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<u>Original Research Article</u> Body Size Variation in *Pterostichus montanus* Motch. (Coleoptera, Carabidae) in Altitudinal Gradient

Abstract. We studied morphometric variation in Ground Beetles populations inhabiting plots at different altitudes of the Barguzin mountain transect (Russia, Buryatia). Sample size was 1200 specimen, six measurements were analyzed. Beetles size did not differ in altitude gradient. Multivariate analysis showed that population structure was not the same at different altitudes with variation in factors loading on the studied traits. PCA extracted two factors that affected body size variation in species studied in altitudinal gradient: the first – climatic conditions of the shore and high altitudes (false subbald and high mountains belts, correspondingly), the second – altitude gradient.

Keywords: altitude gradient, discriminant analysis, Ground Beetles, morphometric variation, PCA.

1. INTRODUCTION.

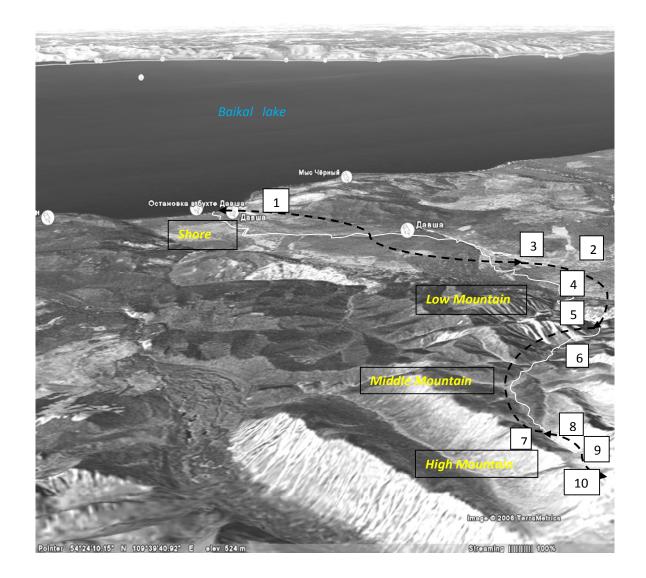
Many insect species are broadly distributed along elevation gradients. The populations living at the upper and lower elevation extremes experience different environmental conditions that affect them. As the rule researchers study such changes on the community level not paying attention to the intrapopulation variation of morphometric traits. Body size is a key trait involved in adaptation because it affects the physiological and life history traits of an organism. Geographical variation in body size is widely observed, the most common pattern being for increasing body size with latitude, which is called Bergmann's rule [1]. This pattern is observed in many endotherms and in some ectotherms such as insects [2, 3, 4]. Because the body surface-volume ratio generally decreases with increasing body size, body size plays an important role in the thermoregulation of endotherms, and in starvation resistance and desiccation resistance in ectotherms. In contrast, clinal body size variation in arthropods often follows the converse of Bergmann's rule [5, 6]. In univoltine insects, which can only overwinter at a particular developmental stage, their developmental time is restricted by habitat temperature. The decrease in body size in cooler habitats can be explained by selection for a shorter developmental time, which results in smaller body size. Therefore, the converse of Bergmann's rule is considered a result of climatic adaptation in univoltine arthropods [5, 7]. Such adaptations can predict communities alterations when climate changes. Traditional morphometrics was characterized by the application of the multivariate statistical methods to sets of variables such as length, width, and height. With these approaches, covariation in the morphological measurements could be quantified, and patterns of variation within and among samples could be assessed. Statistical analyses typically included principal components analysis (PCA), factor analysis, canonical variates analysis (CVA), and discriminant function analysis. But these methods are rarely used in body size variation researches in altitudinal gradient.

40 The aim of this study was to reveal altitudinal body size and population structure variation in
 41 carabid species *Pterostichus montanus*.
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2. MATERIAL AND METHODS

44 **2.1. Study Sites**

We conducted our study in north-east region of Baikal Lake (N 54° 20'; E 109° 30') (Barguzin Reserve
National Park "Zapovednoe Podlemorie", Republic of Buryatia, Russian Federation) (Fig. 1). Beetles were
sampled in 30 -km high-altitude transect.



- Altitude belts: Shore (450 m up level sea)
- 1. BILBERRY CEDAR
- ◆ Low Mountain (460 m 635 m)
- ◆ 2. ASPEN-ABIES
 - ♦ 3. BLUEBERRY LARCH
 - ♦ 4. RED BILBERRY PINE
- 5. BERGENIA CEDAR
 - Middle Mountain (720 m– 1000 m)
 - 6. BERGENIA ASPEN
 - ♦ 7. BILBERRY ABIES
 - ◆ High mountain (1300 m− 1700 m)

Fig. 1 Region, where study in *P. montanus* took place.

