

# New studies on *Karkenian incurva* ARCHANG. from the Early Cretaceous of Argentina. Evolution of the seed cone in Ginkgoales

by

GEORGINA M. DEL FUEYO and SERGIO ARCHANGELSKY,  
Buenos Aires (Argentina)<sup>\*)</sup>

With 5 plates and 1 text-figure

## Abstract

New material of the ginkgoalean seed cone *Karkenian incurva* ARCHANG. from the Lower Cretaceous Baqueró Formation of Patagonia is described. The seed cone gross morphology is similar to that of the holotype. Study of the megaspore membrane with SEM and TEM shows that ovules of *K. incurva* at time of fossilization were mature. Also, the ultrastructure of this membrane has a striking resemblance to that of *Ginkgo biloba*. Evolution of the seed cone in Ginkgophytes is discussed, and it is suggested that Paleozoic Dicranophyllales could be the ancestral stock from which Mesozoic Ginkgoales evolved.

Key words: Gymnosperms, Ginkgoales, seed cone, morphology, cuticle, megaspore membrane, ultrastructure, evolution, Baqueró Formation, Santa Cruz Province, Patagonia.

## Zusammenfassung

Neue Funde des Samenzapfens *Karkenian incurva* ARCHANG. (Ginkgoales) aus der Baqueró Formation der frühen Kreidezeit in Patagonien werden beschrieben. Die generelle Morphologie des Samenzapfens entspricht der des Holotyps. Mit Hilfe von SEM und TEM wird die Megasporenmembran untersucht. Die Ultrastruktur dieser Membran zeigt, daß die Samenanlagen von *K. incurva* zum Zeitpunkt der Versteinierung zur vollen Reife entwickelt waren.

Fossile Samenzapfen der Ginkgoales einschließlich der Samenanlagen der heute vorkommenden Art *Ginkgo biloba* werden verglichen. Die Evolution des Samenzapfens innerhalb der Ordnung wird diskutiert.

Schlüsselwörter: Gymnospermen, Ginkgoales, Samenzapfen, Morphologie, Kutikula, Megasporenmembran, Ultrastruktur, Evolution, Baqueró Formation, Provinz Santa Cruz, Patagonien.

## Table of contents

|   |     |
|---|-----|
| Introduction .....  | 112 |
| Materials and methods .....   | 112 |
| Systematics .....   | 112 |
| Description .....   | 114 |
| Comparisons .....   | 115 |
| Compared morphology of Mesozoic Ginkgophyte seed cones and extant <i>Ginkgo biloba</i> .. | 116 |
| Relationships within the Ginkgophyta .....  | 117 |
| Distribution .....  | 118 |
| Conclusions .....   | 118 |
| Acknowledgements .....  | 119 |
| References .....  | 119 |
| Explanation of plates .....   | 121 |

<sup>\*)</sup> Authors' address: División Paleobotánica. Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". Av. Angel Gallardo 470, (1405) Buenos Aires, Argentina.

## Introduction

*Karkenian incurva* is a ginkgoalean seed cone that was described by ARCHANGELSKY (1965) from the Lower Cretaceous of Patagonia. New specimens of this fossil were collected during recent field excursions. In the present paper this material which has better preserved cuticles is described. New data, especially about the ultrastructural features of the megaspore membrane are added to the original diagnosis of *K. incurva*. Comparisons are also made at the ultrastructural level with other fossil taxa and the living *Ginkgo biloba*.

Ginkgoalean remains first appear in the Carboniferous and become widely distributed during the Mesozoic, in both the southern and northern hemispheres (TRALAU 1968). Fossil leaves attributed to the group have been described in numerous reports (FLORIN 1936, KRÄUSEL 1943 a, b, REYMONOWNA 1985, CZIER 1998). In Patagonia, the record begins in the Late Paleozoic (FERUGLIO 1942, CÚNEO 1987), and is continuous throughout the Mesozoic (FRENGUELLI 1946, AZCUY & BALDONI 1990, VILLAR DE SEOANE 1997).

Ovulate reproductive organs of several ginkgoalean taxa from different fossiliferous sites have been reported, confirming that the group was well established during the Mesozoic (ZHOU 1991). To date *Karkenian* is the largest genus with five species characterized by multiovulate reproductive organs (KRASSILOV 1982, SCHWEITZER & KIRCHNER 1995). *K. incurva* is the only known ginkgoalean seed cone in which ultrastructural features of the megaspore membrane are preserved.

## Materials and methods

The specimens described here were found in the Baqueró Formation latest Barremian to early Aptian in age (ARCHANGELSKY 1967), in Santa Cruz province, Argentina. Bajo Tigre is the locality where the type specimen and new material were collected (ARCHANGELSKY 1965).

Some ovules were removed from the matrix and treated with 40% nitric acid followed by 5% ammonium hydroxide. Other ovules were cleared with sodium hydroxide. Finally, ovules removed from the matrix using only hydrofluoric acid showed the best features for observation.

The integument of mature ovules of *Ginkgo biloba* was macerated following BOODLE (1916). For optical observation cuticles and megaspore membrane of *K. incurva* and *G. biloba* ovules were mounted in glycerine jelly. For scanning electron microscopy isolated ovules of *K. incurva* were dehydrated in an ethyl alcohol series, placed on a double face, scotch tape attached to a stub and, after drying, coated with gold-palladium. For transmission electron microscopy the ovules were treated following the technique used by ARCHANGELSKY & DEL FUEYO (1989).

The specimens and the microscope slides are deposited in the Paleobotanical collection of the Buenos Aires Natural Sciences Museum "Bernardino Rivadavia": BA PB and BA PB Pm respectively. The description of the stomata follows VAN COTTEM (1970).

## Systematics

### Order Ginkgoales

Family: *Karkeniaceae* KRASSILOV 1970

Genus: *Karkenian* ARCHANGELSKY 1965

Type species: *Karkenian incurva* ARCHANGELSKY, 1965.

*Karkenian incurva* ARCHANGELSKY 1965 emend.

Plates 1-5; Text-fig. 1

Emended diagnosis: Seed cone up to 8 cm long and 1.3 cm wide, tapering gradually towards base and apex. Cone compact, up to 100 ovules present, overlapping each other and irregularly disposed.

