Review of Palaeobotany and Palynology 120 (2002) 1–15

Review of Palaeobotany & Palynology

www.elsevier.com/locate/revpalbo

First evidence of epidermal structures of *Ginkgo* from the Mediterranean Tertiary

Thomas Denk [a,*], Dimitrios Velitzelos [b]

[a] *Swedish Museum of Natural History, Department of Palaeobotany, P.O. Box 50007, 10405 Stockholm, Sweden*
[b] *University of Tübingen, Institute and Museum of Geology and Palaeontology, Sigwartstrasse 10, 72076 Tübingen, Germany*

Received 26 July 2001; accepted 18 January 2002

Abstract

Tertiary leaf compressions of a *Ginkgo* plant with cuticle displaying all taxonomically important epidermal features are reported for the first time from Greece, and for the Mediterranean area as a whole. The fossils are from Upper Miocene sediments of Vegora, north-western Greece, and assigned to *Ginkgo adiantoides* (Unger) Heer. The most conspicuous cuticle characteristics are prominent papillae on the subsidiary cells that sometimes completely cover the stomatal apertures. Such papillae also occur in leaves of *G. adiantoides* from the Pliocene of eastern Central Europe, but are absent in leaves from the Pliocene of Germany also assigned to this species. We observed a high variability in the degree of papillosity of subsidiary cells of stomata in leaves of living *Ginkgo biloba* that exhibited prominent papillae in mature sun leaves and a total lack of papillae in shade leaves of a potted plant. This may suggest that differences in epidermal characters between the fossil leaves from Greece and Germany are due to ecotypical/intraspecific variability and not to genetically fixed specific differences. It also suggests that a number of Tertiary *Ginkgo* species from Eurasia that were distinguished from *G. adiantoides* by having papillate epidermis cells and subsidiary cells of the abaxial cuticle should be included within *G. adiantoides*. Two lineages of *Ginkgo* can be distinguished for the Tertiary of the Northern Hemisphere. From the Palaeogene high latitudes leaves were reported that exhibit upper epidermis cells with prominent papillae similar to those of older Mesozoic *Ginkgo*, whereas *G. adiantoides* lacks the papillae of the upper epidermis, a feature which is shared with the living *G. biloba*. In general, the size of stomata is larger in extant *Ginkgo* leaves than in those of the fossils.

Keywords: evolution; Eurasia; fossil; cuticle; morphological variability; *Ginkgo*

1. Introduction

The genus *Ginkgo* (maidenhair tree, Ginkgoaceae, Ginkgoales) represents a distinct and ancient lineage of gymnosperms. At present *Ginkgo* is represented by a single species, *Ginkgo biloba* L., native to China, but the genus has an extensive fossil history ranging back into the Jurassic (Zhou, 1997). During the Jurassic, Cretaceous and the Early Tertiary, *Ginkgo* had a circumpolar distribution in both hemispheres (Tralau, 1968; Pole and Douglas, 1999; Hill and Carpenter, 1999), but it disappeared from the Southern Hemisphere

---

* Corresponding author. Fax: +46-8-5195-4221.
E-mail address: thomas.denk@nrm.se (T. Denk).

PII: S0034-6667(02)00076-3

after the Palaeogene and became confined to Eurasia by the end of the Miocene. In the Northern Hemisphere the genus shifted from high latitudes to lower latitudes from the Paleocene and onward, and disappeared completely from Europe by the Latest Pliocene (Samylina, 1967; Tralau, 1968).

The first description of *Ginkgo* leaves from the Tertiary of Europe was by Unger (1845), who ascribed some poorly preserved leaf imprints from the Late Miocene of north-eastern Italy to the genus *Salisburia* Smith, and established a new species, *Salisburia adiantoides* Unger. The formal description of the species was provided by Unger in 1850 (Unger, 1850). Later, Heer (1878) recognised *S. adiantoides* as belonging to the genus *Ginkgo*, and included a number of leaf imprints from the Miocene of Sakhalin to the new combination *Ginkgo adiantoides* (Unger) Heer. Subsequently, several Early Tertiary ginkgos from the Arctic and Northern Europe have been assigned to this species (Greenland: Heer, 1878; Isle of Mull: Gardner, 1886; Spitsbergen: Nathorst, 1919). The species was also reported from Frankfurt, Germany, by Florin (1936). The German material was preserved as compressions and Florin was able to prepare cuticles and for the first time provided information on epidermal features in material assigned to *G. adiantoides*. Furthermore, Florin (1936) recognised that leaves assigned to *G. adiantoides* from the Eocene of the Isle of Mull were distinct from the leaves from Frankfurt in having conspicuously papillate upper epidermis cells. Based on these differences in epidermal features, Florin established a new species, *G. gardneri* Florin for the Mull material. Later, Manum (1966) observed that *Ginkgo* leaves from the Paleocene of Spitsbergen were also distinct from those of *G. adiantoides* in having subsidiary cells with prominent papillae, and established a new species, *Ginkgo spitsbergensis* Manum, for his material. McIver and Basinger (1993) reported *G.* cf. *spitsbergensis* from the Paleocene of Canada.

Because the type material of *Ginkgo adiantoides* from Italy lacked any cuticular features, Samylina (1967) argued that there could not be certainty about the assignment of the Frankfurt material to this species. Therefore, she established a new species name for the specimens of Frankfurt, *Ginkgo florinii* Samylina. From Greece, leaf impressions of *G. adiantoides* were reported by Knobloch and Velitzelos (1987) and Velitzelos (1993).

In the present paper *Ginkgo* leaves with epidermal structures preserved are described for the first time from Greece. The finding is also the first report of cuticle features of *Ginkgo* for the Mediterranean region. Comparative study of the fossil material from Greece and other Central and eastern European Late Tertiary deposits yielding cuticles, as well as leaves of the living *Ginkgo biloba* was undertaken. Based on this, we discuss species delimitation and possible evolutionary and ecological trends of *Ginkgo* during the Tertiary.

2. Material and methods

The fossil *Ginkgo* leaves described here were found in whitish–grey diatomaceous sediments in the limnic Upper Miocene browncoal basin of Vegora, northern Greece. The material comprises a single slab with two compressed leaves. The leaf compressions were removed from the sediment slab and stored in glycerin. Small fragments were treated with Schulze's solution (HNO_3 and $KClO_3$) and KOH (5%), and washed with water. For light microscope (LM) observations cuticles were stained with safranin (red). Standard procedures were followed for scanning electron microscopy (SEM). Samples were fixed to a stub and coated with gold. The original material of *Ginkgo adiantoides* (Unger) Heer emend. Florin (Florin, 1936) from Frankfurt, Germany (micro-preparations stained with safranin; housed in the Department of Palaeobotany of the Swedish Museum of Natural History, Stockholm, Sweden, S), was studied under LM. In addition, untreated leaf fragments from Frankfurt which had been mounted between two slides in glycerin gelatin were treated with Schulze's solution and prepared for SEM. Leaves of extant *Ginkgo biloba* were prepared for LM and SEM in the same way as fossils. Cuticle preparations of both fossil and living specimens are stored in S. For comparison, material of *Ginkgo spitsbergensis* Manum, *G. gardneri* Florin, and *G. coriacea* Florin, stored in S has been studied.

3. Results

3.1. *Ginkgo leaves from the Late Miocene of Vegora*

The two leaves are flabelliform and not dissected in their preserved apical parts. They are about 40 mm long and 45 mm wide. The petiole is incomplete. The leaf base is cuneate. Venation is dichotomous, fine and densely spaced (about 18 veins per cm). Secretory bodies 800–900 μm long and 150–250 μm wide are scattered between the veins (Plate I, 1 and 2).

3.1.1. *Adaxial cuticle*

Cells over veins are narrow rectangular, or elongate with acute ends, 100–150 μm long and 10–40 μm wide. Anticlinal cell walls are undulate, sinuses occurring with a frequency of 8–9/100 μm length of the cell. Cells of intercostal areas are isodiametric to broad rectangular, triangular or polygonal, from about 50×50 to 90×35 μm. Anticlinal cell walls are undulate, the sinuses occurring with a frequency of 7–8/100 μm length of the cell (Plate II, 1 and 2). Periclinal cell walls are slightly convex. No stomata or trichome bases are present.

