New fossil evidence of the early diversification of bees: *Paleohabropoda oudardi* from the French Paleocene (Hymenoptera, Apidae, Anthophorini)

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Phylogenetic relationships among and within the major groups of bees (Apoidea Apiformes) were recently reconsidered using extensive molecular and morphological datasets. The next step in the study of bee evolution will consist in estimating the antiquity of the nodes within the inferred topologies. We describe here the third oldest bee fossil, *Paleohabropoda oudardi* gen. n. sp. n. (Apidae, Apinae, Anthophorini) from the Paleocene of Menat (France, Puy-de-Dôme; 60 Myr). Phylogenetic analysis of 17 morphological characters and morphometric analysis of the wing shape were used to recover, respectively, its taxonomic position and morphological affinities. Our results indicate that *Paleohabropoda oudardi* gen. n. sp. n. clearly belongs to the Anthophorini. *Paleohabropoda* is therefore the oldest fossil that can be confidently date and place to an extant tribe. Its wing shape is surprisingly close to the extant genus *Habropoda*. The discovery of *Paleohabropoda oudardi* gen. n., sp. n. brings further evidence for the Cretaceous diversification of major lineages of bees and for strong constancy of wing shape within the Anthophorini.

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Introduction

Bees (Apoidea Anthophila) are a monophyletic group of pollen-eating taxa derived from carnivorous apoid wasps (formerly 'Sphecidae') (Michener 1944; Brothers 1975; Alexander 1992; Brothers 1998; Ronquist *et al*. 1999; Engel 2001a; Danforth *et al*. 2006a,b). They likely arose during the mid-Cretaceous (~120 Myr) concomitantly with the diversification of the flowering plants (Angiosperms) (Grimaldi 1999; Engel 2001a; Crepet *et al*. 2004; Grimaldi & Engel 2005). Seven contemporary families are usually acknowledged in Apoidea: Andrenidae, Apidae, Colletidae, Halictidae, Melittidae, Megachilidae, and Stenotritidae, including ~1200 genera and ~20 000 species (Discover Life Apoidea species guide, Ascher *et al*. 2007; Michener 2007). Two fossil families are also described: Paleomelittidae from Eocene Baltic amber and a stem-group, Melittosphecidae, from Cretaceous Myanmar amber (Engel 2001a; Poinar & Danforth 2006). The usual phylogenetic hypothesis associated with this classification pictures the Colletidae as the sister family of the other bees while Apidae (including eusocial species such as the honey bee *Apis mellifera* L.) is considered as highly derived (Michener 1944; Engel 2001a; Grimaldi & Engel 2005). Using an extensive dataset (molecular + morphological), Danforth *et al*. (2006a,b) obtained a very different topology of bee phylogeny and classification, first proposed by McGinley (1980). These authors suggested that the Melittidae *s.l.* constitute a basal group from which other groups of bees derive. Moreover, Danforth *et al*. (2006b) recognized nine extant families, the additional ones following the split of Melittidae *s.l.* into Dasypodaidae, Meganomiidae and Melittidae

s.str. (Fig. 6). The next step in the study of the evolution of bees consists in validating and dating the newly inferred topology through additional fossil evidence.

When documenting the minimal ages of taxa, fossils are of particular interest for dating topologies. They can also be used for molecular clock calibration (further discussion see Rutschmann *et al.* 2007). Worldwide, four main deposits of bee fossils are known: Dominican amber from the Miocene (20 Myr), Florissant shale from the Eocene-Oligocene boundary (34 Myr), Eckfeld/Messel shales and Baltic amber from the middle Eocene (*c.* 45 Myr). These sources have produced sizeable bee paleofaunas showing unexpected taxonomic Paleogene bee diversity (Zeuner & Manning 1976; Poinar 1999; Engel 2001a,b; Wappler & Engel 2003). Cretaceous, Paleocene and early Eocene bee fossils are much rarer. Only five specimens have been found in layers older than 50 Myr. (1) *Halictus*? *saveneyi* Engel & Archibald 2003 is from the Ypresian (lowermost Eocene) of British Colombia, ~53 Myr (Engel & Archibald 2003). (2) *Paleomacropis eocenicus* Michez & Nel 2007 is also ~53 Myr and was isolated from amber in Oise (France) (Michez *et al.* 2007). (3) *Probombus hirsutus* Piton 1940 is a compression from the Paleocene found in Menat (France, ~60 Myr). *P. hirsutus* was recently revised and attributed to Megachilidae (Nel & Petrulevicius 2003). (4) *Cretotrigona prisca* (Michener & Grimaldi 1988) obviously belongs to corbiculate-Apidae (Apinae). *C. prisca* was described from New Jersey amber and attributed to the late Maastrichtian (~65–70 Myr) (Engel 2000). However, age of this fossil is uncertain because the specimen was not adequately labelled (Rasnitsyn & Michener 1991). (5) The oldest bee fossil *Melittosphex burmensis* Poinar & Danforth 2006 was discovered in the Upper Albian of the early Cretaceous (~100 Myr). It represents a stem group (Melittosphecidae) of Anthophila sharing only a few synapomorphies with the extant bees (Poinar & Danforth 2006).

Because the bee fossil record remains dramatically fragmentary, especially for the Cretaceous and Paleocene periods, additional records are of significant interest to evaluate the origin of the major groups and the rates of their diversification and extinction. Hereafter, *Paleohabropoda oudardi* gen. n., sp. n. is described from the Paleocene of Menat (France, ~60 Myr). This fossil, from the same deposit as *Probombus hirsutus*, is the third oldest fossil bee and the oldest fossil that can be confidently date and place to an extant tribe. We used phylogenetic (maximum parsimony) and geometric morphometric (wing shape) methods to ascertain its taxonomic identity and morphometric affinity.

