

Antiquity of cleptoparasitism among bees revealed by morphometric and phylogenetic analysis of a Paleocene fossil nomadine (Hymenoptera: Apidae)

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Abstract. Cleptoparasitism is a way of life involving the theft of resources by one animal from another. This behaviour occurs in many bee tribes but its origin and evolution remain obscure, particularly owing to the relative scarcity of bees in the fossil record. Hitherto, no fossil evidence has been recorded to trace the origin of cleptoparasitim among bees. In the current study, we present the first cleptoparasitic bee fossil, providing analyses of its taxonomic affinities and a complete description. The specimen also happens to be one of the earliest bee fossils, having been discovered in the spongo-diatomitic volcanic paleolake of Menat (Paleocene) in France. We employed geometric morphometrics of the forewing shape to assess the taxonomic affinities of the fossil with modern apoid tribes. Our dataset included 979 specimens representing 50 tribes and 225 extant species. Based on linear and geometric morphometrics, we demonstrate that the fossil's forewing shape is similar to that of Apidae, and particularly to that of the tribe Epeolini (Nomadinae). The fossil is described as *Paleoepeolus micheneri* gen.n., sp.n. and provides the first direct evidence on the antiquity of cleptoparasitism among bees.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid: zoobank.org;pub:BBBE2217-6CAE-48F9-851F-716B813DFEBF.

Introduction

Parasitism, characterized by the benefit of one species to the detriment of another, is perhaps the most widespread mode of life on Earth, at least in terms of sheer species numbers (Thompson, 1994). More than one half of all known species are parasitic at some stage of their development (Anderson & May, 1982; Windsor, 1998). Among animals, parasites in all of their varieties can be found in virtually every phylum with the exception of the Echinodermata (Price, 1980). This life history is manifest in varied forms, ranging from endoparasitism,

Price (1980) for definitions]. Among these, cleptoparasitism is a strategy involving the theft of resources, typically food or nesting materials, by one animal from another (Price, 1980). Cleptoparasites are found among bony fishes (Davis & Dill, 2012), birds (Brockmann & Barnard, 1979), mammals (Honer *et al.*, 2002), spiders (Rypstra, 1979), and, of course, various lineages of insects (Roques, 1976; Weaving, 1989; Spofford & Kurczewski, 1990). Successful parasitism necessitates a series of specializations in behaviour, phenology, morphology, physiology and even development (Price, 1980).

ectoparasitism and mesoparasitism, to cleptoparasitism [see

Cleptoparasitism and social parasitism have appeared numerous times across most clades of bees, and parasites greatly outnumber social lineages both in numbers of species and in independent origins (Rozen, 2000; Michener, 2007; Cardinal

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et al., 2010; Cardinal & Danforth, 2011; Litman et al., 2013). Unlike some clades of social bees where there are reversals to solitary living (Michener, 2007), there is no known case of reversion to free living from within an originally cleptoparasitic clade. In total, 26 tribes of bees contain cleptoparasites. These are distributed across the families Colletidae, Halictidae, Megachilidae and Apidae (Rozen, 2000; Michener, 2007). With the host dispatched, the cleptoparasite then feeds on the host's provisions before pupating and later emerging as an adult. Females of free-living bees have several morphological and behavioural modifications for the collection and transport of pollen, nectar and plant oils (e.g. scopae or corbiculae for the transport of pollen; elongate mouthparts to forage on nectar from floral nectaries; elongation of forelegs in Rediviva Friese for collecting oils) (Michener, 2007), and these specializations have putatively fuelled the diversification of Eudicots during the Cretaceous (Michener, 1979; Engel, 2001; Cardinal & Danforth, 2013). Naturally, female cleptoparasitic bees do not forage for pollen, but will visit flowers for nectar, and accordingly lack the typical anatomical specializations for the handling and transport of pollen (Michener, 2007). The result of this loss of pollen-collecting behaviours results in a generalized syndrome found across adult cleptoparasitic bees, namely an overall loss of pilosity and pollen-transporting apparatuses, and a frequently thicker cuticle (to protect against the potential sting of a defending host) (Michener, 1996, 2007).

Direct evidence regarding the origins and antiquity of parasitism among bees has been lacking, although molecular-based estimates have suggested that cleptoparasitism in bees is at least of Late Cretaceous age, c. 95 Ma (Cardinal et al., 2010). Unfortunately, the entire fossil record of bees is meagre when compared with other insect lineages, with only about 200 described fossil species (Michez et al., 2012). Hitherto, only one fossil species, Protomelecta brevipennis (Cockerell) (Cockerell, 1908; Zeuner & Manning, 1976), from the Eocene-Oligocene boundary (c. 35 Ma) of Colorado, has been tentatively described as cleptoparasite. The assumption of parasitism for P. brevipennis was based on an apparent lack of a metafemoral scopa, but this may reflect the degree of preservation as the hindlegs are largely obscured in the holotype (M.S. Engel, personal observation), and perceived similar habitus on the part of its original describer to modern melectine bees. It remains to be determined whether or not P. brevipennis is a cleptoparasite or has any affinity to modern parasitic bee lineages (Michez et al., 2012).

