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Source: *International Journal of Plant Sciences*, Vol. 174, No. 3, Special Issue: Conceptual Advances in Fossil Plant Biology Edited by Gar Rothwell and Ruth Stockey (March/April 2013), pp. 406-424

Published by: [The University of Chicago Press](#)

Stable URL: <http://www.jstor.org/stable/10.1086/668221>

Accessed: 20/03/2013 14:11

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LEAF CUTICLE ANATOMY AND THE ULTRASTRUCTURE OF *GINKGOITES TICOENSIS* ARCHANG. FROM THE APTIAN OF PATAGONIA

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The leaf cuticle of the *Ginkgoites ticoensis* Archang. type material from the Aptian Anfiteatro de Ticó Formation in Patagonia, Argentina, is fully characterized with additional scanning and transmission electron microscopic observations. Many new anatomical and ultrastructural cuticular features are identified in the four-lobed leaf of *G. ticoensis*: the leaf shows a hypostomatic and papillate laminae, straight and pitted anticlinal and granulate periclinal walls, actinocytic stomata with between five and seven papillate, striate subsidiary cells, and guard cells with anticlinal smooth walls. The TEM studies on ordinary epidermal cells, papillae, subsidiary cells, and guard cells reveal general ultrastructural features of Ginkgoaceae: an outer polylamellate layer A made with A1 and a granular inner layer A2; A1 with an upper part A1U with continuous and straight translucent lamellae; a lower part A1L with significantly disrupted and waving translucent lamellae; and the fibrillar cuticular layer B1 as the innermost part. Ten ultrastructural characters are detailed and ranked by the use of confidence intervals based on 30 statistical measurements. A three-dimensional reconstruction of the cuticle is also provided. Because of the anatomical and ultrastructural fine details shown in the *G. ticoensis* cuticle, new elements are given to suggest its probable family affinity and to enhance the specificities of *Ginkgo* and *Ginkgoites*.

Keywords: *Ginkgoites ticoensis*, leaf cuticle, anatomy, ultrastructure, Ginkgoales, Aptian, Argentina.

Introduction

The Ginkgophytes in Argentina were part of the plant assemblages in the Carboniferous and extending into the Mesozoic, until their complete disappearance by the middle Eocene. The group is well documented both by diverse foliage types and by different complete ovuliferous structures (Archangelsky 1965; Jain and Delevoryas 1987; Archangelsky and Leguizamón 1980; Archangelsky and Cúneo 1990; Morel et al. 1999; Cúneo et al. 2010). Among fossil leaves, *Ginkgoites* Seward is perhaps the most frequently represented genus, with several species ranging back to the late Paleozoic and occurring mainly in Patagonia. Impressions of *Ginkgoites eximia* (Feruglio) Cúneo and *G. feruglioi* Cúneo, which has a remarkable resemblance to juvenile leaves of the extant *Ginkgo biloba* L., were recorded from the Lower Permian Río Genoa Formation in the Chubut Province (Feruglio 1942; Cúneo 1987).

In the Mesozoic, *Ginkgoites* become more diverse, with several taxa inhabiting Patagonia alone. Of these, the leaf gross morphology of *G. truncata* Frenguelli was described from the Potrerillos Formation of Upper Triassic age in the Mendoza Province (Frenguelli 1946). The Group El Tranquilo is of the same age and outcrops in the Patagonian Province of Santa Cruz, holding imprints of *G. dutoitii* Anderson

& Anderson, *G. palmata* (Ratte) Gnaedinger & Herbst, and *G. waldeckensis* (Anderson & Anderson) Gnaedinger & Herbst (Azcuy and Baldoni 1990; Gnaedinger and Herbst 1999).

Jurassic records in southern South America of *Ginkgoites* or other ginkgoalean foliage types have not been found to date, as a result of either preservation or taphonomy; this is in marked contrast to the abundant and well-preserved forms of the same age that have been recovered from the northern latitudes (Watson et al. 1999; Guignard and Zhou 2005; Yang et al. 2008; Nosova et al. 2011).

Leaves of *Ginkgoites* from Cretaceous strata can also be found in Patagonia, and in several cases, the presence of these species also extends into Antarctica (Archangelsky 1965; Baldoni and Medina 1989). Particularly, the taxa recovered from Patagonia are characterized by the presence of very well-preserved epidermal structures. For example, *G. skottsbergii* Lund. from the Albian Kachaike Formation was established for its leaf cuticle features (Lundblad 1971; Del Fueyo et al. 2006). One of the better-known Patagonian species of *Ginkgoites* is *G. tigrensis* Archang. from the Aptian Anfiteatro de Ticó Formation (Archangelsky 1965). The first leaf cuticular ultrastructure analysis of a ginkgoalean foliage type was carried out on this taxon by Taylor et al. (1989). Later, the leaf cuticle of *G. tigrensis* was fully investigated by means of both SEM and TEM by Villar de Seoane (1997), who demonstrated a certain resemblance of the fossil leaf cuticle to that of the extant *G. biloba* L. It is worth noting that the seeds of *Karkenian incurva* Archang., a multiple ovuliferous organ at-

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Manuscript received March 2012; revised manuscript received July 2012.

tached to short shoots with *G. tigrensis* leaves, were found with several cuticular layers, including the megaspore membrane; these layers were also ultrastructurally characterized (Del Fuego and Archangelsky 2001). Seeds from another locality and from the same stratigraphical level were also studied by Archangelsky (1965); in particular, these seeds belong to the putative ginkgoalean *Allicospermum patagonicum* Archang. and exhibited preserved cuticular features.

In this article, we report new evidence of the anatomy and ultrastructure of the *G. ticoensis* Archang. leaf epidermis based on additional observations with light and electron (scanning and transmission) microscopic techniques. *Ginkgoites ticoensis* was first described with light microscopy by Archangelsky (1965) from the well-known Aptian Anfiteatro de Ticó Formation in the Santa Cruz Province. Here, we reexamine the holotype and paratypes of *G. ticoensis* to characterize in detail its foliar epidermal anatomy and cuticle ultrastructure and to compare it more accurately with that of other species of *Ginkgoites* and other *Ginkgo*-like foliage. We also propose a relationship between the two genera, and we reveal the probable family affinity of *G. ticoensis*.

Material and Methods

Material

The leaves of *Ginkgoites ticoensis* are compressions with well-preserved cuticles; they were collected by one of the authors, S. Archangelsky, during the years 1958–1959 at the eastern side of the Anfiteatro de Ticó locality (Estancia La Magdalena in the Santa Cruz Province, Argentina; fig. 1), where several fossiliferous levels are known to have abundant plant remains, such as *Cladophlebis tripinnata* Archang., *Brachyphyllum mirandai* Archang., *Ticoa harrisii* Archang., and *Ptilophyllum longipinnatum* Archang. *Ginkgoites ticoensis* was found in the lenticular (~5 m wide and 0.5 m high) *C. tripinnata* fossiliferous bed and was associated with seeds of *Allicospermum patagonicum* Archang. The leaves occur in the compact sediments, such as the dark-brown mudrocks of the Anfiteatro de Ticó Formation, and the basal unit (followed toward the top by the Bajo Tigre and Punta del Barco Formations) of the Baqueró Group (Cladera et al. 2002). According to Limarino et al. (2012), the fossiliferous level where *G. ticoensis* occurs is included between the middle-upper sections of depositional sequence 1 and is considered equivalent to stratigraphic levels 4 and 5. The Anfiteatro de Ticó Formation is of Early Aptian age, 118.56 ± 1.4 Ma, based on the radiometric dating made by Corbella (2001). Recently, the upper Punta del Barco Formation was dated as Late Aptian age, 114.67 ± 0.18 Ma (Césari et al. 2011).

Methods

The leaf cuticles of the holotype and paratypes were removed from the matrix and oxidized in 40% nitric acid followed by 5% ammonium hydroxide. Some cuticles were cleared with sodium hypochlorite. The cuticles intended for light microscopy (LM) and SEM investigations were prepared in accordance with the procedures outlined in Passalia et al. (2010). The LM observations were made with a Leitz Diaplan, while the

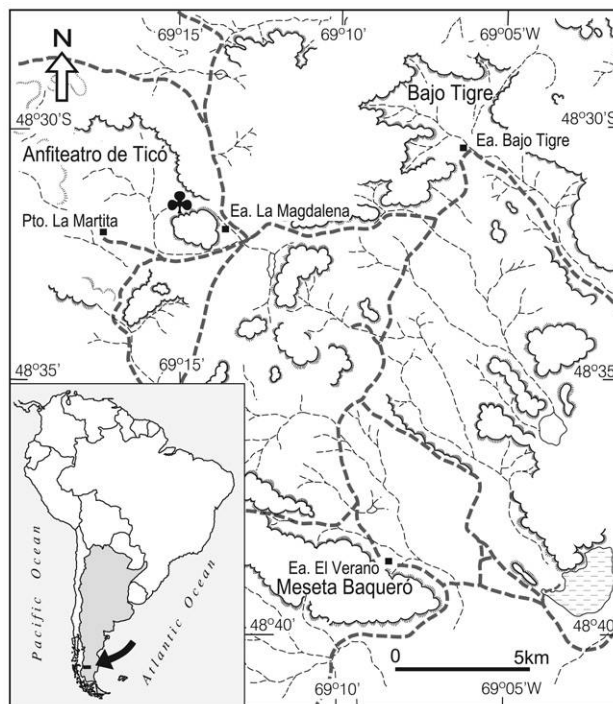


Fig. 1 Location map showing the Anfiteatro de Ticó locality (club symbol), Anfiteatro de Ticó Formation, Santa Cruz Province, Argentina.

micrographs were captured with a Leica DFC 280. The SEM observations were made in a Philips XL30 TMP SEM at 15.1 kV at the Argentine Natural Sciences Museum Bernardino Rivadavia.

The samples for TEM were prepared following Lugardon's (1971) technique, which has been used for fossil pollen and spores as well as for living plant cuticles (Bartirolo et al. 2012). Ultrathin sections were observed and photographed with a Philips CM 120 TEM instrument at 80 kV, at the Centre de Technologie des Microstructures (CT μ) of University Lyon 1. In total, four pieces of material were embedded in Epon resin blocks, and 50 uncoated, 300-mesh copper grids were prepared (40 as transverse sections, i.e., perpendicular to the leaf length, and 10 as longitudinal sections, i.e., parallel to the leaf length).

The fossil specimens and the microscope slides reside in the Palaeobotanical Collection of La Plata Natural Sciences Museum, while the samples for SEM are housed in the Palaeobotanical Collection of the Argentine Natural Sciences Museum Bernardino Rivadavia (under the acquisition numbers LP 5800–5803, LP 5805, LP Pm 21–25, BA Pb MEB 252–254, respectively). The resin blocks and the TEM negatives are stored in the University Lyon 1. The terminology of Holloway (1982) and Archangelsky (1991) was used for the ultrastructural analysis.

