

This article was downloaded by: [Universite De Mons-Hainaut], [Denis Michez]

On: 27 March 2012, At: 08:07

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Systematic Palaeontology

Publication details, including instructions for authors and subscription information:
<http://www.tandfonline.com/loi/tjsp20>

Taxonomic affinity of halictid bee fossils (Hymenoptera: Anthophila) based on geometric morphometrics analyses of wing shape

Thibaut De Meulemeester^a, Denis Michez^a, Ahmet Murat Aytekin^b & Bryan Nicholas Danforth^c

^a University of Mons, Laboratory of Zoology, Place du Parc 20, 7000, Mons, Belgium

^b Hacettepe University, Department of Biology, 06800, Beytepe, (Ankara), Turkey

^c Cornell University, Department of Entomology, Comstock Hall, Ithaca, NY, 14853-0901, USA

Available online: 27 Mar 2012

To cite this article: Thibaut De Meulemeester, Denis Michez, Ahmet Murat Aytekin & Bryan Nicholas Danforth (2012): Taxonomic affinity of halictid bee fossils (Hymenoptera: Anthophila) based on geometric morphometrics analyses of wing shape, *Journal of Systematic Palaeontology*, DOI:10.1080/14772019.2011.628701

To link to this article: <http://dx.doi.org/10.1080/14772019.2011.628701>



PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Taxonomic affinity of halictid bee fossils (Hymenoptera: Anthophila) based on geometric morphometrics analyses of wing shape

Thibaut De Meulemeester^a, Denis Michez^{a*}, Ahmet Murat Aytekin^b and Bryan Nicholas Danforth^c

^aUniversity of Mons, Laboratory of Zoology, Place du Parc 20, 7000 Mons, Belgium; ^bHacettepe University, Department of Biology, 06800 Beytepe (Ankara), Turkey; ^cCornell University, Department of Entomology, Comstock Hall, Ithaca, NY 14853-0901, USA

(Received 5 January 2011; accepted 8 March 2011; printed XXXX)

Taxonomic assignment of fossils can be difficult because descriptions and phylogenetic analyses are often based on a limited number of discrete visible morphological characters. Quantitative, morphometric analyses can help to assign fossil specimens to modern groups, especially when two-dimensional features, such as insect wings, provide easily identifiable landmarks that are not likely to be deformed in the fossil specimens. Here we study taxonomic attribution of bee fossils by analysing wing shape of fossil and contemporary taxa. Our study focuses on the bee family Halictidae (Hymenoptera: Halictidae: Halictinae). Halictinae is a well-known cosmopolitan group including more than 2500 species in five tribes described from robust molecular and morphological analyses. We analysed 202 specimens of 48 species of Halictinae and Nomiinae. To analyse wing shape, we performed a Geometric Morphometrics analysis by using 19 2D-landmarks. Ordination methods, such as canonical variates analysis (CVA) and linear discriminant analysis (LDA), were used to discriminate tribes based on wing shape. CVA and LDA were both powerful enough to recover tribes previously delimited by adult morphology and DNA sequences. We then assigned fossils in CVA and LDA groups based on Mahalanobis distances. The ‘morphometric affinity’ of the two fossil taxa did not confirm their previous taxonomic attribution. By re-examining the fossil description we propose a new taxonomic attribution for *Electrolictus antiquus*.

Keywords: bees; wing; geometric morphometrics; molecular phylogeny; characters; evolution

Introduction

Fossils are extremely important for providing insights into the early evolution and diversification of many animal and plant lineages (Jackson & Erwin 2006). They can provide insights into the evolutionary relationships among extant organisms (e.g. Mecoptera and Siphonaptera) and they can provide information on the antiquity of major groups. Unfortunately, fossil specimens are not always easily assigned to modern groups because the specimens can be fragmentary, damaged, or, in some cases, important morphological details may be obscured. This is particularly important for organisms with soft parts, such as insect or other arthropod fossils, where only external morphological data are available and where examination of internal anatomy and hidden structures (genitalia, hidden sterna and terga) is not possible (Kinchloe Roberts *et al.* 2008). One approach to associating insect fossil taxa that has not been widely exploited is the use of quantitative (morphometric) methods for analysing two-dimensional structures, such as wings. In this paper we use wing geometric morphometrics to more accurately place two fossil halictid bees within the classification of modern Halictinae.

Bees (Apoidea Anthophila) are a clade of pollen and nectar feeding insects derived from a paraphyletic group of predatory apoid wasps (formerly ‘Sphecidae’) (Brothers 1998; Danforth *et al.* 2006; Michener 2007). Hypotheses on the origin and diversification of bees are mainly based on the bee fossil record (Michener & Grimaldi 1988; Poinar & Danforth 2006), the plant fossil record (Hu *et al.* 2008) and phylogenetic analyses including relaxed-clock dating (Danforth *et al.* 2004). Bees are generally thought to have arisen during the mid-Cretaceous (~120 Ma), concomitantly with the origin and diversification of the Eudicots (Danforth 2007). Bee fossils are very rare (Grimaldi & Engel 2005; Michener 2007). Worldwide, four main deposits of bee fossils are known: Dominican amber from the Miocene (20 Ma; Michener & Poinar 1996), the Florissant shale from the Eocene–Oligocene boundary (34 Ma; Engel 2002), and the Eckfeld/Messel shales and Baltic amber from the middle Eocene (c.45 Ma; respectively Wappler & Engel (2003) and Engel (2001)). Cretaceous, Paleocene and early Eocene bee fossils are much rarer. Only six specimens have been found in layers older than 50 Ma (Engel & Archibald 2003; Michez *et al.* 2007, 2009). The oldest fossils of each major clade are

*Corresponding author. Email: denis.michez@umons.ac.be

listed by Grimaldi & Engel (2005) and Michez *et al.* (2009).

To analyse wing shape of fossil and contemporary taxa we used landmark based Geometric Morphometrics methods (Bookstein 1991). Wings show many methodological advantages in comparison with other organs. They are a 2D structure that can easily be compared among species. They are rigid and often taxon-specific in morphology, and there are a large number of useful landmarks formed by the homologous intersections of veins. Furthermore, insect wings are often well preserved in both amber and compression fossils. Historically, the study of wing shape variation has concentrated on using interlandmark distances, as well as angles and distance ratios (e.g. Louis 1970; Ito 1985). This approach has been gradually replaced by modern geometric morphometric methods (Rohlf & Marcus 1993). The geometric morphometric method is aimed at comparison of the shapes themselves in a Kendall's space (see Adams *et al.* 2004) and produces more informative data in separating groups (Monteiro *et al.* 2002).