3.1.2. *Abaxial cuticle*

Cells over veins are elongated with acute to rectangular ends, and about 100 μm long. Anticlinal walls are straight to (sometimes) slightly undulate (Plate II, 3). Periclinal walls are convex, often with papillae developed at the ends of the cells (Plate II, 4). Cells of intercostal areas are broadly rectangular to polygonal, about 70×30 and 60×60 μm, respectively, and commonly papillate. Stomata are 22–33 μm long and 18–23 μm wide. Subsidiary cells are complete to incomplete amphicyclocytic. The inner ring of subsidiary cells is strongly papillate, the papillae often covering most of the stomatal aperture. The outer ring displays radiating cuticular striae (Plate II, 5–8).

3.2. *Comparison with modern Ginkgo biloba*

Gross morphology as encountered in the fossil leaves is also found in *Ginkgo biloba*. Modern leaves, however, display high variability in leaf size, shape, and dissection of the apical leaf margin. Also, the size of the secretory (resin) bodies is much more variable in modern *Ginkgo*. Nevertheless, only a few micromorphological characteristics distinguish the Greek from modern leaves. Stomata are absent on the adaxial epidermis of the fossil leaves (hypostomatic), whereas they may occur sparsely in living *G. biloba* (amphistomatic). Trichomes are absent in the fossils, but occur over veins on the abaxial epidermis and

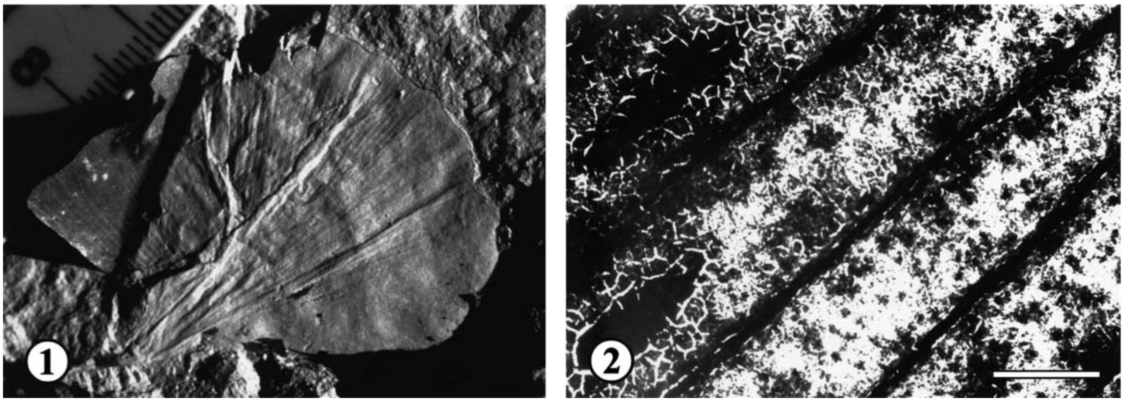


Plate I. *Ginkgo adiantoides* (Ung.) Heer from the Late Miocene of Vegora, north-western Greece.

1. Hand-specimen containing a leaf compression/impression. Note the entire apical leaf margin.
2. Part of naturally macerated leaf compression. Two secretory bodies can be seen. Specimen no. S 116311. Scale is 500 μm.

close to the leaf base on the adaxial epidermis in *G. biloba*. Subsidiary cells of the stomata are strongly papillate in the fossils (Plate II, 4–8), whereas they may be with or without papillae in modern *Ginkgo* (Plate IV, 3–8, Plate V, 4–6). Finally, stomata tend to be slightly larger in living *Ginkgo* leaves than in fossil ones.

3.3. Comparative morphology of Late Tertiary Ginkgo leaves from Europe and modern Ginkgo biloba

Leaf gross morphology, including entire or only slightly dissected leaf margin and density of veins per cm, is remarkably consistent in several Tertiary specimens from Eurasia. This applies also to Unger's type specimens of *Ginkgo adiantoides* (Table 1). Secretory bodies are more or less the same size in specimens from Germany and Hungary assigned to *G. adiantoides*, and in the Greek specimens studied here. In living *Ginkgo biloba* secretory bodies are much more variable in size, ranging from 500 to over 3000 μm in length (character 1 in Table 2).

3.3.1. Comparative cuticle anatomy

Twenty-five leaf epidermal characters were studied comparatively for the Late Miocene *Ginkgo* from Greece, the original material of *Ginkgo adiantoides* from the Pliocene of Germany, and extant *Ginkgo biloba* (Table 2). In addition, data from the Early Pliocene of Hungary obtained from both SEM and LM studies (Hably and Kvaček, 1997) were included in Table 2.

Features of the adaxial cuticle were very similar for all specimens (characters 2–12 in Table 2). Anticlinal cell walls are undulate in fossil as well as living *Ginkgo*; however, they may be thicker in living (sun) leaves, and therefore the undulation may not be clearly seen under LM, whereas it is always seen under SEM (Plate IV, 1 and 2: sun leaf, versus Plate V, 1 and 2: shade leaf). The sinus frequency in the anticlinal cell walls was more or less the same in fossil and living ginkgos (characters 5 and 9 in Table 2). Furthermore, in extant *Ginkgo biloba* narrow and elongate epidermis cells over veins may also have very straight anticlinal cell walls.

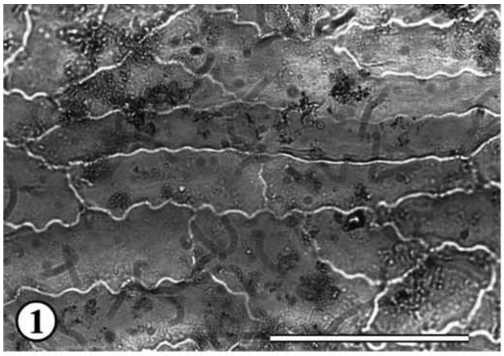
All fossil samples lacked stomata on the adaxial epidermis, whereas stomata may occasionally occur adaxially in living *Ginkgo*. The same applies to trichomes.

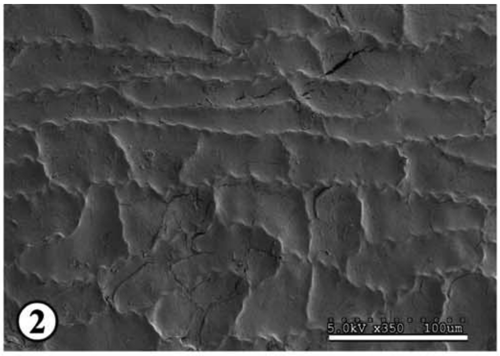
In contrast to the adaxial cuticle, the abaxial cuticle is more variable among the fossil specimens, as well as extant *Ginkgo biloba* (characters 13–25 in Table 2). Epidermal cells over veins exhibit papillate periclinal cell walls in ginkgos from northern Greece and Hungary (Plate II, 4), as well as in sun leaves of extant *G. biloba*, whereas they lack papillae in the material from Frankfurt and

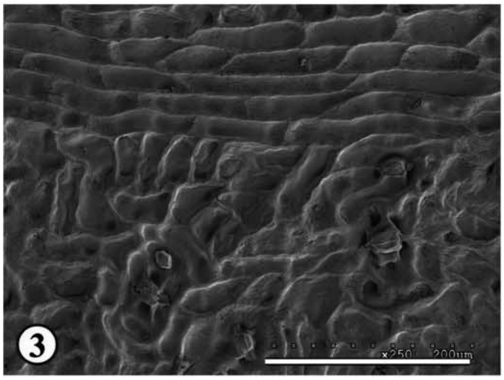
Plate II. *Ginkgo adiantoides* (Ung.) Heer from the Late Miocene of Vegora, north-western Greece.

