells varies in wider limits but the average value is lower (Table 1).

The lower epidermis (Fig. 4) is made up of polygonal cells with rounded corners and 4 to 6 sides covered with a highly sclerenchymous cuticle (10 μm in thickness). The variability limits, both the length of the cell and its width are higher, whereas the average values are different: considerably equal for the length of the cells, and higher for their width (Table 1; Fig. 5). In some cases, there are free spaces between the median lamina of three adjacent cells. The stomata (Fig. 2) oriented in different directions display 2-3 annexed cells. The length of the stomata length varies slightly, the mean value being smaller (Table 1).

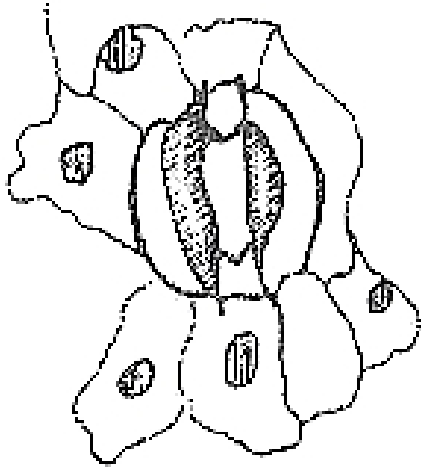


Fig. 1 - *Pseudocycas dunkeriana* – lower epidermis (according to Givulescu, 1998).

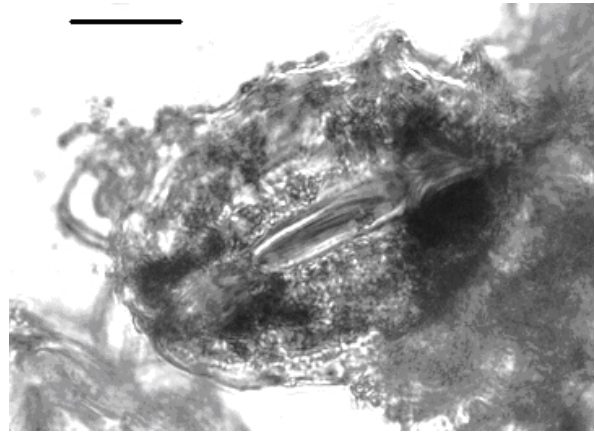


Fig. 2 - *Cycas revoluta* – stomata on lower epidermis (scale bar = 10 μ m).

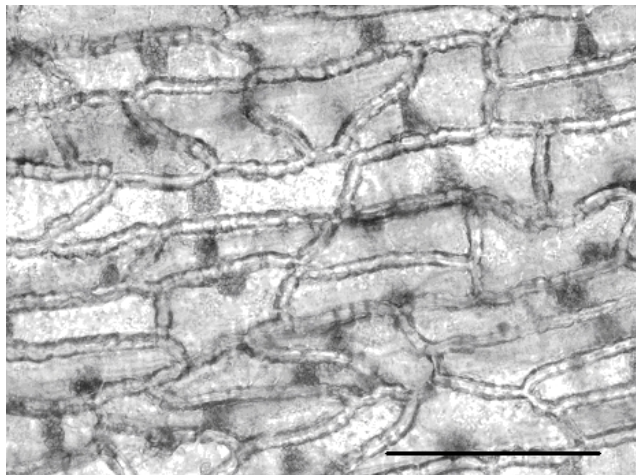


Fig. 3 - *Cycas revoluta* – upper epidermis (scale bar = 50 μ m).

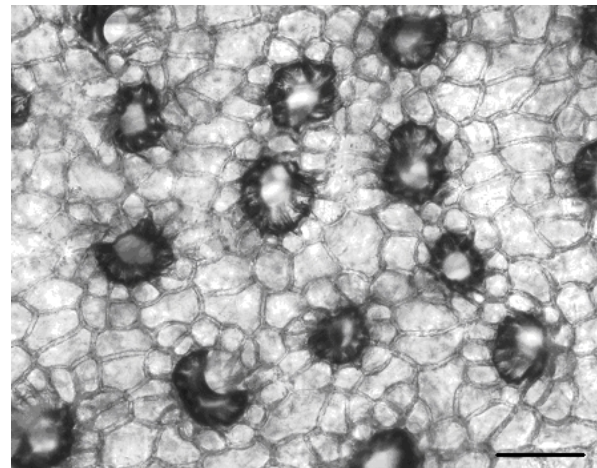


Fig. 4 - *Cycas revoluta* – lower epidermis (scale bar = 50 μ m).

The Bennettitales (Cycadeoids) present pinnately compound leaves whose surface is very similar to that of cycad leaves. The epidermis analysis reveals two different aspects: similar values for the length of epidermis cells in both species (Table 1) and different values for the length of the stomata and the cuticle thickness. The morphological resemblance of the leaves may be the result of a convergent process (quite frequent in the living realm), while differences in other morphological features of the epidermis (the length of the stomata and the cuticle thickness) may be the result of a process of adaptation to environmental conditions.

The Bennettitales were propounded as possible ancestors of flowering plants, given their bisexual reproductive structures (Leubner, 2009). The existence of the dioicy process in present-day Cycads is underlined by the presence of heterosomes (sexual chromosomes) implied in the sexual determinism (Khoshoo, 1961; Segawa et al., 1971; Kokubugata et Kondo, 1996). With regard to *Cycas revoluta*, the presence of sexual determinism of the *Drosophyla* type was ascertained, the male plant displaying two types of chromosomes (X and Y) and the female plants only one single chromosome type (XX). Although Cycads offer a small number of species, the Mesozoic Bennettitales have been well represented – as far as the number of species and their distribution on

Earth is concerned. In this extinct group of plants evincing a dioicy process, the sexual chromosomes of very old origin play an important role in the evolution of the species.

Moreover, researches performed by Kono and Tobe (2007), underlined that *Cycas revoluta* appears to use an initial mode of animal pollination; furthermore, a specific substance in pollinating insects exists both in the male and the female cone nitidulin. With regard to Bennettitales, the study performed by Labandeira et al. (2007) revealed the presence of pollinating insects in Mesozoic gymnosperms too.

Tsuga europaea, Upper Miocene, Chiuzbaia (Givulescu, 1990, 2003).