We combined a dataset of digitized wings from a diverse sample of genera, tribes and subfamilies of halictid bees with digitized images of fossil wings from previously described fossil halictid bees in order to develop a better understanding of the affinities of the fossil taxa with modern groups. We sought to answer the following questions: (1) do morphological data discriminate tribes previously defined by molecular data? (2) with what modern tribes do the halictid fossil wings show the clearest similarities? and (3) can we provide a more accurate taxonomic assignment of the fossils to modern groups based on these new analyses?

Material and methods

Taxa studied and material

Contemporary Halictinae include more than 2500 species and it is the most diverse subfamily among the four subfamilies of Halictidae (Danforth *et al.* 2008). Halictinae are cosmopolitan and live in virtually all ecosystems from Chilean and Californian deserts, to Asian and African tropical forests (Michener 2007). In order to study their wing shapes, we sampled 238 specimens from 59 species from the Cornell University Insect Collection, Cornell University Ithaca, NY (<http://www.entomology.cornell.edu/CUIC/>). Specimens were chosen when to represent morphological diversity and geographical ranges of tribes (Online Supplementary Material Table 1). All specimens were females to avoid the likely effect of sexual dimorphism in sampling (Pretorius 2005). After first sampling we had to delete some specimens with missing landmarks (e.g. some translucent veins in some *Lasioglossum* species). To reduce the likely bias of allometry we also had to delete species which deviated obviously in size from the sample, excluding the

five biggest species. Lastly, after a preliminary UPGMA cluster analysis, we deleted single specimens that did not cluster with the overall sample of conspecific individuals. Re-examination of the wings in these specimens indicated that they were deformed or poorly mounted on microscope slides. The final dataset of contemporary taxa included 195 specimens from 47 species of Halictinae, and five specimens from one species of the sister subfamily (Nomiinae) as a comparison group (Online Supplementary Material Table 1).

Halictid fossils are relatively abundant in comparison with other bee families (Engel 2002). From the 25 described specimens we selected two well-preserved fossils for our analysis: *Electrolictus antiquus* Engel, 2001 and *Halictus petrefactus* Engel & Peñalver, 2006. *Electrolictus antiquus* is from Eocene Baltic amber (Engel 2001). *Halictus petrefactus* is from laminated mudstones of Early Miocene age from Rubielos in the Mora Basin (Engel & Peñalver 2006). Both were described originally based on only one female specimen and as belonging to the tribe Halictini (Online Supplementary Material Table 2). The wing morphology of another important halictine fossil, *Halictus savenyi* from the early Eocene of Canada (Engel & Archibald 2003), is not well preserved enough for morphometric analysis.

Shape analysis

The left forewings of female bees obtained from the Cornell University Insect Collection were initially removed from the body and photographed by the first author (DM) using a Zeiss SV11 stereomicroscope coupled with a Sony DSC camera. In fossil specimens, we used wing photographs or drawings from the original publications (Engel 2001; Engel & Peñalver 2006). Photographs were input to tps-UTILS 1.38 (Rohlf 2006a) and Cartesian coordinates of landmarks were digitized with tps-DIG 2.05 (Rohlf 2006b). We selected 19 landmarks to capture the shape (Fig. 1) (Aytekin *et al.* 2007). Wing veins and their intersections are unambiguously homologous among bees. Landmark configurations were scaled, translated and rotated against the consensus configuration using the GLS Procrustes superimposition method (Bookstein 1991). We performed the superimposition with tps-RELW 1.44 (Rohlf 2006c). Further statistical analyses were performed on landmark configurations projected in the Euclidean tangent space approximate to

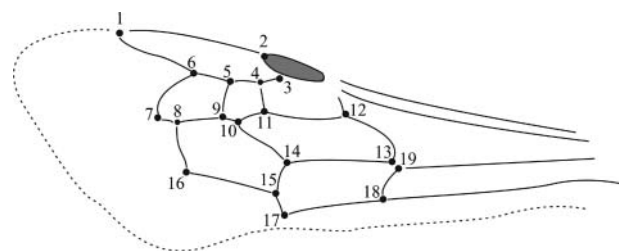


Figure 1. Left forewing of Halictinae female with 19 landmarks.

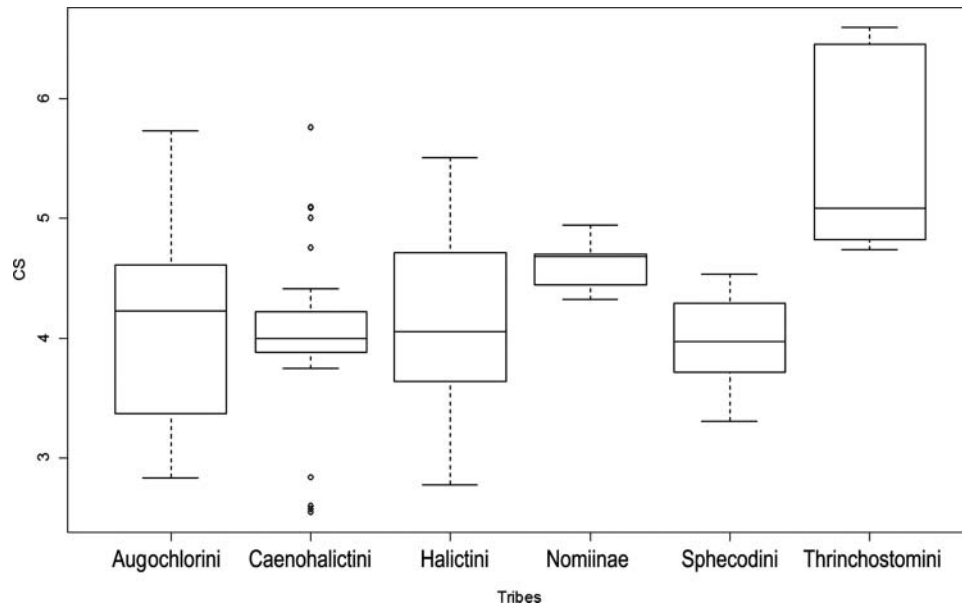


Figure 2. Box-plot of centroid size for halictid bees (median and percentiles 25–75%).

Kendall's shape space, which is curved. This approximation is permitted when the amplitude of variation in shape in the dataset is small enough. To determine this point, we calculated the least-squares regression slope and the correlation coefficient between the two distances (Euclidean and Procrustes distances between pairs of specimens) with tps-Small 1.2 (Rohlf 2003).

A measure of centroid size (CS) was computed as the square root of the sum of squared distances from all landmarks to the centroid of the landmarks configuration (Bookstein 1991). The CS measure was calculated with tps-RELW 1.44 (Rohlf 2006c). The CS variation for each tribe, including Nomiinae, is shown with a box-plot (Fig. 2). We tested significance of wing size variation among tribe by analysis of variance (ANOVA).