Materials and methods
Description and systematics
The morphological terminology follows Michener (2007) for the body description and Engel (2001a) for the wing description. For phylogenetic hypotheses we refer to Michener (2007) and Danforth *et al.* (2006b).

Phylogenetic analysis
Roig-Alsina & Michener (1993) and Dubitzky (2007) were the primary sources of morphological characters. We added four characters to the 13 obtained from the previous works, yielding a 17-character dataset (Table 1). *Macropis steironematis* Robertson 1891 (Melittidae *s.str.*) was used as an outgroup of the long-tongued bees according to the most recent phylogenetic hypothesis (Danforth *et al.* 2006a,b). The 10 other taxa were sampled as representatives of some major lineages in Apidae (*Centris*, *Bombus*) and especially in Anthophorini (*Amegilla*, *Anthophora*, *Deltoptila*, *Elaphropoda*, *Habropoda*, *Habrophorula* and *Pachymelus*) (Table 1).

PAUP 4.0b10 (Swofford 2001) was used to perform an exhaustive tree search based on the morphological dataset (Table 1). Both 50% majority rule and strict consensus of the

Table 1 Matrix of data for the cladistic analysis

Taxa	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17
Outgroup																	
Macropis steironematis	?	?	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0
Centris sp.	2	0	1	0	2	0	1	0	1	0	1	0	0	0	0	0	0
Bombus terrestris	2	0	0	0	1	1	2	0	0	0	1	0	0	0	0	0	0
Ingroup																	
Amegilla quadrifasciata	1	0	0	0	2	0	0	0	1	1	1	1	1	1	?	1	?
Anthophora plumipes	1	0	0	1	1	0	1	0	1	0	1	1	1	0	1	1	?
Deltoptila elefas	1	1	1	0	1	1	0	0	1	0	1	?	1	1	1	0	1
Elaphropoda moelleri	0	1	1	0	1	1	2	0	1	0	0	0	1	0	0	0	2
Habrophorula nubilipennis	0	1	1	0	1	1	2	0	1	0	0	0	1	0	0	0	2
Habropoda tarsata	0	1	1	0	0	1	0	0	1	1	1	1	1	1	0	1	0
Pachymelus radovae	1	1	1	0	1	2	2	1	1	2	1	0	1	0	1	1	?
Paleohabropoda oudardi	0	1	0	0	1	1	2	0	1	2	0	1	1	?	?	?	?

most parsimonious trees were computed and compared. The mapping of characters on the consensus topology was performed in Winclada 1.00.08 (Nixon 2002).

Morphometric analysis

As the previous phylogenetic analysis was based on a small dataset of available morphological characters, we performed a morphometric analysis of the wing shape of *Paleohabropoda oudardi* sp. nov. This morphometric analysis allows us to evaluate the similarity of the fossil wing to contemporary wings. Compared with other organs, wings show many methodological advantages, such as 2D structure, rigidity, species specificity and good conservation in fossil specimens (Pavlinov 2001). Moreover, wing veins and their intersections are unambiguously homologous among bees (Ross 1936).

The sampled taxa are equivalent to those considered in the phylogenetic analysis. We excluded *Macropis* because its wing shape (with two submarginal cells) is too different from the sampled Apidae. When the material was available, we considered three specimens in each species and several species in each genus to evaluate, respectively, the interspecific and the intergeneric variations of shape. We assembled a dataset of 42 specimens representing 17 species and 10 genera of Apidae: (i) Anthophorini: *Amegilla albigena* Lepeletier 1841, *Am. quadrifasciata* de Villers 1789, *Anthophora aestivalis* (Panzer 1801), *A. bimaculata* (Panzer 1798), *A. quadrimaculata* (Panzer 1798), *Deltoptila elefas* (Friese 1917), *Elaphropoda moelleri* Lieftinck 1966, *E. percarinata* (Cockerell 1930), *Habropoda tarsata* (Spinola 1838), *H. zonatula* Smith 1854, *Habrophorula nubilipennis* (Cockerell 1930), *Pachymelus ocularis* Saussure 1890, *P. radovae* Saussure 1890, *P. unicolor* Saussure 1890 and *Paleohabropoda oudardi* sp. n.; (ii) Bombini: *Bombus mendax* Gerstaecker 1869; (iii) Centridini: *Centris* sp. All specimens of contemporary species are conserved in the University of Mons-Hainaut Laboratory of Zoology (Belgium) and the University of Kansas Natural History Museum (USA).

To analyse the wing shape, we adapted the geometric morphometric method as detailed in Aytekin *et al.* (2007). We used 17 landmarks in the left forewing (Fig. 2A). Wings were photographed by a single experimenter (TD) using an Olympus SZH10 microscope coupled with a Nikon D200 camera. Photographs were input to tps-UTILS 1.38 (Rohlf 2006a) and Cartesian coordinates of landmarks were digitized with tps-DIG 2.05 (Rohlf 2006b). Landmark configurations were scaled, translated and rotated against the consensus configuration using the GLS Procrustes superimposition method (Bookstein 1991). The coordinates were analysed using tps-RELW 1.44 (Rohlf 2006c) to calculate eigenvalues for each principal warp. We tested the amplitude of variation inside our dataset with tps-Small 1.2 (Rohlf 2003). Finally, we processed a relative warps analysis, which is technically a PCA based on the landmarks.

Additionally, a matrix of similarity based on Euclidean distance of Cartesian coordinates was used to compute a Neighbour-joining tree (Saitou & Nei 1987) with Ntsys-Pc2.1 (Rohlf 2000). The Neighbour-joining tree was rooted with a specimen of *Bombus mendax*.