Tsuga europaea is a taxon that can be found both in the Great Lakes Region of North America and in Europe (in Upper Miocene deposits, at Chiuzbaia - Givulescu, 1990; in the Pliocene of Alsace, Teodoridis et al., 2009). The epidermis leaves features in the fossil species *Tsuga europaea* and the present-day species *Tsuga canadensis* are quite similar.

We have recorded only a few biometrical values on the lower epidermis of the fossil species *Tsuga europaea*: the length of the stomata apparatus varies

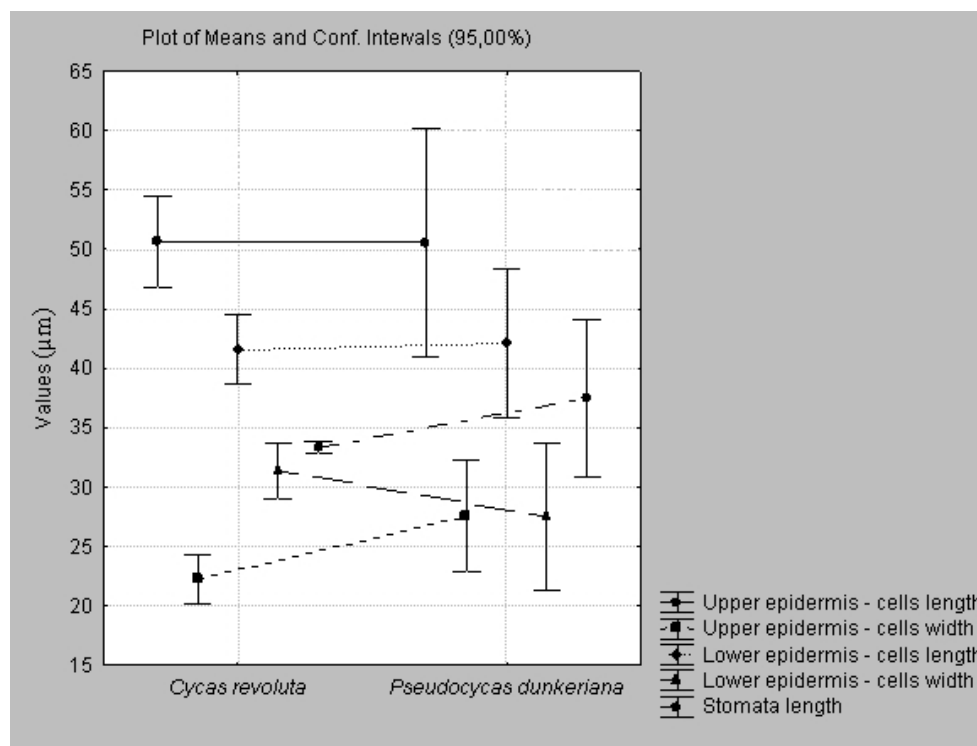


Fig. 5 - Variation limits and mean of some cytotaxonomical features in *Pseudocycas dunkeriana* and *Cycas revoluta*.

between 56 µm and 70 µm and its width between 34 µm and 36 µm, the length of the stomata varying from 34 µm and 36 µm (Givulescu, 2003). The absence of biometrical values referring to the size of the epidermal cells may be attributed to the length of the cells in the present-day species (and probably in fossil species as well) and to the particular ecological conditions affecting the growth of this species (along rivers and in meadows).

According to Givulescu (1999), the *Tsuga canadensis* represents the present-day correspondent of the fossil species *Tsuga europaea*.

As far as *Tsuga canadensis* is concerned, its upper epidermis is made up of cells whose shape and size resemble those of the cells without stomata of the lower epidermis. The very elongated polygonal cells have numerous punctuations and are covered by a thick cuticle with 4 (sometimes even 5) sides. The recorded biometrics values are displayed in Table 1. The lower epidermis is represented by two sectors of the cells: (A) rows of cells without stomata, situated near the nervure and on both sides of the leaf, and (B) rows of cells with stomata, shorter and polygonal in shape. The same characteristic structure also occurs in other fossil species (e.g. *Taxodium dubium* by Kunzmann et al., 2009). The regions without stomata are constituted of 4-5 rows of parallelepiped cells with 4-5 sides, with an almost straight cell wall covered by a thin cuticle. The regions with stomata contain polygonal elongated cells, with 4-5 sides, shorter than the cells from adjacent regions.

The stomata (3-8) are all grouped in the longitudinal transverse sector of the leaves, separated by 1-2 (or 3) short epidermal cells – similar in length with the annexed stomata cells). The biometric values recorded for the epidermal and stomata cells are displayed in Table 1.

Compared to *Tsuga europaea* fossil species that displays stomata with a length of 34.0 µm to 36.0 µm

(mean of 35.00 µm ± 0.89 µm), *Tsuga canadensis* present-day species displays very elongated stomata (with variability limits ranging from 48.0 µm to 64.0 µm and an average of 54.27 µm ± 4.06 µm), the difference being thus very significant (Fig. 6).

The difference in the stomata length between the fossil and the present-day *Tsuga* species may be accounted for by the results of the investigations made by different authors. The stomata length in conifers may be attributed to their ontogenetic development stage or to the amount of CO₂ in the air. Kouwenberg et al. (2004) have established that the length and stomata density of conifers (*Tsuga heterophylla*) change during the ontogenetic development of the leaf. Thus, in studies performed on leaf ontogenetic stages, the differences recorded between the fossil *Tsuga europaea* and the present-day *Tsuga Canadensis* were diminished.

In other researches concerning four conifer species (*Tsuga heterophylla* included), Kouwenberg et al. (2003) established the capacity of conifers to adjust their stomata frequency to changes in their CO₂ mixing ratios, emphasizing the fact that a negative correlation exists between the stomata frequency and the amount of CO₂ in the atmosphere.

