



Sex- and size-dependent variation in wing morphology of the cuckoo wasp *Trichrysis cyanea* (Hymenoptera: Chrysididae)

DAVID FRÖHLICH¹ , LUKAS ZANGL^{1,2} , GÜNTHER RASPOTNIG¹  and STEPHAN KOBLMÜLLER¹ 

¹ Institute of Biology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria; e-mails: david.froehlich@uni-graz.at, lukas.zangl@uni-graz.at, guenther.raspotnig@uni-graz.at, stephan.koblmueller@uni-graz.at

² Universalmuseum Joanneum, Studienzentrum Naturkunde, Weinzöttlstraße 16, 8045 Graz, Austria; e-mail: lukas.zangl@museum-joanneum.at

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Abstract. Intraspecific variation and sexual dimorphism in wing shape and size is common in winged insects. The exact patterns, however, differ among taxa and are related to the selection pressure acting on specific traits. Cuckoo wasps (Chrysididae) are hymenopterans that have evolved a complex parasitoid or cleptoparasitic life-style. For the first time, we studied the intraspecific variation in wing shape and size in the model species, *Trichrysis cyanea*, a common Palearctic cuckoo wasp. This involved geometric morphometrics combined with a novel, non-invasive way of obtaining images, to study the shape and size of the forewings of males and females. We found clear sexual dimorphism in both wing shape and size, possibly related to fecundity (as wing size typically correlates with body size in many insects) and increased manoeuvrability of females for searching for their host's nesting holes. This study increases our knowledge of the biology of *T. cyanea* in terms of a better understanding of the evolution of parasitism and corresponding adaptations in this hymenopteran family.

INTRODUCTION

In pterygote insects, wing shape, size and venation vary considerably at all taxonomic levels down to populations (Grimaldi & Engel, 2005). These traits are under strong natural selection, as they are directly related to flight performance and thus determine foraging and dispersal ability (Wootton, 1992; Bots et al., 2009; Johansson et al., 2009). In addition, sexual selection also plays a role in some taxa (e.g. Outomuro et al., 2016) and in particular at the intraspecific level, genetic variability and environmental factors potentially also affect wing morphology (Hoffmann & Shirriffs, 2002; Loh et al., 2008; Outomuro et al., 2013). Consequently, wing morphology and flight behaviour can and do differ between closely related species (DeVries et al., 2010; Suárez-Tovar & Sarmiento, 2016), among populations (Hassall, 2015) and between sexes (Breuker et al., 2007).

Most studies on variation in wing shape in Hymenoptera report at least some degree of sexual dimorphism in wing size and/or shape between males and females, but sex-dependent wing-size and the trajectories of sex-specific differences in wing shape differ among species (e.g. Pretorius, 2005; Benítez et al., 2013; Petrović et al., 2014). Chrysididae, or cuckoo wasps, is a worldwide distributed family of parasitoid and cleptoparasitic Hymenoptera with

around 3000 described species. They parasitize sawflies, aculeate wasps and bees and in some cases also silk moths and stick insects (Kimsey & Bohart, 1990). So far, there are no reports of sexual dimorphism and/or intra-sexual variation in wing size and morphology of any chrysidid wasp. In the present study, we focus on one species of Chrysididae, *Trichrysis cyanea* (Linnaeus, 1758). This is a palearctic species and unlike many other cuckoo wasps, has a broad range of hymenopteran hosts, belonging mainly to Pompilidae (*Trypoxylon*) and Crabronidae (*Auplopus* and *Deuteraenia*) (Pauli et al., 2019; Wiesbauer et al., 2020). A recent study (Fröhlich et al., 2022) reports sexual dimorphism in cuticular hydrocarbons in *T. cyanea*. Furthermore, female *T. cyanea* express several markedly different profiles of cuticular hydrocarbons, the reason of which is still unclear, although adaptations (mimicry) to different host species or signalling of the females' reproductive status have been hypothesized.

Here, using geometric morphometrics on photographs of forewings taken with the aid of a newly developed tool that can be used on type-material as it does not require the removal of wings from specimens, we studied intraspecific variation in wing morphology and size in *T. cyanea* from eastern Austria. These findings are discussed in the context of the current state of knowledge of this species' biology.