Results

Leaf Morphology

Ginkgoites ticoensis Archangelsky has a simple, lobed, hypostomatic, and petiolate leaf. It is flabelliform, measuring

up to 4 cm long and 3 cm wide, and possesses a slender petiole, measuring up to 1 cm long and 0.1 cm wide, in which four parallel veins can be observed (fig. 2A, 2B).

The lamina is divided into four linear to oblong lobes, measuring 1.5 cm long and 0.5 cm wide, with rounded apices and straight margins. The lamina shows a deep incision that forms two first-order lobes. The second-order lobes originate 0.7 cm from the petiole base. The veins are radially positioned from the lamina base. They are subparallel and dichotomously forked, with a concentration of approximately four veins at the leaf base and eight to 10 veins in each segment (fig. 2A, 2B).

General Cuticular Structure

Ginkgoites ticoensis shows both adaxial and abaxial surface lobes formed by epidermal cells with different forms, sizes, and dispositions (figs. 2C, 2D, 2E, 3A). On the middle portion of each segment, the veins are formed by six to eight rows of cells. They are rectangular (measuring 70–90 μm long and 15–18 μm wide), with an anticlinal straight and strongly pitted wall of 1–2.5 μm in width. The periclinal walls evident in the internal view are granulate (fig. 3H).

The epidermal cells between the veins are irregularly disposed and composed of polygonal to isodiametric cells measuring 40–70 μm long and 20–35 μm wide. Their walls are slightly thickened (2.5 μm wide) and markedly pitted (figs. 2G, 2H, 3I).

The papillae are densely distributed in both epidermis layers (fig. 2D, 2E); however, they are nearly absent on the veins (fig. 3A). Externally, the papillae have a smooth surface, a width of 18–20 μm , and a height of 11–15 μm (figs. 2E, 3C). Internally, the papillae are observed as an excentric granulate hollow (fig. 3I).

The stomata are irregularly disposed between the veins, and the pits are oval to circular in shape and haphazardly oriented (figs. 2D, 2E, 3A, 3B). The stomatal density is 80–100 per mm^2 (figs. 2D, 3A). The stomatal apparatus is circular and up to 70 μm in diameter; additionally, it is monocyclic and actinocytic with five to seven subsidiary cells (figs. 2G, 2H, 2I, 3E). Generally, the subsidiary cells have an isodiametric (14 \times 12 μm) to rectangular (21.5 \times 14 μm) shape (fig. 2G, 2H). Their anticlinal flanges are straight, granulated, and 1.5 μm thick (fig. 3E, 3G). Each subsidiary cell has a subcircular to elongated papilla measuring 15 μm high and 10 μm wide; the papillae overarch the pit and form an oval- to circle-shaped (82 μm long and 64 μm wide) suprastomatal aperture. The surfaces of some papillae are striate to rugulate (fig. 3C). The guard cells are sunken and reniform and measure 30 μm long and 10 μm wide. Their walls are smooth and thicker around the pit (fig. 3D, 3E, 3G).

Cuticular Ultrastructure

The cuticular membranes observed in *G. ticoensis* are of four types: ordinary epidermal cell (figs. 4, 5) and papillae (fig. 7) or subsidiary or guard cell of the stomatal apparatus (fig. 6). They all consist of an outer cuticle proper (A = outer polylamellate layer A1 [A1U in the upper part with continuous, straight, and translucent lamellae + A1L in the lower part with significantly disrupted, wavy, translucent la-

mellae going downward] and a granular inner layer A2 often containing some fibrils in its lowermost part) and an innermost fibrillar cuticular layer CL (=B1) with rather heterogeneous constituents (figs. 4, 6E–6K, 7). The well-preserved cuticle of *G. ticoensis* demonstrates the great variability that can exist at this ultrastructural level, particularly in the B1 layer (fig. 4D–4F). Statistical measurements were performed on each of the four types of cuticles (table 1). Ten characters were taken into account, including the total cuticular membrane thickness (CM), five characters of the cuticle proper (A) thickness, thickness of the B layer (=B1), thicknesses of the opaque and translucent lamellae of the A1 layer, and, finally, number of translucent lamellae.

The ordinary epidermal cell and papilla cuticles (figs. 4, 5, 7) have a similar total thickness (1.02 and 1.20 μm in mean thickness, respectively), while the subsidiary cell cuticle (fig. 6) is the thickest (1.47 μm in mean thickness), and the guard cell cuticle is the thinnest (0.46 μm in mean thickness). The ordinary epidermal cell cuticle has the highest percentage of cuticle proper A (41.2%) and the lowest percentage of cuticular layer B (58.8%; fig. 4); conversely, the three other types (i.e., the papillae, the subsidiary, and the guard cells) have a lower and rather similar percentage of cuticle proper (33.3%, 31.3%, and 34.8%, respectively) and the highest percentage of cuticular layer (66.7%, 68.7%, and 65.2%, respectively; figs. 6, 7). At high magnification, the A1 layer of the cuticle proper A is quite similar with respect to its ordinary epidermal cells (fig. 5) and papilla cuticles (fig. 7A–7F; 0.052 and 0.047 μm in mean thickness, respectively); a similar result exists for the A2 layer (0.368 and 0.353 μm in mean thickness, respectively). However, these layers are very different with respect to the stomatal apparatus (A1 = 0.055 vs. 0.022 μm in mean thickness for the subsidiary cell and the guard cell cuticles, respectively, as shown in fig. 6C–6K; similarly, A2 = 0.405 vs. 0.138 μm , respectively). Enlargements of the A1 layer show that A1U is rather similar in its ordinary epidermal cells and papilla cuticles (0.023 and 0.016 μm in mean thickness, respectively; figs. 5A–5K, 7D–7F), while it is much more variable in the stomatal apparatus cuticles (0.038 and 0.013 μm in mean thickness, respectively, for the subsidiary cells and the guard cell cuticles; fig. 6H–6K). The percentages vary within the same range. The translucent lamellae of the A1 layer are twice as thick in the ordinary epidermal cell versus the papilla cuticles (5.2 vs. 2.6 nm in mean thickness, respectively; figs. 5A–5K, 7D–7F). These lamellae are significantly similar in the stomatal apparatus (4.8 and 4.3 nm in mean thickness, respectively, for the subsidiary cells and the guard cell cuticles; fig. 6H–6K). Their number decreases regularly from the ordinary epidermal cell to the papilla and then to the subsidiary cell and guard cell cuticles (5.8, 5.3, 4, and 3.4 nm, respectively). The opaque lamellae vary greatly in thickness (22.5, 6.6, 5.77, and 9.23 nm, respectively; figs. 5A–5K, 6H–6K, 7D–7F).

Discussion

Identity of *Ginkgoites* versus *Ginkgo*

In their recent paleobotanical synthesis, Taylor et al. (2009, p. 747) have noted the difficulties in distinguishing among foliar ginkgophytes, particularly with respect to the leaves

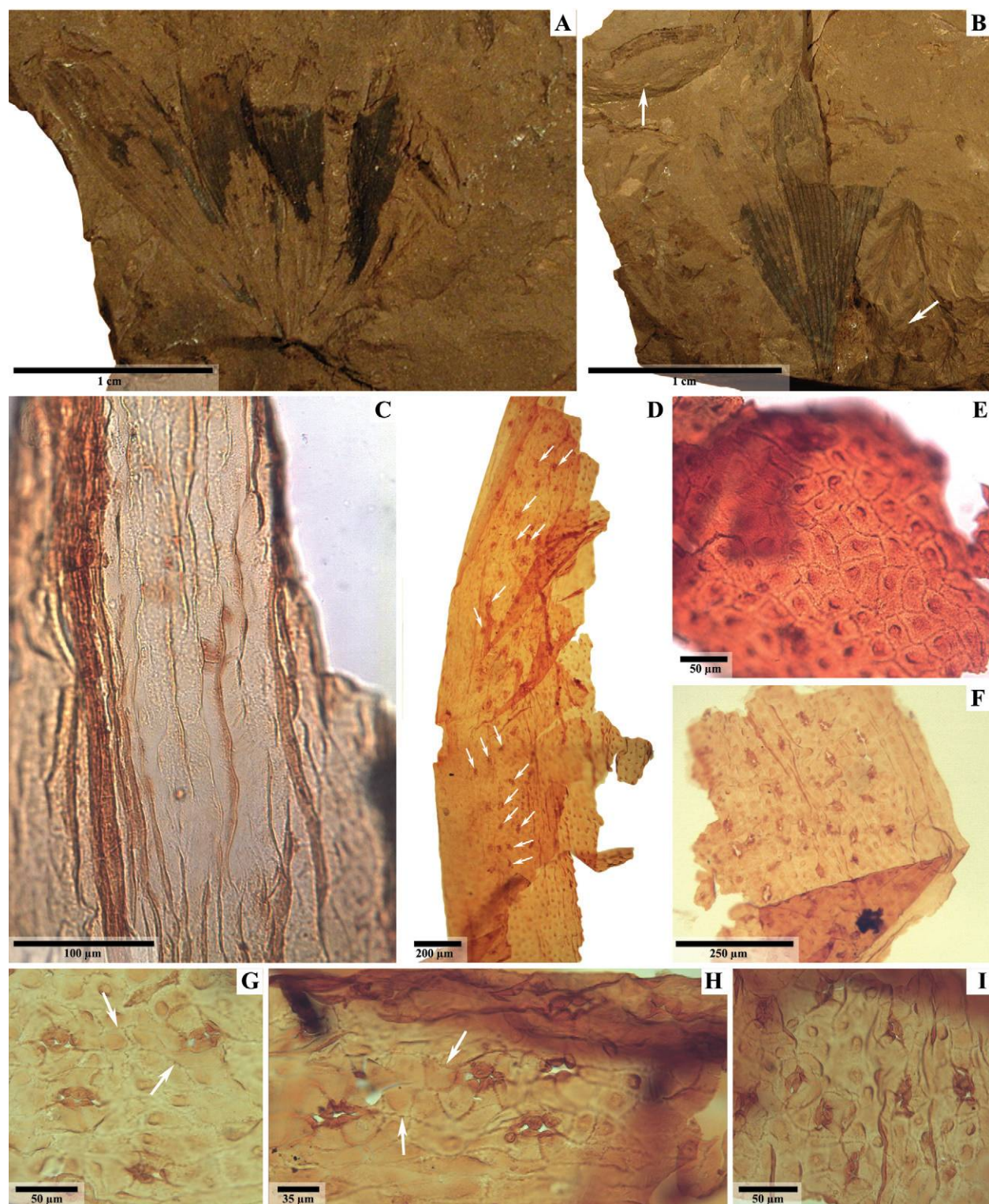


Fig. 2 *Ginkgoites ticoensis* Archang. Stereo and light microscope. A, Leaf gross morphology. Paratype LP 5805. B, Detail of leaflets. Note the entire margin and veins. Arrows indicate associated seed of *Alliospermum patagonicum* Archang. and a frond of *Cladophlebis tripinnata* Archang. Paratype LP 5801. C–I, Leaf cuticle under light microscope. C, Adaxial epidermis devoid of stomata. LP Pm 22. D–I, Abaxial epidermis. D, General view showing papillae and stomata (arrows). LP Pm 24. E, Detail of papillae. LP Pm 23. F–I, Stomata. F, Random disposition of stomata. LP Pm 24. G–I, Details of stomatal apparatus. Note papillate subsidiary cells and anticlinal pitted walls (arrows). All LP Pm 24.

