Unsupervised learning for detection of possible sexual dimorphism in larvae of *Belgica antarctica* Jacobs (Diptera, Chironomidae)

Pavlo Kovalenko[1*], Svitlana Serga[2,3], Daniel Einor[4], Volodymyr Gorobchyshyn[1,2], Vladlen Trokhymets[2], Oleksandra Protsenko[2,3], Iryna Kozeretska[3]

[1]*Institute for Evolutionary Ecology, National Academy of Sciences of Ukraine, Lebedeva str. 37, Kyiv, Ukraine*
[2]*Taras Shevchenko National University of Kyiv, Volodymyrska str. 64/1, Kyiv, Ukraine*
[3]*National Antarctic Scientific Center of Ukraine, Taras Shevchenko b. 16, Kyiv, Ukraine*
[4]*University of South Carolina, Bull str. 945, Columbia, USA*

Abstract

Belgica antarctica is one of the two native chironomid species of the Antarctic Peninsula. In this species, adult males and females are considerably different, yet the question of larval sex differences in morphometric parameters remains unanswered. In this paper, we analyze five morphometric parameters: head capsule length, head capsule width, mandible width, mandible length, mentum length of 140 fourth-instar larvae of *B. antarctica* from seven study plots in the south of Petermann Island, Wilhelm Archipelago, Antarctic Peninsula. To infer possible sexual dimorphism, we use the methods of unsupervised analysis (PCA and hierarchical clusterization). Our results suggest that the selected morphometric features of width and length are highly intercorrelated and cannot differentiate sex in the sampled larvae of *B. antarctica*.

Introduction

Body size sex dimorphism is known to influence the population biology of insects (Esperk et al. 2007, Stillwell and Fox 2007, Benbow 2008). Because secondary production is calculated using biomass, as organism density multiplied by individual body

------

size, sexual dimorphism may be inherently important to standing stock biomass and secondary production estimates of insect groups where sex dimorphism is common (Benbow 2008).

Male and female insects are often distinguishable morphometrically, by size (Blanckenhorn 2007). Females are generally larger than males, while males have lower phenotypic plasticity (Stillwell et al. 2010, Vea et al. 2021). Phenotypic plasticity is induced by ecological and environmental variables (Stillwell and Fox 2007). Atchley and Martin (1971) and Atchley (1971) provided larval morphometric analyses between sexes of several species of the Chironomus genus.

Belgica antarctica Jacobs (Diptera: Chironomidae) is a wingless insect with complete metamorphosis from the Orthocladiinae subfamily endemic to the western shore of the Antarctic Peninsula (Usher and Edwards 1984, Convey and Block 1996, Kovalenko et al. 2021, Kozeretska et al. 2022). In recent years, this midge has been studied actively in numerous research on its genomic, phylogenetic, physiological, biochemical, ecological, and life cycle features (*e.g.*, Benoit et al. 2009, Allegrucci et al. 2012, Harada et al. 2014, Kelley et al. 2014, Kawarasaki et al. 2019, Finch et al. 2020, Potts et al. 2020, Ajayi et al. 2021, Michailova et al. 2021a, Spacht et al. 2021, Yoshida et al. 2021). In addition, recently, Michailova et al. (2021b) described the external morphology of the 4th instar larvae of this species.

The larval stage includes four developmental periods with different sizes and pigmentation. Atchley and Davis (1979) reported that the 4th larval stage exhibits sexual dimorphism at the cytological (sex-linked inversion) (Atchley and Davis 1979) and morphological (sex apparatus) levels (Wülker and Götz 1968). Atchley and Hilburn (1979) carried out a large morphometric study of the eldest larval stage *B. antarctica* more than 40 years ago, covering 16 localities on ten islands in the vi-

cinity of the Anvers Island (Antarctic Peninsula). Nine parameters of the larval head capsule had been recorded including frons length, frons width, clypeus width, mandible length, central labial tooth (Cl) length, central labial tooth (Cl) width, antennal segment 1 length, antennal segment 1 width, and antennal segment 2 length. Atchley and Hilburn (1979) reported high between-sample variation in the expression of sexual dimorphism. Some samples showed no sexual dimorphism whereas others exhibited highly complex patterns of sexual dimorphism by the selected parameters such as mandible length, frons length, Cl width, and antennal segment 1 width. Nevertheless, measurements of larvae of *B. antarctica* head capsule length in a few long-standing works show the absence of sexual dimorphism (*e.g.*, Sugg et al. 1983, Usher and Edwards 1984). Usher and Edwards (1984) did not indicate the bimodal distribution of head capsule length within 4th instars of larvae *B. antarctica* from two different populations. Moreover, the bimodal distribution is not observed visually for larval head capsule length in all instars, according to the data presented by Sugg et al. (1983). Also, Sugg et al. (1983) concluded that the head capsule length could vary between different populations. Moreover, they suggested that the result was a consequence of both nutrient and thermal variations. That is why the question about the presence of sexual dimorphism in larvae of *B. antarctica* is still relevant.