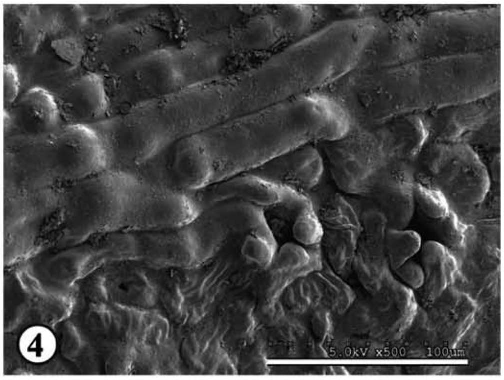
1. Adaxial cuticle displaying elongate cells over a vein, and polygonal cells in intercostal areas. Anticlinal walls are undulate (LM, specimen no. S̲ 116311-01).
2. Inner surface of adaxial cuticle showing the same features as in 1 (SEM, specimen no. S̲ 116311-02).
3. Inner surface of abaxial cuticle displaying elongate straight to slightly undulate cells over a vein, polygonal slightly undulate cells in intercostal areas, and stomata with (incomplete) amphicyclocytic subsidiary cells (SEM, specimen no. S̲ 116311-03).
4. Outer surface of abaxial cuticle showing elongate papillate cells over a vein, and two stomata with prominently papillate subsidiary cells. Note the striation of the outer ring of subsidiary cells (SEM, specimen no. S̲ 116311-03).
5. Abaxial cuticle with four irregularly oriented stomata with papillate subsidiary cells (LM, specimen no. S̲ 116311-01).
6. Inner surface of adaxial cuticle showing a stoma with the cavities of the inner ring of papillate subsidiary cells, and the incomplete outer ring of subsidiary cells (SEM, specimen no. S̲ 116311-03).
7. Detail of a stoma showing five papillae and thickened inner walls of four lateral subsidiary cells (LM, specimen no. S̲ 116311-01).
8. Outer cuticle surface showing a stoma with the stomatal aperture almost completely covered by papillae of subsidiary cells (SEM, specimen no. S̲ 116311-03).

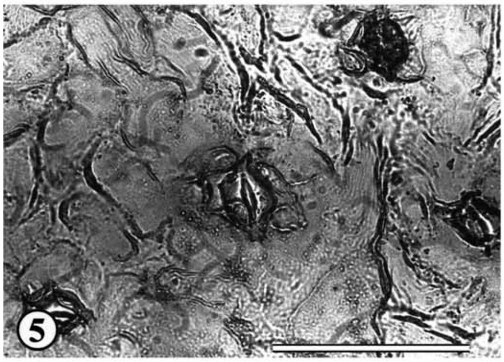
Scale is 100 μm in figs. 1, 2, 4, and 5, 200 μm in fig. 3, and 50 μm in figs. 6–8.

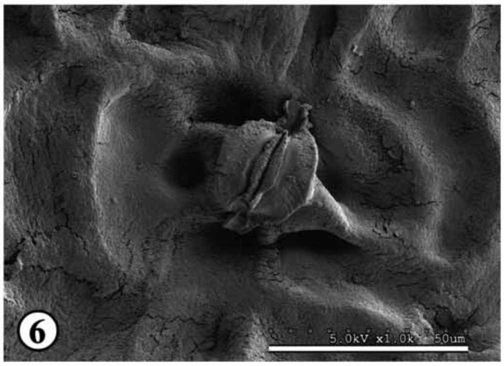

1

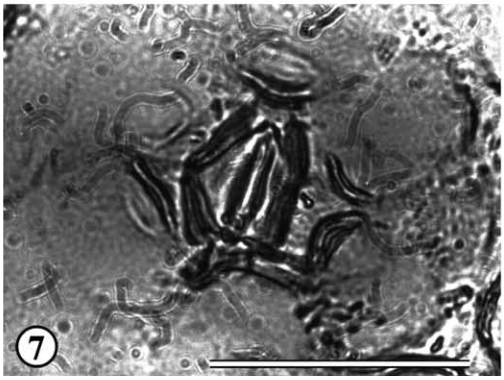

2
5.0kV x350 100um

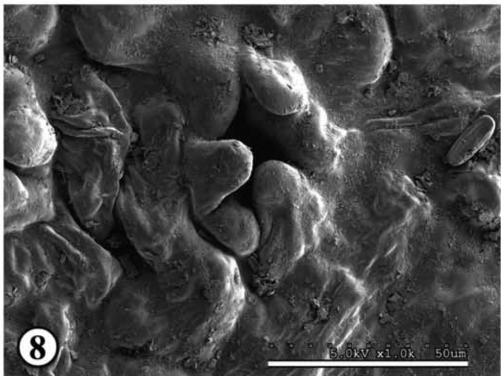

3
x250 200um


4
5.0kV x500 100um


5


6
5.0kV x1.0k 50um


7


8
5.0kV x1.0k 50um

in shade leaves of *G. biloba* (Plate III, 4 and 5, and Plate V, 4). Even more conspicuous is the papillosity found in subsidiary cells of the leaves from northern Greece. Papillae originating from the inner ring of subsidiary cells may almost completely cover the stomatal aperture (Plate II, 4–8). The same is observed for *Ginkgo adiantoides* from the Pliocene of Hungary (Hably and Kvaček, 1997, pl. 2 and 3). In living *G. biloba* papillae of the subsidiary cells, though clearly present, are not as prominent as in the two fossil ginkgos (Plate IV, 3–8), or may be absent in shade leaves (Plate V, 6). Interestingly, the material from Frankfurt lacks papillae on the subsidiary cells and instead the subsidiary cells of the inner ring fuse laterally to form a single ring around the guard cells (Plate III, 4, 5, 7, and 8). When observed as imprints on the inner surface of the cuticle, the stomatal cells in the Pliocene specimens from Frankfurt and those of shade leaves of a potted extant *Ginkgo* are almost indistinguishable (Plate III, 6 and Plate V, 5, respectively). Epidermal cells of the intercostal areas may have papillae-like convex periclinal cell walls also in the specimens from Frankfurt (Plate III, 4). Size of stomata (character 20 in Table 2) tends to increase from Late Miocene specimens (northern Greece) to Pliocene and extant specimens. This difference may reflect an evolutionary trend, but could also be due to different environmental conditions. Epicuticular wax, which is present in living *G. biloba*, is absent or was not preserved in fossil cuticles.

4. Discussion

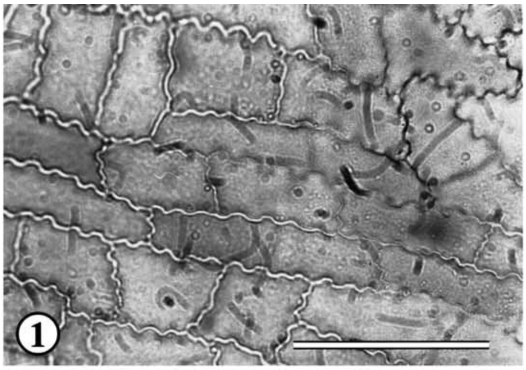
4.1. Systematics

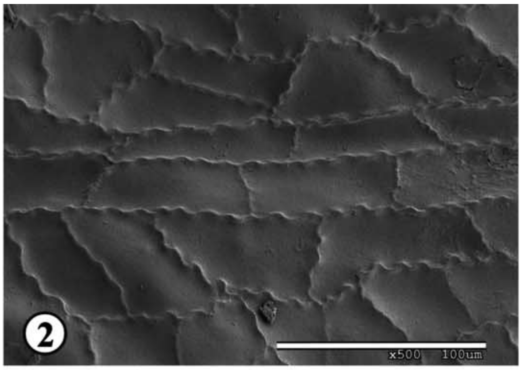
The specimens from Vegora are similar or identical to the material of *Ginkgo adiantoides* from Frankfurt in almost all of the epidermal characteristics, as well as in macromorphological features (leaf size, only slightly dissected apical leaf margin, Mädler, 1939; see Table 1). The only marked difference is the strong papillosity of the subsidiary cells in the Greek specimens. The degree of papillosity of subsidiary cells, however, was found to be highly variable in extant *Ginkgo* (Plates IV and V). Furthermore, clinally connected morphotypes displaying very pronounced papillae of subsidiary cells (Vegora), pronounced papillae (Hungary and sun leaves of extant *Ginkgo biloba*), and non-papillate subsidiary cells (Frankfurt, and shade leaves of *G. biloba*), suggest that this character is not diagnostic at the species level, but susceptible to ecotypic variability. Thus, we assign the specimens from Vegora to *G. adiantoides*. Unger's (1845, 1850) type material of *G. adiantoides* from Senigallia (Late Miocene, north-eastern Italy) is lacking epidermal features, but specimens from Senigallia, figured in Massalongo and Scarabelli (1859), also display undissected leaf margins, and are the same size as leaves from Vegora and Frankfurt. Samylina (1967) argued that the compression fossils from Frankfurt that formed the basis for Florin's emendation of *G. adiantoides* could not be directly