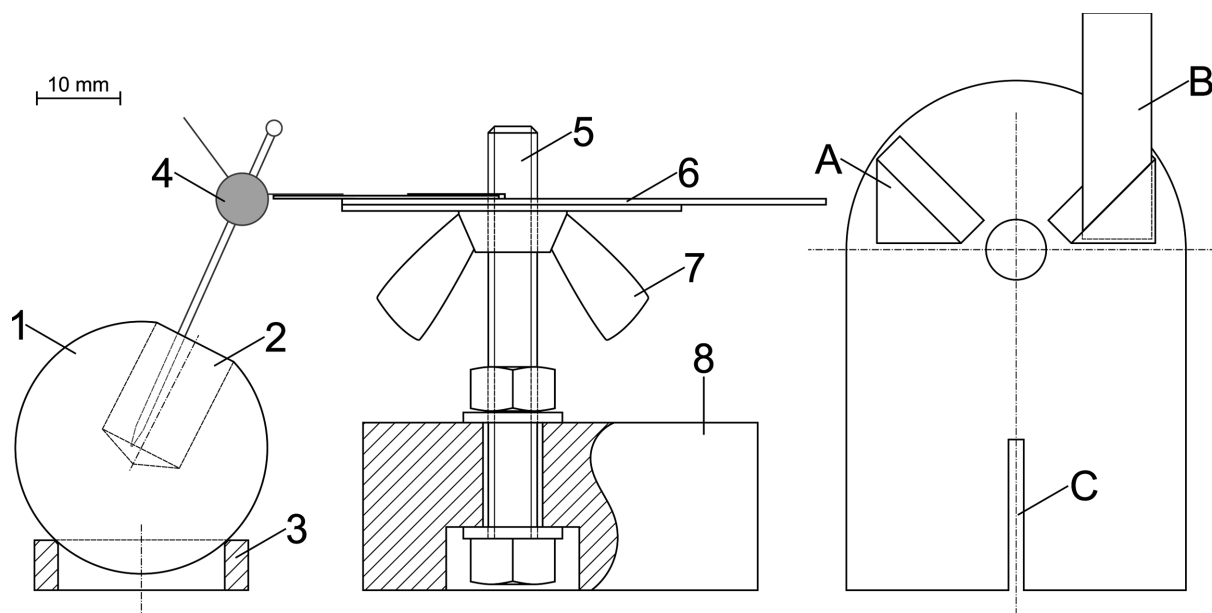


Fig. 1. Detailed sketch of WOMBAT. Items 1–3 are already commonly used by entomologists to fix a pinned insect for examination, e.g. under a stereo microscope and to position wings horizontally: 1 – iron sphere (\varnothing 30 mm), 2 – drilled hole (\varnothing 10 mm) filled with foam, 3 – short piece of pipe (inner \varnothing 20 mm); 4 – pinned insect. Items 5–8 were used to adjust the height of the paper platform: 5 – screw (M6), 6 – paper platform (detailed top view on the right with letters A–C), 7 – butterfly nut stuck to a thick disc made of paper, 8 – wooden block. The paper platform (A–C) is used to completely cover the iron sphere or to mount differently shaped and coloured strips, which can be adjusted as background for imaging the wings and can help with adjusting them: A – photograph mounting corners for the paper strip, B – paper strip for, C – slot for complete covering of the iron sphere.

MATERIAL AND METHODS

Fifty-one individuals of *T. cyanea* were collected in 2017 and 2018 in Eastern Austria and their cuticular hydrocarbons and DNA barcodes were analysed (Fröhlich et al., 2022). Thus, detailed information on the specimens and the 14 sites sampled can be found in Fröhlich et al. (2022), on BOLD (www.boldsystems.org; project-ID: BACHR), and in Fig. S1. The individuals were collected from deadwood, insect nesting aids and tree trunks in gardens, grassland or at the edges of forests. Only one of these specimens of *T. cyanea* (sample ID: DF014) was not used in the present study because its wings were badly misshapen and folded. Overall, 27 males and 24 females were investigated.

The left forewing was digitized using a VHX-5000 digital microscope (Keyence, Belgium) fitted with a VHX-J20T-objective. Magnification was $\times 50$ and the photographs had a resolution of 1600×1200 pixels and included an internal scale. To circumvent the removal of the forewings, we designed a new tool, Wing Orientation & Manipulation and Bracket for Afterimage-Tool (WOMBAT), that enables the in situ photographing of wings for geometric morphometric studies (Figs 1 and S2).

An iron sphere filled with foam on a short piece of pipe is generally used by entomologists to adjust pinned insects for examination and determination. This item is combined with a second tool we designed to have a height-adjustable platform providing a standardized background and that allows for the wings to be aligned horizontally. A similar device was used by Perrard et al. (2012), but differed in that we did not use glass slides that were clamped together. Instead, we used a platform, which is continuously variable, to support the wings from underneath.

