Morphological variability of *Bembidion varium* (Coleoptera, Carabidae) in gradient of soil salinity

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Abstract

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This article discusses the effect of soil salinity on the variability of *Bembidion (Notaphus) varium* (Oliver, 1795). The authors of the article collected imagoes of this species in five ecosystems that differed in soil salinity; 13 linear characteristics, one angular characteristic and 6 morphometric indices were measured. Significant changes in six linear parameters of *B. varium* (body length, head length and width, width of prothorax between front angles, maximum width of prothorax and elytra width) and two morphometric indices are observed in the considered ecosystems. Soil salinity probably has the most impact on the variability of these characteristics. However, the influence of other important natural and anthropogenic factors is not ruled out. Significant differences between the sexes are observed for all linear parameters: females of *B. varium* are larger than males. There are no differences between males and females in morphometric indices. The head width, prothorax length and width, elytra length and width depend on the body length of *B. varium* individuals. The morphological variability of *B. varium* under the influence of other environmental factors needs further research.

Keywords

morphometrics, population variability, riparian beetles, sexual dimorphism

Introduction

Understanding the patterns of morphological changes and their evolutionary causes is one of the goals of evolutionary biology (PIE and TRANIELLO, 2007). The morphological variability of species is the result of the combined effects of genes and the environment (LUPI et al., 2015; LANGRAF et al., 2017). Insect populations accumulate (summarize) the effects of factor exposure over a certain period of time. Environmental stress can lead to a decrease in metabolic rate, which can be reflected in increased variability of morphological characters (ELEK et al., 2014). At present, it remains unclear at what stage of ontogenesis the influence of environmental factors is the most significant and which of these factors are decisive at a particular case (KOMLYK and BRYGADYRENKO, 2019b). It is assumed that a change in even one environmental factor is the cause of interpopulation morphological differences in beetles (PALMER, 2002).

Morphological variability within populations is a reflection of the plasticity of the development of a species, and in different populations is a measure of adaptive plasticity of a species (KAWANO, 2006, 2016). There are articles devoted to the influence of the type of food objects (BONAL et al., 2011; MARTYNOV and BRYGADYRENKO, 2018; MARTINKOVÁ et al., 2019), the temperature and nature of the substrate (ERNSTING and ISAAKS, 1997), competition (OKUZAKI and SOTA, 2018), biogeography (influence of altitude, climate) (SUKHODOLSKAYA and SAVELIEV, 2016) and anthropogenic factors (VICIAN et al., 2018; SOWA

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and SKALSKI, 2019) on the morphological variability of beetles. Little research has been devoted to the influence of environmental factors on the morphological variability of ground beetles (Coleoptera, Carabidae). In previous articles (BRYGADYRENKO, 2015a, 2015b, 2016; BRYGADYRENKO and KOROLEV, 2015), we examined the influence of various natural and anthropogenic factors in riparian ecosystems on different species of ground beetles, among which the salinity of the soil solution was also analyzed.

Soil salinization is the accumulation of mobile salts in the soil layers above a certain level, which negatively affects the state of the environment. Worldwide, more than 800 million hectares of land are subject to salinization (PICHU, 2016). Soil salinization is intensified by human activities (intensive agriculture and other land use). Soil salinity affects the biotopic distribution of hygrophilous beetles. Many of them prefer saline areas, as they are mostly open, well lit and warm. Soil salinization contributes to the suppression of growth and the ability to infect hosts in fungi pathogenic for ground beetles (DANGALLE et al., 2013). The level of soil salinity affects the duration of developmental stages and phenotypic plasticity of insects (CLARK et al., 2004), as well as the process of laying eggs (SPOMER et al., 2015). We previously examined the effect of soil salinity on Bembidion minimum (Fabricius, 1792) (KOMLYK and BRYGADYRENKO, 2019b). In this article we want to consider in more detail the effect of soil salinization on the morphological variability of another species of the same subgenus – B. (Notaphus) varium (Oliver, 1795).