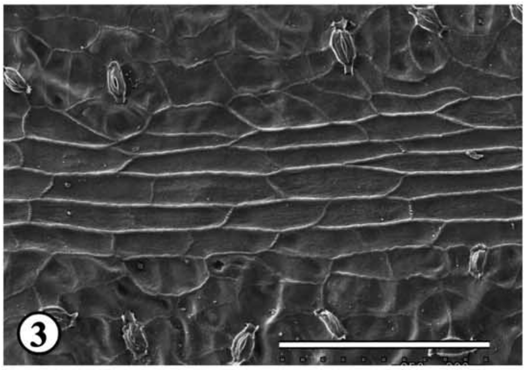
Plate III. *Ginkgo adiantoides* (Ung.) Heer emend. Florin (= *Ginkgo florinii* Samyl.) from the Pliocene of Frankfurt, Germany.

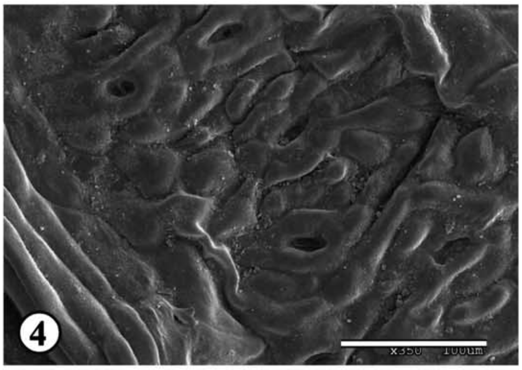
1. Adaxial cuticle displaying elongate cells over a vein, and polygonal cells in intercostal areas. Anticlinal walls are undulate (LM, specimen no. S 116317-01).
2. Inner surface of adaxial cuticle showing the same features as in 1 (SEM, specimen no. S 116317-01).
3. Inner surface of abaxial cuticle displaying elongate cells over a vein, polygonal slightly undulate cells in intercostal areas, and stomata with (incomplete) amphicyclocytic subsidiary cells (SEM, specimen no. S 116317-02).
4. Outer surface of abaxial cuticle showing elongate convex cells over a vein, and stomata surrounded by a ring wall made up of fused convex subsidiary cells. Cells in intercostal areas with convex periclinal walls (SEM, specimen no. S 116317-01).
5. Abaxial cuticle with irregularly oriented stomata (LM, specimen no. S 116314; preparation made by R. Florin).
6. Inner surface of adaxial cuticle showing a stoma with the inner ring of subsidiary cells. Note the absence of cavities observed in the material of Vegora (SEM, specimen no. S 116317-02).
7. Detail of a stoma showing a thickened ring wall formed by subsidiary cells (LM, specimen no. S 116317-01).
8. Outer cuticle surface showing a stoma and subsidiary cells without any papillae (SEM, specimen no. S 116317-02).

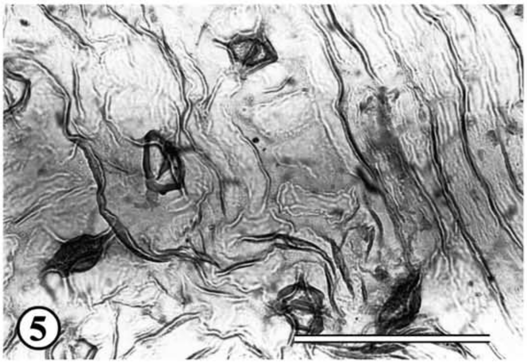
Scale is 100 μm in figs. 1, 2, 4, and 5, 200 μm in fig. 3, and 50 μm in figs. 6–8.

