

A LEAFCUTTER BEE TRACE FOSSIL FROM THE MIDDLE EOCENE OF PATAGONIA, ARGENTINA, AND A REVIEW OF MEGACHILID (HYMENOPTERA) ICHNOLOGY

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Abstract: The ichnospecies *Phagophytichnus pseudocircus* isp. nov. is described to include trace fossils characterized by leaf-margin excisions showing eccentricity values of 0.35–0.65 and more than 270 degrees of an arc, a non cusped margin and vein stringers or necrotic flaps of tissue along the margin. A method for determining ellipse eccentricity was performed on leaf discs obtained from the nests of the modern leafcutter bee *Megachile rotundata* (Hymenoptera: Megachilidae), which provided objectively obtained values

comparable to the trace fossil from the middle Eocene of Argentina and other world-wide ichnological records, historically and subjectively considered to be ‘circular’ trace fossils and attributed to leafcutter bees. The material described herein represents the first evidence for fossil Megachilidae from the Southern Hemisphere.

Key words: trace fossil, leafcutter bee, plant-insect associations, Río Pichileufú flora, Patagonia, Argentina.

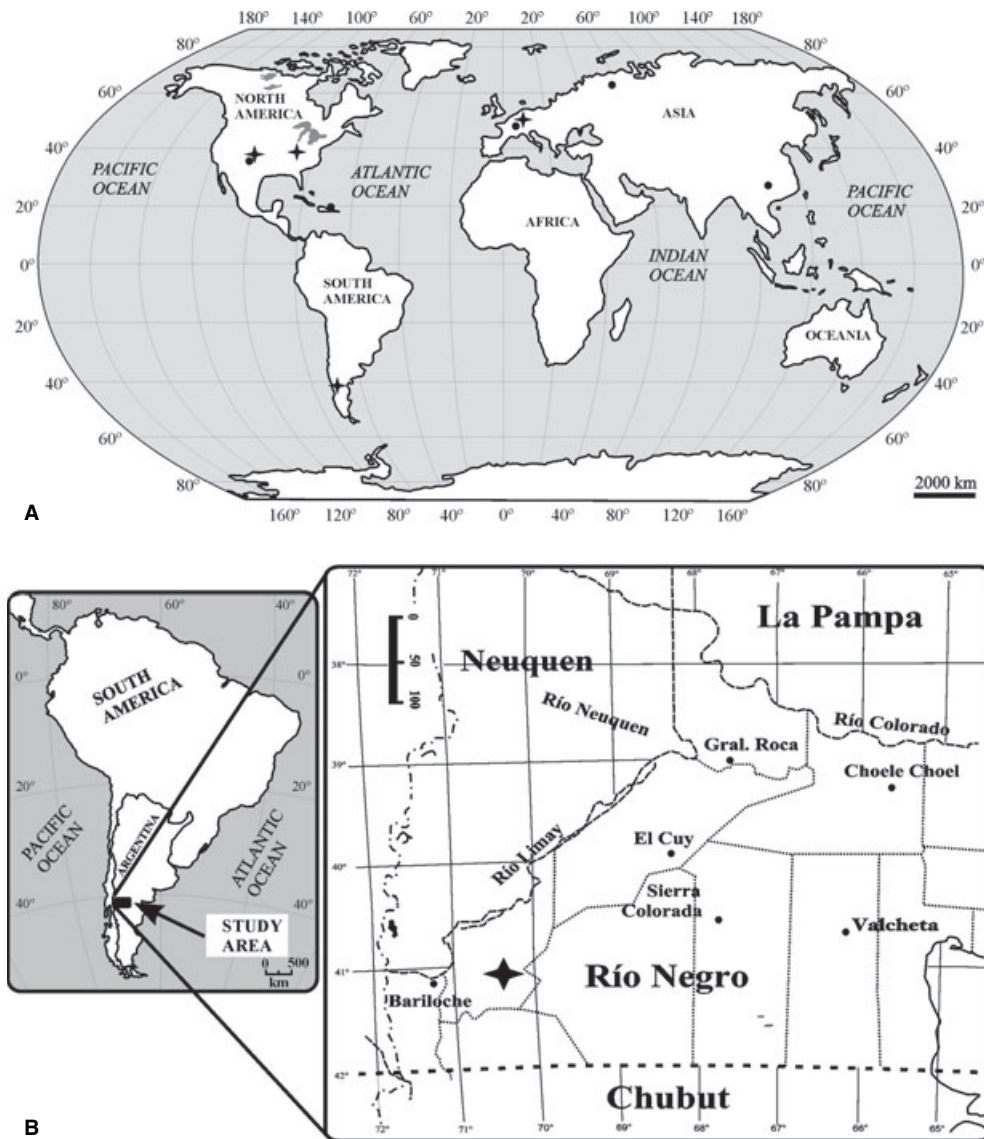
THE family Megachilidae comprises about 3000 extant species worldwide, which are included in four subfamilies: Pararhophitinae, Fideliinae, Liturginae and Megachilinae. One of the most common genera, *Megachile*, contains numerous species, particularly in xeric regions of the Old World (Michener 1979; Engel 1999; Baker and Engel 2006; Engel and Baker 2006). Adults are distinctive because they carry pollen in a metasomal structure called the ventral scopa. Nest building is an extensive activity among megachilids, for which females of some *Megachile* species use rounded leaf or petal fragments for construction, resulting in the presence of distinctive excision patterns along the margin leaves (Michener 1953, 2000; Horne 1995; Petit 2002) (Text-fig. 2A–B).

