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The utility of wing morphometrics for assigning type specimens to cryptic bumblebee species

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Abstract. Since the beginning of taxonomy, species have been described based on morphology, but the advent of using semio-chemicals and genetics has led to the discovery of cryptic species (i.e. morphologically similar species). When a new cryptic species is described, earlier type specimens have to be re-evaluated, although this process can be challenging as only nondestructive methods ought to be used in order to preserve the integrity of the type specimens. Methods should allow comparison with recently collected specimens clustered based on chemical, ethological and/or genetic traits with old specimens (i.e. type specimens) where only morphological traits are available. Here we develop a method based on geometric morphometric analyses of wing shape for a taxonomically challenging group of bumblebees, the subgenus *Alpinobombus* Skorikov. We consider nine monophyletic taxa (including several cryptic species) to assess the accuracy of this method to discriminate the taxa based on their wing shape and then to attribute type specimens using a leave-one-out cross-validation procedure. We show that, for these bees, wing shape is taxon-specific, except for two sister taxa for which the species status is still debated. Moreover, for most of the taxa, type specimens were correctly attributed with high posterior probabilities of attribution, except for a few type specimens corresponding to the same two sister taxa where taxa delimitation based on wing shape was previously the subject of discussion. Our study highlights the potential of geometric morphometric analyses to help in the re-attribution of type specimens when the existence of cryptic species is revealed.

Introduction

Cryptic species are defined as phylogenetically closely related species that are especially difficult to diagnose based on their morphology (Murray *et al.*, 2008; Williams *et al.*, 2012a). They are likely to be an important part of the global biodiversity (Bickford *et al.*, 2007). Morphological similarity among cryptic, closely related species may result from high intraspecific phenotypic variation or stasis in morphological evolution (Hebert *et al.*, 2003). Several novel methods have been developed to define and recognize cryptic taxa, including ethological, molecular or chemical methods (Moritz & Cicero, 2004; Murray *et al.*, 2008; Martinet *et al.*, 2019). These methods are very useful for exploring new traits in relatively freshly collected specimens (Jörger & Schrödl, 2013), but old type material can be more challenging. The traits can be lost (e.g. chemical/DNA degradation) or not available for the type material (e.g. ethological trait). Moreover, most of the methods are partly destructive and sometimes not replicable. Even if several nondestructive DNA extraction methods have been developed, they are time-consuming and use toxic or corrosive products (e.g. Pons *et al.*, 2006; Gilbert *et al.*, 2007). These should be avoided for unique type material in order to preserve its integrity. However, nomenclature depends on associating a name with type material (according the International Code of Zoological Nomenclature;https://www.iczn.org/the-code/the-international-code-of-zoological-nomenclature/the-code-online/), leading to the

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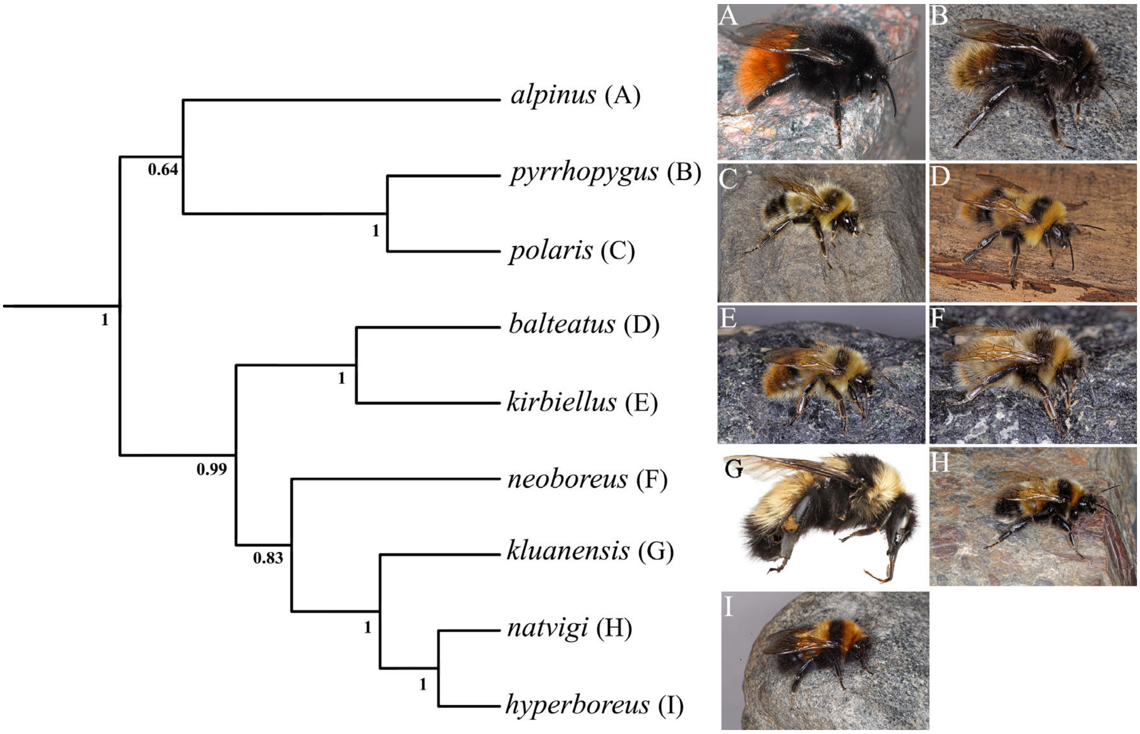