Attempts to find any relationship between morphometric variation and geographic and ecological features were generally unsuccessful. The general population seemed to split into sex-specific and non-sex-specific subpopulations and no correlation was found with geographical parameters or environmental conditions. The reliability of the above-mentioned traits for detection of sexual dimorphism in these insects remains unclear, also considering high morphometric plasticity of females (Stillwell et al. 2010).

Differentiating between males and females in chironomids gets complicated, especially in the field, as it was demonstrated by inconsistent differences found in *B. antarctica* by Atchley and Hilburn (1979), although Atchley and Martin (1971) and Atchley (1971) were able to demonstrate sexual dimorphism in chironomid larvae. Documenting traits for rapid sex determination would be beneficial for the research where one needs to collect crucial data from an extremely rare biological specimen without risking the loss of fragile material due to manipulations. Studies of the fauna living in severe habitats like Antarctica are a stark example of such research; nonetheless, reliable morphometric features in *B. antarctica* larvae that could be used *in situ* are not described yet (reviewed in Kozeretska et al. 2022). Potentially such indirectly measured traits can be as well-validated on the spot by observation in the controlled environment.

To visually assess for patternization in the parameters that could be attributed to unlabeled subgroups within the general population, we applied methods of unsupervised machine learning, such as principal component analysis (PCA) (Jolliffe 2002) and cluster analysis (hierarchical clustering) (Hartigan and Wong 1979). Both methods are widely used in ecological research where identification of main drivers of variation and markers of divergence is of key importance (Xue et al. 2011). PCA is carried by an orthogonal transformation of multivariate data (in other words, reduces data of many dimensions to a single plane with axes parallel to the directions of general variation). The method allows to evaluate the contribution of the general variability pattern by known factors (*e.g.*, environmental: insolation, water, soil quality, *etc*.) as well as to visualize unexplained variability (Jolliffe 2002). Therefore, if a population has a dimorphism actually explainable by a sex factor, then even if the researcher did not label the specimens by sex, the dimorphism patterns are expected to be observable as points aggregated around two or more centroids (clusters). PCA-based evaluation is used to determine possible sexual dimorphism in populations of insects (beetles) (Benítez et al. 2020), other animals (frogs) (Fathinia et al. 2012), and even for studies in human populations (Shui et al. 2017). Current software algorithms allow evaluation of the cluster resolving power using cubic clustering criterion (CCC, measure of within-cluster to total-sample ratio of sums of squares) and pseudo-F statistic (PSF, ratio of between-cluster to within-cluster sums of squares). A dimorphism, if manifested by any feature, would be inferred from local maximums in CCC and PSF values and will be clearly visible on the PC diagram as well ([1]-R Core Team 2018).

In our study, we test if the larvae of *B. antarctica* at their latest developmental stage exhibit a dimorphism in certain head capsule parameters, which would be detectable by an unsupervised machine learning algorithm. For this, we analyzed 5 morphometric features of the head capsule of the 4th instar larvae of *B. antarctica* from 7 study plots on the Petermann Island population, which were collected in 2007. To infer for patterns of dimorphism potentially attributable to a sex factor, we applied PCA, and to test the grouping strength we used hierarchical clustering.

Material and Methods

Study plots

B. antarctica larvae were collected from randomly chosen seven study plots around the Petermann Island of the Wilhelm Archipelago on March 21 – 23, 2007, being a task for the XII Ukrainian Antarctic Expedition (Fig. 1, Table 1).
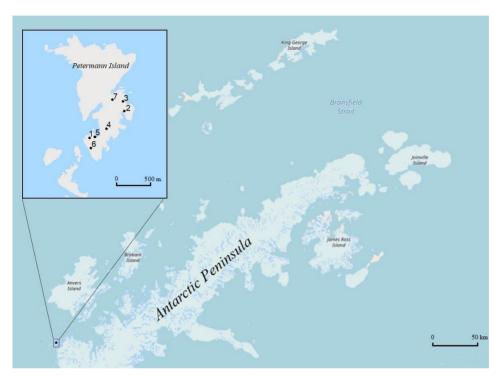

Fig. 1. Sites of collection of *B. antarctica* on the Petermann Island.

