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4 1 **Narrow versus broad: sexual dimorphism in the wing form of western European**  
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6 2 **species of the subgenus *Avaritia* Fox, 1955 (*Culicoides*, Ceratopogonidae)**  
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9 4 **ABSTRACT**  
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13 6 While wing form is known to differ between males and females of the genus  
14 7 *Culicoides* Latreille, 1809, detailed studies of sexual dimorphism are lacking. In this  
15 8 study we analyze sex-specific differences in the wing form of five species of the  
16 9 subgenus *Avaritia* Fox, 1955 using geometric morphometrics and comparative  
17 10 phylogenetic methods. Our results confirm the existence of marked sexual dimorphism  
18 11 in the wing form of the studied species and reveal for the first time that while there is a  
19 12 shared general pattern of sexual shape dimorphism within the subgenus, sexual size  
20 13 dimorphism and particular features of sexual shape dimorphism differ among species.  
21 14 Sexual shape dimorphism was found to be poorly associated to size and the  
22 15 evolutionary history of the species. The tight association of sexual shape dimorphism  
23 16 with aspect ratio suggests that the shape of the wing is optimized for the type of flight of  
24 17 each sex, i.e. dispersal flight in females vs aerobatic flight in males. Moreover, the fact  
25 18 that interspecific shape differences are greater and more strongly associated to aspect  
26 19 ratio in males than in females might be indicating that in males the selective pressures  
27 20 affecting flight performance characteristics are more heterogeneous and/or stronger than  
28 21 in females among the studied species.  
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42 23 **Keywords:** aspect ratio, *Culicoides*, sexual dimorphism, shape, size  
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## 24 INTRODUCTION

25 Variation in life history, morphology and behavior of male and female of the same  
26 species has long fascinated evolutionary biologists. Charles Darwin drew attention to  
27 this sexual dimorphism (SD) and postulated that it might be the result of a specific  
28 mode of selection, which he termed sexual selection (Darwin 1871). Darwin's  
29 hypothesis proposes that SD evolves when traits that confer an advantage in  
30 reproductive success are selected for within one sex (Hedrick & Temeles 1989). He  
31 distinguished two forms of sexual selection: competition for mates, or 'intrasexual  
32 selection', and mate choice, or 'intersexual selection' (Berns 2013). Despite the  
33 continuing popularity of this hypothesis, it is acknowledged that other mechanisms,  
34 such as competition between sexes for limited resources or intrinsic differences between  
35 males and females in their reproductive roles, might be involved in the evolution of SD  
36 (Hedrick & Temeles 1989). Additional mechanisms driving the evolution of SD are still  
37 under discussion, but it has been suggested that these mechanisms are not necessarily  
38 mutually exclusive, but can operate synergistically or antagonistically to shape different  
39 and complex SD patterns (Berns 2013).

40 Insects are often used in studies of SD, particularly in the case of model species  
41 such as *Drosophila melanogaster* Meigen, 1830 where underpinning genomics can be  
42 manipulated (Chown & Gaston 2010). Less attention has been paid to SD in insect  
43 vectors of pathogens, despite divergence in olfaction, blood feeding behavior and many  
44 other traits. *Culicoides* Latreille, 1809 (Diptera, Ceratopogonidae) is a highly diverse  
45 genus of over 1400 species that includes biological vectors of important arboviruses  
46 affecting domestic livestock (Purse *et al.* 2015). Sexual dimorphism occurs across the  
47 genus and includes morphological traits that have formed the basis of taxonomic keys  
48 (Kremer 1965).

49 Sexual dimorphism has been reported for the wing form of some *Culicoides*  
50 species (Muñoz-Muñoz *et al.* 2014, 2016), but a detailed study of sexual size  
51 dimorphism (SSD) and sexual shape dimorphism (SShD) in *Culicoides* is lacking.  
52 SShD has generally received less attention than SSD, despite the fact that shape is  
53 related to function, and thus its analysis could provide insights into selective processes  
54 underpinning divergence (Gidaszewski *et al.* 2009; Berns 2013). Examining size and  
55 shape together also provides a much more complete quantification of morphological

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4 56 SD, as the two components are related through allometry (Gidaszewski *et al.* 2009). In  
5 57 addition, studies analysing SShD are usually performed without an explicit phylogenetic  
6 58 framework (Gidaszewski *et al.* 2009).

9 59 In this study we analyze sex-related differences in the wing form of five species  
10 60 of biting midges of the subgenus *Avaritia* Fox, 1955 with the aim of improving our  
11 61 knowledge of SD in this subgenus, and more broadly in the genus *Culicoides*. To  
12 62 analyze wing form changes, we apply landmark-based geometric morphometrics, which  
13 63 has proven to be a powerful tool for describing the shape of *Culicoides* wings (Muñoz-  
14 64 Muñoz *et al.* 2011, 2014, 2016). An important advantage of geometric over traditional  
15 65 morphometrics is that it allows a straightforward separation of size and shape (Zelditch  
16 66 *et al.* 2004), two traits that have a different genetic background (Carreira *et al.* 2011)  
17 67 and might be under different selective pressures (Jugovic *et al.* 2018). Moreover,  
18 68 geometric morphometrics allows the precise study of allometric shape changes, which  
19 69 might represent an important source of SShD (Gidaszewski *et al.* 2009). Consequently,  
20 70 an objective of this study is to accurately quantify SSD and SShD of the wing, as well  
21 71 as the contribution of size to SShD through allometric variation. We apply a  
22 72 phylogenetic approach with the aim to determine the role of shared ancestry in SD  
23 73 within the subgenus *Avaritia*. Traditional morphological measurements, such as aspect  
24 74 ratio (AR), also yield fundamental data to underpin aerodynamic theory, helping to  
25 75 understand the effect of wing form on flight performance (Ray *et al.* 2016).  
26 76 Accordingly, a further aim of this study is to assess intersexual and interspecific  
27 77 differences in AR as well as the association of this measurement with wider shape  
28 78 changes in the subgenus *Avaritia*.