The discovery of a Paleocene (c. 60 Ma) fossil bee that can be placed among a subordinate clade of obligate cleptoparasitic bees is therefore the first direct fossil evidence of a cleptoparasitic lineage. This fossil can be placed among the parasitic tribe Epeolini, itself a derived group of a larger subfamily of cleptoparasitic bees, the Nomadinae (Michener, 2007). Phylogenetic and geometric morphometric analyses of forewing shape were employed to assess the similarity and affinity of the fossil among modern bee groups. We assembled a comprehensive sampling of modern taxa forewings, representing 225 species, 135 genera, 50 tribes, 18 subfamilies, and all seven families of bees. We investigated the same dataset by using two alternative analyses: geometric morphometrics of forewing shape and a heuristic phylogenetic landmark analysis under parsimony. The fossil reveals the earliest evidence of cleptoparasitism in bees, and, owing to the phylogenetic position of epeolines among Anthophila, corroborates prior hypotheses of an even deeper origin and diversification of cleptoparasitism across bees.

Materials and methods

Description, terminology and repositories

For the systematic analyses and descriptions, comparative morphological terminology follows that of Engel (2001), while the classification adopted follows that of Michener (2007) and Danforth et al. (2013). The type material is deposited in the Museum National d'Histoire Naturelle (Paris, France). The taxonomic actions are registered under ZooBank LSIDs urn:lsid:zoobank.org:act:B457A7B9-D47C-46C4-9FE3-DEF73 D4E65AB [for generic name] and urn:lsid:zoobank.org:act:98E7 C6C3-6F9D-4A71-AD1D-554D5EC09680 [for specific epithet]. Representative specimens were sampled from the following collections: Laboratoire de Zoologie (University of Mons, Belgium); Département d'Entomologie Fonctionnelle et Evolutive (University of Liège, Belgium); Royal Museum of Central Africa (Tervuren, Belgium); Royal Belgian Institute of Natural Sciences (Brussels, Belgium); the Natural History Museum (London, United Kingdom); Naturalis Biodiversity Center (Leiden, the Netherlands); Zoologische Staatssammlung München (Munich, Germany); Oberösterreichisches Landesmuseum (Linz, Austria); University of Neuchâtel (Neuchâtel, Switzerland); Museum of Comparative Zoology (Cambridge, MA); American Museum of Natural History (New York, NY); University of Kansas Natural History Museum (Lawrence, KS); and York University (Toronto, Canada).

Geological setting

The basin deposit of Menat (Puy-de-Dôme, France), a spongo-diatomitic volcanic paleolake, is composed of shale and clay soil impregnated with iron oxide (Russel, 1967, 1982). The palaeoenvironment is inferred to be a forest of oak and willow distributed around a crater lake (Piton, 1940). The fauna is represented by few vertebrates [mainly 'fishes', but also crocodiles (Crocodylidae) and mammals (Mammalia)] and diverse Insecta. Insects are mainly represented by mantises (Mantodea), cockroaches (Blattaria), beetles (Cerambycidae, Buprestidae, Curculionoidea, etc.), dragonflies and damselflies (Odonata), and true bugs (Hemiptera), and the taxa generally confirm a forest paleoenvironment and a warm and wet paleoclimate (Piton, 1940; Guth, 1962; Russel, 1967; Nel & Roy, 1996; Nel et al., 1997; Matsumoto et al., 2013). The deposit has also produced trace fossil evidence for the activity of leafcutter bees, although body fossils remain to be discovered (Wedmann et al., 2009).



Fig. 1. Left forewing of *Bombus terrestris* (Linnaeus, 1758), with the 18 landmarks used in phylogenetic analyses and indicated to describe the shape in geometric morphometric analyses.

Phylogenetic analysis of landmarks under parsimony

Wings are useful for morphometric studies because they are rigid, comparatively simple two-dimensional structures, often species-specific, and well preserved in fossils (Pavlinov, 2001). Moreover, forewing veins and intersections are probably homologous among bees in taxa with three submarginal cells (Ross, 1936; Michener, 2007). We assembled a dataset of specimens sampled broadly across extant tribes of bees with three submarginal cells in the forewing. The tribal dataset included 979 female specimens representing seven families, 18 subfamilies, 50 tribes, 135 genera and 225 species (Table 1). In order to maximize the morphological diversity of the dataset, we sampled four species per tribe and five specimens per species, when possible.

Forewings were photographed using a Nikon D200 camera coupled with an Olympus SZH10 microscope. Photographs were input in TPS-UTIL 1.56 (Rohlf, 2013a). The left forewing shape of the specimens was captured from photographs by digitizing two-dimensional Cartesian coordinates of 18 landmarks on the wing veins (Fig. 1) with TPS-DIG v2.17 (Rohlf, 2013b). The landmarks configurations of the reference datasets were superimposed using the GLS Procrustes superimposition (Rohlf & Slice, 1990; Bookstein, 1991). This was done using the package 'geomorph' on the software R version 3.0.2 (Adams & Otárola-Castillo, 2013; R Development Core Team, 2013). The closeness of the tangent space to the curved shape space was tested by calculating the least-squares regression slope and the correlation coefficient between the Procrustes distances in the shape space with the Euclidean distances in the tangent space (Rohlf, 1999) using the software TPS-SMALL v1.25 (Rohlf, 2013c). Landmark coordinates resulting from digitization of wing shape of the fossil and of representative extant specimens are available in Tables S1 and S2.