All sites are located on the southern side of the island, yet vary in altitude, variability of avian species, and water availability. Sites 1, 5, and 6 are located on the western shore of the Petermann Island (there is no big land from this side, only open water and two small islands opposite to site 6); while sites 2, 3, and 7 – on the eastern shore (separated from the continent by Penola Strait: approximately, 2300 – 2400 m to Antarctic Peninsula); site 4 is located in the middle of the southern part of this island. All studied localities characterized appreciable ornithogenic influence. It is necessary to note that we could not take samples from the northern part of the Petermann Island due to this territory was already covered by a thick layer of snow (also, this part of the island is higher). Another reason was the height of the nothern part.

Region	№ (code)	Location	Altitude [m]	Number of accessions	Date of collection
The very south of the Peterman Island, Wilhelm Archipelago; on hills where *Pygoscelis papua* nest, relatively near a nesting site of *Phalacrocorax atriceps*	1 (2B)	S 65°10.630' W 64°08.711'	0	20	3/23/2007
North of the southern part of the Petermann Island, Wilhelm Archipelago; in a lowland area where *Pygoscelis papua* nest	2 (4B)	S 65°10.428' W 64°08.080'	1	20	3/22/2007
North of the southern part of the Petermann Island, Wilhelm Archipelago; in a lowland fed by meltwater from the hills, nesting site of *Pygoscelis papua*	3 (7B)	S 65°10.354' W 64°08.082'	4	20	3/22/2007
In the south of the southern part of the Petermann Island, Wilhelm Archipelago; on hills where *Pygoscelis papua* nest	4 (0B)	S 65°10.561' W 64°08.400'	1	20	3/23/2007
	5 (251A)	S 65°10.626' W 64°08.618'	0	20	3/23/2007
	6 (0A)	S 65°10.703' W 64°08.669'	0	20	3/22/2007
North of the southern part of the Petermann Island, Wilhelm Archipelago; nesting site of *Pygoscelis papua* and *Pygoscelis adeli*	7 (0Z)	S 65°10.340' W 64°08.270'	13	20	3/21/2007

Table 1. Study plots of *B. antarctica* larvae used for morphometrical analysis.

Methods

Part of the live material (collected on sites 5 and 6) was fixed in 96% ethanol, part (collected on sites 1, 2, 3, 4, and 7) – in ethanol-acetic acid solution (3:1). To study morphometric variability, we used individuals of the 4th larval stage (20 specimens from every study plot). Sex was not identified.
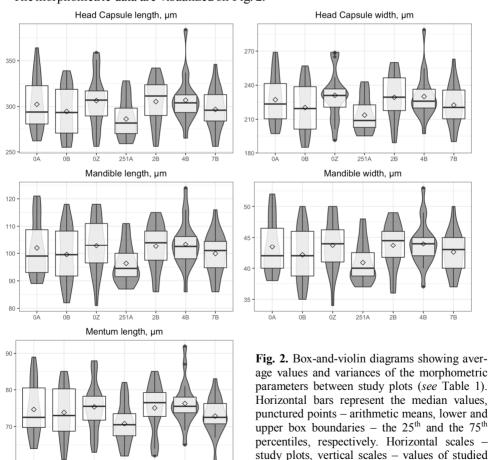
The five chosen morphometric features included head capsule length, head capsule width, mandible width, mandible length, mentum length. The mandible length feature was the only parameter cross-used from Atchley and Hilburn (1979), while the head capsule length was cross-used from Sugg et al. (1983) and Usher and Edwards (1984).

The images were captured via a digital single-lens reflex (DSLR) Sigeta MCMOS 5100 5.1Mp USB 2.0 (South Korea) camera mounted on MBS-10 stereo microscope (Russian Federation) with further profiling using TopView software (TopTek Photonics, v3.5).

Statistical analysis

Data transformation and analysis were carried using base R v3.6.2([1]-R Core Team 2018) with the relevant libraries for visualization (ggplot2) as well as SAS University Edition software package (SAS Institute Inc., US, 2019). The normality of every parameter's distribution was checked individually for each site using the Kolmogorov-Smirnov test (PROC UNIVARIATE). Analysis of variance of the parameters by site and by collection coordinates was done using ANOVA (PROC GLM) with the Tukey test for multiple comparisons. The sexual dimorphism was inferred for by the results of cluster analyses (Centroid Hierarchical Cluster Analysis, PROC CLUSTER; K-means clustering, PROC FASTCLUS) comparing the clustering power within the array of 2 to 7 clusters (by the number of sites) and visualization of the principal components (PCA, *R/prcomp*).

Results

The morphometric data are visualized on Fig. 2.

Fig. 2. Box-and-violin diagrams showing average values and variances of the morphometric parameters between study plots (*see* Table 1). Horizontal bars represent the median values, punctured points – arithmetic means, lower and upper box boundaries – the 25th and the 75th percentiles, respectively. Horizontal scales – study plots, vertical scales – values of studied parameters (µm).