As landmarks 16 and 17 are weakly perceptible in the fossil specimen, we compiled a subset of data without these landmarks and processed the same analyses. We compared the results from both datasets.

Results

Systematic paleontology

Family Apidae Latreille 1802
Subfamily Apinae Latreille 1802
Tribe Anthophorini Dahlbom 1835

Genus Paleohabropoda *Michez & Rasmont gen. n*

Type species. Paleohabropoda oudardi

Etymology. From the Greek *Paleo*, referring to the Paleocene origin of the fossil, and *-habropoda* referring to the similar widespread extant genus *Habropoda* (see discussion).

Generic diagnosis

Female (Figs 1 and 2F). Robust anthophoriform body. Pterostigma small, not tapering beyond vein r-s, parallel-sided. Three submarginal cells, the first longer than the second. Apex of marginal cell pointed. Distal costal part of wing papillate. Hind femur nearly hairless. Basitibial plate present on hind tibia. Scopa well developed on hind tibia and hind basitarsus. Hind basitarsus shorter than hind tibia. Pygidial fimbria and pygidial plate present.

Male. Unknown.

Paleohabropoda oudardi *Michez & Rasmont spec. nov*

Holotype. MNHN-LP-R 63891 (female, print with organic matter), Laboratoire de Paléontologie, Muséum national d'Histoire naturelle, Paris, France.

Etymology. Named after Jacques Oudard, who collected the type specimen conserved in the collections of the MNHN. In acknowledgement of his contribution to Paleoentomology.

Type strata and locality. Paleocene, spongo-diatomitic volcanic paleolake (maar) deposit, Menat (46°06′N 02°54′E), Puy-de-Dôme, France (Russel 1967, 1982).

Specific diagnosis: see diagnosis of the genus.

Description

Female. Head (Fig. 1A): Upper part of vertex visible. Lower part of head hidden under mesosoma. Right antenna visible;
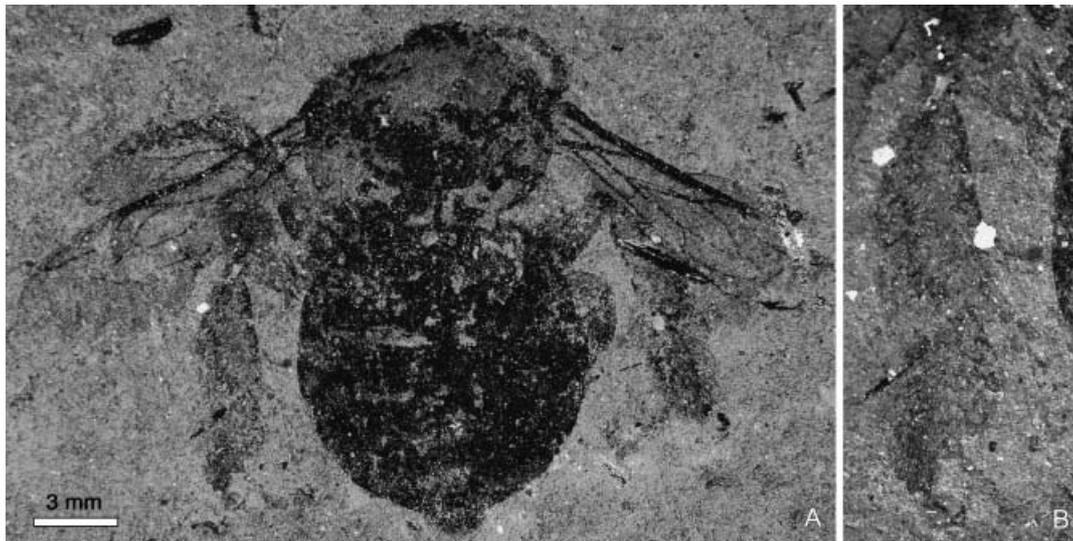
Fig. 1 A, B. *Paleohabropoda oudardi* gen. n. sp. n. —A. General habitus. —B. Detail of hind leg.
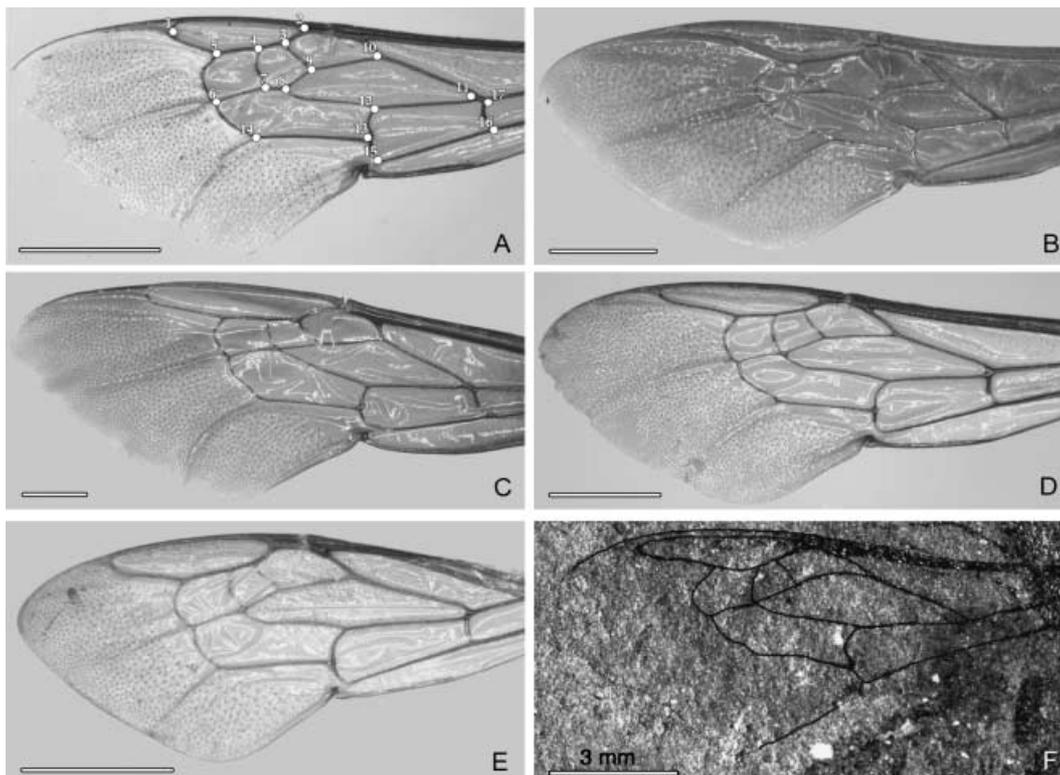
Fig. 2 A–F. Left forewings of Anthophorini (scale = 2 mm).—A. *Amegilla quadrifasciata* with 17 landmarks. —B. *Centris* sp. —C. *Pachymelus radovae*. —D. *Deltoptila elephas*; —E. *Habrophorula nubilensis*. —F. *Paleohabropoda oudardi* gen. n. sp. n., detail of wing with line drawing overlapping veins.

