



Components of morphological variation in Baikalian endemic cyclopoid *Acanthocyclops signifer* complex from different localities

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Abstract

Use of traditional methods for morphological studies only permits the analysis of a small part of the information embodied in morphological structures. Besides comparing populations using the mean values of characters which allows one to estimate their morphological similarity, analysis of variation among individuals within a population can be informative. Variation among individuals consists of factorial and stochastic components. The factorial component is an upper estimate of genetic heterogeneity and thus permits one to evaluate the population's adaptability. The stochastic component (estimated by fluctuating asymmetry, i.e. random deviations from perfect bilateral symmetry), being a measure of developmental stability, is an indicator of a population's fitness. Assessment of measurement error is necessary for assessment of the true value of the stochastic component and for selection of the most informative characters. Such analysis allows one to extract additional information from morphological data in comparison with methods traditionally used on copepods. This approach was applied to an analysis of morphological variation in the study of the Baikalian endemic cyclopoid *Acanthocyclops signifer* (Mazepova) from three different isolated localities. Characters typically used in studies of taxonomy of this group are considered here. Measurement error was rather high (more than 50% of the stochastic component), which can be explained by technical difficulties of measuring the characters. All populations differ in the mean values of the characters. This shows the taxonomic heterogeneity of this group and reveals the necessity of its taxonomic revision. Populations also differ in the level of stochastic and factorial components of the total variance. The data are interpreted from the point of view of taxonomy and the possible evolution of the group.

Introduction

Much attention traditionally has been paid to morphological variation in studies of taxonomy and evolution of the Copepoda. Copepods have many morphological structures with complex shapes and these are rather convenient for different analyses. However, the morphological methods only permit analysis of a small part of the information embodied in morphological structures. A significant part of the information is not available for analysis. The goal of the present study is to apply some new approaches that permit extraction of additional information from morphological data tra-

ditionally used for the study of a Baikalian endemic copepod, *Acanthocyclops signifer* Mazepova, 1978.

A. signifer is a common species inhabiting both the near-shore and deep water zones of Lake Baikal. Probably, it is a meiobenthic species that inhabits biotopes with various sediments (Mazepova, 1978). *A. signifer* exhibits high morphological diversity, which is typical also for many other Baikalian endemic invertebrates: other Cyclopoida (Mazepova, 1978), Amphipoda (Bazikalova, 1945), Ostracoda (Mazepova, 1989), Nematoda, Turbellaria (Tsalolikhin, pers. comm.). Special attention was paid to explaining the high level of morphological variability within these groups. Possible reasons for the geographical and biotopical isolation that are typical of Baikal fauna were suggested by Mazepova (1978). But until now, no experimental

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verification of this point of view has been done. In particular, it is unclear whether the high diversity is due to within – or among – population heterogeneity.

The term ‘morphological variation’ commonly refers to the differences in body shape between groups of organisms. In the Copepoda, such kind of analysis is usually provided by various morphological indices. Developing a set of informative indices is time-consuming and thus good indices exist primarily for well-studied groups. Differences in the body shape between species traditionally are used in taxonomic arguments. It is also possible to discuss variation in the values of the characters among individuals within a population. This variation reflects the genetic differences among individuals and the diversity of the environment. Genotypic variation reflects the ability of a population to adapt to the environmental changes and, thus, is a measure of a population’s potential to adapt. While individuals exist under somewhat different environmental conditions during their development, environmental diversity results in additional variation among individuals, which can be called modification.

There is, also, within-individual variation which is manifested in the differences between genetically identical structures that have developed under the same environmental conditions. This was first studied by Astauroff (1930) and was called a ‘stochastic variation’. Astauroff wrote: “Any process of formation in an organism has some self-dependent variation, which can be reduced neither to genotypic differences, nor to the direct effect of environment. This stochastic variation is inherited in intrinsic conditions of the character formation and in small spontaneous alterations coming both from outside, from fluctuations of environment, and inside, from organic structure itself.” (1974, 103 pp., translation from Russian by D. L. and V. A.). At present, it is usually proposed that this kind of variation results from developmental instability, which reflects the ability of an organism to develop the same phenotype under the same environmental conditions. Developmental stability can be considered as a component of fitness; it decreases under either environmental or genetic stress and, thus, can be used as an effective estimate of stress (Zakharov, 1989; Graham et al., 1993; Møller & Swaddle, 1997). Developmental stability is commonly measured as fluctuating asymmetry, i.e. variance in random deviations from perfect bilateral asymmetry. Because it is an informative and sensitive indicator of stress, fluctuating asymmetry has become a rather popular parameter to measure.

Besides fluctuating asymmetry, there are also two

other kinds of asymmetry – directional asymmetry and antisymmetry. In directional asymmetry, both the presence of asymmetry and its manifestation (on the left or right sides of the body) are inherited. When only the presence of asymmetry, but not its direction is inherited, it is antisymmetry. In fluctuating asymmetry, neither the presence of asymmetry nor its direction are under genomic control (Palmer & Strobeck, 1986).

When one discusses variation of non-symmetric structure, two kinds of variation, heterogeneity among individuals and intra-individual variation, impact the total morphological variation and it is impossible to analyze them separately. There are different ways of partitioning the total phenotypic variance into genotypic, modification and stochastic components. Most require special experimentation and are very difficult to apply to populations of outbreeding species in natural conditions. While differences between bilaterally symmetrical structures are due to stochastic variation, it is possible to divide the total variance of bilateral characters into stochastic and factorial components using a special index of fluctuating asymmetry. The factorial component includes genotypic variation, environmental effects and their interaction; hence, it can be considered the maximum estimate of genetic heterogeneity (Kozhara, 1994; Lajus, 1996).