Bembidion varium is a Palearctic species that lives on the shores of European water bodies: Albania (GUEORGUIEV, 2007), England (LUFF, 2007), Bulgaria (JOCQUE et al., 2016), Denmark (LINDROTH, 1985), Finland (LINDROTH, 1985), France (PETILLON et al., 2007), Greece (HIEKE and WRASE, 1988), Latvia (BUKEJS and TELNOV, 2007), Lithuania (TAMUTIS et al., 2011), the Republic of Macedonia (HRISTOVSKI and GUEORGUIEV, 2015), the Netherlands (DEN BOER, 1970), Norway (LINDROTH, 1985), Russia (Caucasus, Siberia) (KRYZHANOVSKIJ et al., 1995), Romania (HIEKE and WRASE, 1988), Slovakia (HURKA, 1996), Sweden (LINDROTH, 1985), European Turkey (GUEORGUIEV, 2011), the Czech Republic (KOPECKY, 2007). The species is also common in Dagestan (ABDURAKHMANOV et al., 2010), Azerbaijan (ATAMEHR, 2013), Egypt (ABDEL-DAYEM, 1998), Pakistan (KAZI et al., 2016), Turkestan (GUEORGUIEV and GUEORGUIEV, 1995) and Mongolia (LINDROTH, 1985). Bembidion varium is also found on islands, for example, on the Canary Islands (GUEORGUIEV and GUEORGUIEV, 1995). In Ukraine, the species is widespread in the Transcarpathian Lowlands, the Carpathians, Polesye, the broad-leaved forest, foreststeppe and steppe zones (PUTCHKOV, 2012).

The species is hygrophile (SZENTKIRALYI et al., 2005). It prefers humid clay soils, especially the banks of stagnant water bodies (ZHEREBTSOV, 2000). The species is heliophile, it is active in the daytime, especially in sunny weather (LINDROTH, 1985). It is a halotolerant species (SCHULTZ, 2000), found on both saline and non-saline soils (LINDROTH, 1985; PETILLON et al., 2007). *Bembidion varium* is found in bare areas lacking vegetation, as well as

with a small number of plants (LINDROTH, 1974; TURIN, 2000) and on salt marshes (LUFF, 2007).

Bembidion varium is a monomorphic macropterous species, characterized by high flight activity of both sexes at the adult stage (HURKA, 1996; MATALIN, 2003). The maximum activity in adults is noted in spring and summer (LINDROTH, 1985). Breeding occurs in spring (LINDROTH, 1985). The peak of summer migration is observed in the first half of July, a new generation migrates in search of new biotopes with a stable humid regime (MATALIN, 1998). *Bembidion varium* often falls into light traps (JOCQUE et al., 2016), and flies more to polarized than to unpolarized light (SZENTKIRALYI et al., 2005). It feeds on nematodes and beetle larvae (LINDROTH, 1985). Studies in the Canary Islands revealed that *B. varium* is the host of *Laboulbenia vulgaris* Peyrit. (Ascomycota) (ARNDT and SANTAMARIA, 2004).

We previously examined the morphological variability of *B. varium* under the influence of anthropogenic factors (SLINKO et al., 2008). The morphological variability of *B. varium* under the influence of natural factors has not yet been studied. The purpose of this article is to evaluate the morphological variability of *B. varium* and to determine which parameters among the linear characteristics and morphometric indices are more variable in the gradient of soil salinity.

Materials and methods

The research was carried out in five ecosystems in Novomoskovsk, Pokrovka, Pavlograd and Vasilkovka districts of Dnipropetrovsk region of Ukraine (Table 1). The ecosystems differed in mechanical composition, salinity and pH of the soil, and in the degree of anthropogenic load.

The methodology for determining the total salinity and pH of the soil is described in our previous article (BRYGADYRENKO and SLYNKO, 2015). The pH of the aqueous extract in all examined areas was weak-alkaline. The salinity of the soil solution varied within 0.37–0.99 and, in our opinion, is decisive among the influencing factors.

The adults of *B. varium* were collected 10–16 May 2016 (from 10 a.m. to 1 p.m.) manually using an aspirator during pouring water on the soil surface. A total of 107 adults (45 males and 62 females) were collected (Table 1). The beetles were frozen during 24 hours in a refrigerating chamber, laid onto cotton mats, straightened in advance of examination (to maintain proportions, we monitored the orientation of the head and prothorax). The dried out insects were photographed through a binocular microscope using a digital camera of 5 megapixels resolution. Each beetle was assigned a serial number, including the ecosystem number and sex of the specimen (female, male). The measurements were performed using digital photographs in the TpsDig 2.17 program.