PERMIAN

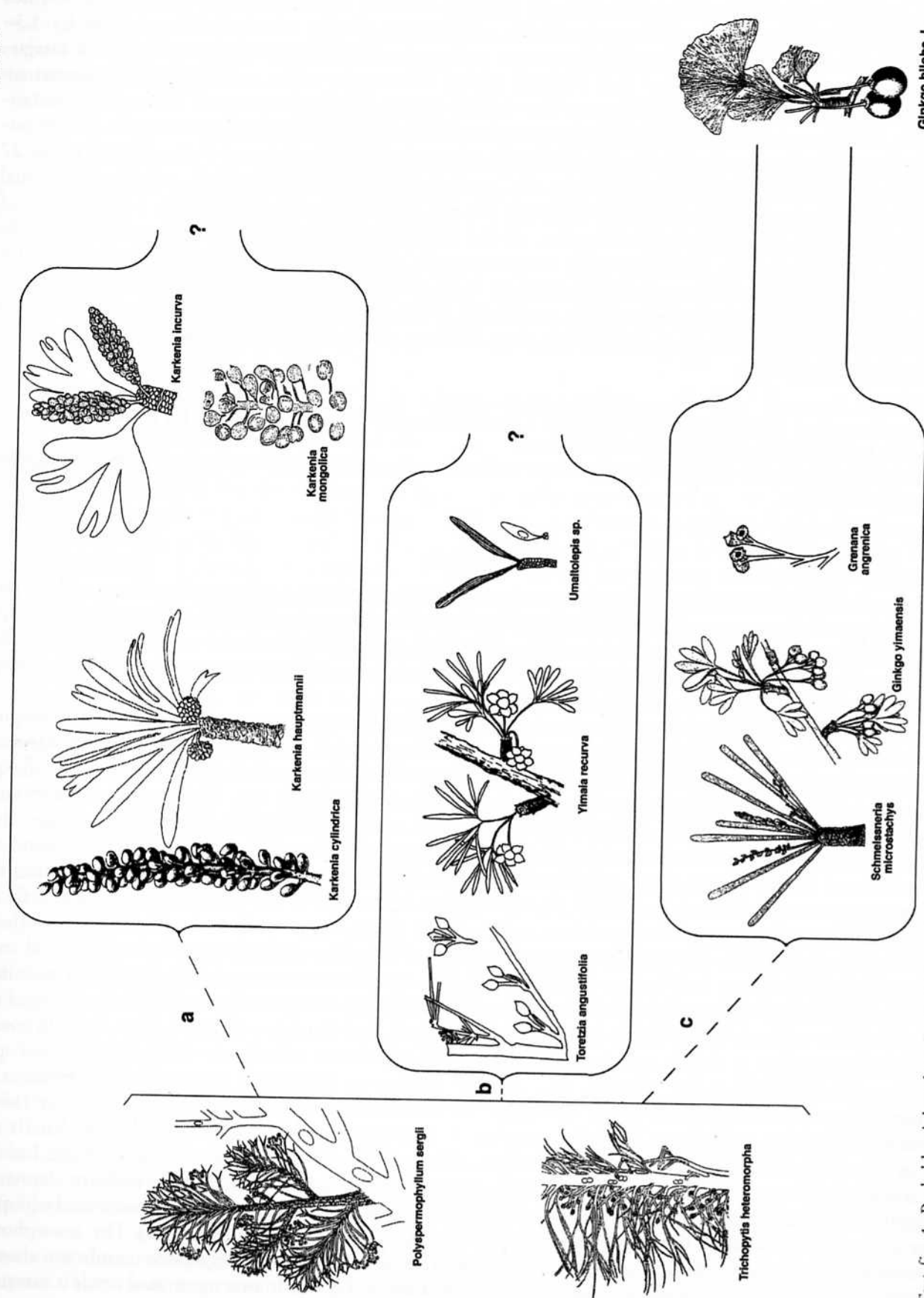
TRIASSIC

JURASSIC

CRETACEOUS

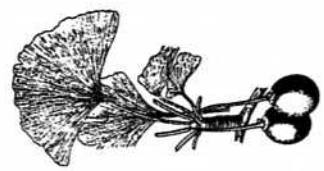
TERTIARY

RECENT



Text-fig. 1. Probable ginkgoalean lineages through geological time. According to ZHOU (1997) three Mesozoic lineages are represented; a. lineage including the family Karkeniaceae (*Karkenia* spp.); b. lineage including Yimaiaaceae (*Yimaia*) and Umaltolepideaceae (*Umaltolepis* and *Toraitzia*), both families become extinct after the Cretaceous; c. lineage including Schmeissneriaceae (*Schmeissneria*) and Ginkgoaceae (*Ginkgo* and *Grenana*) that have persisted to our time. Paleozoic Dicranophyllales (*Polyspermophyllum* and *Trichopteryx*) are suggested to be the ancestral stock from which Mesozoic Ginkgoales evolved.

**Ginkgo biloba L.**



attached by delicate funicule, 3.5–4 mm long by 0.3 mm wide to central axis, 0.5–2 mm wide. Ovules anathropous with micropylar end directed towards central axis. Ovules ovoid, 3–4.5 mm long by 1.3–2.5 mm wide, composed of several cuticle layers and a megaspore membrane. Outer epidermis of integument differentiated into micropylar and chalazal zones. Micropylar zone consisting of isodiametric-rectangular cells, 26–57  $\mu\text{m}$  long, 13–39  $\mu\text{m}$  wide, stomata absent. Chalazal zone with isodiametric-rectangular cells, 50–59  $\mu\text{m}$  long, 20–27  $\mu\text{m}$  wide. Stomata irregularly disposed, sunken, anomocytic. Neighbouring cells 5 to 6 forming a cuticular ridge above the stoma, 4  $\mu\text{m}$  thick. Suprastomatal pit, elliptical ovate, 27  $\mu\text{m}$  long by 17  $\mu\text{m}$  wide. Stomatal index, 20 per  $\text{mm}^2$ . Surface of epidermal cells slightly granulose, anticlinal walls 3  $\mu\text{m}$  thick, not pitted. Resiniferous idioblasts present, 57  $\mu\text{m}$  long, 31  $\mu\text{m}$  wide. Inner epidermis of integument probably extending to near base of ovule. Nucellus epidermis extending to near base, cells rectangular, 63–100  $\mu\text{m}$  long, 10–18  $\mu\text{m}$  wide, surface smooth, anticlinal walls 2  $\mu\text{m}$  thick. At top nucellus protruding as a short acute micropylar beak. Megaspore membrane 1  $\mu\text{m}$  thick, densely granular, granules 2–7  $\mu\text{m}$  wide. Ultrastructure consisting of a sculptured and a foot layer. Sculptured layer composed of elongate bacula 0.28–0.9  $\mu\text{m}$  long, 0.14–0.20  $\mu\text{m}$  wide, following transverse and oblique courses. Foot layer 0.07  $\mu\text{m}$  thick, amorphous.

