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Early Cretaceous Angiosperm Leaves from Southern South America

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Early angiosperm leaves from the Aptian (113 to 119 million years ago) Baqueró Formation of Patagonia have been found in a fossil flora dominated by more than 100 species of gymnosperms and pteridophytes. They may be the first early Cretaceous angiosperm leaves to be reported from southern South America and one of the few reported in the Southern Hemisphere. The leaves are large, lobate, craspedodromous, and dentate (A-1 teeth) and have ramified tertiary veins and random fourth-order venation. Several of these features have been found in coeval and younger strata elsewhere, but not in the same combination. They were probably a marginal component of the flora.

RECENT YEARS HAVE WITNESSED the discovery or reevaluation of Cretaceous fossil fruits, flowers, cuticles, leaf imprints, and pollen grains that have provided insights into the early evolution of angiosperms. Critical reevaluation has resulted in rejection of most reports of pre-Cretaceous angiosperms (*1*), establishing the oldest unequivocal record of the group in the Barremian (*1–4*).

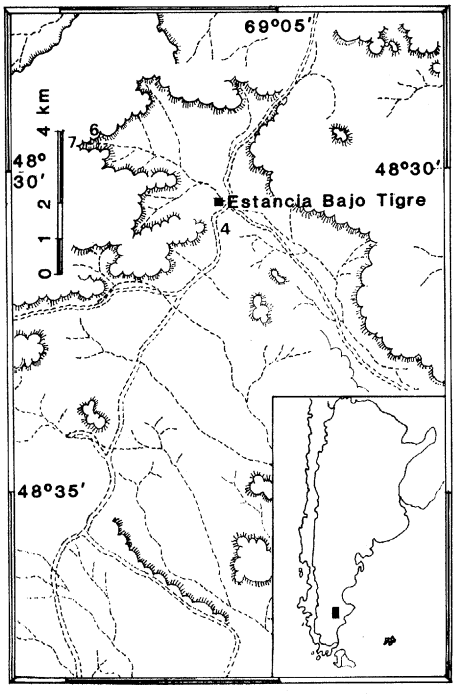

Fig. 1. Map of the fossil locality.

Descriptions of several Albian and Cenomanian flowers (*5*) support the idea that the main lines of evolution within the angiosperms were being differentiated at that time. Early angiosperm leaves are rare. Barremian or Aptian remains have been described only from the Soviet Union and eastern North America (*6–11*).

Characteristic Mesozoic floras occur in several formations in Patagonia. Among them, the floras preserved in the Baqueró Formation (*12*), reported as Barremian to Aptian in age, have been intensively studied. Younger floras, dominated by angiosperms, are those from Mata Amarilla and Cerro Dorotea formations, probably of Coniacian to Maastrichtian age (*13*). We now describe the first angiosperm leaf imprints in the Baqueró flora—the first description of early Cretaceous angiosperm leaves from South America and one of the few in the Southern Hemisphere (*14*).

The Baqueró Formation crops out in an extensive area in Santa Cruz Province, Argentina (*12*) (Fig. 1). It consists mainly of tuffaceous, fluvial, and minor lacustrine deposits, together with paleosols. It is divided into two members, with the lower containing a rich assortment of fossil compressions. In the Estancia Bajo Tigre area the formation is 140 m thick and contains kaolin and laminated silts with plant remains at the base. Plant fossils are usually concentrated in lenses. Angiosperm leaves were found first by one of us (S.A.) at site 7 (*12*), but were not reported. More and better preserved angiosperm leaves were collected in 1983. At site 7 (*12*) two levels (named after the most abundant genus) have been recognized: (i) *Brachyphyllum*, with only fragments of angiosperm leaves, and (ii) *Ptilophyllum*, where the best specimens of angiosperms occur. At this level, angiosperms are found in poorly laminated brown tuffs 15 to 20 cm thick containing limonite veins; these poorly laminated tuffs rest on laminated brown tuffs that include abundant organic matter, twigs, roots, and coal fragments. These lie in turn on a conspicuous layer with abundant *Ptilophyllum* remains.