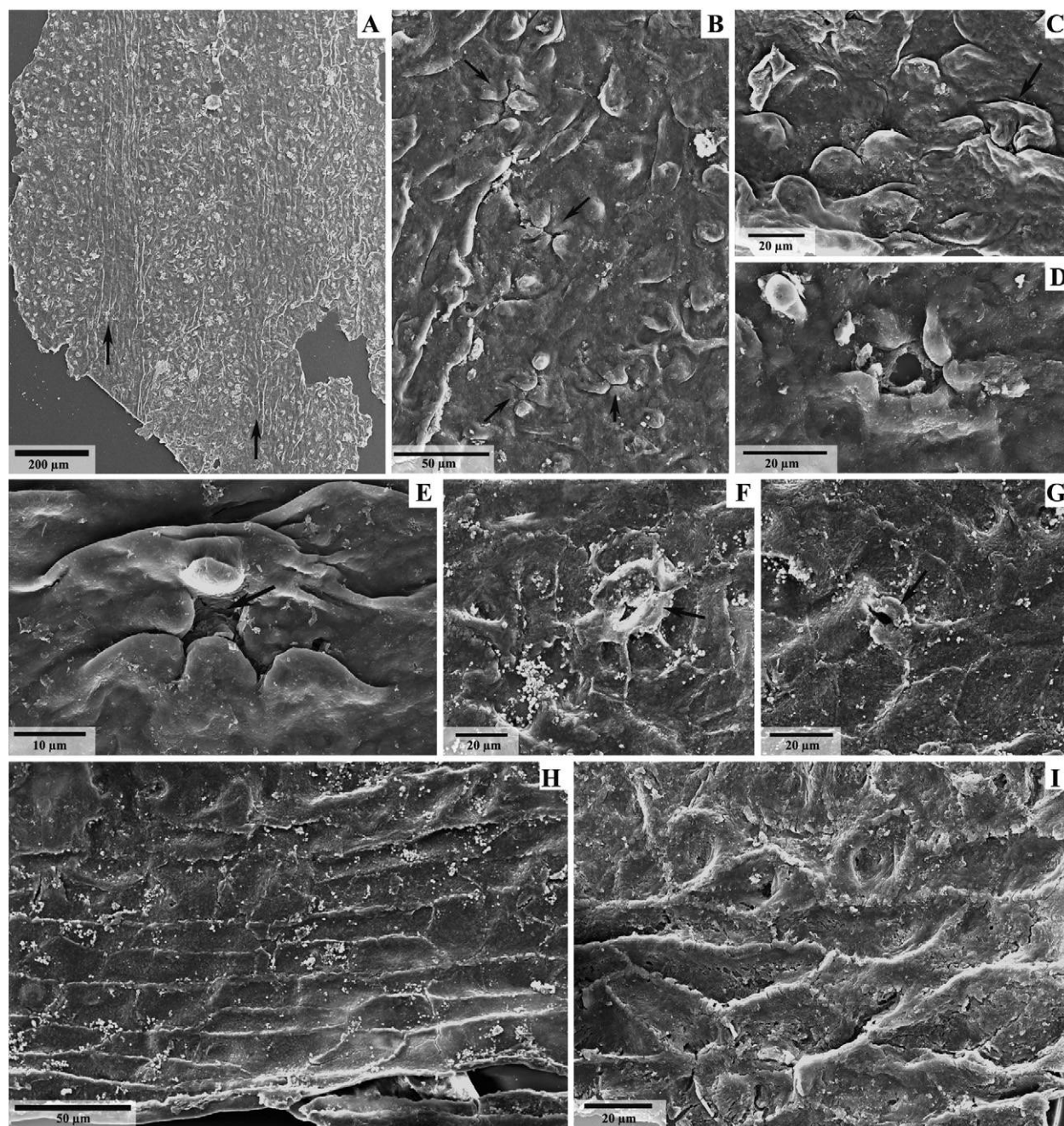


Fig. 3 *Ginkgoites ticoensis* Archang. Abaxial leaf epidermis under scanning electron microscope. A–E, Epidermis outer surface. A, Epidermis in general view. Note papillate stomata between veins (arrows). BA Pb MEB 253. B, Cuticle showing stomata randomly located (arrows). BA Pb MEB 253. C, Detail of papillate cells. Arrow indicates stoma. BA Pb MEB 252. D, E, Details of stomata. D, Papillate subsidiary cells over arching stoma aperture. BA Pb MEB 253. E, Almost nearly closed subsidiary cells. Arrow shows suprastomatal chamber. BA Pb MEB 252. F–I, Epidermis inner surface. F, G, Stomata inner view. Arrows indicate guard cells. F, BA Pb MEB 254. G, BA Pb MEB 253. H, Elongate rectangular cells area on vascular strain. BA Pb MEB 254. I, Papillate epidermal cells area. Note anticlinal walls strongly pitted. 37-BA Pb MEB 252.

of the extant *Ginkgo biloba* L. versus those of *Ginkgoites* Seward. Over the years, the name of this latter fossil taxon has been ambiguously used because there have been different interpretations regarding its generic definition (Seward 1919; Florin 1936; Tralau 1968). For these reasons, Harris and Millington (1974) have argued for the abandonment of *Ginkgoites* in fa-

vor of *Ginkgo* as a result of the great variability that exists in the leaf morphology of both the fossil and extant genera, making it typically impossible to distinguish between them. Nevertheless, Zhou and Zhang (1989) and Zhou (1997, 2009) have proposed retaining *Ginkgoites* in the sense originally used by Seward, that is, as a generic name for *Ginkgo*-like leaves that

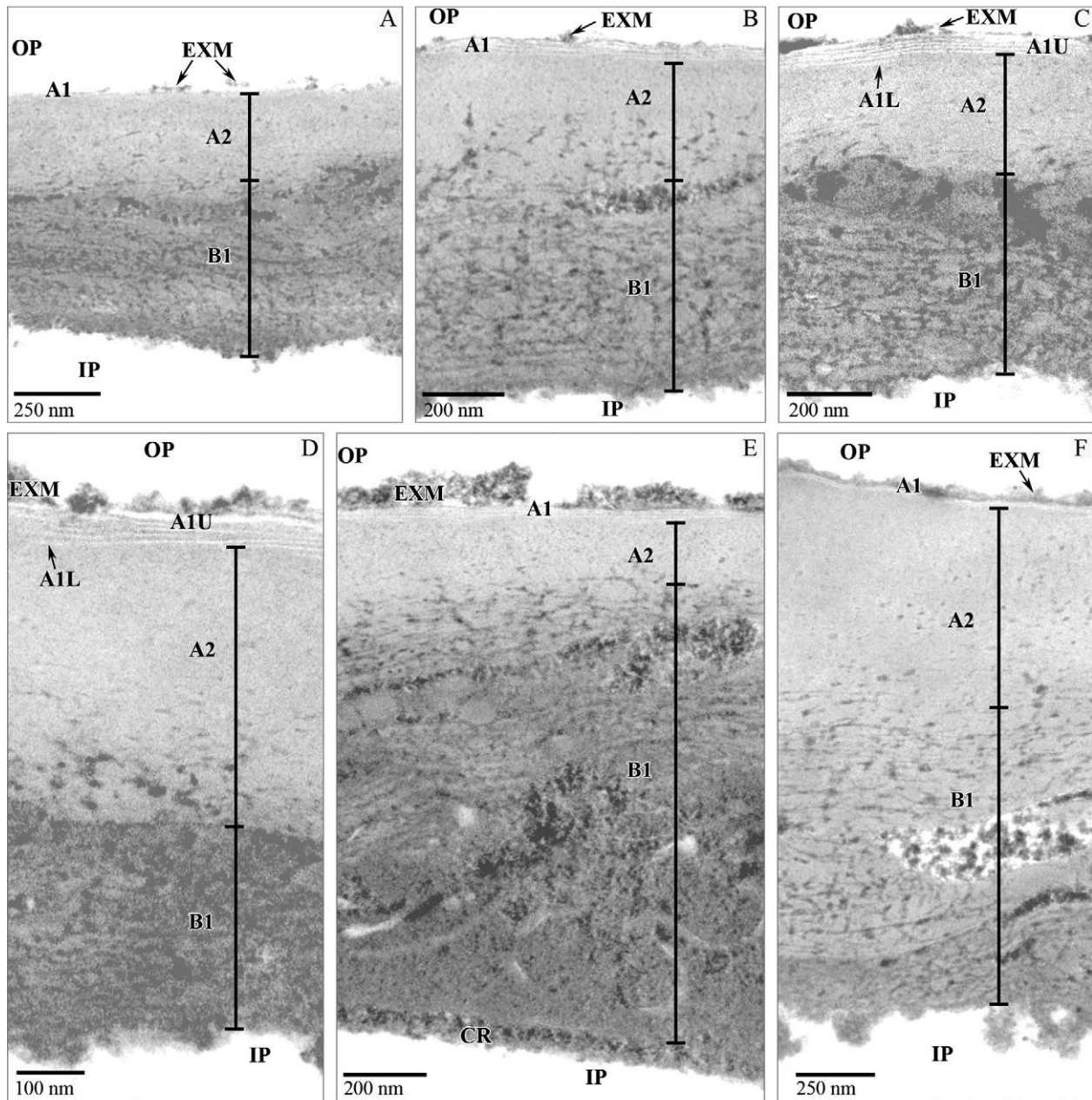


Fig. 4 *Ginkgoites ticoensis* Archang. A–F, Ultrathin transversal sections of ordinary epidermal cell cuticles, general views. Transmission electron microscope. OP = outer part of the cuticle; IP = inner part of the cuticle; EXM = extracuticular material; CR = cell residues. At these low magnifications, the A1 outermost layer is more or less visible, made with only A1U or with both A1U and A1L parts. The different sections show the variations of thickness of the layers A1, A2, and B1. They also show the various consistencies of their materials, with A2 having some fibrils in some lowermost parts in contact with the cuticular layer B. It is especially obvious for the B1 fibrilous layer, mainly with fibrils oriented parallel to the outer sides of the cuticle but also with perpendicular or waving fibrils (in E) or even with big holes (F) in some rare cases. A, ECGV46/13/12/10; B, ECGV30/25/11/10; C, ECD20/13/12/10; D, ECD21/13/12/10; E, ECD50/13/12/10; F, ECGV15/25/11/10.

cannot be attributed to any natural genus with certainty. On the other hand, Watson et al. (1999) have readopted the status of *Ginkgoites* for fossil *Ginkgo*-like foliage lacking associate reproductive structures.