The types of variation listed above are the attributes of biological objects themselves. Any one measurement always leads to some measurement error (ME). Low ME in component characters (i.e. size of left and right sides) does not ensure low ME in the derived trait (i.e. value of fluctuating asymmetry) (Møller & Swaddle, 1997). Swaddle et al. (1994) illustrate this point on birds showing that measurements of left and right bones can be significantly repeatable, while the value of asymmetry between them is not repeatable. If ME is non-systematic, it does not affect the magnitude of the mean value, but only influences its accuracy and hence the significance of the results of comparing populations using the mean values. However, when one is analyzing variation itself, ME leads to an increase of the variation. Being stochastic by its nature, ME impacts only the stochastic variation and has no effect on the factorial variation.

Thus, the observed estimate of stochastic variation is a sum of true stochastic variation and ME. Sources of ME may be some of the following: instrumental error, alterations of position of specimen during measurements (for morphometric characters) and counting (for meristic characters), mistakes of records, alterations in accuracy of operator during measurements,

differences between operators. To assess ME, it is necessary to make replicate measurements of the same specimen and to calculate its contribution to the total variance (Møller & Swaddle, 1997). Subtraction of ME from observed stochastic variation allows one to find the true stochastic variation. Palmer & Strobeck (1986) showed that the contribution of ME to fluctuating asymmetry, according to the published data, is sometimes surprisingly high, and ranges from 10% to 76%. Recently, several papers, especially devoted to the analysis of ME in the studies of the fluctuating asymmetry and suggesting great impact of ME on the fluctuating asymmetry were published (Merilä & Bjorklund, 1995; Hubert & Alexander, 1995). Møller (1997) considers ME to be one of the most important criteria of the studies on fluctuating asymmetry to be included in meta-analysis. The studies of copepod morphology necessitate special attention to ME because of the small size of animals and difficulties of measurement.

A multilevel approach for analysis of morphological variation is carried out here. Data on morphological variation on different levels (population, individual, within-individual) supplement each other well and can be informative for understanding the diversity of the group and its adaptive potential. Comparing populations using the mean values of characters allows one to estimate their morphological similarity. Analysis of variation among individuals within a population, providing an upper estimate of genetic heterogeneity (Kozhara, 1994), permits one to judge the population's adaptability. Within-individual variation, being a measure of developmental stability (and a component of biological fitness), is thought to be an indicator of a population's state of adaptation (Jones, 1987). Analysis of measurement error is necessary for assessment of the true value of the stochastic component and for choosing of the most informative characters.

Materials and methods

Specimens were collected from 26 to 31 July 1994 in Lake Baikal during the expedition of the research vessel 'Academik Obruchev'. Samples of bottom sediments were obtained with a benthic net from near shore localities (depths 12–50 m). Live animals were collected from the sediments immediately after sampling. Adult females were preserved in 70% ethanol or frozen. Three localities where *A. signifer* is

abundant were chosen: 1. the biological station of the Limnological Institute Bolshie Koty, 2. Peschannaya Bay and 3. the Ushkanii Islands (Figure 1).

In the laboratory, each specimen was dissected in glycerine. At least three specimens of the species from each locality were drawn in detail with a camera lucida. The number of specimens in the samples was 16, 19 and 21, respectively. Characters were measured on left and right sides of the body with a graduated eye-piece. All the measurements were made by one operator (V. A.). For the analysis we have chosen characters which often are used in taxonomic studies on Cyclopidae species (Monchenko, 1974). Most characters were taken from the abdominal segments since the shape of the cephalothorax often is compressed by a cover glass. The list of utilized characters is as follows:

1. Length of the furcal branch from the proximal origin of the branch to the point of insertion of the outermost terminal seta.
2. Width of the furcal branch of the lateral seta insertion.
3. Length of the furcal branch from the beginning of the branch to the place of insertion of the lateral seta.
4. Length of the outer seta.
5. Length of the inner seta.
6. Length of the outer medial seta.
7. Length of the inner medial seta.
8. Length of the dorsal seta.
9. Length of the lateral seta.
10. Length of the distal segment of the endopodite of the 4th pair of the swimming leg.
11. Width of the distal segment of the endopodite of the 4th pair of the swimming legs.
12. Length of the distal spine of the endopodite of the 4th pair of swimming leg.
13. Length of the inner distal spine of the endopodite of the 4th pair of swimming leg.
14. Length of the outer seta of the distal segment of the endopodite of the 4th pair of swimming legs.
15. Length of the 5th swimming leg.
16. Width of the 5th swimming leg.
17. Length of the distal spine of the 5th swimming leg.
18. Length of the distal seta of the 5th swimming leg.
19. Length of the proximal seta of the 5th swimming leg.

