Zoologischer Anzeiger 293 (2021) 163–167

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Assessing the shape plasticity between Russian biotopes in *Pterostichus dilutipes* (Motschulsky, 1844) (Coleoptera: Carabidae) a geometric morphometric approach

Hugo A. Benítez [a,*], Raisa A. Sukhodolskaya [b], Rodrigo Órdenes-Claveria [a], Dmitriy N. Vavilov [b], Tatiana Ananina [c,d]

[a] *Laboratorio de Ecología y Morfometría Evolutiva, Centro de Investigación de Estudios Avanzados del Maule, Universidad Católica del Maule, Talca, Chile*
[b] *Research Institute for Problems of Ecology and Mineral Wealth Use of Tatarstan Academy of Sciences, Kazan, Tatarstan, 420000, Russia*
[c] *FSBE Zapovednoe Podlemorie, Ulan Ude, 670045, Russia*
[d] *Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences, Ulan-Ude, 670047, Russia*

ARTICLE INFO

Article history:
Received 19 April 2021
Received in revised form
30 May 2021
Accepted 11 June 2021
Available online 15 June 2021

Keywords:
Morphometrics
Shape variation
Sexual dimorphism
Biotopes
Pterostichus dilutipes

ABSTRACT

Different biotopes or environments produce dissimilar stress level in insects, which can be reflected on the capacity of an individual to overcome these pressures and survive. Morphological plasticity of an organism is expressed by the ability of the species to produce different phenotypes depending on the environmental conditions. The following research evaluate the use of geometric morphometrics (GM) tool to evaluate the effect in the development of multiple phenotypes on a native beetle *Pterostichus dilutipes* Motschulsky, 1844 inhabiting different biotopes from the Baikal Lake coastal in Russia. GM results provided a powerful graphical visualization to detect small variation in morphology from beetles collected in an altitudinal gradient with a special plastic variation from warmer to colder biotopes. GM are very useful tool to future studies where the integration of research areas could allow researcher to identify with clarity of the morphological body plan product of natural selection.

1. Introduction

Phenotypic plasticity is the ability of an organism to express different phenotypes depending on the environmental conditions (Agrawal 2001; West-Eberhard 1989). Such plasticity is therefore the consequence of the interaction between environmental variability and the developmental program (genotypic × environmental interaction) and is thus, one solution to the problem of adaptation to heterogeneous environments (DeWitt & Scheiner 2004; DeWitt et al. 1998; Ernande & Dieckmann 2004).

Body size in beetles has been used for studies of sexual dimorphism and population differentiation and taxonomical differentiation for decades where in many genus like *Carabus*, *Pterostichus* or *Ceroglossus* there are some biogeographically trends that body size decreases related to latitude (Benítez et al. 2020b; Juache et al. 2018; Sukhodolskaya & Saveliev 2017). They differently response to anthropogenic factor (Sukhodolskaya 2013) and which is very noticeable, to biotope characteristics where they inhabit. The development of a morphological quantitative toolkit known as geometric morphometrics (GM) have allowed to researcher to make better morphological descriptions, particularly to identify trait variation that could be hard to be distinguished by simple sight (Adams et al. 2013; Zúniga-Reinoso & Benítez 2015). The GM allows studying shape, defined as the remaining geometric properties after removing the effects of scale, rotation and translation of an object (Adams & Funk 1997; Benítez & Püschel 2014; Rohlf & Slice 1990).

The ground beetle species *Poecilus cupreus* (Linnaeus, 1758) in agrocenoses are characterized by a significant difference in the width of the elytra and pronotum. It can be due to the environment characteristics when certain body size and shape allows them to find the appropriate habitats and be better competitors in certain agricultural landscapes (Sukhodolskaya 2016; Sukhodolskaya & Saveliev 2017). On the other hand, this GM has been used for identify sexual shape dimorphism in secondary sexual traits, which are difficult to identify using traditional morphometrics. An example was found in the genus *Ceroglossus* where a trend was

* Corresponding author.
E-mail address: hbenitez@ucm.cl (H.A. Benítez).

https://doi.org/10.1016/j.jcz.2021.06.008
0044-5231/

observed to keep a “similar” body shape between sexes and species due to characteristics associated with sexual selection of the group in which the lack of outstanding morphological characters does not restrict reproduction capacity (Benítez et al. 2013b). The biotopes characteristic could be affected by environmental stress, which are reflected in degrees of developmental instability or plasticity which are the ability of an individual to overcome the effects of stress (Benítez et al. 2020a; Palmer 2004).