only a few segments distinguishable. Antenna 2.9 mm long, 0.2 mm wide. Mesosoma (Fig. 1A): 3 mm long, 3.1 mm wide (between tegulae). Punctures, sculpture and segments not visible. Legs (Fig. 1B): forelegs hidden. Right mid femur and tibia partly visible. Left mid leg visible: femur ~1.0 mm long (half part visible); tibia ~1.6 mm; tarsus ~1.3 mm. Both hind legs visible: femur ~1.9 mm long (half part visible), without scopal hairs; tibia ~3.2 mm long. Basitibial plate well developed;

basitarsus 1.9 mm long, with dense penicillus. Scopa well developed on hind tibia and basitarsus. Fore- and hind wings (Fig. 2F): left forewing 9.4 mm long, well preserved. Right forewing 8.4 mm in length, partly deformed. First free abscissa of M straight. Cu-a crossvein meeting M + Cu vein at the intersection of M- and Cu vein. Three submarginal cells, first 0.7 mm long (maximal length), second 0.4 mm long, third 0.5 mm long. Vein 1 m-cu joining the second submarginal near its apex. Pterostigma small, not tapering beyond crossvein r-rs, parallel-sided. Marginal cell 1.6 mm long; apex pointed. 2 m-cu crossvein straight. Distal and costal parts of forewing papillate. Metasoma (Fig. 1A): 9.4 mm long, 7.4 mm wide. Six exposed terga. Pygidial fimbria and pygidial plate present. Pygidial plate triangle shaped. Pilosity: nearly not visible except few setae on metasoma and scopa on hind leg.

Male. Unknown.

Position of **Paleohabropoda oudardi** *sp. n. in Apoidea*

Supra-generic taxa (tribes, subfamilies) in bees are mainly distinguished according to morphology of their mouthparts (Michener 2007). The classification of *Paleohabropoda oudardi* sp. n. is therefore difficult because of its hidden labiomaxillary complex. However, the overall morphology, the shape of the antenna, the wing venation and the shape of the scopa allow us to assign it to family, subfamily and tribe.

First, *Paleohabropoda oudardi* sp. n. does not display the typical extension of antenna observed in Stenotritidae. At the same time, the anterior wing in *Paleohabropoda oudardi* sp. n. displays three submarginal cells (Fig. 2F), which distinguishes this specimen from a number of other high-level taxa with only two submarginal cells such as Dasypodaidae, Euryglossinae (Colletidae), Hylaeinae (Colletidae), Macropidini (Melittidae *s.str.*), Megachilidae, Paleomelittidae (most) Panurginae (Andrenidae), and Xeromelissinae (Colletidae). In addition, the basal vein of the forewing is straight (unlike in most Halictidae) and the second recurrent vein is not S-shaped as is typically the case in some Colletinae.

The scopa of *Paleohabropoda oudardi* sp. n. is clearly restricted to its hind tibia and hind basitarsus (Fig. 1B) unlike in extant Andreninae (Andrenidae), Colletinae (Colletidae), and most of the Halictidae, which display additional collecting structures (e.g., on hind femur). Scopal hairs restricted to the hind tibia and hind basitarsus are only similar to Melittidae *s.l.* and nonparasitic, noncorbiculate Apidae (Thorp 1979). The short prestigma and the general anthophoriform morphology (Fig. 2F) prevent the placement of *Paleohabropoda oudardi* sp. n. in Melittidae *s.str.* and Meganomiidae and support placement of the species into Apidae as advocated here.

Position of **Paleohabropoda oudardi** *sp. n. in Apidae*

Three subfamilies are acknowledged in Apidae: Xylocopinae, Nomadinae, and Apinae (Roig-Alsina & Michener 1993; Michener 2007). *Paleohabropoda oudardi* sp. n. can be distinguished from Xylocopinae (i.e., Allodapini, Ceratinini, Manueliini, and Xylocopini) by several morphological traits: (1) small pterostigma, (2) hind basitarsus shorter than hind tibia, (3) pygidial plate present. In addition, according to the preceding description, *Paleohabropoda oudardi* sp. n. shows well developed scopae on the hind legs, suggesting that the species was probably not cleptoparasitic like Nomadinae and a number of other groups in Apinae: *Coelioxoides*, Ericrocidini, Isepeolini, Melectini, Osirini, Protepeolini, Rhathymini.