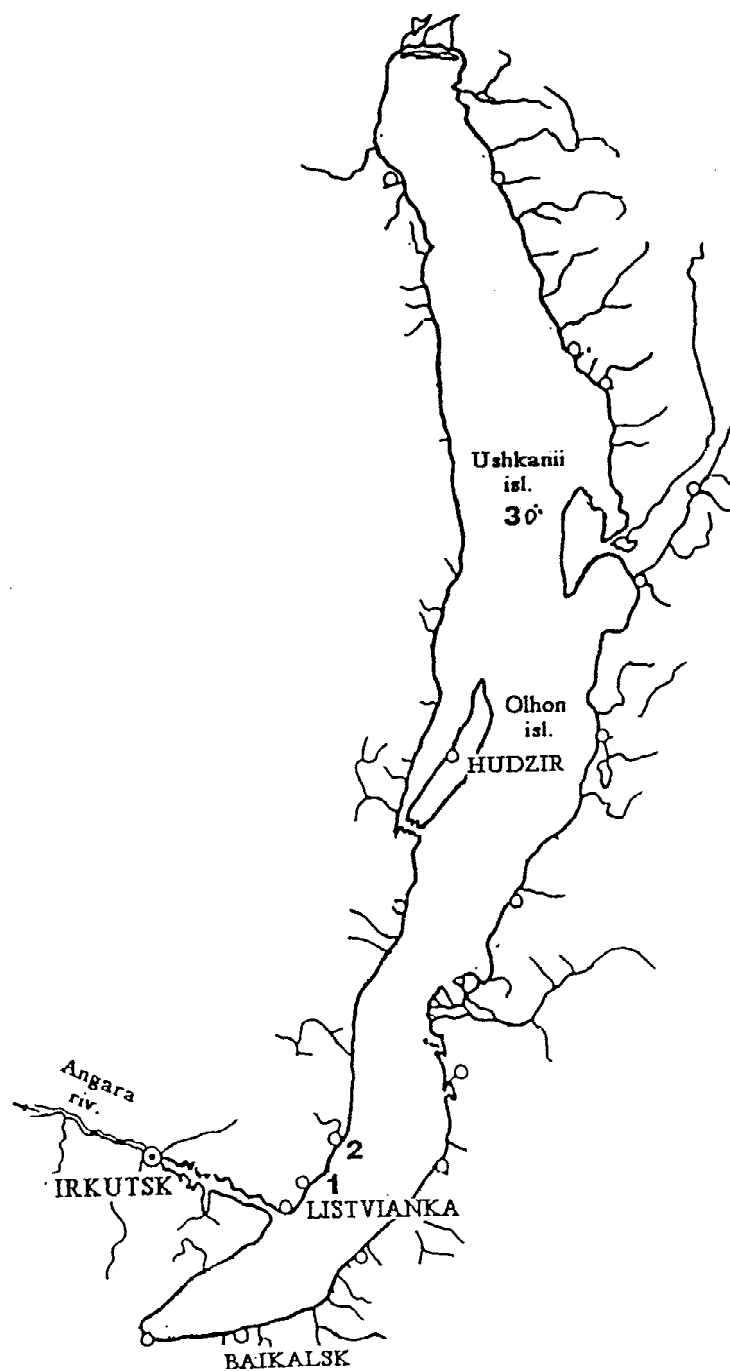


Figure 1. Sampling localities in Lake Baikal.

Inter-population variation

On the basis of the mean values of all characters, a 'size coefficient' of the individuals in the samples was calculated, i.e. relation of sample 2 and sample 3 to

sample 1, averaged across all characters. Thus the 'size coefficient' indicates how much 'bigger' samples 2 and 3 were than sample 1. All data on samples 2 and 3 subsequently were divided by the 'size coefficient'.

These data were used for comparison of samples using the body shape.

Moreover, we also compared samples using indices which are typically used in morphological studies of Cyclopidae: 1. ratio of the furcal length to its width (1/2); 2. length of the inner to outer seta (4/5); 3. length of the distal segment of the endopodite of the 4th pair of swimming leg to its width (10/11); 4. length of the inner distal spine of the endopodite of the 4th pair of swimming leg to the length of the inner spine (12/13); 5. length of the 5th swimming leg to its width (15/16); 6. length of the distal spine of the 5th swimming leg to the length of the 5th leg (15/17); 7. width of the 5th swimming leg to length of the distal spine on the 5th leg (16/17); 8. length of the distal seta of the 5th swimming leg to the length of the distal spine of this pair (17/18).

Within-population variation and measurement error

The procedure for calculation of size-independent indices of variation includes:

1. Calculation of the first principal component (PC1) based on all characters (it was considered as generalized size).
2. Regression of each character on PC1.
3. Dividing residuals by predicted values.

Data obtained were size-independent residuals. They were used for computations of the total variance (δ^2) and its stochastic component (observed) (δ_s^2), based on the factor model of the bilateral trait, according to the method of Kozhara (1989, 1994):

$$\delta^2 = \Sigma(X_i - M)^2 / 2(n - 1),$$

$$\delta_s^2 = \Sigma(R - L)^2 / 2n,$$

where X_i is both left and right manifestations of character, M is mean value, R is right and L is left manifestation of the character, n is number of individuals.

For the assessment of the level of fluctuating asymmetry, we also used the following individual index:

$$FA = (|R - L|) / 0.5(R + L).$$

The mean values of these individual indices for each character were used as population characteristics.

To evaluate measurement error we made 3 series of additional replicate measurements on 11 specimens from sample 2 for 12 characters (1, 2, 3, 4, 7, 9, 10, 11, 13, 15, 18, 19) on 10th, 25th and 45th

days after preparation of the specimens. Thus, we have three assessments of the measurement error obtained from comparison of the sequential replicates. For calculation of the magnitude of the measurement error, after calculations of size-independent indices (see above), we applied the formula similar to that used for calculation of stochastic component:

$$ME = \Sigma(X_1 - X_2)^2 / 2n,$$

where X_1 and X_2 are replicate measurements, n is the number of measurements. This estimate of measurement error is compatible with the total variance and the stochastic component. The true stochastic component was estimated as the difference between the observed stochastic component and the measurement error.

It is necessary to note that this method gives *a priori* independent estimates of stochastic component and ME. Thus, due to chance, these estimates can exceed total variance and stochastic component respectively (Kozhara, 1989). The overall estimate of the level of variation of a population was assessed using a sum of ranges (Zakharov, 1989).

Results and discussion

Measurement error

There was a clear trend in decrease of the magnitude of the measurement error from the first (comparing replicates 1 and 2) to the last (comparing replicates 3 and 4) estimates (Table 1). We assume that decrease of the measurement error was due to the change in the position of the specimens on the slides due to the coverglass depression. At first, the position of the specimen is unstable and then progressively stabilizes; repeatability of measurements increases and hence the measurement error decreases. For the assessment of stochastic variation, left and right structures are measured simultaneously. Theoretically, the best way to assess the measurement error is to make replicate measurements simultaneously; however, this is impossible. Moreover, the replicate measurements have to be independent by definition (i.e. operator does not remember result and way of the obtaining the first replicate while making the second one). This is very difficult to achieve when intervals between replicates are minimal. Thus, one has to wait for a stable position of the specimen. Evidently, the most accurate estimate of the measurement error is that of the minimal value, i.e. the last one. Nevertheless, we suspect that even