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## 45 80 **MATERIAL AND METHODS**

### 46 81 *Sample Composition and Preparation*

47 82 Five different species of the subgenus *Avaritia* were analyzed in this study:  
48 83 *Culicoides obsoletus* (Meigen), 1818; *Culicoides scoticus* Downes and Kettle, 1952; *C.*  
49 84 *chiopterus* (Meigen), 1830; *C. dewulfi* Goetghebuer, 1936; and *C. imicola* Kieffer,  
50 85 1913. A total of 242 (150 females and 92 males) specimens were analyzed. Specimens  
51 86 originated from 24 different sites in four European countries: Sweden, United Kingdom  
52 87 (UK), Germany and Spain (Table 1). Specimens were dissected for geometric  
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4 88 morphometric analysis and DNA extraction. Three legs were separated for DNA  
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6 89 extraction, while the remaining body parts were mounted on glass microscope slides  
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8 90 following the procedure described in Muñoz-Muñoz *et al.* (2011).  
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#### 11 92 *Species Identification*

13 93 Specimens were initially identified under the stereomicroscope according to their  
14 94 pattern of wing pigmentation (Kremer, 1965). As species of the *Obsoletus* Group are  
15 95 challenging to differentiate solely by wing pigmentation, males were identified to  
16 96 species level by examination of their genitalia (Kremer 1965), while females were  
17 97 identified by species-specific PCR assays (Pagès & Sarto i Monteys 2005; Nolan *et al.*  
18 98 2007).  
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#### 25 100 *Morphological Data Acquisition*

26 101 Digital images of wings were obtained using a Nikon Eclipse 90i microscope  
27 102 equipped with a 4x Plan Fluor Nikon objective lens and a Nikon DXM 1200F camera  
28 103 (Tokyo, Japan). The use of left and right wings was randomized as selection of one side  
29 104 may bias the results in case of differential directional asymmetry between groups.  
30 105 Images were scaled in order to avoid variation due to picture resolution in the analysis  
31 106 of wing size. A set of thirteen landmarks (LMs; Figure 1) covering the wing surface was  
32 107 selected and recorded on each wing using the tpsDig PC program (Rohlf 2001).  
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#### 40 109 *Geometric Morphometrics and Variation Analyses*

41 110 The wing form defined by each set of LMs was decomposed into size and shape  
42 111 components, which were analyzed separately. Size was defined as centroid size (CS),  
43 112 calculated as the square root of the sum of squared distances of each landmark to the  
44 113 centroid of the landmarks configuration (Bookstein 1991). A factorial ANOVA with sex  
45 114 and species as categorical predictors and the logarithm of CS (logCS) as the dependent  
46 115 variable was conducted to detect the effect of both factors on wing size. To test for  
47 116 differences between males and females of the same species an ANOVA with sex as  
48 117 categorical factor and logCS as dependent variable was performed separately for each  
49 118 species. Shape information was obtained from configurations of landmarks by  
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56 119 generalized Procrustes superimposition and projection onto tangent space (Rohlf &  
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4 120 Slice 1990). The effects of species, sex and size on shape were tested in the whole  
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6 121 sample with a MANOVA, in which the first two factors were entered as categorical  
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8 122 predictors and logCS as a covariable (Gidaszewski *et al.* 2009).  
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#### 11 124 *Allometry Analyses*

12  
13 125 The significant interaction between species and logCS detected in the MANOVA  
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15 126 (see Results) suggested interspecific differences in allometry, i.e. shape changes  
16  
17 127 associated to size. In order to evaluate allometry, multivariate regressions of shape  
18  
19 128 (Procrustes coordinates) on logCS were computed separately for each species and sex  
20  
21 129 (Monteiro 1999). Statistical significance was tested using permutation tests with 10,000  
22  
23 130 iterations under the null hypothesis of no allometric relationship (Good 1994; Monteiro  
24  
25 131 1999). Vectors of allometric coefficients were compared between males and females of  
26  
27 132 the same species, among males of different species, and among females of different  
28  
29 133 species, following the approach proposed by Adams and Collyer (2007). As vectors,  
30  
31 134 they may differ in two geometric attributes, orientation, and length. Differences between  
32  
33 135 sexes and among species in the length and orientation of allometric trajectories were  
34  
35 136 tested, respectively, by calculating the absolute difference in vector lengths (distances)  
36  
37 137 and the angle between vectors (Collyer & Adams 2013). The angle was computed  
38  
39 138 considering that the cosine of the angle is the correlation between two vectors, which is  
40  
41 139 calculated as the inner product between normalized vectors (Sheets & Zelditch 2013).  
42  
43 140 To test the null hypothesis that the observed angle between trajectories is not larger than  
44  
45 141 might be expected by chance, a permutation test of the residuals of the reduced model  
46  
47 142 with 10,000 iterations was applied (Sheets & Zelditch 2013). These statistical analyses  
48  
49 143 were performed using the advanced.procD.lm function in the geomorph package  
50  
51 144 (Adams *et al.* 2017) in R v 3.5.1 (R Core Team, 2016).  
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#### 54 146 *Differences in SShD*

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56 147 A discriminant function analysis with sex as classifier was carried out independently  
57  
58 148 for each species in order to visualize and test for SShD. Morphological distances among  
59  
60 149 sexes were quantified in terms of pairwise Procrustes distances. Statistical significance  
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62 150 of the Procrustes distances resulted from permutation tests with 10,000 permutation  
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64 151 rounds (Good 1994; Klingenberg 2011). These analyses were performed with global

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4 152 shape data and with non-allometric shape data with the aim to visualize the effect of  
5 153 allometry in SShD. The residuals obtained from the multivariate regressions of shape  
6 154 onto logCS pooled by sex were used as non-allometric shape data. These analyses were  
7 155 performed with MorphJ 234 v.1.07d.

8 156 To test for differences in SShD among species, we used an approach in which  
9 157 shape changes between two levels of a variable are defined as a vector (Adams &  
10 158 Collyer 2007). Following this approach, we considered each sex of the same species as  
11 159 a distinct level, so that the differences in shape between the two sexes of the same  
12 160 species defined a shape change vector (Adams & Collyer 2007, 2013; Collyer & Adams  
13 161 2007), which we termed the SShD vector. Thus, we obtained five SShD vectors, one for  
14 162 each species, which we compared regarding orientation and length in the same way as  
15 163 we did for allometric trajectories (see *Allometry Analyses*). In order to visualize  
16 164 differences in SShD vectors, a principal component analysis (PCA) based on covariance  
17 165 matrix of group means was computed, and the projection of vectors on the first two  
18 166 principal components (PC1 and PC2) was plotted (Adams & Collyer 2007; Collyer &  
19 167 Adams 2013). These statistical analyses were performed using the trajectory.analysis  
20 168 function in the geomorph package in R (Adams *et al.* 2017) in R v 3.5.1 (R Core Team  
21 169 2016).

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### 23 171 *Phylogenetic Signal*

24 172 To evaluate the effect of phylogeny on SShD, we followed the methods described by  
25 173 Gidaszewski *et al.* (2009), and Klingenberg and Gidaszewski (2010), and implemented  
26 174 in MorphoJ (Klingenberg 2011). Firstly, SShD for each species was computed as the  
27 175 mean vector for female shape minus the mean vector for male shape. Afterwards, a PCA  
28 176 was applied to the covariance matrix of the SShD vectors for the five species  
29 177 (Gidaszewski *et al.* 2009). The five species were plotted in a shape space defined by the  
30 178 two first principal components of SShD, which represents an optimal illustration in two  
31 179 dimensions accounting for the maximal amount of SShD variation among species  
32 180 (Klingenberg & Gidaszewski 2010).