Ichnological evidence for fossil leafcutter bees has been reported by several authors, indicating that the use of leaves in nesting behaviour by these insects is old (Cockerell 1910; Berry 1931; Brooks 1955; Johnston 1993; Lewis 1994; Wappler and Engel 2003). These contributions document examples from the Northern Hemisphere that expand the past evolutionary history of the group (Text-fig. 1A). Historically, these trace fossils were described as semicircular excisions along the margin of angiosperm, and rarely *Ginkgo*, leaves (Lewis 1994; La-

bandeira 2002a, b), and were attributed to leafcutter bees because of their rounded shape as evaluated by visual inspection.

For South America, the Río Pichileufú (RP) palaeoflora of central Patagonia, from where the trace fossil in this contribution originates (Text-fig. 1B), comprises a diverse assemblage of plants. Early studies on this site were focused on plant diversity and taxonomy (Berry 1925, 1928, 1935a–c, 1938); more recently they have concentrated on geological setting, palaeoenvironment, and palaeoclimate (Romero 1978, 1986; Aragón and Romero 1984; Wilf *et al.* 2005a).

There have been only a few studies on fossil leaf damage in South America hitherto. Durango De Cabrera and Rodriguez De Sarmiento (1995), Rodriguez De Sarmiento and Durango De Cabrera (1995) and Rodriguez De Sarmiento *et al.* (1998) identified fungal attack on leaves from the Eocene Laguna del Hunco Flora. From the same locality, Wilf *et al.* (2005b) analysed the plant diversity and plant-insect associations, and Sarzetti *et al.* (2007) mentioned elevated levels of plant diversity and plant-insect associations. The only contributions that include an ichnotaxonomic analysis of plant-insect associations in Patagonia have been those of Genise (1995) and Genise and Hazeldine (1995), who identified insect



TEXT-FIG. 1. A, Map of occurrences of fossil *Megachile P. pseudocircus* (stars) and body fossil evidence (filled circles). B, maps of Río Negro Province and the Río Pichileufú locality (star).

borings in wood and described new ichnogenera and ichnospecies from Jurassic and Late Cretaceous localities.

