Morphometric exploration of diversity of the Eurasian minnow *Phoxinus phoxinus*: a case study of a widely distributed Palaearctic fish

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The Eurasian minnow *Phoxinus phoxinus* is widely distributed across most of Europe and northern Asia. It displays considerable morphological diversity and variable colouration. Despite this it is still believed that its whole distribution holds only one monotypic species. There are no previous conclusive morphometric analyses of samples from different parts of its distribution area. This paper presents results of multidimensional analyses of morphometric characters of 14 widely scattered populations of the species. Our analyses show considerable inter-population differences. Morphometric evidence suggests that populations from the Amur River system and central Asia may represent new, distinct species or subspecies. The status of the remaining populations remains unclear.

Introduction

The Eurasian minnow *Phoxinus phoxinus* is a small cyprinid fish with an extensive Palaearctic distribution. It is distributed from the Pyrenees and the British Isles in the west to northern Asia, the Chukchi Peninsula, northern China, and the Korean Peninsula. Despite displaying considerable morphological variation across its distribution, until recently only one species — *P. phoxinus* — was recognized in this vast area (Řepa & Pivnička 1980, Tsepkin 2002, Kottelat & Freyhof 2007). At present, however, seven distinct species of the former 'Eurasian minnow' are distinguished in Europe (Kottelat 2007). The

Asian populations have not been thoroughly and adequately revised, but preliminary data in Kottelat (2006) and Bogutskaya *et al.* (2008) suggest that their morphological and genetic differentiation is so great that they should be classified into at least a few separate species. This pertains especially to forms from the upper sections of the Irtysh, Ob and Yenisey river systems and from the undrained basin of the great lakes of northwestern Mongolia and the Amur basin (Kottelat 2006, Bogutskaya *et al.* 2008).

Results in Imoto *et al.* (2013) concerning the mitogenomic phylogeny of Leuciscinae indicate strong divergence between the sample populations of *P. phoxinus* from Italy and Mongolia.



Fig. 1. Geographic distribution (light grey zone) and sampling sites (see Table 1) of Phoxinus phoxinus (dots).

They estimate the time of divergence of these forms between 24 and 9.5 Mya, i.e., during the Miocene. Given the most frequently used scaling of the rate of evolution of different mtDNA genes, such a deep diversity seems to be at least equivalent to the level of differences between two independent species. This supports the supposition of Kottelat (2006) and Bogutskaya et al. (2008) and allows us to assume that in the area of Eurasia, we deal with unexplored cryptoradiation of P. phoxinus. Unfortunately, diagnostic characters used by the authors in describing the new European species appear ambiguous and not very convincing (Kottelat 2007, Kottelat & Freyhof 2007). According to these authors the basis for distinguishing new species have mainly been differences in the pattern of the breast scale patches, while there is a lack of comparative analyses concerning the variability of this character between P. phoxinus populations from different parts of its range. Despite this, high differentiation of populations and the vast distribution of the Eurasian minnow allow one to reasonably assume the existence of at least a few distinct forms of species rank, especially in the Asian part of the range.

This paper is a first attempt at a broad morphometric analysis of variation of Eurasian minnow populations from various localities across its vast distributional range. Our main goal was to identify morphologically distinct forms which may correspond to formal taxa. Based on the literature, we assume that morphometric distinctiveness may concern at least the population from the Amur basin (Kottelat 2006, Bogutskaya *et al.* 2008).

Material and methods

Sample localities and data collection

The material included a total of 222 Eurasian minnow specimens representing 14 populations from the river systems of Danube (1 population), Vistula (1), upper Irtysh (5), Amur (1), upper Kolyma (1) undrained basin of Lake Balkhash (3) and the coasts of White (1) and Okhotsk Seas (1) (Fig. 1 and Table 1). Samples consisted of both sexes, which were analysed together. Assuming that the sex ratios in our samples are close to those found in particular populations and are also similar in all studied populations, the results of the analyses should be equally averaged for males and females. This can probably affect the accuracy of the results but not the general pattern of diversity. It was not possible to determine the values of 5 of the 26 studied characters for 8 of the 14 individuals from the Pischevoy population. They were replaced with group means.