After data acquisition and validation of amplitude variation, the landmark coordinates were analysed using tps-RELW 1.44 (Rohlf 2006c) to calculate eigenvalues for each principal warp. We processed a relative warps analysis with specimens of modern species, which is technically a PCA based on the landmarks coordinates (Fig. 3). We estimated the effect of size on the relative warps by linear regression. As grouping of tribes was not robust, we simplified the shape variation by calculating species wing mean shapes using the software Morpheus (Slice 1998). The final dataset included the 48 mean shapes of modern species. Afterwards we used both linear discriminant analysis (LDA) and canonical variate analysis (CVA) with tribe a priori grouping based on molecular affinities (Fig. 4). We used R statistical language 2.0 (<http://cran.r-project.org/>) and IMP CVAGen6o (Sheets 2003) to perform respectively the LDA and the CVA (Fig. 4). An assignment procedure

based on Mahalanobis distances in the LDA space was used to test the effectiveness of discriminant analysis to separate groups. Based on their scores in the discriminant space, Mahalanobis distances were calculated between each 48 species shapes and each six mean-groups. Then each species was assigned to the nearest group in the discriminant shape (Tables 1, 2). Pairwise MANOVA Goodals F-test was also performed on aligned landmark coordinates using IMP TwoGroups6h (Sheets 2003) to estimate the shape difference between tribes (Table 2).

Finally, clustering analysis techniques were employed for reconstructing phenetic relationships among tribes based on their wing shape. As MANOVA shows significant shape differences among tribes (Table 2), we used procrustes distances of tribe mean shape to perform a neighbour-joining clustering rooted on *Nomia*. We compared shape based clustering with a previously published (Danforth *et al.* 2004) molecular phylogeny (Fig. 5).

Table 1. Linear discriminant analysis of species mean wing shape. Mahalanobis distances from mean shape contemporary species to tribe centroid. Min. = Minimum; 1st Q and 3rd Q = first and third quartil; Med = Median; Max. = Maximum. Aug = Augochlorini; Caе = Caenohalictini; Hal = Halictini; Sph = Sphecodini; Tri = Thrinchostomini.

	Min.	1st Q	Med	Mean	3rd Q	Max.
Aug	0.84	1.52	1.94	1.90	2.17	3.15
Caе	1.22	1.61	1.92	1.87	2.12	2.36
Hal	1.10	1.71	1.99	2.11	2.41	4.20
Sph	1.39	1.58	1.77	1.90	1.90	2.85
Tri	0.56	1.62	2.68	2.08	2.85	3.01

Table 2. Linear discriminant analysis of species mean wing shape. Reassignment test is indicated in diagonal (original groups are along the rows, predicted groups are along the columns). The p -values of pair-wise Manova are above the main diagonal and Mahalanobis distances are below. Fossil taxa were included in the analysis as unknown attribution. Posterior probabilities of fossil taxa assignment are above and Mahalanobis distances are below. Aug = Augochlorini; Cae = Caenohalictini; Hal = Halictini; Sph = Sphecodini; Tri = Thrinchostomini; Nom = Nomiinae; Fos1 = *Electrolictus antiquus*; Fos2 = *Halictus petrefactus*.

	Aug	Cae	Hal	Sph	Tri	Nom
Aug	7	0.0002	1.4e-07	2.5e-015	4.8e-015	0
Cae	16.42	8	1.2e-05	1.8e-015	1.2e-012	0
Hal	12.13	9.08	24	0	0	0
Sph	21.95	11.34	13.52	5	0	0
Tri	43.05	40.86	45.09	42.83	3	0
Nom	36.96	32.72	35.41	30.99	35.14	1
Fos1	0	2.8e-296	0	2.80e-110	1	0
Fos2	44.29	40.20	45.23	43.33	15.91	27.49
	1	6.4e-97	1.1e-60	0	3.9e-218	0
	20.26	29.22	26.25	37.26	57.77	54.24

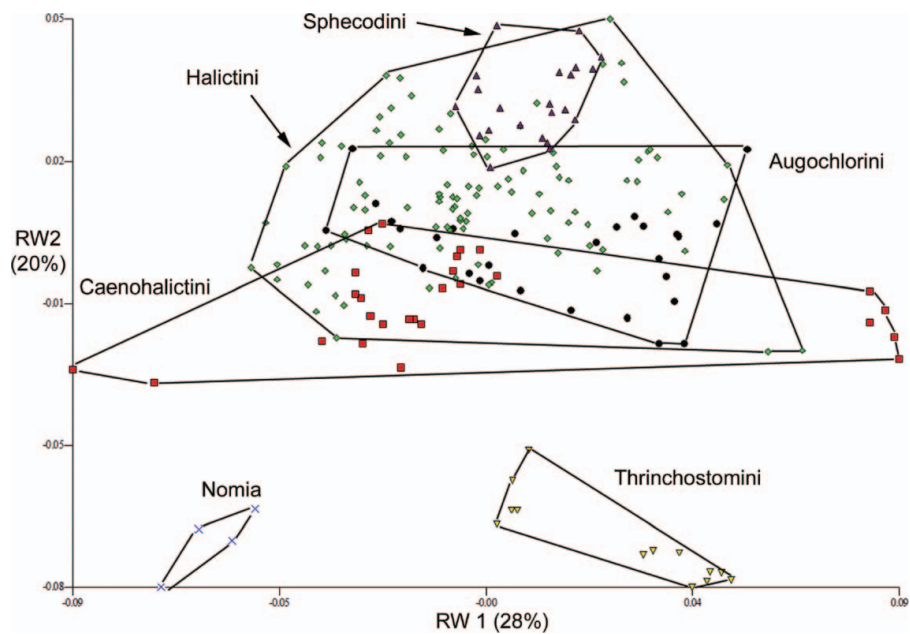


Figure 3. Distribution of the 200 examined specimens of modern halictid bee species, along the first two RWs. RWA of tangent space coordinates derived from GPA of the original coordinates digitized from the wing.

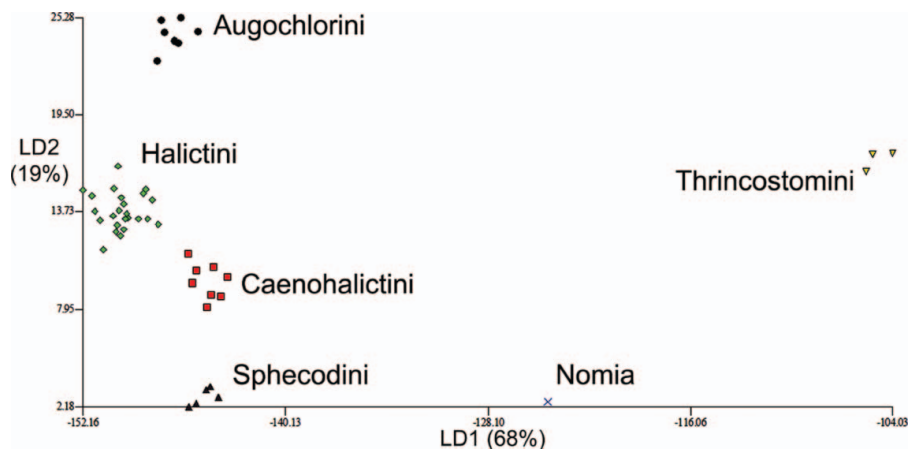


Figure 4. Ordination of the modern halictines in the space of the first two axes of the linear discriminant analysis (LD1 and LD2) based on the species mean shapes.