Fig. 1. Estimate of phylogeny for species of the subgenus *Alpinobombus* based on COI-barcode and Phosphoenolpyruvate carboxykinase (PEPCK) sequences modified from Williams *et al.* (2015). Values below the nodes are Bayesian posterior probabilities showing branch support. (Photographs are by P. Rasmont.) [Colour figure can be viewed at wileyonlinelibrary.com].

difficulty of how to associate old type material (for a valid name) with cryptic taxa that may be recognized from molecular, behavioural or chemical traits. Noninvasive methods like geometric morphometrics could be an especially useful alternative because geometric morphometrics is a quantitative morphological method that is cheap, nondestructive, quick and has been proved to be more successful in diagnosing closely related taxa in some groups compared with traditional descriptive morphology (e.g. Tatsuta *et al.*, 2018). We explore this method as applied to a taxonomically challenging group of bumblebees.

Bumblebees are primitive eusocial bees that are important pollinators in temperate and cold ecosystems (Heinrich, 1979). Currently, the genus *Bombus* includes *c*. 260 species (Williams, 1998) divided into 15 subgenera (Williams *et al.*, 2008). Bumblebee systematics has a long history, with explicit reviews on species-diagnosing characteristics, e.g. in the works of Radoszkowski (1884), Vogt (1909, 1911), Krüger (1920), Pittioni (1939) and Løken (1973). However, despite being well studied, their systematics remains challenging. Bumblebee morphology is relatively homogenous among species but often with a high level of intraspecific variation (Williams, 1998; Williams, 2007; Williams *et al.*, 2016). Their colour patterns are rarely species-specific, because sympatric species often converge towards a locally similar colour pattern (Williams, 2008, e.g. in Corsica, Lecocq *et al.*, 2015) and a single species can display different colour patterns in different places (regional colour forms) (Reinig, 1970; Lecocq *et al.*, 2015). Unfortunately, colour coat is nonetheless often used as an important character for diagnosing species, which can lead to taxonomic and nomenclatural confusion, with poorly defined cryptic species and species complexes (e.g. Williams, 1998; Benton, 2006; Rasmont *et al.*, 2008), and potentially negative decision in conservation and trade (Williams *et al.*, 2012b; Lecocq *et al.*, 2016). We focused our study on the taxonomically difficult subgenus *Alpinobombus*. Based on genetic (Cameron *et al.*, 2007) and morphological features (Richards, 1968; Ito, 1985), this subgenus is supported as a monophyletic taxon but the number of species is still debated mainly because authors follow different species concepts and/or methodologies (e.g. Williams *et al.*, 2016, 2019; Martinet *et al.*, 2018;). Recent revisions on skeletal morphology, pheromones (i.e. chemical traits) and genetic markers have concluded that there are from four to nine species, including cryptic species, in the last hypothesis (Martinet *et al.*, 2018; Williams *et al.*, 2019; Fig. 1).