Morphometric data (i.e. landmark coordinates) were used to place the fossil in an evolutionary framework using phylogenetic morphometric methods (Catalano *et al.*, 2010; Goloboff & Catalano, 2011; Catalano & Goloboff, 2012). In this paper as well as in previous works (e.g. De Meulemeester *et al.*, 2012; Wappler *et al.*, 2012), it was shown that there is a structure of the individuals for each tribe in the shape space of a linear discriminant analysis (LDA). In other words, the wing shapes of a given tribe constitute a well-defined unit in the shape space. Given the computational cost of phylogenetic analyses including all of the 979 sampled specimens, the tribal unit was used by summarizing

Table 1. Reference dataset of Anthophila with three submarginal cells for the geometric morphometric and heuristic phylogenetic analyses.

Family	Subfamily	Tribe	N1	N2
Andrenidae	Andreninae	Andrenini	4	20
Apidae		Euherbstiini	3	20
	Oxaeinae		4	20
	Panurginae	Melitturgini	4	19
		Nolanomelissini	1	10
		Protandrenini	5	20
	'Non-parasitic'	Ancylaini	7	20
		Apini	4	20
		Bombini	4	20
		Centridini	4	20
		Emphorini	4	20
		Eucerini	4	20
		Euglossini	4	20
		Exomalopsini	6	20
		Tapinotaspidini	5	20
		Tetrapediini	5	20
	'Parasitic'	Anthophorini	6	20
		Brachynomadini	5	20
		Ericrocridini	4	20
		Epeolini	7	20
		Isepeolini	4	20
		Melectini	6	20
		Nomadini	4	20
		Osirini	5	20
		Protepeolini	4	20
		Rhathymini	3	20
	Xylocopinae	Ceratinini	4	20
		Manuelinii	3	20
~ ~ ~ ~ ~	~ ~ ~ ~ ~	Xylocopini	4	20
Colletidae	Callomelittinae	~	3	12
	Colletinae	Colletini	4	20
	Neopasiphaeinae Diphaglossinae	~	4	20
		Caupolicanini	7	20
		Diphaglossini	3	20
		Dissoglotini	3	20
Halictidae	Halictinae	Augochlorini	4	20
		Caenohalictini	5	20
		Halictini	5	20
		Sphecodini	5	20
		Thrinchostomatini	4	20
	Nomiinae		4	20
	Nomioidinae	~	5	20
	Rophitinae	Conanhalictini	6	20
		Penapini	4	19
		Rophitini	6	20
		Xeralictini	3	20
Megachilidae	Fideliinae	Fideliini	3	20
Melittidae	Meganomiinae		6	20
G	Melittinae	Melittini	6	20
Stenotritidae			8	19

This sampling includes 979 specimens from 225 species, 135 genera, 50 tribes, 18 subfamilies and seven families of Anthophila. *N1*, number of species; *N2*, number of specimens.

for each tribe their median wing shape. We also estimated the difference in forewing shape between and within the different tribes of bees. The difference in forewing shapes between tribes explained most of the wing shape variation. The variation within tribes was estimated by the mean squares of the residuals of a linear model of the tribe factor on the forewing shape data. It

corresponded to 1.13% of the variation between tribes that was estimated by the mean squares of the tribe effect in the same model. Considering the low forewing shape variation within tribes and the computational cost of adding taxa in the phylogenetic analysis including landmark data, we decided to perform the heuristic phylogenetic analysis using only one forewing per tribe. Analyses were performed on the median wing of each of the 50 tribes of bees, the fossil wing and one outgroup forewing. Median wing shape was calculated by using the function find-MeanSpec from the package 'geomorph' in R version 3.0.2 (Adams & Otárola-Castillo, 2013; R Development Core Team, 2013), meaning that for each tribe the specimen lying closest to the estimated mean shape was identified. The outgroups were the crabonid wasps (Apoidea: Crabronidae), selected for their bee-like morphology among spheciforms and their phylogenetic relatedness with bees in recent studies of Apoidea (Alexander, 1992; Prentice, 1998; Melo, 1999; Ohl & Bleidorn, 2006; Pilgrim et al., 2008; Debevec et al., 2012). Analyses were performed with the software TNT version 1.5 (Goloboff & Catalano, 2016). Parameters include the number of replicates, the accuracy of the grids used to reconstruct the ancestral landmark positions, the number of nested grids used to refine the estimation of the ancestral landmark position, the number of cells covered by the nested grids during the refinement step and the use of dynamic realignment. Landmarks analyses were performed using eight cell grids with a four nested grid search of 200%. Tree search was performed using 'New Technology' algorithms including Sectorial search, Ratchet, Drift and Tree fusing, with minimal length found four times. The search started using landmark data from the Procrustes superimposition. The superimposition was then modified during the search according to dynamic realignment methods minimizing the landmark displacement along the tree (Catalano & Goloboff, 2012). Supports for nodes (i.e. group frequencies resulting from landmark resampling) were assessed using landmark resampling of 1000 repetitions (Fig. 2). The results of the present phylogenetic analysis are merely heuristic given that they are based solely on median wing shape, and do not attempt to integrate character data from other sources (e.g. mouthparts, genitalia), owing to an inability to code most pertinent traits from the fossil. Thus, these results are best when considered in conjunction with the results of the geometric morphometric analysis and for ascertaining the fossils' most likely placement among Apidae.