Holotype: ARCHANGELSKY, S. 1965, p. 132, pl. 1, fig. 10; pl. 2, figs. 11, 14, 16, 18; pl. 5, figs. 9–32; text-figs. 13–19.

Material studied: BA PB 1022; 10724 a, b; 10725 a, b; BA PB Pm. 380–393; BA PB MEB. 65; BA PB MET. 168, 176.

Locality: Bajo Tigre, Santa Cruz Province, Argentina.

Stratigraphic position: Baqueró Formation, lower member (latest Barremian to earliest Aptian in age), *Ginkgoites tigrensis* fossiliferous Bed (BTG).

### Description

The seed cone is elongated, cylindrical, tapering gradually towards base and apex, 8 cm long and 1.1 cm wide. It is dense and compact with the ovules overlapping each other and irregularly disposed on a slender central axis (Plate 1, Figs. 2–4). Ovules are anathropous with rounded chalazal end narrowing towards the micropyle, which is directed towards a central axis, the funicule is free from integument of ovule emerging at approximately 50° from the axis (Plate 2, Figs. 1–4).

Several cuticles and part of the megaspore membrane of ovules were recovered. Externally, the integument cuticle is differentiated into both micropylar and chalazal zones. In the area of the micropylar end stomata are absent (Plate 3, Fig. 5). The cuticle between the chalaza and middle portion of ovule is characterized by having anomocytic stomata randomly disposed (Plate 3, Fig. 1). They are sunken and guard cells are difficult to measure due to poor preservation while neighbouring cells which surround the stoma are indistinguishable from those remaining in the epidermis. These neighbouring cells vary in number from 5 to 6 and form a conspicuous cuticular ridge 4  $\mu\text{m}$  thick above the stoma and form an elliptical to ovate suprastomatal pit 27  $\mu\text{m}$  long and 17  $\mu\text{m}$  wide (Plate 3, Figs. 2, 3). The surface of epidermal cells is slightly granulose and the 3  $\mu\text{m}$  thick anticlinal walls are not pitted. Some epidermal cells are distinguished as idioblasts, probably with resiniferous content. They are of irregular shape tending to be polygonal and measure about 57  $\mu\text{m}$  long and 31  $\mu\text{m}$  wide (Plate 3, Fig. 4). The next inner cuticle found is a nucellar epidermis composed of rectangular cells 63 to 100  $\mu\text{m}$  long and 10 to 18  $\mu\text{m}$  wide, tending to be arranged in longitudinal files with smooth and very thin anticlinal walls of 2  $\mu\text{m}$  thickness (Plate 4, Figs. 2, 3). In most specimens the nucellus is closely attached to a megaspore membrane, which seen with light microscope appears to be densely granular (Plate 4, Fig. 5). The megaspore membrane has a double structure: a sculptured layer and a foot layer (Plate 4, Fig. 6). This membrane seen with SEM shows better these features (Plate 5, Figs. 1, 2). With TEM the sculptured layer appears to be formed by elongate bacula of more or less similar thickness, 0.28 to 0.9  $\mu\text{m}$  long and 0.14 to 0.20  $\mu\text{m}$  wide (Plate 5, Fig. 3). Some bacula have rounded and truncated distal ends narrowing towards the base and others have uniform diameter throughout their total length while a few are constricted distally. Most bacula run in a transverse and oblique course from the foot layer to the upper surface of the megaspore wall (Plate 5, Figs. 5, 6). The amorphous foot layer shows the same electronic density as the patterned layer. Another megaspore membrane shows completely disorganized bacula and a complete foot layer (Plate 5, Fig. 4). In one macerated ovule a massule

composed of several kinds of pollen grains (*Podocarpidites*, *Araucariacites* and *Ginkgocycadophytus* types), was recovered together with an outer cuticle of integument (Plate 4, Fig. 4). In some ovules remains of carbonized tissue were observed and probably belong to the megagametophyte or the inner fleshy tissue of the integument (Plate 5, Figs. 3, 5).

#### Outer integument of the mature ovule in *Ginkgo biloba*

*Ginkgo biloba* has an outer integument epidermis divided into chalazal and micropylar areas. The chalazal area is characterized by generally isodiametric epidermal cells 33  $\mu\text{m}$  long and 24  $\mu\text{m}$  wide, polygonal resiniferous idioblasts 33  $\mu\text{m}$  long and 23  $\mu\text{m}$  wide, and randomly disposed stomata that tend to become absent near the micropyle. Stomata are anomocytic with 5 to 6 neighbouring cells, which have a cuticular ridge 3  $\mu\text{m}$  thick and form a suprastomatal pit 12  $\mu\text{m}$  wide and 19  $\mu\text{m}$  long. Guard cells are sunken 51  $\mu\text{m}$  long and 19  $\mu\text{m}$  wide, and have polar thickenings. Stomatal index is 8 stomata per  $\text{mm}^2$  (Plate 3, Fig. 6). In the micropylar area the epidermis is devoid of stomata and resiniferous idioblasts and the cells are isodiametric to rectangular 47  $\mu\text{m}$  long and 25  $\mu\text{m}$  wide, with strongly pitted anticlinal walls.

#### Comparisons

Specimens studied in this paper show only a few morphological differences from the holotype. One difference is that the holotype is shorter, up to 4.5 cm long and up to 1.3 cm wide. Also, the ovules are slightly shorter, only 3 mm long. Other features such as the nucellus and the megaspore membrane are similar. There seems to be little doubt that the closely associated *Ginkgoites tigrensis* leaves may be possibly referred to the same plant that bore *K. incurva* cones. Large blocks, 0.50 m  $\times$  0.50 m (Plate 1, Fig. 1) show densely packed *G. tigrensis* leaves and abundant cones as sole components of the taphocoenosis. This association also suggests that cones and leaves were shed approximately at the same time.