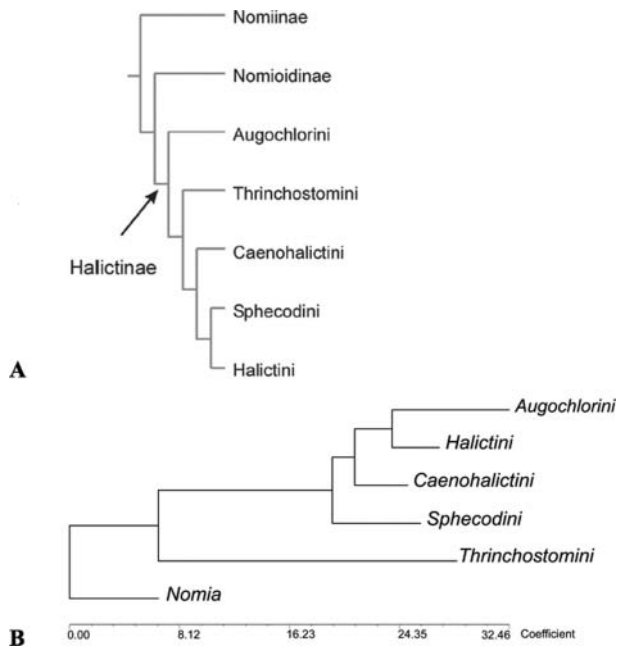


Figure 5. **A**, molecular phylogeny of Halictinae (according to Danforth *et al.* 2004); **B**, rooted NJ tree based on procrustes distances of tribe mean shapes.

Fossil assignment

We reused the previous LDA based on modern species mean shape to assign both fossil species (*Electrolictus antiquus* and *Halictus petrefactus*). We included fossil specimens in the computed discriminant space as ‘unknown’ groups. Assignment of the fossils was estimated by calculating the Mahalanobis distance between ‘unknowns’ and group mean of contemporary tribe (Table 2). We also calculated posterior probabilities of membership to confirm the assignment to one group (Table 2). Given the observed scores of an ‘unknown’, the posterior probability equals the probability of the unit to belong to one group compared to all others. The unit is consequently assigned to that group for which the posterior probability is the highest (Huberty & Olejnik 2006). We used the scores of the ‘unknown’ specimens in the discriminant space to visualize affinity of the fossil to a modern tribe (Fig. 6). Lastly we assessed a neighbour-joining clustering based on Mahalanobis distances between tribe means and fossil specimens (Fig. 7).

Results

Shape analysis

The tps-Small test reveals a regression coefficient close to 1 (0.999003 using all specimens; 0.999061 using species mean shape) and a high correlation coefficient (1.00 using both all specimens and species mean shape) between the two distances, which means a very small deviation from a

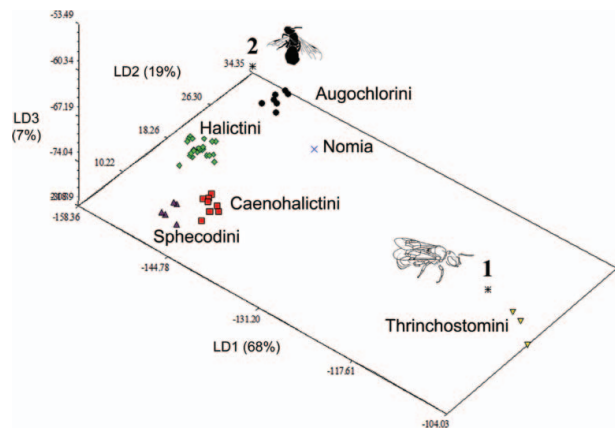


Figure 6. Distribution in halictines of the 48 mean modern shapes and the two fossil shapes (1 = *Electrolictus antiquus*; 2 = *Halictus petrefactus*) along the three first LDA axes.

linear relationship and which allows us to be confident in the variation amplitude of our dataset. One-way ANOVA on CS between tribes shows that at least one group differ significantly in size from the others when considering all specimens (F-value = 10.797; p -value = $3e-09$). Moreover the p -values of the coefficient of linear regression between CS and the three first RW axes ($p = 4.7e-08$, $p = 1.1e-07$ and $p = 0.755$) show a significant effect of size on the two first RW axis.

The two first axes of the relative warp analysis account for just 49% of the variation in the data. The first axis does not isolate any tribe while Thrinchostomini and *Nomia* are well separated along the second axis. The other four tribes (Augochlorini, Caenohalictini, Sphecodini and Halictini) are poorly discriminated (Fig. 3).

Both CVA and LDA based on species mean shape discriminate the six tribes in five distinct axes. All MANOVA between pairs of tribes revealed significant mean

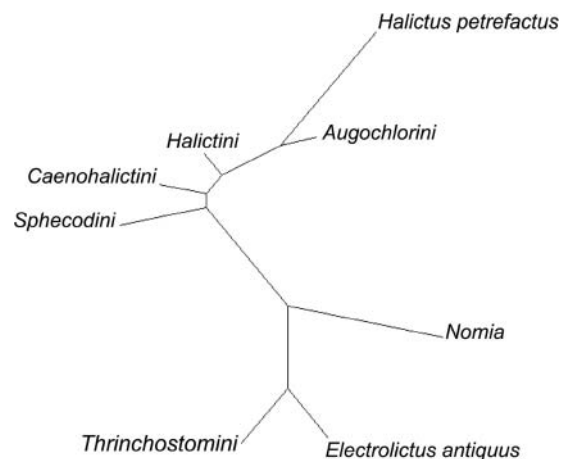


Figure 7. Unrooted NJ tree based on Mahalanobis distances including modern tribes and fossil taxa of halictines.

differences (Table 2). The plot of the specimens along the first two canonical axes (CV1-eigenvalue 138.2 and CV2-eigenvalue 39.4) and the first two linear discriminant axes (LD1–68% and LD2–19%) do not show overlapping of samples (Fig. 4). The first axis discriminates Nomiinae and Thrinchostomini from the other Halictinae. The second axis provides a good distinction along the different tribes within Halictinae. Assignment procedure reveals an unambiguous separation of the groups as all specimens are assigned to the correct group (Table 2).