Nevertheless, because of variation (adaptation) over time to a specific environment, ecological pressures and geographic distances affect the biotopes locally and thus their associated fauna (Alibert et al. 2001; Benítez et al. 2011).

The following article aims to evaluate the efficiency of geometric morphometrics tools to differentiate populations of *P. dilutipes* from the different level of altitude and biotopes.

2. Material and methods

2.1. Sampling

More than 140 individuals of *P. dilutipes* were collected from three different biotopes using pitfall traps from the Barguzin State Reserve, at the western shore of Lake Baikal. These population were separated between 2 and 3 km from each other in a transect from the low-mountainous to the high-altitude, nevertheless the environmental characteristic from the different biotopes were evaluated in order to quantify the relationship between shape and environment (Table 1).

The biotopes are: 1 - Blueberry larch N 54° 21′ 10,8"; E 109° 30′ 12,3" (518 m. a.s.l.), 2 - Redbillberry pine N 54° 23′ 58,5"; E 109° 41′ 06,2" (535 m. a.s.l.), 3 - Bergenia cedar N 54° 23′ 37,5"; E 109° 42′ 58,1" (635 m. a.s.l.). Blueberry larch and Redbillberry pine are separated from each other by wide meadows and marshes. Redbillberry pine and Bergenia cedar are separated from each other by the Davshe River and the Birch Grove biotope forbs.

2.2. Morphometrics analyses

For the morphometrics analyses 16 individuals per biotope were used, (other individuals were discarded due to the damage in the collection methods) the dorsal and ventral view of *P. dilutipes* were used in order to analyze the shape using 2D geometric morphometrics, every specimen was photographed using a Nikon D5000100 digital camera. For the ventral and dorsal view 19 and 14 landmarks (Figs. 1 and 2) were digitized using the software tpsDig2 (Rohlf 2013), respectively following the literature on beetles and geometric morphometrics (Benítez et al. 2020b; Benítez et al. 2010; Espinoza-Donoso et al. 2020). All statistical and morphometric

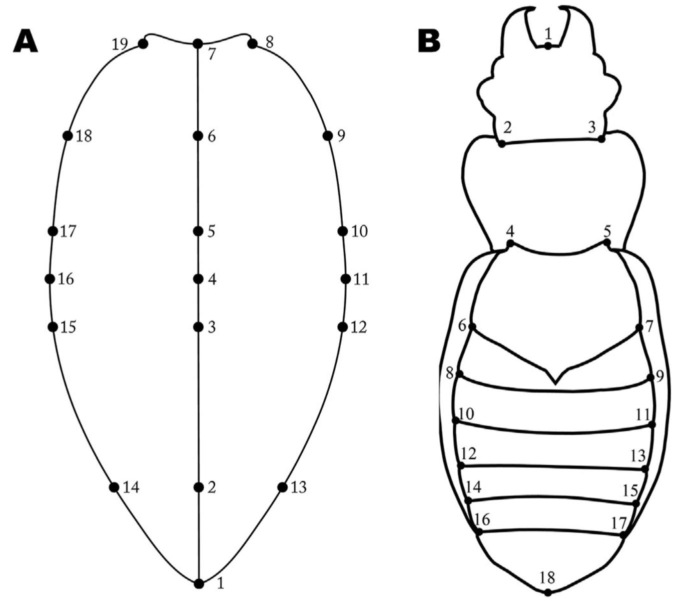

Fig. 1. Graphical representation of the *Pterostichus dilutipes* A: Dorsal (Elytral) view with 14 landmarks and the B: Ventral view with 18 landmarks.