Ginkgoalean seed cones occur mostly in Mesozoic strata. Several other species of *Karckenia* differ in some characters compared with *K. incurva*. *K. cylindrica* was described by SCHWEITZER & KIRCHNER (1995) from Jurassic strata of Iran and is almost identical to *K. incurva* in morphology, arrangement and shape of ovules. However, it differs in length (12 cm long), width of the central axis (0.8 mm) and number of ovules (57 per cone). *K. hauptmannii* from Liassic beds of Germany (KIRCHNER & VAN KONIJNENBURG-VAN CITTERT 1994) differs in its spherical shape, size (35 mm long by 27 mm wide) and a central axis that bears up to 30 ovules that are 4–9 mm long by 2–5 mm wide. KRASSILOV (1982) described *K. mongolica* from the Lower Cretaceous of Mongolia. It is a compression of a fragmentary cone, 3.5 cm long and 2 cm wide, bearing 40 ovules, each of which is 5 mm long by 4 mm wide. The structure of the outer epidermis of the integument of *K. mongolica* differs from that of *K. incurva* by having polygonal cells 40–42  $\mu\text{m}$  long by 30–36  $\mu\text{m}$  wide, amphicyclic stomata and elliptical stomatal pits 10 to 20  $\mu\text{m}$  long. Specimens of *K. asiatica* described by KRASSILOV (1970, 1972) from the Upper Jurassic of Siberia, are rounded and compact, 5 cm long by 4.5 cm wide, with oval ovules and an outer epidermis of integument that lacks stomata. *Schmeissneria microstachys* (KIRCHNER & VAN KONIJNENBURG-VAN CITTERT 1994) from the Liassic of Germany, is different from *K. incurva*. It is 8 cm long and only the upper part is covered with pedunculate or sessile, spirally arranged cupules. Each cupule bears one ovule, 3 mm long by 2 mm wide, which is winged when mature.

Dispersed seeds attributed to *Ginkgo buttonii* were described from Jurassic strata of Yorkshire by HARRIS & MILLINGTON (1974). The outer epidermis of their integument does not have stomata and some cells show a thin pale circular patch, which probably is a hair base. Several isolated forked axes bearing seeds have been found in association with *Ginkgo rajmahlensis* leaves, from Upper Jurassic beds of Rajmahal Hills of India (ZEBBA-BANO et al. 1979). Ovules are similar to *G. biloba*, but differ in the absence of the collar. *Allicospermum* HARRIS (1935) is another isolated seed recovered in a few floras in association with *Ginkgoites* leaves (Jurassic of Yorkshire and Cretaceous of Baqueró). These seeds are 5 mm long and 3 mm wide, and are larger than those of *K. incurva*. The relationship of *Allicospermum* and the Ginkgoales remains obscure, although they are found in association to *Ginkgoites* leaves in several plant horizons in Baqueró Formation, Argentina.

In Jurassic strata of China two fertile ginkgoalean taxa were recovered from the Yima Formation. They are together with our species the only ginkgoalean seed cones with ultrastructural features of the megaspore membrane well preserved. Both have a different morphology that distinguishes them from *K. incurva*. *G. yimaensis* (ZHOU & ZHANG 1989) has short shoots bearing bifurcated pedicels carrying orthotropous ovules, 10–15 mm long by 8–12 mm wide, with a cup-shaped collar. The outer epidermis of the integument has isodiametric to rectangular cells, 30 by 65  $\mu\text{m}$  and irregularly distributed anomocytic stomata with 6–7 subsidiary cells, while the nucellar epidermis has rectangular cells 40–150  $\mu\text{m}$  long by 7.5–15  $\mu\text{m}$  wide. *Yimaia recurva* is the other Chinese taxon described by ZHOU & ZHANG (1992). It is based on a globular pedunculated seed cone, bearing 8–9 orthotropous ovules, with the outer epidermis of the integument having few irregularly distributed anomocytic stomata with 3–6 neighbouring cells. The megaspore membrane structure is the most similar feature shared between *K. incurva* and the two Chinese genera. In optical view they are all finely and densely granular, with granules of variable thickness. Ultrastructural studies of the megaspore membrane made by ZHOU (1993) show that at this level both Chinese taxa are almost similar with *K. incurva*. The foot layer has the same amorphous and thin structure in the three species, although in *G. yimaensis* and *Yimaia recurva* the bacula of the sculptured layer are densely arranged and thicker than those seen in the ovule of *K. incurva*. Most of the bacula in *Yimaia recurva* run horizontally from the bottom to the surface of the sculptured layer. According to ZHOU (1993), the differences in structural configuration of these megaspore membranes are due to the stage of development of the ovules at the time of fossilization.

The morphology of the mature ovule of *G. biloba* clearly differs from *K. incurva*. In the former, ovules are larger (3 cm long and 2 cm wide), orthotropous and have a collar. Nevertheless, both taxa share general characters including a micropylar epidermis lacking stomata, a chalazal epidermis showing anomocytic stomata and resiniferous idioblasts. ZHOU (1993) recognized several stages of development of the megaspore membrane in mature ovules of *G. biloba*, the last of which shows a striking resemblance to *K. incurva* because the bacula of the patterned layer of the megaspore membrane are reduced in number, irregularly arranged and thicker and more widely separated from one another. This resemblance confirms that the ovules of *K. incurva* were in the latest stage of development at the time they were buried and fossilized.

### Compared morphology of Mesozoic ginkgophyte seed cones and extant *Ginkgo biloba*

During the Mesozoic most ginkgophytes produced seed cones and two types of ovuliferous structures co-existed: multiovulate cone-like forms (*Karkenian*, *Yimaia*, *Schmeissneria*) and structures with one or two ovules (*Ginkgo yimaensis*, *Umaltolepis*, *Toretzia*) as in the extant condition of *G. biloba*.

The variation in number of ovules in Mesozoic ginkgophytes may have led to their aggregation in cone-like structures, which may be either elongated (*K. incurva*, *K. cylindrica*, Text-fig. 1) or globose (*K. hauptmannii*, *Yimaia recurva*, Text-fig. 1). The youngest ovuliferous structure is that of *G. biloba* with one or few ovules which in abnormal cases are sessile on slightly modified leaves.

FLORIN (1949) and ZHOU (1993), suggested that seed cones evolved in a sense toward a reduction in number and enlargement of ovules. The reduction that has taken place in the reproductive organs of Ginkgoales has been a common process in the plant kingdom through geologic time. An analogous example is the Mesozoic podocarp multiovulate cones from Gondwana; *Rissikia* and *Mataia* (TOWNROW 1967) and *Squamastrobis* (ARCHANGELSKY & DEL FUEYO 1989) where a reduction process may have affected them toward to a single ovule stage as present in living podocarps (DEL FUEYO 1999).