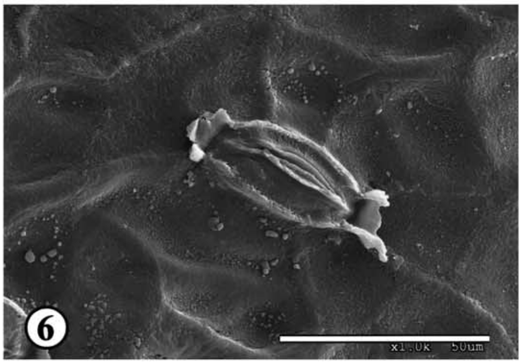

1

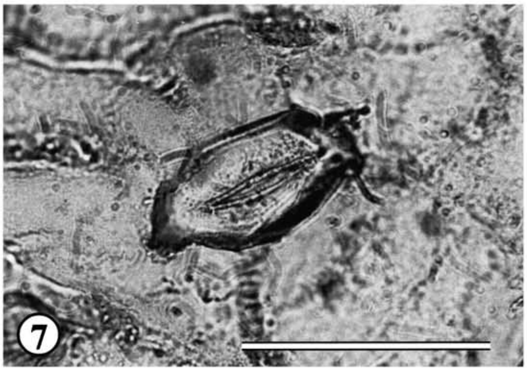

2
x500 100um

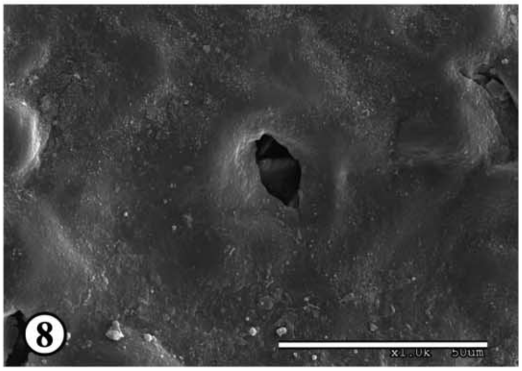

3

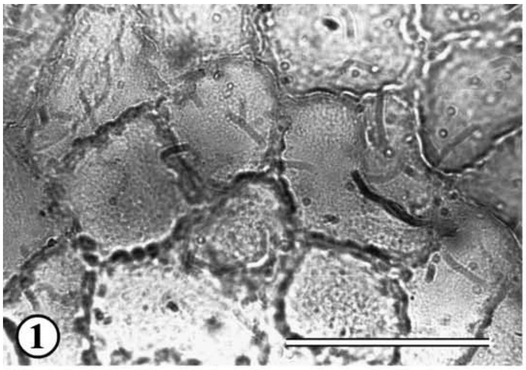

4
x350 100um

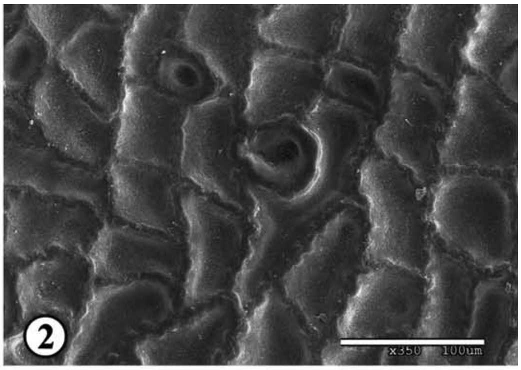

5

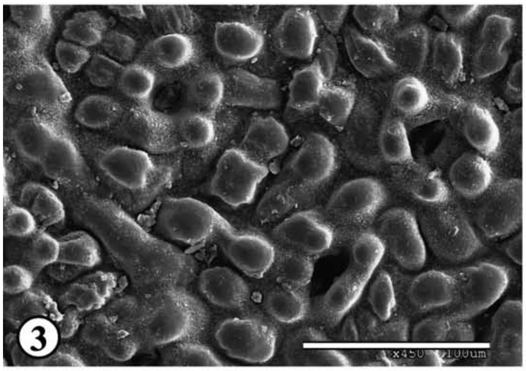

6
x1.0k 50um

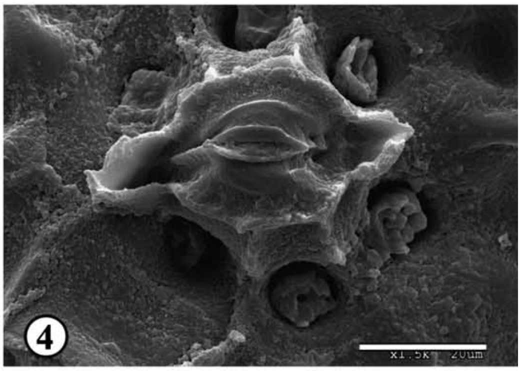

7

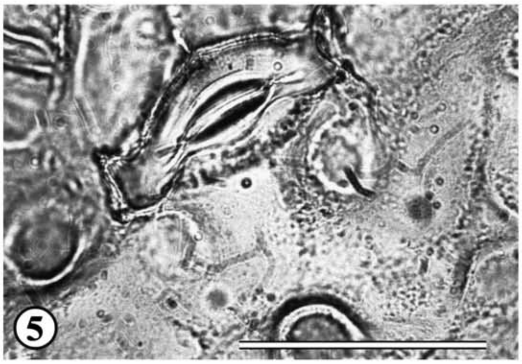

8
x1.0k 50um

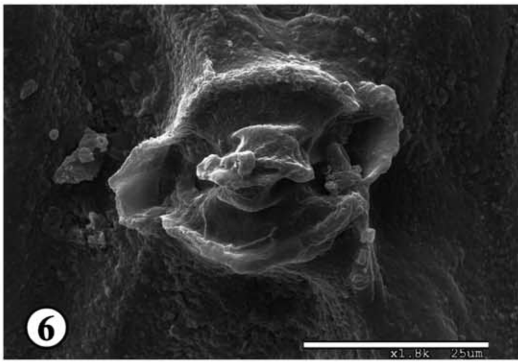

1

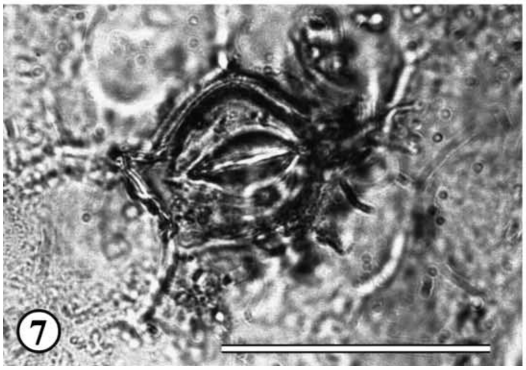

2
x350 100um

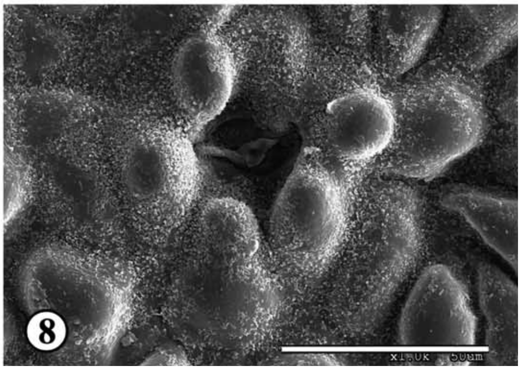

3
x450 100um


4
x1.5k 20um


5


6
x1.8k 25um


7


8
x1.0k 50um

compared to leaves from Senigallia, which lack any cuticular features. She therefore re-named the specimens from Frankfurt *Ginkgo florinii*.

Several Pliocene and Miocene *Ginkgo* leaves assigned to *Ginkgo adiantoides* and yielding cuticles show a high degree of macromorphological and micromorphological homogeneity (Table 1). The variability of the papillosity of subsidiary cells observed for several fossil leaves is similar to that of modern *Ginkgo biloba*. Both our material and Unger's type material of *G. adiantoides* belong to the Late Miocene 'Florenkomplex' Likudi-Vegora (= 'Florenkomplex' Senigallia; Mai, 1995). These fossil assemblages have many taxa in common including *Acer tricuspidatum* Bronn, *A. palaeosaccharinum* Stur, *A. monspessulanum*-like leaves, Juglandaceae (*Juglans*, *Carya*, *Pterocarya*), Lauraceae (*Sassafras*, *Cinnamomum*-like leaves), *Zelkova zelkovaefolia* (Ung.) Buzek and Kotl., and, very typically, *Fagus gussonii* Mass. and various species of *Quercus*. In addition, a number of conifers of swamp forests and of mesic forests are characteristic.

In light of macromorphological similarities of the Greek material to the Italian type material, the geographic, stratigraphic, and palaeofloristic close proximity of the Italian type locality and Vegora, as well as the homogeneity in both macromorphological and epidermal features in several European Miocene and Pliocene *Ginkgo* leaves, we strongly suggest that Pliocene and Miocene impression/compression fossils be treated as one single species, *Ginkgo adiantoides*, contrary to the view of Samylina (1967) and Boulter and Kvaček (1989). An increasing number of *Ginkgo* leaves yielding cuticles may serve as a geographic and stratigraphic framework for the delimitation of the species *G. adiantoides*. Some specimens, mostly from the Late Tertiary of Europe, based on their gross morphology only, are included here (e.g. Kolakovski, 1964, Abkhasia; Kovar-Eder, 1988, Austria; Table 1).

Based on our results, a few Early Tertiary specimens from Arctic regions and the Far East also fall within the variability of *Ginkgo adiantoides*. *Ginkgo spitsbergensis* from the Paleocene of Spitsbergen was distinguished from *G. adiantoides* by displaying less pronounced undulation of the anticlinal walls of the upper epidermis cells, and subsidiary cells with prominent papillae typically covering the stomatal aperture (Manum, 1966). Given a high variability in the degree of papillosity in *G. adiantoides*, and the undulation of the anticlinal walls of the upper epidermis cells in living *Ginkgo biloba*, *G. spitsbergensis* is here included in *G. adiantoides* (Table 1). The same applies to *Ginkgo wyomingensis* Manum from the Paleocene of Wyoming. This species differs from *G. spitsbergensis* in its slightly larger amplitude of the sinuses formed by the anticlinal cell walls on the upper epidermis (Manum, 1966). Very similar leaves from the Early Paleocene of the Far East were described by Samylina (1967) as *Ginkgo tzagajanica* Samylina, and also reported from the Paleocene/Eocene of north-eastern Japan by Horiuchi and Kimura (1986). Again, this spe-

Plate IV. *Ginkgo biloba* L., 'Goethe-tree' Weimar, Germany, coll. E.M. Friis September 2000.

1. Adaxial cuticle displaying relatively thick-walled polygonal cells in intercostal areas. Anticlinal walls are undulate (LM, preparation stored in S).
2. Inner surface of adaxial cuticle showing the same features as in 1 (SEM, specimen stored in S).
3. Outer surface of abaxial cuticle showing papillate epidermis cells and stomata with papillate subsidiary cells. Incomplete outer rings of subsidiary cells sometimes are slightly papillate and display striation (SEM, specimen stored in S).
4. Inner surface of abaxial cuticle showing a stoma with the cavities of the inner ring of papillate subsidiary cells (SEM, specimen stored in S).
5. Stoma with only slightly papillate subsidiary cells (LM, specimen stored in S).
6. Inner surface of abaxial cuticle showing a stoma without papillate subsidiary cells (SEM, specimen stored in S).
7. Stoma with weakly papillate subsidiary cells and thickened inner walls of subsidiary cells (LM, specimen stored in S).
8. Outer surface of abaxial cuticle showing a stoma with papillate subsidiary cells and epicuticular wax (SEM, specimen stored in S).