All samples were normally distributed (Kolmogorov-Smirnov test, p > 0.05), except for mandible width in site 5 (Kolmogorov-Smirnov test, p = 0.02).

Parametric models did not find differences in the variations of all studied parameters for different collection sites ($F_{6.133} \leq 1.3$, $P > 0.1$). Morphometric measurements were also not correlated with the geographical coordinates ($F_{5.134} \leq 0.1$, $P > 0.9$), suggesting that the specimens from all study plots belong to the same population. Further clustering could not segregate the population into statistically significant subgroups comprising different study plots (PROC CLUSTER, n_cluster ≤ 7, $R^2 << 0.1$, Fig. 3).
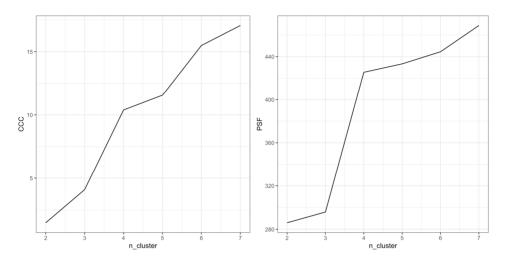

Fig. 3. Analysis of the clustering power of morphometric features of *B. antarctia* showing the relationship of cubic clustering criterion (CCC, left) and Pseudo F-statistic (PSF, right) and the number of clusters into which the data were divided during K-means clustering (PROC FASTCLUS). The absence of a local maximum in the first cluster shows poor segregation power of the morphometric data when dividing into two groups of hypothetical males and females.

Since we did not see any difference in morphometric parameters in larvae from different study plots, we pooled them together. Within this pool, the data could not be differentiated into two groups (n_cluster = 2, $R = 0.072$) (Fig. 3). Only one significant principal component was defined by the analysis – size (Table 2).

	PC1	PC2	PC3
Head capsule length	-0.45188	0.211914	0.207294
Head capsule width	-0.44684	0.180538	0.740384
Mandible length	-0.44941	-0.45585	-0.18521
Mandible width	-0.44683	-0.56079	-0.18931
Mentum length	-0.44103	0.632626	-0.582
Eigenvalue	2.190865	0.331766	0.249811
% of total variation	0.96	0.02201	0.01248

Table 2. Principal components (PC) analysis of the *B. antarctica* morphometrics.

PCA results are presented on a biplot (Fig. 4), showing no patterns of bimodal distribution typical for sexual dimorphism. A similar analysis, conducted on the publicly available data (Laiolo et al. 2013) for comparison, demonstrated the expected patterns of variation due to sex differences that are as significant as due to geo-climatic characteristics of the environment (*see* Supplementary Material).

Therefore, the larvae from the Petermann Island population could not be grouped by sex by any of the studied parameters.
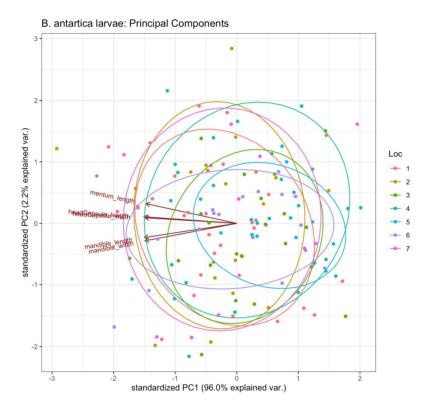

Fig. 4. PCA of the pooled morphometric data. 96% of the overall variation is described by one principal component (PC1). The arrows indicate features' axes relative to the principal components. Ellipses group the samples by location.

Discussion

The results of analyses of the head capsules of the 4th instar larvae of *B. antarctica* (Petermann Island, Wilhelm archipelago on the Antarctic Peninsula) demonstrate that the morphometric features are characterized by unidirectional variance. Given this and the fact that all sampling plots were on the same side of a single island, and despite some differences in the protection against winds (sites 2, 3, and 7 are more protected: they are covered from the northwest by the upper part of the island and they are located farther from the open water), we can confidently assume that all samples belong to the same highly ecologically uniform population.