Geometric morphometric analysis

Geometric morphometrics of forewing shape were performed on the same sampled specimens (Table 1) to explore in an alternative method the taxonomic affinity of the fossil. Geometric morphometrics provides quantification of the shape of a structure (Bookstein, 1991; Rohlf & Marcus, 1993; Adams *et al.*, 2004). It is used in paleontology for diagnosing fossil taxa at different levels, and for estimating their taxonomic affinities (i.e. similarities) with extant taxa (Roberts, 2008; Michez *et al.*, 2009; De Meulemeester *et al.*, 2012). After phylogenetic and exploratory geometric morphometric analyses indicated affinity to the cleptoparasitic Apinae and especially the tribe Epeolini, we analysed a second dataset sampling more extensively across genera of this tribe, as well as the closely related Brachynomadini and Nomadini according to the phylogeny of Cardinal *et al.* (2010), and the closely related tribe Melectini according to our heuristic phylogenetic results (Fig. 2). We sampled 21 specimens of Brachynomadini (three genera and five species), 20 specimens of Nomadini (one genus and four species), 27 specimens of Melectini (four genera and nine species), and 146 specimens of Epeolini (eight genera and 25 species), accounting for a total of 214 specimens (Table 2).

Shape variation within the reference dataset and discrimination of the different taxa was assessed by LDAs of the projected aligned configuration of landmarks at family, subfamily and tribal levels as a priori grouping by using the software R. The LDA effectiveness was assessed by the percentages of individuals correctly classified to their original taxon (hit-ratio, HR) in a leave-one-out (LOO) cross-validation procedure based on the posterior probabilities (PPs) of assignment (Tables S3-S6). Given the observed scores of an 'unknown', the PP is equal to the probability of the unit belonging to one group compared with all others. The unit is consequently assigned to the group for which the PP is the highest (Huberty & Olejnik, 2006). Taxonomic affinities of the fossil were first assessed based on the score in the predictive discriminant space of shapes. Aligned coordinates of the specimens from the reference dataset were used to calculate the LDA. We included a posteriori the fossil specimen in the computed LDA space as 'unknown' specimen and calculated its score. Assignment of the fossil was estimated by calculating the Mahalanobis distance between 'unknown' and the group mean of each taxa (Tables S7-S10). We also calculated PPs of assignment to confirm the assignment to one taxon. Assignments of the fossil were performed in three consecutive analyses corresponding to different taxonomic levels of a priori grouping for the reference dataset: family, subfamily and tribe.

Results

Similarity and phylogeny of wing shape

In the strict consensus topology of a heuristic phylogenetic analysis of forewing shape space, the fossil was recovered within a group of cleptoparasitic apid tribes consisting of Epeolini, Ericrocridini, Melectini, Nomadini and Rhathymini (Fig. 2). These results indicate placement of the fossil as well-nested within the Nomadinae, a subfamily of exclusively cleptoparasitic bees. Contemporary taxa of bees (families, subfamilies and tribes) are well discriminated based on their forewing shape, with a global hit-ratio of 95.21% for family a priori grouping, 95.20% for subfamily a priori grouping, and 96.32% for tribe a priori grouping (Tables S3-S5). In the three discriminant analyses the fossil was assigned to Apidae [Mahalanobis distance (MD) = 4.28; PP = 1], among the 'parasitic Apidae' (MD = 6.52; PP = 1), as well as the tribe Epeolini (MD = 11.10;PP = 1), respectively (Tables S7–S9). Based on these results, the fossil was analysed more extensively against the forewing shape



Fig. 2. Resampled, heuristic phylogenetic tree based on landmarks analysis under parsimony of forewing landmarks (resampling of 1000 repetitions). Support for tree nodes are group frequencies from landmarks resampling. Cleptoparasitic tribes are highlighted in black and non-cleptoparasitic tribes in grey, and the position of *Paleoepeolus micheneri* gen., sp.n. is indicated by a black star. Note that this analysis is based solely on forewing shape and does not integrate other forms of critical character data (e.g. mouthparts, genitalia) owing to an inability to code such traits from the fossil. It is therefore meant solely as a heuristic tool in ascertaining the general affinities of the fossil among Apidae, and corroborates placement within the cleptoparasitic Nomadinae.

Table 2. Reference dataset of Brachynomadini, Epeolini, Melectini and Nomadini for the geometric morphometric analyses.