The fish were caught with nets or by electrofishing and preserved in 4% formaldehyde in horizontally positioned plastic containers so that the specimens maintained their natural shape. In the laboratory, the specimens were rinsed in running water for two hours and then prepared to be photographed. Each photographed fish was placed on its right side and pinned to a cork board using thick entomological needles in such a way that the sagittal plane of the specimen was parallel to the board surface. Thinner entomological needles were used to mark the landmarks. This facilitated identification of the land-



Fig. 2. Landmark locations and "truss network" scheme of measurements (c1–c26 are the "truss network" characters). I = anterior-most point of premaxillary bone; II = posterior margin of posterior naris; III = posterior-most point of mandible; IV = base of the last (interior) ray of the pectoral fin; V = posterior margin of the supraoccipital bone; VI = base of the first ray of the dorsal fin; VII = base of the first ray of the ventral fin; VIII = base of the first ray of the dorsal fin; X = base of the last ray of the dorsal fin; X = base of the last ray of the anal fin; IX = base of the last ray of the first ray base) and XII = origin of ventral lobe of the caudal fin (anterior-most point of the first ray base).

marks in the photographs. A millimetre scale was placed at the level of the sagittal plane of each specimen for the purpose of later calibration of the measurements taken from the photographs. Prepared specimens were photographed with a digital Nikon D80 camera. Only non-deformed and straight specimens were used for the photographs and measurements. Twelve landmarks were designated on each specimen according to the Truss Network scheme (Strauss & Bookstein 1982). The landmarks (*see* Fig. 2) were chosen so as to favour intermediate distances. This provided a necessary compromise between measurement error (higher error for shorter distances) and problems with too long distances (variation difficult to associate with a particular body region in an individual).

Distances between the landmarks that correspond to 26 variables used in our analyses as well as the 15 mm scale for each of the photographed specimens were measured with the programme SigmaScan Pro 5.0 (SPSS Inc.).

Statistical analyses

According to Allometric Burnaby's Method (Bookstein 1991, Reyment 1991, Rohlf 2010),

Site no.	Site	Drainage basin (region)	Sampling date	п	
1	Czadeczka	Dunaj River	27.V.2007	31	
2	Brzózowka	Vistula River	15.IX.1982	23	
3	Belaya	White Sea region	30.VIII.2002	10	
4	Lepsy	Balkhash Lake	29.V.2009	11	
5	Sarkand	Balkhash Lake	18.VIII.2010	13	
6	Ayagoz	Balkhash Lake	23.V.2009	29	
7	Ulken Boken	Irtysh River	27.VII.2009	15	
8	Oskemen	Irtysh River	20.08.2007	8	
9	Solonovka	Irtysh River	3.VIII.2009	13	
10	Kurchum	Irtysh River	20.VII.2009	13	
11	Zharma	Irtysh River	20.VII.2009	14	
12	Birobidzhan	Amur River	8.VIII.2008	19	
13	Bystry	Magadan region	5.VII.2004	11	
14	Pischevoy	Kolyma River	9.VII.2004	12	

Table 1. Sampling site locations, sampling dates and number of the *Phoxinus phoxinus* individuals analysed.