Atchley and Hilburn (1979) reported sexual dimorphism in *B. antarctica* larvae sampled from several different islands by morphometrical parameters of the head capsules. For all parameters they had measured, females had higher values than males. Actually, in half of the sites the dimorphism was manifested by two principal components, such as the shape (interaction between several head capsule features) and size (single feature) while in 11 out of 15 sites the dimorphism was seen at least by the size. The patterns of dimorphism did not statistically correlate with geographical coordinates or ecological factors or the soil parameters. Unfortunately, it was not possible to infer for the exact parameters by which the males and females varied in a given site using only the aggregated results of the principal component method. This makes it improbable to make a detailed geographical comparison of direct measurement data and allows only to compare the regularities of the morphological traits and their interaction, and to check the expectation of the sexual dimorphism. The differences in the results of our work and the work of Atchley and Hilburn (1979) could be caused by several reasons. Firstly, limited spatial sampling in our work (the samples were collected in several sites but only from one island). Secondly, hypothetical genetic differences between populations due to sufficiently isolation – it is necessary to note that *B. antarctica* possibly survived multiple glacial cycles in Pleistocene Epoch or even earlier (Allegrucci et al. 2012, Chown and Convey 2016); moreover, this insect is flightless (perhaps the only way to settle this midge is to transfer it by birds together with the nesting material (Peckham 1971, Parnikoza et al. 2018). And thirdly, possible differences between environmental conditions in the experimental locations. It should be also noted that in the work of Atchley and Hilburn (1979), the sexual dimorphism in larvae was founded not for all populations: three of them (from Cormorant Island (AA1), Ike's Island (AF1), and one of four examined from Litchfield Island (AO4)) showed the absence of morphometric differences between males and females by all three selected principal components in their research.

Concerning head capsule length, our results also show the absence of sexual dimorphism in this parameter, similar to the work of Sugg et al. (1983) and Usher and Edwards (1984).

In our work, the unidirectionality of the morphometric parameters (Fig. 4) and statistically insignificant second axis of the principal components (Table 2) suggest that the selected set of morphometric parameters played little to no role in variation of the shape of the head capsule which also has a negative effect on the expectation of sexual dimorphism in the general sample. One should note that 4 out of 5 parameters measured in our work had not been analyzed in *B. antarctica* larvae before. The single parameter the studies have in common is the mandible length; by Atchley and Hilburn (1979), it belongs to one of four parameters showing "significant locality by sex interaction". We did not find any similar effect for this feature in our data.

In this study we employed an unsupervised method of detection of dimorphic patterns. Morphological data, as visualized by PCA, did not suggest presence of a significant principal component other than size, neither it could show any detectable motifs of differences. These results can be explained by the low power of the chosen method over chosen features, or by the actual absence of sex differences in these traits in these larvae as well.

Therefore, according to our results, the differences between sexes in the studied parameters of the larvae from the Petermann Island population were much less profound than in the samples collected by Atchley and Hilburn (1979). Meanwhile, our results do correspond to those authors' research and evidence of no noticeable ef-

fect of the environmental factors on the morphometrical parameters of the head capsules of *B. antarctica* larvae at the 4th larval stage. What then could explain the results obtained by our predecessors? Recent studies have shown that plasticity in body size can differ quite remarkably between sexes, generating intraspecific variation in sexual size dimorphism (Teder and Tammaru 2005, Stillwell and Fox 2007). One could suppose that the differences found by Atchley and Hilburn (1979) between larvae from different populations could have been caused by the higher phenotypic plasticity of females which is common for insects (Stillwell et al. 2010) in response to different habitats, since their study encompassed 16 different populations from multiple islands. As Atchley and Hilburn (1979) did not provide specific numeric, so it is not possible to evaluate statistical approaches in their work, we decided to check whether there is a difference in morphometric parameters or not. After all, in the above research paper, there is no clear answer (not all populations exhibited sexual dimorphism in fourth-instar larvae). According to our results, sexual dimorphism is absent in studied morphometric parameters. We also propose using the PCA method to detect possible sexual dimorphism in some cases where it is difficult to identify sex in organisms by external features. Thus, the problem of sexual dimorphism of the *B. antarctica* as detectable by head capsule at the latest larval stage remains unclear. Nevertheless, our results suggest that the head capsule length, head capsule width, mandible width, mandible length, mentum length of the 4th larval stage do not differ much in larvae of different sexes, at least in the Petermann Island population.

References

AJAYI, O. M., GANTZ, J. D., FINCH, G., LEE, R. E., DENLINGER, D. L. and BENOIT, J. B. (2021): Rapid stress hardening in the Antarctic midge improves male fertility by increasing courtship success and preventing decline of accessory gland proteins following cold exposure. *Journal of Experimental Biology*, 224(14): jeb242506. doi: 10.1242/jeb.242506

ALLEGRUCCI, G., CARCHINI, G., CONVEY, P. and SBORDONI, V. (2012): Evolutionary geographic relationships among orthocladine chironomid midges from maritime Antarctic and sub-Antarctic islands. *Biological Journal of the Linnean Society*, 106(2): 258-274. doi: 10.1111/j.1095-8312.2012.01864.x

ATCHLEY, W. (1971): Components of Sexual Dimorphism in Chironomus Larvae (Diptera: Chironomidae). *The American Naturalist*, 105(945): 455-466. doi: 10.1086/282737