BrachynomadiniBrachynomada margaretae (Rozen, 1994)5Brachynomada roigella (Michener, 1996)2Paranomada nitida Linsley & Michener, 19375Paranomada velutina Linsley, 19394Triopasites penniger (Cockerell, 1894)5EpeoliniDoeringiella arechavaletai (Brethes, 1909)Doeringiella baeri (Vachal, 1904)5Doeringiella bipunctata (Friese, 1908)5Doeringiella gayi (Spinola, 1851)5
Brachynomada roigella (Michener, 1996)2Paranomada nitida Linsley & Michener, 19375Paranomada velutina Linsley, 19394Triopasites penniger (Cockerell, 1894)5Doeringiella arechavaletai (Brèthes, 1909)5Doeringiella baeri (Vachal, 1904)5Doeringiella bipunctata (Friese, 1908)5Doeringiella gayi (Spinola, 1851)5Encolus acompetitu Creacer, 18725
Paranomada nitida Linsley & Michener, 1937SParanomada velutina Linsley, 19394Triopasites penniger (Cockerell, 1894)5Doeringiella arechavaletai (Brèthes, 1909)5Doeringiella baeri (Vachal, 1904)5Doeringiella bipunctata (Friese, 1908)5Doeringiella gayi (Spinola, 1851)5Encolus competitu Cracere, 18725
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Doeringiella bipunctata (Friese, 1908)5Doeringiella gayi (Spinola, 1851)5Encolus competito Graces 18725
Doeringiella gayi (Spinola, 1851) 55
Encolus compostus Crossen 1979
<i>Epeotus compactus</i> Cresson, 18/8
Epeolus cruciger (Panzer, 1799) 5
Epeolus pusillus Cresson, 1864
Epeolus scutellaris Say, 1824
Odyneropsis armata (Friese, 1900)
Odyneropsis batesi Cockerell, 1916
Odyneropsis sp. 1
Pseudepeolus bizonata Holmberg, 1886 5
Pseudepeolus cingillata Moure, 1954
Pseudepeolus gayi (Spinola, 1851) 5
Pseudepeolus holmbergi (Schrottky, 1913) 5
Pseudepeolus singularis (Friese, 1908)
Rhinepeolus rufiventris (Friese, 1908)
Rhogepeolus bigibbosus Moure, 1955 7
Rhogepeolus plumbeus (Ducke, 1910) 5
Thalestria spinosa (Fabricius, 1804) 5
Thalestria sp. 1
Triepeolus circumculus Rightmyer, 2008 5
Triepeolus concavus (Cresson, 1878) 5
Triepeolus tristis (Smith, 1854)
Triepeolus verbesinae (Cockerell, 1897) 5
Melectini Melecta albifrons (Forster, 1771)
Melecta luctuosa (Scopoli, 1770)
Melecta punctata (Fabricius, 1775) 5
Thyreus abdominalis (Friese, 1905) 55
Thyreus orbatus (Lepeletier, 1841)
Thyreus nitidulus Gibodo, 1884 5
Tetralonioidelia hymalayana Bingham, 1897 4
Tetralonioidelia pendleburyi Cockerell, 1926 1
Xeromelecta californica (Cresson, 1878) 4
Nomadini Nomada fabriciana (Linné, 1767) 5
Nomada flava Panzer, 1798 5
Nomada fucata Panzer, 1798 5
Nomada goodeniana (Kirby, 1802) 5

This sampling includes 214 specimens from 43 species, 16 genera and four tribes of Anthophila. *N*, number of specimens.

of a diverse set of genera from Epeolini, as well as the tribes Brachynomadini, Melectini and Nomadini (Fig. 3). The four tribes are perfectly discriminated based on their forewing shape, with a global HR of 100%. Based on genus *a priori* grouping, the genera are well discriminated based on their forewing shape, with a global HR of 97.66% (Table S6). The Menat fossil was again assigned to Epeolini (Epeolini, MD = 4.50, PP = 1; Nomadini, MD = 10.22, pp = $6.53e^{-20}$; Brachynomadini, MD = 10.84, PP = $8.96e^{-23}$; Melectini, MD = 14.56, PP = $3.07e^{-43}$), but outside of any of the sampled genera (Table S10; Fig. 4). The



Fig. 3. Tribal taxonomic affinity of *Paleoepeolus micheneri* gen.n., sp.n. based on forewing shape similarity. Ordination of the new fossil with Brachynomadini, Epeolini, Melectini and Nomadini along the first two axes (PC1 and PC2) of the principal components analysis. [Colour figure can be viewed at wileyonlinelibrary.com].

most similar genera in terms of forewing shape space were *Rhogepeolus* Moure (MD = 13.40; PP = 1.00), *Paranomada* Linsley & Michener (MD = 14.17, PP = $1.99e^{-5}$), *Doeringiella* Holmberg (MD = 14.44; PP = $8.75e^{-7}$) and *Rhinepeolus* Moure (MD = 14.67; PP = $3.07e^{-8}$).

Systematics

Family Apidae Latreille Subfamily Nomadinae Latreille Tribe Epeolini Robertson

Paleoepeolus Dehon, Engel, & Michez, gen.n.

The genus is registered under ZooBank LSID http://zoobank .org/urn:lsid:zoobank.org:act:B457A7B9-D47C-46C4-9FE3-DEF73D4E65AB (Fig. 4, Figures S1 and S2).

Type species. Paleoepeolus micheneri Dehon, Engel, and Michez, **sp.n.**

Etymology. The generic name is a combination of *palaios* (Greek, 'ancient') and *Epeolus*, type genus of the Epeolini. The gender of the name is masculine.