It was situated in Davsha river valley and stretched from the coast of Baikal Lake to the bald Barguzin zone. The transect was divided into three plots designated as low (5-17 km from Baikal Lake shore, 500-700 m altitude), medium (20-25 km, 701-1300 m) and high part of the range (25-30 km, 1301 -1700 m). Besides beetles were sampled at the plot, adjacent to the lake. It was so-called pseudo-subbald belt (coastal stretch of Baikal Lake). Some features of north seaside regions are specific to it. Slow seasons change and phenological

69 inversions occur here, bald belt flora grow (e. g. creeping cedar) [8, 9]. Among glacial relicts are Nebria frigida, 70 Nebria nivalis, Elaphrus lapponicus, Curtanotus alpinus, Harpalus nigritarsis. Bottom part of the mountain 71 forest zone (Low Mountain) included biotopes with Blueberry larch, Red bilberry pine. Middle mountains 72 biotopes included Bergenia cedar, Bergenia aspen and Bilberry abies, High mountains - Birch park, Blueberry 73 tundra, Lichen tundra. We had conducted the previous research in this region when the majority of 74 environmental factors had been investigated with thermographs, precipitation cylinderes, the soil thermometers: 75 snow depths had been measured in altitude belts before melting (in March) [10]]. We had concluded that 76 environment surroundings were less optimal for Ground Beetles at high altitude.

2.2. Study Organism

Pterostichus (Petrophilus) montanus (Motschulsky, 1844) is distributed in Mongolia and Russia
 (Amur; Buryat Republic; Chita Area; East Sayan; Irkutsk Area; Khamar-Daban; Krasnoyarsk; N Ural mts.;
 Tuva; Yakutiya). It is univoltine and larvae develope from spring to summer. In studied area it occurs
 throughout a wide range of elevation with the highest number in low mountains. Species abundance correlated
 positively with the mean winter temperatures, hydrothermal coefficient in September. Negative correlation
 occurred between species abundance and minimal soil temperatures and the sum of summer atmospheric
 condensation [10].

2.3. Study design

Our study took place in 1988 – 2010. Ten pitfall traps were set per site in a transect with traps separated
from each other by a minimum of 15 m to ensure independence of samples. Each trap was 10 cm diameter, 15
cm in depth and contained approximately 3 cm of silicate-free ethylene glycol as a killing and preserving agent.
Traps were open between 8 June and 24 August. Trap contents were collected every 2 weeks and stored in 70%
ethanol until processed.

91 **Morphometric analysis** was made with a Leitz RS stereoscopic dissecting microscope at a 92 magnification of 10 diameters, using a calibrated ocular grid with a scale interval of 0.1 mm. For each of 93 specimens six variables were measured, including: elytra length and width, pronotum length and width, head 94 length and distance between eyes. In total 1200 specimens from 8 local populations were measured. All 95 measurements were log-transformed for analysis.

96 Statistical analysis was made in Software Statistica 6.0. We applied discriminant, correspondence and 97 principal component analyses to identify the patterns of morphological variation within the populations at 98 different elevation based on data of the similarity matrix and to reveal the role of different traits in beetles 99 adaptation to different altitudes.

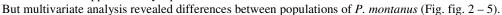
101 **3. RESULTS**

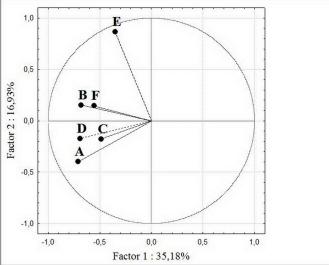
102Body size did not differ in the populations at different altitudes. Means of the six studied traits and their103standard deviations were approximately equal.

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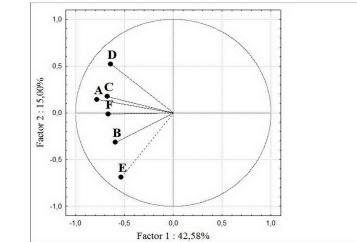


106Factor 1 : 35,18%107Fig.2. Principal component analysis of the morphometric traits in the coastal populations of *P. montanus*108(A - elytra length, B - elytra width, C - pronotum length, D - pronotum width, E - head length, F - distance109between eyes).

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113 114 115 Fig. 3. Principal component analysis of the morphometric traits in the low mountains populations of P. montanus (A – elytra length, B – elytra width, C – pronotum length, D – pronotum width, E – head length, F – distance between eyes).

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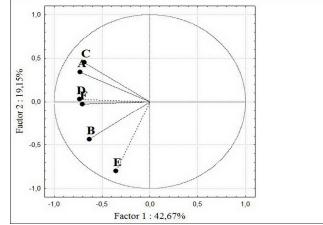


Fig. 4. Principal component analysis of the morphometric traits in the middle mountains populations of P. montanus (A - elytra length, B - elytra width, C - pronotum length, D - pronotum width, E - head length, F - distance between eyes).

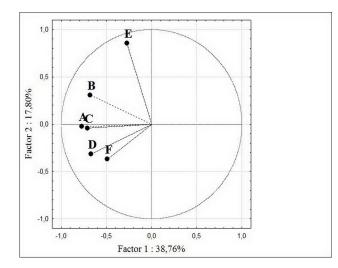
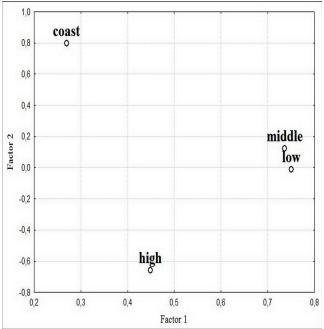


Fig. 5. Principal component analysis of the morphometric traits in the high mountains populations of *P*.
 montanus (A – elytra length, B – elytra width, C – pronotum length, D – pronotum width, E – head length, F – distance between eyes).