The angiosperm leaves are covered by small gypsum crystals that fill the areoles bordering the veins. Fine details are obscure, with only the primary and secondary veins well marked. Cuticles are not present on the leaves. Apart from the species described here, abundant fragmentary leaves have been found in the same bed. Considering them all reveals the presence of other angiosperm species, some with entire margins and others with more complicated teeth. However, their poor preservation (Fig. 2F) does not warrant a description.

The other plants described so far from the Bajo Tigre area are all ferns and gymnosperms (*12, 15*). The only angiosperm pollen grain reported from the formation is the small, monosulcate *Clavatipollenites hughesii* (*16*).

Originally, the age of this formation was considered to be Barremian or Aptian (*12*). Recently, this unit was assigned a late Barremian to early Aptian age on the basis of its showing of a transition from *tectifera-corrugatus* to *Antulsporites-Clavatipollenites* pollen zones (*17*).

Five fairly complete specimens of angiosperm leaves described here have simple, symmetrical laminae (*18*), are pinnately lobed, and are 7 to 14 cm long and 6 to 13 cm wide (mesophyll size class). Fragmentary remains show that the leaves may also be palmately lobed, with two to three pairs of small lobes. Probably both types and intermediate forms were present in the fossil species. The apex of each lobe is obtuse; the leaf base is badly preserved, but is probably truncate or cordate. The margin is dentate and serrate, with obtuse, simple, regular, A-1 (convex-convex) teeth separated by angular sinuses. The principal vein of each tooth is a branch of a tertiary vein that enters the tooth slightly eccentrically. No accessory veins were observed. Nor was any glandular-

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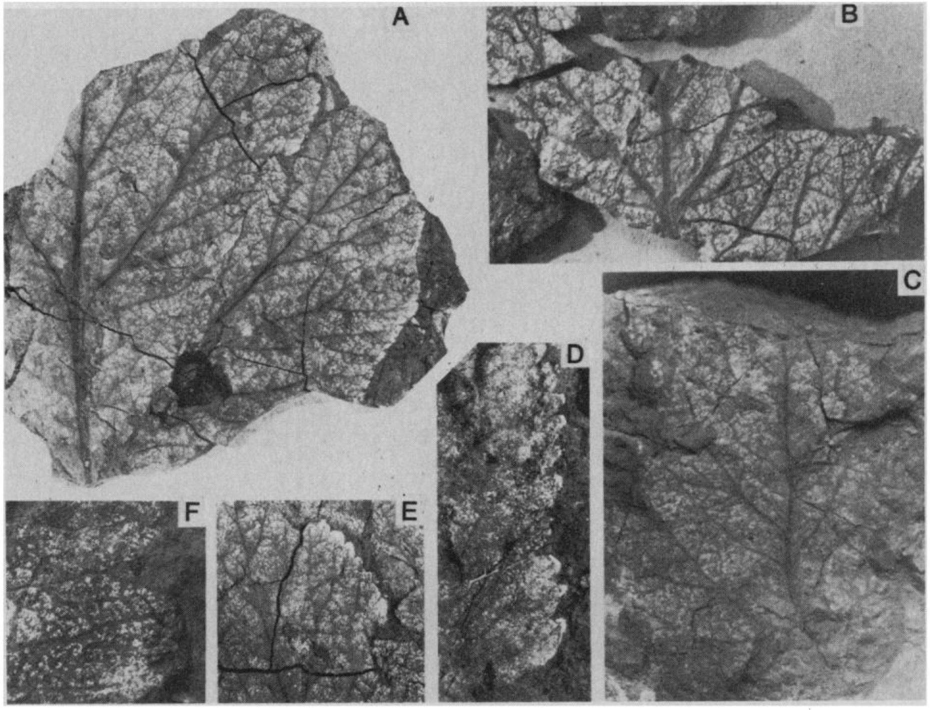

Fig. 2. (A) Large leaf. Note the primary vein separated into several strands, decurrent secondaries, and exmedially ramified tertiaries. (B) Part of a larger leaf, showing high-order venation. (C) Smaller leaf with sinuous primary vein, decurrent secondaries, and strong intersecondaries. (D) Teeth with a deposit of gypsum along their apex. (E) Enlarged margin of the specimen in (A), showing deposit of gypsum on teeth, distal branching of tertiary veins, and slightly eccentric principal vein. (F) Isolated marginal part with more complicated, composite teeth, but the same pattern of marginal venation. Original magnifications: (A to C), ×1, and (D to F), ×2.

ity observed, but a deposit of gypsum usually present along the tooth apex (Fig. 2, A, D, and E) may represent the remnant of a glandular area. The petiole was not preserved. Texture is probably chartaceous, since the imprint is never deep.