Fifteen landmarks and 43 semilandmarks on the photographs of the wings were digitized (Fig. S3, Table S1).

All these steps were done by the same observer and in random order. Nomenclature of the wing venation follows Wiśniowski (2015). Semilandmarks were located around the outline and along

the veins. Only the outline between landmarks 4, 5 and 15 was not covered with semilandmarks, because this edge was often slightly damaged or not clearly visible in the photographs. All steps in the geometric morphometric analysis were done using tps-software packages (Rohlf, 2015). For digitizing, we used tpsDig2 ver.2.31, with the photographs in random order. Afterwards, calculation of consensus, partial warps and relative warps was done using tpsRelw ver1.69. Wing area was calculated using tpsUtil 1.76. As the sample size is relatively small and the number of (semi)landmarks (58 in total) is large, a principal component analysis (PCA) was used to reduce the dimensions (equals relative warp analysis). Differences due to sex, locality and female chemotype were tested using one-way PERMANOVA (Euclidean similarity index) or t-tests. For calculating the p-value, 9999 permutations were used. Only the two most common chemotypes sensu Fröhlich et al. (2022) were included, as for the third chemotype only two samples were available. This and further statistical analyses were done using Past 3.17 (Hammer et al., 2001). If not stated otherwise, the default settings were used for computation.

To examine the effect of allometry on changes in shape and whether this effect differs among sexes, we conducted a multivariate analysis of covariance (MANCOVA) using tpsRegr ver1.45 and landmarks as dependent variables, centroid size as covariance and sex as the grouping factor. A significant sex \times centroid size interaction indicates that allometric slopes differ between sexes (Zelditch et al., 2004).

RESULTS

The results indicate that male and female *T. cyanea* differ in both the size and shape of their wings and that both sex and size influence wing shape and the extent to which size effects wing shape differs between males and females. In detail, the relative warp scores (Fig. 2A) for wing shape separated, albeit with some overlap, the sexes