Ovules in *Karkenian* are curved, a condition known to occur among putative Paleozoic ginkgophytes as *Trichopitys* or *Polyspermophyllum* (Text-fig. 1). Orthotropous ovules similar to *G. biloba* were described in Mesozoic taxa: *Yimaia*, *Umaltolepis* and *G. yimaensis*. ZHOU (1994) suggested that the functional sessile ovule directly connected with the peduncle in *G. biloba* and also in *G. adiantoides* (CRANE et al. 1990) would have originated through a *G. yimaensis* type ovule organ by successive steps in reduction of pedicels and number of ovules together with an enlargement of their size. According to ZHOU (1994), the intermediate *G. biloba* type organs may have occurred prior to the Tertiary: i.e., ovulate organs and foliage leaves of *G. adiantoides* have been found in the Late Cretaceous as well as Paleocene strata.

*Ginkgo biloba* ovules have a collar that is not present in *Yimaia* and *Umaltolepis* orthotropous ovules. The erect insertion of ovules is related to the collar appearance as a secondary feature (FLORIN 1949). Although, KRASSILOV (1970) suggested that the bract in the *Umaltolepis* species he studied, may correspond to the collar. It should be noted that this Russian material is too poorly preserved to be used as positive evidence of this theory.

The anatomical and ultrastructural resemblance of the megaspore membrane of the fossil *K. incurva* and *G. biloba* suggests that this structure has not changed substantially since Mesozoic times. A similar static condition was previously observed in other fossil plants from Baqueró Formation, e.g., in the pollen wall of the fossil bisaccate grains of *Squamastrobis* (ARCHANGELSKY & DEL FUEYO 1989) and *Morenoa* (DEL FUEYO et al. 1990) that have alveolate sexine and a lamellated nexine, identical to the living podocarp species (DEL FUEYO 1996). The stable internal structure of ginkgophyte ovules, as described in this paper, contrasts with the diversified organization shown by the seed cones. Protection given by most sporophytes to gametophytic generations was completely developed within Ginkgoales perviously to the Jurassic, needing no modification during phylogeny.

### Relationships within the Ginkgophyta

The relationship of the different taxa within the Ginkgophyta is presently based on the organization of the ovule bearing structure (ZHOU 1991, 1997). Using only the morphology and structure of leaves of Northern latitudes TRALAU (1968) considered the families Ginkgoaceae, Torelliaceae, Glossophyllaceae and Phoenicopsidaceae to be main phylogenetic groups within Ginkgoales. On the other hand, KRASSILOV (1970) presented a classification of Mesozoic Ginkgoalean plants from Siberia, including both vegetative and reproductive structures in which he recognized three families: Ginkgoaceae, Karkeniaceae and Pseudotorelliaceae. According to KRASSILOV (1970) the Paleozoic genus *Trichopitys* has more characters in common with progymnosperms than with true Ginkgoales. This view, however, is not shared by many paleobotanists who consider that *Trichopitys* is better placed within Ginkgoales (FLORIN 1949, TAYLOR & TAYLOR 1992 and ZHOU 1997). KRASSILOV (1970) also finds that single terminal fertile structure of *Umaltolepis* is analogous with the two terminal ovules present in *G. biloba*.

Basing his findings on cladistic analysis, ZHOU (1991) proposed a different classification of Mesozoic ginkgoales. In it the Trichopityaceae is considered to be a primitive family from which three independent Mesozoic lineages arose: 1) the Karkeniaceae, characterized by multiovulate seed cones, 2) the Umaltolepidaceae (including *Toretzia* STANISLAVSKY 1973 and *Umaltolepis*) with greatly reduced ovule-bearing organs, each with only one ovule and 3) the Ginkgoaceae, a lineage that includes *Yimaia* and *Ginkgo yimaensis* and has structures resembling the ovule-bearing organs of *G. biloba*.

Recently ZHOU (1997) updated his analysis using 14 characters of ovules as well as leaves. In this new approach, *Trichopitys* was considered to be an archetype and used as an outgroup. Although a rather small number of characters are used, cladograms show *Karkenia* as a distinct lineage among Mesozoic ginkgoaleans and much nearer to *Trichopitys* (ZHOU 1997). *Toretzia*, *Umaltolepis* and *Yimaia* are placed in one group, while *Schmeissneria* and *Grenana* appear to be related to the *Ginkgo* clade. On the other hand, the closest member to *Ginkgo* is *Grenana*, a Jurassic genus described by SAMYLINA (1990) from Middle Asia and originally assigned to Lyginopteridopsida, although the resemblance to the extant *Ginkgo* in its paired cupules and deeply divided leaves was pointed out by ZHOU (1997). In this regard ZHOU (1997) has underscored that relationships among taxa in each group are rather poorly resolved, because some characters used, are not phylogenetically informative and therefore his analysis still remains preliminary. Nevertheless, using results of his cladistic analysis ZHOU (1997) distinguished six families in the order Ginkgoales, viz. Karkeniaceae (*Karkenia*), Umaltolepidaceae (*Umaltolepis* and *Toretzia*), Schmeissneriaceae (*Schmeissneria*), Yimaiceae (*Yimaia*), Trichopityaceae (*Trichopitys*) and Ginkgoaceae (*Grenana* and *Ginkgo*).

In this paper we share the opinion of ARCHANGELSKY & CÚNEO (1990) that there are Paleozoic plants, that may have integrated a stock from which Mesozoic representatives of Ginkgoales evolved. These putative candidates may have been the Trichopityaceae (*Trichopitys*), while Dicranophyllaceae (*Polysper-*

*mophyllum*, *Dicranophyllum*) may be another. ARCHANGELSKY & CÚNEO (1990) suggested that both families may well belong to the order Dicranophyllales, characterized by sessile or pedunculate ovules placed on leaves, a character seen in abnormal cases of *G. biloba*, or axillary to them. These taxa may represent a widely distributed primitive type of gymnosperms in both Carboniferous and Permian strata. It is reasonable to suppose that the ancestor of *Karckenia* may have well belonged to the Dicranophyllales (ARCHANGELSKY & CÚNEO 1990). Sporangial trusses of *Polyspermophyllum* or *Trichopitys* type of fructification may have been reduced during phylogeny becoming multiovulate seed cones similar to *Karckenia*. *Karckenia* ARCHANGELSKY (1965) is a unique multiovulate type of seed-cone among Mesozoic Ginkgoales that was the most successful as far as number of species and geographic distribution are concerned.

The general cone morphology and possible relationships of the taxa above discussed are plotted in Text-fig. 1, in which arrangement of the three Mesozoic lineages suggested by ZHOU (1997) are illustrated from a geochronological perspective. Present knowledge suggests that lineages containing the families Karckeniaceae, Umaltolepidiaceae and Yimaiaceae became extinct during the Cretaceous. In contrast the lineage including the Schmeissneriaceae and Ginkgoaceae has persisted to the present. Distribution of taxa presented in Text-fig. 1 is based on only a few genera, and better support is needed by discovering new fossil evidence. So far the inclusion of *Umaltolepis* and *Grenana* within Ginkgoales may be questionable. Only Karckeniaceae are well enough represented in time and space to be confidently defined as a natural group within the order and deserving a family rank.