Diagnosis. Q (Fig. 4, Figures S1 and S2). Epeoliform body, i.e. similar to *Epeolus* Latreille, *Triepeolus* Roberston or *Doeringiella*, more robust than nomadiform and without scopa (sensu Michener, 2007), apparently without metallic colouration, without maculation; metatibia flared apically such that apex is more than twice the width of metabasitarsus; pubescence sparse, with scattered appressed setae on sterna. Forewing with elongate basal vein, 1cu-a postfurcal and in line with first abscissa Cu such that second abscissa Cu slightly below level of M + Cu; 2cu-a short (thus Cu prior to 2 m-cu shifted posteriorly); angle of Cu and 2 m-cu inside second medial cell obtuse



Fig. 4. *Paleoepeolus micheneri* **gen.n., sp.n.** (A–D), holotype, female. (A) General habitus of part (Oli 431) with segments, tergites and sternites labelled, ventral view; (B) general habitus of counterpart (Oli 436) with segments and sternites labelled, dorsal view; (C) detail of the left forewing of part; (D) reconstruction of the left forewing venation, with membranous cells and longitudinal sectors and cross-veins labelled, based on Engel (2001) (photographs by A. Nel and R. Garrouste). Labels: S, sternite; T, tergite; sm cell, submarginal cell; LF, left forewing; RF, right forewing. [Colour figure can be viewed at wileyonlinelibrary.com].

(as in higher Epeolini); basal vein (1 M) elongate, straight, meeting 1Rs slightly basad position of pterostigmal base; pterostigma short, broad, length less than 2.5 times width; three submarginal cells, first largest but shorter than combined length of second and third; second submarginal cell greatly narrowed anteriorly; 1rs-m and 2rs-m arched toward wing apex; 1 m-cu entering second submarginal cell, 2 m-cu arched and entering third submarginal cell; marginal cell of medium size, apex acutely rounded, very slightly offset from anterior wing margin and well distant from wing apex. Wing membrane not papillate or infuscate; marginal cell with dense setae in anterior half and radial cell lacking dense setae of any kind.

Paleoepeolus micheneri Dehon, Engel, & Michez, sp.n.

The species is registered under ZooBank LSID http://zoobank .org/urn:lsid:zoobank.org:act:98E7C6C3-6F9D-4A71-AD1D-554D5EC09680 (Fig. 4, Figures S1 and S2). *Holotype.* 'Holotype, Q, Oli 436 (part) and Oli 431 (counterpart), female, France, Puy-de-Dôme, spongo-diatomitic volcanic paleolake (maar) of Menat, Paleocene (60 Ma). Deposited in the Muséum National d'Histoire Naturelle, Paris, France.

Etymology. The specific epithet honours the late Charles D. Michener (1918–2015), the world's authority on bees, member of the National Academy of Sciences, and dear mentor and friend.

Diagnosis. Owing to monotypy, the diagnosis for the species is identical to that of the genus (*vide supra*).

State of preservation. Specimen preserved in lateral orientation in respect to head and mesosoma, with one forewing

extended obliquely out and downward, while other wings extended obliquely backward and away from body (Fig. 4, Figures S1 and S2). Metasoma twisted down and away from body, with ventral surface twisted outward, thereby largely exposing sterna, while overlapping portions of associated terga can be seen. Left tergal margins more extensively exposed than those of the right, as orientation of preservation is somewhat oblique. Five well-developed metasomal tergal margins observed, along with portions of a sixth apically, and at least five (perhaps six) sterna. Sternal graduli discernible, easily misinterpreted as segmental margins owing to an apical bend in metasoma resulting in partial contraction of segments. Small, linear remnants situated in an area of greater preserved sclerotization appear to preserve portion of sting and its associated sclerites. Left metatibia, metabasitarsus, and one or two subsequent tarsomeres probably present over portion of mesosoma and metasoma and just posterior to wing.

Description. Female (Fig. 4, Figures S1 and S2). A compressed specimen with both forewings preserved and outstretched. Part (Oli 431) in ventral view and counterpart (Oli 436) in dorsal view. Total body length as preserved 9.98 mm; head height 2.89 mm as preserved; mesosomal height 3.31 mm as preserved. Integument largely taphonomically altered, but apparently without maculation; head and mesosoma black as preserved, metasomal terga dark brown to black, apparently lighter in pre-gradular areas (evident basolaterally on at least terga III-V), sterna lighter as preserved (sterna are largely impressions with most cuticle lacking, where present integument is dark brown). Head in profile (integumental details difficult to discern but owing to overlapping positions of right and left scapes and flagella, the head appears to be in profile rather than twisted to the side in either direction); antenna slightly above mid-height of head; scape slender, approximately 3.5 times as long as wide; flagellum preserved but distinction between flagellomeres only discernible in a few places; surface of clypeus and part of mandible, right compound eye and part of left compound eye preserved. Mesosoma with mesosoma and mesoscutellum low, gently arched, forming continuous line between notal sclerites; metanotum not discernible but apparently short and declivitous owing to step drop posterior to mesoscutellum, and therefore basal area of propodeum likely comparatively short and declivitous. Left forewing 5.49 mm long as preserved, 1.78 mm wide. Forewing membrane not infumate or papillate; marginal cell with dense setae in anterior half; radial cell lacking dense setae of any kind; costal edge of wing setose; 1cu-a postfurcal and in line with first abscissa Cu such that second abscissa Cu is slightly below level of M+Cu; basal vein (1 M) elongate, much longer than 1Rs, straight, nearly in line with 1Rs, meeting 1Rs slightly basad pterostigmal base; 1Rs arising near pterostigmal base; pterostigma short, broad, length less than 2.5 times width, length 0.50 mm, width 0.21 mm; marginal cell of medium size, length 1.48 mm, apex acutely rounded, very slightly offset from anterior wing margin and well distant from wing apex; three sub-