33 181 The phylogenetic tree that we used for testing the phylogenetic signal of SShD  
34 182 in the subgenus *Avaritia* is based on the molecular phylogeny obtained by Augot *et al.*  
35 183 (2017). Although in their tree, the phylogenetic relationships between *C. scoticus*,

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4 184 *C. obsoletus* and *C. chiopterus* were not resolved, we consider *C. chiopterus* as the  
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6 185 sister species of a clade composed of *C. scoticus* and *C. obsoletus* based on classic  
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8 186 taxonomy and recent molecular data (Ander *et al.* 2013; Muñoz-Muñoz *et al.* 2014). In  
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10 187 this way, the cladogram that we use to test for phylogenetic signal of SShD represents,  
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12 188 in our opinion, a plausible phylogenetic hypothesis considering the current molecular  
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14 189 and morphological knowledge (Figure 2). This phylogenetic tree was projected into the  
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16 190 morphometric space using the criterion of squared-change parsimony to reconstruct the  
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18 191 internal nodes from the shape averages of terminal taxa. The phylogenetic signal of  
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20 192 morphometric data was tested by comparing the length of the phylogenetic tree  
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22 193 projected into the morphometric space with the lengths of 10,000 trees obtained by  
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24 194 randomly permuting the shape means among the species (Klingenberg & Gidaszewski  
25  
26 195 2010).

#### 27 196 *Aspect Ratio*

28 197  
29 198 The aspect ratio (AR) was calculated by dividing the length by the width of the wing,  
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31 199 which was the distance from the arculus to M1 and the distance from Cu2 to the vein  
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33 200 margin of r2 (Figure 1). A factorial ANOVA with species and sex as factors was  
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35 201 performed to assess the effect of both variables on the AR of the wing. Only certain  
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37 202 pairwise comparisons had biological interest, so separate ANOVAs were performed  
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39 203 instead of post-hoc tests to evaluate differences in AR values between sexes and  
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41 204 between species. Intersexual differences were assessed by performing a separate  
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43 205 ANOVA for each species with sex as the categorical factor and AR as the dependent  
44  
45 206 variable. To test for specific differences, an ANOVA with species as the categorical  
46  
47 207 factor and AR as the dependent variable was performed separately for each sex. For  
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49 208 evaluating differences between pairs of species, a Tukey's Honestly Significant  
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51 209 Difference (HSD) multiple comparison test for unequal sample size was used in these  
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53 210 ANOVAs (Spjotvoll & Stoline 1973).

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55 211 To evaluate the extent that SShD and shape changed between species due to changes  
56  
57 212 in slenderness, regressions of shape onto AR were computed in two ways. Firstly, the  
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59 213 PC1 from the PCA computed on the covariance matrix of the whole sample was used as  
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61 214 the shape variable to evaluate the association between SShD and AR. Secondly, the PC1  
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63 215 from the PCAs calculated on the covariance matrices of males and females separately



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4 216 were used as shape variables to assess the degree to which shape difference between  
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6 217 species is associated with changes in the AR. Statistical significance was tested using  
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8 218 permutation tests with 10,000 iterations under the null hypothesis of no allometric  
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10 219 relationship (Good 1994; Monteiro 1999).

11 220 Those morphometric analyses in which the software is not specifically indicated  
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13 221 were conducted with the MorphoJ package (Klingenberg 2011).

14 222

## 16 223 **RESULTS**

### 18 224 *Size and Shape Variation*

19  
20 225 The ANOVA performed to detect the effect of species and sex on logCS  
21  
22 226 highlighted that the only factor having a significant effect on wing size was the species  
23  
24 227 ( $F = 30.9$ ;  $df = 4$ ;  $P < 0.001$ ). A significant interaction was found between species and  
25  
26 228 sex ( $F = 7.8$ ;  $df = 4$ ;  $P < 0.001$ ), however, indicating that SSD varies among species of  
27  
28 229 the subgenus *Avaritia*. Significant intersexual differences in logCS values were detected  
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30 230 in all species, with the exception of *C. chiopterus* (Table 2). However, while in  
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32 231 *C. dewulfi* and *C. imicola* the wings of the females were bigger than the wings of males,  
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34 232 the opposite occurred in *C. obsoletus* and *C. scoticus* (Figure 3). The MANOVA  
35  
36 233 revealed that there was also significant interaction between species and sex with regards  
37  
38 234 to SShD, and that this parameter varies between species (Table 3). A significant effect  
39  
40 235 of logCS was also detected, indicating that allometry contributes to the differences in  
41  
42 236 wing shape observed. The significant interaction between species and logCS, but not  
43  
44 237 between sex and logCS (Table 3), suggests that while allometric patterns vary between  
45  
46 238 species they do not vary between sexes. However, a significant triple interaction was  
47  
48 239 also obtained (Table 3), which might suggest that dimorphism in the patterns of  
49  
50 240 allometry differ among species. In fact, significant intersexual differences in the  
51  
52 241 orientation of the vectors of allometric coefficients were only detected in one of the five  
53  
54 242 species examined (*C. dewulfi*, see section below).

51 243

### 52 244 *Allometry Analyses*

53  
54 245 Multivariate regressions detected a significant effect of wing shape on size in all  
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56 246 species except for *C. imicola* (Table 4), further confirming the existence of allometric  
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58 247 shape changes detected in the MANOVA. Percentages of shape changes associated with  
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4 248 size ranged between 4.9% in *C. imicola* females and 26.7% in *C. chiopterus* males  
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6 249 (Table 4). Despite this considerable variation in allometry, the comparison of vector  
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8 250 lengths indicated that the rate of allometric shape changes (amount of shape changes per  
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10 251 unit of size) do not differ between sexes within species (Table 5), or among species  
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12 252 within sexes (Table 6). The analyses of angles revealed that SD in the orientation of  
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14 253 vectors of allometric coefficients are only present in *C. dewulfi* (Table 5). When  
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16 254 comparing species, the analyses of angles revealed that most species do not differ in the  
17  
18 255 orientation of vectors of allometric coefficients. In females, significant differences were  
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20 256 detected between three species (*C. scoticus*, *C. dewulfi*, and *C. imicola*), whereas in  
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22 257 males, significant differences were only detected between *C. chiopterus* and three  
23  
24 258 species, *C. obsoletus*, *C. dewulfi*, and *C. imicola* (Table 7).

#### 25 260 *Differences in SShD*

26  
27 261 Discriminant function analyses confirmed the existence of SShD in the five species  
28  
29 262 (Figure 4). Procrustes distances ranged from 0.100 to 0.128 when calculated from  
30  
31 263 global shape data, and from 0.099 to 0.125 when calculated from non-allometric shape  
32  
33 264 data. The results were very similar when employing both types of data, indicating that  
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35 265 allometric shape changes do not play a primary role in SShD. In both cases, the species  
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37 266 with the lowest and the highest SShD were *C. chiopterus* and *C. scoticus*, respectively,  
38  
39 267 while the other three species showed intermediate values (Figure 4). Among the five  
40  
41 268 species, SShD was similar and mainly consisted of a narrowing and an elongation,  
42  
43 269 especially of the proximal part, of the wing in males when compared to females (Figure  
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45 270 4).