Generally, all the above considerations have referred mainly to characters of the leaf gross morphology. However, the results presented here indicate that the leaf general morphology,

along with the leaf cuticular structure and ultrastructure features observed in *Ginkgoites ticoensis*, are quite distinct from those observed in other described fossil species of *Ginkgoites* or *Ginkgo*, including the living *G. biloba*. The following comparisons among selected taxa using these three character levels may also contribute toward establishing an enhanced real identity of *Ginkgoites* versus *Ginkgo*.

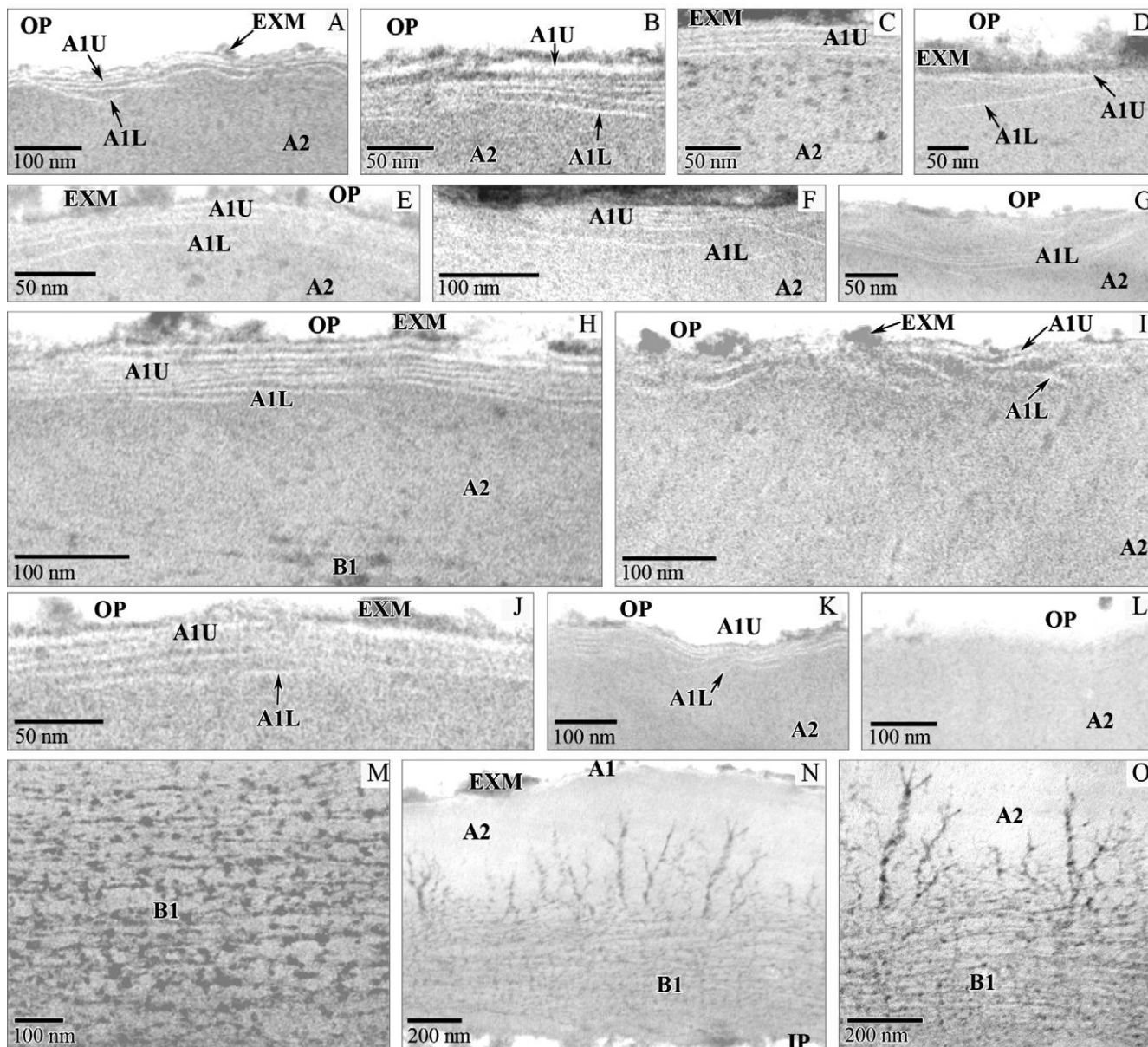


Fig. 5 *Ginkgoites ticoensis* Archang. Ultrathin sections of ordinary epidermal cell cuticles, details. Transmission electron microscope. All photos, except N and O (longitudinal sections), are transversal sections. OP = outer part of the cuticle; IP = inner part of the cuticle; EXM = extracuticular material. A–L, Outer parts of the cuticle. Note the A1 layer, composed of two parts: a straight A1 upper polylamellate part with condensed and regularly arranged polylamellae, only rarely present, as in C; the A1 lower polylamellate part has more dispersed polylamellae and makes various schemes, particularly developed as in G, where A1U is absent. A, ECD51/23/11/10; B, ECD11/13/12/10; C, ECD14/13/12/10; D, ECD12/25/11/10; E, ECD60/23/11/10; F, ECD14/25/11/10; G, ECD28/25/11/10; H, ECD25/13/12/10; I, ECD27/13/12/10; J, ECD01/25/11/10; K, ECD27/04/01/11; L, ECD25/25/11/10. M–O, Inner parts of the cuticle. The innermost B1 layer can be heterogeneous as in fig. 4, but it is more often made with fibrils arranged parallel. In very rare parts, as in N and O, B1 fibrils are oriented transversally in their uppermost parts. M, ECD58/13/12/10; N, ECD57/03/01/11; O, ECD52/03/01/11.

Morphological and anatomical leaf comparisons among most relevant Mesozoic species of Ginkgoites and Ginkgo L. We have selected those *Ginkgoites* and *Ginkgo* species, recorded from the Mesozoic of both the Northern and Southern Hemispheres, that have been created based on entire specimens and for which the morphology and cuticular anat-

omy have been fully and properly described (tables 2, 3). The majority of the species of both *Ginkgoites* and *Ginkgo* are characterized by the presence of a flabelliform leaf divided into lobes (a few to several) and possessing radially disposed dichotomous veins, which sometimes makes difficult the intraspecific or interspecific recognition, as noted above. How-

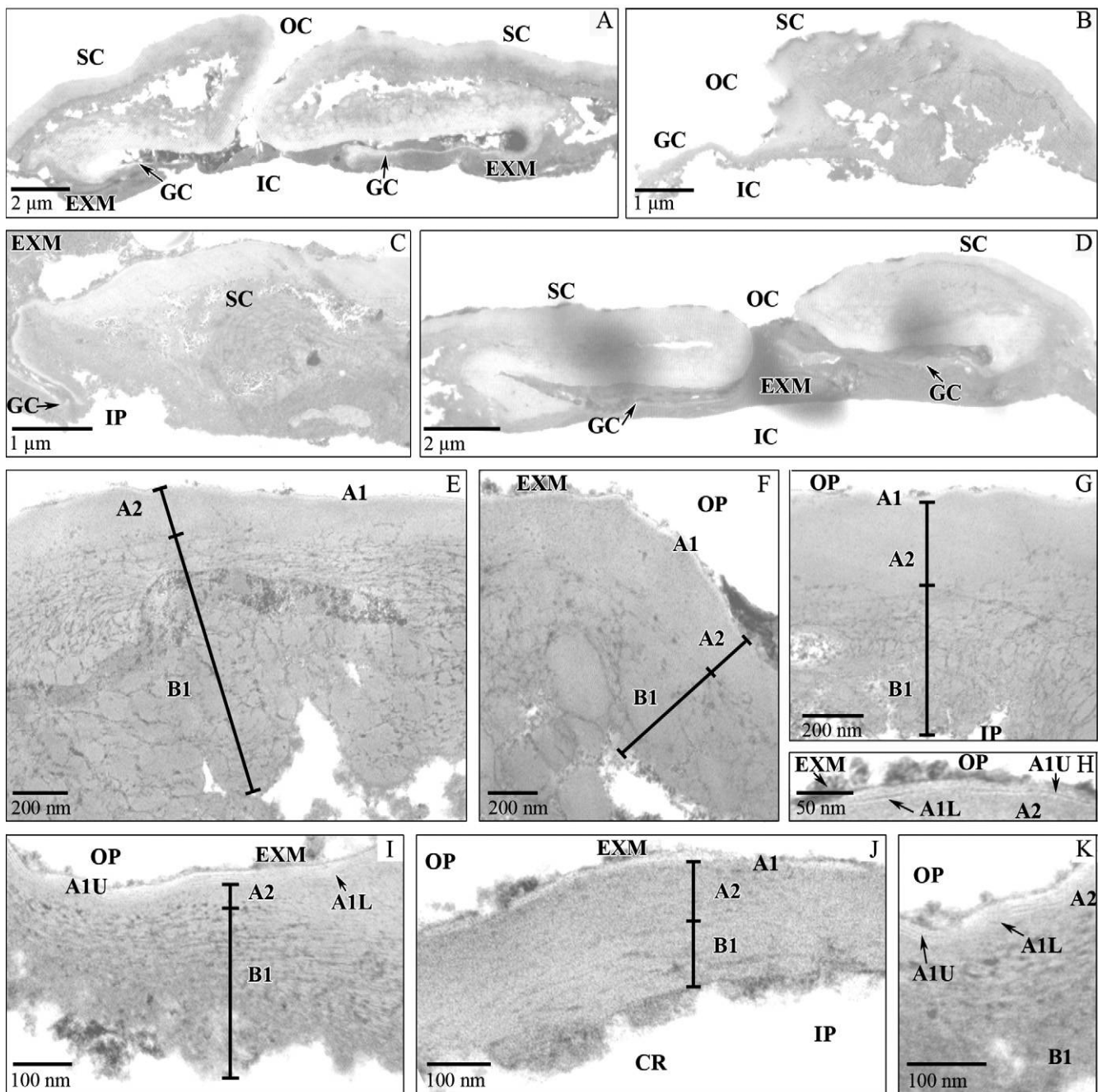


Fig. 6 *Ginkgoites ticoensis* Archang. Ultrathin sections of stomatal apparatus cuticles. Transmission electron microscope. *B–H* are longitudinal sections; *A* and *I* are transversal sections. OP = outer part of the cuticle; IP = inner part of the cuticle; EXM = extracuticular material; CR = cell residues; SC = subsidiary cell cuticle; GC = guard cell cuticle; OC = outer chamber of the stomatal apparatus; IC = inner chamber of the stomatal apparatus. *A–D*, General views, *A* and *D* being whole stomatal apparatus, *B* and *C* being half stomatal apparatus. Note the differences of the sections. *A*, S2/2/03/01/11; *B*, S4/13/04/01/11; *C*, S5/51/04/01/11; *D*, S3/35/03/01/11. *E–H*, Details of subsidiary cell cuticles. Note the differences of consistency of the B1 fibrilous layer, with *H* showing translucent details of the A1 upper and lower parts of the A1 polylamellate layer. *E*, S4/21/04/01/11; *F*, S2/13/03/01/11; *G*, S2/28/03/01/11; *H*, S5/04/01/11. *I–K*, Details of guard cell cuticles. Note that the A1 layer is in some cases devoid of polylamellae, as in *J*, while the B1 fibrilous layer is very heterogeneous in thickness. *I*, S4/15/04/01/11; *J*, S1/35/13/12/10; *K*, S416/04/01/11.