marginal cells, first largest but shorter than combined length of second and third; second submarginal cell greatly narrowed anteriorly; cell metrics: first submarginal cell 0.73 mm long (as measured from origin of Rs + M to juncture of r-rs and 2Rs), 0.37 mm high (as measured from Rs + M to pterostigma); second submarginal cell 0.49 mm long (as measured from juncture of Rs + M-2M to juncture of 2M and 1rs-m), 0.51 mm high (as measured from midpoint on 2M between 1 m-cu and 1rs-m to juncture of r-rs and Rs); third submarginal cell 0.46 mm long (as measured from juncture of 1rs-m and M to juncture of M and 2rs-m), 0.61 mm high (as measured from juncture of M and 2 m-cu to juncture of 2rs-m and Rs); first medial cell 2.16 mm long (as measured from juncture of M+Cu and Cu to juncture of 1 m-cu and M), 0.57 mm high (as measured from juncture of M and Rs + M to midpoint on Cu between M + Cu and 1 m-cu); second medial cell 1.19 mm long (as measured from origin of Cu1 to juncture of 2 m-cu and M); 1rs-m and 2rs-m arched toward wing apex; 1 m-cu arched, entering second submarginal cell between midpoint and juncture of 1rs-m and M; 2 m-cu arched and entering third submarginal cell near midpoint; first abscissa of Rs very slightly curved, 0.43 mm long; 2cu-a short (thus Cu prior to 2m-cu shifted posteriorly); angle of Cu and 2 m-cu inside second medial cell obtuse. Basal part of first cubital cell not preserved, most of costal cell preserved, radial cell incompletely preserved. Right forewing 5.46 mm long and 1.57 mm wide as preserved; first submarginal cell and basal part of second submarginal cell preserved; first submarginal cell $0.74 \text{ mm} \log$ (as measured from origin of Rs + M to the juncture of r-rs and Rs), 0.36 mm high (as measured from Rs + M to pterostigma); first medial cell 2.15 mm long (as measured from juncture of M + Cu and Cu to juncture of 1 m-cu and M), 0.55 mm high (as measured from juncture of M and Rs + M to midpoint on Cu between M + Cu and 1 m-cu); first and second cubital cells visible, second medial not entirely preserved; radial and costal cells preserved; pterostigma 0.52 mm long, 0.22 mm wide; marginal cell length 1.45 mm; first abscissa of Rs very slightly curved, 0.45 mm long. Basal area of right hindwing visible, length 3.02 mm and width 0.74 mm as preserved; Sc + R, M+Cu, A, and cu-a are preserved; cu-a oblique, apparently slightly longer than 2 M + Cu; the cubital and radial cells visible. Trace of right metatibia preserved, apex flared (as in Epeolini); metabasitarsus less than one-half width of metatibial apex. Metasoma 5.84 mm long and 4.25 mm in height as preserved; six terga and sterna visible (female); short trace of sting present, length 0.46 mm.

Discussion

The forewing shape geometric morphometric analyses consistently indicate that the shape is diagnostic at higher taxonomic levels (i.e. tribe, subfamily and family), confirming previous studies on such wing shapes as applied to fossil bees (Michez *et al.*, 2009; De Meulemeester *et al.*, 2012; Kotthoff *et al.*, 2011, 2013; Wappler *et al.*, 2012; Dehon *et al.*, 2014; Dewulf *et al.*, 2014), although the phylogenetic signal

of this similarity from such analyses alone is difficult to discern. Accordingly, phylogenetic placement of the wing shape data supports placement among cleptoparasitic apids and corroborates the analyses of shape similarity. Most importantly, the analyses associate Paleoepeolus micheneri with the tribe Epeolini. Modern Epeolini are found on all continents except Australia (Michener, 2007), and, like other Nomadinae, are exclusively cleptoparasites. Further evidence corroborating the cleptoparasitic biology of the present fossil is the general absence of body pubescence and pollen-transporting apparatuses, although thicker cuticle could not be observed on the fossil as preserved. Sparse pilosity on the metasoma does occur in various free-living bees, such as species of Ceratina Latreille (Apidae: Xylocopinae) or Hylaeus Fabricius (Colletidae: Hylaeinae), but in these cases this is associated with nesting in stems and a more elongate metasomal form, while the fossil lacks this shape and has a broader, shorter metasoma (Fig. 4, Figures S1 and S2).