Here, we present an original study to associate old type material with recent specimens using the wing shape through geometric morphometric analyses. Geometric morphometric analyses have been widely used to discriminate bumblebees between castes (Gérard *et al.*, 2015), or different taxonomic levels (Aytekin *et al.*, 2007; Kozmus *et al.*, 2011) as well as fossil specimens (Dehon *et al.*, 2019), often with a high level of discrimination (but see Lecocq *et al.*, 2015). We aim to assess: (i) if wing shape can be used as a taxonomically diagnostic trait;

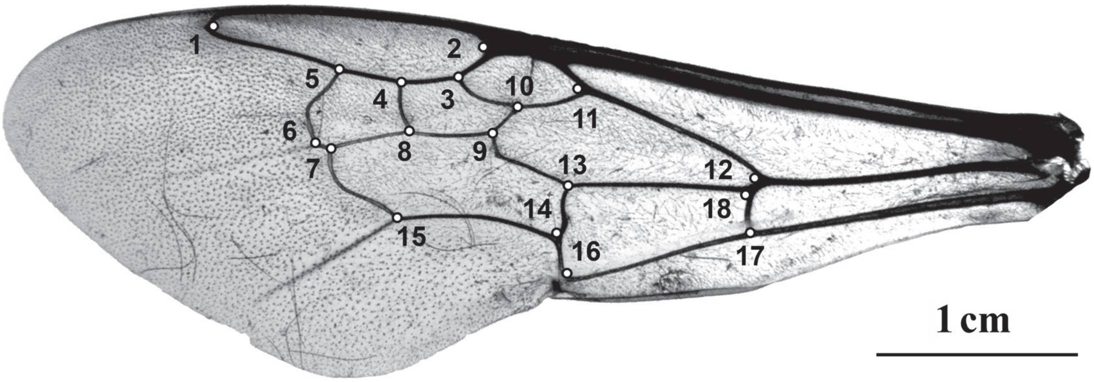

Fig. 2. Wing shape of an *Alpinobombus* species (i.e. *Bombus balteatus*) and the 18 landmarks.

and (ii) if older named type specimens can be associated with taxa recognized using other characters.

Materials and methods

Dataset

We considered the nine taxa recently recognized by Williams *et al.* (2019) in the subgenus *Alpinobombus* (Table S1): *Bombus alpinus* ($n = 69$), *Bombus balteatus* ($n = 73$), *Bombus hyperboreus* ($n = 41$), *Bombus kirbiellus* ($n = 82$), *Bombus kluanensis* ($n = 24$), *Bombus natvigi* ($n = 37$), *Bombus neoboreus* ($n = 82$), *Bombus polaris* ($n = 66$), *Bombus pyrrhopygus* ($n = 64$). Specimens were identified based on morphology, molecular (i.e. COI barcode) and/or chemical (i.e. male cephalic gland secretion) data published by Williams *et al.* (2015, 2019) and Martinet *et al.* (2018) (see Table S1 for detailed information). When possible, we sampled 60 specimens for each taxon from five different populations (i.e. 20 males, 20 workers and 20 queens) because specimens of different sex and caste can show different wing shape (Gérard *et al.*, 2015) and 20 specimens are enough to capture a specific mean shape (e.g. Dewulf *et al.*, 2014). For *B. hyperboreus* and *B. natvigi*, there are only two castes (i.e. queens and males) as they are inquiline species. In addition, we did not have enough *B. kluanensis* males to compute the analysis for this caste in the species. Overall we studied 538 specimens from four different collections (University of Mons, Natural history Museum London, Williams's research collection, and Museum für Naturkunde Berlin). Our dataset also includes type specimens used for the assignment: one queen of *B. balteatus*, one queen of *B. hyperboreus*, one queen of *B. kirbiellus*, one queen of *B. natvigi*, two queens of *B. neoboreus*, four queens of *B. polaris*, one queen, one worker and four males of *B. pyrrhopygus* (Table S1).

Geometric morphometric analyses

We photographed left forewings using an Olympus SZH10 microscope (Tokyo, Japan) combined with a Nikon D200 camera (Tokyo, Japan). We uploaded the pictures in the software TPSUTIL v.1.69 (Rohlf, 2013a). We digitized the wing shape with two-dimensional Cartesian coordinates of 18 homologous landmarks on the wing veins and cells with TPSDIG v.2.27 (Rohlf, 2013b) based on Owen (2012) (Fig. 2). We then superimposed the landmarks using GLS Procrustes superimposition using R v.3.6.1. (Rohlf & Slice, 1990; R Development Core Team, 2019). We evaluated the closeness of the tangent space to the curved space by calculating the least-squares regression slope and the correlation coefficient between Euclidean distances and Procrustes distances (Rohlf, 1999) using TPSSMALL v.1.25(Rohlf, 2013c).