Table 1. Measurement error (ME) assessed by three estimates based on four repeated measurements of 11 specimens from the Peschannaya Bay population. The third estimate is defined for further calculations. Contribution of this value of ME to stochastic variation and contribution of stochastic variation in total variance (stochastic and total variance are calculated from first replicate) for three populations of *Acanthocyclops signifer* from Bolshie Koty (1), Peschannaya Bay (2) and Ushkanii Islands area (3)

| Char-acter | ME | | | Contribution of ME to stochastic variation | | | Contribution of stochastic variation in total variance | | |
|------------|-----------------------|---------|---------|--|---------|---------|--|---------|----------|
| | Repeated measurements | | | Locality | | | Locality | | |
| | 1×2 | 2×3 | 3×4 | 1 | 2 | 3 | 1 | 2 | 3 |
| 1 | 0.00112 | 0.00056 | 0.00027 | 0.59962 | 0.26454 | 1.92735 | 0.08226 | 0.21512 | −0.05607 |
| 2 | 0.00166 | 0.00091 | 0.00004 | 0.05831 | 0.04054 | 0.02097 | 0.30517 | 0.42928 | 0.51779 |
| 3 | 0.00156 | 0.00059 | 0.00048 | 1.19142 | 0.17849 | 1.03602 | −0.02786 | 0.34603 | −0.00785 |
| 4 | 0.00217 | 0.00094 | 0.00061 | 0.28021 | 0.36968 | 0.49490 | 0.26874 | 0.20915 | 0.22131 |
| 7 | 0.00111 | 0.00139 | 0.00054 | 0.65412 | 0.54840 | 1.75135 | 0.08743 | 0.07525 | −0.11852 |
| 9 | 0.00404 | 0.00050 | 0.00117 | 0.26651 | 0.35751 | 0.52822 | 0.66357 | 0.37525 | 0.08661 |
| 10 | 0.00039 | 0.00027 | 0.00023 | 2.13182 | 0.54535 | 0.78167 | −0.18845 | 0.19708 | 0.09905 |
| 11 | 0.00143 | 0.00109 | 0.00046 | 1.04108 | 0.37547 | 0.60273 | −0.01798 | 0.40706 | 0.37960 |
| 13 | 0.00095 | 0.00125 | 0.00023 | 0.33245 | 0.15699 | 0.31398 | 0.24358 | 0.37098 | 0.13226 |
| 15 | 0.00416 | 0.00588 | 0.00228 | 0.51388 | 0.41925 | 0.36541 | 0.61990 | 0.36130 | 0.58675 |
| 18 | 0.00151 | 0.00209 | 0.00081 | 0.50331 | 0.15821 | 0.65471 | 0.26035 | 0.38822 | 0.10262 |
| 19 | 0.00456 | 0.00272 | 0.00048 | 0.28578 | 0.06957 | 0.24987 | 0.32690 | 0.64668 | 0.37525 |

Table 2. Parameters of variation of morphological characters in *Acanthocyclops signifer* from three localities in Lake Baikal: Bolshie Koty (1), Peschannaya Bay (2) and Ushkanii Islands area (3)

| Char-acter | Mean value (μm) | | | Size-corrected mean value (μm) | | | Stochastic component | | | Mean fluctuating asymmetry | | | Factorial component | | |
|------------|------------------------------|-------|-------|---|-------|-------|----------------------|---------|---------|----------------------------|---------|---------|---------------------|---------|---------|
| | locality | | | locality | | | locality | | | locality | | | locality | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 1 | 175.3 | 176.9 | 246.7 | 175.3 | 186.0 | 200.3 | 0.00045 | 0.00102 | 0.00014 | 0.02116 | 0.03536 | 0.00854 | 0.00156 | 0.00125 | 0.00075 |
| 2 | 40.8 | 42.6 | 43.1 | 40.8 | 44.8 | 35.0 | 0.00073 | 0.00105 | 0.00203 | 0.02642 | 0.03347 | 0.00162 | 0.00092 | 0.00049 | 0.00171 |
| 3 | 125.7 | 125.3 | 188.1 | 125.7 | 131.8 | 152.7 | 0.00040 | 0.00267 | 0.00046 | 0.02094 | 0.04528 | 0.02016 | 0.00289 | 0.00284 | 0.00101 |
| 4 | 146.3 | 136.5 | 127.4 | 146.3 | 143.5 | 103.4 | 0.00219 | 0.00166 | 0.00124 | 0.03932 | 0.04372 | 0.03184 | 0.00287 | 0.00400 | 0.00307 |
| 5 | 558.6 | 537.8 | 617.9 | 558.6 | 565.4 | 501.5 | 0.00052 | 0.00006 | 0.00008 | 0.02653 | 0.00952 | 0.01012 | 0.00252 | 0.00159 | 0.00119 |
| 6 | 796.7 | 771.4 | 806.8 | 796.7 | 811.1 | 654.9 | 0.00080 | 0.00058 | 0.00006 | 0.03018 | 0.01976 | 0.01308 | 0.00299 | 0.00318 | 0.00130 |
| 7 | 185.6 | 173.5 | 198.8 | 185.6 | 182.4 | 161.4 | 0.00083 | 0.00099 | 0.00031 | 0.03434 | 0.03324 | 0.01582 | 0.00209 | 0.00466 | 0.00249 |
| 8 | 142.7 | 126.4 | 220.3 | 142.7 | 132.9 | 178.8 | 0.00193 | 0.00315 | 0.00076 | 0.05968 | 0.05424 | 0.03219 | 0.00283 | 0.00924 | 0.00373 |
| 9 | 70.0 | 69.5 | 96.3 | 70.0 | 73.1 | 78.2 | 0.00440 | 0.00328 | 0.00222 | 0.07198 | 0.05872 | 0.04852 | 0.00156 | 0.00339 | 0.00439 |
| 10 | 105.0 | 100.5 | 119.8 | 105.0 | 105.7 | 97.3 | 0.00011 | 0.00043 | 0.00030 | 0.00912 | 0.02114 | 0.01728 | 0.00073 | 0.00052 | 0.00050 |
| 11 | 51.5 | 49.1 | 51.8 | 51.5 | 51.7 | 42.0 | 0.00044 | 0.00122 | 0.00076 | 0.02378 | 0.04534 | 0.02472 | 0.00075 | 0.00086 | 0.00093 |
| 12 | 126.5 | 121.6 | 107.3 | 126.5 | 127.9 | 87.1 | 0.00020 | 0.00076 | 0.00064 | 0.01594 | 0.02902 | 0.02767 | 0.00052 | 0.00170 | 0.00394 |
| 13 | 86.4 | 83.6 | 126.0 | 86.4 | 87.9 | 102.2 | 0.00068 | 0.00144 | 0.00072 | 0.03082 | 0.04294 | 0.02846 | 0.00117 | 0.00203 | 0.00343 |
| 14 | 161.7 | 149.9 | 153.7 | 161.7 | 157.6 | 124.8 | 0.00153 | 0.00107 | 0.00030 | 0.03914 | 0.03558 | 0.02366 | 0.00029 | 0.00511 | 0.00198 |
| 15 | 22.5 | 19.9 | 25.0 | 22.5 | 20.9 | 20.3 | 0.00443 | 0.00543 | 0.00623 | 0.07444 | 0.08754 | 0.06228 | 0.00110 | 0.00545 | 0.00390 |
| 16 | 13.3 | 12.9 | 18.1 | 13.3 | 13.5 | 14.7 | 0.00547 | 0.00545 | 0.01608 | 0.09518 | 0.07244 | 0.13848 | 0.00303 | 0.00361 | 0.00747 |
| 17 | 11.9 | 10.6 | 23.6 | 11.9 | 11.2 | 19.2 | 0.00479 | 0.00701 | 0.01376 | 0.06136 | 0.08313 | 0.12083 | 0.01710 | 0.02108 | 0.01538 |
| 18 | 113.6 | 102.7 | 131.2 | 113.6 | 108.0 | 106.5 | 0.00160 | 0.00509 | 0.00123 | 0.04908 | 0.08566 | 0.04132 | 0.00221 | 0.00649 | 0.00368 |
| 19 | 105.0 | 97.3 | 151.5 | 105.0 | 102.3 | 123.0 | 0.00167 | 0.00686 | 0.00191 | 0.05434 | 0.08788 | 0.05454 | 0.00279 | 0.00386 | 0.00446 |