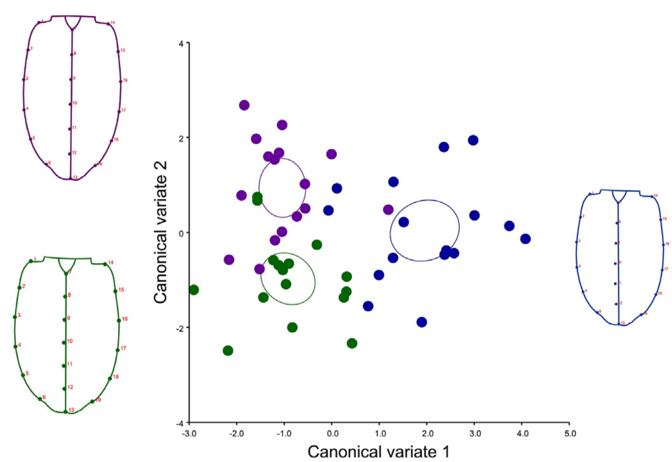

Fig. 2. Scatterplot of the Canonical variate analysis of the dorsal (elytral) view in *P. dilutipes* with their respectively average shape outline. The colors legend represents the three different biotopes: purple: Blueberry larch; green: Redbillberry pine; and blue: Bergenia cedar. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Soil characteristic from the three different biotopes where the *P. dilutipes* was collected.

	Blueberry larch	Redbillberry pine	Bergenia cedar
Height ab. s. l., m	520	535	635
Abundance, ind. /per 100 traps /day	13,7	22,1	16,5
Soil temperature, H = 5 cm	10,1	9,6	9,5
Soil temperature, H = 10 cm	9,0	8,9	9,3
Average annual Min t°C on the soil surface	−7,6	−3,8	−6,2
Min t°C on the soil surface, July	11,5	13,9	13,5
Duration of snow cover, days	174	188	198
Snow cover height, cm	60	68	79
Soil moisture (June–August),%	32,9	31,8	48,1
Mosses, projective cover,%	85	35	70
Lichens, projective cover,%,	5	40	10

analyses were performed using MorphoJ software, version 1.06 d (Klingenberg, 2011). Shape information was extracted with a Procrustes fit analysis from all landmark configurations (Rohlf & Slice 1990). This methodology removes non-shape information produced by scale, orientation and size, by standardizing each specimen to a unit centroid size (Dryden & Mardia 1998; Rohlf & Marcus 1993). A Principal Component Analysis (PCA) based on the covariance matrix of the individual shape was performed in order to analyze shape space and quantify the levels of variation at the different dimensions (eigenvalues). In order to magnify the variation between biotopes a Canonical variate analysis was performed as a discriminant analysis that modify the axes of the PCA using the maximun variation to create new axes and organize the individuals by the selected groups. Finally, to evaluate the sexual dimorphism and the influence of allometry (shape influence on size), a multivariate regression was performed using the shape as a dependent variable and the centroid size as an independent variable.

3. Results

The PCA calculated for the dorsal view (elytra) showed for the first three PCs accounted for 83,7% of the total shape variation (PC1 = 54,07%; PC2 = 20,5%; PC3 = 9.13%), The shape variation was clear distinguished between populations by the movement of the landmark 1 and 14, this landmark is localized at the top of the elytra where the population from the Redbillberry pine (green) showed a wider elytra in comparison with the other 2 biotopes (also noticed by the expansion of the symmetric landmarks 3–4 and 16–17) which both are thinner than the Redbillberry pine but between them the population collected in Blueberry larch (purple) results to have a more elongated elytra than the Bergenia cedar (blue) population noticed by the projection of the landmark 7 to the upper central section of the elytra shape. The scatterplot of the CVA showed a clear separation of the population where the CV1 separate the Redbillberry pine biotope to the left section of the axe and the Bergenia cedar biotope to the right section of the CV1 and CV2 showed the Blueberry larch biotope separated to the other 2 population the upper left section of the scatterplot (Fig. 2).

For the ventral view the shape variation was lower for the first three PCs in comparison to the dorsal view accounting for the 73,9% (PC1 = 42,3%; PC2 = 23,4%; PC3 = 8,1%). The ventral shape of *P. dilutipes* was less variable to the dorsal where again the population of Redbillberry pine had a elongated shape of the abdomen in contrast to the wider elytra by the elongation to the landmark 18 to the lower center. A noticeable differentiation was found in the scatterplot of the CVA for the three populations, nevertheless there was not much intrapopulation shape variation but a clear interpopulation differentiation, Its important to notice that the individuals from the Blueberry larch and Bergenia cedar population had similar shape in comparison to the dorsal view (Fig. 3).

In order to discard if there any allometry effect in the data a multivariate regression of the shape into centroid size showed a permutation test after 10,000 randomization rounds found a significant (P: 0.0068) allometry of the 9% of the size effect on the shape for the dorsal view (Fig. 4A) and a lower percentage 6% but also significant (P: 0,01%) allometry effect in the ventral view, in which a noticeable sexual shape dimorphism was visualized where females are bigger than males in both views (Fig. 4B).