ever, when the cuticular features in those taxa appear well preserved, they allow for an unequivocal specific identification of the specimens. With respect to the epidermal characters,

at first, the papillate stomatal apparatus is present, followed by the papillae in the majority of the *Ginkgoites* and *Ginkgo* species. Nevertheless, the combinations of these data along

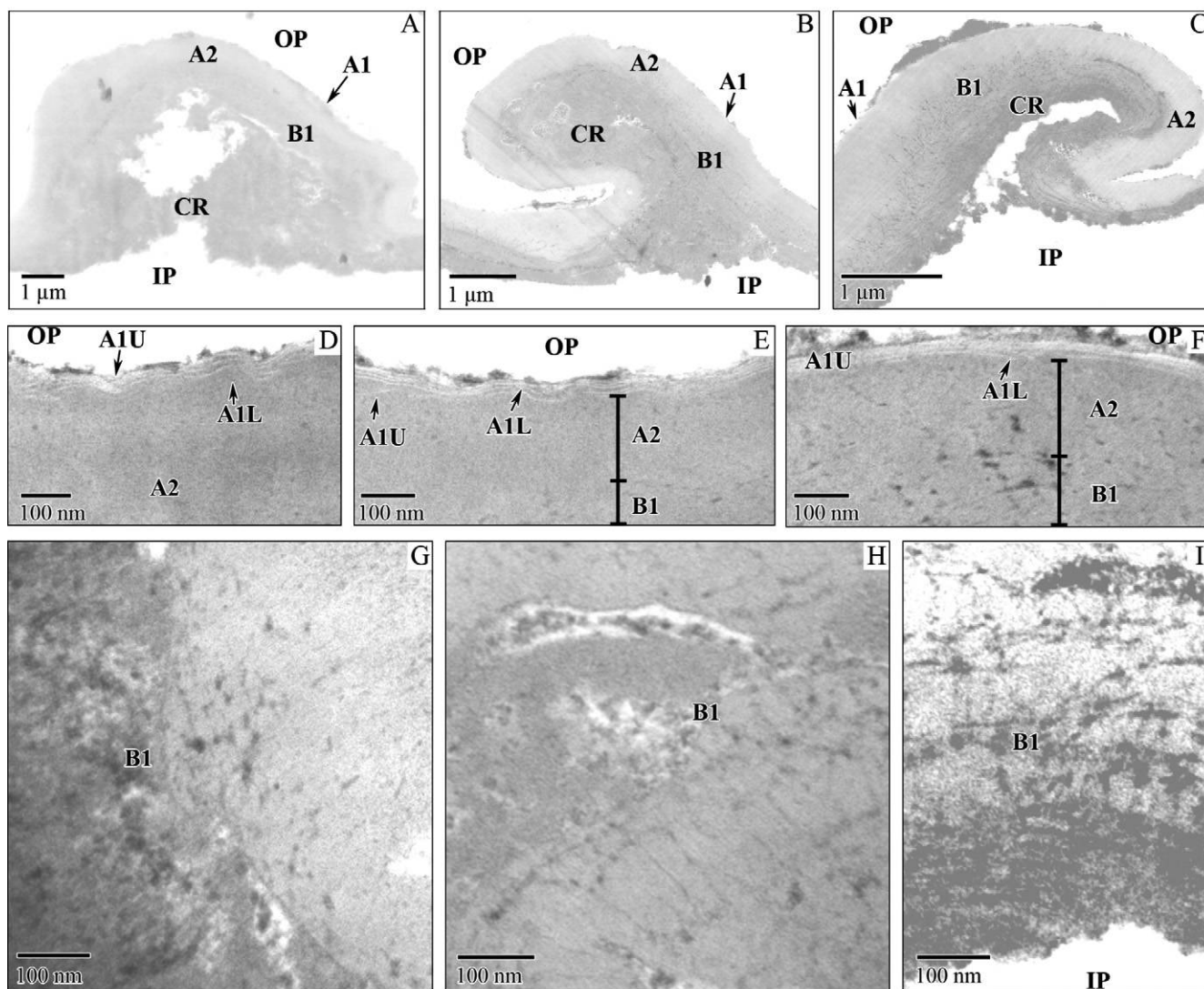


Fig. 7 *Ginkgoites ticoensis* Archang. Ultrathin sections of epidermal cell papilla cuticles. Transmission electron microscope. *B*, *G*, and *H* are longitudinal sections; all others are transversal sections. OP = outer part of the cuticle; IP = inner part of the cuticle; CR = cell residues. *A*–*C*, General views of somewhat flattened or prominent papillae cuticles, more or less full in their inner part with cell residues. The A1 uppermost layer is hardly visible at this low magnification. *A*, ECPP3/31/25/11/10; *B*, ECPP6/35/04/01/11; *C*, ECPP4/04/13/12/10. *D*–*F*, Details of the outer part of the cuticle, with the straight A1 upper polylamellate part with condensed and regularly arranged polylamellae, while the A1 lower polylamellate part has more dispersed translucent polylamellae. *D*, ECPP1/44/23/10/10; *E*, ECPP1/45/23/11/10; *F*, ECPP1/46/23/11/10. *G*–*I*, Details of the inner part of the cuticle, with B1 a more or less heterogeneous layer, fibrils not always oriented in the same direction and even with holes (*H*) in some rare cases. *G*, ECPP6/37/04/01/11; *H*, ECPP6/39/04/01/11; *I*, ECPP4/5/13/12/10.

with the amphistomatic or hypostomatic laminae are the key features used to distinguish these two taxa (tables 2, 3).

Tables 2 and 3 are synthesized by considering the particular foliar morphology and the cuticular anatomy characters observed in *G. ticoensis*, such as the size (3 cm wide × 4 cm long) and number of lobes (4), hypostomatic laminae, papillae in both epidermises, and actinocytic stomata with five to seven papillate subsidiary cells; it is shown that this taxon can clearly be identified as a *Ginkgoites* species (tables 2, 3).

Cuticular ultrastructure comparisons among species of Ginkgoites and Ginkgo. Villar de Seoane (1997) and, more recently,

Del Fueyo et al. (2006) noted more precisely that the fine structure of the foliar cuticle of living *G. biloba* and fossil *G. tigrensis* are rather similar. However, these remarks concern low-magnification features that fit perfectly with the general ultrastructural features observed in the Ginkgophytes leaves, such as the presence of a cuticle proper A, which is generally composed of two or more sublayers, and the cuticular layer B (Guignard and Zhou 2005).

In the present detailed ultrastructural study made on the leaf cuticle of *G. ticoensis*, many differences appear compared with those described for the two ginkgos (Ginkgoa-

Table 1
***Ginkgoites ticoensis* Leaf Cuticle Statistics Based on 30 Measurements**

	Ordinary epidermal cell cuticle			Papilla cuticle			Subsidiary cell cuticle			Guard cell cuticle		
	Mean	Min.-max. (SD)	Percentage	Mean	Min.-max. (SD)	Percentage	Mean	Min.-max. (SD)	Percentage	Mean	Min.-max. (SD)	Percentage
CM	1.02	.69-1.60 (.29)	100	1.20	.62-1.73 (.31)	100	1.47	.83-2.51 (.4)	100	.46	.15-1.20 (.37)	100
CP (A)	.42	.22-.69 (.13)	41.2	.40	.18-.72 (.15)	33.3	.46	.16-.94 (.2)	31.3	.16	.03-.58 (.160)	34.8
A1	.052	.02-.11 (.02)	5.1	.047	.03-.08 (.02)	3.9	.055	.04-.06 (.01)	3.73	.022	.017-.024 (.002)	4.8
A1 _U	.023	0-.045 (.014)	2.25	.016	.01-.043 (.008)	1.3	.038	.02-.05 (.01)	2.59	.013	.011-.014 (.001)	2.8
A1 _L	.029	0-.086 (.022)	2.85	.031	.016-.072 (.015)	2.6	.016	0-.025 (.007)	1.14	.009	.005-.011 (.003)	2
A2	.368	.17-.65 (.13)	36.1	.353	.11-.68 (.15)	29.4	.405	.112-.89 (.2)	27.6	.138	.0135-.56 (.159)	30
CL (B)	.6	.32-1.04 (.23)	58.8	.80	.42-1.19 (.20)	66.7	1.01	.66-2.02 (.31)	68.7	.3	.1-.78 (.22)	65.2
OL (nm)	22.5	6-55 (16.44)		6.6	3-12 (2.97)		5.77	4-11 (1.83)		9.23	4-15 (3.99)	
TL (nm)	5.2	3-8 (1.42)		2.6	2-3.5 (.53)		4.8	4-6 (.71)		4.3	3-5 (.65)	
TL (no.)	5.8	2-9 (2.02)		5.3	3-9 (1.84)		4	3-5 (.74)		3.4	3-4 (.5)	

Note. The cuticular membrane (CM) is made of a cuticle proper (A = outer polylamellate layer A1 [A1_U + A1_L] and granular inner layer A2) and a fibrillar cuticular layer (CL; =B1). All values are in micrometers, except opaque lamellae (OL) and translucent lamellae (TL) of the A1 layer.

Table 2
Morphological and Anatomical Leaf Comparisons among Mesozoic Selected Species of *Ginkgoites*

Species	Period	Origin	Laminae	Morphology				Anatomical characters				
				Size width × length (cm)	Lobes	Veins/cm	Petiole width (cm)	Adaxial cells	Abaxial cells	Stomata	Subsidiary cells	
<i>G. skottsbergii</i> (Lundblad 1971)	Albian	Argentina	Lobed amphistomatic	4 × 5	8–12	Smooth	Smooth	Actinocytic	Actinocytic	6–8 papillate
<i>G. ticoensis</i> Archang. (this article)	Aptian	Argentina	Lobed hypostomatic	3 × 4	4	12	.1	Papillate	Papillate	Actinocytic	Actinocytic	5–7 papillate
<i>G. tigrensis</i> Archang. 1965	Aptian	Argentina	Lobed amphistomatic	5 × 4.5	3–6	18–20	.1	Smooth	Smooth	Tetracytic-actinocytic	Tetracytic-actinocytic	4–6 striated
<i>G. myrio-neurus</i> (Yang 2004)	Barremian	China	Lobed hypostomatic	8 × 3.8	8–18	20–30	.1–.2	Papillate	Papillate	Actinocytic bicyclic	Actinocytic bicyclic	6–8 papillate
<i>G. pluripartita</i> Seward (Watson et al. 1999)	Lower Cretaceous	Germany	Lobed hypostomatic	1.5–10 × 2.5	4–10	20–30	.1–.2	Papillate	Papillate	Actinocytic	Actinocytic	4–8 papillate
<i>G. brauniana</i> (Watson et al. 1999)	Lower Cretaceous	Germany	Lobed hypostomatic	1.5–10 × 2.5	9–17	18–20	.1–.2	Smooth	Papillate	Actinocytic	Actinocytic	5–7 papillate
<i>G. multiloba</i> (Douglas 1970)	Lower Cretaceous	Australia	Lobed hypostomatic	2.5–3.5	6–8	13–15	.2	Haired	Haired	Actinocytic	Actinocytic	5–7 papillate
<i>G. buttonii</i> Heer (Tralau 1968)	Middle Jurassic	England	Lobed amphistomatic	...	2–12	Smooth	Papillate	Actinocytic	Actinocytic	6 papillate
<i>G. regnellii</i> (Tralau 1968)	Middle Jurassic	Sweden	Lobed hypostomatic	.8–6 × 6.7	3–8	10	.1	Smooth	Smooth	Actinocytic monocytic	Actinocytic monocytic	4–6 papillate
<i>G. sibirica</i> Seward (Tralau 1968)	Middle Jurassic	Siberia	Lobed hypostomatic	...	8–10	10–14	...	Papillate	Papillate	Actinocytic	Actinocytic	Papillate

Table 3
Morphological and Anatomical Leaf Comparisons among Selected Species of *Ginkgo* L.