The following 13 linear characteristics that we have already used before (BRYGADYRENKO and KOROLEV, 2015) were evaluated: length of body (Lb), head (from

front edge of clypeus to articulation with prothorax) (Lc), prothorax (Lp), elytra (Le), width of head with eyes (Sc), width of prothorax between front angles (Sp1) and size of the back angles (Sp2), maximum width of prothorax (Sp3), maximum width of elytra (Se), distance from the base of the left and right elytra to the first setae (L11, L1r), distance between setae on the left and right elytra (L2l, L2r). The back angles of the prothorax (B) were determined on the right and left parts of the body, for the further calculations their arithmetic mean value was used. Six morphometric indices were calculated: ratio of arithmetic mean value of the width of head, prothorax and elytra to body length ((Sc+Sp+Se)/(3Lb)), ratio of prothorax length to its maximum width (Lp/Sp), ratio of elytra length to prothorax length (Le/Lp), ratio of maximum width of elytra to maximum prothorax width (Se/Sp), ratio of prothorax width at the back edge to its maximum width (Sp2/Sp3), and ratio of elytra length to their width (Le/Se).

The results were processed by standard methods using Statistica software (version 8, StatSoft, USA). The effects of sex and ecosystems on morphological characteristics and indices were evaluated using MANOVA. The differences between samples were considered reliable at P < 0.05 (taking into account the Bonferroni correction). Variability of morphometric characteristics is represented by Box-Whisker plots. The dependence of morphometric characteristics on body length was evaluated using linear regression analysis. The reliability of the regression equation was estimated by r-square.

Results

The following results of MANOVA (Table 2) for the morphometric characteristics of the studied *B. varium* populations indicate that the differences between males and females are significant for 13 linear measurements (Lb, Lc, Lp, Le, Sc, Sp1, Sp2, Sp3, Se, L2l, L1l, L2r and L1r), but they are not significant for angular measurements (the value of the posterior angle of the prothorax B).

The ecosystem condition affects 7 measured characteristics (Lb, Lc, Sc, Sp1, Sp3, Se, L2r). The remaining characteristics (Sp2, Le, Lp, B, L2l, L1l, L1r) are unchanged in the different ecosystems.

According to the results of MANOVA (Table 3), the habitat conditions significantly affect the ratio of arithmetic mean value of the width of head, prothorax and elytra to body length (Sc+Sp+Se)/(3Lb) and ratio of elytra length to their width (Le/Se). Sex affects the ratio of maximum width of elytra to maximum prothorax width (Se/Sp). Sex and ecosystems jointly influence the ratio of prothorax width at the back edge to its maximum width (Sp2/Sp3).

With the increasing salinity of the soil solution (from the first to the fifth sampling area), the differences between males and females of *B. varium* (Figs 1–3) do not change for any of the measured characteristics or for any of the morphometric indices. The linear sizes of *B. varium* individuals within the sexual groups do not have a significant tendency to change within the soil salinity

strative Ecosyste	Mechanical	Salt content in	pH of soil		Degree of	Predominant type of
tes	composition of soil	soil solution (g L ⁻¹)	solution	Litter composition	anthropogenic load	anthropogenic load
5''N 2''E	Sandy loam	0.37	8.17	Litter is absent, large number of algae and mollusk shells	High	Watering place for cattle, household waste, railway
3´N 4´E	Sandy loam	0.49	7.83	Litter is absent	Low	Recreational load and cattle grazing are absent
3′`N 9′'E	Loam	0.69	7.98	Litter is absent	High	Watering place for cattle, household waste, mines
'N 1°E	Loam	0.73	8.07	Litter is absent, there are stones, algae on the surface of the soil	High	Watering place for cattle, household waste
)`N 5'E	Loam	0.99	8.25	Litter is absent	Medium	Recreational load, watering place for cattle

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Characteristic	Factor	F	Р
	Ecosystem	2.70	0.0348
Lb	Sex	69.87	< 0.0001
	Ecosystem * Sex	1.87	0.1215
	Ecosystem	5.53	0.0005
Lc	Sex	7.65	0.0068
	Ecosystem * Sex	1.69	0.1593
	Ecosystem	1.80	0 1344
Lp	Sex	46.89	< 0.0001
-r	Ecosystem * Sex	1.23	0.3029
	Ecosystem	0.83	0 5089
Le	Sex	60.58	< 0.0001
	Ecosystem * Sex	1.37	0.2485
	Ecosystem	4 75	0.0015
Sc	Sex	47.57	< 0.0001
	Ecosystem * Sex	0.37	0.8308
	Ecosystem	3.69	0.0077
Sp1	Sex	65.33	< 0.0001
1 -	Ecosystem * Sex	0.34	0.8474
	Ecosystem	2.40	0.0556
Sp2	Sex	48.92	< 0.0001
-	Ecosystem * Sex	1.34	0.2608
	Ecosystem	3.13	0.0182
Sp3	Sex	46.40	< 0.0001
	Ecosystem * Sex	0.40	0.8073
	Ecosystem	4.71	0.0016
Se	Sex	78.20	< 0.0001
	Ecosystem * Sex	1.10	0.3591
	Ecosystem	1.62	0.1766
В	Sex	0.21	0.6515
	Ecosystem * Sex	1.79	0.1367
	Ecosystem	2.25	0.0691
L21	Sex	6.25	0.0141
	Ecosystem * Sex	0.71	0.5889
	Ecosystem	1.15	0.3371
L11	Sex	37.49	< 0.0001
	Ecosystem * Sex	0.97	0.4273
I O	Ecosystem	3.45	0.0112
L2r	Sex Fcosystem * Sev	9.03	0.0034
	LUSYSICIII SEA	0.55	0./15/
т 1	Ecosystem	1.42	0.2333
LIT	Sex Ecosystem * Sex	29.77	<0.0001 0.0725