The present contribution has three principal objectives. The first is to describe a new trace fossil and provide other examples of a distinctive type of plant damage attributed to leaf-cutter bees within an ichnotaxonomic context, thus contributing to our knowledge of plant-insect associations in the fossil record. The second is to provide a method, using an explicit shape analysis of ellipse eccentricity of leaf margins and holes, such that 'circular' trace-fossil excisions can be attributed to leaf-cutter bees by comparison with similar excisions from extant bees. The third is to place the documented occur-

rences in their broader spatiotemporal context of early bee biogeography and phylogeny.

GEOLOGICAL SETTING

The Río Pichileufú (RP) locality is located in the Río Negro Province of southern Argentina (Text-fig. 1B), and occurs in exposures of volcanic lake deposits of the Ventana Formation (Berry 1938; Aragón and Romero 1984). The palaeoflora comprises many leaves but also includes compressions of insects, flowers, seeds and fruits. Its age was first suggested to be Miocene (Berry 1938), but recently $^{40}\text{Ar}/^{39}\text{Ar}$ determinations from volcanic ashes

(Wilf *et al.* 2005a) intimately associated with the flora have dated it as 47.46 ± 0.5 Ma (middle Eocene, Lutetian), indicating that the RP flora was within the initial phase of a global cooling interval, post-dating the Paleocene–Eocene Thermal Maximum (Zachos *et al.* 2001; Wing *et al.* 2005).

Palaeobotanical research at this locality by Berry (1925, 1935a–c, 1938) revealed a broad spectrum of plant taxa. Berry described approximately 130 species of plants, but his assignments are problematic today because very few of his specimens have been re-examined in the light of current palaeobotanical knowledge (Wilf *et al.* 2005a). Extensive new collections were made in 2002 and 2005 (Wilf *et al.* 2005a), especially from a single quarry known as RP3. These are housed at the Museo Paleontológico ‘Asociación Paleontológica Argentina’ in Bariloche (Río Negro Province). Observations on this material and the type collections at the National Museum of Natural History (NMNH), in Washington, DC, indicate a diverse assemblage of angiosperms and to a lesser extent, ferns and gymnosperms (Wilf *et al.* 2005a; Sarzetti *et al.* 2007).

Previously, Romero (1978, 1986) considered the RP palaeoflora to be akin to a ‘mixed flora’ of heterogeneous composition and origin, with some species adapted to either tropical or subtropical conditions and others to a more temperate climate typical of a Gondwanan or other Austral realm. Thus, Romero (1978) concluded that the flora represented a stable community lacking a modern analogue.

Observations from Recent studies, both correlative and experimental, have shown that insect diversity is often associated with plant diversity (Murdoch *et al.* 1972; Siemann *et al.* 1998; Hawkins and Porter 2003), suggesting that the richness of the RP flora may also have a positive relationship with the diversity of foliar damage types in the ichnofauna.

MATERIAL AND METHODS

The extant species *Megachile rotundata* Fabricius was selected for comparison with the potential producer of the trace fossil described herein. Michener (2000) divided the genus *Megachile* into three groups, defined by distinctive external morphology and behaviour among subgenera. The species of Group 1 generally use leaf fragments to line cells in soil substrates or in hollows left by wood-boring beetles (Text-fig. 2C). According to Michener (2000) these species use oblong leaf fragments for the cell bases and lateral walls, and circular leaf discs for cell closures and separation between adjacent cells (Text-fig. 2D).

Nests of extant *M. rotundata* were used in this study to analyse shapes of leaf discs for comparison with the simi-

lar fossil excisions from RP and other traces described in the literature. *Megachile rotundata* was introduced to Argentina to improve the pollination of alfalfa (also known as lucerne, purple medick or trefoil: Fabaceae, *Medicago sativa* L.), at agricultural experimental stations (Arretz Vergara and Martínez 1988). The nests analysed were obtained from INTA (Instituto Nacional de Tecnología Agropecuaria) where the nesting bees are reared in cavities within woody substrates.