The largest epeoline genera are Epeolus and Triepeolus, with 109 and 142 species, respectively, and are the only two genera that today occur in the Old World (Rightmyer, 2004; Michener, 2007). Interestingly, the basal subtribe of Epeolini, the Odyneropsina (Rightmyer, 2004), is exclusively New World. As in many nomadine species, P. micheneri has 1Rs arising near the pterostigmal base, while 1cu-a is postfurcal and in line with the first abscissa of Cu such that the second abscissa of Cu is slightly below the level of M + Cu (particularly in Epeolini). Like the more derived groups of Epeolini, P. micheneri has the shorter and broader pterostigma of that clade, in contrast to the much more elongate pterostigma of Odyneropsis Schrottky, basal lineage of Epeolini (Rightmyer, 2004). In addition, the angle of Cu and 2m-cu in the second medial cell is nearly orthogonal for Odyneropsina, while this angle is pronouncedly obtuse in the other clades. Accordingly, details of the forewing shape correspond to the subtribes Rhogepeolina, Epeolina and Thalestriina. The marginal cell apex is broader and more truncate or oblique in the South American Rhogepeolina, and differs from the acutely rounded apex of the fossil and other Epeolini (Alexander, 1990; Roig-Alsina, 1996). Importantly, the lack of dense setae in the radial cell supports placement among the Epeolina + Thalestriina clade, this feature being one of several synapomorphies for the clade (Rightmyer, 2004). The short 2cu-a is most similar to many Epeolus, Doeringiella, and Triepeolus, and contrasts with the longer form observed in taxa such as Thalestria, while the straight 1cu-a differs from most Triepeolus and is closer in form to that observed in many Epeolus. Accordingly, P. micheneri does not correspond to any modern genus of Epeolina or Thalestriina, and we cannot with confidence place it among either of these subtribes. It is possible that the fossil genus is basal within one of these groups, or is a stem group to the combined clade. Further placement of the fossil will require the discovery of additional material and further character information.

Where known, hosts of species of *Epeolus* are in the genus *Colletes* Latreille (Colletidae), while *Triepeolus*

are cleptoparasitic in the nests of various bee groups [e.g. Anthophora Latreille, Centris Fabricius, and Melitoma Lepeletier & Audinet-Serville (Apidae: Apinae); Ptiloglossa Smith (Colletidae: Diphaglossinae); Protoxaea Cockerell & Porter (Andrenidae: Oxaeinae); Dieunomia Cockerell (Halictidae: Nomiinae)] but mostly of Eucerini (Apidae: Apinae) (Michener, 2007). Naturally, it is impossible to speculate as to a host association, but there are free-living bee species documented from the same locality: the anthophorine Paleohabropoda oudardi Michez & Rasmont (Apidae: Apinae) (Michez et al., 2009) and the megachiline Probombus hirsutus (Piton) (Nel & Petrulevičius, 2003), as well as trace evidence of leafcutter bees (Wedmann et al., 2009). This is not to say that P. micheneri was cleptoparasitic upon those species, but it does document the presence of a representative of a tribe parasitized by at least one modern epeoline clade, albeit marginally so. Paleoepeolus micheneri is the first definitive fossil of a cleptoparasitic bee, representing the earliest documented occurrence of this prominent mode of life among bees. Given the c. 60 Ma age of P. micheneri along with its nested placement among Nomadinae, and particularly Epeolini, there must have been considerable prior cladogenesis, at least partially corroborating model-based hypotheses indicating an early appearance of cleptoparasitism among apid bees, as estimated by Cardinal & Danforth (2013) (i.e. ~75 Ma). Much like the more widely studied social behaviour of bees, cleptoparasitism appeared early in bee diversification, representing a rather quick evolution to a parasitic lifestyle.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12230

Figure S1. Part (Oli 431) of *Paleoepeolus micheneri* **gen.n.**, **sp.n.** (A) General habitus. (B) Detail of the metasoma with sternites and tergites indicated. (C) Detail of the left forewing. (D) Detail of the right forewing (photographs by A. Nel and R. Garrouste). Labels: S, sternite; T, tergite; LF, left forewing; RF, right forewing.

Figure S2. Counterpart (Oli 436) of *Paleoepeolus micheneri* gen.n., sp.n. (A) General habitus. (B) Detail of the prosoma with eyes, clypeus, mandible and part of left antenna indicated. (C) Detail of the left forewing. (D) Detail of the right forewing (photographs by A. Nel and R. Garrouste).). Labels: L, left; LF, left forewing; R, right; RF, right forewing.

Table S1. Landmarks coordinates of the reference dataset of

 Anthophila and the fossil.

Table S2. Landmarks coordinates of Brachynomadini, Epeolini, Melectini and Nomadini.

 Table S3. Specimen assignments in families using the cross-validation procedure in the LDA of forewing shape.

Original groups are along the rows, predicted groups are along the columns. The hit-ratio (HR%) is given for each taxa.

Table S4. Specimen assignments in subfamilies using the cross-validation procedure in the LDA of forewing shape. Original groups are along the rows, predicted groups are along the columns. The hit-ratio (HR%) is given for each taxa.

Table S5. Specimen assignments in tribes using the cross-validation procedure in the LDA of forewing shape. Original groups are along the rows, predicted groups are along the columns. The hit-ratio (HR%) is given for each taxa.

Table S6. Specimen assignments in genera using the cross-validation procedure in the LDA of forewing shape. Original groups are along the rows, predicted groups are along the columns. The hit-ratio (HR%) is given for each taxa.

Table S7. Mahalanobis distances (MD) between the fossil and families' centroids.

Table S8. Mahalanobis distances (MD) between the fossil and subfamilies' centroids.

Table S9. Mahalanobis distances (MD) between the fossil and tribes' centroids.

 Table S10. Mahalanobis distances (MD) between the fossil and genera' centroids.

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