We first explored the wing shape variation using principal component analysis (PCA) to visualize clustering and detect outliers. We then assessed the discrimination of the different taxa based on wing shape using a linear discriminant analysis (LDA) of the projected aligned configuration of landmarks. Due to a potential effect of sexual and caste polymorphism on the taxa assignment using wing shape, we computed analyses based on the global dataset including all castes and then within each caste specifically. We therefore performed four LDA analyses, based on the full data set (Fig. 3), workers, queens and males. We assessed the effectiveness of the LDA to discriminate the taxa using the percentage of individuals correctly identified to their original taxa (i.e. hit ratio, HR) using a leave-one-out (LOO) cross-validation procedure. This accuracy of attribution is based on the posterior probabilities of assignment (PP). This PP is calculated as the probability of an unknown specimen belonging to one group as compared with all others. Consequently, the specimen is attributed to the group with the highest PP of assignment (Huberty & Olejnik, 2006).

In order to assign type specimens, we first visualized shape affinities of the type material within the *Alpinobombus* using a PCA. We assessed taxonomic affinities of the type material by calculating the score in the predictive discriminant shape space of the four different aforementioned LDAs. We assessed the assignment of each type specimen using the Mahalanobis distance (Claude, 2008) between each type specimen and group mean of each taxon. We then assigned the type specimen to the nearest group in the discriminant shape space of the LDA. All the analyses have been performed using R (R Development Core Team, 2019).

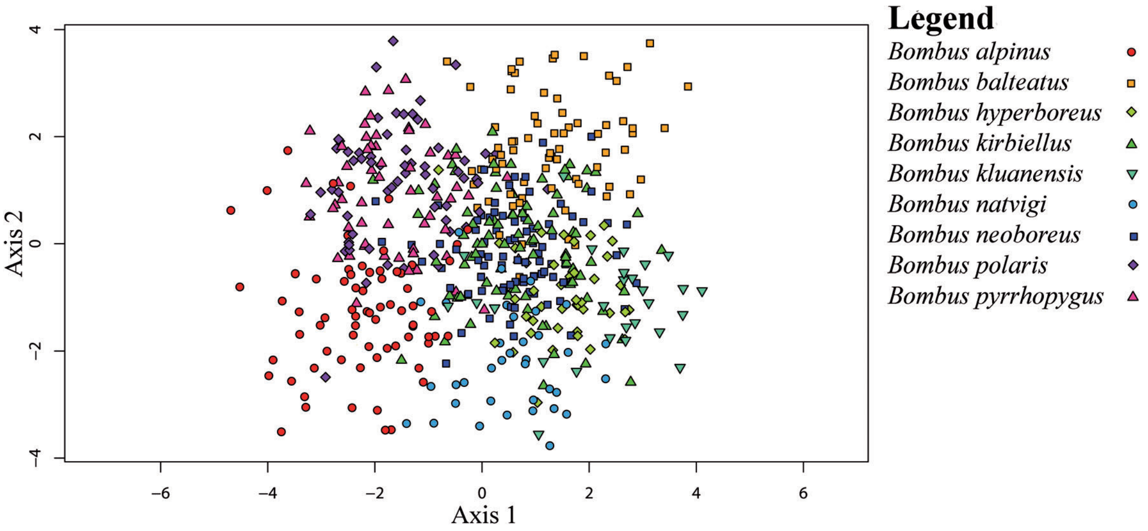

Fig. 3. Ordination of the nine species of *Alpinobombus* along the two first axes of the linear discriminant analysis (the first and the second axes explain 31.9% and 13.5% of the total variance, respectively). [Colour figure can be viewed at wileyonlinelibrary.com].

Results

Wing shape discrimination of the different species

Based on the analysis of the full dataset (i.e. all castes; Fig. 3), most of the taxa were well separated in the LDA (global HR = 78%). Based on the assignment by cross-validation, *B. alpinus* (HR = 87%), *B. balteatus* (HR = 90%), *B. hyperboreus* (HR = 83%), *B. kirbiellus* (HR = 80%), *B. kluanensis* (HR = 81%) and *B. natvigi* (HR = 84%) were well identified. Conversely, *B. neoboreus* was less correctly identified (HR = 74%); *B. polaris* and *B. pyrrhopygus* had the lowest HR values (69% and 59% respectively). The most common attribution mistakes were: *B. pyrrhopygus* identified as *B. polaris* (16 specimens; 26% of the total *B. pyrrhopygus*; Fig. 4), *B. neoboreus* identified as *B. kirbiellus* (14 specimens; 18% of the total *B. neoboreus*) and *B. polaris* identified as *B. pyrrhopygus* (13 specimens; 23% of the total *B. polaris*).