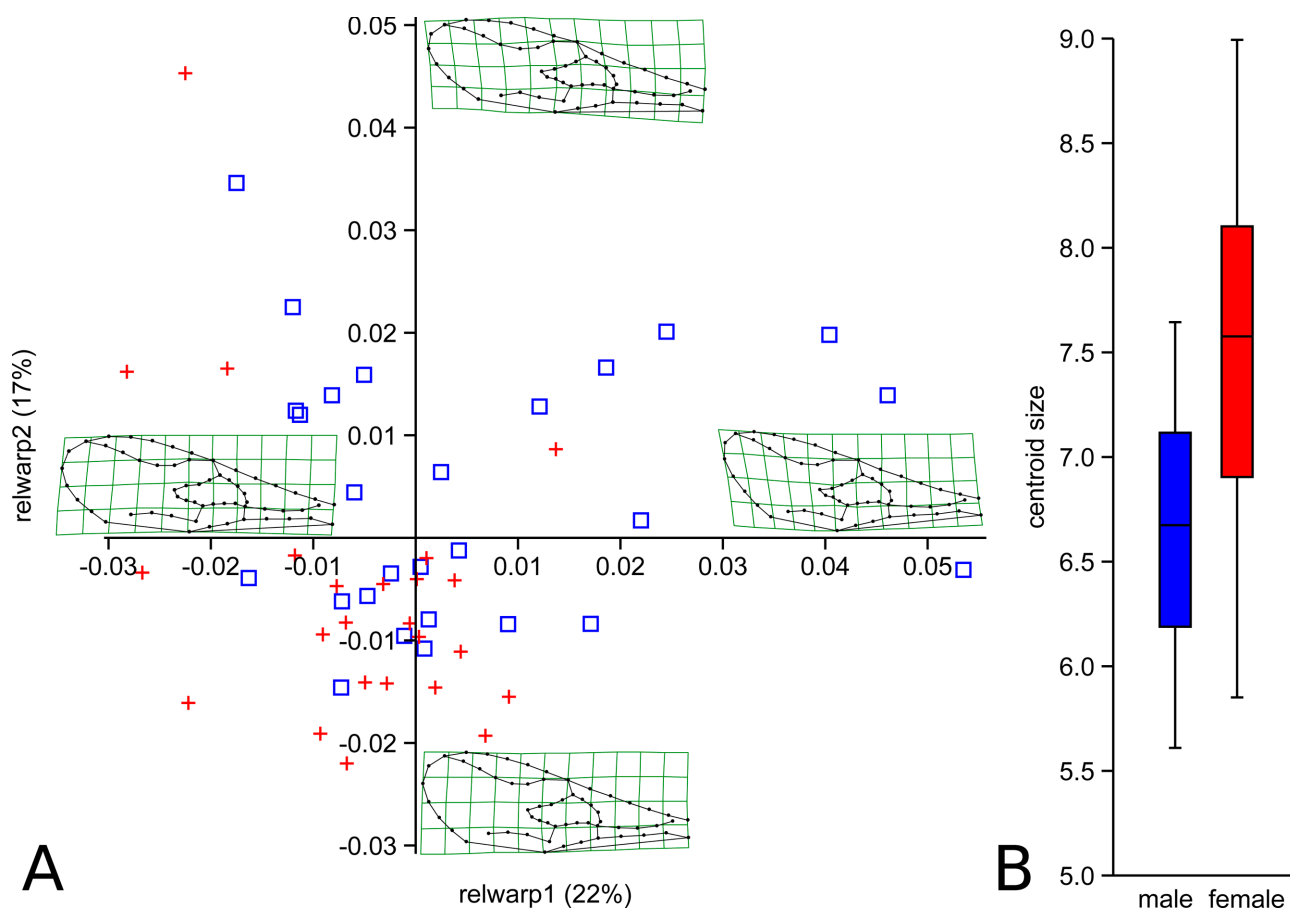


Fig. 2. A – Plot of the relative warp scores 1 against 2. Males are indicated by blue squares, females by red crosses. The deformation grids show the extreme values of relwarp1 (–0.0282, 0.535) and relwarp2 (–0.0220, 0.0453). B – Boxplots showing the differences in the centroid sizes of the sexes of *Trichrysis cyanea* (blue – males; red – females).

(PERMANOVA: $p = 0.002$ using relative warps 1 & 2). The centroid size of the wings also differed, with males being generally smaller than females (t-test: $p < 0.001$) as shown in Fig. 2B). The variance in centroid size is larger for females (0.512) than males (0.354), but the differences are not significant ($p = 0.362$). The same results were obtained for the analysis of wing area.

Analysis of the variation in shape and venation associated with variation in size of wings revealed a shift in the position of central veins towards the distal area in large wings and a relative extension of the discal cell, whereas the discal cell is smaller relative to wing size and shifts proximally in small wings (Fig. 3).

In addition, the pterostigma is broader in larger wings as the distance between landmark seven and the outline increases.

In general, a females' wing is more oval-shaped than that of males. The male wing tapers towards the distal front

edge whereas that of the female does not (Fig. 4). The results of the multivariate test for allometry can be found in Table 1. These results show, that the differences in wing shape and venation are due to both size and sex, as well as their interaction.

There were no significant differences in wing shape recorded for the two main regions sampled; eastern and southern Austria (PERMANOVA: $p = 0.162$ using relwarp 1 & 2, excluding the single inner alpine individual) nor among different female chemotypes (PERMANOVA: $p = 0.201$ using relwarp 1 & 2) based on Fröhlich et al. (2022).

DISCUSSION

Flight ability is determined by a number of morphological components, such as absolute body mass, the ratio of body mass to wing area (= wing loading) and wing shape, in particular the length of the wings relative to wing area (= aspect ratio) (Betts & Wootton, 1988; Jenkins et al., 2007; Dudley, 2018). Elongated wings (high aspect ratio) are generally more efficient for long distance flights (Wootton, 1992), whereas broader wings with blunter tips (low aspect ratio) experience less mechanical stress and increase manoeuvrability (Combes & Daniel, 2001). While such aspects seems to be universal in birds (e.g. Vágási et al., 2016), there is conflicting evidence for insects, with some

Table 1. Results of the MANCOVA test of wing shape associated with centroid size, sex and their interaction.

Effect	Wilks' Lambda	F	p
Sex & centroid size	0.09559140	1.997	0.0101
Sex	0.24361922	2.866	0.0058
Centroid size	0.29988737	2.155	0.0313