46  
47 271 The analysis of SShD vectors also revealed differences among species. The  
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49 272 comparison of vector lengths confirmed that SShD in *C. scoticus* was significantly  
50  
51 273 greater than in the other four species, even than in its sibling, *C. obsoletus* (Table 8 and  
52  
53 274 Figure 4). This species had the second highest SShD and showed significant differences  
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55 275 when compared with the other species. *Culicoides dewulfi* and *C. imicola* showed  
56  
57 276 intermediate SShD, while *C. chiopterus* was the species with the less marked SShD.  
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59 277 However, the length of the SShD vector of these three species did not differ  
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278 significantly, indicating that they display a similar degree of SShD. The comparison of  
279 the orientation of SShD vectors also revealed significant differences between species

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4 280 (Table 9 and Figure 5). While the angles were not high (ranging between 6.23° and  
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6 281 16.23°) the majority were significantly different than would be expected by chance,  
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8 282 indicating that some of the shape features differentiating males and females vary  
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10 283 between species. The only two non-significant angles were between *C. scoticus* and  
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12 284 *C. obsoletus*, and between *C. dewulfi* and *C. chiopterus* (Table 9).  
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### 15 286 *Phylogenetic Signal*

16 287 The first two principal components (PC1 and PC2) obtained from the PCA  
17  
18 288 performed on the covariance matrix of the SShD vectors jointly explained 87.5% of  
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20 289 variation (Figure 6). When the phylogenetic tree was projected on the shape space  
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22 290 defined by these two PCs, it was observed that the branches of the tree do not intersect  
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24 291 and that, in general, distant species occupy separate regions of the shape space,  
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26 292 suggesting the existence of a phylogenetic structure in SShD. However, in some cases,  
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28 293 sister clades showed sharp changes in the direction of the reconstructed trajectories of  
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30 294 evolutionary change, as occurred with *C. imicola* and *C. dewulfi*, and with *C. chiopterus*  
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32 295 and the clade formed by *C. obsoletus* and *C. scoticus*. The tree length obtained by  
33  
34 296 projecting the phylogeny into the SShD space was 0.046 (in units of squared Procrustes  
35  
36 297 distance). When comparing this tree length with the lengths of the random trees, it was  
37  
38 298 observed that while most of the permutations resulted in longer tree lengths, 6.5% of the  
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40 299 random trees were shorter (corresponding to  $P = 0.065$ ), providing no statistical support  
41  
42 300 for a phylogenetic signal in SShD.  
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### 45 302 *Aspect Ratio*

46 303 The factorial ANOVA indicated that sex, species, and their interaction have a  
47  
48 304 significant effect on AR (sex:  $F = 6265.3$ ;  $df = 1$ ;  $P < 0.001$ ; species:  $F = 19.6$ ;  $df = 4$ ;  $P$   
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50 305  $< 0.001$ ; sex\*species:  $F = 9.7$ ;  $df = 1$ ;  $P < 0.001$ ). Further analyses corroborated these  
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52 306 results and indicated that, while significant intersexual differences in AR values are  
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54 307 consistent across all species ( $P < 0.001$  in all cases; Figure 7), differences between  
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56 308 species depend on the sex, being more marked in males than in females. Thus, while the  
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58 309 factor species significantly affected AR values both in males ( $F = 13.9$ ;  $df = 4$ ;  $P <$   
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60 310  $0.001$ ) and females ( $F = 2.9$ ;  $df = 4$ ;  $P < 0.05$ ), significant differences between pairs of  
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species were only detected in males (Table 10).

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4 312 Linear regressions showed that shape changes are significantly associated with  
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6 313 changes in AR. However, while 98.4% of intersexual shape differences were explained  
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8 314 by AR ( $P < 0.0001$ ), the percentage of shape changes between species explained by the  
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10 315 AR differed considerably between males and females; 36.5% of shape changes between  
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12 316 species were predicted by changes in AR in males compared to 4.6% in females ( $P <$   
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14 317  $0.0001$  in both cases).  
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## 319 **DISCUSSION**

18 320 The present study shows for the first time that while there is a shared general pattern  
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20 321 of SShD within the *Avaritia* subgenus of the genus *Culicoides*, SSD and some specific  
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22 322 features of SShD differ among species. Allometry was an important contributing factor  
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24 323 to shape variation but did not contribute significantly to SShD. In addition, the absence  
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26 324 of a significant phylogenetic pattern in SShD indicates that the differences have evolved  
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28 325 independently in each species. The tight association of SShD with AR suggests that the  
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30 326 shape of the wing is optimized to acquire the best performance, depending on the type  
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32 327 of flight of each sex, i.e. dispersal flight in females vs aerobatic flight in males. The fact  
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34 328 that interspecific shape differences are greater and more strongly associated with AR in  
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36 329 males than in females might indicate that in males the selective pressures affecting  
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38 330 flight performance characteristics are more heterogeneous and/or stronger than in  
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40 331 females among the studied species.

39 332 Our study detected significant wing SSD in all studied species of the subgenus  
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41 333 *Avaritia* except *C. chiopterus*. In the remaining four species, however, the pattern of  
42  
43 334 SSD was not homogeneous. While in *C. obsoletus* and *C. scoticus* males have larger  
44  
45 335 wings than females, the contrary occurs in *C. dewulfi* and *C. imicola*. Sexual size  
46  
47 336 dimorphism is the result of different selection pressures acting on males and females  
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49 337 (Lande 1980). Since male and female body sizes are highly correlated (Fairbairn 1997),  
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51 338 SSD must result from a balance between selection pressures acting jointly or  
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53 339 independently in both sexes. Accordingly, differences between species in SSD indicate  
54  
55 340 that the intensity and/or the direction with which the selective pressures act in each  
56  
57 341 species vary; or even that each species is under the effect of a different set of selective  
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59 342 pressures. Wing size strongly depends on body size (Nijhout & Callier 2015), and so  
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343 selective pressures acting on body size may also affect wing SSD. In insects, females

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4 344 are usually bigger than males because of more intense selection for large size (Jarošik &  
5 345 Honěk 2007). Thus, female fecundity is more closely correlated with body size than the  
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7 346 major components of male reproductive success, such as mating ability (Honěk 1993;  
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9 347 Jarošik & Honěk 2007). However, in small flying insects, a reduced body size offers  
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11 348 greater manoeuvrability and, consequently, a greater success to males mating in swarms  
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13 349 (Crompton *et al.* 2003). This is the case for most *Culicoides* species (Downes 1955,  
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15 350 1969), including those of the subgenus *Avaritia* (González *et al.* 2017). Moreover,  
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17 351 because males tend to emerge before females in many *Culicoides* species (Blackwell *et*  
18  
19 352 *al.* 1992), selection for faster development may also result in smaller males (Jarošik &  
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21 353 Honěk 2007). The combination of these selective pressures would be enough to explain  
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23 354 the SSD observed in *C. dewulfi* and *C. imicola*, in which the females are bigger than  
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25 355 males. However, it would not explain the SSD observed in *C. scoticus* and *C. obsoletus*.  
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27 356 In these two species, male-biased SSD must be explained by other factors, such as  
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29 357 increased plasticity for body size in males (Rohner *et al.* 2017), increased fertility  
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31 358 success in bigger males (Simmons & Parker 1992), or higher capacity to carry larger  
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33 359 loads, e.g. females during mating flight, in bigger males (Petersson 1995). Regardless of  
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35 360 the factors driving the observed patterns of SSD, our results show that these patterns can  
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37 361 differ even among closely related species. This is likely because in each species SSD is  
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39 362 the result of complex interactions between several selective pressures that depend on the  
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41 363 particular biology, genetic constitution, and ontogenetic history of each sex.