Foliar disc shape was analysed according to the following procedure. Each piece of leaf within the nests was separated using forceps and the more ‘circular’ pieces were selected, which meant selecting discs from the entrance of the cells as well as several from the base (Text-fig. 2D). These discs were subsequently mounted on paper and optically scanned to obtain a digital image analyzable by computer software. Analysis of the shape of these leaf discs, in addition to the trace fossil, was made using Corel Draw. One hundred discs of alfalfa leaves used by *M. rotundata* for their nests were analysed. An ellipse was redrawn over the perimeter of each disc to obtain the major and minor axes. Twelve potential ellipses were redrawn using the same methodology for the leaf from the RP site as well as previously documented trace fossils from the literature to obtain the average value of the minimum and maximum axial diameters.

These values were then used to evaluate the eccentricity of the leaf discs, using the general formula of an ellipse: $a^2 = b^2 + c^2$, where a is the major axis, b the minor axis, and c represents the distance between the centre and the focus. The eccentricity is represented by $e = c/a$ and $e = \sqrt{a^2 - b^2}/a$, whereby e is 0 when it is equivalent to a circle and 1 when it is equivalent to a line. Intermediate values represent ellipsoidal figures ranging from circles to ellipses. The unaided eye can distinguish more accurately between circles and ellipses when the values are, respectively, closer to 0 or 1.

Potential errors inherent to the methodology should be noted. The margin of leaf discs could inadvertently be broken when being separated manually, resulting in the lowest values of eccentricity obtained, between 0.06 and 0.10. Moreover, it was difficult to distinguish in some cells between those discs located at the base from adjacent pieces of the lateral wall that were progressively more oval. This, in turn, may result in the highest values obtained of around 0.67. Additional error may be introduced when redrawing the ellipses over the discs, producing subtle differences between the shapes of the ellipses and discs.

To evaluate these measurement errors, 25 discs were chosen at random from the entire sample ordered in rows and columns. The first specimen selected was placed on the upper-left corner, and subsequently a disc from each

of four discs was chosen. Four possible ellipses were redrawn over each of these 25 discs and four eccentricity values obtained for each disc. The minimum value of these was subtracted from the average of the four. Twenty-five values were so obtained and the average of these values resulted in the error.

SYSTEMATIC PALAEOLOGY

Ichnogenus PHAGOPHYTICHNUS van Amerom, 1966

Type ichnospecies *Phagophytichnus ekowskii* van Amerom, 1966, by monotypy.

Diagnosis. Semicircular, oval or round-oblong excisions cutting into the leaf margin. Along the border of these chewed edges are more or less pronounced thickened ridges. These borders are characterized by distinctive dark areas. Veins and other tissues with stiffened parts are often removed but some usually remain as stringers of structural or conductive tissue projecting from the chewed margin into the void area (translated from van Amerom 1966).

Remarks. Van Amerom (1966) included only the ichnospecies *Phagophytichnus ekowskii* in his initial establishment of the ichnogenus. Later, Straus (1977) described six new ichnospecies including margin and hole feeding traces, changing the original diagnosis of the ichnogenus. Givulescu (1984) described additional ichnospecies and, following Strauss, also included margin- and hole-feeding foliar damage under *Phagophytichnus*. Recently, Vasilenko (2006) erected the ichnogenus *Pinovulnus*, proposing to use plant hosts as valid ichnotaxobases. He included in it two ichnospecies from two unrelated host plants, one assigned to the Pinales and the other to the Ginkgoales. In addition, he did not establish the taxonomic rank of plants to which this arrangement should be made. This practice of establishing ichnotaxa of plant-insect associations based on plant-host affiliation should be discouraged because it conflates insect behavioural patterns and plant damage morphology with the logically separate issue of plant-host taxonomy.

Phagophytichnus pseudocircus isp. nov.

Text-figure 2A

- 1910 'Leafcutter bee trace'; Cockerell, p. 429.
- 1955 'Leafcutter bee trace'; Brooks, p. 2, pl. 1, fig. 1–1a.
- 2002 'Leafcutter bee trace'; Wappler and Engel, p. 346.
- 2003 'Leafcutter bee trace'; Wappler and Engel, p. 909, fig. 1.
- 2004 'Leafcutter bee trace'; Engel, p. 21, fig. 13.