Taxodium dubium was a forest tree widely spread in Europe, Asia and America during the Upper Oligocene, as well as in the Miocene (Givulescu, 1996). The fossil specimens in Romania are present in Upper Oligocene deposits from Petroşani Basin (Lonea-Jieţ, Petrla, Lupeni, Livezeni; Givulescu 1996). Pending on the organs analyzed in order to establish their phylogenetic relationships (leaves or cones), *Taxodium distichum* is considered to be the present-day correspondent species ((L. Rich.) of *Taxodium mucronatum* Tennessee (Givulescu, 1996; Vickulin et

SOME MORPHOLOGICAL FEATURES OF THE LEAF EPIDERMIS IN FOSSIL SPECIES

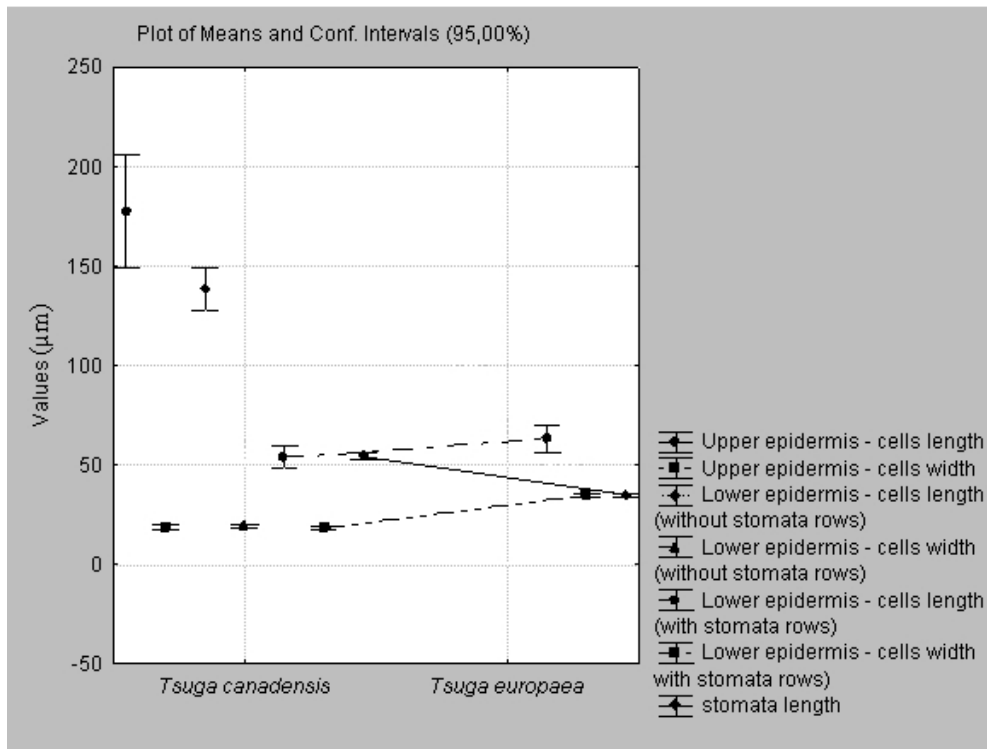


Fig. 6 - Variation limits and mean of some cytotaxonomical features in *Tsuga europaea* and *Tsuga canadensis*.

al., 2003). Kusumi et al. (2000) have analyzed the relationships between the *Taxodiaceae* and the *Cupressaceae* resorting to four chloroplasts genes and an intron. The resulting phylogenetic schemes suggest a much closer relationship between *Taxodium mucronatum*, *Taxodium distichum* var. *imbricatum* and *T. distichum* var. *distichum*, which derive from a common ancestor.

A detailed analysis of the structural features of the epidermis was offered by Kunzmann et al. (2009) for numerous specimens of the *Taxodium dubium* species from the Paleogene and Neogene of Central Europe. They observed the existence of a certain degree of variability between different genotypes regarding the stomata presence on the leaf surface. Thus, in *Taxodium mucronatum* species, the stomata are disposed relatively equal on both sides of the leaf, while in both varieties of *Taxodium distichum* species the stomata are present in a great number on the abaxial side of the leaf. In *Taxodium dubium*, the stomata are usually absent at the same location. The differences regarding the presence and disposition of the stomata cannot emphasize the ecological niche influence since all the studied species are present in the same region. The stomata disposition constitutes a morphological feature used in the identification of some genotypes.

The *Taxodium dubium* analyzed by Kunzmann et al. (2009) presented the following features: the upper epidermis (Fig. 7, according to Kunzmann et al., 2009) is made up of polygonal, elongated cells with 4 sides. If the short walls of the cells are in an oblique position, the cell edges are rounded. Although the stomata are generally absent on the upper epidermis, however in some cases short rows display a few stomata, usually located on the middle leaf or on the leaf basis. Just like in the case of *Tsuga europaea* species, the lower epidermis (Fig. 8, according to Kunzmann et al., 2009) displays rows of cells

with stomata and rows of cells without stomata. The elongated epidermal cell of parallelepiped shape has 4 sides (sometimes even 5 sides). The stomata are oriented usually on the length of the leaf. The cuticle leaf is thin. In the study performed by Kunzmann et al. (2009), on fossil remains of *Taxodium dubium* from Upper Eocene to Lower Miocene (Rupelian) from Germany, Czech Republic and Russia, specific differences were not underlined (or these were very slightly noticeable) concerning leaf morphology or leaf epidermal anatomy.

The study performed by Worobiec (1995) in the brown coal mine "Belchatów" (Central Poland) on Lower Miocene specimens of *Taxodium dubium* evidenced the presence of two morphological cell types on the abaxial side of the leaf, corresponding to the rows of cells with and without stomata in the present-day species *Taxodium distichum*. The epidermal cells presented a length between 19.7 µm and 40.0 µm, which is inferior to the values recorded in the present-day species *Taxodium distichum*.

Taxodium distichum. The upper epidermis made up of long cells with 4 sides, the cells situated on the exterior of the leaf being usually enlarged, with slightly rounded corners. The cells are disposed in orderly rows (Fig. 9), usually without stomata. Rarely, a few stomata can be present in the transition region from the abaxial to the adaxial sides of the leaf. The variability limits for the length and width of the cells are very broad (the cell length varies from 50.0 µm to 145.0 µm and the cell width from 12.5 µm to 27.5 µm; see Table 1).

The lower epidermis presents areas with stomata and areas without stomata (Fig. 10). The areas situated near the nerve are characterized by the absence of stomata. The epidermal cells are very long, have a

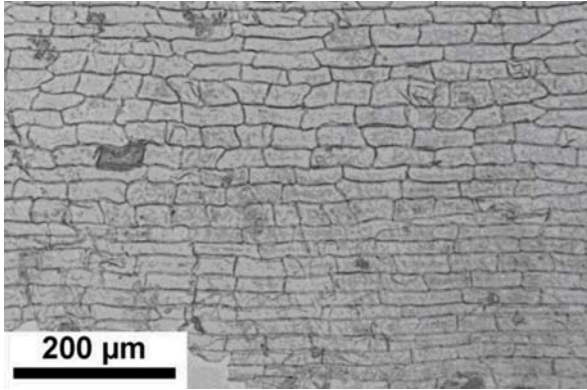


Fig. 7 - *Taxodium dubium* – upper epidermis (reprinted from Review of Palaeontology and Palynology, 153 (1-2), Kunzmann L., Kvaček Z., Mai D.H., Walther H., The genus *Taxodium* (*Cupressaceae*) in the Paleogene and Neogene of Central Europe, pp. 153-183, 2009, Copyright 2010, with permission from Elsevier).