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43 364 All species of the subgenus *Avaritia* analyzed in this study show significant  
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45 365 wing SShD, but despite some specific differences, the five studied species share a  
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47 366 common SShD pattern. Interestingly, AR explained a huge percentage of SShD,  
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49 367 indicating that shape differences between males and females are mainly due to changes  
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51 368 in the slenderness of the wing. Specifically, males have more slender wings than  
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53 369 females, due to a global narrowing of the wing and a relative elongation of the proximal  
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55 370 part of it. AR values, which are around 25% higher in males than in females, further  
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57 371 corroborated differences in slenderness. Because AR has a marked effect on important  
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59 372 flight characteristics (Ray *et al.* 2016), differences in slenderness between males and  
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373 females are probably the result of divergent selective pressures arising from differential  
374 functional requirements in both sexes. Thus, the more slender wings of males probably  
375 offer them greater maneuverability by increasing tangential acceleration and maximum

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4 376 turn rate, and decreasing turn radius (Ray *et al.* 2016). As seen above, a greater  
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6 377 maneuverability might represent an advantage to males mating in swarms (Downes  
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8 378 1969; Blackwell *et al.* 1992; Yuval 2006). In contrast, more rounded wings produce  
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10 379 greater lift, mechanical efficiency, and moment of inertia (Ray *et al.* 2016), which might  
11  
12 380 be advantageous when flight is mainly used to disperse and find hosts and suitable  
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14 381 oviposition sites, behaviors associated with *Culicoides* females (Logan *et al.* 2010). In  
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16 382 fact, similar shape changes between males and females have already been described in  
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18 383 other species of dipterans in which males have a swarming behavior (Virginio *et al.*  
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20 384 2015). A positive and strong association between specific values of AR and wing size  
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22 385 was detected in males, but not in females (results not shown). Thus, in bigger males the  
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24 386 loss of manoeuvrability due to increased size (Crompton *et al.* 2003) is compensated by  
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26 387 increased AR. These results suggest that competition among males for females taking  
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28 388 place in swarms is a key driver of wing SD in the studied species of the subgenus  
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30 389 *Avaritia*.

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32 390 Interspecific differences in the amount of SShD and in the particular shape changes  
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34 391 between males and females were detected. Although differences in SShD seemed to be  
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36 392 somewhat affected by shared ancestry, the absence of a significant phylogenetic  
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38 393 relationship indicates that SShD has evolved independently in each species, as shown  
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40 394 for other groups of dipterans (Gidaszewski *et al.* 2009). Procrustes distances and  
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42 395 differences in vector lengths concurred, with *C. scoticus* showing the highest and *C.*  
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44 396 *chiopterus* the lowest SShD. Although a considerable amount of global shape variation  
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46 397 is associated with size changes, allometry does not significantly contribute to the  
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48 398 patterns of SShD within species. Thus, morphological distances between sexes  
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50 399 calculated with total and non-allometric shape changes were very similar or even  
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52 400 identical. Moreover, the comparison of the vectors of allometric coefficients showed  
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54 401 almost no differences between sexes. On the one hand, the length of the vectors did not  
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56 402 differ between sexes in any species, which indicates that the amount of shape change  
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58 403 per unit of size is equal. On the other hand, the orientation of the male and female  
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60 404 vectors only differed in *C. dewulfi*, indicating that only in this species the shape features  
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406 associated with size differ between sexes.

## 407 CONCLUSION

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4 408 In the subgenus *Avaritia*, SD on wing shape is mainly the result of different types of  
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6 409 flight in both sexes, with aerobatic flight in males mating in swarms being an important  
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8 410 driver of such differentiation. Interspecific differences in SShD are non-allometric and  
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10 411 not associated to phylogeny, and probably result from a complex balance of intra-sexual  
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12 412 competition and several other selective forces acting with different intensity in each sex  
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14 413 and on several interrelated traits, such as body size, wing size, and wing shape. Thus,  
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16 414 the supposed loss of efficacy for a specific function in one trait may be compensated by  
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18 415 changes in another trait. Overall, the results of the present study demonstrate that  
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20 416 mechanisms prompting SD can operate synergistically or antagonistically to shape  
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22 417 different and complex SD patterns (Berns 2013), which in addition are modulated by  
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24 418 the patterns of integration among traits.  
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543 **Table 1.** Specific and geographic composition of the sample.

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Country	Site	Coordinates	Sample (n <sub>females</sub> ; n <sub>males</sub> ) <sup>†</sup>
Spain	Anglès	41°57'N, 02°38'E	Co (4;0)
	Aramunt	42°12'N, 00°59'E	Co (2;0), Cs (1;0), Cd (1;0)
	Bonastre	41°13'N, 01°25'E	Ci (1;0)
	Caldes de Malavella	41°50'N, 02°50'E	Cs (0;7), Ci (7; 11)
	Cellera de Ter	41°58'N, 02°37'E	Co (2;0)
	Colunga	43°29'N, 05°21'W	Co (2;0), Cch (2;3), Cd (8;7)
	Corbera de Llobregat	41°24'N, 01°55'E	Ci (1;0)
	Garcia	41°08'N, 00°38'E	Co (1;0), Ci (1;0)
	La Almoraima	36°17'N, 05°26'W	Ci (1;3)
	Montagut	42°14'N, 02°36'E	Co (8;0)
	Piera	41°30'N, 01°45'E	Co (2;0), Ci (2;0)
	Proaza	43°12'N, 06°04'W	Co (1;7), Cs (1;7), Cd (0;6)
	Quintos de Mora	39°24'N, 04°07'W	Ci (0;1)
	St. Feliu de Buixalleu	41°48'N, 02°34'E	Cs (2;0), Cd (0;1)
	St. Iscle de Vallalta	41°38'N, 02°33'E	Ci (1;0)
	Susqueda	41°59'N, 02°32'E	Co (1;0), Cs (13;0), Cd (2;1)
	Vilanova de la Muga	42°18'N, 03°02'E	Ci (1;0)
Germany	Friedberg	50°22'N, 08°55'E	Co (9;0), Cs (12;8), Cch (4;3)
	Giessen	50°35'N, 08°45'E	Co (0;8)
	Waldsiefersdorf	52°33'N, 14°05'E	Co (2;0)
Sweden	Torestorp	57° 23'N, 12°39'E	Co (18;0)
	Torsås	56° 21'N, 15°49'E	Cs (15;0)
UK	Cumbria	55° 04'N, 02°46'W	Cd (5;0)
	Devon	51° 11'N, 03°56'W	Co (8;5), Cs (0;5), Cch (9;4), Cd (0;5)

545 <sup>†</sup> The number of specimens of each species trapped in each sampling site is indicated546 within brackets. n<sub>females</sub>, number of females; n<sub>males</sub>, number of males; Co, *C. obsoletus*;547 Cs, *C. scoticus*; Cch, *C. chiopterus*; Cd, *C. dewulfi*; Ci, *C. imicola*.