Figure 1(B) does not display any evidence of corbiculate-like organization of the scopae. There are long hairs visibly covering the hind tibia and basitarsus. On this basis and the obvious *Anthophora*-like habitus, the species cannot reasonably be associated with the corbiculate group (Apini, Bombini, Euglossini and Meliponini).

There are eight tribes in Apidae with the *Anthophora*-like habitus: Ancylini, Anthophorini, Centridini, Ctenoplectrini, Emphorini, Eucerini, Tapinotaspidini and Exomalopsini.

A handful of characters can exclude a few groups from possible tribe associations. First, the inner Tb3 of *Paleohabropoda oudardi* sp. n. is narrow, without a comb, which prevents the species from being placed in Ctenoplectrini. Second, the shape of pterostigma (small, not tapering beyond r-s crossvein, and parallel-sided) and the papillate distal part of the wing indicate that *Paleohabropoda oudardi* sp. n. could belong to Anthophorini or Centridini, other tribes displaying a broader pterostigma. The ratio of first (larger) and second (smaller) submarginal cells (Fig. 2F) leads us to consider that *Paleohabropoda oudardi* sp. n. is an anthophorine.

Seven extant genera are included in the tribe Anthophorini: *Anthophora* (worldwide, ~350 species), *Amegilla* (Old World, ~250 species), *Deltoptila* (Central America, ~10 species), *Elaphropoda* (Eastern Asia, ~11 species), *Habrophorula* (Oriental, ~3 species), *Habropoda* (Old and New World, ~60 species), *Pachymelus* (Southern Africa and Madagascar, ~20 species) (Dubitzky 2007). *Paleohabropoda* can easily be distinguished from *Anthophora* and *Amegilla* by the branching position of the first recurrent vein (1 m-cu) in the second submarginal cell. In *Paleohabropoda*, crossvein 1 m-cu joins the second submarginal near its apex unlike in the group *Anthophora* + *Amegilla*. Moreover, crossvein 1 cµ-a is longer and more oblique than in *Habropoda*. No other character allows us to determine if this species is more closely related to one of the other four genera of Anthophorini. Given the antiquity of the present fossil and its dissimilarities with other genera, we propose to describe an original genus.
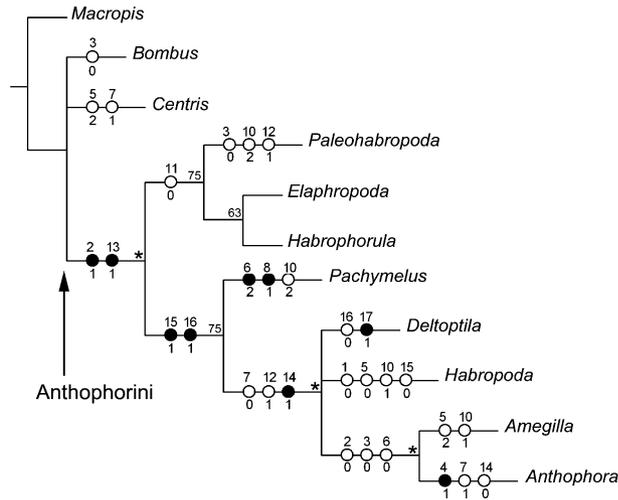
Fig. 3 Majority rule consensus tree from heuristic search yielding 16 shortest trees (L = 40; CI = 0.57; RI = 0.54) with characters states (black dots = apomorphies; white dots = homoplasies) and frequency of occurrence (* = 100%).

Fig. 4 Distribution of the 42 examined specimens along the first two PCs. PCA of tangent space coordinates derived from GPA of the original coordinates digitized from the wing.

Cladistic analysis

The maximum parsimony analysis of the 17 morphological characters (Table 1) yields 16 shortest trees (L = 40; CI = 0.57; RI = 0.54) displaying similar topologies and matching strongly with the Dubitzky (2007) hypothesis. Our analysis recovers the monophyly of Anthophorini including *Paleohabropoda* (supported by characters 2 and 13) (Fig. 3). Other Apidae (*Centris* + *Bombus*) are their sister group. Observing the majority rule consensus of the above topologies, *Paleohabropoda* branches as the sister genus of (*Elaphropoda* + *Habrophorula*) within Anthophorini. This latter tribe includes two monophyletic groups: (*Paleohabropoda* (*Elaphropoda* + *Habrophorula*)) and (*Pachymelus*, *Habropoda*, *Deltoptila* (*Amegilla* + *Anthophora*)). The 16 shortest trees differ mainly in the branching of the genera associated in the latter group. The divergences between our trees and those obtained by Dubitzky (2007) must be interpreted as the result of the absence of several key-characters linked to the preservation of the fossil studied. The consensuses (50% majority rule and strict) of these 16 trees differ from the previous Dubitzky hypothesis only in the relative position of *Habropoda* and *Pachymelus*.

Morphometric analysis

The tps-Small test reveals a high correlation coefficient (0.999997), which allows us to be confident in the variation amplitude of our dataset. Moreover, the complete dataset (17 landmarks) and the subdataset (15 landmarks) lead to exactly the same results. Hereafter we discuss the results from the complete dataset.

The relative warps analysis (Fig. 4) and the neighbour-joining tree (Fig. 5) largely corroborate the result of the phylogenetic analyses. In both morphometric analyses (Figs 4, 5), the Anthophorini are quite separate from other Apidae (*Centris* sp. and *Bombus mendax*). *Paleohabropoda oudardi* sp. n. is clearly included in the Anthophorini and more precisely within the group of *Habropoda s.l.* This group includes the Anthophorini sharing 1 m-cu vein terminating near the apex of the second submarginal cell (Fig. 2C–F): *Deltoptila*, *Elaphropoda*, *Habrophorula*, *Habropoda* and *Pachymelus*. Brooks (1988) considered this group as a tribe (Habropodini). The *Anthophora sensu lato* (*Anthophora* and *Amegilla*) constitutes the second group, which is characterized by vein 1 m-cu joining the second submarginal cell near the middle.