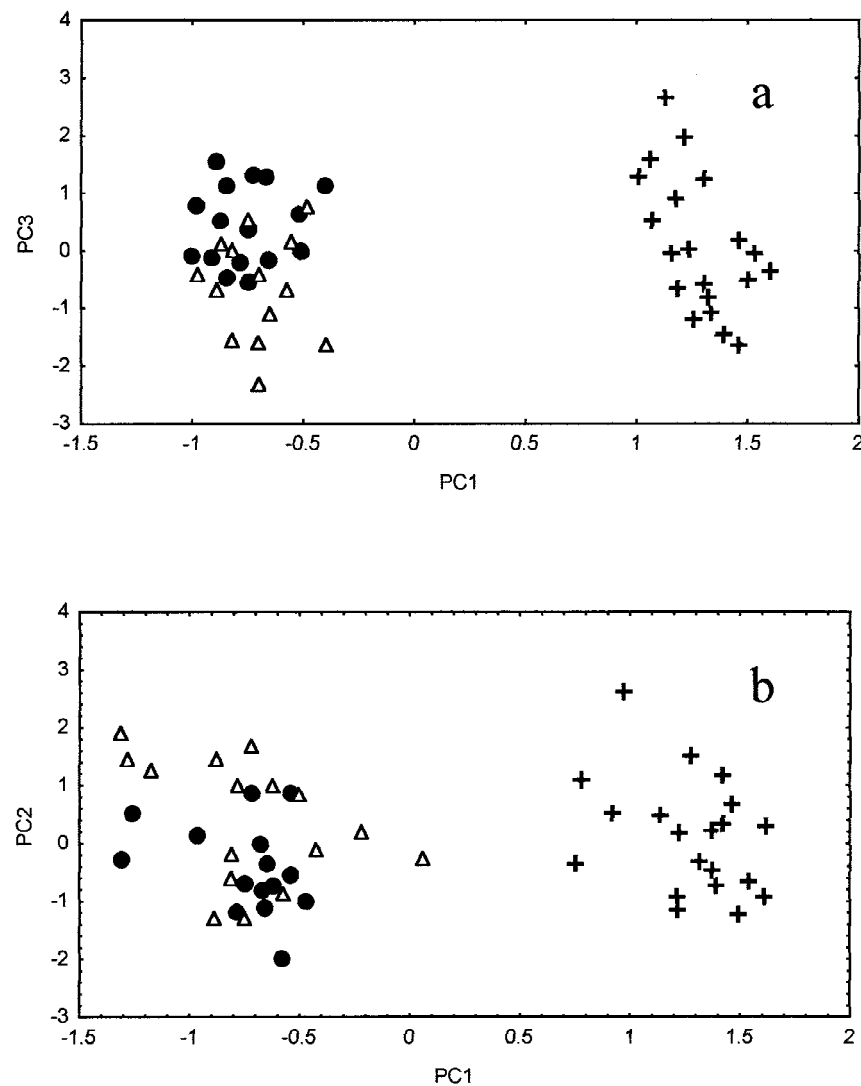


Figure 2. Distribution of individuals in coordinates PC1, PC2 and PC3, calculated on the base of all characters (a) and several indices (see text) (b) for samples from Bolshie Koty (filled circles), Peschannaya Bay (open triangles) and Ushkanii Islands area (crosses).

after a long time, changes in position are possible. Thus, our estimates of the measurement error may be higher than its true value.