Scale is 100 μm in figs. 1–3, 20 μm in fig. 4, 50 μm in figs. 5, 7, and 8, and 25 μm in fig. 6.

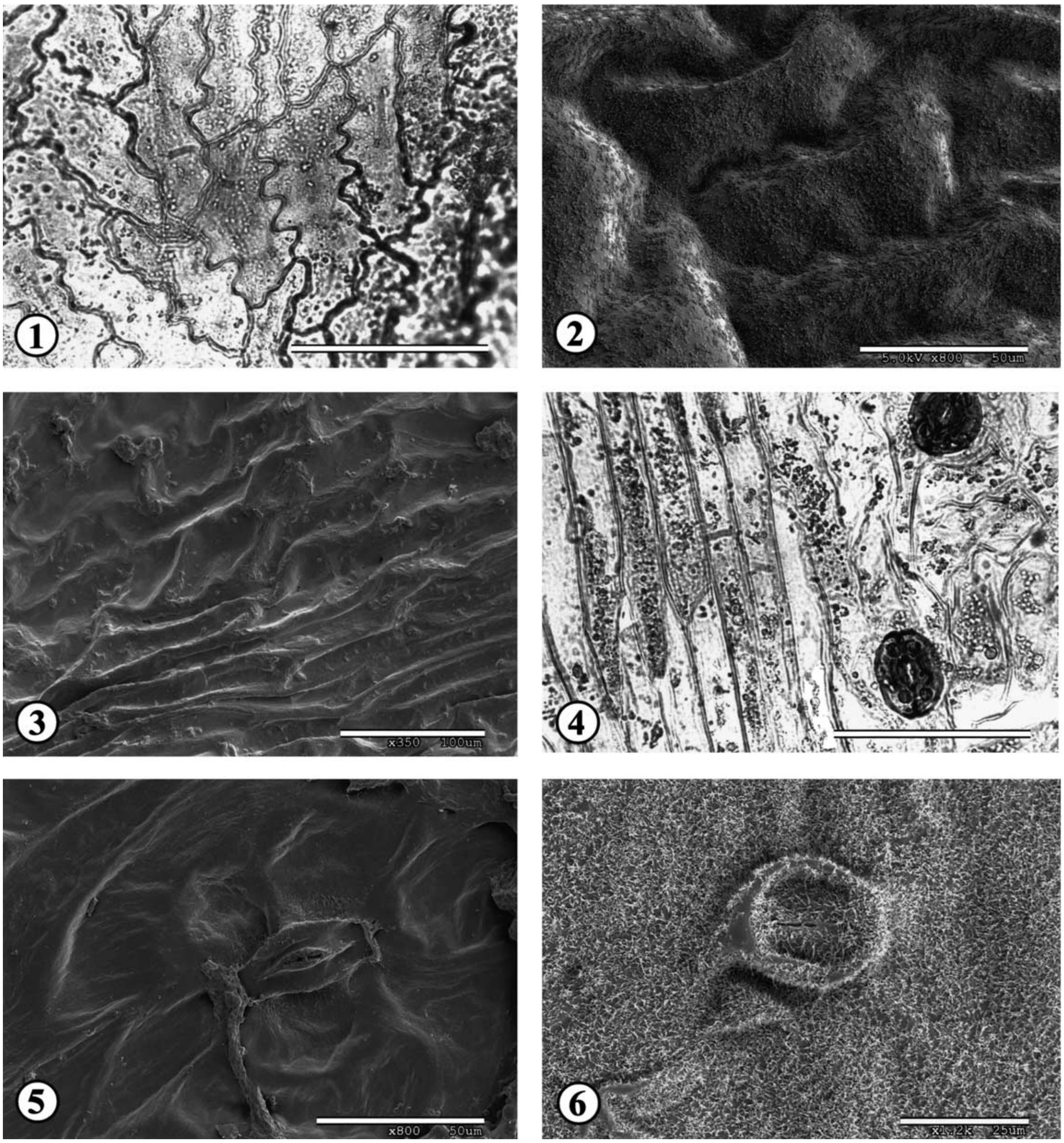


Plate V. *Ginkgo biloba* L., potted plant, 'shade leaf', S.

1. Adaxial cuticle with undulate epidermis cells (compare to figure 4A, 'sun leaf') (LM, specimen stored in S).
2. Outer surface of adaxial cuticle showing the same features as in 1 (SEM, specimen stored in S).
3. Adaxial cuticle with elongate straight cells over a vein, and slightly undulate polygonal cells in intercostal areas (SEM, specimen stored in S).
4. Abaxial cuticle with elongate straight cells over a vein, undulate cells in intercostal areas, and two stomata lacking papillae (LM, specimen stored in S).
5. Inner surface of abaxial cuticle with a stoma and incomplete amphicyclocytic subsidiary cells (SEM, specimen stored in S).
6. Outer surface of abaxial cuticle showing a stoma lacking papillate subsidiary cells, but displaying a thickened ring wall around the guard cells. Untreated leaf with fine epicuticular wax ornamentation (SEM, specimen stored in S).

Scale is 100 μm in figs. 1, 3, and 4, 50 μm in figs. 2 and 5, and 25 μm in fig. 6.

Table 1

Tertiary *Ginkgo* of Eurasia included in this study within *Ginkgo adiantoides* (Ung.) Heer: above the broken line are specimens with cuticle features preserved, below are some specimens that are included into *G. adiantoides* based on macromorphological features only.

Ginkgo adiantoides (Ung.) Heer in Eurasia	Apical leaf margin	Veins per cm[a]	Epidermal features preserved	Subsidiary cells papillate	Reference
Ginkgo spitsbergensis Manum Paleocene, Spitsbergen; southwestern Saskatchewan	Slightly dissected	∼20; 12–15	+	+	Manum, 1966; McIver and Basinger, 1993
Ginkgo wyomingensis Manum Paleocene, Wyoming	?	∼20	+	+	Manum, 1966
Ginkgo tzagajanica Samyl. Paleocene, River Bureya, Far East; Paleocene–Eocene, northeastern Japan	Entire or slightly dissected	∼20[b]; 17	+	+	Samylina, 1967 Horiuchi and Kimura, 1986
Ginkgo orientalis Samyl. Oligocene, Sakhalin	Entire	12–14	+	Weakly	Samylina, 1967
Ginkgo occidentalis Samyl. Late Miocene, Zakarpatian region, Ukraine; North Caucasus	Entire, one central notch	∼20[b]	+	+	Samylina, 1967
Ginkgo adiantoides (Ung.) Heer Late Miocene, Vegora, Greece	Entire	16–18	+	+	This study
Ginkgo adiantoides (Ung.) Heer Miocene, Stare Gliwice, Poland	Entire	17	+	Weakly	Szafer, 1961
Ginkgo adiantoides (Ung.) Heer Early Pliocene, Gerce, Hungary	Entire	?	+	+	Hably and Kvaček, 1997
Ginkgo adiantoides (Ung.) Heer = *Ginkgo florinii* Samyl. Pliocene, Frankfurt-Niederrad, Germany	Mostly entire or one notch	18–20	+	–	Florin, 1936 Samylina, 1967
Ginkgo adiantoides (Ung.) Heer Pliocene, Chiuzbaia, Transylvania, Romania	?	?	+	+	Givulescu, 1973
Ginkgo biloba L. *fossilis* Pliocene, Gocedelcevsko, Bulgaria	Entire, one central notch	∼13	+	–	Jordanov and Kitanov, 1963
Ginkgo biloba L. Late Pliocene, Koriyama Formation in Daiwa, Kagoshima Prefect., southwestern Japan	Entire, one central notch	?	+	+	Uemura, 1997
Salisburia adiantoides Unger (nomen) Late Miocene, Senigallia, northeastern Italy	Entire	?	–	?	Unger, 1850
Ginkgo adiantoides (Ung.) Heer (?) Miocene, Sakhalin	Mostly entire	16–20	–	?	Heer, 1878
Ginkgo adiantoides (Ung.) Heer Late Miocene, Grossenreith, etc., Austria	?	∼15	–	?	Kovar-Eder, 1988
Ginkgo adiantoides (Ung.) Heer Late Miocene, Kodor, Abkhasia	Entire	16–18	–	?	Kolakovski, 1964

[a] According to Horiuchi and Kimura (1986) vein density is 12–20 veins per cm in modern *G. biloba*.

