



## Elevation Changes of Morphometric Traits Structure in *Pterostichus montanus* Motch. (Coleoptera, Carabidae)

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### Authors' contributions

*This work was carried out in collaboration between both authors. Author TA designed the study, wrote the protocol, managed the analysis of study. Author RS performed the statistical analysis, wrote the first draft of the manuscript, managed the literature searchers. Both authors read and approved the final manuscript.*

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### 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 (coast) and high altitudes (false subbald and high mountains belts, correspondingly), the second – altitude gradient.

**Keywords:** Altitude gradient; discriminant analysis; ground beetles; morphometric variation; PCA.

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## 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 thermoregulation of endotherms, 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. Data on body size variation in altitudinal gradient in insects is contradictory: some of them increased in size in high altitudes [8-12]. Sometimes the only one trait of organism changed, but another remained the same in altitudinal gradient [13]. Our previous research in that region showed that another carabid species –*Carabus odoratus*–monotonically decreased in size with increasing altitude [14].

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.

The aim of this study was to reveal altitudinal body size and population structure variation in carabid species *Pterostichus montanus* Motch.

## 2. MATERIALS AND METHODS

### 2.1 Study Sites

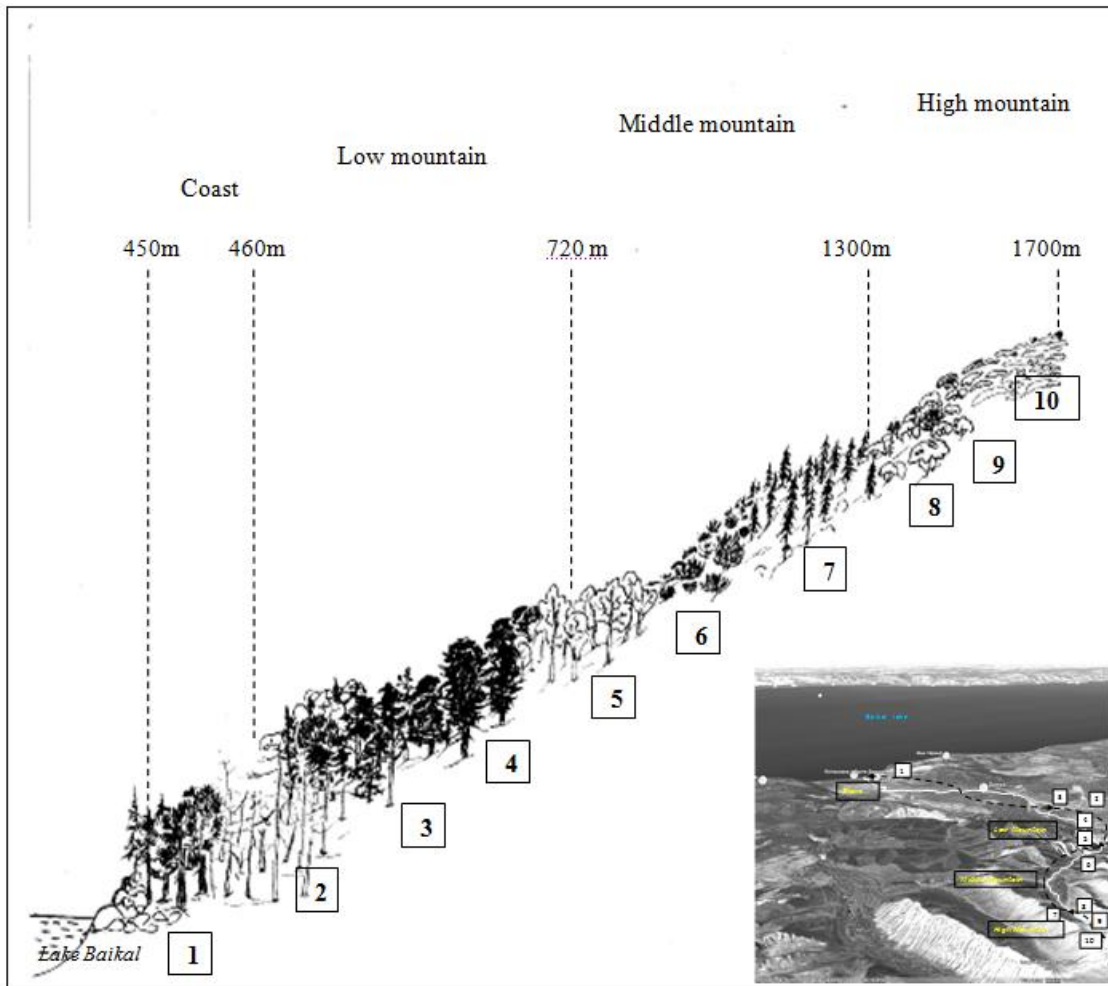
We conducted our study in north-east region of Baikal Lake (N 54° 20'; E 109° 30') (Barguzin State Nature Biosphere Reserve, Republic of Buryatia, Russian Federation). Beetles were sampled in 30 -km transect of Barguzin mountain range in Davsha river valley (Fig. 1).