Table 2. MANOVA results for morphometric characteristics of the studied populations of B. varium

Lb, length of body; Lc, length of head; Lp, length of prothorax; Le, length of elytra; Sc, width of head with eyes; Sp1, width of prothorax between front angles; Sp2, width of prothorax between back angles; Sp3, maximum width of prothorax; Se, maximum width of elytra; B, back angles of prothorax; L11, distance from the base of the left elytra to the first setae; L1r, distance from the base of the right elytra to the first setae; L21, distance between setae on the left elytra; L2r, distance between setae on the right elytra.

Characteristic	Factor	F	Р
	Ecosystem	4.41	0.0028
(Sc+Sp+Se)/(3Lb)	Sex	2.59	0.1095
	Ecosystem * Sex	0.92	0.4928
Lp/Sp	Ecosystem	0.71	0.5883
	Sex	0.14	0.7080
	Ecosystem * Sex	1.71	0.1546
Le/Lp	Ecosystem	1.19	0.3221
	Sex	0.14	0.7138
	Ecosystem * Sex	1.54	0.1956
Se/Sp	Ecosystem	0.24	0.9158
	Sex	4.37	0.0392
	Ecosystem * Sex	1.18	0.3252
	Ecosystem	2.21	0.0741
Sp2/Sp3	Sex	0.39	0.5362
	Ecosystem * Sex	2.93	0.0243
	Ecosystem	4.95	0.0011
Le/Se	Sex	3.63	0.0598
	Ecosystem * Sex	0.22	0.9293

Table 3. MANOVA results for morphometric indices of the studied populations of B. varium

(Sc+Sp+Se)/(3Lb) – ratio of arithmetic mean value of the width of head, prothorax and elytra to body length; Lp/Sp – ratio of prothorax length to its maximum width; Le/Lp – ratio of elytra length to prothorax length; Se/Sp – ratio of maximum width of elytra to maximum prothorax width; Sp2/Sp3 – ratio of prothorax width at the back edge to its maximum width; Le/Se – ratio of elytra length to their width.

gradient. The most constant morphometric index (Fig. 3) is the ratio of prothorax length to its maximum width (Lp/Sp).

After combination of all the measured individuals of *B. varium* into one sample and their distribution depending on body length (Fig. 4*a*-4*m*), we found that the characteristics most closely associated with body size of the beetles are Le ($r^2 = 0.854$), Se ($r^2 = 0.799$), Lp ($r^2 = 0.723$), Sc ($r^2 = 0.703$), Sp1 ($r^2 = 0.677$), Sp2 ($r^2 = 0.674$) and Sp3 ($r^2 = 0.699$). At the same time, none of the six considered morphometric indices (Fig. 4*n*-4*s*) correlates with an increase in body size: r^2 is in the range 0.002–0.038.

Discussion

Changes in environmental factors affect size of insects (DREYER and SHINGLETON, 2011). The nature of the substrate and the presence of vegetation largely determine the size and form of the body of ground beetles. This is especially true of ground beetles in hygrophilous biotopes, where habitat conditions are diverse and unstable. There is a relationship between body form and ecological characteristics of the habitats of species of the tribe Bembidiini (ANDERSEN, 1985). Body size data for *B. varium* is limited to total body length. It is known from sources in the literature that body length varies mainly within 3.0–6.5 mm (Table 4). In our research, the body

length of individuals varies in the range of 4.17–5.25 mm, averaging 4.75 mm.

The ability of insects to fly is a key mechanism in the settlement of new biotopes. It is increasingly recognized that physiological and behavioural mechanisms control the flying ability of beetles (IVERSEN et al., 2017). Various authors indicate that *B. varium* is a macropterous species (HURKA, 1996; MATALIN, 2003). Our study also confirms that fact.