[b] Values based on measurements for 5 mm have been twiced. According to Horiuchi and Kimura (1986) the so obtained values are somewhat higher than the actual ones.

Table 2
Comparative morphology of three Late Tertiary European ginkgos and extant *G. biloba* L.

		Ginkgo adiantoides (Ung.) Heer	*Ginkgo adiantoides* (Ung.) Heer	*Ginkgo adiantoides* (Ung.) Heer emend. Florin	*Ginkgo biloba* L.
	Origin	Vegora, Greece	Gerce, Hungary	Frankfurt, Germany	China; worldwide cultivated
	Age	Late Miocene	Early Pliocene	Pliocene	Recent
		(This study)	(Hably and Kvaček, 1997)	(This study and Florin, 1936)	(This study and Florin, 1936)
1	**Secretory bodies**	150–250 μm × 800–900 μm Scattered	150 μm × 800 μm Scattered	150 μm × 700–800 μm Scattered	150 μm × 500–3000+ μm Scattered
	Adaxial cuticle				
2	Epidermis cells over veins	Narrow rectangular or elongate with acute ends	Elongate	Narrow rectangular or elongate with acute ends	Narrow rectangular or elongate with acute ends
3	Dimensions	10–40 × 100–150 μm	15–25 × 100–250 μm	10–20 × 60–120 μm	30–60 × (75) 150–250 μm
4	Anticlinal cell walls	Undulate	Slightly wavy	Undulate	Undulate to straight
5	Sinus frequency/100 μm	8–9	Not mentioned	Ca. 9	Ca. 8 (5–6 in shade leaves)
6	Epidermis cells of intercostal areas	Isodiametric to broad rectangular, triangular or polygonal	Trigonal to polygonal, rarely isodiametric	Isodiametric to broad rectangular, triangular or polygonal	Broad rectangular to polygonal
7	Dimensions	50 × 50 or 35 × 90 μm	50–100 × 50–100 μm	50–60 × 50–70 μm	40–90 × 40–90 μm
8	Anticlinal cell walls	Undulate	Finely undulating	Undulate	Undulate
9	Sinus frequency/100 μm	7–8	Not mentioned	Ca. 9	Ca. 10 (6 in shade leaves)
10	Periclinal cell walls	Slightly convex	Smooth	Slightly convex	Slightly convex
11	Stomata	Absent	Not mentioned	Absent	Present, but rare
12	Trichome bases	Absent	Not mentioned	Absent	Rare, restricted to leaf base
	Abaxial cuticle				
13	Epidermis cells over veins	Elongate with acute to rectangular ends	Elongate	Elongate with acute to rectangular ends	Elongate with acute to rectangular ends
14	Dimensions	15–30 × 100 μm	25 × 60+ μm	20–35 × 90–200 μm	20–40 × 80–120 μm
15	Anticlinal cell walls	Straight to slightly undulate	Straight	Straight	Straight
16	Periclinal cell walls	Convex, often with papillae developed at ends of cells	Papillate	Convex	Convex, often one to three papillae
17	Epidermis cells of intercostal areas	Broad rectangular to polygonal	Not mentioned	Broad rectangular to polygonal	Roundish to polygonal
18	Dimensions	70 × 30 and 60 × 60 μm	50 × 50 μm	70 × 30 μm	50–70 × 25–50 μm
19	Papillae	Often papillate	Sometimes papillate	Often weakly papillate	Often papillate
20	Anticlinal cell wall	Straight to slightly undulate	Straight or wavy	Straight or wavy	Straight (sun leaves) or undulate in shade leaves
	Stomata				
21	Size	22–33 μm long, 18–23 μm wide	25–38 μm long	27–39 μm long	29–41 μm long
22	Type of subsidiary cells	Complete to incomplete amphycyclocytic	Not mentioned	Complete to incomplete amphycyclocytic, 4–8 subs.	Complete to incomplete amphycyclocytic

Table 2 (*Continued*).

	Ginkgo adiantoides (Ung.) Heer	*Ginkgo adiantoides* (Ung.) Heer	*Ginkgo adiantoides* (Ung.) Heer emend. Florin	*Ginkgo biloba* L.
Origin	Vegora, Greece	Gerce, Hungary	Frankfurt, Germany	China; worldwide cultivated
Age	Late Miocene	Early Pliocene	Pliocene	Recent
	(This study)	(Hably and Kvaček, 1997)	(This study and Florin, 1936)	(This study and Florin, 1936)
23 Inner ring of subsidiary cells	Strongly papillate	Thickened and papillate	Not or weakly papillate	Papillate (in sun leaves, ?mature leaves), or not papillate (in shade leaves, ?young leaves)
24 Outer ring of subsidiary cells	With radiating cuticular striae	Not mentioned	Sometimes weakly striate	Often papillate with radiating cuticular striae on the papillae
25 Trichome bases	Absent	Not mentioned	Sometimes in intercostal areas	Rare over veins
26 Leaves amphi/hypostomatic	Hypostomatic	Hypostomatic	Hypostomatic	'Amphistomatic'

cies was based on the conspicuous papillosity of its subsidiary cells and on its small amplitude of sinuses of anticlinal walls on the upper epidermis. The only argument to consider *G. tzagajanica* as distinct from *G. spitsbergensis* and *G. wyomingensis* was that the latter two were based on a too small number of specimens (Horiuchi and Kimura, 1986). Thus, it seems reasonable that Krassilov (1976) referred specimens of *G. tzagajanica* to *G. spitsbergensis*. Based on our results and on the description and illustrations given in Samylina (1967) and Horiuchi and Kimura (1986) these specimens are considered conspecific with *G. adiantoides*. The same applies to *G. orientalis* Samyl. from the Oligocene of Sakhalin (Table 1).

Markedly different epidermal features are reported only from the Early Tertiary (Eocene) of the Isle of Mull (*G. gardneri*). The most typical characteristic of this species, its pronounced papillae of the adaxial epidermis, is not found in either the modern *Ginkgo biloba* nor *Ginkgo adiantoides*, but is found in various Mesozoic species, and therefore it should be treated as a distinct species (but compare Boulter and Kvaček, 1989).

Uemura (1997) figured cuticular features of *Ginkgo biloba* from the Late Pliocene of Japan. This specimen is indistinguishable from *Ginkgo adiantoides*. Moreover, it displays stomata that are around 37 μm long, falling into the variability of both the living and the Late Tertiary species (see Table 2).

4.2. Character evolution in Ginkgo of the late Mesozoic and Tertiary

The genus *Ginkgo* can be traced back until the Jurassic (Zhou, 1997, China; Brik, 1953, eastern Uzbekistan, Fergana). Based on leaf shape, branching patterns of veins, and epidermal features, Tralau (1968) distinguished two morphotypes in the Jurassic, corresponding to two species, *Ginkgo digitata* (Brgn.) Heer *sensu* Tralau and *Ginkgo huttoni* (Sternb.) Heer *sensu* Tralau. These species are generally more lobed and more strongly dissected than species from the European Tertiary. Trichomes occur frequently on upper and lower leaf surfaces, and epidermis cells are polygonal; blunt papillae may be present on the abaxial epidermis. From the Early Cretaceous onwards, morphotypes occur that combine various characteristics typical of *Ginkgo adiantoides* and extant *Ginkgo biloba*, such as papillate subsidiary cells, together with features not found in either of these species, such as polygonal epidermis cells, conspicuously papillate upper epidermis cells, and trichomes on the upper leaf surface (*G. pluripartita* (Schimper) Heer, *G. polaris* Nath., *G. coriacea* Florin). Moreover, these species still are more dissected than the Tertiary *G. adiantoides*. At the same time, leaves from the Early Cretaceous that resemble *G. adiantoides* and *G. biloba* by gross morphology but are clearly distinguishable from them by epidermal features (trichomes and papillae on the upper epidermis) have been described as *G. paradiantoides* Samyl. and *G. pilifera* Samyl. from Eastern Siberia (Samylina, 1967).