ATCHLEY, W. R., DAVIS, B. L. (1979): Chromosomal Variability in the Antarctic Insect, *Belgica antarctica* (Diptera: Chironomidae). *Annals of the Entomological Society of America*, 72(2): 246-252. doi: 10.1093/aesa/72.2.246

ATCHLEY, W. R., HILBURN, L. R. (1979): Morphometric variability in larvae of the Antarctic fly, *Belgica antarctica* (Diptera: Chironomidae). *Canadian Journal of Zoology*, 57(12): 2311-2318. doi: 10.1139/z79-300

ATCHLEY, W. R., MARTIN, J. (1971): A morphometric analysis of differential sexual dimorphism in larvae of *Chironomus* (Diptera). *Canadian Entomologist*, 103(3): 319-327. doi: 10.4039/Ent103319-3

BENBOW, M. E. (2008): Role of larval sexual dimorphism, biased sex ratios, and habitat on the energetics of a tropical chironomid. *Environmental Entomology*, 37(5): 1162-1173. doi: 10.1093/ee/37.5.1162

BENÍTEZ, H. A., SUKHODOLSKAYA, R. A., ÓRDENES-CLAVERÍA, R., AVTAEVA, T. A., KUSHALIEVA, S. A. and SAVELIEV, A. A. (2020): Measuring the inter and intraspecific sexual shape dimorphism and body shape variation in generalist ground beetles in Russia. *Insects*, 11(6): 361. doi: 10.3390/insects11060361

BENOIT, J. B., LOPEZ-MARTINEZ, G., ELNITSKY, M. A., LEE, R. E. and DENLINGER, D. L. (2009): Dehydration-induced cross tolerance of *Belgica antarctica* larvae to cold and heat is facilitated by trehalose accumulation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 152(4): 518-523. doi: 10.1016/j.cbpa.2008.12.009

BLANCKENHORN, W. U. (2007): Case studies of the differential-equilibrium hypothesis of sexual size dimorphism in two dung fly species. *In*: D. J. Fairbairn, W. U. Blanckenhorn, T. Szekely (eds.): Sex, size and gender roles: evolutionary studies of sexual size dimorphism. Oxford University Press, Oxford, UK, pp. 106–114. doi: 10.5167/uzh-19273

CHOWN, S. L., CONVEY, P. (2016): Antarctic Entomology. *Annual Review of Entomology*, 61: 119-137. doi: 10.1146/annurev-ento-010715-023537

CONVEY, P., BLOCK, W. (1996): Antarctic Diptera: Ecology, physiology and distribution. *European Journal of Entomology*, 93(1): 1-13.

ESPERK, T., TAMMARU, T., NYLIN, S. and TEDER, T. (2007): Achieving high sexual size dimorphism in insects: Females add instars. *Ecological Entomology*, 32(3): 243-256. doi: 10.1111/j.1365-2311.2007.00872.x

FATHINIA, B., RASTEGAR-POUYANI, N., DARVISHNIA, H., MOHAMADI, H. and FAIZI, H. (2012): Sexual size dimorphism in *Rana* (Pelophylax) *ridibunda ridibunda* Pallas, 1771 from a population in Darr-Shahr Township, Ilam Province, western Iran. *Amphibian and Reptile Conservation*, 5(1): 92-97 (e44).

FINCH, G., NANDYAL, S., PERRETTA, C., NANDYAL, S., ROSENDALE, A., HOLMES, C., GANTZ, J. D., SPACHT, D. E., BAILEY, S. T., CHEN, X., OYEN, K., DIDION, E. M., CHAKRABORTY, S., LEE, R. E., DENLINGER, D. L., MATTER, S. F., ATTARDO, G. M., WEIRAUCH, M. T. and BENOIT, J. B. (2020): Multi-level analysis of reproduction in an Antarctic midge identifies female and male accessory gland products that are altered by larval stress and impact progeny viability. *Scientific Reports*, 10: 19791. doi: 10.1038/s41598-020-76139-6

HARADA, E., LEE, R. E., DENLINGER, D. and GOTO, S. (2014): Life history traits of adults and embryos of the Antarctic midge *Belgica antarctica*. *Polar Biology*, 37(8): 1213-1217. doi: 10.1007/s00300-014-1511-0

HARTIGAN, J. A., WONG, M. A. (1979): A K-Means Clustering Algorithm. *Applied Statistics*, 28(1): 100-108. doi: 10.2307/2346830

JOLLIFFE, I. T. (2002): Principal Component Analysis, 2nd ed. Springer-Verlag, New York, USA, 2002, 488 p. doi: 10.1007/b98835