Interpopulation variation

Morphological characters from the three localities in Lake Baikal showed clear differences in the mean values (Table 2). The overall size (i.e. the mean values of most characters) is maximal in the population from the Ushkanii Islands area, whereas the population from Peschannaya Bay shows the smallest values for most of the characters. The calculation of the

‘size coefficient’ for the mean value of the characters at location 1 (Bolshie Koty) was defined to be equal to 1. At location 2 (Peschannaya Bay), the ‘size coefficient’ was equal to 0.951 and for location 3 (Ushkanii Islands area) it was 1.232. Size corrected individual values were used to compare the locations using principal component analysis (PCA). This analysis showed that 58.1% of the total variation was attributed to PC1, 9.5% to PC2 and 7.0% to PC3. The remaining principal components described less than 5% of the variation for each. Significant differences were found between populations for PC1 and PC3 (Figure 2a).

Difference between the group of populations from the southern part of Lake Baikal (locations 1 and 2) and the population from the northern part of the lake were related to PC1 ($df=54$; $p<0.001$). Characters exhibiting maximal (0.81–0.96) factor loading on PC1 were 12, 11 and 4 (negative), and 8, 3, 13 (positive). These characters showed the most pronounced differences between the populations from different parts of the lake, although nearly all other characters also showed significant differences.

The two samples from the southern part of Lake Baikal were morphologically similar to each other. They differed significantly ($df=33$; $p<0.01$) in PC3 (maximal factor loading (0.53–0.49) for characters 18 (positive) and 1 (negative)). Characters showing significant differences are 2, 1, 15, 3, 8.

We also used principal component analysis to analyze indices. The observed results (considering the negative values) were quite similar to those obtained using the analysis of the size-corrected values (Figure 2b). PC1 explained 70.4% of the total variation and PC2 12.9%. Maximal loading on the PC1 (greater than 0.9) was observed for indices 13/12, 17/15 and 1/2. Maximal loading on PC2 showed the indices 15/16 and 16/17. For paired comparisons, differences between groups (samples 1 and 2 on the one hand and sample 3 on the other) were significant (t test, $df=35$ for comparing samples 1 and 3, $df=38$ for comparing samples 2 and 3, $p<0.05$) using all indices (they were maximal for indices 13/12 and 1/5). Samples 1 and 2 differed significantly ($df=33$; $p<0.01$) for one index (15/20).

Therefore, the analysis of characters normally used in Cyclopidae studies permitted separation of *A. signifer* for different locations, and hence may be useful for morphological studies of this complex. Principal component analysis clearly indicated that all characters do not universally discriminate the samples. Characters which showed differences between a pair of samples, did not show differences with others. Therefore, for the analysis of morphological variation of the whole group, it is necessary to use different characters. Discrimination of the samples is better, when initial data (Figure 2a), but not indices (Figure 2b) are compared. Thus the use of the indices does not give advantages in comparison with the application of the principal component analysis, which seems to be simpler to apply for groups where indices are not usually used.

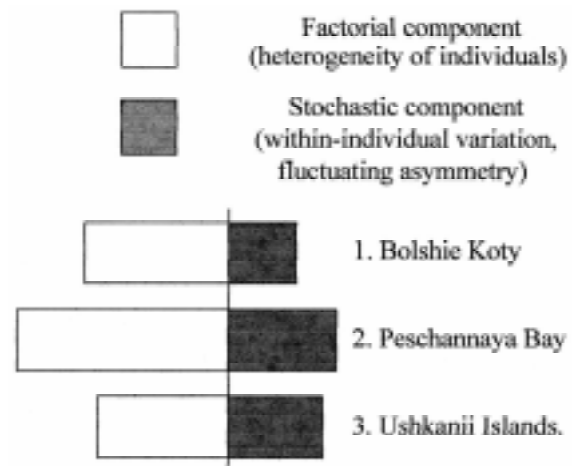


Figure 3. Components of morphological variation: factorial (variation among individuals within population) – white, and stochastic (within-individual's variation) – gray.

Within-population variation

Factorial component (heterogeneity of individuals)

The heterogeneity of individuals in the population from Peschannaya Bay is notably higher than in the other two populations, which do not differ considerably from each other (variation is somewhat higher in the population from Bolshie Koty) (Table 1, Figure 3). The difference between the populations from Peschannaya Bay and Bolshie Koty was significant by sign test ($p<0.01$).

Stochastic component of the total variation

(within-individual variation, fluctuating asymmetry)

Partitioning the total variance into its components is correct for fluctuating asymmetry, but not for anti-symmetry or directional asymmetry. Therefore, it is necessary to justify that the asymmetry under study is fluctuating asymmetry. Our preliminary treatment indicated that all (R–L) samples in this study were normally distributed (sequential Bonferroni test, Rice, 1989), no significant p -values). No significant directional differences were found between left and right (t -test). Thus, we concluded that we were dealing with fluctuating asymmetry.

The highest level of stochastic variation was found in the population from Peschannaya Bay and the lowest in the population from Bolshie Koty (Table 1, Figure 3). Differences between the populations from the southern part of the lake were close to significance (the asymmetry was higher in the population from Peschannaya Bay in 13 of 19 cases for both in-

dices of fluctuating asymmetry). Differences between the populations from Bolshie Koty and Ushkanii Islands area were significant for the mean-asymmetry index ($df=35$; $p<0.05$), but not for the levels of the stochastic component (10 cases). Differences between the populations from Peschannaya Bay and Ushkanii Islands area were larger and significant for the mean-asymmetry index ($df=38$; $p<0.01$). As regards the stochastic component, the difference was close to significance. Thus, for comparison of the last populations it is necessary to continue the analysis using larger samples.

The paired comparison of the mean value of asymmetry index $|R-L|/0.5(R+L)$ of the characters by t -test showed significant differences for the following cases. The population from Peschannaya Bay showed higher level of asymmetry compared to the population from Bolshie Koty in characters 10, 11 and 18, and in characters 1, 2, 11 and 18 when compared to the population from Ushkanii Islands area. For the other two populations significant differences are observed in the characters 1, 2, 5, 6, 7, 8 (in all cases asymmetry in the sample from Bolshie Koty was higher).