### Distribution

The ginkgophytes were widely distributed and taxonomically diverse during Mesozoic and early Tertiary times. Although they are especially abundant in northern latitudes (SEWARD & GOWAN 1900), they also occur in several regions of the southern hemisphere (BERRY 1938, FRENGUELLI 1946, ARCHANGELSKY 1965, TRONCOSO & HERBST 1997). The decline of the group began in the Oligocene and was accelerated from Miocene times onwards until the group was reduced to a single taxon *G. biloba* in its present-day East Asiatic home (TRALAU 1968). Several theories have been proposed for this decline. SEWARD & GOWAN (1900) suggested that unfavourable climatic conditions may have been the cause of a gradual reduction of the group. FLORIN (1949) suggested that the whole fertile apparatus of ginkgophytes had less evolutionary potential compared to conifers that retained a cone type of reproductive structure. Conifers evolved parallel to Ginkgoales in time and space, but were more successful, surviving in abundance to the present day. During the Mesozoic a seed-cone organization was common to some gymnosperms such as the conifers, cycads and ginkgos, but ginkgos lost this structure at the end of the Era. In contrast cycads and conifers maintained a seed-cone organization to the present day, having both lignification of their sporophylls (in cycads) and bracts and sporophylls (in conifers). This type of protection, that may persist for long periods before seed dispersal, could well be an additional survival factor.

Other factors also could account for the decline of the ginkgophytes. For example, TAYLOR & ARCHANGELSKY (1985) considered the reproductive biology of Mesozoic seed ferns and suggested that a large number of small seeds may be a passive dispersal agent that allowed these plants to effectively establish seedlings at new sites. In this regard, Mesozoic Karckeniaceae produced a large number of seeds that were smaller than those known in the extant *Ginkgo*. On the other hand ROTHWELL & HOLT (1997) suggested that big seeds of *Ginkgo* may have been dispersed by herbivorous reptiles far from the parent tree assuring a wide geographic distribution of these plants. Extinction of dinosaurs and large reptiles during Late Mesozoic, could have severely limited the ability of *Ginkgo* to colonize areas by this mechanism resulting in a decline of diversity and geographical distribution of ginkgophytes during the Tertiary. This also may have been the case with other plant groups such as Bennettitales, Pteridospermae and Cycadales.

### Conclusions

In some parts of our planet such as Patagonia, ginkgophytes are abundantly represented in Late Paleozoic and Mesozoic strata. Leaves and fertile structures are known in Carboniferous through Tertiary



times. Most Jurassic and Cretaceous ginkgophytes had ovuliferous multiovulate and cone-like structures. However, structures with one or two ovules are also known in the Jurassic and Cretaceous.

In order to better understand the role of Ginkgophyta in time and space, we suggest that Paleozoic Dicranophyllales could be an ancestral stock from which Mesozoic Ginkgoales evolved (Text-fig. 1). Reduction and consolidation of the fertile structure in *Trichopitys* and *Polyspermophyllum* may have led to a compact cone-like ovuliferous organ in Mesozoic Karkeniaceae or Yimaiaceae. Similarly, webbing and planation of sterile appendages of Paleozoic Dicranophyllales led to a Mesozoic variety of ginkgophyte leaves. Reduction of Mesozoic seed-cones resulted in simple structures and ovule enlargement.

In spite of a reduced ovuliferous structure, the anatomical and ultrastructural resemblance of the megaspore membrane of *G. biloba* to that of the fossil *Karkenia incurva*, suggests that protection given by most sporophytes to the gametophytic generation did not change during phylogeny of the group.

Distribution of Ginkgophyta was wide during the Mesozoic. A severe decline of the group at the end of that era was probably due to the disappearance of several important dispersal features such as numerous small seeds, dinosaurs and large reptiles extinction and paleoclimatic changes. The decline parallels what happened in Bennettitales, Pteridospermae and Cycadales at the end of the Mesozoic.

Considering different phylogenetic analyses performed by others authors (ARCHANGELSKY 1965, KRASSILOV 1970, ZHOU 1991, 1997) cone-like ovuliferous structures are common to several Mesozoic taxa referred to the Karkeniaceae family. Ginkgoaceae are known since the Jurassic and form a clade that includes several taxa (ZHOU 1997). A third group, the Umaltolepidaceae is a somewhat doubtful clade considering its incomplete geologic record. Studies performed in recent years on ginkgoalean fossils recovered from different areas and ages permit a new perspective on phylogenetic relationships so that is now possible to envisage several taxonomic groupings within the order and to recognize probable links with other gymnosperms such as dicranophylls.

### Acknowledgements

This study was partially funded by grants: PEI -CONICET N° 0085/97 and BID -802/PID CT N° 163. We are grateful to the Botanical Institute Darwinion for the use of scanning electron microscope facilities and to LUIS LEZAMA for helping design Text-fig. 1. Also, we want to thank an anonymous reviewer for useful comments, which have improved this manuscript.