KAWARASAKI, Y., TEETS, N. M., PHILIP, B. N., POTTS, L. J., GANTZ, J. D., DENLINGER, D. L. and LEE, R. E. (2019): Characterization of drought-induced rapid cold-hardening in the Antarctic midge, *Belgica antarctica*. *Polar Biology*, 42(6): 1147-1156. doi: 10.1007/s00300-019-02503-6

KELLEY, J. L., PEYTON, J. T., FISTON-LAVIER, A. S., TEETS, N. M., YEE, M. C., JOHNSTON, J. S., BUSTAMANTE, C. D., LEE, R. E. and DENLINGER, D. L. (2014): Compact genome of the Antarctic midge is likely an adaptation to an extreme environment. *Nature Communications*, 5: 4611. doi: 10.1038/ncomms5611

KOVALENKO, P., TROKHYMETS, V., PARNIKOZA, I, PROTSENKO, YU., SALGANSKIY, O., DZHULAI, A., DYKYY, I., NABOKIN, M., KOZERETSKA, I. and GOROBCHYSHYN, V. (2021): Current status of *Belgica antarctica* Jacobs, 1900 (Diptera: Chironomidae) distribution by the data of Ukrainian Antarctic Expeditions. *Ukrainian Antarctic Journal*, 2: 76-93. doi: 10.33275/1727-7485.2.2021.679

KOZERETSKA, I., SERGA, S., KOVALENKO, P., GOROBCHYSHYN, V. and CONVEY, P. (2022): *Belgica antarctica* (Diptera: Chironomidae): A natural model organism for extreme environments. *Insect Science*, 29(1): 2-20. doi: 10.1111/1744-7917.12925

LAIOLO, P., ILLERA, J. and OBESO, J. (2013): Data from: Local climate determines intra- and interspecific variation in sexual size dimorphism in mountain grasshopper communities. Dryad, Dataset. doi: 10.5061/dryad.c5097

MICHAILOVA, P., ILKOVA, J., KOVALENKO, P., DZHULAI, A. and KOZERETSKA, I. (2021a): Long-term retainment of some chromosomal inversions in a local population of *Belgica antarctica* Jacobs (Diptera, Chironomidae). *Czech Polar Reports*, 11(1): 16-24. doi: 10.5817/CPR2021-1-3

MICHAILOVA, P., ILKOVA, J., KOVALENKO, P. A., GOROBCHYSHYN, V. A., KOZERETSKA, I. A. and CONVEY, P. (2021b): External morphology of larvae of *Belgica antarctica* Jacobs, 1900 (Diptera, Chironomidae) obtained from two locations in Maritime Antarctica. *Insects*, 12(9): 792. doi: 10.3390/insects12090792

PARNIKOZA, I., ROZHOK, A., CONVEY, P. , VESELSKI, M., ESEFELD, J., OCHYRA, R., MUSTAFA, O., BRAUN, C., PETER, H.-U., SMYKLA, J., KUNAKH, V. and KOZERETSKA, I. (2018): Spread of Antarctic vegetation by the kelp gull: comparison of two maritime Antarctic regions. *Polar Biology*, 41(6): 1143-1155. doi: 10.1007/s00300-018-2274-9

PECKHAM, V. (1971): Notes on the chironomid midge *Belgica antarctica* Jacobs at Anvers Island in the Maritime Antarctic. *Pacific Insects Monograph*, 25: 145-166.

POTTS, L. J., GANTZ, J. D., KAWARASAKI, Y., PHILIP, B. N., GONTHIER, D. J., LAW, A. D., MOE, L., UNRINE, J. M., McCULLEY, R. L., LEE JR., R. E., DENLINGER, D. L. and TEETS, N. M. (2020): Environmental factors influencing fine-scale distribution of Antarctica's only endemic insect. *Oecologia*, 194(4): 529-539. doi: 10.1007/s00442-020-04714-9

SHUI, W., ZHOU, M., MADDOCK, S., HE, T., WANG, X. and DENG, Q. (2017): A PCA-Based method for determining craniofacial relationship and sexual dimorphism of facial shapes. *Computers in Biology and Medicine*, 90: 33-49. doi: 10.1016/j.compbiomed.2017.08.023

SPACHT, D. E., GANTZ, J. D., DEVLIN, J. J., LEE JR., R. E., DENLINGER, D. L. and TEETS, N. M. (2021): Fine-scale variation in microhabitat conditions influences physiology and metabolism in an Antarctic insect. *Oecologia*, 197(2): 373-385. doi: 10.1007/s00442-021-05035-1

STILLWELL, R. C., BLANCKENHORN, W. U., TEDER, T., DAVIDOWITZ, G. and FOX, C. W. (2010): Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annual Review of Entomology*, 55: 227-245. doi: 10.1146/annurev-ento-112408-085500