The variability of half of the linear characteristics of B. varium (body length, head length and width, width of prothorax between front angles, its maximum width and elytra width) and two morphometric indices ((Sc+Sp+Se)/ (3Lb), Le/Se) are observed in the considered ecosystems. We suggest that soil salinization has the greatest impact on the variability of these parameters. However, the influence of other equally important environmental factors (food availability during the larval stage, rivalry, infectious disease and parasites, temperature and mechanical composition of soil and anthropogenic factors) is not excluded. The variability of these linear characteristics and morphometric indices in gradient of soil salinity was previously described by us for B. minimum (KOMLYK and BRYGADYRENKO, 2019b). Changes in the remaining body parameters are not significant, which makes them useless for biological indication of the habitat conditions of B. varium. The size of the back angles of the prothorax does



Fig. 1. Variability of morphometric characteristics of the head and prothorax of *B. varium* in the studied populations: for names of the characteristics see Table 2; the figure shows Box-Whisker plots with the whiskers indicating minimum and maximum, the box describing the range between first and third quartile, the square indicating the median and outliers shown by dots and crosses.



Fig. 2. Variability of the posterior angles of prothorax and morphometric characteristics of elytra of *B. varium* in the studied populations: for names of the characteristics see Table 2, Fig. 1.



Table 4. Sizes of Bembidion varium from various sources

Country	Size (mm)	Source
Armenia	3.0–4.5, usually 4.0–4.5	IABLOKOV-KHNZORYAN, 1976
Great Britain	4.1–5.1	Lindroth, 1974
Egypt	4.8–6.5	Abdel-Dayem, 1998
Tatarstan	3.8–5.0	ZHEREBTSOV, 2000
Fennoscandia, Denmark	4.1–5.1	Lindroth, 1985
Czech Republic, Slovakia	3.4–5.2 (4.5)	Hurka, 1996
Ukraine	4.17-5.25, averaging 4.75	This article

not change in the different ecosystems in *B. varium* unlike *B. minimum* (KOMLYK and BRYGADYRENKO, 2019b).

In most ground beetles, females are larger than males in most linear parameters (SUKHODOLSKAYA et al., 2016). In our studies females of *B. varium* are larger than males in all the considered linear parameters. Differences between males and females in size of the back angles of the prothorax are not significant. Similar data were obtained for other species of ground beetles of the genus *Bembidion: B. articulatum* (Panzer, 1796) (BRYGADYRENKO and SLYNKO, 2015), *B. aspericolle* (Germar, 1829) (KOMLYK and BRYGADYRENKO, 2019a), *B. minimum* (KOMLYK and BRYGADYRENKO, 2019b). Differences between sexes in *B. varium* are not significant for all morphometric indices except Se/Sp. Females of *B. varium* have wider and longer elytra as in most species of ground beetles (BRYGADYRENKO and RESHETNIAK, 2016). However, females of *B. articulatum* (BRYGADYRENKO and SLYNKO, 2015), *B. aspericolle* (KOMLYK and BRYGADYRENKO, 2019a), *B. minimum* (KOMLYK and BRYGADYRENKO, 2019b) are characterized by an isometric increase in body size, i.e. body proportions remain unchanged with a change in absolute size of beetles.

The increase in the salinity of the soil solution, as a determining environmental factor in the considered ecosystems does not cause changes in dimorphism between males and females for all linear measurements and morphometric indices; similarly no changes are recorded within the sex groups. The ratio of prothorax length to its maximum width is the most stable index for *B. varium* as for *B. minimum* (KOMLYK and BRYGADYRENKO, 2019b), i.e. differences in the form of



Fig. 4. Scatter diagram of morphometric characteristics and indices of males and females of *B. varium* depending on their body length: for names of characteristics and indices see Tables 2, 3.

prothorax between males and females are not significant. *Bembidion varium* is characterized by a close relationship between head width, prothorax length and width, elytra length and width, and total body length of the individuals.

Conclusions

Despite the existence of research on the morphological variability of ground beetles, it still remains unclear which of the environmental factors are determining and at what stages of ontogenesis their impact is more significant. The results presented in this research expand knowledge about the morphological variability of riparian ground beetles and support the hypothesis that certain environmental factors can influence some important morphometric characters of insects. This research is consistent with our previous research of B. minimum. The same linear characteristics and morphometric indices vary. Thus, we might conclude that the salinity is one of the environmental factors influencing the life history and population variability of the hygrophilous beetles. The results obtained suggest further study of this issue on the example of ground beetle species, which will help to further identify the causes and mechanisms of species stability in natural and anthropogenic transformed ecosystems.

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