Derivation of name. Greek, *pseudo*, false, and Latin, *circus*, circle.

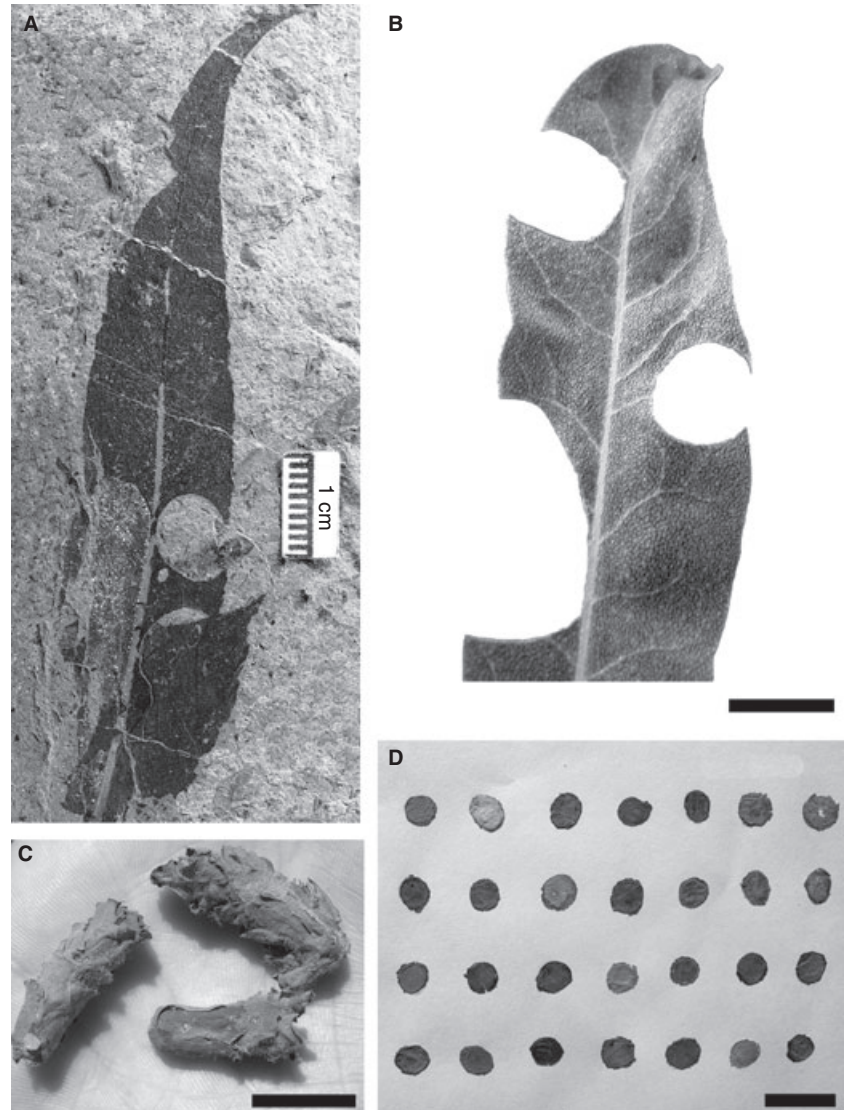
Holotype. Part and counterpart of specimen, BAR 4740a–b (Text-fig. 2A), from the RP quarry, occurring respectively in a part and counterpart of a leaf housed in the Museo Paleontológico 'Asociación Paleontológica Argentina', Bariloche, Río Negro Province (BAR 4739a–b).

Diagnosis. A rounded excision, showing eccentricity values from 0.35 to 0.65, cut along the margin of a leaf. The lamina of the leaf is prolonged in two triangular projections along the margin, which are not connected, resulting in an arc with values of more than 270 degrees. All the vein orders, except the midrib, are removed. Non-cusped margin and vein stringers or necrotic flaps of tissue are present along the margin.