The average contribution of the measurement error in stochastic variation for the three populations was rather high, exceeding 56% (Figure 4). In some characters, it exceeds stochastic variation (Table 1) that can only be explained by the small sample size (while the estimates of stochastic variation and the measurement error are independent) and also by change of position of specimens over time between making replicate measurements (see above). The contribution of the measurement error decreases with the increasing size of characters ($r=-0.42$, $p>0.05$) i.e. in comparatively large characters fluctuating asymmetry might be measured more accurately. No association of the magnitude of the measurement error with the type of characters (setae, spines or size of some more consistent parts of the body) has been observed. The contribution of the measurement error to the stochastic variation (fluctuating asymmetry) in our study exceeds that of most estimates in the literature (Palmer & Strobeck, 1986). The high value of the measurement error can be explained by the small size of structures measured and by the changes in their positions on the slide. In general, the high levels of the measurement error suggest that special attention should be paid to this problem in morphological studies of the Copepoda. Evaluation of the measurement error for the assessment of the mean values of the characters allows choosing characters that can be measured more precisely. For the assessment of

fluctuating asymmetry, evaluation of the measurement error seems to be necessary for obtaining reliable data.

With data on the measurement error, it is possible to estimate the contribution of stochastic variation to the total variance, and to assess not only observed but true magnitude of stochastic variation, which ranges in most of cases from 0.2 to 0.5 (average is 0.27) (Figure 4). The level of the variation (both stochastic and factorial) of characters was negatively correlated with the mean values, which has been observed also in other organisms (Soulé, 1982; Lajus, 1997, 1998).

In general, the results of our study allowed us to draw the following conclusions. Our data showed notable differences in the mean values of taxonomic characters between populations of *A. signifer* from the distant parts of Lake Baikal. A taxonomic revision of the *A. signifer* species complex may be necessary. Diversity of this group probably can be explained by its taxonomic heterogeneity. Previous analyses have revealed similar heterogeneity for the *Eucyclops* species complex from Lake Baikal, that permitted description of a new species *E. arcanus* (Alekseev, 1990). Evidently, the endemic cyclopoid fauna of Lake Baikal is more complicated than previously thought and is in need of substantial revision. Such revision with the description of new taxa within those described earlier will decrease the level of within-species variability. Our study suggests that, at least in the *A. signifer* species complex, high degrees of morphological variation are probably explained by a scarcity of taxonomic data. Most likely, this can also explain the high variability in other groups of Baikalian endemics.

As to the inter-relationships among the studied samples, the morphological distinction of the Ushkanii Islands population is probably explained by their long-term geographical isolation. Deep waters surrounding the archipelago act as a geographical barrier for the near-shore *A. signifer*. Other invertebrates of this part of Lake Baikal also exhibit local endemism. According to Starobogatov (1970) the Ushkanii Islands area belongs to the East-Baikalian zoogeographical province, whereas sampling sites in Bolshie Koty and Peschannaya Bay belong to the South-Baikalian province. Morphologically, copepods from the two last sites are considerably more similar to each other, however, not identical, differing in the shape of the furca and the lengths of the fifth swimming leg and dorsal seta.

It is interesting to note that contrary to the mean value data, level of variation in the population from Bolshie Koty is more similar to the level of vari-

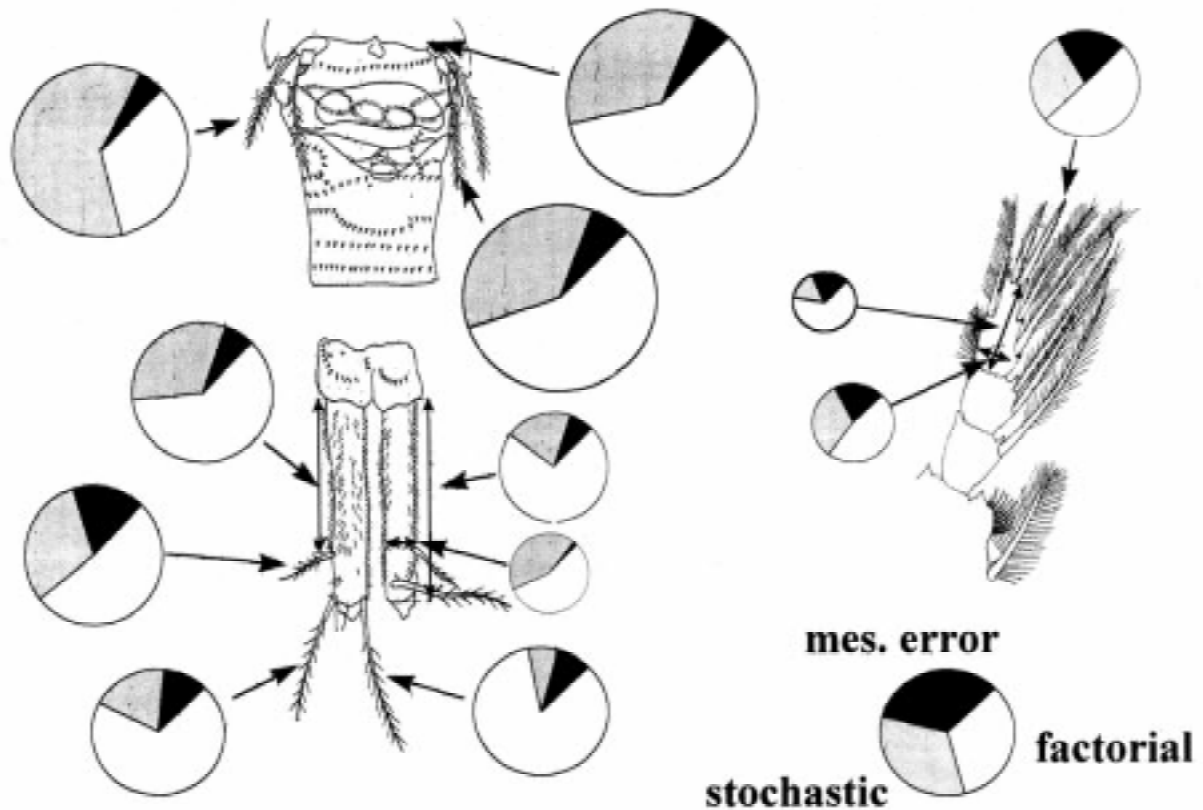


Figure 4. Magnitude of morphological variation and contribution of different components in it: factorial (white), stochastic (gray), measurement error (black). Area of circles is proportional to total variance.