While CVA and LDA robustly recover the tribal associations of species, Mahalanobis distances (MD) among tribe centroids are not equivalent to their respective phylogenetic relationships (Fig. 5, Table 2). For example, MD between Sphecodini and Halictini is relatively high despite their close phylogenetic relationships (Table 2). Moreover, neighbour-joining clustering based on procrustes distances among mean shape of tribes does not recover their phylogenetic relationships based on molecular data (Fig. 5). Auglochlorini is the sister group to all other Halictinae in the phylogenetic analysis but their mean wing shape is more similar to Halictini in the geometric morphometrics analysis. Sphecodini is sister to Halictini in the phylogenetic analysis but the mean shape of the wing is quite different from the morphocluster (Caenohalictini + Halictini + Augochlorini), possibly because Sphecodini includes exclusively cleptoparasites.

Fossil assignment

In the LDA space *Electrolictus antiquus* is clearly close to the extant members of Thrinchostomini (Fig. 6). Assignment test, posterior probabilities and clustering based on Mahalanobis distance (MD) confirm the association of *E. antiquus* with the contemporary Thrinchostomini (Fig. 7, Table 2). MD between *E. antiquus* and Thrinchostomini centroid is 15.91, while this distance is more than double with the Sphecodini centroid (40.20), the second closest halictine tribe (Fig. 6, Table 2). However, tribe assignment of *E. antiquus* has to be faced with intra- and inter-tribe values of MD. MD between contemporary Thrinchostomini species and Thrinchostomini centroid is a maximum of 3.01 (Table 1), and MD among tribes could be under 16 (Table 2). From a morphometric point of view, *E. antiquus* is therefore as far from Thrinchostomini than Augochlorini is from Halictini in the discriminant space.

Halictus petrefactus is closer to the extant members of Augochlorini in the LDA space (Fig. 6). Assignment test, posterior probabilities and clustering support this result (Fig. 7, Table 2). However, MD between *H. petrefactus* and Augochlorini centroid (20.26) is quite in the same order size than MD between *H. petrefactus* and Halictini centroid (26.25) or Caenohalictini centroid (29.22). Moreover *H. petrefactus* is farther from Augochlorini than Augochlorini is from Halictini (Table 2).

Discussion

Geometric morphometrics versus molecular phylogeny

Many studies have demonstrated the potential close accordance between morphoclusters based on shape similarities and clades from molecular phylogenies (e.g. Monteiro *et al.* 2002; Moraes *et al.* 2004; Patterson & Schofield 2005). But morphological clustering can obviously differ from phylogeny in two ways: (1) lack of correct clustering (false negative) and (2) homoplastic clustering (false positive). Firstly if strong morphological modification occurs in one (or some) element(s) of a clade because of plesiomorphic trait retention (e.g. Cardini 2003), natural selection (e.g. Azevedo *et al.* 1998; Cardini & Tongiorgi 2003; Gilchrist *et al.* 2000; Guill *et al.* 2003), sexual selection (Vencl 2004), genetic drift (e.g. Cardini 2003), abiotic factors (e.g. Aytekin *et al.* 2009) or allometry (e.g. Astua 2009), its shape could diverge independently more quickly than its sequenced genes. Strong independent divergence of shape in one group can produce therefore a ‘false negative’ morphoclustering (valid clade with unmorphoclustered elements). This problem could be of less importance in a taxonomy because all terminal clusters are potentially monophyletic, as in the present study. Secondly, homoplastic shapes can be driven by the same six previous factors but in this case the morphology of unrelated clades converge to similar shapes. Clusters would therefore include unrelated clades. This bias is very problematic because morphoclusters are paraphyletic or polyphyletic groups.

As it partly defined flight performance and therefore foraging success (e.g. Hepburn *et al.* 1998), bee wing shape would probably not be a neutral trait. Many studies have highlighted polymorphy of wing shape likely driven by: (1) environmental pressures such as latitude (Alpatov 1929), altitude (Verma *et al.* 1994; Hepburn *et al.* 2000) and climate (Hepburn *et al.* 2001; Radloff *et al.* 2005a, b; Tan *et al.* 2008); (2) sexual selection (Radloff *et al.* 2003) and (3) abiotic factors such as temperature (Soose 1954) and season (Mattu & Verma 1984). But these analyses considered mainly a few linear independent features (not shape as a whole) and did not analyse wing shape in an evolutionary context. Without a broad evolutionary study on bee wing shapes it is impossible to discuss their potential homoplasy. We can say that variation in bee wing shape clearly allows discrimination of taxa at different levels: specimens (e.g. Kastberger *et al.* 2003), populations (e.g. Quezada-Euán *et al.* 2007), subspecies (e.g. Alpatov 1929; Tofilski 2008) and species (e.g. Aytekin *et al.* 2007). Based on these studies we cannot evaluate the phylogenetic signal in the wing shapes of bees.

Michez *et al.* (2009) and the present work compare for the first time wing shape polymorphy of bees and phylogeny at suprageneric level. Both studies showed discordance between phylogeny and morphoclustering. In the present

study divergent rates of wing shape evolution among tribes could explain the incorrect clustering at supratribal level (Fig. 5). In this hypothesis wing shapes of Spheco-dini, Thrinchostomini and Caenohalictini would appear to diverge faster than Augochlorini and Halictini. However, a hypothesis of homoplastic convergence seems unlikely in view of the equivalent sizes, worldwide sampling and high taxonomic scale (tribe). Homoplastic shapes would imply unlikely equivalent selection pressure on a worldwide scale and through geological time.

In conclusion, geometric morphometrics analyses of wing shape are powerful enough to discriminate among tribes of contemporary halictid bees based on a priori grouping, but supratribal morphoclustering and phylogeny do not give identical results. Geometric morphometrics of wing shape will probably not become an equal to molecular phylogenetics in the study of bee phylogeny. However, when phylogenetic study is not possible, geometric morphometrics becomes a powerful tool for discriminating taxa at intra- and supra-specific levels, and for discussing the taxonomic affinities of fossils.