The venation is pinnate and simple craspedodromous. The primary vein is weak, straight to slightly sinuous, and occasionally separated into two or three strands (Fig. 2, A and C). Secondary veins are arranged in two to four pairs, which form the principal veins of the leaf lobes. Strong basal secondaries occur in some fragmentary palmate specimens. Secondary veins of moderate thickness, decurrent along the primary vein for 1 to 2 cm, hold a straight course to the apex of the lobe and form an angle of about 40° with the primary. Intersecondary veins are present.

Wide tertiary veins, not very different in size from the secondaries, emerge at about 30°, or sometimes at a wider angle, but then curve and become straight at about 30°. Tertiary veins are exmedially ramified, usually subdichotomously, with the branch closest to the secondary remaining straight or nearly so and the other branch departing at an acute angle, leaving room for the next branch. Usually only two bifurcations occur, with the last one reaching the apex of the tooth. Fourth-order veins (Fig. 2B), although badly preserved, are well differentiated from the tertiaries in the middle part of the leaf, but are poorly so toward the margins; they are randomly oriented. Fifth-order veins were not observed, possibly because of poor preservation. The smallest areas of leaf tissue surrounded by veins, about 2 mm across, are imperfect and incompletely closed. Poor preservation makes it difficult to determine whether these are really areoles.

These leaves belong to a "high first-rank" organization (*19*) because they have decurrent secondary veins, separate strands in parts of the primary, and various angles of emergence of secondaries. However, they also have secondary veins of relatively regular courses and intercostal areas of uniform size and shape, which are features of second-rank leaves.

Among the fossil leaves hitherto described, none closely resembles that from the Baqueró Formation, although many of the individual features are known from leaves of similar age. Palmately lobed leaves are present in *Vitiphyllum multifidum* (*20*), convex-convex (A-1) teeth in *Quercophyllum tenuinerve* and *Proteaephyllum dentatum* (*7, 8*), large laminae (as long as 20 cm) in *Ficophyllum crassinerve* and *F. tenuinerve* (*20*), and craspedodromous venation in leaf type 1 (*21*), all from zone I of the Potomac group in eastern North America [Barremian through Aptian in age (*7–9*)]. Lobate shape is also present in *Dicotylophylum bilobatum* (*2*), from Albian deposits in western Kazakhstan. These species differ from the Patagonian fossil in almost all their other features, but they show that a set of shared primitive features was present in a worldwide complex of primitive angiosperm leaves during Barremian and Aptian times. The unique combination of features in the Patagonian fossil, including very primitive ones, prevents any close comparison with living plants or inclusion in one of the subclasses of dicotyledons.

Among living plants, simple, toothed, and pinnately or palmately lobed leaves are basic to some Magnoliidae, Hamamelididae, Rosidae, and the "palmate" Dilleniidae (*22*). Leaves of the last two subclasses differ the most from the fossil because of their semicraspedodromous venation and their dissimilar teeth. The other two subclasses share the type of tooth known as chloranthoid (*22*). The tooth type found in our fossil is similar to the chloranthoid type, although the characteristic lateral veins found in this form were not seen in our material. Magnoliidae present intersecondary veins, but usually have pinnate brochidodromous or acrodromous venation, or, when actinodromous, have numerous primary veins radiating from the petiole. They are not basically craspedodromous (*22*). Hamamelididae, on the other hand, may have palmately lobed leaves and actinodromous or pinnately craspedodromous venation (*22, 23*). Secondaries may branch, as do the tertiaries in the fossil leaves described above. In *Tetracentron*, an expanded gland is present in the teeth, similar to the gypsum deposit found in the fossil. Therefore, although the fossil cannot be included in any of the subclasses, if it is compared with the basic type of leaves of any of them it is most similar to those of Hamamelididae. Comparisons below the subclass level remain highly speculative.