Remarks. *Phagophytichnus pseudocircus* differs from the type ichnospecies, *P. ekowskii* van Amerom (1966), by its rounded shape and the presence of triangular projections of lamina on the leaf margin. Straus (1977) described *P. circumsecans* for rounded traces that could be present both on the margin and inside the lamina because he considered that *Phagophytichnus* included both hole and margin feeding traces. However, the specimens of *P. circumsecans* occurring in the leaf margin lack the triangular projections. Other ichnospecies defined by Straus (1977), Givulescu (1981) and Vasilenko (2006) are morphologically very different from *P. pseudocircus* in not presenting a broadly circular feature in the margin of the leaves.

Among the trace fossils documented in the literature and assigned to *Megachile* that were surveyed by us (Cockerell 1910; Berry 1931; Brooks 1955; Johnston 1993; Lewis 1994; Wappler and Engel 2003), only three are attributable to *P. pseudocircus*. These are marginal excisions, and bear the distinctive triangular projections on the leaf margin and a compatible range of eccentricity values (Cockerell 1910; Brooks 1955; Wappler and Engel 2003). The trace fossils described by Cockerell (1910) are three similar specimens occurring on the lamina of an undetermined dicotyledonous leaf from late Eocene (late Priabonian) deposits of Florissant, Colorado (USA). Only two specimens, having an eccentricity average value of 0.35, show the triangular projections, being the lowest value of all the trace fossils analysed and consequently indicating the roundest shape. Brooks (1955) described four trace fossils along the margin of a leaf identified as *Nectandra pseudocoriacea* (Lauraceae) from Early Eocene deposits in Tennessee (USA). Only one specimen exhibits the well-preserved triangular projections of megachilid damage, and shows an eccentricity value of 0.48. Finally, Wappler and Engel (2003) recorded the first, and currently only, example of *P. pseudocircus* from the Old World. These authors identified three trace fossils similar

TEXT-FIG. 2. A, Holotype of *P. pseudocircus*, BAR 4740a. B, extant leaf with a megachilid trace. C, nest of *Megachile* sp. D, leaf discs from closures and bases of *M. rotundata* cells. Scale bars represent 1 cm.



in shape and size in the lamina of an unidentified leaf from the Early Eocene Messel beds, near Darmstadt, Germany. Their trace fossils are not equally preserved, so only one specimen preserving the triangular projections was measured. This trace fossil shows an eccentricity value of 0.45. From the four trace fossils that Lewis (1994) illustrated on the same leaf, at least one is rounded, but it lacks triangular projections of the leaf margin, whereas for those described by Berry (1931) and Johnston (1993), it is difficult to distinguish a rounded shape.

A rounded trace fossil with an eccentricity value of 0.26 is present on a dicotyledoneous, probable lauraceous, leaf (specimen 1034a) from another highly diverse Palaeogene locality (site LH13) in Patagonia, Laguna del Hunco (early Eocene, Chubut) (Labandeira, pers. obs. 2005). This trace fossil occurs inside the lamina and, impor-

tantly, lacks a connecting incision to the leaf margin, which excludes it from *Phagophytichnus pseudocircus* and also from *Phagophytichnus* as defined originally by van Amerom (1966).

Description. The holotype occurs along the margin of a lanceolate, unidentified dicotyledoneous leaf, in the central region of the lamina and extending to the midrib. No clear reaction tissue or cuspluate chew marks, vein stringers or necrotic flaps of tissue were observed along the cut margin. The maximum chord length is 9.4 ± 0.2 mm and the minimum chord length is 7.3 ± 0.2 mm. The total area removed is 56 mm^2 and the perimeter is 26.8 mm. The trace fossil shows an average eccentricity of 0.56. A second similar trace, partly covered by a superimposing leaf, is present on the same leaf a few millimeters from the holotype. The small, uncovered part of this leaf has the same curvature and shape as the holotype.