In the relative warps analysis, the genus *Pachymelus* is well isolated from the *Habropoda s.l.* As members of this genus are clearly bigger than members of the other Anthophorini, we suspected an influence of the size on the shape as previously shown in other bees (Danforth 1989). We therefore processed a linear regression using R software (R Development Core Team 2004) to estimate the effect of the size on the shape (relative warps). The shape is not highly correlated with the size in axis 1 (t-test on slope of regression, P-value = 6.39 10^{-3}). However, axis 2 is strongly correlated with the size (t-test on slope of regression, P-value = 8.22 10^{-8}) partly explaining the position of *Pachymelus* in the relative warps analysis (Fig. 4).

Discussion

Anthophorini systematics

With about 700 described species, the Anthophorini is one of the most diverse tribes in Apidae (Michener 2007). Marikovskaya (1976) and Brooks (1988) successively proposed to split the Anthophorini into two different tribes: Anthophorini (i.e., *Anthophora* and *Amegilla*) and Habropodini (including the five
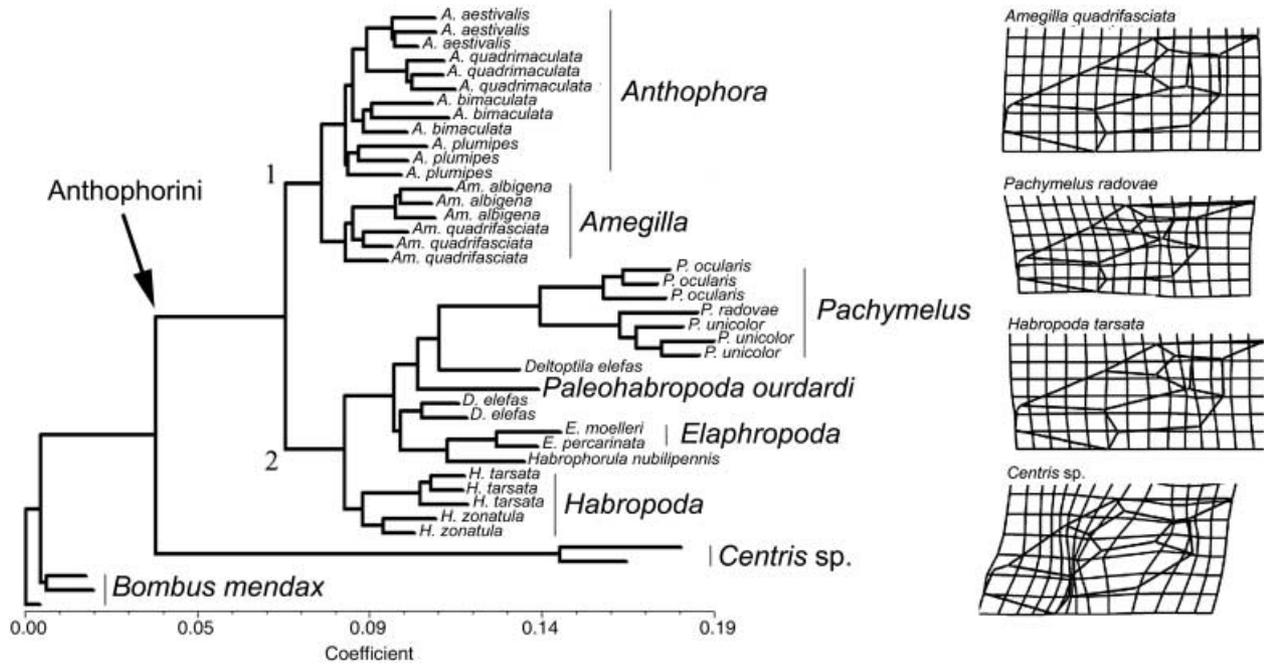
Fig. 5 Neighbour-joining tree rooted by one specimen of *B. mendax*. 1 = *Anthophora sensu lato*; 2 = *Habropoda sensu lato*. On the right, four deformation grids extracted from the relative warps analysis.

other genera). However, Michener (2007) as well as Dubitzky (2007) concluded that the Habropodini *sensu* Marikovskaya (1976) constitute a basal paraphyletic group from which the other Anthophorini emerge. Both hypotheses, one or two tribes, match with the present topologies obtained, respectively, by phylogenetic and morphometric analyses (Figs 3 and 5). The morphological similarity (phenetic argument) allows us to recognize two groups but the polarization of the characters (cladistic argument) shows only one monophyletic group. As we used a strict cladistic approach acknowledging only genealogical relationships to define taxa, we consider one tribe: the Anthophorini.

Palaeobiogeographical and palaeoenvironmental implications

Distributions and phylogeny of extant taxa suggested an Asian origin for Anthophorini (Dubitzky 2007). First, genera *Elaphropoda* + *Habrophorula* are the sister group of the remaining Anthophorini. Second, these genera and three additional ones (*Habropoda*, *Amegilla*, and *Anthophora*) exhibit their greatest diversity in the Oriental region (South-eastern Asia). However, the record of *Paleohabropoda oudardi* sp. n. from the Paleocene in Europe challenges this hypothesis and suggests that the extant South-eastern Asian distributions could be relics. In fact, Anthophorini could have originated from anywhere (East, Central or West) in the Palaearctic region during the tropical eras of the late Cretaceous or the early Paleocene.