129 In the coastal and high mountains populations factor loadings on the elytra length, pronotum and head 130 width were very similar. So we concluded that morphometric structure of those populations was affected by the 131 same factor. In low- and middle mountains populations factor loadings on the all traits were similar (with the 132 exception of pronotum width). So we concluded that environmental factors, which influenced population 133 structure at those territories, were similar.

Elytra length is thought to be the main trait that control body size. So we took data sets concerning the only elytra length variation in studied species and conducted correspondence analysis (Fig. 6).



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Fig. 6. CCA results in elytra length variation in the populations of *P. montanus* at different altitudes

140Elytra length variation was very similar in the populations of low- and middle mountains141corresponding to the first and to the second factor as well. According to the second factor clear altitudinal142gradient in elytra length variation was revealed.

143 Discriminant analysis revealed confident differences in morphometric structure between populations at 144 different altitudes (Fig. 7). Though Wilk's λ was high and cohesiveness of values was low, significant values of 145 squared Mahalanobis distances proved structural differences in *P. montanus* populations in altitude gradient 146 (Table 1).

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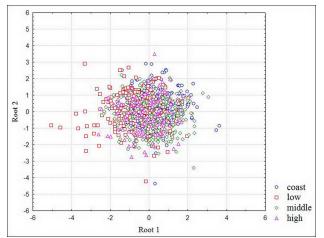




Fig. 7. Results of discriminant analysis traits in the populations of *P. montanus*



Table 1. Squared Mahalanobis distances between centroids of traits distribution in the populations of *P*. *montanus* (Wilks' Lambda: .8281887 approx. F (18.2670) = 10.22530 p < 0.0000)

monianus (wilks	Lambua: ,62	oloo/ applox.	F(18,2070) = 10	,22330 p < 0,0000
	С	Low	Middle	High
	oast	Mountains	Mountains	Mountains
Coast	0, 000000 8	0,85321	0,363623	0,46426 1
Low Mountains	0, 853218 0	0,00000	0,812780	0,32656 8
Middle Mountains	0, 363623 0	0,81278	0,000000	0,17177 2
High Mountains	0, 464261 8	0,32656	0,171772	0,00000 0

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4. DISCUSSION

Field experiments showed that insects body size could increase in altitude gradient [11, 12] and decrease as well [13, 14]. One of the dominant species in Barguzin mountain decreased in body size in high altitudes [15], studied species – *P. montanus* – did not show any alterations in size in altitude gradient. In both species factor loadings at all altitudes fell to the elytra and pronotum traits, but in *P. montanus* those loadings were smaller. On our opinion it was due to the genus peculiarities. According PCA2 in *P. montanus* the main loading always fell to the "head length" trait, but in *C. odoratus* in high mountains – to the "pronotum width".

161 Discriminant analysis results differed also. In C. odoratus population morphometric structure have been 162 changing gradually: squared Machalanobis distance between "low mountains" and "middle mountains" was 163 less compared with "low mountains" and "high mountains". In P. montanus four populations were under study. 164 Besides various altitude plots, coastal one was researched. Structures of coastal and high mountains populations 165 were most similar. We explained such the pattern by Barguzin mountain location. The deep and cold reservoir – 166 Baikal Lake – is situated at its foot. Unique false subbald belt (coastal belt of Baikal Lake) is situated there with 167 the climate similar to the north seas shore regions: low season changes, phonological inversions, glacial 168 vegetation. In such severe climate of North Baikal region temperature conditions are of great importance. 169 Previous studies showed that temperature affected Ground Beetles abundance there indirectly - through the non 170 frosty season duration, which influenced reproductive activity, and through Selyaninov hydrothermal coefficient 171 [9].

Our study did not aim to realize the genetic determination of difference in population structure of *P*. *montanus* at various altitudes. We are only oriented to the studies of *Carabus tosanus* body size variation at different altitudes [16]. The authors sampled beetles at various altitudes and reared them in laboratory. Variation in body size due to temperature effects (phenotypic plasticity) was small compared to the interpopulation differences, which suggests substantial genetic differences between populations (subspecies) at different altitudes.

178 Such genetic differences in population structure can be the result of adaptation to different temperatures and 179 are important for the process of incipient speciation because body size differences can contribute to premating 183

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180 reproductive isolation. This view coincides with the results of other researches in the field of phenotypic 181 variation in insects in geographic and ecological gradients [17, 18, 19, 20]. 182

5. CONCLUSION

184 Studied species of carabid did not change in size in elevation gradient, its but populations structure did. 185 We consider such consistent pattern to be adaptation to the environmental fluctuations from low- to high 186 mountains.

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