4. Discussion

The following research describes the shape variation using geometric morphometric in two different views (dorsal and ventral) in *P. dilutipes* a native beetle from the Baikal Lake coastal biotopes in Russia.

Independently of the closeness of the different biotopes, clear differences were noticeable in the different populations where

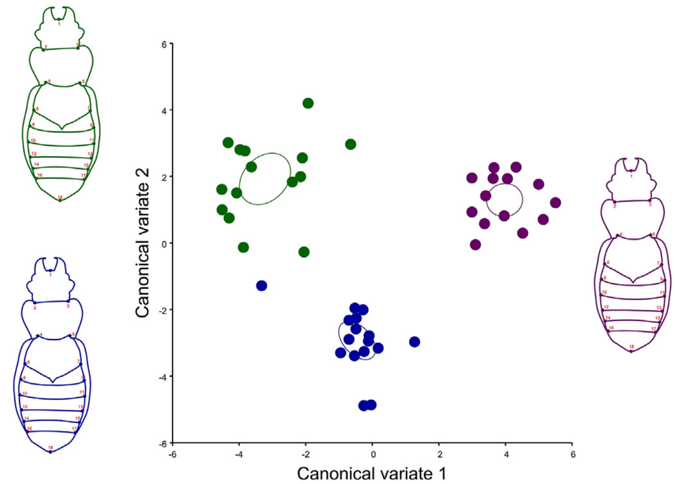

Fig. 3. Scatterplot of the Canonical variate analysis of the ventral view in *P. dilutipes* with their respectively average shape outline. The colors legend represents the three different biotopes: purple: Blueberry larch; green: Redbillberry pine; and blue: Bergenia cedar. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

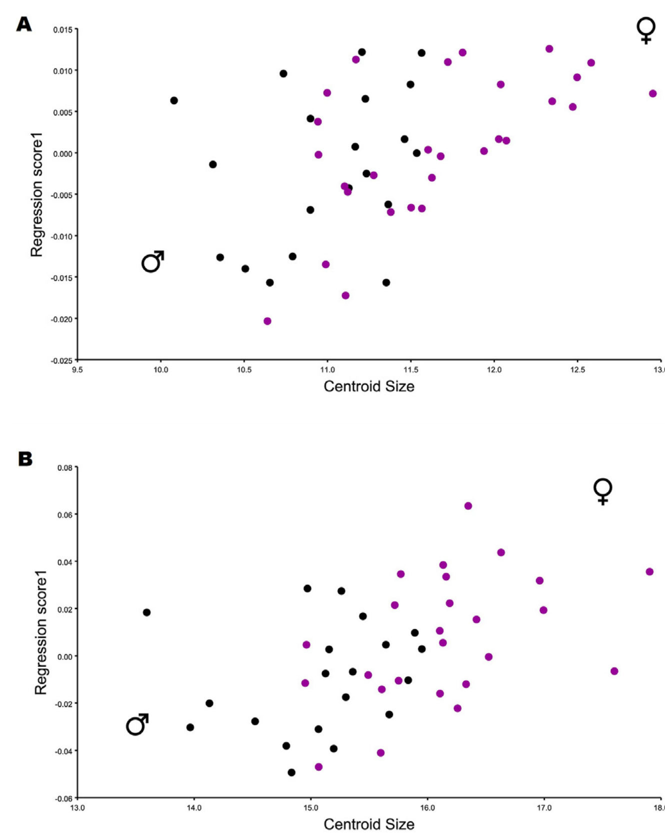

Fig. 4. Multivariate regression of shape (dependent variable) on centroid size (independent variable) of *P. dilutipes*, the purple represents females and the black point represent the males. A: Dorsal (elytral) view and B: ventral view. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the most plastic population was found to be one inhabiting in the Redbillberry pine biotope. The Redbillberry biotope in contrast to the other two biotopes, is considered the warmer biotope with a very thick vegetational layer with higher abundance of lichens and other plants providing a richest environment for food availability which translates into more reproduction events. The other two biotopes Blueberry larch at 518 m.a.s.l and Bergenia cedar 635 m.a.s.l, however, result to have very stable environmental condition for the ground beetles which results in not having much differences in phenotype.