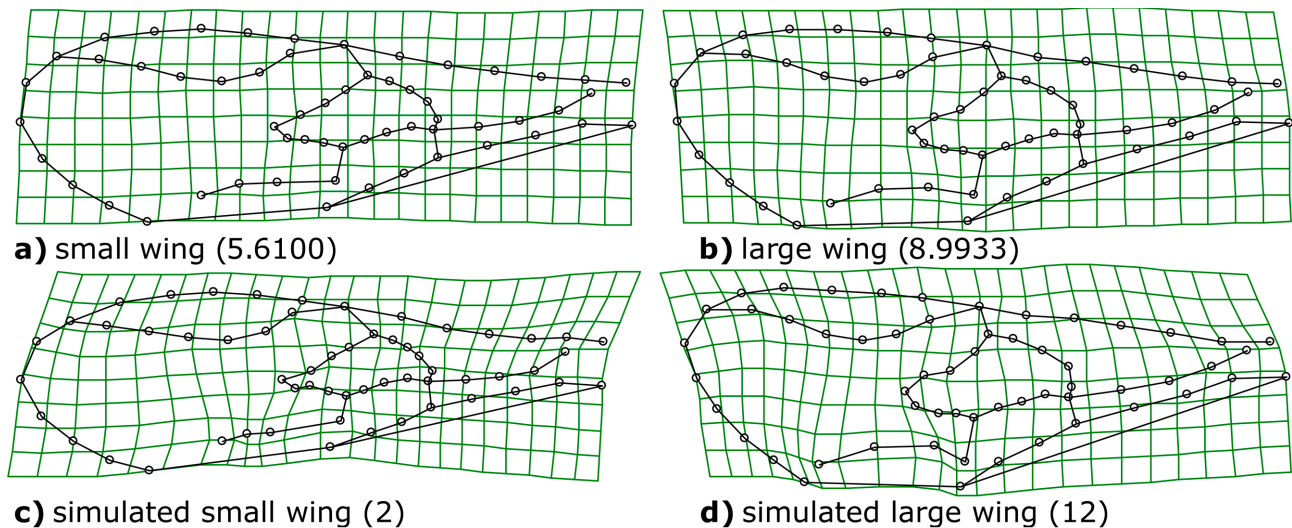


Fig. 3. Regression analysis of size-dependent and sex-independent differences in forewing shape and venation in *Trichrysis cyanea*, based on observed extreme (a, b) and simulated (c, d) values of wing centroid sizes. Centroid sizes are given in parentheses.

studies indicating that low aspect ratios are beneficial for dispersal at least in some taxa (Hassall, 2015 and references therein). In other taxa like Coleoptera and Lepidoptera, elongated wings are related to the demands of better long distance/migratory flight performance (e.g. Mikac et al., 2013). Elongated wings are more common in males, possibly because of the longer distances they need to fly in search of mates (e.g. Kingsolver, 1983; Hernández-L. et al., 2010; Jeratthitikul et al., 2014). On the other hand, shorter and broader wings, often observed in females, are regarded as an adaptation to slow flight, e.g. for searching for host plants (e.g. Jeratthitikul et al., 2014).

In *T. cyanea*, the wings are elongated and tapered in males and broad with blunter tips in females (Fig. 4). Species of chrysidids are known to visit exposed positions (trunks, piles of stones, walls etc.) and repeatedly land on exactly the same spot (Linsenmaier, 1997; Wiesbauer et al., 2020). These spots may represent marked landing

sites (possibly by scent), which are often visited while flying around. They may represent waypoints which are used for seeking out intruders or potential mating partners. As outlined above, the slender wings in males are considered more efficient for long-distance flights in search of mates or dispersal. Unfortunately, there are no population genetic studies on cuckoo wasp species that might shed light on potential sex-biased dispersal, but capture and recapture experiments on another species of chrysidids, *Caenochrysis tridens* (Lepeletier, 1825), indicate no movement between populations, but did not exclude the possibility of dispersal of newly emerged individuals (Trexler, 1984). Correspondingly, the broader and blunter wings of females recorded in our study imply an increased manoeuvrability. Indeed, high manoeuvrability could be beneficial to females when searching for the nest holes of their hosts. Thus, it is likely that the observed divergence in wing morphology between males and females is not due to some specific selective