DISCUSSION

Phagophytichnus was defined for cusped excisions on the margin of pinnules and leaves, and included only the ichnospecies *P. ekowskii* (van Amerom 1966; van Amerom and Boersma 1971). Later, Straus (1977) erected six new ichnospecies: *P. nervillo-reliquens*, *P. circumsecans*, *P. marginis-folii*, *P. nervos-mutans*, *P. nigromarginatus* and *P. catellarius*, which included both margin- and hole-feeding traces. Similarly, Givulescu (1984) included in this ichnogenus margin- and hole-feeding trace fossils, creating the new ichnospecies *P. uvaeformis* and *P. gastropodinus*, and identifying two more that were unnamed. However, the difference between margin and hole feeding should be regarded as ichnotaxonomically important considering that it involves two distinct morphological patterns, which reflect clear behavioural differences from potentially different groups of insect producers. Usually insects that feed on leaf margins avoid the interior of the lamina because non-marginal leaf feeders need distinctive anatomical modifications (Edwards and Wratten 1980; Scott *et al.* 1992). In addition, the fossil evidence described hitherto has indicated that marginal feeding appeared earlier than hole-feeding (Scott *et al.* 1992; Stephenson and Scott 1992; Labandeira 1998; Labandeira and Allen 2007). Marginal feeding appeared during the Middle/Late Mississippian boundary interval on *Triphylopteris austrina*, a tree fern of small stature (Iannuzzi and Labandeira in prep.), and became prominent principally on various types of medullosan tree ferns during the Middle Pennsylvanian (van Amerom and Boersma 1971; Scott *et al.* 1992). By contrast, hole feeding was rare during the late Middle Pennsylvanian, but became prominent during the Early Permian, principally on giantopterid seed-fern taxa (Beck and Labandeira 1998; Glasspool *et al.* 2003).

By the mid Mesozoic, many lineages of non-angiospermous seed plants displayed a wide variety of external foliage feeding, particularly hole and margin feeding (Ash 1999; Rasnitsyn and Krassilov 2000; Scott *et al.* 2004). During the Early Cretaceous, gymnosperm floras dominated most environments, including that of Baissa in Transbaikalia (Russia), which is of Barremian age. It is from this flora that Vasilenko (2006) established *Pinovulnus* to include *P. regularis*, *P. serpentiformis* and *P. erectus* from leaves of Pinales and Ginkgoales. According to him, *Pinovulnus* differs from *Phagophytichnus* in its plant-host taxonomic affiliation. It is proposed herein to maintain *Phagophytichnus* only for margin-feeding traces as defined originally. We strongly advocate that plant-insect association ichnotaxa should be defined by their morphology and underlying behaviour, rather than by their plant-host taxonomic assignments. A possible

exception concerns highly specific associations between a herbivore and the host-plant, such as gall insects, wherein developmental or structural features of the host-plant are co-opted by the production of physiologically plant-mimicking hormones.

The attribution of *P. pseudocircus* to leafcutter bees required a particular type of analysis. Earlier authors (Cockerell 1910; Berry 1931; Brooks 1955; Johnston 1993; Lewis 1994; Wappler and Engel 2003) attributed trace fossils in leaves to megachilids based on their apparently circular shape. However, evaluation of the degree of circular expression involved a subjective analysis of the outline of these fossil excisions as well as of leaf fragments cut by extant megachilids. Herein, the eccentricity of the ellipse fitted to the cut margin was used as a test to evaluate objectively the circularity of the trace fossils as well as the extant traces. More than 60 per cent of the values of the entire sample (N = 100) of discs cut by *Megachile rotundata* had eccentricity values that ranged from 0.35 to 0.63, indicating that, in fact, the discs approximate more to an ellipse than to a circle. The resulting eccentricity average was 0.42 ± 0.10 . The eccentricity value of the holotype of *P. pseudocircus* (0.56) and material reported by Brooks (1955), Cockerell (1910) and Wappler and Engel (2003), fall within the range of the values obtained from excisions of extant megachilids, indicating, with a high degree of confidence, that these trace fossils are the result of the nesting activity of a leafcutter bee. Additionally, Horne (1995) demonstrated that megachilid bees usually cut several discs from the same leaf, as seen in the leaves of *P. pseudocircus* considered herein and in previous studies (Cockerell 1910; Brooks 1955; Wappler and Engel 2003).