Species	Period	Origin	Laminae	Size width × length (cm)	Morphology				Anatomical characters		
					Lobes	Veins/cm	Petiole width (cm)	Adaxial cells	Abaxial cells	Stomata	Subsidiary cells
<i>G. biloba</i> L. (Villar de Seoane 1997)	Recent	China	Lobed hypostomatic	8 × 4	2	10–20	.1	Smooth	Papillate	Actinocytic	6–7 papillate
<i>G. cranoi</i> (Zhou et al. 2012)	Paleocene	United States	Entire hypostomatic	3.2–11.3 × 2.8–8.3	...	11–14	...	Smooth	Papillate	Incomplete bicyclic	4–7 papillate
<i>G. patagonica</i> Berry (Traverso 1964)	Eocene	Argentina	Lobed hypostomatic	4 × 5	4	14	.3	Striate	Striate	Tetracytic-actinocytic	4–6 papillate
<i>G. coriacea</i> Florin (Sun 1993)	Lower Cretaceous	China	Lobed hypostomatic	3.8–8.0 × 2.0–4.3	4	15–20	.1–.2	Papillate	Papillate	Actinocytic monocyclic	5–6 papillate
<i>G. longifolius</i> Harris (Sun et al. 2008)	Middle Jurassic	China	Lobed amphistomatic	12 × 6–7	4	14–20	.1–.2	Smooth	Smooth	Actinocytic	4–6 papillate
<i>G. shiguanensis</i> (Sun et al. 2008)	Middle Jurassic	China	Lobed amphistomatic	6–7 × 3–4	4	20–24	.1–.2	Smooth	Smooth	Actinocytic	4–6 papillate
<i>G. yimaensis</i> Zhou et Zhang 1989	Lower Jurassic	China	Lobed hypostomatic	4–13 × 3.3–8.0	4–8	7–18	.1–.3	Smooth	Smooth	Anomocytic	6–7 papillate

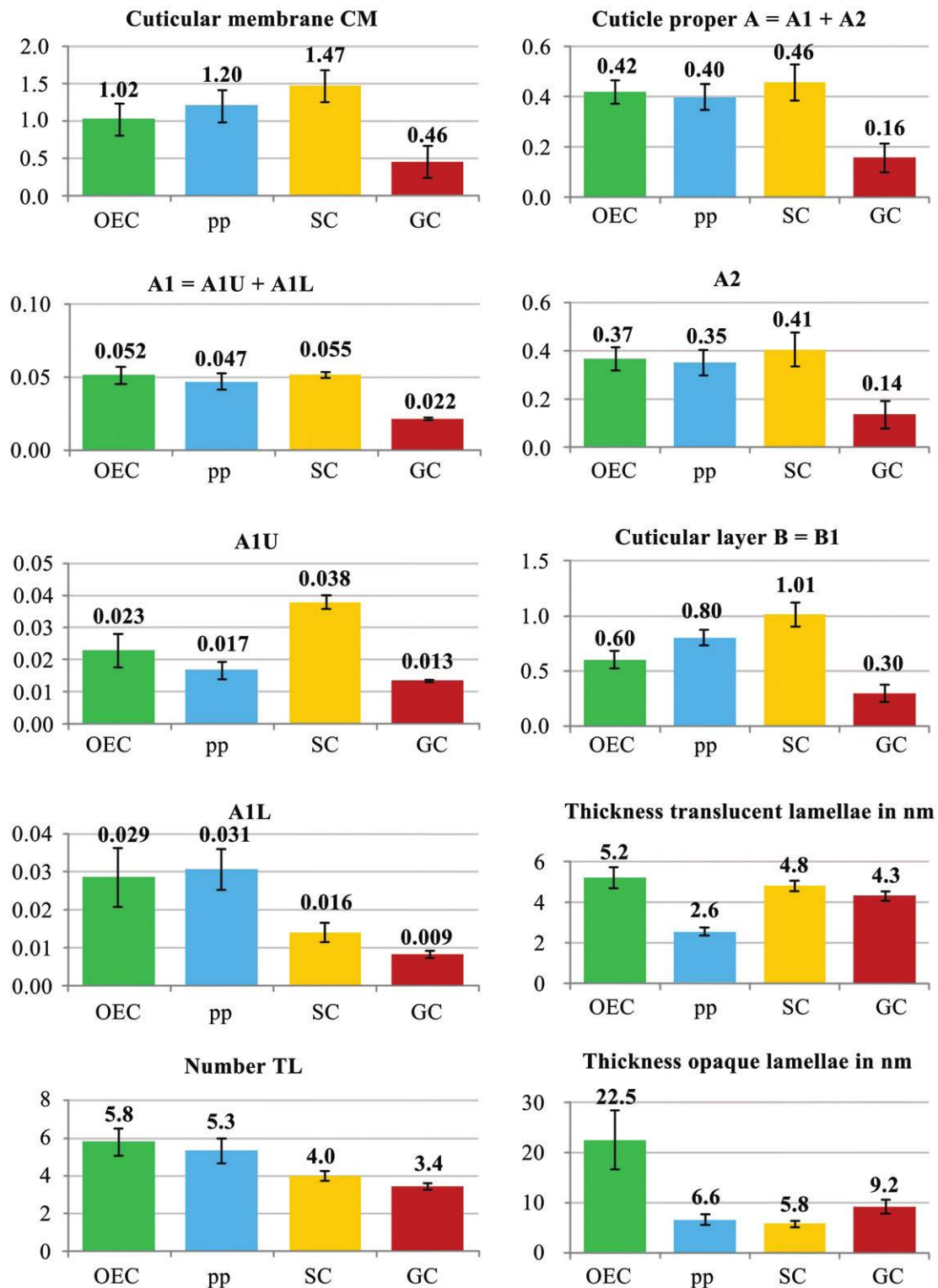


Fig. 8 *Ginkgoites ticoensis* Archang. Mean and confidence interval (CI = $\bar{x} \pm [\text{var}/n]^{1/2} \times 1.96$, giving 95% α risk) for each of the four types of cell cuticles. The values represent mean \pm CI. The cuticular membrane (CM) is made up of cuticle proper (=A = A1U + A1L zones, + A2 layer) and cuticular layer (CL; =B = B1 layer). The A1 layer (=A1U + A1L zones) is composed of alternate lamellae of opaque layer (OL) and translucent layer (TL). All measurements are in micrometers, except for the very thin OL and TL, which are measured in nanometers. The number of translucent lamellae TL (number TL) is also evaluated with CI.

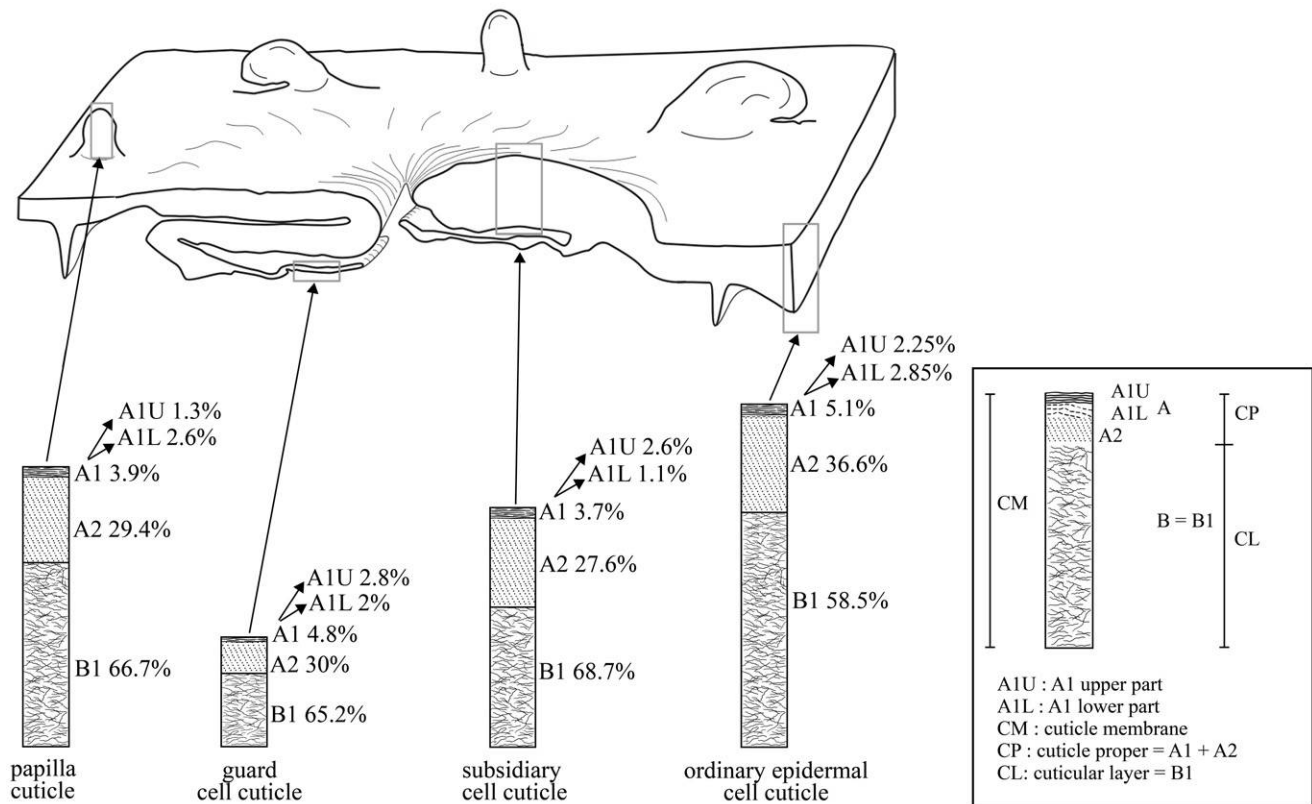


Fig. 9 *Ginkgoites ticoensis* Archang. Three-dimensional reconstruction for each of the four types of cuticles, using the percentages of each layer and zone.