Electrolictus antiquus

Based on the new morphometric definition of halictid tribes we re-examined the taxonomic attribution of *Electrolictus antiquus*. While Engel (2001) originally placed the female of *E. antiquus* in Halictini based on discrete morphological characters, our morphometric analysis shows its similarity with Thrinchostomini (Fig. 7). Engel (2001) considered *E. antiquus* to be most similar to the halictine genus *Patellapis*, an Old World tropical group. He considered it similar in some ways to Thrinchostomini but felt that placement in this group was not warranted because *Electrolictus* lacks certain features he considered to be typical of Thrinchostomini. Firstly, it lacks an elongate head and expanded genal region. However, not all members of Thrinchostomini have an expanded genal area (e.g. the subgenus *Eothrinchostoma*). Secondly, it lacks a dense patch of hairs on the first transverse cubital vein. However, this patch of hairs is absent in the subgenus *Eothrinchostoma* (Michener 2007), and in the other two subgenera (*Thrinchostoma* s.s. and *Diagonozus*) it is weakly developed in females. Thirdly, *E. antiquus* lacks the laterally directed, apical bands of setae on the abdominal terga that are common in many species of extant Thrinchostomini. Again, this feature can be variable and examination of species of Thrinchostomini indicates that several lack distinct setal bands on the terga. A morphological feature that seems to strongly support placement of *Electrolictus* within Thrinchostomini is the intersection of both the first and second recurrent veins (crossveins m-cu) in the third submarginal cell. This feature was mentioned by Engel (2001) as suggesting affinities with Thrinchostomini. One feature that would not seem to accord well with placement of *E. antiquus* in Thrinchostomini is the morphology of the inner hind tibial spur. Engel (2001) described the

inner hind tibial spur as pectinate (see his fig. 15) but extant Thrinchostomini bear an inner hind tibial spur that is typically finely to coarsely serrate.

The association of *E. antiquus* with Thrinchostomini is certainly plausible given the current distribution and ecology of Thrinchostomini. They are diverse in Old World subtropical areas, including tropical forested areas of Africa and tropical Asia (Danforth *et al.* 2008). This is similar to the subtropical palaeoclimate that occurred in the Baltic region during the middle Eocene (Grimaldi 1996; Engel 2001).

In conclusion, we are confident that *E. antiquus* is taxonomically closer to the tribe Thrinchostomini rather than Halictini, as originally proposed by Engel (2001). But some features such as the pectinate inner hind tibial spur and the relatively high MD in *E. antiquus* relative to the mean shape of contemporary Thrinchostomini could indicate that *Electrolictus antiquus* belongs to a stem group of the contemporary Thrinchostomini rather than the crown group of Thrinchostomini. A careful analysis of thrinchostomine phylogeny based on extant and fossil taxa is needed to test this hypothesis.

Halictus petrefactus

Engel & Peñalver (2006) originally placed the female of *Halictus petrefactus* in Halictini based on the suite of morphological features visible in the compression fossil. Features that seemed to support placement in Halictini included the lack of metallic coloration and the insertion of the first recurrent vein in the second (rather than third) submarginal cell. Definitive placement of a modern specimen in either Augochlorini or Halictini requires examination of the details of the female T5 (whether divided by a slit or not), the degree of fusion of hind tibial segments, and the structure of the male genitalia, none of which are visible in the compression fossil. Two features that might support placement in Augochlorini are the slightly emarginate compound eyes and the shiny black integument. While most Augochlorini are metallic, there are several non-metallic genera (e.g. *Neocorynura*). It is difficult to know if the shiny black integument described by Engel & Peñalver (2006) is indicative of a hint of metallic coloration in the compression fossil. It is also difficult to reconcile the current distribution of Augochlorini (New World, with the greatest diversity in tropical Central and South America) with the presence of the fossil *Halictus petrefactus* in Spain. All other augochlorine fossils have been described from Miocene deposits in Dominican amber (Online Supplementary Material Table 2). We are therefore sceptical of the association of this fossil with Augochlorini based exclusively on wing morphometric analysis. Wing deformation in a compression fossil is certainly possible and this could lead to spurious results. A re-examination of the fossil is warranted given the possibility of association with Augochlorini.

As with placement of *Electrolictus antiquus* in the stem group of Thrinchostomini, *Halictus petrefactus* could be included in the stem group of Halictini. This stem group would show peculiar wing shape close to the extant Augochlorini.

Evolution of halictid bees

The assignment of *Electrolictus antiquus*, a fossil from the Baltic amber, within the Thrinchostomini provides a new evidence of halictid diversity in the early Cenozoic and confirms the hypothesis of an early diversification of Halictinae during the Late Cretaceous or Early Cenozoic (Danforth *et al.* 2004). Body fossils of Halictinae are now distributed as follows: the oldest Halictinae is described from the Early Eocene of Quilchena (~53 Ma; Engel & Archibald 2003), the oldest Thrinchostomini from Baltic amber (~48 Ma; present study), and the oldest Augochlorini and Caenohalictini from Dominican amber (~20 Ma; Engel 2000 and Michener & Poinar 1996, respectively). There are no fossil Sphecodini. Fossilized nests of halictine origin are recorded from the Cenomanian (*c.* 89 Ma) of North America but their attribution is still questionable (Elliot & Nations 1998).

Acknowledgements

This project was supported by grants from the Fulbright Program (Commission for Educational Exchange between Belgium and USA), the National Belgian Funds (FNRS) and the University of Mons. The research of T. De Meulemeester is funded by the FRIA (FNRS, Belgium).