STILLWELL, R. C., FOX, C. W. (2007): Environmental effects on sexual size dimorphism of a seed-feeding beetle. *Oecologia*, 153(2): 273-280. doi: 10.1007/s00442-007-0724-0

SUGG, P., EDWARDS, J. S. and BAUST, J. (1983): Phenology and life history of *Belgica antarctica*, an Antarctic midge (Diptera: Chironomidae). *Ecological Entomology*, 8(1): 105-113. doi: 10.1111/j.1365-2311.1983.tb00487.x

TEDER, T., TAMMARU, T. (2005): Sexual size dimorphism within species increases with body size in insects. *Oikos*, 108(2): 321-334. doi: 10.1111/j.0030-1299.2005.13609.x

USHER, M. B., EDWARDS, M. (1984): A dipteran from south of the Antarctic Circle: *Belgica antarctica* (Chironomidae), with a description of its larvae. *Biological Journal of the Linnean Society*, 23(1): 19-31. doi: 10.1111/j.1095-8312.1984.tb00803.x

VEA, I. M., WILCOX A., FRANKINO, W. A. and SHINGLETON, A. W. (2021): Sex-specific plasticity explains genetic variation in sexual size dimorphism in *Drosophila*. *bioRxiv*, Article 448738. doi: 10.1101/2021.06.16.448738

WÜLKER, W., GÖTZ, P. (1968): Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von *Chironomus*-Larven (Dipt.). *Zeitschrift für Morphologie der Tiere*, 62(4): 363-388. (In German). doi: 10.1007/BF00401562

XUE, J., LEE, C., WAKEHAM, S. and ARMSTRONG, R. (2011): Using principal components analysis (PCA) with cluster analysis to study the organic geochemistry of sinking particles in the ocean. *Organic Geochemistry*, 42(4): 356-367. doi: 10.1016/j.orggeochem.2011.01.012

YOSHIDA, M., LEE, R. E., DENLINGER, D. L. and GOTO, S. G. (2021): Expression of aquaporins in response to distinct dehydration stresses that confer stress tolerance in the Antarctic midge *Belgica antarctica*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 256: 110928. doi: 10.1016/j.cbpa.2021.110928
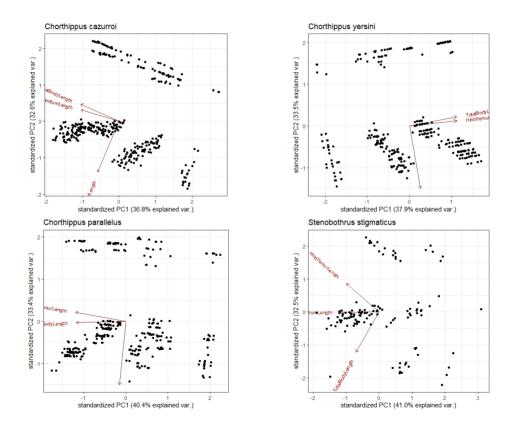
Web sources / Other sources

[1] R Core Team (2018): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
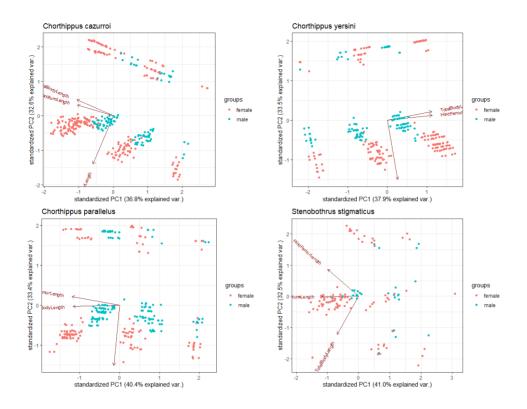
Supplementary Material

Principal Component Analysis of the expected sexual dimorphism in grasshoppers (*Chorthippus* sp.) using three morphometric parameters (Total Body Length, Femur Length, Pronotum Length). On Supplementary Fig. 1, the labeling by sex is purposefully omitted, to replicate the view on the data the same way as it was analyzed in the presented manuscript. Orthogonality of the eigenvectors (loadings) indicates that the underlying traits already significantly contribute to at least two principal components. Unlike in our data, a significant clustering here can be observed with a naked eye, but the role of sex is not obvious simply by visual analysis of the unlabeled data. The following figure (Supplementary Fig. 2) uncovers that besides sex, there might be other significant factors in explaining variation in insect morphometry (authors provide coordinates, temperature data, insolation, and rainfall readings as significant explanators of the seen patterns).

Supplementary Fig. 1. PCA of the pooled morphometric data; labeling by sex is omitted.

Supplementary Fig. 2. PCA of the pooled morphometric data; labeling by sex is included.