### References

- ARCHANGELSKY, S. (1965): Fossil Ginkgoales from Ticó flora, Santa Cruz Province, Argentina. - Bull. Brit. Mus. (Nat. Hist.), Geology, **10**: 119-137; London.
- , (1967): Estudio de la Formación Baqueró, Cretácico Inferior de Santa Cruz, Argentina. - Rev. Mus. La Plata, Secc. Paleontol., **5**: 63-171; La Plata.
- ARCHANGELSKY, S. & CÚNEO, R. (1990): *Polyspermophyllum*, a new Permian gymnosperm from Argentina, with considerations about the Dicranophyllales. - Rev. Palaeobot. Palynol., **63**: 117-135; Amsterdam.
- ARCHANGELSKY, S. & DEL FUEYO, G.M. (1989): *Squamastrobis* gen. n., a fertile podocarp from the Early Cretaceous of Patagonia, Argentina. - Rev. Palaeobot. Palyn., **59**: 109-126; Amsterdam.
- AZCUY, C. L. & BALDONI, A. M. (1990): La flora triásica del Grupo El Tranquilo, Pcia de Santa Cruz (Patagonia) Parte III. Ginkgoales. - Actas V Congreso Argentino de Paleontología y Bioestratigrafía, **7**: 109-115; Tucumán, Argentina.
- BERRY, E. W. (1938): Tertiary Flora from the Río Pichileufú, Argentina. - Geol. Soc. Amer., **12**: 1-140; Baltimore.
- BOODLE, L. A. (1916): A method of macerating fibres. - Royal Bot. Gard. Kew Bull. Miscellaneous Inf., **4**: 108-110; Kew.
- CRANE, P. R., MANCHESTER, S. R. & DILCHER, D. L. (1990): A preliminary survey of fossil leaves and well preserved reproductive structures from the Sentinel Butte Formation (Paleocene) near Almont, North Dakota. - Fieldiana (Geology), New Series, **20**: 1-63; Chicago.
- CÚNEO, R. N. (1987): Sobre la presencia de probables Ginkgoales en el Pérmico inferior de Chubut, Argentina. - Actas VII Simposio Argentino de Paleobotánica y Palinología: 47-49; Buenos Aires.
- CZIER, Z. (1998): *Ginkgo* foliage from the Jurassic of the Carpathian basin. - Palaeontology, **41** (2): 349-381; London.
- DEL FUEYO, G. M. (1996): Microsporogenesis and microgametogenesis of the Argentinian species of *Podocarpus* (Podocarpaceae). - Bot. J. Linn. Soc., **122**: 171-182; London.
- , (1999): Cone and ovule development in the *Podocarpus* species from Argentina. - Phytomorphology, **49** (1): 49-60; Delhi.
- DEL FUEYO, G. M., ARCHANGELSKY, S. & TAYLOR, T. N. (1990): Una nueva podocarpácea fértil (Coniferal) del Cretácico Inferior de Patagonia, Argentina. - Ameghiniana, **27** (1-2): 63-73; Buenos Aires.

- FERUGLIO, E. (1942): La flora liásica del valle del Río Genoa (Patagonia). Ginkgoales et "Gymnospermae" incertae sedis. - Not. Mus. La Plata 7. Pal., 40: 93-109; La Plata.
- FLORIN, R. (1936): Die fossilen Ginkgophyten von Franz-Joseph-Land nebst Erörterungen über vermeintliche Cordaitales mesozoischen Alters. - Palaeontographica Abt. B, 81: 71-173; Stuttgart.
- (1949): The morphology of *Trichopitys heteromorpha* SAPORTA, a seed-plant of Palaeozoic age, and the evolution of the female flowers in the Ginkgoinae. - Acta Horti Bergiani, 15: 79-109; Uppsala.
- FRENGUELLI, J. (1946): Contribuciones al conocimiento de la flora del Gondwana Superior en Argentina. 33. Ginkgoales de los estratos de Potrerillos en la Precordillera de Mendoza. - Notas del Museo de La Plata (Serie Paleontológica), 11 (87): 100-127; La Plata.
- HARRIS, T.M. (1935): The fossil flora of Scoresby Sound, East Greenland, IV. Ginkgoales, Coniferales, Lycopodiales and isolated fructifications. - Medd. Grønland, 112: 1-176; København.
- HARRIS, T.M. & MILLINGTON, W. (1974): The Yorkshire Jurassic Flora. IV, 1. Ginkgoales. - British Museum (Nat. Hist.), 78 p.; London.
- KIRCHNER, M. & VAN KONIJNENBURG-VAN CITTERT, J.H. (1994): *Schmeissneria microstachys* (PRESL, 1833) KIRCHNER et VAN KONIJNENBURG-VAN CITTERT, comb. nov. and *Karkenienia hauptmannii* KIRCHNER et VAN KONIJNENBURG-VAN CITTERT, sp. nov., plants with ginkgoalean affinities from the Liassic of Germany. - Rev. Palaeobot. Palyn., 83 (1-3): 199-215; Amsterdam.
- KRASSILOV, V.A. (1970): Approach to the classification of Mesozoic "ginkgoalean" plants from Siberia. - Palaeobotanist, 18: 12-19; Lucknow.
- (1972): The Mesozoic flora of the Bureja River (Ginkgoales and Czekanowskiales). - 151 p.; (Akad. Nauka S.S.S.R.) Moscow.
- (1982): Early Cretaceous Flora of Mongolia. - Palaeontographica Abt. B, 181: 1-77; Stuttgart.
- KRÄUSEL, R. (1943a): Die Ginkgophyten der Trias von Lunz in Nieder-Österreich und von Neue Welt bei Basel. - Palaeontographica Abt. B, 87: 59-93; Stuttgart.
- (1943b): *Furcifolium longifolium* (SEWARD) n. comb. eine Ginkgophyte aus dem Solenhofener Jura. - Senckenbergiana, 26: 426-433; Frankfurt.
- REYMONOWNA, M. (1985): *Mirofia szaferi* gen. et sp. nov. (Ginkgoales) from the Jurassic of the Kraków region, Poland. - Acta Palaeobot., 25 (1-2): 3-129; Kraków.
- ROTHWELL, G.W. & HOLT, B. (1997): Fossils and phenology in the evolution of *Ginkgo biloba*. - In: T. HORI et al. (eds.): *Ginkgo biloba*. A global Treasure. - pp. 223-230 (Springer Verlag) Tokyo.
- SAMYLINA, V.A. (1990): *Grenana* - a new genus of seed ferns from the Jurassic deposits of Middle Asia. - Bot. Zh., 75 (6): 846-850; St. Petersburg. (in Russian)
- SCHWEITZER, H. J. & KIRCHNER, M. (1995): Die Rhäto-Jurassischen Floren des Iran und Afghanistans: 8. Ginkgophyta. - Palaeontographica Abt. B, 237: 1-58; Stuttgart.
- SEWARD, A. C. & GOWAN, J. (1900): The Maidenhair Tree (*Ginkgo biloba* L.). - Ann. Bot., 14 (53): 111-154; London.
- STANISLAVSKY, F.A. (1973): The new genus *Toretzia* from the Upper Triassic of the Donetz Basin and its relation to the genera of the order Ginkgoales. - Paleontol. Zh., 1: 88-96; Moscow. (in Russian)
- TAYLOR, T.N. & ARCHANGELSKY, S. (1985): The Cretaceous pteridosperms *Rufloiria* and *Ktalenia* and implications on cupule and carpel evolution. - Amer. J. Bot., 72 (12): 1842-1853; Columbus, Ohio.
- TAYLOR, T.N. & TAYLOR, E. (1992): The Biology and Evolution of fossil plants. - 982 p.; (Prentice Hall) New Jersey.
- TOWNROW, J.A. (1967): On *Rissikia* and *Mataia* podocarpaceous conifers of the lower Mesozoic of southern lands. - Pap. Proc. R. Soc. Tasmania, 101: 103-136; Tasmania.
- TRALAU, H. (1968): Evolutionary trends in the genus *Ginkgo*. - Lethaia, 1: 63-101; Oslo.
- TRONCOSO, A.A. & HERBST, R. (1997): Ginkgoales del Triásico del Norte de Chile. - Actas X Simposio Argentino de Paleobotánica y palinología: 5. Mendoza; Argentina.
- VAN COTTEM, W.R.J. (1970): A classification of stomatal types. - Bot. J. Soc. Linn., 63: 235-246; London.
- VILLAR DE SEOANE, L. (1997): Comparative study between *Ginkgoites tigrensis* ARCHANGELSKY and *Ginkgo biloba* LINN. - Palaeobotanist, 46 (3): 1-12; Lucknow.
- ZEBA-BANO, MAHESHWARI, H. K. & BOSE, M. N. (1979): Some plant remains from Pathargama, Rajmahal hills, Bihar. - Palaeobotanist, 26: 144-156; Lucknow.
- ZHOU, Z. (1991): Phylogeny and evolutionary trends of Mesozoic ginkgoaleans - a preliminary assessment. - Rev. Palaeobot. Palyn., 68: 203-216; Amsterdam.
- (1993): A comparative ultrastructure of fossil and living ginkgoalean megaspore membranes. - Rev. Palaeobot. Palyn., 78: 167-182; Amsterdam.
- (1994): The heterochronic origin of *Ginkgo biloba*-type ovule-bearing organs. - Acta Palaeontol. Sinica 33 (2): 131-137; Beijing.
- (1997): Mesozoic ginkgoalean megafossils: A systematic review. - In: T. HORI et al. (eds.): *Ginkgo biloba*. A global Treasure. - pp. 183-206. (Springer Verlag) Tokyo.
- ZHOU, Z. & ZHANG, B. (1989): A Middle Jurassic *Ginkgo* with ovule-bearing organs from Henan, China. - Palaeontographica Abt. B, 211: 113-133; Stuttgart.
- (1992): *Baieria hallei* SZE and associated ovule-bearing organs from the Middle Jurassic of Henan, China. - Palaeontographica Abt. B, 224: 151-169; Stuttgart.