ceae) studied by Guignard and Zhou (2005). These authors found that the living *G. biloba* leaves from male and female trees and fossil *G. yimaensis* leaves from the Jurassic Yima Formation maintain a close resemblance with respect to their cuticle ultrastructures. However, when they are compared with *G. ticoensis*, very different schemes are observed, revealing the respective specificities of each taxon (cf. the present three-dimensional reconstruction of fig. 9 and that of fig. 3, p. 154, in Guignard and Zhou 2005). Among the main differences (see tables 2–4, pp. 146–148, in Guignard and Zhou 2005), (1) the most striking differences are the proportions of the A and B layers, always smaller and higher, respectively, in the present *G. ticoensis*, whereas they are highly variable in ginkgos; (2) all total mean values of cuticular membrane thickness are different between the two taxa (except for the ordinary epidermal cell and the subsidiary cell cuticles of fossil *G. yimaensis*, which are very similar to the present material, 1110 and 1492 μm in mean, respectively); (3) the A1 polylamellate layer has a small proportion in the present *G. ticoensis* (3.7% and 5.1%), whereas it is often much higher in ginkgos (up to 17% in *G. yimaensis*, 12% in living *G. biloba* female, and 7% for male leaf cuticles); (4) the proportions of A1U and A1L zones of the A1 layer are nearly 50 : 50 for the present *G. ticoensis*, whereas they are much more variable in the *Ginkgo* cuticles, in which A1L is usually much thicker than A1U; (5) the translucent lamellae of the A1 layer are thicker in the present fossil material (2.6 and 5.2 nm in

mean), whereas they are usually much thinner (about half that value) for the *Ginkgo* species, which never reach those mean values. Thus, the ultrastructural features observed in the foliar cuticle of *G. ticoensis* are significantly different from those observed in other ginkgophyte leaves, reinforcing the specificity of this Patagonian taxon.

Family Affinity of *Ginkgoites ticoensis*

Morphological and anatomical evidence. In his systematic treatment of the Ginkgophytes, Zhou (1997, 2009) recognized six families: the Trichopityaceae, Karkeniaceae, Umaltolepideaceae, Schmeissneriaceae, Yimaiaceae, and Ginkgoaceae. However, according to Taylor et al. (2009), the Trichopityaceae are not considered as such a higher rank within the group. In three of these families, the *Ginkgoites*-type foliage was usually recorded to be associated with the ovuliferous structures of Karkeniaceae, Ginkgoaceae, or Yimaiaceae (Archangelsky 1965; Zhou 2009). The defining feature of Karkeniaceae is the pluriovulate elongate fructification of *Karkenia* with orthotropous incurved ovules, whereas that of Ginkgoaceae is the biovulate, pedicelate structure of *Ginkgo* with orthotropous ovules surrounded by a collar, and that of Yimaiaceae is the pluriovulate spherical fructification of *Yimaia* with sessile and orthotropous ovules (Archangelsky 1965; Krassilov 1970; Zhou et al. 2007).

			CM 1		
			A1U 2,3%	→	Ordinary
			B 59%		epidermal
		A1L 2,6-2,9%	OL 22,5 TLth. 5,2		cell cuticle
		TLnb. 5,3-5,8	→	CM 1,2	
			A1U 1,3%		
			B 67%	→	Papilla cuticle
			OL 6,6, TLth. 2,6		
A 0,40-0,46	→		CM 1,5		
A1 0,05-0,06		A1L 1,1%	A1U 2,6%	→	Subsidiary cell
A2 0,35-0,40		TLnb 4	B 69%		cuticle
			OL 5,8, TLth. 4,8		
A 0,16	→		CM 0,5		
A1 0,02		A1L 2%	A1U 2,8%		
A2 0,14		TLnb 3,4	B 65%	→	Guard cell
			OL 9,23, TLth. 4,3		cuticle

Fig. 10 Key for the identification of each of the four types of *Ginkgoites ticoensis* cuticles. The distinctions of measurements with the confidence interval ($CI = \bar{x} \pm [\text{var}/n]^{1/2} \times 1.96$, giving 95% α risk) are calculated with 30 measurements. Except for translucent lamellae thickness (TLth.) and opaque lamellae thickness (OL), which are given in nanometers, all other mean values are indicated in micrometers, percentages (%), or numbers (TLnb).

It is noteworthy that the unique record of Karkeniaceae in which *Karkeniania* (*Karkeniania incurva*) was closely related to the *Ginkgoites*-type foliage is the Cretaceous *G. tigrensis* (Archangelsky 1965; Del Fueyo and Archangelsky 2001). This latter taxon has been treated as belonging to the Karkeniaceae ever since (Villar de Seoane 1997). Moreover, as mentioned above, *G. tigrensis* was recovered from the same Patagonian Aptian formation where the leaves of *G. ticoensis* are also known to occur. However, within Karkeniaceae, several Jurassic and Cretaceous species of *Karkeniania* from the Northern Hemisphere (e.g., *Karkeniania cylindrica* and *Karkeniania henanensis*, among others) were found in association with *Sphenobaiera*- and *Eretmophyllum*-type leaves (Krassilov 1970; Schweitzer and Kirchner 1995; Zhou et al. 2002).

However, it is well known that, during the Mesozoic, within Ginkgoaceae the *Ginkgoites*-type leaves associated with *Ginkgo*-type ovulate or pollen organs were found worldwide, with the current exception being South America and Africa (Zhou 2009). Meanwhile, the Yimaiceae were restricted to the Jurassic of Eurasia and have only a single taxon, *Yimaia capituliformis* from Inner Mongolia, which was recovered in association with *Ginkgoites*-type leaves (Zhou et al. 2007).

The material of *G. ticoensis* studied in this article was found in close association with isolated ginkgoalean seeds of *Allicospermum patagonicum* (fig. 2B). The cuticular study made with LM by Archangelsky (1965) on the integument outer cuticle revealed that it is composed of isodiametric cells, 10–15 μm in diameter, with thick and straight anticlinal walls and an absent stomata; these features were not observed in the present leaf cuticle of *G. ticoensis*. However, in the Jurassic of Scoresby Sound, Greenland, seeds of *Allicospermum xistum* Harris were strongly linked with *G. taeniata* Harris because the cuticle of both integument and leaves share the presence of resin bodies and similar stomatal apparatus, suggesting that they were produced by the same ginkgophyte plant (Harris 1935).

With respect to the other ginkgoalean foliage genera, the *Allicospermum*-type seeds appear to be heterogeneous in origin because they were related to at least three types of repro-

ductive structures and therefore to three different lineages. It is believed that the *Allicospermum*-type seeds are the dispersed mature ovules of *Karkeniania* (Karkeniaceae; Zhou 2009), *Yimaia* (Yimaiceae; Zhou et al. 2007), and *Ginkgo ginkgoidea* (Ginkgoaceae; Yang et al. 2008).

Taking into account this evidence and despite the different cuticular anatomy showed between *G. ticoensis* and *A. patagonicum*, it could be suggested that these two taxa were most likely produced by the same ginkgoalean sporophyte. Although our taphonomic evidence, based on the close association between *G. ticoensis* and *A. patagonicum*, is stronger than that revealed through the cuticular anatomy, there have not yet been records of Ginkgoaceae or Yimaiceae in the Mesozoic of Patagonia of the type that would relate *G. ticoensis* to either of these two lineages with certainty. In this regard, the leaves of *G. ticoensis* might be more related to Karkeniaceae than to the Ginkgoaceae or Yimaiceae.

Leaf cuticular ultrastructural evidence. Within Ginkgophytes, the ultrastructure leaf cuticle of the Ginkgoaceae is characterized by having a cuticle proper A (divided into A1 and A2 layers, the first one also divided into upper [A1U] and lower [A1L] zones) and a cuticular layer B (=B1) identical to those observed in the present *G. ticoensis*. For example, the foliar cuticles of the living *G. biloba* and the fossil Chinese *G. yimaensis* share a remarkably similar ultrastructural organization with *G. ticoensis* (Guignard and Zhou 2005).

Conversely, the cuticular membrane of *G. ticoensis* is different from that of another Ginkgoales family already investigated, the Karkeniaceae. In *Sphenobaiera huangii* (most likely belonging to this family, as established above), a granulate cuticle proper A (=A1) + a fibrillar cuticular layer B (=B1) have been recognized (Wang et al. 2005).

The ultrastructural details described by Taylor et al. (1989) in the Karkeniaceae *G. tigrensis* leaf cuticle have shown that it shares with *G. ticoensis* an innermost fibrillar B1 cuticular layer and a well-developed polylamellate A1U cuticle proper layer; however, it differs by having four to eight lucent lamellae, each 2–6 nm in thickness. Additionally, the A1L + A2 cuticle proper layers recognized in *G. ticoensis* are absent in *G. tigrensis*.

Therefore, the ultrastructural characteristics observed in the leaf of *G. ticoensis* reinforce its assignment to the family Ginkgoaceae. Moreover, there is also a distinction between the cuticle of *G. ticoensis* and those of other taxa belonging to several orders. The differences are as follows: in the leaf cuticles of two Czekanowskiales, *Arctobaiera* and *Phoenicopsis*, a cuticle proper A (=A2) + a cuticular layer B differentiated in two sublayers are recognized (=B1 + B2; Zhou and Guignard 1998). In the Pteridospermales, the genera *Komlopteris* (Guignard et al. 2001) and *Dichopteris* (Thévenard et al. 2005) present only the granular layer A2, while the genus *Pachypteris* (Corystospermaceae, Umkomasiaceae; Guignard et al. 2004) has an upper cuticle composed of A (=A1 upper + A1 lower + A2) and B (=B1) and a lower cuticle with A (=A1 + A2) and B (=B1). In the Bennettitales, three other types occur: one type for the genera *Otozamites*, *Zamites*, and *Dictyozamites* (outer layer lamellate, inner layer alveolate, 0.90–1.70 μm in width); a second type for *Ptilophyllum* and *Pterophyllum* (outer layer alveolate or reticulate, inner layer lamellate-reticulate, 2.45–3.25 μm in width); and the third type for *Cycadolepis* and *Williamsonia* (outer layer reticulate, inner layer lamellate, 2.35–5.75 μm in width; Villar de Seoane 1999, 2001, 2003). In Coniferales, until recently, only in the fossil family Cheirolepidiaceae had the cuticular ultrastructure been characterized; it has a cuticle proper A (very typical with wavy A1 + A2) and a cuticular layer B (with B1 + B2; Yang et al. 2009 for *Pseudofrenelopsis*; Guignard et al. 1998 for *Hirmeriella*).