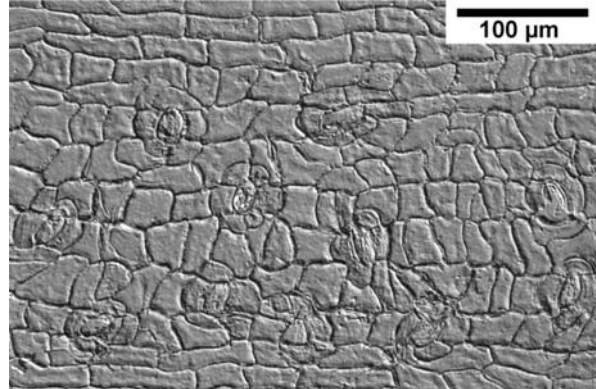


Fig. 8 - *Taxodium dubium* – lower epidermis (reprinted from Review of Palaeontology and Palynology, 153 (1-2), Kunzmann L., Kvaček Z., Mai D.H., Walther H., The genus *Taxodium* (*Cupressaceae*) in the Paleogene and Neogene of Central Europe, pp. 153-183, 2009, Copyright 2010, with permission from Elsevier).

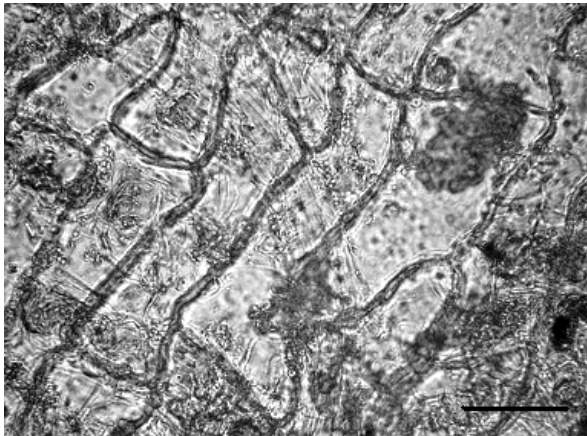


Fig. 9 - *Taxodium distichum* – upper epidermis (scale bar = 50 µm)

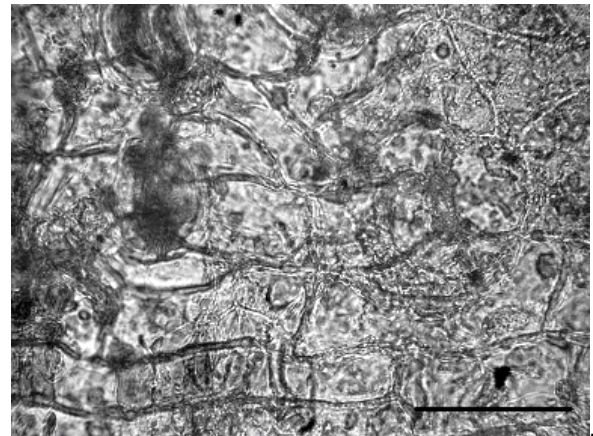


Fig. 10 - *Taxodium distichum* – lower epidermis (scale bar = 50 µm).

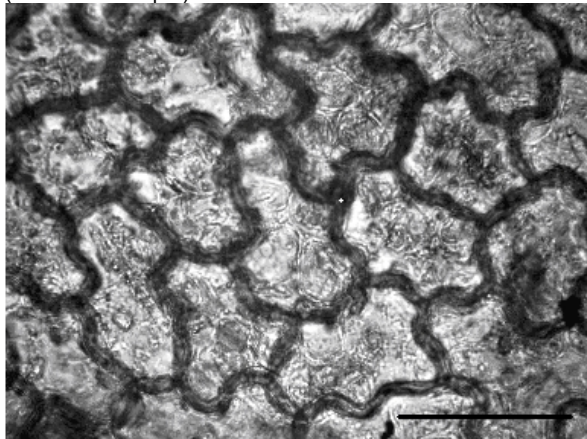


Fig. 11 - *Platanus hybrida* – upper epidermis (scale bar = 50 µm)

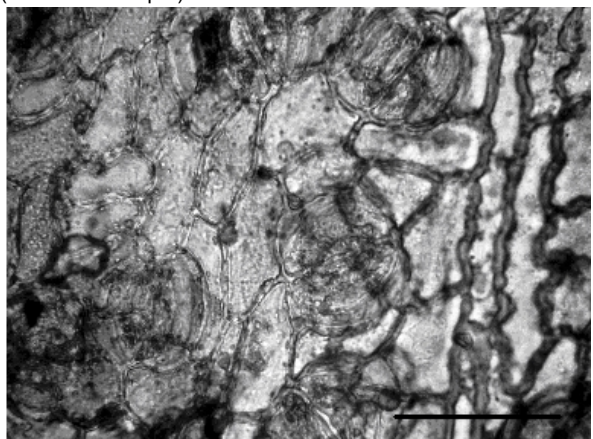


Fig. 12 - *Platanus hybrida* – lower epidermis (scale bar = 50 µm).

parallelepiped shape, and display relatively straight walls with rounded necks. The size of these cells is slightly inferior to the upper epidermis cells size (Table 1).

In the adjacent areas with stomata, the epidermis cells are shorter, sometimes with a quadrangular shape (Table 1). The stomata with 2-3 annexed cells are disposed usually with their ostiole slightly inclined towards the nervure. The stomata length ranged from 32.5 µm to 40.0 µm with the average value of 35.29 µm to ± 2.12 µm.

Platanus neptuni, Late Miocene, Delureni, Givulescu (1997)

Platanus neptuni (Ettingsh. 1877; Bůžek, Holý & Kvaček 1967) developed under conspicuously warmer temperatures as compared to subtropical elements during Late Eocene to Late Miocene in European floras. According to Kvaček & Manchester (2004), the *Platanus neptuni* complex included taxa with different types of leaves: simple, trifoliolate, or quinquefoliate.