Similarly, based on the analysis of the queen dataset, HR strongly differed between species (global HR = 80%). Most of the species had high HR values (i.e. *B. alpinus*, 90%; *B. balteatus*, 91%; *B. hyperboreus*, 90%; *B. kirbiellus*, 84%; *B. natvigi*, 90% and *B. neoboreus*, 88%) except for *B. kluanensis*, *B. polaris* and *B. pyrrhopygus* which had 70%, 55% and 53% of correct identification, respectively. Most of the misidentification consisted in *B. polaris* identified as *B. pyrrhopygus* (eight specimens, 42% of the total queens of *B. polaris*) and *B. pyrrhopygus* identified as *B. polaris* (eight specimens, 40% of the total queens of *B. pyrrhopygus*).

Regarding the analysis of the worker dataset, the reliability based on wing shape also depended on the species (global HR = 84%). The HR values for *B. balteatus*, *B. kirbiellus*, *B. kluanensis* and *B. neoboreus* were all above the 80% correct attributions. However, the HRs of *B. alpinus* (74%), *B. polaris* (74%) and *B. pyrrhopygus* (74%) were slightly lower. The most common misclassification was *B. pyrrhopygus*, identified as *B. polaris* (four specimens; 21% of the total workers of *B. pyrrhopygus*).

Finally, based on the analysis of the male dataset, the HR again strongly differed between species; the global HR was also the lowest of all the castes (73%). The HR was only higher than 80% for *B. alpinus* (96%). Hit ratios of *B. balteatus*, *B. hyperboreus*, *B. natvigi* and *B. neoboreus* were all between 70% and 80% of correct attributions. Finally, HRs of *B. kirbiellus* (56%), *B. polaris* (61%) and *B. pyrrhopygus* (45%) were the lowest. The most common misclassifications were *B. pyrrhopygus*, identified as *B. polaris* (five specimens; 25% of the total males of *B. pyrrhopygus*), and *B. kirbiellus*, identified as *B. neoboreus* (five specimens; 28%).

Assignment of the type material

When using the full dataset, 13 out of 16 types were assigned to the group defined based on traditional morphology (Table 1). The neotype of *B. balteatus* was correctly attributed (PP = 99%), as well as the holotype of *B. hyperboreus* (97%), the lectotype of *B. kirbiellus* (PP = 90%), the holotype of *B. natvigi* (PP = 99%) and the two lectotypes of *B. neoboreus* (PP = 95% and 99%, respectively). Concerning the four types of *B. polaris*, three out of four types were correctly attributed but only the holotype had a PP of attribution > 90%. The two other *B. polaris* types were correctly attributed with PPs of 88% [lectotype *kirbiellus pyrrhopygus*; the closest taxa was *B. pyrrhopygus* (PP = 5%)] and 35% [lectotype *kincadii*; the closest taxa were *B. hyperboreus* (PP = 34%), *B. balteatus* (PP = 20%) and *B. pyrrhopygus* (PP = 9%)]. The only misidentified *B. polaris* type (lectotype *polaris*) was attributed to *B. kirbiellus* (PP = 60%) and the closest taxa was *B. polaris* (PP = 28%). Finally, four out of the six *B. pyrrhopygus* types were correctly