After the cooler Cenozoic, South-eastern Asia would have constituted a tropical refuge for most Anthophorini and not their place of origin. This scenario has already been demonstrated for several mammalian communities (e.g., Marivaux *et al.* 2006).

This hypothesis is supported by the palaeoenvironment of *Paleohabropoda oudardi* sp. n. This fossil species probably lived in a wet and very warm climate. The area of Menat was characterized ~60 Myr ago by a forest of oak and willow distributed around a crater lake (Piton 1940). The fauna included crocodiles, very numerous large Mantodea: Chaeteessidae, Blattodea, Coleoptera: Buprestidae and Cerambycidae, Odonata: Megapodagrionidae, and very diverse Hemiptera: Fulgoroidea, all indicative of a warm palaeoclimate and a forest palaeoenvironment (Piton 1940; Nel & Roy 1996; Nel *et al.* 1997). Moreover, extant taxa of Anthophorini are partly associated with wet and rather warm areas, and three genera are strictly endemic in this kind of climate (the mesoamerican genus *Deltoptila* and the Asian basal group of *Elaphropoda* + *Habrophorula*). The current palaeoclimatic interpretation for Menat is therefore congruent with the hypothesis that the association of Anthophorini with wet and warm climates could be an ancestral feature. The colonization of drier areas, as for the genera *Anthophora* and *Amegilla*, could be a derived characteristic.

Fossil records and early diversification of Apidae

Apidae has one of the best fossil records of any bee family. However, the relative abundance of records varies strongly
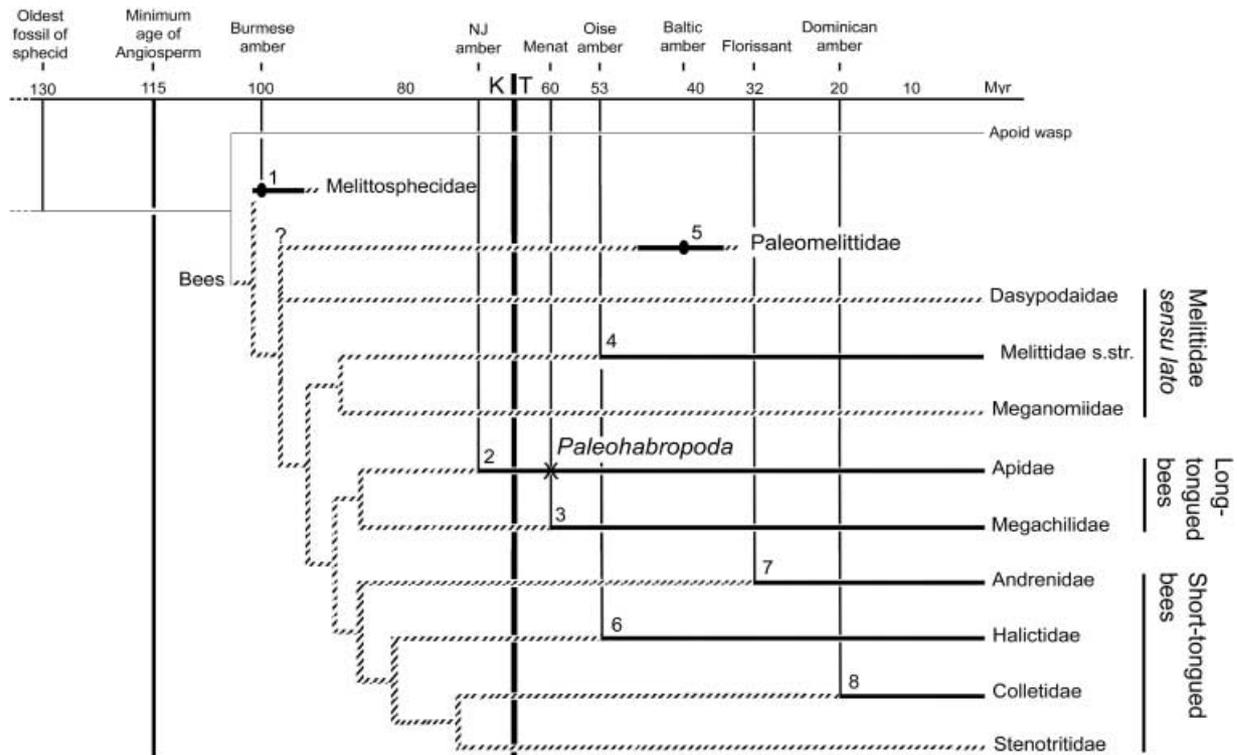
Fig. 6 Chronogram of the bee families with mapping of oldest Apoidea fossils. Cretaceous divergence of families according to Danforth *et al*. (2004). Phylogeny according to Danforth *et al*. (2006b), the position of Paleomelittidae Engel 2001 is not resolved. Age of the oldest fossil of sphecid according to Grimaldi & Engel 2005; minimum age of Angiosperm according to Crepet *et al*. 2004. 1 = *Melittosphex burmensis* Poinar & Danforth 2006 (~100 Myr); 2 = *Cretotrigona prisca* (Michener & Grimaldi 1988) (~65 to 70 Myr); 3 = *Probombus hirsutus* Piton 1940 (~60 Myr); 4 = *Paleomacropis eocenicus* Michez & Nel 2007 (~53 Myr); 5 = *Paleomelitta nigripenis* Engel 2002 (~45 Myr); 6 = *Halictus? saveneyi* Engel & Archibald 2003 (~53 Myr); 7 = *Andrena* spp., (~32 Myr); 8 = *Chilicola gracilis* Michener & Poinar 1996 and *Chilicola electrodominica* Engel 1999 (~20 Myr).

among the three subfamilies. Most records consist of taxa obviously belonging to the corbiculate Apinae (Engel 2001b; Patiny *et al*. 2007). No extinct Nomadinae has been described so far and only a few Xycolopinae have been recorded. The oldest specimens in that latter subfamily are the three species of *Boreallodape* Engel 2001 from Baltic amber (~45 Myr) (Engel 2001a).