The remains of the angiosperm leaf described from Barremian to Aptian sediments of Patagonia are significant in relation to the evolution of the group as well as for lower Cretaceous biology. First, they are the earliest angiosperm megafossil so far described from well-dated sediments in the Southern Hemisphere. If the West Gondwana origin of angiosperms is accepted (*24*) as suggested by the pollen record, the Patagonian leaves would argue for a rapid dispersal of the group during its early evolution, since they lived at fairly high southern latitudes. Second, although many individual features in the Patagonian leaves are known from features of contemporary Northern Hemisphere leaves, they differ from all earlier reports in their combination of characteristics. They therefore support the notion that the evolution of the group might have followed different morphological trends in different areas. After an origin in tropical areas, different lineages migrated to higher latitudes and fairly diverse dicotyledons attained an essentially worldwide distribution by 115 million years ago, that is, 10 million years after the first appearance of pollen in the fossil record. Third, the isolated, almost monospecific remains, in an association dominated by gymnosperms and pteridophytes, as well as the nature of the sediments (fluvial and volcanic, that is, from unstable environments) seem to indicate that the angiosperms were a marginal component of the flora during their early radiation. Primitive angiosperms probably were opportunists related to unstable environments (*7*) or successional elements in the first steps toward an assemblage dominated by gymnosperms and pteridophytes.

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Separation of Large DNA Molecules by Contour-Clamped Homogeneous Electric Fields

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Electric fields can be manipulated by a method in which multiple electrodes are arranged along a closed contour and clamped to predetermined electric potentials. This method may be applied to a broad range of problems in the separation of macromolecules by gel electrophoresis. DNA molecules as large as 2 megabases can be well separated with a contour-clamped homogeneous electric field alternating between two orientations 120° apart. The pattern of separation is independent of position in the gel, which is an advantage over previous methods. DNA less than 50 kilobases can be separated without distortion even at high voltage with a nonalternating contour-clamped homogeneous field. Decreased band broadening in DNA less than 200 bases can be achieved with a contour-clamped inhomogeneous field.

GEL ELECTROPHORESIS IS CAPABLE of separating macromolecules such as proteins and nucleic acids on the basis of size, charge, or conformation. Most applications use a single pair of electrodes to generate the electric field. Such a field is necessarily constrained to be uniform and oriented in a single direction. Conventional techniques are therefore limited in many respects. For example, they cannot resolve DNA fragments much larger than 50 kb (*1*).

The recent introduction of new electrode configurations that generate electric fields in alternating orientations has allowed the separation of large DNA molecules up to 2 megabases (Mb) in size (*2–4*). The technique has been used to separate chromosomes from yeast and other organisms. A problem with these methods is that the electric field is not uniform, so that DNA molecules migrate with mobilities and trajectories that depend on where in the gel the samples are loaded. Comparison of multiple samples across the gel is therefore difficult.

These limitations can be overcome by applying a contour-clamped homogeneous electric field (CHEF) that alternates between two orientations. The electric field is generated by a method in which multiple electrodes are arranged along a polygonal contour and clamped to predetermined electric potentials. The method applies the principles of electrostatics to gel electrophoresis. In particular, the electric field vector is confined to two dimensions and has two components, $E_x(x,y)$ and $E_y(x,y)$. To simplify the problem, the electric field may be expressed as the negative gradient of a single function, the scalar potential field $\phi(x,y)$ (*5*),

$$E_x(x,y) = -\partial\phi(x,y)/\partial x \qquad (1a)$$

$$E_y(x,y) = -\partial\phi(x,y)/\partial y \qquad (1b)$$

A homogeneous electric field is generated by two parallel, infinitely long electrodes. If one electrode is located along the x axis ($y = 0$) and the other is separated by a fixed distance ($y = a$), the potential field between the electrodes is

$$\phi(x,y) = \phi_0 y/a \qquad (2)$$

where ϕ_0 is the voltage applied across the electrodes. Substitution of Eq. 2 into Eq. 1 shows that the corresponding electric field is homogeneous and oriented perpendicular to the electrodes,

$$E_x(x,y) = 0 \qquad (3a)$$

$$E_y(x,y) = -\phi_0/a \qquad (3b)$$

It is impractical to use infinitely long electrodes, but it is possible to produce a homogeneous electric field with a finite system. A solution is to use multiple elec-

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