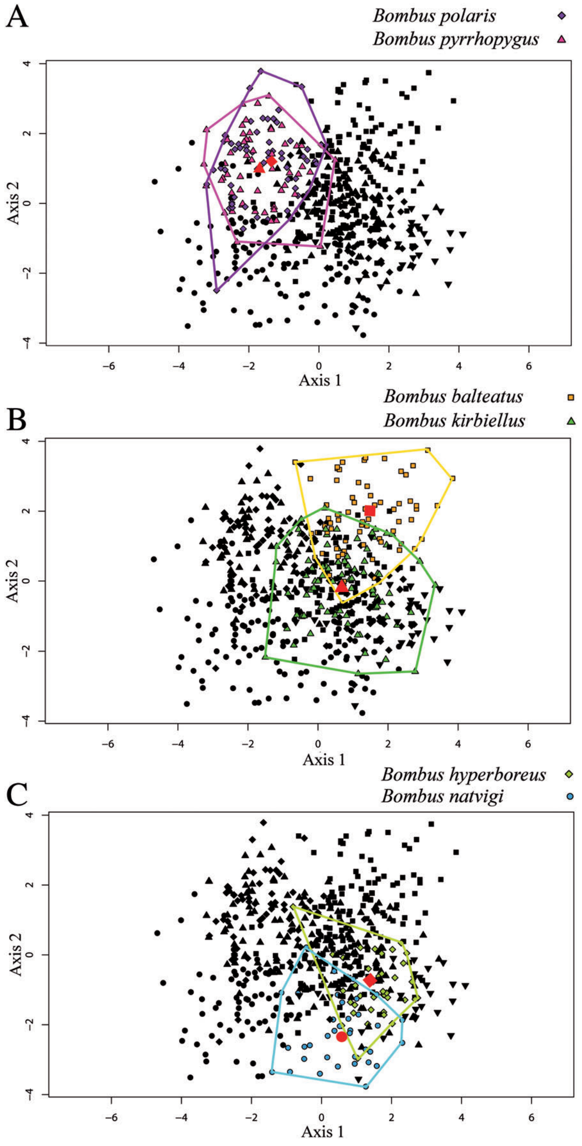

Fig. 4. Ordination of the three pair of *Alpinobombus* sister species along the two first axes of the linear discriminant analysis (LDA; the first and the second axes explain 31.9% and 13.5% of the total variance, respectively). The taxa of interest are in colour in each LDA, and the other taxa are in black. (A) *Bombus polaris* and *Bombus pyrrhopygus*. The red square is the centroid of *B. polaris* while the red triangle is the centroid of *B. pyrrhopygus*. (B) *Bombus balteatus* and *Bombus kirbiellus*. The red square is the centroid of *B. balteatus*, and the red triangle is the centroid of *B. kirbiellus*. (C) *Bombus hyperboreus* and *Bombus natvigi*. The red square is the centroid of *B. hyperboreus*, and the red circle is the centroid of *B. natvigi*. [Colour figure can be viewed at wileyonlinelibrary.com].

attributed. Two *B. pyrrhopygus* types had a PP of attribution > 90% (lectotype *alpinus diabolicus* and holotype *alpinus pretiosus*). The two other correctly attributed types had PPs of 60% [lectotype *kirbyellus cinctus*; the closest taxon was *B. polaris* (PP = 40%)] and 64% [lectotype *kirbyellus cinctellus*; the closest taxon was *B. polaris* (PP = 35%)]. The two misidentified *B. pyrrhopygus* types were attributed to *B. neoboreus* with a PP of 44% [lectotype *kirbyellus semljaënsis*; the closest taxa were *B. natvigi* (PP = 26%) and *B. pyrrhopygus* (PP = 19%)] and to *B. polaris* with a PP of 73% [lectotype *kirbiellus pyrrhopygus*; the closest taxa was *B. pyrrhopygus* (PP = 27%)].

When we attributed the types in each of their respective caste, only one misidentification persisted: the misidentification of one *B. pyrrhopygus* male as a *B. polaris* male (PP = 98%, the closest taxon was *B. pyrrhopygus*).

Discussion

Diagnosis of Alpinobombus *species*

Our study brings additional morphological evidence (i.e. wing shape similarity) for diagnosing species of the subgenus *Alpinobombus*. Most of the nine taxa were well separated based on wing shape, whether we assessed it within each caste or all castes together. However, it should be noted that the taxa are rarely totally separated and the clusters are overlapping, and thus the wing shape does not allow us to systematically attribute a specimen to its right group. During the last 100 years, authors have recognized between four and nine species (Williams *et al.*, 2019; Table S2) but there is recent consensus for at least five of them: *B. alpinus*, *B. balteatus*, *B. hyperboreus*, *B. neoboreus* and *B. polaris* (Rasmont, 1983; Williams, 1998). However, several authors also recognized *B. kirbiellus*, *B. kluanensis*, *B. natvigi* and *B. pyrrhopygus* based on slight variation of colour pattern (Loken, 1973; Milliron, 1973), morphology (Williams *et al.*, 2016) or molecular trait (Williams *et al.*, 2019). In addition to morphology and genetics, these taxa have also been assessed in terms of cephalic labial gland secretions (CLGS), a pre-mating recognition system in bumblebees (Ayasse & Jarau, 2014). Using this method, *B. polaris* could not be significantly separated from *B. pyrrhopygus*, and *B. hyperboreus* could not be separated from *B. natvigi* (Martinet *et al.*, 2018). Our results corroborate the similarity of one pair of taxa: *B. polaris* and *B. pyrrhopygus*. In the global analysis and even more particularly among queens, HRs of these two species were close to 50%, which is particularly low compared with the other species of the subgenus. Two interpretations of these taxa have been argued: either that this is a case of conspecific taxa as species *in status nascendi* (Martinet *et al.*, 2018) or that these are two separate species in the sense of evolutionarily independent lineages that have not yet been in sympatry, so that substantially divergent mate-recognition systems have not yet evolved (Williams *et al.*, 2019). We confirm that the two species of the other controversial pair (i.e. *B. hyperboreus* and *B. natvigi*) are clearly different based on their wing shape. While CLGS have not diverged between these two taxa, the reason could be that these two sister taxa do not spatially overlap and thus no selective pressure has led to CLGS differentiatio,n as suggested by Williams *et al.* (2019). However, Potapov *et al.* (2019) has also suggested that these two taxa could be conspecific based on genetic evidence, although the multi-rate PTP (Poisson Tree Processes) analytical technique they use appears, from tests of its application to other bumblebee examples, to be unduly conservative.