549 **Table 2.** Results of the ANOVAs performed to test the effect of sex on centroid size in  
 550 each species.

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Species	df <sup>†</sup>	Type III Sum of Squares	Mean Squares	F	P
<i>C. obsoletus</i>	1	0.0889	0.0889	11.95	< 0.001
<i>C. scoticus</i>	1	0.0845	0.0845	7.81	0.007
<i>C. chiopterus</i>	1	0.0047	0.0047	0.71	0.409
<i>C. dewulfi</i>	1	0.0339	0.0339	4.15	0.049
<i>C. imicola</i>	1	0.0600	0.0600	41.02	< 0.001

552 <sup>†</sup>degrees of freedom.

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574 **Table 3.** Results of the MANOVA performed to test the effect of species, sex and size  
 575 on wing shape.

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Effect	Wilk's lambda	F	Effect df <sup>†</sup>	Error df <sup>†</sup>	P
Species	0.391547	2	88	797.3	< 0.001
Sex	0.168327	45	22	201.0	< 0.001
logCS	0.678949	4	22	201.0	< 0.001
Species*Sex	0.521751	2	88	797.3	< 0.001
Species*logCS	0.583315	1	88	797.3	0.031
Sex*logCS	0.883876	1	22	201.0	0.251
Species*Sex*logCS	0.572529	1	88	797.3	0.017

577 <sup>†</sup>degrees of freedom.

579 **Table 4.** Results of the multivariate regressions of shape on the logarithm of centroid  
 580 size.

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Species	Sex	Percentage of allometry	P
<i>C. obsoletus</i>	female	18.1	< 0.001
	male	13.6	0.008
<i>C. scoticus</i>	female	19.1	< 0.001
	male	25.8	< 0.001
<i>C. chiopterus</i>	female	13.8	0.028
	male	26.7	0.014
<i>C. dewulfi</i>	female	12.2	0.046
	male	19.2	< 0.001
<i>C. imicola</i>	female	4.9	0.746
	male	7.3	0.416

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599 **Table 5.** Differences in the length and orientation of allometric vectors between males  
600 and females of the same species.

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Species	$\Delta d^\dagger$	P	$\theta$ (in degrees) $^\ddagger$	P
<i>C. obsoletus</i>	0.001103	0.980	43.0	0.393
<i>C. scoticus</i>	0.038728	0.118	29.8	0.480
<i>C. chiopterus</i>	0.082017	0.093	62.4	0.265
<i>C. dewulfi</i>	0.017576	0.602	74.5	0.003
<i>C. imicola</i>	0.023462	0.735	99.9	0.074

602  $^\dagger$  absolute difference in vector lengths,  $^\ddagger$  angle between allometric vectors



**Table 6.** Differences in the length of allometric vectors among females of different species (above diagonal) and among males of different species (below diagonal).

	<i>C. obsoletus</i>	<i>C. scoticus</i>	<i>C. chiopterus</i>	<i>C. dewulfi</i>	<i>C. imicola</i>
<i>C. obsoletus</i>	0.000000	0.006154ns	0.086276ns	0.022180ns	0.006202ns
<i>C. scoticus</i>	0.070594ns	0.000000	0.092431ns	0.028334ns	0.000048ns
<i>C. chiopterus</i>	0.019202ns	0.051392ns	0.000000	0.064096ns	0.092478ns
<i>C. dewulfi</i>	0.058733ns	0.011861ns	0.039531ns	0.000000	0.028382ns
<i>C. imicola</i>	0.049491ns	0.021104ns	0.030289ns	0.009242ns	0.000000

ns, non-significant.

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4 629 **Table 7.** Differences in the orientation of allometric vectors (in degrees) among females  
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6 630 of different species (above diagonal) and among males of different species (below  
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8 631 diagonal).  
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	<i>C. obsoletus</i>	<i>C. scoticus</i>	<i>C. chiopterus</i>	<i>C. dewulfi</i>	<i>C. imicola</i>
<i>C. obsoletus</i>		57.3ns	79.3ns	48.2ns	40.1ns
<i>C. scoticus</i>	58.3ns		87.5ns	65.5*	56.8*
<i>C. chiopterus</i>	107.9*	87.7ns		68.4ns	72.6ns
<i>C. dewulfi</i>	55.3ns	55.9ns	104.0*		34.2*
<i>C. imicola</i>	44.3ns	43.1ns	102.7*	38.6ns	

21 633 ns, non-significant; \* P < 0.05  
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635 **Table 8.** Length of SShD vector of each species, differences in length ( $\Delta d$ ) among  
 636 species (below diagonal) and associated P-values (above diagonal).

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	Length	<i>C. obsoletus</i>	<i>C. scoticus</i>	<i>C. chiopterus</i>	<i>C. dewulfi</i>	<i>C. imicola</i>
<i>C. obsoletus</i>	0.1179		0.008	0.001	0.036	0.004
<i>C. scoticus</i>	0.1274	0.0096		0.000	0.000	0.000
<i>C. chiopterus</i>	0.1002	0.0176	0.0272		0.113	0.424
<i>C. dewulfi</i>	0.1088	0.0090	0.0186	0.0086		0.426
<i>C. imicola</i>	0.1048	0.0131	0.0226	0.0046	0.0040	

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660 **Table 9.** Differences in the orientation of SShD vectors (in degrees) among different  
 661 species (below diagonal) and associated P-values (above diagonal).

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	<i>C. obsoletus</i>	<i>C. scoticus</i>	<i>C. chiopterus</i>	<i>C. dewulfi</i>	<i>C. imicola</i>
<i>C. obsoletus</i>		0.095	0.009	0.007	< 0.001
<i>C. scoticus</i>	6.23		< 0.001	< 0.001	< 0.001
<i>C. chiopterus</i>	10.65	13.98		0.465	0.024
<i>C. dewulfi</i>	9.47	12.46	6.88		0.029
<i>C. imicola</i>	14.48	16.23	11.05	9.81	

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665 **Table 10.** Probabilities for post-hoc tests evaluating the differences in AR values among  
 666 females of different species (above diagonal) and among males of different species  
 667 (below diagonal).