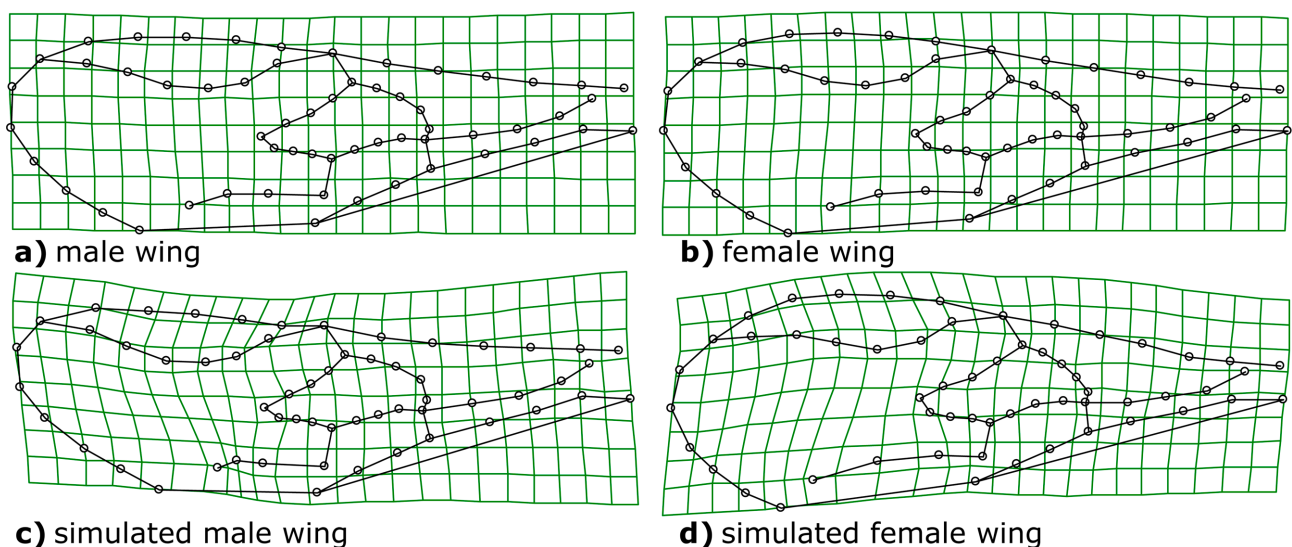


Fig. 4. Regression analysis of the sex-dependent and size-independent differences in the shape and venation of the forewing of *Trichrysis cyanea*, based on observed mean (a & b) and simulated (threefold increased) shapes for both males and females (c & d).

constraints on the male wings, but rather due to a need for increased manoeuvrability in females.

Trichrysis cyanea also exhibits a clear sexual dimorphism in forewing size. In most insects, females are larger than males (Stillwell et al., 2010), a pattern typically attributed to fecundity selection (Honěk, 1993), while cases of male-biased sexual size dimorphism have been related to contest competition (e.g. territoriality) and female choice (Serrano-Meneses et al., 2008). In the present study we did not measure body size of *T. cyanea*, but wing size and body size are highly correlated in most insects. Therefore, wing size is often used as proxy of body size in insects (e.g. Salcedo et al., 2019). Wing size in females was generally larger than in males, indicating larger body sizes of females, which indicates a role in fecundity selection in this species. Consistent with general findings on insects (Rohner et al., 2018), variance in female size in *T. cyanea* appears to be greater than that in males.

In addition to sexual dimorphism in forewing shape and size we found sex-independent size-dependent variation in wing venation (Fig. 3), with allometric patterns being different in males and females. Allometric effects can influence wing shape in Hymenoptera and other insects but there are also examples of allometry-free dimorphism (e.g. Perrard et al., 2012; Mikac et al., 2013; Petrović et al., 2014; Mielczarek et al., 2016 and others). The recorded distal shift of central veins in large wings (large centroid size) and the smaller and proximal shift of the discal cell in small wings is consistent with the findings for other insects (Danforth, 1989; García & Sarmiento, 2012). We also observed a relative broadening of the pterostigma, a pigmented spot close to the leading edge far out on the wing, in large wings. The pterostigma has a greater mass than an equally large wing piece in adjacent wing regions and makes the wing beat of small insects more efficient in slow and hovering flight by passive, inertial, pitch angle control (Åke Norberg, 1972). In most insect groups, the relative size of the pterostigma increases with decrease in body size (Danforth, 1989). Thus, the pattern observed in *T. cyanea* contradicts this general trend, the reason of which remains unknown.

To conclude, we have shown that the cuckoo wasp *T. cyanea* exhibits sexual dimorphism in both wing size and shape, most likely related to the different selective constraints in males and females, and that there is also some degree of sex-independent size-dependent variation in wing morphology. Our study is the first to specifically address intraspecific variation in wing morphology in a species of chrysidids. This study increases our understanding of the biology of *T. cyanea* (and cuckoo wasps in general), but also highlights the urgent need for more baseline data regarding population structure and connectivity as well as morphological adaptations in this and other species of this family of parasitoid Hymenoptera.

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Table S1. Description of the 15 landmarks on the front wing of *Trichrysis cyanea* used in this study and shown in Fig. S3.