The altitudinal transect was stretched from the coast of Baikal Lake to the bald Barguzin zone. It was divided into four belts designated as Coast (0-5 km distance adjacent to the Baikal Lake); Low mountain (6-17 km from Baikal shore), Middle mountain (20-25 km) and High mountain (25-30 km). Ten plots were selected along elevation gradient: Coast (458 m alt., 150 m from the edge of lake); Low mountain – (518 m, 535 m, 635 m), Middle mountain – (721 m, 1004 m, 1279 m); High mountain – (1407 m, 1637 m, 1700 m).

Some features of North Baikal region are specific to it. The climate on the coastal stretch of Lake Baikal is similar to highlands. It is the influence of mountain landscape and huge volume of cooling lake masses. The temperature inversions occur in the shore belt. It is so-called pseudo-subbald belt [15]. There are "the lowered alpine species" meet, e. g. ground beetles – *Pterostichus (Cryobius) brevicornis* (Kirby, 1837), *Bembidion (Plataphodes) crenulatum* (Sahlberg, 1844), *P. montanus*. Bald belt flora grows, e. g. – *Diphysastrum alpinum* (L.) Holub, *Betula nana rotundifolia* (Spach Malyshev., *Alectoria ochroleuca* (Ehrh.) Nyl. [16]. Bilberry cedar is the reference biotope of a northern part of the Lake Baikal coast.

Low mountain belt (bottom part of the mountain forest zone) is presented by mixed light-dark coniferous forest – *Larix chekanowski* Szaf., *Pinus silvestris* L., *Pinus Sibirica* Du Tour.

Middle mountain belt (upper part of the mountain forest zone) is presented by dark coniferous forest – *Abies sibirica* (Ledeb.), *Picea abovata* (Ledeb.), *Pinus sibirica* (Du Tour), *Pinus pumila* (Pall.).



Altitude belts:

**Coast**

1. Bilberry cedar - *Vaccinium myrtillus* L., *Pinus sibirica* Du Tour. (forest).

**Low mountain**

2. Blueberry larch - *Vaccinium uliginosum* L., *Larix czekanowskii* Szaf. (forest).

3. Red bilberry pine - *Vaccinium vitis-idaea* L., *Pinus sylvestris* L. (forest).

4. Bergenia cedar - *Bergenia crassifolia* (L.) Fritsch., *Pinus sibirica* Du Tour. (forest).

**Middle mountain**

5. Bergenia aspen - *Bergenia crassifolia* (L.) Fritsch., *Populus tremula* L. (wood).

6. Cedar creeping - *Pinus pumila* (Pall.) Regel. (forest).

7. Bilberry abies - *Vaccinium myrtillus* L., *Abies sibirica* Ledeb. (forest).

**High mountain**

8. Birch park - *Betula baicalensis* Sukacz. (woodlands).

9. Bilberry tundra - *Vaccinium myrtillus* L. (tundra).

10. Lichen tundra - *Cetraria islandica* (L.) (Ach. Tundra).

**Fig. 1. Western macroslope of Barguzin Range, where study *P. montanus* took place**

High mountain belt (the territory is higher than forest border) is presented by *Pinus pumila* (Pall) Regel, *Betula nana* L. subsp. *exilis* (Sukacz.) Hulten, *Rhododendron aureum* Georgi.

Some species of ground beetles live separately from their main areas. Among "glacial relicts" of High Mountain belt are *Nebria frigida* (Sahlb., 1844), *Nebria nivalis* (Payk., 1798), *Curtonotus*

*alpines* (Payk, 1790), *Harpalus nigritarsis* (Sahlb., 1827).