Such plasticity in traits size must inevitably lead to the shape variation in ground beetles. Some examples can be found in other species in similar environments by means of linear modeling, there has been shown that in the regions with more severe climate the shape of *Pterostichus niger* Schal. became more convex in apical-basal direction (Sukhodolskaya 2013). In *Carabus granulatus* L. in open habitats (meadows, lawns) beetles became more flattened (Sukhodolskaya & Saveliev 2017). Moreover, the significant interaction of Sex x Habitat in *Pterostichus melanarius* indicates a divergence of sexual size dimorphism in different habitats. Sukhodolskaya (2014) after some analysis shows significant effects of sex and all environmental factors on beetles shape. Highly significant Sex x Environmental factors interaction suggested that sexual dimorphism in *P. melanarius* differed considerably in various environment (Sukhodolskaya 2014). Recently studies in Russian beetles found that the sexual shape dimorphism may be variable and the degree of variation depend of the species, nevertheless, the most recognizable trait in shape is the ventral view where other studies in sexual dimorphism using geometric morphometrics in beetles has been studied. An example of abdominal shape variation using a ventral view in Chilean beetles of the genus *Ceroglossus* found that shape could be used as a trait to differentiate sexual dimorphism and population variation (Benítez et al. 2010). Later studies in the same group found that ventral variation could be more informative evaluating the evolution of the trait in a context of sexual selection (Benítez et al., 2013a; Benítez et al., 2013b; Bravi & Benítez, 2013; Juache et al., 2018)Also in the same line of the evolution of shape and size dimorphism same traits where studied in the genus *Nebria* (Palestrini et al. 2012). Other studies take into account the variation in tiger beetles using different sexual and non-sexual traits (Espinoza-Donoso et al. 2020). For *Pterostichus* genus Russian beetles also were studied in their ventral abdominal shape variation in order to detect sexual dimorphism using geometric tools (Benítez et al. 2020b; Sukhodolskaya 2013, 2016; Sukhodolskaya & Saveliev 2017).

Finally, this research confirms that elytra as well as ventral abdominal part of the body in ground beetles can be used as morphological traits to detect shape variation due to different environmental conditions, and suggests that geometric morphometrics techniques provides a powerful visualization to detect slightly morphological variation providing new tools to integrate in the discovering of the hidden world of the phenotypical variation modulated by natural selection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was carried out with partial financial support from the Program of Fundamental Scientific Research (FSR) of the State Russian Academies of Sciences for 2021–2025, project 0271-2021-0001 (FWSM - 2021-0001), No. 1210309000138-8. HB was supported as well by the ANID grant FONDECYT DE INICIACION 11180366 from the Chilean government (Agencia Nacional de Investigación y Desarrollo de Chile).