Number	Description of landmark
1	On the outline of the costal vein at a dent at the base of the wing
2	On the outline of the costal vein next to the costal break
3	Fictive point of contact of the radial sector with the outline
4	Fictive point of contact of the cubital vein with the outline
5	Point of contact of the anal vein with the outline
6	Point of contact of the medio-cubital cross vein with the radial sector and medial vein
7	Point of contact of the radial sector with the pterostigma
8	Point of contact of the medial vein and the radial sector and medial vein
9	Point of contact of the cubital vein and the medio-cubital cross vein
10	Corner of the cubital vein
11	Vein outline at the point of contact of the medial vein and cubital vein
12	Vein outline at the point of contact of the cubito-anal cross vein and cubital vein
13	Point of contact of the sidearm of cubito-anal cross vein and anal vein
14	Point of contact of medial and cubital vein and subcostal vein
15	At the outline of the anal vein at a dent at the base of the wing

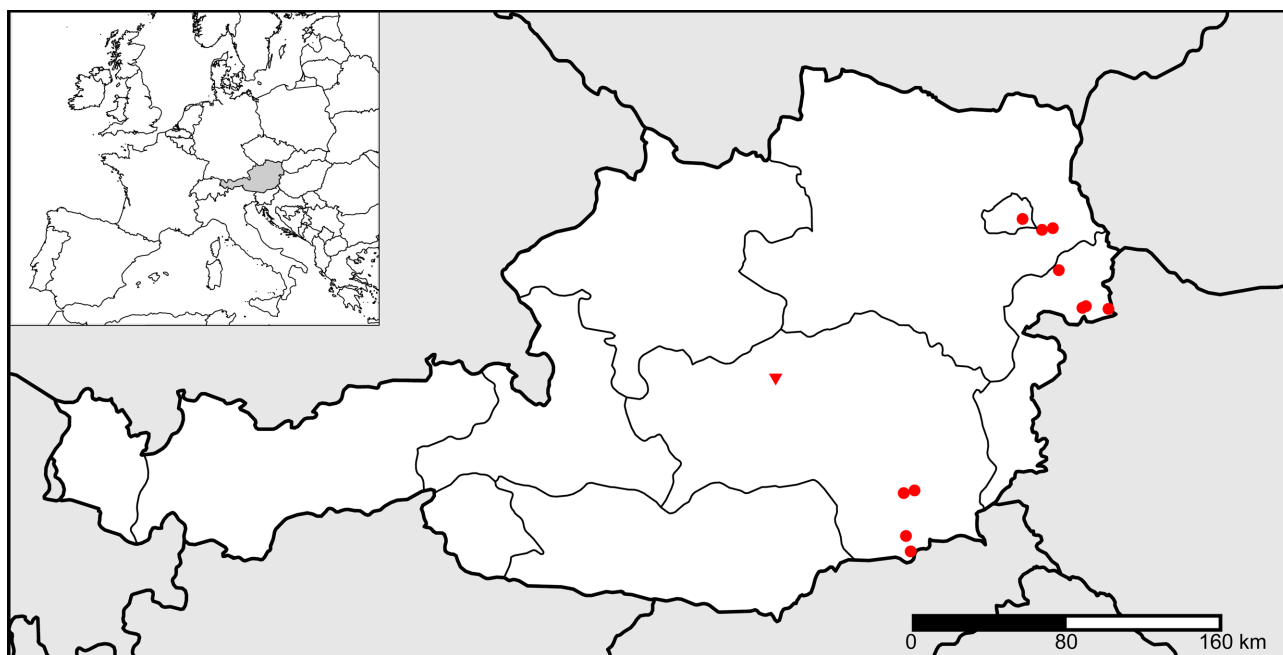


Fig. S1. Map of the sites sampled in eastern Austria. Created with SimpleMappr (<http://www.simplemappr.net/>). Detailed information on samples and sites sampled can be found on BOLD (project-ID: BACHR). The inner alpine individual is from the location marked with an inverted triangle.