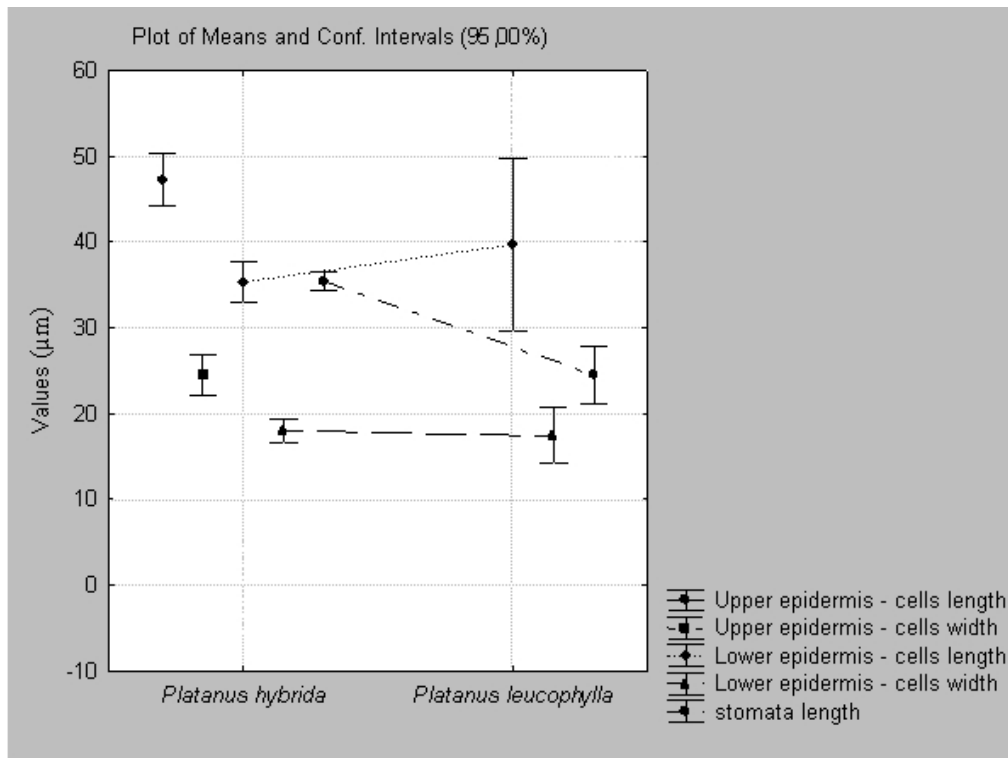


Fig. 13 - Variation limits and mean of some cytotaxonomical features in *Platanus neptuni* and *Platanus hybrida*.

In Romania, *Platanus neptuni* was discovered in Late Miocene deposits at Delureni, Valea de Criş, Corniţel (Givulescu, 1975). *Platanus neptuni* was common in mesic humid subtropical forests on volcanogenic substrates and along seashores. Kvaček & Manchester (2004) described both the anatomical features of the leaf, and the variability limits of the epidermal cells and stomata in *Platanus neptuni*. The upper epidermis is covered by a medium-sized thick cuticle. The cells of lobate-polygonal shape, with corrugated sides, are between 30 µm and 50 µm in length (Kvaček and Manchester, 2004).

The lower epidermis consists of polygonal cells with slightly corrugated walls with lengths varying from 35 µm to 50 µm. Elliptic stomata of anomocytic (only laterocytic) type, present lengths (35 µm–50 µm) resembling those of the epidermal cells (Kvaček and Manchester, 2004).

Givulescu (2003) reported the values of the cell size of the Delureni specimen (Tertiary, Late Miocene), Romania. He referred to the cells from the abaxial side; the epidermal cell lengths were 28 µm, 42 µm, and 49 µm respectively; the widths varied between 14 µm and 21 µm and the stomata length between 21 µm and 28 µm (Givulescu, 2003). These values are slightly lower than the values reported by Kvaček and Manchester (2004) for the size of epidemic cells and they fall in the variability limits for the stomata length (Table 1, Fig. 13). In another species of the same geological age, *Platanus leucophylla* (Late Miocene of eastern Styria, Austria), the cells of the upper epidermis presented very slightly corrugated walls (Kovar-Eder and Hably, 2006).

***Platanus hybrida*, the present-day species.**

Givulescu (2003) considered that the *Platanus hybrida* represents the present-day possible correspondent species of *Platanus neptuni*, while Manchester and Kvaček (2010) adopted a more careful position.

The present-day species of *Platanus* displays a considerable morphological variability; moreover, it is difficult to establish an ancestor or a certain correspondent species for *Platanus neptuni* (Grimm and Denk, 2008). Otherwise, in the *Platanaceae* family there is a single genus, *Platanus*, while “modern species of *Platanus* show a considerable amount of overlapping morphological variability and the morphological characters are difficult to use for phylogenetic reconstitution” (Nixon and Poole, 2003).

Upper epidermis (Fig. 11). The slightly corrugated cells with polygonal lobate shape emphasized longer lengths (variability limits between 30 µm and 65 µm) compared to the *Platanus neptuni* fossil species. The epidermal cells width ranges from 15.0 µm to 37.5 µm (Table 1).

The abaxial side of the leaf limb is divided into sectors with long and thin parallelepiped cells. The epidermal cells of polygonal-quadriateral shape present slightly corrugated and thickened walls. The size of the epidermal cell of the lower epidermis (Fig. 12) is relatively lower compared to the size of the cells of the upper epidermis. The cells length with variability limits between 25.0 µm and 47.5 µm (mean 35.33 µm ± 6.45 µm) stand between the values recorded at Delureni, but they are smaller than the values reported by Kvaček and Manchester (2004). The width of epidermal cells (variability limits between 12.5 µm and 25.0 µm, mean 17.96 µm ± 3.62 µm) falls between the recorded values of the fossil specimen of Delureni.