ation in the population from the Ushkanii Islands area than to that from Peschannaya Bay. We suspect that the relatively low level of stochastic variation and, consequently, relatively high developmental stability shows that populations from the Ushkanii Islands area and Bolshie Koty are rather well adapted to their environment, and demonstrate a 'basic' level of variation for the group, whereas the population from Peschannaya Bay is presumably in a transition stage or lives under stressful environmental conditions. According to Mazepova (1978), *A. signifer* consists of two groups, one of which inhabits the near-shore zone and the other occurs in deep waters. It is quite possible that the sample collected from Peschannaya Bay represents the second group and that deep waters are not a very favourable environment for *A. signifer*.

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References

- Alekseev, V. R., 1990. (*Eucyclops arcanus* sp. n. (Copepoda, Cyclopoida) from waterbodies of Bolshezemelskaia tundra and Prebaikalia). Zool. Z. 69: 133–139 (in Russian).
- Astauroff, B. L., 1930. Analyse der erblichen Störungsfälle der bilateralen Symmetrie im Zusammenhang mit der selbstständigen Variabilität ähnlicher Strukturen. Zeitschrift für induktive Abstammungs- und Vererbungslehre 55 (3): 183–262 (in German). See also translation into Russian: Astaurov, B. L., 1974. (Study of hereditary deviations from bilateral symmetry, associated with variation of metameric structures). In Rokitskii, P. F. (ed.), Nasledstvennost' i razvitie. Moscow: Nauka Press: 54–109.
- Bazikalova, A. Y., 1945. Amphipody ozera Baikal. (Amphipoda of Baikal) Trudy Baik. Limn. Stantsii AN SSSR, v. 11. 370 pp. (in Russian).
- Graham, J. H., D. C. Freeman & J. M. Emlen, 1993. Developmental stability: a sensitive indicator of populations under stress. Envir. Toxicol. and Risk Assessment, ASTM STP 1179. In Glandis, W., J. S. Hughes & M. A. Lewis (eds), American Society for Testing and Materials, Philadelphia: 136–158.
- Hubert, W. A. & C. B. Alexander, 1995. Observer variation in counts of meristic traits affects fluctuating asymmetry. N. am. J. Fish Mgmt: 15: 156–158.
- Jones, J. S., 1987. An asymmetrical view of fitness. Nature 325: 298–299.

- Kozhara, A. V., 1989. On the ratio of components of phenotypic variances of bilateral characters in populations of some fishes. *Genetica (USSR)* 25: 1508–1513 (in Russian).
- Kozhara, A. V., 1994. Phenotypic variance of bilateral characters as an indicator of genetic and environmental conditions in bream *Abramis brama* (L.) (Pisces, Cyprinidae) population. *J. Appl. Ichth.* 10: 167–181.
- Lajus, D. L., 1996. What is the White Sea herring *Clupea pallasii marisalbi* Berg, 1923? a new concept of the population structure. In Lobon-Cerviá, J., B. Elvira & C. Granado-Lorencio (eds), *Proceedings of the VIII Congress Societatis Europaea Ichtiologorum*. Oviedo, Spain, Sept. 26–Oct. 2. Boel. Instit. Nac. Oceanogr. 21: 221–230.
- Lajus, D. L., 1997. Is allomeric effect due to stochastic variation? *J. Morph.* 232: 284.
- Lajus, D. L., 1998. Two-dimensional model of phenotypic variation. *Proc. Zool. Inst. RAS* 267: 107–114.
- Mazepova, G. F., 1978. Tsiklopy ozera Baikal. (Cyclopidae of Baikal) Nauka Press. Novosibirsk. 144 pp. (in Russian).
- Mazepova, G. F., 1989. Ostrakody ozera Baikal. (Ostracoda of Baikal) Nauka Press. Novosibirsk. 344 po. (in Russian).
- Merilä, J. & M. Bjorklund, 1995. Fluctuating asymmetry and measurement error. *Syst. Biol.* 44: 97–101.
- Møller, A. P., 1997. Developmental stability and fitness: a review. *Am. Nat.* 149: 916–932.
- Møller, A. P. & J. P. Swaddle, 1997. *Asymmetry, developmental stability and evolution*. Oxford University Press, Oxford. 292 pp.
- Monchenko, I., 1974. Tchelepnoroty tzyklopopodobni tsiklopi (Cyclopidae). *Kiev* 27 (3): 343 pp. (in Ukrainian).
- Palmer, A. R. & C. Strobeck, 1986. Fluctuating asymmetry: Measurement, analysis, patterns. *Ann. Rev. Ecol. Syst.* 17: 391–421.
- Rice, W. R., 1989. Analysing tables of statistical tests. *Evolution* 43: 223–225.
- Soulé, M. E., 1982. Allomeric variation. I. The theory and some consequences. *Am. Nat.* 120: 751–764.
- Starobogatov, J. I., 1970. *Fauna molluskov i zoogeograficheskoye raionirovanie kontinental'nykh vodoemov zemnogo shara*. Nauka press. Leningrad 435 pp. (in Russian).
- Swaddle, J. P., M. S. Witter & I. C. Cuthill, 1994. The analysis of fluctuating asymmetry. *Anim. Behav.* 48: 986–989.
- Zakharov, V. M., 1989. Future prospects for population phenogenetics. *Sov. Sci. Rev. Sect. F, Physiol. Gen. Biol. Rev.* 4: 1–79.