For the Tertiary Horiuchi and Kimura (1986) recognised a decrease in the development of papillae on the subsidiary and ordinary cells of the abaxial epidermis from Early Tertiary to Late Tertiary species. This conclusion was founded on their interpretation of subsidiary cells of modern *Ginkgo biloba*, and of *Ginkgo adiantoides* from the Pliocene of Frankfurt, both of which they considered to be without papillae. In fact, the specimen of *G. biloba* figured in Horiuchi and Kimura (1986) bears weakly papillate subsidiary and epidermis cells. In the present study (Plate IV, 3) slightly more developed papillae of *G. biloba* are shown. Their papillae nature can be clearly seen on the internal surface of the cuticle where subsidiary cells form conspicuous cavities (Plate IV, 4). At the same time *G. biloba* may bear subsidiary cells without papillae (Plate V). The same observation was made for *G. adiantoides* (Plates II and III). From this we conclude that the degree of papillosity in Tertiary *Ginkgo* may not be a taxonomically (and phylogenetically) significant character as suggested by other authors (Manum, 1966; Samylina, 1967; Horiuchi and Kimura, 1986; McIver and Basinger, 1993).

Based on our results, most of Tertiary *Ginkgo* leaves in the Northern Hemisphere are characterised by upper epidermis cells showing various degrees of undulation and lacking any papillae. Subsidiary and epidermis cells of the lower epidermis may be weakly to conspicuously papillate. *Ginkgo gardneri*, a leaf morphotype with a conspicuous papillate upper epidermis, extends to the Eocene of Mull (Great Britain; Boulter and Kvaček, 1989). This may be the last representative of a mainly Mesozoic lineage having strongly papillate upper epidermis cells.

Acknowledgements

We are thankful to Else Marie Friis, Stockholm, Zlatko Kvaček, Prague, Mike Boulter, London, and James Basinger, Saskatoon, for valuable comments on the manuscript. Special thanks go to Marika Bezhashvili, Stockholm, for translating the paper of Samylina (1967) into English.

References

Boulter, M.C., Kvaček, Z., 1989. The Paleocene flora of the Isle of Mull. Spec. Pap. Palaeontol. 42, 1–149.

Brik, M.J., 1953. The Mesozoic flora of Vostotjno-Fergana coal basin (in Russian). Gosgeoltekh., Moscow.

Florin, R., 1936. Die fossilen Ginkgophyten von Franz-Joseph-Land nebst Erörterungen über vermeintliche Cordaitales mesozoischen Alters. 2. Palaeontographica B 82, 1–72.

Gardner, J.S., 1883–1886. A Monograph of the British Eocene Flora 2. Palaeontogr. Soc. (Monogr.), pp. 1–159.

Givulescu, R., 1973. Die fossilen Koniferen des Fundortes Chiuzbaia F. In: Givulescu, R., Olos, E. (Eds.), Paläobotanische Studien im Tertiär Siebenbürgens. Geolog. Inst. Mem. 19, 31–35.

Hably, L., Kvaček, Z., 1997. Early Pliocene plant megafossils from the volcanic area in West Hungary. In: Hably, L. (Ed.), Early Pliocene Volcanic Environment, Flora and Fauna from Transdanubia, West Hungary. Hungarian Natural History Museum, Budapest, pp. 5–151.

Heer, O., 1878. Miocene Flora der Insel Sachalin. Mém. Acad. Imp. Sci. Saint-Pétersb. Sér. 7 25, 1–61.

Hill, R.S., Carpenter, R.J., 1999. Ginkgo leaves from Palaeogene sediments in Tasmania. Aust. J. Bot. 47, 717–724.

Horiuchi, J., Kimura, T., 1986. *Ginkgo tzagajanica* Samylina from the Palaeogene Noda Group, Northeast Japan, with special reference to its external morphology and cuticular features. Trans. Proc. Palaeontol. Soc. Jpn. 142, 341–353.

Jordanov, D., Kitanov, B., 1963. Njakolko interesni fosilni rastenija ot pliocena Gocedelčevsko. Ann. Univ. Sofia Fac. Biol. Géol. Géochim. 56, 25–37.

Knobloch, E., Velitzelos, E., 1987. New leaf floras in the Neogene of Greece. Vestn. Ustred. Ust. Geol. 62, 157–164.

Kolakovski, A.A., 1964. A Pliocene flora of the Kodor river. Georgian Acad. Sci. Monogr. 1, 1–209.

Kovar-Eder, J., 1988. Obermiozäne (Pannone) Floren aus der Molassezone Österreichs. Beitr. Paläontol. Österr. 14, 19–121.

Krassilov, V.A., 1976. Tsagajanskaya flora of Amurskoy Oblasti (in Russian). Izdat. Nauka, Moscow.

Mai, H.D., 1995. Tertiäre Vegetationsgeschichte Europas. Gustav Fischer, Jena.

Manum, S., 1966. *Ginkgo spitsbergensis* n. sp. from the Paleocene of Spitsbergen and a discussion of certain Tertiary species of Ginkgo from Europe and North America. Norsk Polarinst. Årb. 1965, 49–58.

Massalongo, A., Scarabelli, G., 1859. Studii sulla Flora Fossile e Geologia Stratigraphica del Senegalliese. Tipografia d'Ignazio Galeati e Figlio, Imola.

McIver, E.E., Basinger, J.F., 1993. Flora of the Ravenscrag Formation (Paleocene), Southwestern Saskatchewan, Canada. Palaeontogr. Can. 10, 1–167.

Nathorst, A.G., 1919. *Ginkgo adiantoides* (Unger) Heer im Tertiär Spitzbergens nebst einer kurzen Übersicht der übrigen fossilen Ginkgophyten desselben Landes. Geol. Fören. Stockh. Förh. 41, 233–248.

Pole, M.S., Douglas, J.G., 1999. Bennettitales, Cycadales and Ginkgoales from the mid Cretaceous of the Eromanga Basin, Queensland, Australia. Cretac. Res. 20, 523–538.

Samylina, V.A., 1967. Final stages of the history of the genus *Ginkgo* L. in Eurasia (in Russian). Bot. J. 52, 303–316.

Szafer, W., 1961. Miocene flora from Stare Gliwice in Upper Silesia. Inst. Geol. Prace 33, 1–205.

Tralau, H., 1968. Evolutionary trends in the genus *Ginkgo*. Lethaia 1, 63–101.

Uemura, K., 1997. Cenozoic history of *Ginkgo* in East Asia. In: Hori, T., Ridge, R.W., Tulecke, W., Del Tredici, P., Trémouillaux-Guiller, J., Tobe, H. (Eds.), *Ginkgo biloba*, a Global Treasure: From Biology to Medicine. Springer, Tokyo, pp. 207–221.

Unger, F., 1845. Synopsis Plantarum Fossilium. Leopold Voss, Leipzig.

Unger, F., 1850. Genera et Species Plantarum Fossilium. Wilhelm Braumüller, Vienna.

Velitzelos, E., 1993. Neue paläofloristische Daten zu känophytischen Floren Griechenlands. Doc. Nat. 78, 1–17.

Zhou, Z., 1997. Mesozoic Ginkgoalean megafossils: A systematic review. In: Hori, T., Ridge, R.W., Tulecke, W., Del Tredici, P., Trémouillaux-Guiller, J., Tobe, H. (Eds.), *Ginkgo biloba*, a Global Treasure: From Biology to Medicine. Springer, Tokyo, pp. 183–206.