Stomata with annexed cells are disposed more or less orderly with a length that is smaller than the values reported by Kvaček & Manchester (2004) in the case of *Platanus neptuni*. Compared to the present-day species (variability limits of 27.5 µm to 40.0 µm, mean of 35.42 µm ± 2.67 µm), the Delureni specimen values can be set

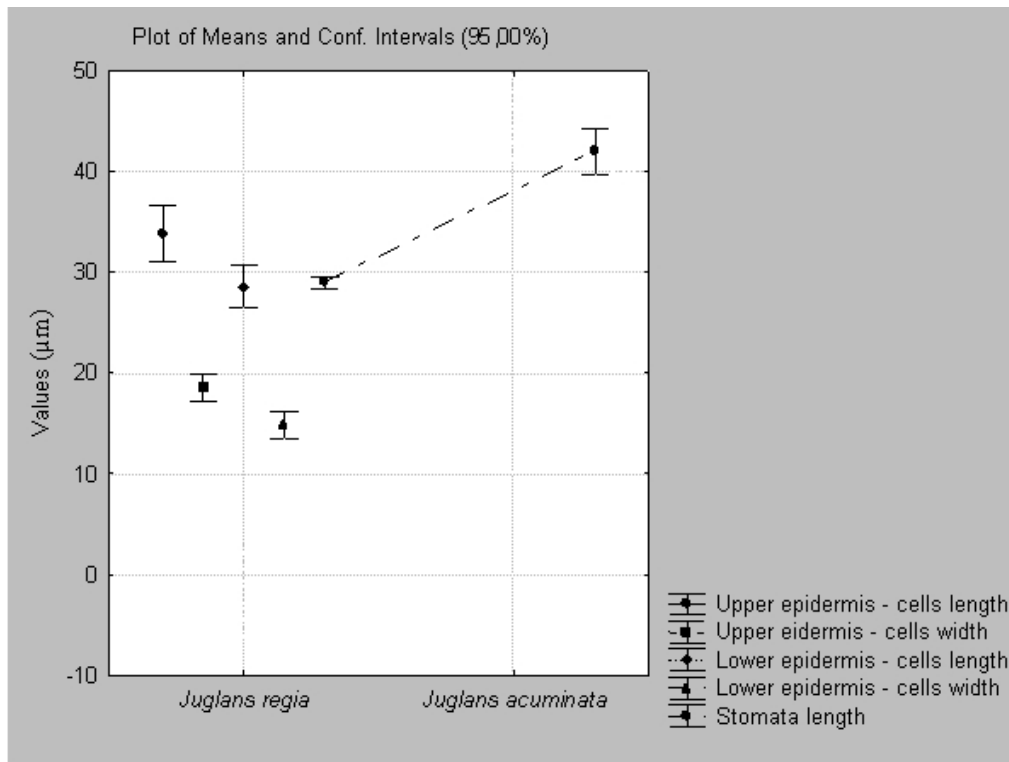


Fig. 14 - Variation limits and mean of some cytotaxonomical features in *Juglans acuminata* and *Juglans regia*.

out in the first half of the registered variability limits (Fig. 13, Table 1).

Juglans acuminata, Lower Sarmatian, Lower Pannonian, Borod-Bihor, Givulescu (1997).

Juglans acuminata was frequently found in Europe during the Miocene and Pliocene (24–2 million years; Givulescu, 1990). Fossil specimens found in Lower Sarmatian and Lower Pannonian deposits from Borod-Bihor area had stomata whose lengths ranged from 40 µm to 44 µm and whose width was 30.8 µm (Givulescu, 1997).

Juglans regia, a present-day correspondent species.

According to Givulescu, the paleontological material assigned to *Juglans acuminata* fossil species, "is totally similar with *Juglans regia* present-day species; there will be no error if the species is going to be given the same name" (Givulescu, 1990). The same opinion was expressed by other researchers too (Zidianakis et al., 2007, a.o).

Aradhya et al. (2006) performed a phylogenetic analysis of the existing *Juglans* species, using five cpDNA intergenic spacer (IGS) sequences in order to elucidate the origin, diversification, historical biogeography and evolutionary relationship within this genus. The results of this study point out that *Juglans regia* of the Old World shows an affinity only with *Juglans sigillata*.

Juglans regia L. is the present-day correspondent species of *Juglans acuminata* fossil species (Givulescu, 1999) and has stomata cells with values between 26.25 µm–32.50 µm in length and an average value of 29.0 µm ± 1.52 µm. The variability limits of the present-day species are narrower as compared to the values reported in fossil species (Fig. 14). On the other hand, the stomata length of the present-day species reaches the upper limit of the

variation recorded for the length of lower epidermal cells (between 17.5 µm–42.5 µm, with an average of 28.58 µm ± 5.55 µm). This aspect makes plausible the length value recorded in *Juglans acuminata*.

The differences in the stomata length of the two taxons (the fossil and the present-day species) may be attributed to different causes, one of which may be the fact that the present-day species does not correspond to the fossil species. Similarities between the two species concerning morphological characteristics of the leaves, but with different values for stomata length, may be determined by various causes as follows: (a) a higher stomata density in the present-day species; (b) modification of the atmosphere composition during geological times; (c) effect of some adaptation processes, as in the case of *Cycas revoluta* that presents shorter stomata as compared to the fossil species, *Pseudocycas dunkeriana*.

CONCLUSIONS

The relationship between the Bennettitales fossil and the present-day Cycads species is demonstrated by many similar features present in *Pseudocycas dunkeriana* fossil species and *Cycas revoluta* existing species: the presence of similar morphological leaves; male and female cones with dioecy process; similar values for the epidermis cell and stomata length, or stomata disposition; the presence of similar pollination insects etc.

The presence of the sex chromosomes (X and Y heterosomes), which play an important role in the sex determination of the present-day Cycads (included in the *Cycas revoluta*), has highlighted the dioecy process, suggesting their presence in the fossil *Pseudocycas*

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dunkeriana species too.

The size (length and width) of the epidermal cells has displayed similar values in both *Tsuga europaea* fossil and the present-day *Tsuga canadensis* species. The recorded difference in the stomata length in fossil and present-day species can be attributed either to a different ontogenetic stage of the analyzed leaves in the two species, or to a different CO₂ amount in the atmosphere.

With regard to *Taxodium dubium* fossil species, depending on the criteria being analyzed (leaves or cones), either *Taxodium distichum* (based on the analysis of the leaves) or *Taxodium mucronatum* (based on the analysis of the cones) could be considered as present-day representative species. Both in the case of *Taxodium dubium* and *Taxodium distichum*, we have noticed the customary absence of the stomata on the ad-axial side of the leaf. A close analysis of the epidermal anatomy of the leaf has evidenced a difference between fossil and present-day species: in fact, with time the edge cells are enlarged in the latter. In comparison, the length of the epidermal cells in *Taxodium dubium* had lower values as compared to *Taxodium distichum*.