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	<i>C. obsoletus</i>	<i>C. scoticus</i>	<i>C. chiopterus</i>	<i>C. dewulfi</i>	<i>C. imicola</i>
<i>C. obsoletus</i>		0.7127ns	0.9999ns	0.3448ns	0.9028ns
<i>C. scoticus</i>	0.0045*		0.9783ns	0.0680ns	0.4826ns
<i>C. chiopterus</i>	0.6824ns	0.0020*		0.2899ns	0.8344ns
<i>C. dewulfi</i>	0.6370ns	0.0001*	0.9972ns		0.8944ns
<i>C. imicola</i>	0.0935ns	0.0001*	0.9440ns	0.6724ns	

669 ns, non-significant; \* P &lt; 0.05

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4 691 **Figure Legends**

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8 693 **Figure 1.** Female wing of *Culicoides scoticus* indicating the position of the 13 recorded  
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10 694 landmarks (open circles). The main veins and cells are indicated. Veins: Arc, arculus;  
11 695 Cu, Cu1, Cu2, cubital and branches; M, M1, M2, medial and branches; R, radial; R-M,  
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13 696 radial-median crossvein. Cells: cu, cubital; r1, r2, first and second radial.

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16 698 **Figure 2.** Phylogenetic tree of the studied species of the subgenus *Avaritia* based on  
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18 699 molecular data.

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21 701 **Figure 3.** Mean values of the logarithm of CS (logCS) in females (circles) and males  
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23 702 (squares) of the five studied species of the subgenus *Avaritia*. Vertical bars indicate  
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25 703 95% confidence intervals.

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28 705 **Figure 4.** Shape changes associated with the total and size-corrected SShD. The shape  
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30 706 changes are shown as the difference between the female average shape (grey outlines  
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32 707 and points) and the average shape for males (black outlines and points). The magnitudes  
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34 708 of total and size-corrected SShD are indicated in units of Procrustes distance (all  
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36 709 significant with  $P < 0.001$ ).

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39 711 **Figure 5.** Scatterplot of PC1 and PC2 with the projection of SShD vectors. Grey dots  
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41 712 correspond to individual values, and white and black dots correspond to mean female  
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43 713 and male values, respectively, in each species. Lines correspond to SShD vectors of  
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45 714 each species (light blue: *C. obsoletus*; green: *C. scoticus*; black: *C. chiopterus*; red: *C.*  
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47 715 *dewulfi*; dark blue: *C. imicola*).

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50 717 **Figure 6.** Projection of the phylogenetic tree on the shape space defined by PC1 and  
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52 718 PC2 from the PCA computed on the covariance matrix of the SShD vectors showing the  
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54 719 phylogenetic signal contained in the divergence of SShD among the species of the  
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56 720 subgenus *Avaritia*.

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4 722 **Figure 7.** Mean values of aspect ratio (AR) in females (circles) and males (squares) of  
5 723 the five studied species of the subgenus *Avaritia*. Vertical bars indicate 95% confidence  
6 724 intervals.  
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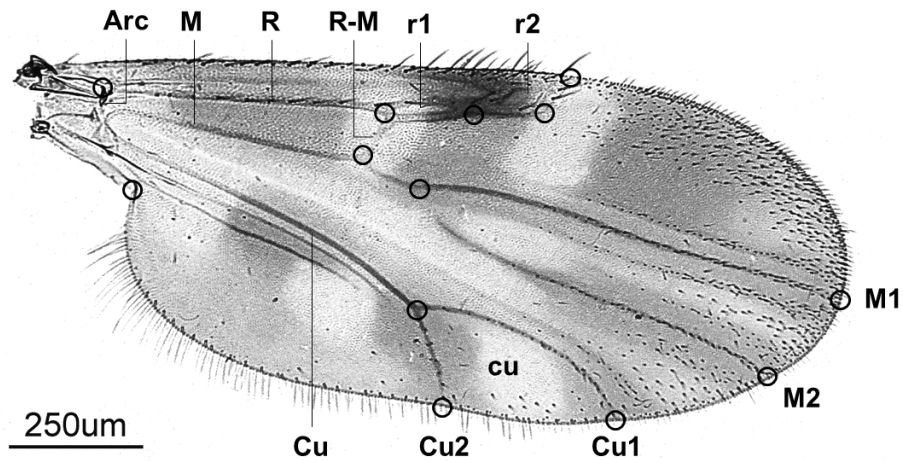


Figure 1. Female wing of *Culicoides scoticus* indicating the position of the 13 recorded landmarks (open circles). The main veins and cells are indicated. Veins: Arc, arculus; Cu, Cu1, Cu2, cubital and branches; M, M1, M2, medial and branches; R, radial; R-M, radial-medial crossvein. Cells: cu, cubital; r1, r2, first and second radial.

310x200mm (96 x 96 DPI)



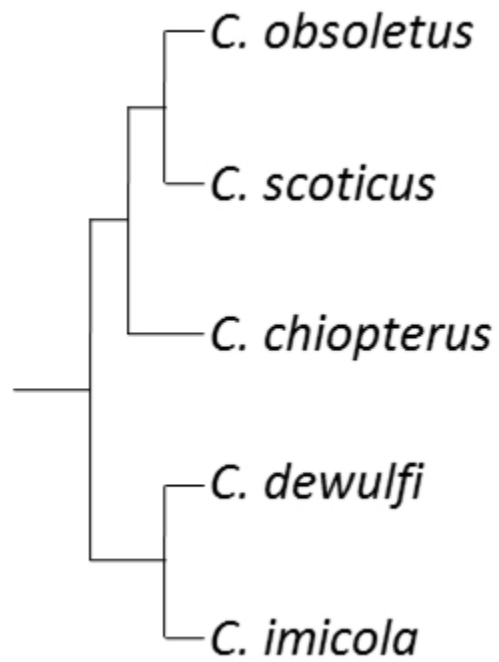


Figure 2. Phylogenetic tree of the studied species of the subgenus *Avaritia* based on molecular data.