We had conducted the previous research in this region when the majority of environmental factors had been investigated with thermographs, precipitation cylinders, the soil thermometers. Snow depths had been measured in altitude belts before melting (in March) [17]. We had concluded that environment surroundings were less optimal for ground beetles at high altitude.

## 2.2 Study Organism

*Pterostichus montanus* is distributed in mountainous areas of Mongolia and Russia (Amur; Buryat Republic; Chita Area; Irkutsk Area; Krasnoyarsk Area; Ural; Tuva; Yakutiya) [18]. In studied area it occurs throughout a wide range of elevation. The largest number *Pt. montanus* is recorded in the Low mountain belt. It's abundance correlated positively with the mean winter temperatures, hydrothermal coefficient in September. Negative correlation noted between 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 in each plot separated from each other by a minimum of 10 m to ensure independence of samples. The glass jars (without lids) were leveled with the surface of the soil. Each trap was 10 cm diameter, 15 cm in depth and contained approximately 3 cm of 4% 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.

## 2.4 Morphometric Analysis

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

## 2.5 Statistical Analysis

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

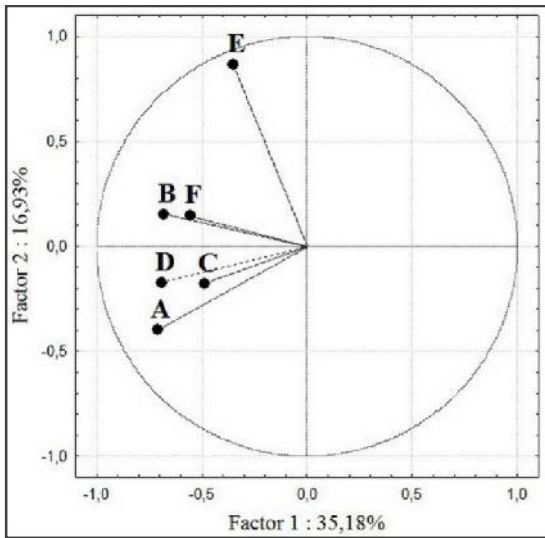
## 3. RESULTS

Body size did not differ in the populations at different altitudes. Means of the six studied traits and their standard deviations were approximately equal (Table 1).

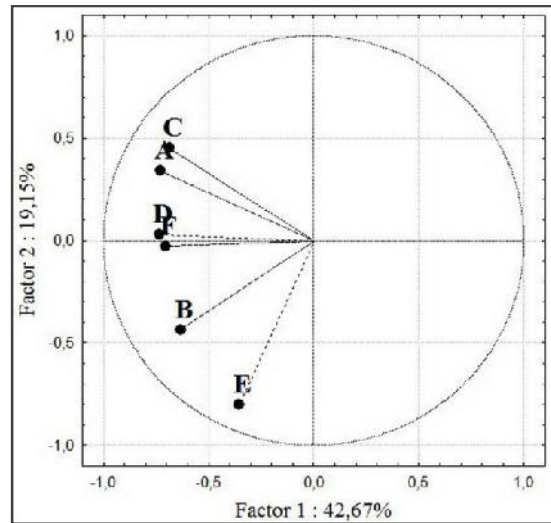
Multivariate analysis revealed differences between populations of *P. montanus* (Figs. 2-5).