The fossil species *Platanus neptuni* and the present-day species *Platanus hybrida* evince several differences with regard to the shape of the epidermal cells on both sides of the leaf, as well as to the stomata size. As far as

the shape of the epidermal cells is concerned, there are greater differences between two fossil species from the Late Miocene, namely, *Platanus neptuni* and *Platanus leucophylla*.

There is a high similarity in leaf morphology between the fossil species *Juglans acuminata* and the present-day correspondent species *Juglans regia*, but some cytological characteristics are clearly different, suggesting that new morphological investigations are required before reaching final conclusions.

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Table 1 - The biometrical values of some morphological features of the leaves, in some fossil and present-day species.

Genotype	Upper epidermis				Lower epidermis					
	Cell length (µm)		Cell width (µm)		Cell length (µm)		Cell width (µm)		Stomata length-µm	
	Var. limits	x ± s _x	Var. limits	x ± s _x	Var. limits	x ± s _x	Var. limits	x ± s _x	Var. limits	x ± s _x
Fossil species										
<i>Pseudocycas dunkeriana</i>	30.8–73.9	50.8±15.0	18.5–37.0	26.7±6.5	30.8–55.4	42.0±8.8	18.5–36.9	27.7±8.7	33.9–41.1	37.5±4.1
<i>Tsuga europaea</i>	-	-	-	-	56.0–70.0	63.0±6.3	34.0–36.0	35.0±0.9	34.0–36.0	35.0±0.9
<i>Taxodium dubium</i>	-	-	-	-	19.0–40.0	-	-	-	-	-
<i>Platanus neptuni</i> ^a	30.0–50.0	-	-	-	35.0–50.0	-	-	-	35.0–50.0	-
<i>P. neptuni</i> ^b	-	-	-	-	28.0–49.0	39.7±9.6	14.0–21.0	17.5±3.1	21.0–28.0	24.5±3.1
<i>Juglans acuminata</i>	-	-	-	-	-	-	-	-	40.0–44.0	42.0±2.2
Present-day species										
<i>Cycas revoluta</i>	32.5–67.5	50.6±10.3	12.5–37.5	22.3±5.6	25.0–65.0	41.5±7.8	20.0–45.0	31.4±6.3	30.0–35.0	33.3±1.4
<i>Tsuga canadensis</i>	72.0–340.0	177.5±75.4	12.0–24.0	19.0±3.3	36.0–90.0	54.1±14.5	16.0–22.0	18.4±1.7	48.0–64.0	54.3±4.1
<i>T. canadensis</i> *	idem	idem	idem	idem	82.0–192.	138.5±28.9	13.0–26.0	19.1±2.4	-	-
<i>Taxodium distichum</i>	50.0–145.0	102.2±21.5	12.5–27.5	18.6±3.4	37.5–75.0	55.1±10.7	12.5–37.5	23.5±5.4	32.5–40.0	35.3±2.1
<i>T. distichum</i> *	idem	idem	idem	idem	37.5–140.	77.5±26.1	6.3–11.3	9.3±1.2	-	-
<i>Platanus hybrida</i>	30.0–65.0	47.3±8.2	15.0–37.5	24.5±6.2	25.0–47.5	35.3±6.5	12.5–25.0	18.0–3.6	27.5–40.0	35.4±2.7
<i>Juglans regia</i>	25.0–60.0	33.8–7.4	12.5–25.0	18.6±3.6	17.5–42.5	28.6±5.6	7.5–20.0	14.8±3.6	26.3–32.5	29.01.52

*rows without stomata; ^a – Kvaček et Manchester; ^b – Givulescu (Delureni).