References

- Adams, D. C., Rohlf, F. J. & Slice, D. E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*, **71**, 5–16.
- Alpatov, W. W. 1929. Biometrical studies on variation and races of the honey bee *Apis mellifera* L. *Quarterly Review of Biology*, **4**, 1–58.
- Arillo, A., Nel, A. & Ortuño, V. 1996. Two fossil bees from the Oligocene of Izarra (Alava, Spain) (Hymenoptera, Apoidea). *Bulletin de la Société Entomologique de France*, **101**(1), 59–64.
- Armbruster, L. 1938. Versteinerte Honigbienen aus dem obermiocänen Randecker Maar. *Archiv für Bienenkunde*, **19**, 1–48, 97–133.
- Astua, D. 2009. Evolution of scapula size and shape in Didelphid Marsupials (Didelphimorphia: Didelphidae). *Evolution*, **63**, 2438–2456.
- Aytekin, S., Aytekin, A. M., & Alten, B. 2009. Effect of different larval rearing temperatures on the productivity (R0) and morphology of the malaria vector *Anopheles superpictus* Grassi (Diptera: Culicidae) using geometric morphometrics. *Journal of Vector Ecology*, **34**(1), 32–42.
- Aytekin, A. M., Terzo, M., Rasmont, P. & Cagatay, N. 2007. Landmark based geometric analysis of wing shape in *Sibiricobombus* Vogt (Hymenoptera: Apidae: *Bombus* Latreille). *Annales de la Société Entomologique de France (n.s.)*, **43**, 95–102.
- Azevedo, R. B. R., James, A. C., McCabe, J. & Partridge, L. 1998. Latitudinal variation of wing: thorax size ratio and wing-aspect ratio in *Drosophila melanogaster*. *Evolution*, **52**, 1353–1362.
- Bachmayer, F., Symeonidis, N. & Theodoropoulos, D. 1971. Einige Insektenreste aus den Jungtertiären Süßwasserablagerungen von Kumi (Insel Euboea, Griechenland). *Annales Géologiques des Pays Helléniques*, **23**, 165–174.
- Bookstein, F. L. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, Cambridge, 435 pp.
- Brothers, D. J. 1998. Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). *Zoologica Scripta*, **28**, 233–249.
- Cardini, A. 2003. The geometry of the marmot (Rodentia : Sciuridae) mandible: Phylogeny and patterns of morphological evolution. *Systematic Biology*, **52**(2), 186–205.
- Cardini, A. & Tongiorgi, P. 2003. Yellow-bellied marmots (*Marmota flaviventris*) 'in the shape space' (Rodentia, Sciuridae): sexual dimorphism, growth and allometry of the mandible. *Zoomorphology*, **122**, 11–23.
- Cockerell, T. D. A. 1906. Fossil Hymenoptera from Florissant, Colorado. *Bulletin of the Museum of Comparative Zoology*, **50**, 33–58.
- Cockerell, T. D. A. 1908. Descriptions and records of bees. XIX. *Annals and Magazine of Natural History*, **8**, 337–344.
- Cockerell, T. D. A. 1909. Two fossil bees. *Entomological News*, **20**, 159–161.
- Danforth, B. 2007. Bees. *Current Biology*, **17**(5), R156–R161.
- Danforth, B. N., Brady, S. G., Sipes, S. D. & Pearson, A. 2004. Single-copy nuclear genes recover Cretaceous-age divergences in bees. *Systematic Biology*, **53**, 309–326.
- Danforth, B. N., Sipes, S. D., Fang, J. & Brady, S. G. 2006. The history of early bee diversification based on five genes plus morphology. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 15118–15123.
- Danforth, B. N., Eardley, C. D., Packer, L., Walker, K., Pauly, A. & Randrianambinintsoa, F. J. 2008. Phylogeny of Halictidae with an emphasis on endemic African Halictinae. *Apidologie*, **39**, 86–101.
- Elliott, D. K. & Nations, J. D. 1998. Bee burrows in the Late Cretaceous (Late Cenomanian) Dakota Formation, northeastern Arizona. *Ichnos*, **5**, 243–253.
- Engel, M. S. 1995. *Neocorymura electra*, a new fossil bee species from Dominican amber (Hymenoptera: Halictidae). *Journal of the New York Entomological Society*, **103**, 317–323.
- Engel, M. S. 1996. New augochlorine bees (Hymenoptera: Halictidae) in Dominican amber, with a brief review of fossil Halictidae. *Journal of the Kansas Entomological Society*, **69**, 334–345.
- Engel, M. S. 1997. A new fossil bee from the Oligo-Miocene Dominican amber (Hymenoptera: Halictidae). *Apidologie*, **28**(2), 97–102.
- Engel, M. S. 2000. Classification of the bee tribe Augochlorini (Hymenoptera: Halictidae). *Bulletin of the American Museum of Natural History*, **250**, 1–90.
- Engel, M. S. 2001. A monograph of the Baltic Amber bees and evolution of the Apoidea (Hymenoptera). *Bulletin of the American Museum of Natural History*, **259**, 1–192.
- Engel, M. S. 2002. Halictine bees from the Eocene-Oligocene boundary of Florissant, Colorado (Hymenoptera: Halictidae). *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **225**, 251–273.