**Table 1. Descriptive statistics of morphometric traits in studied populations**

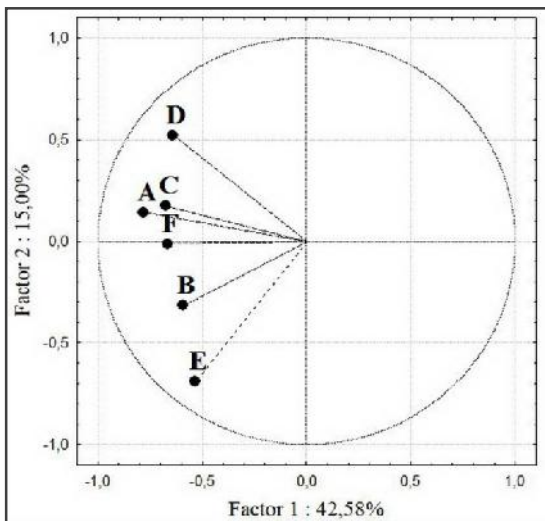
| Sites            | Statistics/Traits | A     | B     | C     | D     | E     | F     |
|------------------|-------------------|-------|-------|-------|-------|-------|-------|
| Coast            | Mean (mm)         | 66,43 | 32,74 | 26,85 | 28,83 | 18,27 | 18,76 |
|                  | std. deviation    | 4,28  | 2,82  | 1,81  | 1,92  | 1,79  | 1,56  |
|                  | std.error         | 0,26  | 0,17  | 0,11  | 0,12  | 0,11  | 0,10  |
| Low mountains    | Mean (mm)         | 68,38 | 34,30 | 28,04 | 29,33 | 19,51 | 19,10 |
|                  | std. deviation    | 4,47  | 3,14  | 2,28  | 2,27  | 1,81  | 1,59  |
|                  | std.error         | 0,28  | 0,20  | 0,14  | 0,14  | 0,11  | 0,10  |
| Middle mountains | Mean (mm)         | 67,19 | 33,35 | 26,56 | 28,25 | 18,65 | 18,42 |
|                  | std. deviation    | 4,10  | 3,03  | 2,03  | 2,15  | 1,72  | 1,39  |
|                  | std.error         | 0,24  | 0,18  | 0,12  | 0,13  | 0,10  | 0,08  |
| High mountains   | Mean (mm)         | 67,79 | 34,23 | 27,13 | 28,85 | 18,99 | 18,66 |
|                  | std. deviation    | 4,14  | 3,37  | 2,02  | 2,42  | 1,33  | 1,51  |
|                  | std.error         | 0,33  | 0,27  | 0,16  | 0,19  | 0,11  | 0,12  |



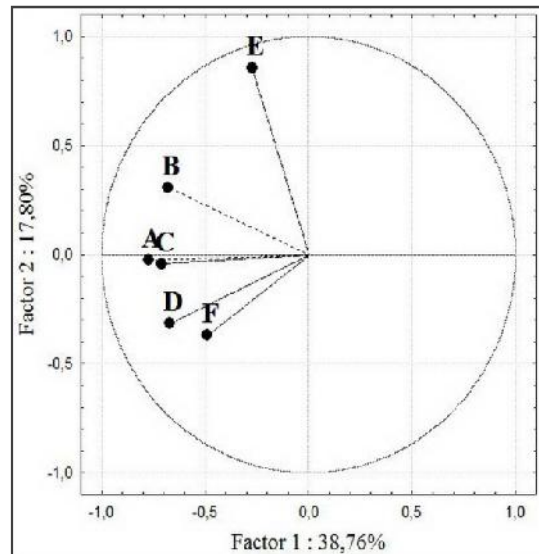
**Fig. 2.** Principal component analysis of the morphometric traits in the coastal populations of *P. montanus* (A – elytra length, B – elytra width, C – pronotum length, D – pronotum width, E – head length, F – distance between eyes)



**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. 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)



**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)

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

Elytra length variation was very similar in the populations of low- and middle mountains corresponding to the first and to the second factor as well. According to the second factor

clear altitudinal gradient, which reflected climate conditions at those plots [15], in elytra length variation was revealed.

Discriminant analysis revealed confident differences in morphometric structure between

populations at different altitudes (Fig. 7). Though Wilk's  $\lambda$  was high and cohesiveness of values was low, significant values of squared Mahalanobis distances proved structural differences in *P. montanus* populations in altitude gradient (Table 2).