REFERENCES

- Aradhya, M.K., Potter, D., Simon, C.J., 2006. Origin, evolution and biogeography of *Juglans*: a phylogenetic perspective. *Proc. Acta Horticulturae*, 705: 85-94.
- Bailey, J.W., Sinnott, E.W., 1915. A botanical index of cretaceous and tertiary climates. *Science*, 41: 831-834.
- Bailey, J.W., Sinnott, E.W., 1916. The climatic distribution of certain types of angiosperm leaves. *Am. J. Bot.*, 42: 24-39.
- Ciocârlan, V., 2009. Flora ilustrata a României. Pteridophyta et Spermatophyta. Edit. Ceres, Bucuresti, 1141 p.
- Corneanu, C.G., Corneanu, M., Bercu, R., 2004. Comparison of some morpho-anatomical features at fossil vegetal species and their present-day correspondent species. *Studia UBB, Geologia*, 49 (2): 77-84.
- Crepet, W.L., 2000. Progress in understanding angiosperm history, success, and relationships: Darwin's abominably "perplexing phenomenon". *Proc. Natl. Acad. Sci.*, 97 (24): 12939-12941.
- Givulescu, R., 1975. Fossile Pflanzen aus dem Pannon von Delureni (Rumänien). *Paleontographica B* 153 : 160-182.
- Givulescu, R., 1990. Flora fosila a Miocenului Superior de la Chiuzbaia. Edit. Academiei Romane, Bucuresti, 237 p.
- Givulescu, R., 1992. Eine mittel und obermiozäne Blattflora aus Tiefbohrungen des Borodar Beckens, Kraiss Bihor, Rumänien. *Docum. Natur.*, 69: 1-21.
- Givulescu, R., 1996. Flora Oligocena Superioara din bazinul Petroșani. Casa Cartii de Stiinta, Cluj-Napoca, 177 p.
- Givulescu, R., 1998. Flora fosila a Jurasicului Inferior de la Anina. Edit. Academiei Romane, Bucuresti, 92 p.
- Givulescu, R., 1999. Flora mica ilustrata a Tertiariului din Romania. Casa Cartii de Stiinta, Cluj, 116 p.
- Grimm, G.W., Denk, T., 2008. ITS evolution in *Platanus* (*Platanaceae*): Homoeologues, Pseudogenes and Ancient Hybridization. *Ann. Botany*, 101: 403-419.
- Grote, J.P., 2008. Use of leaf architecture and anatomy in the study of plant diversity in the Tertiary and Recent of Thailand. Suranarec University of Technology, SUTI-104-43-12-08, 45 p.
- Khoshoo, T.N., 1961. Chromosome numbers in Gymnosperms. *Silvae Genetica*, 10 (1): 1-9.
- Kokubugata, G., Kondo, K., 1996. Differential fluorescent-banding patterns in chromosomes of four species of *Cycas* (*Cycadaceae*). *Bot. J. Linnean Soc.*, 120: 51-55.
- Kono, Masumi, Tobe, Hiroshi, 2007. Is *Cycas revolute* (*Cycadaceae*) wind- or insect-pollinated? *Am. J. Botany*, 94 (5): 847-855.
- Kouwenberg, L.L.R., McElwain, J.C., Kürschner, W.M., Wagner, F., Beerling, D.J., Mayle, F.E., Visscher, H., 2003. Stomatal frequency adjustment of four conifer species to historical changes in atmospheric CO₂. *Am. J. Bot.*, 90 (4): 610-619.
- Kouwenberg, L.L.R., Kürschner, W.M., Visscher, H., 2004. Changes in stomatal frequency and size during elongation of *Tsuga heterophylla* needles. *An. Bot.*, 94: 561-569.
- Kovar-Eder, J., 2004. Die obermiozäne Flora von Mataschen bei Fehring, Steiermark – Blattvergesellschaftungen (The Upper Miocene Flora of Mataschen near Fehring, Styria – Leaf-assemblages). *Joannea Geol. Paläont.* 5: 163–175.
- Kovar-Eder, J., Hably, L., 2006. The flora of Mataschen – a unique plant assemblage from the late Miocene of eastern Styria (Austria). *Acta Palaeobotanica*, 46 (2): 157-233.
- Kunzmann, L., Kvaček, Z., Mai, D.H., Walther, H., 2009. The genus *Taxodium* (*Cupressaceae*) in the Paleogene and Neogene of Central Europe. *Review of Palaeontology and Palynology*, 153 (1-2): 153-183.
- Kusumi, J., Tsumura, Y., Yoshimaru, H., Tachida, H., 2000. Phylogenetic relationships in *Taxodiaceae* and *Cupressaceae* sensu stricto based on *matK* gene *chlL* gene, *trn-L-trnF* IGS region, and *trnL* intron sequences. *Am. J. Bot.* 87 (10): 1480-1488.
- Kvaček, Z., Manchester, S.R., 2004. Vegetative and reproductive structure of the extinct *Platanus neptuni* from Tertiary of Europe and relationships within the *Platanaceae*. *Plant. Syst. Evol.*, 244: 1-29.
- Labandeira, C.C., Kvaček, J., Mostovski, M.B., 2007. Pollination drops, pollen and insect pollination of Mesozoic gymnosperms. *Taxon*, 56 (3): 663-695.
- Leubner, G., 2009. The seeds biology place - seeds evolution. <http://www.seedbiology.de/evolution.asp>
- Manchester, S.R., Kvaček, Z., 2010. Inflorescences and compound leaves of the extinct *Platanus neptuni* complex in the Oligocene of Oregon, USA. *Acta Paleontologica*, 50 (1): 5-15.
- Miller, J.M., 2010. Paleobotany of Angiosperm Origins. Origin of Flowering Plants. <http://www.gigantopteroid.org/html/research.htm>, 57 p.
- Nixon, K.C., Poole, J.M., 2003. Revision of the Mexican and Guatemalan species of *Platanus* (*Platanaceae*). *Lundellia*, 6: 103-137.
- Rothwell, G.W., Crepet, W.L., Stockey, R.A., 2009. Is the anthophyte hypothesis alive and well? New evidence from the reproductive structures of Bennettitales. *Am. J. Botany*, 96 (1): 296-322.
- Segawa, M., Kishi, S., Tatuno, S., 1971. Sex chromosomes of *Cycas revoluta*. *Japan J. Genetics*, 46 (1): 33-39.
- Szafer, W., 1947. The Pleistocene flora of Kroszno in Poland. II. Descriptive Part. *Acad. Pol. Sci., Lettr., Cl. Sci., Mat.-nat. PAU 72 B* (2): 163-375.
- Taylor, T.N., Taylor, E.L., Krings, M., 2009. Paleobotany. The Biology and Evolution of Fossil Plants. Sec. Ed., Academic Press, Burlington – London – San Diego – New York, 1230 p.
- Teodoridis, V., Kvaček, Z., Uhl, D., 2009. Pliocene paleoenvironment and correlation of the Sessenheim-Auenheim floristic complex (Alsace, France). *Paleodiversity*, 2: 1-17.
- Tutin, T.G., Heywood, N.H., Burges, N.A., Valentine, D.H., Walters, S.M., Webb, D.A., 1964. Flora Europaea. Vol 1, Lycopodiaceae to Platanaceae. Cambridge University Press, Cambridge, XXXII + 464 p.
- Upchurch-Jr., G.R., 1984. Cuticle evolution in early Cretaceous Angiosperms from the Potomac group of Virginia and Maryland. *Ann. Missouri Bot. Gard.*, 71: 522-550.
- Vickulin, S.V., LePage, B.A., Shalisko, V.Y., 2003a. Paleogene leaf compressions of *Taxodium mucronatum* Ten. affinity from Pasekovo, Middle Russian Upland, southern European Russia. *Scientia CUCBA*, 5 (1-2): 63-87.
- Vickulin, S.V., Burova, Z.V., Panova, L.A., LePage, B.A., Shalisko, V.Y., 2003b. Mexican affinities of Early Tertiary *Taxodium* from European Russia evidence of last micromorphology, cone scales and palynology. XIV Semana de la investigacion científica, Guadalahara, p. 369-371.
- Wan, Zhihui, 1996. The lower Cretaceous flora of the Gated formation from Western Canada. Doctoral Thesis, University of Saskatoon, Canada, 304 p.
- Worobiec, G., 1995. A preliminary report on the Lower Miocene leaf flora from the brown coal mine "Belchatów" (Central Poland). *Acta Palaeobot.*, 35 (2): 243-251.
- Zidianakis, G., Mohr, B.A.R., Fassoulas, Ch., 2007. A late Miocene leaf assemblage from Vrysses, western Crete, Greece, and its paleoenvironmental and paleoclimatic interpretation. *Geodiversitas*, 29 (3): 351-377.
- ***2010. *Cycad*. En. Wikipedia.org/wiki/Cycad