Table 1. Mahalanobis distances (MD) between type specimens and species centroids as well as the posterior probabilities of attribution (PP) based on the leave-one-out analysis.

	B. kirbellius TQ1		*B. natvigi* TQ1		*B. neoboreus* TQ1		*B. neoboreus* TQ2	
	Centroid MD	Pp	Centroid MD	pp	Centroid MD	PP	Centroid MD	PP
B. alpinus	4.432	< 0.001	6.904	< 0.001	6.5392	< 0.001	5.0913	< 0.001
B. balteatus	2.8642	0.072	7.1316	< 0.001	6.7323	< 0.001	6.4792	< 0.001
B. hyperboreus	5.5171	< 0.001	6.0463	< 0.001	7.8817	< 0.001	7.0687	< 0.001
B. kirbiellus	**1.8716**	**0.904**	5.8678	< 0.001	4.6966	0.005	4.1675	0.005
B. kluanensis	5.3016	< 0.001	7.7042	< 0.001	5.8081	< 0.001	6.0132	< 0.001
B. natvigi	5.2252	< 0.001	**2.9106**	**0.999**	7.3361	< 0.001	5.9938	< 0.001
B. neoboreus	3.3495	0.019	6.2167	< 0.001	**3.3627**	**0.995**	**2.5564**	**0.995**
B. polaris	3.8034	0.002	6.1106	< 0.001	5.3748	< 0.001	5.0238	< 0.001
B. pyrrhopygus	3.8424	< 0.001	6.0063	< 0.001	6.33	< 0.001	5.4606	< 0.001
	B. polaris TQ1		*B. polaris* TQ2		*B. polaris* TQ3		*B. polaris* TQ4	
	Centroid MD	PP	Centroid MD	PP	Centroid MD	PP	Centroid MD	PP
B. alpinus	4.758	< 0.001	4.2174	< 0.001	6.0484	< 0.001	3.9987	0.017
B. balteatus	5.5817	< 0.001	3.7353	0.007	3.6869	0.204	4.8219	< 0.001
B. hyperboreus	6.6944	< 0.001	5.51	< 0.001	3.4926	0.347	6.9065	< 0.001
B. kirbiellus	5.0369	< 0.001	**2.308**	**0.602**	4.7306	0.003	3.8947	0.032
B. kluanensis	6.8437	< 0.001	5.6708	< 0.001	4.9376	< 0.001	5.7213	< 0.001
B. natvigi	6.3743	< 0.001	4.9239	< 0.001	4.4781	0.004	6.3018	< 0.001
B. neoboreus	4.4126	< 0.001	2.964	0.104	5.1413	< 0.001	4.0469	0.017
B. polaris	**2.1682**	**0.987**	2.4904	0.28	**3.3834**	**0.352**	**2.8028**	**0.88**
B. pyrrhopygus	3.6619	0.012	3.6945	0.006	3.8481	0.089	3.6465	0.054
	B. pyrrhopygus TM1		*B. pyrrhopygus* TM2		*B. pyrrhopygus* TM3		*B. pyrrhopygus* TM4	
	Centroid MD	PP	Centroid MD	PP	Centroid MD	PP	Centroid MD	PP
B. alpinus	5.9641	< 0.001	4.6539	0.006	5.7489	< 0.001	5.9476	< 0.001
B. balteatus	4.6679	0.003	5.8193	< 0.001	4.6406	0.009	6.1282	< 0.001
B. hyperboreus	4.8756	< 0.001	7.5861	< 0.001	4.9312	0.001	5.8324	< 0.001
B. kirbiellus	5.769	< 0.001	4.9437	0.002	5.8013	< 0.001	4.6605	0.042
B. kluanensis	6.3395	< 0.001	7.096	< 0.001	6.4576	< 0.001	5.9815	< 0.001
B. natvigi	6.5272	< 0.001	6.6711	< 0.001	6.785	< 0.001	4.116	0.266
B. neoboreus	6.0457	< 0.001	5.8256	< 0.001	6.3644	< 0.001	**4.0462**	**0.442**
B. polaris	3.8735	0.078	3.6284	0.397	3.7378	0.351	4.5054	0.061
B. pyrrhopygus	**3.1532**	**0.918**	**3.4961**	**0.595**	**3.5552**	**0.639**	4.2302	0.19
	B. pyrrhopygus TQ1		*B. pyrrhopygus* TW1		*B. balteatus* TQ1		*B. hyperboreus* TQ1	
	Centroid MD	PP	Centroid MD	PP	Centroid MD	PP	Centroid MD	PP
B. alpinus	4.2728	0.026	5.7517	< 0.001	5.7485	< 0.001	6.1	< 0.001
B. balteatus	6.2126	< 0.001	7.1348	< 0.001	**4.1314**	**0.994**	5.1072	< 0.001
B. hyperboreus	6.1124	< 0.001	7.4301	< 0.001	7.2264	< 0.001	**2.7931**	**0.972**
B. kirbiellus	4.49	0.012	6.6278	< 0.001	5.7722	< 0.001	5.2528	< 0.001
B. kluanensis	6.2598	< 0.001	8.0065	< 0.001	8.2352	< 0.001	4.4676	0.019
B. natvigi	4.6608	0.002	7.5269	< 0.001	8.0255	< 0.001	4.5799	0.001
B. neoboreus	5.1117	< 0.001	7.5245	< 0.001	5.4893	0.002	5.5116	< 0.001
B. polaris	4.4368	0.011	**4.0266**	**0.726**	5.4957	0.001	4.3454	0.006
B. pyrrhopygus	**3.264**	**0.948**	4.2465	0.274	5.4274	0.002	5.0039	< 0.001