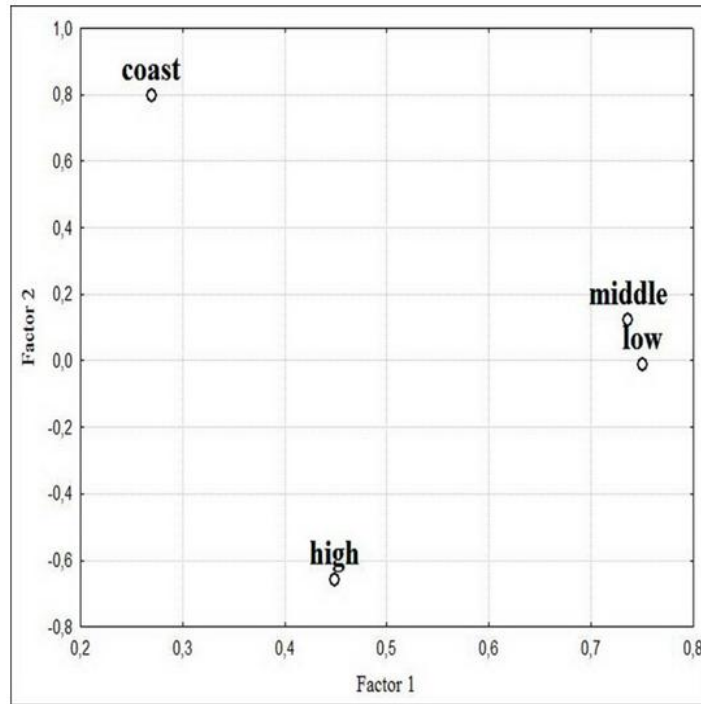


Fig. 6. CCA results in elytra length variation in the populations of *P. montanus* at different altitudes

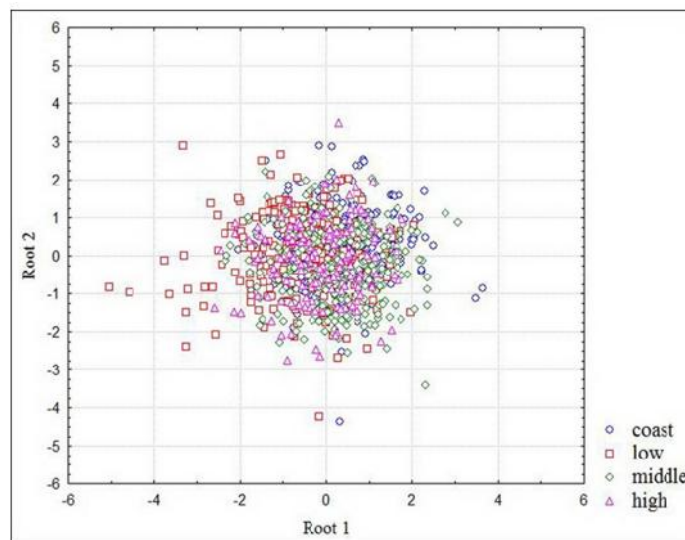


Fig. 7. Results of discriminant analysis traits in the populations of *P. montanus*

**Table 2. 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)**

|                  | Coast | Low mountains | Middle mountains | High mountains |
|------------------|-------|---------------|------------------|----------------|
| Coast            | 0,00  | 0,85          | 0,36             | 0,46           |
| Low mountains    | 0,85  | 0,00          | 0,81             | 0,32           |
| Middle mountains | 0,36  | 0,81          | 0,00             | 0,17           |
| High mountains   | 0,46  | 0,32          | 0,17             | 0,00           |

#### 4. DISCUSSION

Field experiments showed that insects body size could increase in altitude gradient [19,20] and decrease as well [8,9]. We had studied another dominant species - *C. odoratus* - in Barguzin mountain earlier and it had decreased in body size in high altitudes [14]. We have compared those results with studied species – *P. montanus*, which 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* Shil. in high mountains – to the “pronotum width”.

Discriminant analysis results differed also. In *C. odoratus* population morphometric structure have been changing gradually: squared Mahalanobis distance between “low mountains” and “middle mountains” was less compared with “low mountains” and “high mountains”. In *P. montanus* besides various altitude plots, coastal one was researched. Structures of coastal and high mountains populations were most similar. We explained such the pattern by Barguzin mountain location. The deep and cold reservoir – Baikal Lake – is situated at its foot. Unique false subbald belt (coastal belt of Baikal Lake) is situated there with the climate similar to the north seas shore regions: low season changes, phonological inversions, glacial vegetation. In such severe climate of North Baikal region temperature conditions are of great importance. Previous studies showed that temperature affected ground beetles abundance there indirectly – through the non frosty season duration, which influenced reproductive activity, and through Selyaninov hydrothermal coefficient [16].

Our study did not aim to realize the genetic determination of difference in size structure of

the populations in *P. montanus* at various altitudes. We are only oriented to the studies of *Carabus tosanus* body size variation at different altitudes [19]. 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.

Such genetic differences in population structure can be the result of adaptation to different temperatures and are important for the process of incipient speciation because body size differences can contribute to precluding reproductive isolation. This view coincides with the results of other researches in the field of phenotypic variation in insects in geographic and ecological gradients [20-23].

#### 5. CONCLUSION

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

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

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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