As already noticed by Guignard and Zhou (2005) for the *Ginkgo* study, the cuticles of all four types identified in the present *G. ticoensis* are not assignable to any particular cuticle type of living plants, as described by Holloway (1982), and show affinities only with his types 1, 2, or 3. Further, with respect to the cuticle of living *G. biloba*, Villar de Seoane (1997) found a resemblance particularly to type 3 of Holloway.

For the first time in Ginkgoaceae and because of the good quality of the *G. ticoensis* material, 30 measurements (in contrast to the 20 measurements provided for *Ginkgo* species by Guignard and Zhou [2005]) could be performed for each of the 10 characters of each of the four types of cell cuticle details; additionally, the confidence interval was evaluated, significant features were precisely described (fig. 8), and a three-dimensional reconstruction of the cuticle is also provided (fig. 9). Additionally, for the first time, these 10 characters can be arranged by significance, from the most to the least. The most significant are the cuticular membrane (CM), A1U, B, and opaque lamellae (OL) thicknesses, which enable each of the four cuticles to be distinguished. Then, the A1L and translucent lamellae (TL) thicknesses and the TL number enable further distinction between the three sets of cuticles (in two cases, ordinary epidermal cell [OEC] and papilla [PP] cuticles are grouped; in one case, it is OEC and subsidiary cell cuticle [SC]). Finally the A, A1, and A2 thicknesses are the least significant features, enabling a distinction between only two groups (OEC, PP, and SC vs. GC). Considering the types of cell cuticles, the guard cell cuticle is the most different, with 10 significant features, versus the subsidiary cell cuticle, which has six; the ordinary epidermal and papilla cuticles are the most similar with four and five significant features.

This latter remark is congruent with the former investigations made on *Ginkgo* species (Guignard and Zhou 2005) or other taxonomic groups (Cheirolepidiaceae; Guignard et al. 1998; Yang et al. 2009) where the stomatal apparatus could fortunately be observed; this is a rather rare occurrence in TEM sections.

Finally, for the first time in Ginkgoaceae as well, an identification key (fig. 10) can be proposed, enabling the rapid identification of observed cuticle type from the left side to the right side of the key. Three characters (A, A1, A2) separate the guard cell cuticle from the others; then, among the remaining three types, the subsidiary cell cuticle shows its own characteristics with A1L and TLnb (number of translucent lamellae). The two remaining cuticles (ordinary epidermal and papilla cuticles) are easily identified with their own CM, A1U, B, OL thicknesses, and TL thickness.

Paleoenvironment at Anfiteatro de Ticó Locality, Insights into Macro- and Micromorphology, and Ultrastructural Cuticle Data

Entire leaves of *G. ticoensis*, seeds of *A. patagonicum*, and fronds of *Cladophlebis tripinnata* were found in the same fossiliferous bed (fig. 2A), suggesting that the parent plants were close to the site of deposition. The stratigraphic and depositional studies made on the Anfiteatro de Ticó Formation showed that *G. ticoensis* lived in a fluvial sinuous channel system associated with extensive, shallow lakes and that the predominate paleoclimate was hot to temperate (Cladera et al. 2002; Passalia 2009). The presence of a moderately thick cuticle, papillae in both epidermal layers, and a papillate stomata with sunken guard cells in this taxon may indicate that it was adapted to a dry environment. However, the leaves of *G. tigrensis* Archangelsky were also found in sediments of the Anfiteatro de Ticó Formation, although in the Bajo Tigre locality these leaves possess a moderately thick cuticle with epicuticular waxes and do not show any papillae, while the subsidiary cells form a conspicuous ring around the slightly sunken guard cells (Archangelsky 1965; Villar de Seoane 1997). In this particular case, the two Patagonian species of *Ginkgoites* living under the same environmental conditions reveal themselves to have been well adapted through the acquisition of very distinct epidermal characters. In contrast, Denk and Velitzelos (2002) have observed that, in the leaves of *Ginkgo adiantoides* (Unger) Heer from the Miocene of Greece and the Pliocene of Hungary, the subsidiary cells are strongly papillate, like the sun-exposed leaves of extant *G. biloba*, whereas the leaves of *G. adiantoides* from the Pliocene of Germany show nonpapillate subsidiary cells similar to the shade-dwelling leaves of the living taxon. These authors have suggested that the differences in epidermal characters between the fossil leaves are related to environmental influences rather than to genetic differences.

However, the striae occurring in some papillate subsidiary cells of the *G. ticoensis* leaf may have been effective against the supposed dry environment where this taxon was growing. Accordingly, Pott et al. (2007) have already indicated that the surface microrelief, which consists of elevated striae, in the leaf of the Triassic *Glossophyllum florinii* Kräusel from Austria may have an ecological as well as a mechanical func-

tion because of the xeric environment of the Lunzer Sandstein in which this putative ginkgophyte was found. Moreover, these striae may have reduced the leaf wettability or may have facilitated the self-cleaning of the leaf from dust particles, which may cause injuries to the leaf tissues, or may have prevented the formation of a water film on the leaf surface to ensure continuous CO₂ uptake (Pott et al. 2007).

The existence of the xeromorphic structures observed in the two Patagonian ginkgophyte epidermises was also highlighted for most of the taxa recovered from the Baqueró Group (Archangelsky et al. 1995). As has been noted by Archangelsky (2001), the plant community in Patagonia during the Early Cretaceous was growing under conditions of environmental stress caused by extensive and recurrent volcanic activity; therefore, such structures were developed for protection against the high ash particle content in the atmosphere.

Additionally, this interpretation is supported by the recent ultrastructural investigations made by Bartiromo et al. (2012) on the leaf epidermis of the trees of *Pinus halepensis* Mill, which were exposed to persistent volcanic gas fumigation in southern Italy. This study has indicated that the ultrastructure of the cuticular membrane of the particular conifer leaf shows several significant modifications, with the use of a confidence interval, compared with nonexposed trees. One significant modification is that the fibrils of the B1 cuticular layer become more parallel to the cell surface, which has also been observed in *G. ticoensis* (fig. 4A–4C). Another modification is the increment of the oxalate calcium crystal deposits, which could correspond to the electron-transparent areas that appear in the cuticle of *G. ticoensis* (figs. 4E, 4F, 7H), and a third modification is that the epicuticular and epistomatal waxes undergo fusion as they are visible in the cuticle of *G. tigrensis* (Villar de Seoane 1997, pl. 1, fig. 5, pl. 2, fig. 1).

Further, the parallel arrangement of the B1 fibrils in the cuticular membrane (CM) of *G. ticoensis* may reinforce the role of its well-developed polylamellate cuticle proper A1, which is the main barrier to the diffusion of water and solutes across the cuticle layer (Evert 2006). It is noteworthy that the CM of the Jurassic *G. yimaensis*, which may have grown under a high-CO₂ “greenhouse” climate (similar to the high CO₂ atmospheric content recorded for the Ticó flora by Passalia [2009]), and the CM of the extant *G. biloba* cultivated in different worldwide environments (Guignard and Zhou 2005) both show the same layer sequence observed in the CM of the Patagonian *G. ticoensis*; that is, they all exhibit a layer sequence of A1, A2, and B1. In the opinion of Bartiromo et al. (2012), the order in this sequence should be considered a significant diagnostic feature for the Ginkgoaceae because it does not change under different environmental conditions.

Conclusions

Because the foliar cuticle of *Ginkgoites ticoensis* Archang. from the Aptian of Patagonia is well preserved, a number of new features were revealed with the use of scanning and transmission electron microscopic techniques; further, these new features allowed for the unequivocal identification of this taxon. It is worth noting that, among the numerous species belonging to such a worldwide genus as *Ginkgoites*, *G.*

ticoensis is at present the taxon that has been most completely described both anatomically and ultrastructurally. Additionally, for the first time in a ginkgoalean leaf, it was possible to recognize and measure 10 cuticular characters, including the thickness of the cuticular membrane (CM), the thickness of five cuticle proper (A) features, the thickness of cuticular layer B1, the thicknesses of the opaque and translucent lamellae of the A1 layer, and the number of translucent lamellae.

The heterophylly in the *Ginkgo*-like leaves and those of the *Ginkgoites* type is very common in the fossil record, making distinctions between the two taxa difficult. However, the cuticular analysis made in *G. ticoensis* was determined to be of great systematic value. Accordingly, the peculiar foliar morphology and the new anatomical and ultrastructural features plus the TEM statistical data found in this Patagonian taxon have shown that it can be differentiated from other species of *Ginkgoites* and those of *Ginkgo* with high certainty. Therefore, the uniqueness of *G. ticoensis* is highlighted here.

The leaves of the *Ginkgoites*-type and *Allicospermum*-type seeds are generally related to three ginkgoalean families, the Karkeniaceae, Ginkgoaceae, and Yimaiceae. Of these families, the Karkeniaceae is the only lineage that has been identified to date in the Mesozoic of Patagonia. Further, *G. ticoensis* was recovered in close association with the isolated seeds of *Allicospermum patagonicum*, suggesting that they were most likely produced by the same plant. In this particular case, the Karkeniaceae would be the putative family to which both organs would belong, although they do not share the same cuticular anatomy. However, the ultrastructural features observed in the leaves of *G. ticoensis* are identical to those observed in ginkgos; thus, this Patagonian taxon may have a stronger potential affinity for the family Ginkgoaceae than for the Karkeniaceae.

At present, two *Ginkgoites*-type leaves, *G. ticoensis* and *G. tigrensis*, have been recorded in the Aptian of Patagonia, with the former belonging most likely to Ginkgoaceae and the latter to Karkeniaceae. The available evidence appears to favor such an assumption; therefore, it appears that during the Mesozoic two ginkgoalean lineages already inhabited that area, including *G. ticoensis*, the first member of Ginkgoaceae to be recorded for that time period in Patagonia.

The leaves of these two *Ginkgoites* species appear to have developed xeromorphic features, which most likely functioned as protective mechanisms against the stressful environmental conditions induced by the volcanic activity that persisted during the Lower Cretaceous in Patagonia, rather than as a response to an arid environment. However, further studies of the leaf anatomy and particularly of the ultrastructure of the cuticular membrane in other Ginkgophyte taxa from Patagonia and elsewhere will determine the key characters for a more complete understanding of plant adaptation.

Acknowledgments

We dedicate this contribution to Thomas N. Taylor on his seventy-fifth birthday for all his inspiring work in paleobotany and especially for being one of the pioneers in the study of fossil plant cuticle ultrastructures. This article was supported in part by grants CONICET PIP 679 and PICT 433/07. We thank E. Morel of the Palaeobotanical Collection of La Plata Natural Sciences Museum for the loan of *Ginkgoites*

ticoensis type material. We are grateful to the editors and two anonymous reviewers for all their valuable comments, which have undoubtedly improved the manuscript. We thank Orlando Cárdenas for technical assistance in sample process-

ing and Amalia González for drafting figure 1. We also wish to thank the technical staff of the University Lyon 1, Centre Technologique des Microstructures CT μ , and Sophie Cros for all the technical work on the TEM figures.

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