Pre-Cenozoic fossil bee evidence comes from Halictidae nests described by Elliott and Nations (1998) from the Cenomanian Dakota Formation of the USA and by Genise *et al.* (2002) from the Santonian–Campanian Laguna Palacios Formation of Argentina. Until late 2006, the oldest documented bee body-fossil was *Cretotrigona prisca* Michener and Grimaldi (1988), originating from Late Cretaceous amber from New Jersey (USA) (Michener and Grimaldi 1988; Engel 2000). However, its derived position within the superfamily Apoidea suggests a substantially earlier origin for the entire group and that significant stages of bee evolution had already occurred by the Late Cretaceous (Engel and Perkovsky 2006). In a report from late 2006 (Poinar and Danforth 2006), a significantly earlier and smaller bee specimen was described from Myanmar (Burmese) amber, dated as late Albian or latest Early Cretaceous. This specimen was assigned to a new family, Melittosphecidae. More recently, however, Ohl and Engel (2007) have indicated that this insect body fossil is not of a bee; moreover, they stated that

Melittosphex burmensis is a synonym of *Cretospilomena* (Hymenoptera: Crabroninae), also described from Burmese amber (Antropov 2000). For the Megachilidae, which lack Cretaceous body fossils, the trace- and body-fossil record only becomes relatively abundant for the mid Palaeogene. The evidence for fossil megachilids in North America comes from the late Eocene Florissant Formation in Colorado (Cockerell 1906, 1908, 1911, 1913, 1914, 1917, 1923, 1925a; Engel 2001; Meyer 2003), and from amber of the early Miocene (Aquitania) La Toca Formation in the Dominican Republic (Engel 1999). In Europe, by contrast, major fossil occurrences are of an older Palaeogene age. *Megachilid* body fossils have been documented from amber from the late Eocene (Priabonian) Obukhov Formation in the Rovno Region of the Ukraine (Engel and Perkovsky 2006), the middle Eocene (mid Lutetian) Prussian Formation of the Baltic Region (Cockerell 1909a, b; Engel 2001), the upper Oligocene (Chattian) brown coals of Rott, in Schwaben, Germany (von Heyden 1862; Meunier 1920; Statz 1936), the Miocene (probably Aquitania) of the Kudia River, near Amagu in the Far East of Russia (Cockerell 1925b), and late Miocene deposits of Oeningen, Germany (Heer 1849, 1865; Cockerell 1909a; Zeuner and Manning 1976). For Asia there is evidence for the presence of megachilid bees from the middle Miocene Shanwang Formation, near Linqu, Shandong, China (Zang 1989a, b).

The subfamily Megachilinae is over-represented among fossil Megachilidae when compared with the Recent bee fauna (Engel and Perkovsky 2006). For South America, body-fossil bee evidence comes only from a fossil bee assigned to a corbiculate apid from Late Paleocene deposits in north-west Argentina (Petrulevicius 2002). The Megachilidae as a whole is regarded as a derived group based on phylogenetic analysis (Roig-Alsina and Michener 1993); it probably originated during the Paleocene or latest Cretaceous (Engel and Perkovsky 2006). Since derived megachilids produce a distinctive leaf-excision pattern along the margin of leaves, the trace-fossil evidence presented herein demonstrates that a more advanced clade of Megachilini was already present in South America by the early Eocene some 47.5 myr ago, thus indicating a significantly earlier origin for the Megachilidae.

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