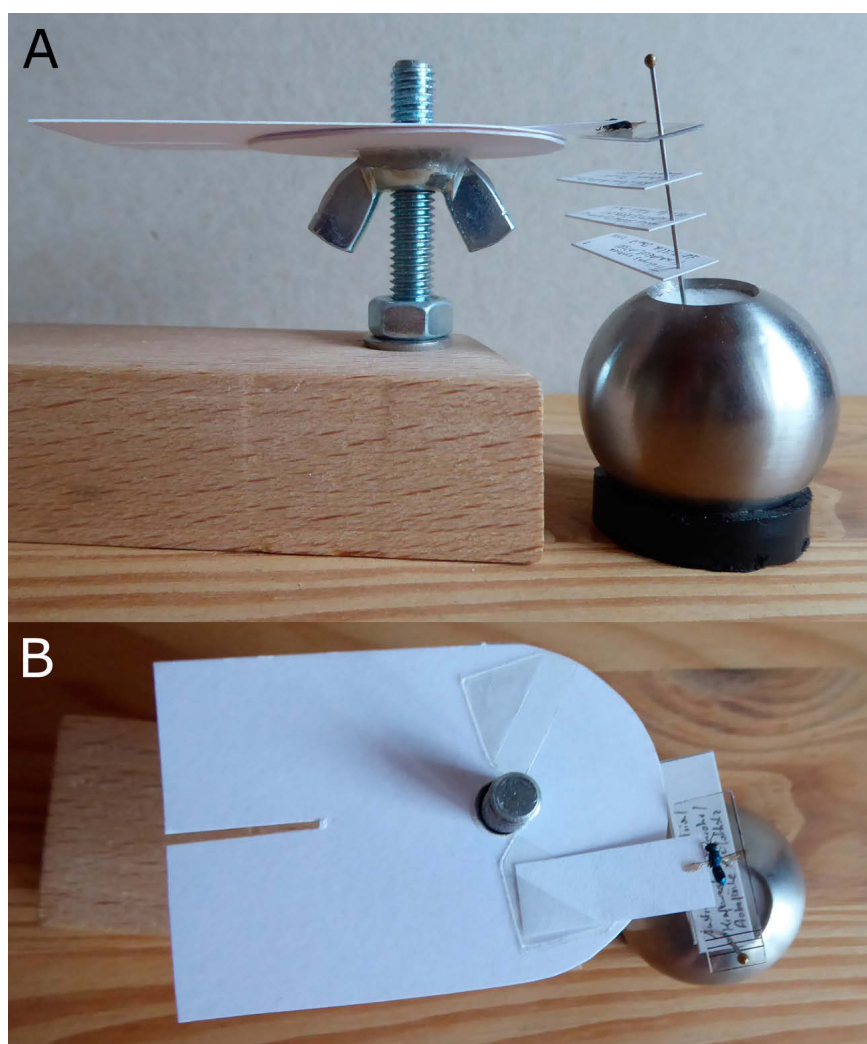


Fig. S2. Detailed pictures of WOMBAT. A – side view, B – top view.

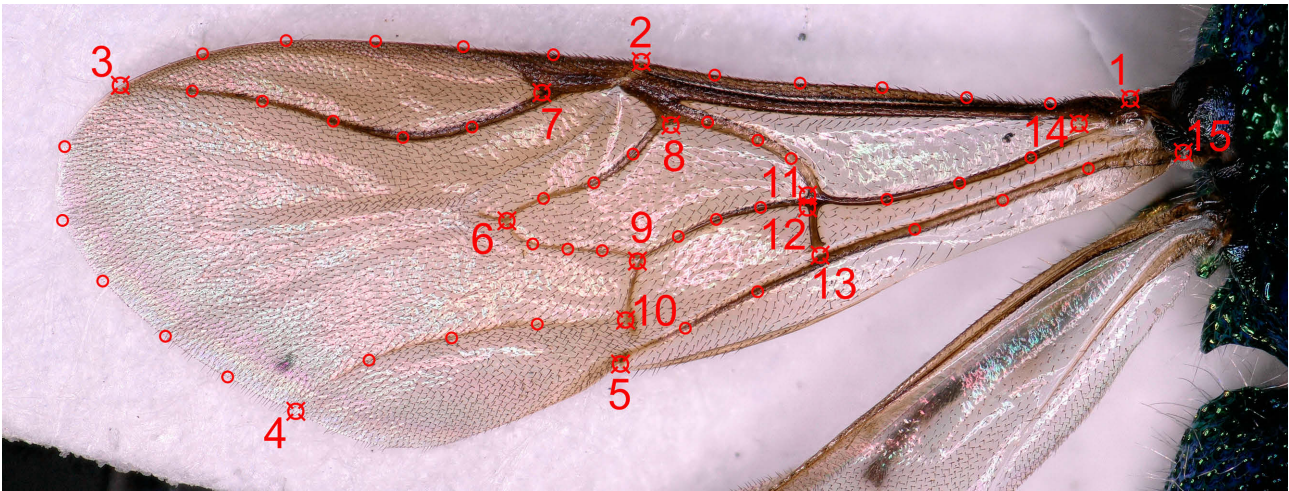


Fig. S3. Set of landmarks (cross-hairs) and semilandmarks (circles) on the veins and the outline of a fore wing. The wing remained attached to the body of the dry-mounted specimens. A description of the landmark positions can be found in Table S1.