M, male; Q, queen; T, type specimen; W, worker. Numbers in bold represent the attributions.

Type material association

Associating specimens from morphologically cryptic species to old type material is particularly challenging. The description of new cryptic species based on genetic and/or pheromonal analyses can question the specific attribution of the previously described type specimens. Based on traditional descriptive morphology, older type specimens can be difficult to attribute to these newly discovered cryptic species, particularly if the cryptic species co-occur (e.g. Polasek *et al.*, 2019). In this framework, the quantification of the wing shape through geometric morphometrics can be particularly helpful but is largely understudied. Using this set of techniques, attribution attempts of a single specimen to particular groups (or specimens for which morphological features are the only information available) have mostly been used in palaeontology until now (e.g. De Meulemeester *et al.*, 2012; Wappler *et al.*, 2012; Dehon *et al.*, 2014, 2019). In this field of research, the results of this quantitative analysis can either confirm those obtained using traditional morphological features or refine their attribution (Dehon *et al.*, 2019). In our study, 13 out of the 16 types were attributed to the initial assessment based on descriptive morphology, with high PP values (> 85%) and even 15 out of 16 types were correctly attributed when only using datasets corresponding to their caste. Most of the misidentifications were associated with phylogenetically close taxa for which the species status is still debated (i.e. *B. polaris* and *B. pyrrhopygus*; Fig. 1). Whether or not they are distinct species, the selective pressure at specific level on wing shape is probably too weak to lead to systematic wing differentiation between two taxa that do not share the same geographical range. However, in this case, the taxa *B. polaris* and *B. pyrrhopygus* are only known to occur on separate continents so that identification should be possible from the locality data alone.

Our study sheds light on the potential of geometric morphometrics both to help in the attribution of newly collected specimens to old type material and to reassess the attribution of old type material when cryptic species have been discovered with genetic or semio-chemical methods. This assertion is particularly true when only high PPs of attribution are considered. Indeed, the quantification of a morphological trait (e.g. wing shape) is not always adequate to discriminate species and it has to be tested in preliminary analyses (e.g. Lecocq *et al.*, 2015). The applicability of this method could obviously benefit a wider number of taxa than these bees if a morphological trait can capture taxonomic information and be digitized using homologous landmarks.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Overall dataset including 538 specimens. Type specimens are in red.

Table S2. *Alpinobombus* species based on taxonomical revision. The asterisk (*) indicates that a new species has been described by the authors.

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