## Explanation of plates

### Plate 1

*Karkenian incurva* ARCHANGELSKY emend.

- Fig. 1. Seed cones (arrows) in close association with *Ginkgoites tigrensis* leaves (arrows head); BA PB 1022. Scale = 2.5 cm.  
Figs. 2, 3. Seed cone (part and counterpart) showing compact arrangement of ovules. Note *Ginkgoites tigrensis* leaves (arrow); Fig. 2 BAPB 10724 a. Fig. 3 BAPB 10724 b. Scale bar = 1 cm.  
Fig. 4. Distal and incomplete portion of a seed cone. Note the central axis (arrow) of the cone and funicle of the ovule (arrow head); BAPB 10725 a. Scale bar = 0.5 cm.

### Plate 2

*Karkenian incurva* ARCHANGELSKY emend.

- Figs. 1–4. Details of the ovules and their disposition on the cone axis; Figs. 1, 2. Most ovules are seen in frontal view; Figs. 3, 4. Ovules showing funicle and chalaza in lateral view; Figs. 1, 2, 4 BAPB 10724 a; Fig. 3 BAPB 10724 b. All scale bars = 0.25 cm. chalaza (c); funicle (f); micropyle (m).

### Plate 3

*Karkenian incurva* ARCHANGELSKY emend. and *Ginkgo biloba* L.

- Figs. 1–6. Outer epidermis of the integument; Figs. 1–4, 6. Chalazal zone; Fig. 5. Micropylar zone.

*Karkenian incurva* ARCHANGELSKY emend.

- Fig. 1. Cuticle with stomata; BAPB 10725a; BAPB Pm 383, × 125.  
Fig. 2. Close view of stomata. Note thick cuticular ridge surrounding the mouth of stomata; BAPB 10725a; BAPB Pm 383, × 200.  
Fig. 3. Detail of two stomata and epidermal cells. Note inside the stomatal mouth remnants of guard cells at bottom; BAPB 10725a; BAPB Pm 383, × 400.  
Fig. 4. Idioblast (arrow) showing middle part of the cell crushed, probably because of contents; BAPB 10725a; BAPB Pm 380, × 400.  
Fig. 5. Cuticle of micropyle without stomata; BAPB 10725a; BAPB Pm 383, × 125.

*Ginkgo biloba* L.

- Fig. 6. Integument of the chalazal area with stomata and idioblasts (arrows), × 100.

### Plate 4

*Karkenian incurva* ARCHANGELSKY emend.  
(all from BAPB 10725 a)

- Figs. 1, 2. Nucellus from two ovules; Fig. 1. Note attached megaspore membrane (arrow); BAPB Pm 383, × 200; Fig. 2. Note associated *Podocarpidites*-type pollen grain (arrow head); BAPB Pm 384, × 125.  
Fig. 3. Detail of nucellar cells. Note striations on elongated nucellus cells; BAPB Pm 384, × 300.  
Fig. 4. Pollen grains associated with the outer integument of the ovules: *Podocarpidites* (arrow), *Aracariacites* (arrow head) and *Ginkgocycadophytus* type (bar); BAPB Pm 382, × 250.  
Fig. 5. Megaspore membrane showing granular appearance with light microscope; BAPB PM 382, × 125.  
Fig. 6. Foot layer of the megaspore membrane with Nomarski contrast; BAPB Pm 382, × 100.

### Plate 5

*Karkenian incurva* ARCHANGELSKY emend.  
(megaspore membrane; all from BAPB 10725 a)

- Figs. 1, 2. Surface views; Fig. 1. Sculptured layer and foot layer (arrow), × 3000; Fig. 2. Detail of sculpture layer, × 2000. Both from BAPB MEB 65.  
Figs. 3–6. Sections through the megaspore membrane seen with TEM: Fig. 3. Slightly oblique section showing foot layer (l) and different shape and arrangement of bacula (b). Note carbonized tissue (arrows), × 18000; Fig. 4. Megaspore membrane in oblique section showing disorganized bacula, arrow indicates carbonized tissue, × 8000; Fig. 5. Detail of bacula (b) and foot layer (l), arrows show carbonized tissue, × 16000; Fig. 6. Irregularly arrangement of bacula, × 64000. (all from BAPB MET 168).