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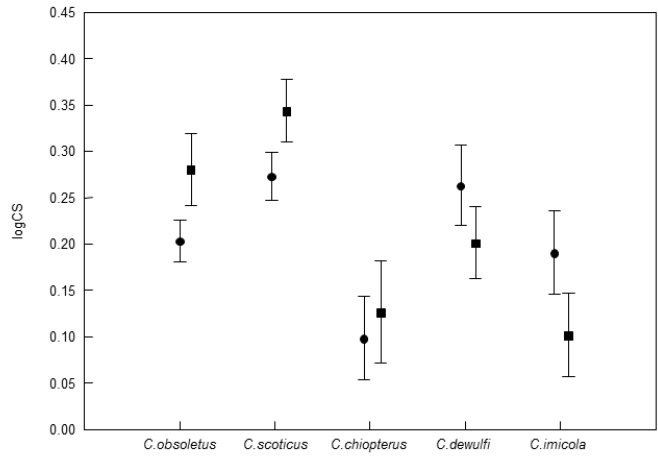
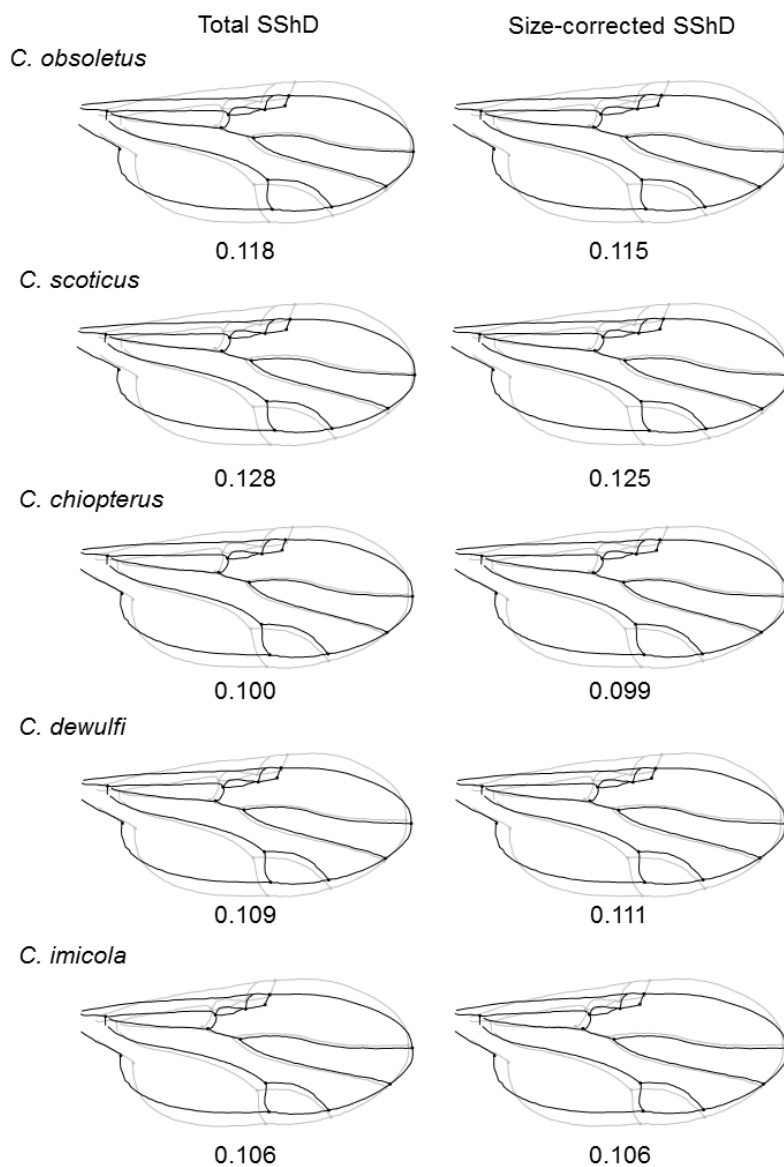


Figure 3. Mean values of the logarithm of CS (logCS) in females (circles) and males (squares) of the five studied species of the subgenus *Avaritia*. Vertical bars indicate 95% confidence intervals.

254x190mm (96 x 96 DPI)



45 Figure 4. Shape changes associated with the total and size-corrected SShD. The shape changes are shown  
46 as the difference between the female average shape (grey outlines and points) and the average shape for  
47 males (black outlines and points). The magnitudes of total and size-corrected SShD are indicated in units of  
48 Procrustes distance (all significant with  $P < 0.001$ ).

49 190x275mm (96 x 96 DPI)

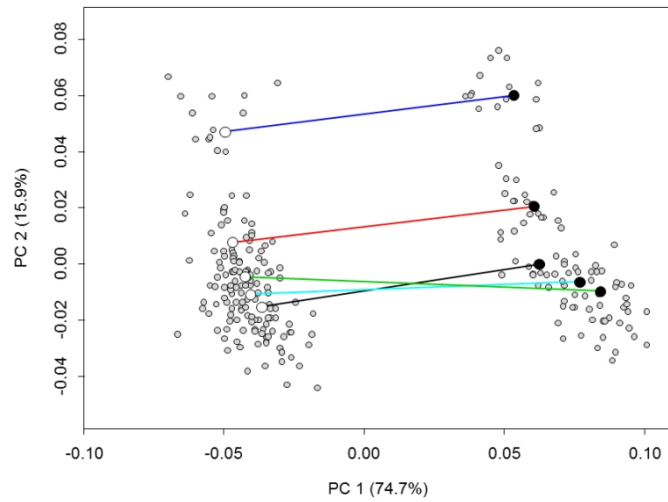


Figure 5. Scatterplot of PC1 and PC2 with the projection of SShD vectors. Grey dots correspond to individual values, and white and black dots correspond to mean female and male values, respectively, in each species. Lines correspond to SShD vectors of each species (light blue: *C. obsoletus*; green: *C. scoticus*; black: *C. chiopterus*; red: *C. dewulfi*; dark blue: *C. imicola*).

338x190mm (96 x 96 DPI)

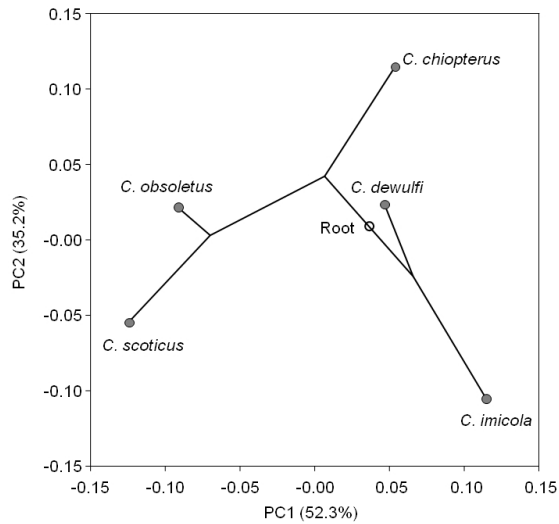


Figure 6. Projection of the phylogenetic tree on the shape space defined by PC1 and PC2 from the PCA computed on the covariance matrix of the SShD vectors showing the phylogenetic signal contained in the divergence of SShD among the species of the subgenus *Avaritia*.

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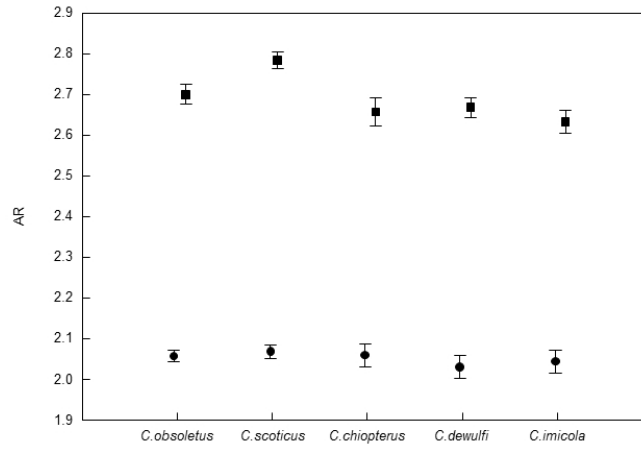


Figure 7. Mean values of aspect ratio (AR) in females (circles) and males (squares) of the five studied species of the subgenus *Avaritia*. Vertical bars indicate 95% confidence intervals.

254x190mm (96 x 96 DPI)