- Engel, M. S. 2009. Two new Halictine bees in Miocene amber from the Dominican Republic (Hymenoptera, Halictidae). *Zookeys*, **29**, 1–12.
- Engel, M. S. & Archibald, S. B. 2003. An Early Eocene bee (Hymenoptera: Halictidae) from Quilchena, British Columbia. *The Canadian Entomologist*, **135**, 63–69.
- Engel, M. S. & Peñalver, E. 2006. A miocene Halictine bee from Rubielos de Mora Basin, Spain (Hymenoptera: Halictidae). *American Museum Novitates*, **3503**, 1–12.
- Engel, M. S. & Rightmyer, M. G. 2000. A new augochlorine bee species in Tertiary amber from the Dominican Republic (Hymenoptera: Halictidae). *Apidologie*, **31**(3), 431–436.
- Gilchrist, A. S., Azevedo, R. B. R., Partridge, L. & O'Higgins, P. 2000. Adaptation and constraint in the evolution of *Drosophila melanogaster* wing shape. *Evolution & Development*, **2**(2), 114–124.
- Grimaldi, D. 1996. *Amber: window to the past*. American Museum of Natural History, New York, 216 pp.
- Grimaldi, D. & Engel, M. S. 2005. *Evolution of the insects*. Cambridge University Press, Cambridge, 755 pp.
- Guill, J. M., Hood, C. S. & Heins, D. C. 2003. Body shape variation within and among three species of darters (Perciformes: Percidae). *Ecology of Freshwater Fish*, **12**(2), 134–140.
- Hepburn, H. R., Youthed, C., Illgner, P., Radloff, S. E. & Brown, R. E. 1998. Production of aerodynamic power in mountain honeybees (*Apis mellifera*). *Naturwissenschaften*, **85**, 389–390.
- Hepburn, H. R., Radloff, S. E. & Oghiakhe, S. 2000. Mountain honeybees of Africa. *Apidologie*, **31**(2), 205–221.
- Hepburn, H. R., Radloff, S. E., Verma, S. & Verma, L. R. 2001. Morphometric analysis of *Apis cerana* populations in the southern Himalayan region. *Apidologie*, **32**(5), 435–447.
- Hu, S., Dilcher, D. L., Jarzen, D. M. & Taylor, D. W. 2008. Early steps of angiosperm–pollinator coevolution. *Proceeding of the National Academy of Sciences of the United States of America*, **105**, 240–245.
- Huberty, C. J. & Olejnik, S. 2006. *Applied MANOVA and Discriminant Analysis. Second Edition*. Wiley, New Jersey, 488 pp.
- Ito, M. 1985. Supraspecific classification of bumblebees based on the characters of male genitalia. *Contributions from the Institute of Low Temperature Science, Series B*, **20**, 1–143.
- Jackson, J. B. C. & Erwin, D. H. 2006. What can we learn about ecology and evolution from the fossil record? *Trends in Ecology & Evolution*, **21**, 322–328.
- Kastberger, G., Radloff, S. & Kranner, G. 2003. Individuality of wing patterning in Giant honey bees (*Apis laboriosa*). *Apidologie*, **34** (3), 311–318.
- Kinchloe Roberts, A., Smith, D. M., Guralnick, R. P., Cushing, P. E. & Krieger, J. 2008. An outline morphometric approach to identifying fossil spiders: A preliminary examination from the Florissant formation. Pp. 105–116 in H. W. Meyer & D. M. Smith (eds) *Paleontology of the Upper Eocene Florissant Formation, Colorado. Geological Society of America Special Paper*, **435**.
- Louis, J. 1970. Etudes sur les ailes des Hyménoptères. II. L'aile de l'abeille domestique (*A. mellifica* L.). *Apidologie*, **1**(3), 309–328.
- Mattu, V. K. & Verma, L. R. 1984. Morphometric studies on the Indian honeybee, *Apis cerana indica* F. – effect of seasonal variations. *Apidologie*, **15**(1), 63–74.
- Michener, C. D. 2007. *The bees of the world. Second edition*. The Johns Hopkins University Press, Baltimore, 913 pp.
- Michener, C. D. & Grimaldi, D. 1988. The oldest fossil bee: Apoid history, evolutionary stasis, and antiquity of social behavior. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 6424–6426.
- Michener, C. D. & Poinar, G., Jr 1996. The known bee fauna of Dominican amber. *Journal of the Kansas Entomological Society*, **69**(4 Supplement), 353–361.
- Michez, D., Nel, A., Menier, J.-J. & Rasmont, P. 2007. The oldest fossil of a melittid bee (Hymenoptera : Apiformes) from the Early Eocene of Oise (France). *Zoological Journal of the Linnean Society*, **150**, 701–709.
- Michez, D., De Meulemeester, T., Nel, A., Rasmont, P. & Patiny, S. 2009. New fossil evidence of the early diversification of bees: *Paleohabropoda oudardi* from the French Paleocene (Hymenoptera, Apidae, Anthophorini). *Zoologica Scripta*, **38**, 171–181.
- Miller, D. F. & Morgan, A. V. 1982. A postglacial coleopterous assemblage from Lockport gulf, New York. *Quaternary Research*, **17**, 258–274.
- Monteiro, L. R., Diniz, J. A. F., dos Reis, S. F. & Araujo, E. D. 2002. Geometric estimates of heritability in biological shape. *Evolution*, **56**, 563–572.
- Moraes, E. M., Spressola, V. L., Prado, P. R. R., Costa, L. F. & Sene, F. M. 2004. Divergence in wing morphology among sibling species of the *Drosophila buzzatii* cluster. *Journal of Zoological Systematics and Evolutionary Research*, **42**(2), 154–158.
- Nel, A. & Petrulevicius, J. F. 2003. New Palaeogene bees from Europe and Asia. *Alcheringa*, **27**, 227–293.
- Patterson, J. S. & Schofield, C. J. 2005. Preliminary study of wing morphometry in relation to tsetse population genetics. *South African Journal of Science*, **101**, 132–134.
- Poinar, G. O. J. 2004. Evidence of parasitism by Strepsiptera in Dominican amber. *Biocontrol*, **49**(3), 239–244.
- Poinar, G. J. & Danforth, B. N. 2006. A fossil bee from early Cretaceous Burmese amber. *Science*, **314**, 614.
- Pretorius, E. 2005. Using geometric morphometrics to investigate wing dimorphism in males and females of Hymenoptera – a case study based on the genus *Tachysphex* Kohl (Hymenoptera: Sphecidae: Larrinae). *Australian Journal of Entomology*, **44**, 113–121.
- Quezada-Euan, J. J. G., Paxton, R. J., Palmer, K. A., Itza, W. D. M., Tay, W. T. & Oldroyd, B. P. 2007. Morphological and molecular characters reveal differentiation in a Neotropical social bee, *Melipona beecheii* (Apidae : Meliponini). *Apidologie*, **38**(3), 247–258.
- Radloff, S. E., Hepburn, H. R. & Koeniger, G. 2003. Comparison of flight design of Asian honeybee drones. *Apidologie*, **34**, 253–358.
- Radloff, S. E., Hepburn, H. R., Hepburn, C., Fuchs, S., Otis, G. W., Sein, M. M., Aung, H. L., Pham, H. T., Tam, D. Q., Nuru, A. M. & Ken, T. 2005a. Multivariate morphometric analysis of *Apis cerana* of southern mainland Asia. *Apidologie*, **36**(1), 127–139.
- Radloff, S. E., Hepburn, R. & Fuchs, S. 2005b. The morphometric affinities of *Apis cerana* of the Hindu Kush and Himalayan regions of western Asia. *Apidologie*, **36**(1), 25–30.
- Rohlf, F. J. 2003. *tpsSMALL*. Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf, F. J. 2006a. *tpsUTIL, file utility program*. Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf, F. J. 2006b. *tpsDIG, digitize landmarks and outlines*. Department of Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf, F. J. 2006c. *tpsRELW, relative warps analysis*. Department of Ecology and Evolution, State University of New York at Stony Brook.

- Rohlf, F. J. & Marcus, L. F.** 1993. A revolution in Morphometrics. *Trends in Ecology & Evolution*, **8**(4), 129–132.
- Sheets, H. D.** 2003. *CVAGen6*. Canisius College, Buffalo [updated at <http://www3.canisius.edu/~sheets/morphsoft.htm>].
- Slice, D. E.** 1998. *Morpheus et al.: Software for morphometric research. Revision 01-30-98*. Department of Ecology and Evolution, State University of New York, Stony Brook.
- Soose, E.** 1954. Effect of temperature on the wing index and chitin colour of the honeybee. *Archive für Bienenkunde*, **31**, 49–66.
- Tan, K., Hepburn, H. R., Radloff, S. E., Fuchs, S., Fan, X., Zhang, L. J. & Yang, M. X.** 2008. Multivariate morphometric analysis of the *Apis cerana* of China. *Apidologie*, **39**(3), 343–353.
- Timon-David, J.** 1944. Insectes fossiles de l'Oligocène inférieur Camoins (Bassin de Marseille) II. Hyménoptères. *Bulletin de la Société Entomologique de France*, **49**, 40–45.
- Tofilski, A.** 2008. Using geometric morphometrics and standard morphometry to discriminate three honeybee subspecies. *Apidologie*, **39**(5), 558–563.
- Vencl, F. V.** 2004. Allometry and proximate mechanisms of sexual selection in *Photinus* fireflies, and some other beetles. *Integrative and Comparative Biology*, **44**(3), 242–249.
- Verma, L. R., Mattu, V. K. & Daly, H. V.** 1994. Multivariate morphometrics of the Indian honeybee in the northwest Himalayan region. *Apidologie*, **25**, 203–223.
- Wappler, T. & Engel, M. S.** 2003. The middle Eocene bee faunas of Eckfeld and Messel, Germany (Hymenoptera: Apoidea). *Journal of Paleontology*, **77**, 908–921.
- Zeuner, F. E. & Manning, F. J.** 1976. A monograph on fossil bees (Hymenoptera: Apoidea). *Bulletin of the British Museum (Natural History), Geology Series*, **27**, 149–268.