Within Apinae, Cenozoic corbiculate fossils were associated with the four modern tribes: Apini, Bombini, Meliponini, Euglossini, as well as in three extinct groups: Electrapini, Electrobombini and Melikertini. One compression of Eucerini was formerly described from the upper Oligocene (*Eucera mortua* Meunier 1920; for discussion of systematic see Statz 1936; Kelner-Pillault 1969; Zeuner & Manning 1976; and Engel 2001a) and another putative eucerine has been described from the middle Eocene of Germany (Wappler & Engel 2003). Only two badly preserved fossils of Anthophorini have been described so far: *Anthophora melfordi* Cockerell 1908 from Florissant shale (early Oligocene) and *Anthophora effosa* (Heyden 1862) from Rott, West-Germany (Oligocene).

because of the poor quality of these specimens, their association with the tribe Anthophorini is questionable (Zeuner & Manning 1976; Brooks 1988). *Paleohabropoda oudardi* sp. n. is consequently also the first fossil obviously belonging to Anthophorini.

Together with the previously described Cretaceous Apidae (*Cretotrigona prisca*), the results of the present study underline that (1) Apidae is an old lineage; (2) Two extant tribes (Meliponini and Anthophorini) were already differentiated ~60 Myr ago; (3) Several of the oldest lineages are tropical. Moreover, the morphology of both fossils is surprisingly close to the extant taxa. The present morphometric study shows that the wing shape is very highly conserved, at least in the Anthophorini clade. Further studies would be warranted to explore the phylogenetic signal of the wing shape in other bee groups. If this phylogenetic signal is strong enough in other bee groups as it is in Anthophorini, morphometric methods of the present article could be developed in a global review of bee fossil compressions (e.g., *Halictus? saveneyi*) bringing new evidences of their association with extant taxa.

Broader significance in bee evolution

It is noteworthy that the oldest bee fossils known so far belong to the group 'Melittidae + long tongued bees (i.e., Apidae + Megachilidae)' (Fig. 6). The oldest nonmelittid short tongued bee is *Halictus? saveneyi* (Halictidae) from the early Eocene of Canada (Engel & Archibald 2003). Conversely, the known representatives of the other families are much more recent: *Andrena* spp. (Andrenidae, Florissant, 32 Myr), *Chilicola gracilis*, and *Chilicola electrodominica* (Colletidae, Dominican amber, 20 Myr; Michener & Poinar 1996; Engel 1999). The resulting temporal distribution of the fossil archives for bees provides additional support to the hypothesis by Alexander & Michener (1995) and Danforth *et al.* (2006a,b) designating the 'Melittidae + long tongued bees' group as the most basal group of Apoidea (Fig. 6). However, absence of data cannot be considered conclusive, as one cannot prove a negative. Given the overall rarity of bee fossils continued exploration at deposits throughout the world will be the only arbiter for resolving the earliest phases of bee evolution.

Acknowledgements

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Appendix 1

Characters for the phylogenetic analyses.

Forewing

1. Second submarginal cell: (0) distinctly shorter than the first and the third submarginal cells; (1) shorter than the first, about as long as the third; (2) longer than the first and as long as the third.

2. First recurrent vein (1m-cu) joining second submarginal cell: (0) near middle; (1) near apex.

3. Front margin of the second submarginal cell: (0) about half as long as hind margin or shorter; (1) distinctly longer than half the length of hind margin.

4. Third submarginal cell: (0) front margin distinctly shorter than hind margin; (1) front margin about as long as hind margin.

5. Cu-V vein meeting M+Cu-vein: (0) behind M-vein; (1) at the intersection of M- and Cu-veins; (2) before M-vein.

6. Margin cell: (0) short, about four times as long as broad; (1) medium long, about five times as long as broad; (2) long, nearly seven times as long as broad.

7. Distance from apex of marginal cell to wing tip: (0) about as long to slightly longer than marginal cell; (1) distinctly longer than marginal cell; (2) distinctly shorter than marginal cell.

8. Pterostigma: (0) well-developed, at least as long as broad; (1) nearly absent, minute, broader than long.

9. Distal part of wing: (0) not papillate; (1) papillate.

Hind wing

10. Vein Cu-V: (0) distinctly shorter (about half as long) than second abscissa of M+Cu-vein; (1) about as long as second abscissa of M+Cu-vein; (2) distinctly longer than second abscissa of M+Cu-vein.

11. Vein cu-v: (0) nearly transverse, at angle of 50° or more to first abscissa of M+Cu; (1) conspicuously oblique, at angle of 45° or less to first abscissa of M+Cu.

Other body parts

12. Antennal segment 3 (female): (0) distinctly shorter than scape; (1) about as long as scape.

13. Hind basitarsus penicillus (female): (0) hind basitarsus giving rise to second tarsomere at apex; (1) projecting distal above articulation of second tarsomere but no penicillus.

14. Median disc of S7 (male): (0) absent, apical and basal apodemal region joining each other without an interspace; (1) elongate, broadly separating apical and apodemal parts.

15. Apex of gonostylus (male): (0) distinctly protruding beyond apex of penis valvae; (1) not or only slightly beyond apex of penis valvae.

16. Ventral lobe of gonocoxite (male): (0) absent; (1) distinctly developed.

17. Shape of dorsal gonostylus (male): (0) slender, strongly spatulate; (1) oval; (2) truncate, weakly spatulate to slightly oval.