References

Adams, D.C., Funk, D.J., 1997. Morphometric inferences on sibling species and sexual dimorphism in *Neochlamisus bebbianae* leaf beetles: multivariate applications of the thin-plate spline. Syst. Biol. 46, 180–194.
Adams, D.C., Rohlf, F.J., Slice, D.E., 2013. A field comes of age: geometric morphometrics in the 21st century. Hystrix 24, 7–14.
Agrawal, A.A., 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294, 321–326.
Alibert, P., Moureau, B., Dommergues, J.L., David, B., 2001. Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C-nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. Zool. Scripta 30, 299–311.
Benítez, H.A., Avaria-Llautureo, J., Canales-Aguirre, C.B., Jerez, V., Parra, L.E., Hernandez, C.E., 2013a. Evolution of sexual size dimorphism and its relationship with sex ratio in carabid beetles of Genus *Ceroglossus* Solier. Current Zoology 59, 769–777.
Benítez, H.A., Briones, R., Jerez, V., 2011. Intra and Inter-population morphological variation of shape and size of the Chilean magnificent beetle, *Ceroglossus chilensis* in the Baker River Basin, Chilean Patagonia. J. Insect Sci. 11.
Benítez, H.A., Lemic, D., Villalobos-Leiva, A., Bažok, R., Órdenes-Claveria, R., Pajač Živković, I., Mikac, K.M., 2020a. Breaking symmetry: fluctuating asymmetry and geometric morphometrics as tools for evaluating developmental instability under diverse agroecosystems. Symmetry 12, 1789.
Benítez, H.A., Püschel, T.A., 2014. Modelando la Varianza de la Forma: morfometría Geométrica Aplicaciones en Biología Evolutiva. Int. J. Morphol. 32, 998–1008.
Benítez, H.A., Sanzana, M.-J., Jerez, V., Parra, L.E., Hernandez, C.E., Canales-Aguirre, C.B., 2013b. Sexual shape and size dimorphism in carabid beetles of the genus *Ceroglossus*: is geometric body size similar between sexes due to sex ratio? Zool. Sci. 30, 289–295.
Benítez, H.A., Sukhodolskaya, R.A., Órdenes-Clavería, R., Avtaeva, T.A., Kushalieva, S.A., Saveliev, A.A., 2020b. Measuring the inter and intraspecific sexual shape dimorphism and body shape variation in generalist ground beetles in Russia. Insects 11, 361.
Benítez, H.A., Vidal, M., Briones, R., Jerez, V., 2010. Sexual dimorphism and morphological variation in populations of *Ceroglossus chilensis* (eschscholtz, 1829)(Coleoptera: Carabidae). J. Entomol. Res. Soc. 12, 87–95.
Bravi, R., Benítez, H.A., 2013. Left-right asymmetries and shape analysis on *Ceroglossus chilensis* (Coleoptera: Carabidae). Acta Oecologica-International Journal of Ecology 52, 57–62.
DeWitt, T.J., Scheiner, S.M., 2004. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford University Press.
DeWitt, T.J., Sih, A., Wilson, D.S., 1998. Costs and limits of phenotypic plasticity. Trends Ecol. Evol. 13, 77–81.
Dryden, I.L., Mardia, K.V., 1998. Statistical Shape Analysis. Wiley, Chichester.
Ernande, B., Dieckmann, U., 2004. The evolution of phenotypic plasticity in spatially structured environments: implications of intraspecific competition, plasticity costs and environmental characteristics. J. Evol. Biol. 17, 613–628.
Espinoza-Donoso, S., Angulo-Bedoya, M., Lemic, D., Benítez, H.A., 2020. Assessing the influence of allometry on sexual and non-sexual traits: an example in *Cicindelidia trifasciata* (Coleoptera: Cicindelinae) using geometric morphometrics. Zool. Anz. 287, 61–66.
Juache, A., Ordenes, R., Benítez, H.A., 2018. Quantifying the shape variation of the elytra in Patagonian populations of the ground beetle *Ceroglossus chilensis* (Coleoptera: Carabidae). Zool. Anz. 274, 123–126.
Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. 11, 353–357.
Palestrini, C., Roggero, A., Hernández Nova, L.K., Giachino, P.M., Rolando, A., 2012. On the evolution of shape and size divergence in *Nebria* (*Nebriola*) ground beetles (Coleoptera, Carabidae). Syst. Biodivers. 10, 147–157.
Palmer, A.R., 2004. Symmetry breaking and the evolution of development. Science 306, 828–833.
Rohlf, F.J., 2013. TPSdig, v. 2.17. State University at Stony Brook, NY.
Rohlf, F.J., Marcus, L.F., 1993. A revolution in morphometrics. Trends Ecol. Evol. 8, 129–132.
Rohlf, F.J., Slice, D., 1990. Extensions of the Procustes methods for the optimal superimposition of landmarks. Syst. Zool. 39, 40–59.

Sukhodolskaya, R., 2013. Intraspecific body size variation in ground beetles (Coleoptera, Carabidae) in urban-suburban-rural-natural gradient. Acta Biol. Univ. Daugavp. 13, 121–128.
Sukhodolskaya, R., 2014. Variation in body size and body shape in ground beetle *Pterostichus melanarius* Ill.(Coleoptera, Carabidae). Journal of Agri-Food and Applied Sciences 2, 196–205.
Sukhodolskaya, R., 2016. Intra-specific body size variation of ground beetles (Coleoptera: Carabidae) in latitudinal gradient. Period. Biol. 118, 273–280.
Sukhodolskaya, R.A., Saveliev, A.A., 2017. Impact of environmental factors on the body shape variation and sexual shape dimorphism in *Carabus granulatus* L.(Coleoptera: Carabidae). Zoological Systematics 42, 71–89.
West-Eberhard, M.J., 1989. Phenotypic plasticity and the origins of diversity. Annu. Rev. Ecol. Systemat. 249–278.
Zúniga-Reinoso, Á., Benítez, H.A., 2015. The overrated use of the morphological cryptic species concept: an example with *Nyctelia* darkbeetles (Coleoptera: Tenebrionidae) using geometric morphometrics. Zoologischer Anzeiger-A Journal of Comparative Zoology 255, 47–53.