data were log-transformed and the effect of body size of the specimens was removed by projection of the character set on the plane orthogonal to the size vector (the first eigenvector from the variance-covariance matrix of logarithmic data). In order to find patterns of inter-population variation, the number of variables (characters) was subjected to reduction in data matrices freed from the effect of the first eigenvector, using multidimensional methods: Canonical Variate Analysis (CVA) and Principal Component Analysis (PCA). Both analyses were based on the covariance matrix. CVA is a more appropriate method due to minimalisation of the ratio of intra-group to inter-group variance, but requires assumptions of normal distributions of characters within populations and homogeneity of their respective variances. PCA does not consider intra-group variance: it only maximises the individual variance and does not require the assumption of homogeneity of variance. In cases for which the assumptions of parametric tests are only partly met (see Results), similar results obtained with the two methods (parametric and non-parametric) increase confidence in the patterns observed. For each population, normality of the distribution of characters was tested with Shapiro-Wilk's test, the homogeneity of variance with Levene's test, and correlations of character means with variances using Pearson's correlation (Dytham 2007). The Minimum Spanning Tree (MST) was graphically combined with the distribution of populational means along the first, second and third canonical vectors. Data matrices used in the analyses are available on request. Statistical calculations were conducted using Microsoft Excel, NTSYS 1.8 (Rohlf 1996), NTSYS 2.211 (Rohlf 2010), R 2.12.1 (R Development Core Team 2010) and STATISTICA 9.1 (StatSoft Inc.).

Results

The pattern of morphometric differentiation in *P. phoxinus*

Shapiro-Wilk's test showed that for most empirical distributions, the hypothesis of a normal distribution could not be rejected (26 characters in

14 populations gave 364 empirical distributions, of which only 30 [ca. 8%] were not normal). Levene's test indicated that the hypothesis of homogeneity of variance could not be rejected for 10 of the 26 characters. The correlations of variances with means were, however, not high and not statistically significant for the majority of characters (23 of 26). Since these correlations violated the assumptions of variance analysis the most (Dytham 2007), and the distributions of characters in most cases were compatible with normality assumptions, multidimensional discriminant and canonical analyses were performed. Moreover, we conducted the non-parametric PCA to test the general pattern of variability obtained from the parametric analyses. Average character values for all populations studied (after Burnaby's correction for size) are presented (Fig. 3).

Wilks' λ for the general model resulting from the canonical analysis of the 14 populations of *P. phoxinus* was 0 at $p \le 0.01$, indicating strong and statistically significant discrimination power of the model. The percentage of correct classification (based on generalised distances for particular specimens from the cluster centroids) for the data matrix we used to compute the model was high and ranged from ca. 67% to 100% for individual populations. On average, ca. 89% of specimens from the population were correctly classified within the model (Table 2). The first nine canonical vectors were significant at the $p \le$ 0.05 level, and the first three explained 69% of the total variation.

Discrimination of the studied populations in three-dimensional space based on the first, second, and third canonical vectors was used to depict tendencies in morphometric similarity of the populations (Fig. 4). The extreme positions along vector I were occupied by populations from Czadeczka (smallest vector values) and Pischevoy and Ayagoz (greatest vector values). An intermediate position between the population from Czadeczka and the cluster of central Asian populations was occupied by the population from Brzozówka, and that between the population Ayagoz and the remaining central Asian populations was occupied by the Ulken Boken population. Vector II discriminated the Birobidzhan population (smallest vector values) from



Fig. 3. Average character values for all studied populations (after Burnaby's correction for size); bars show 95% confidence intervals, c1–c26 are the "truss network" characters.

most other populations, and at least the position of the populations Bystry and Pischevoy could be regarded as intermediate. With respect to the mean of vector II, the Oskemen population was close to most populations with higher values of that vector (Fig. 4B). However, the range of variation of the vector values assumed by individual specimens of this population (Fig. 4A) placed it close to the Bystry and Pischevoy populations. The greatest values of vector III were those for populations of Pischevoy, Bystry, and Solonovka and the smallest were for Birobidzhan and Zharma (Fig. 4B).

The combined effect of the first three canonical vectors distinguished the following extreme populations or clusters, which were the most distinct from all the remaining populations: (1) typical group including populations from the Danube (Czadeczka) and Vistula (Brzozówka) basins; (2) population from the Amur basin (Birobidzhan) and (3) the central Asian group (Lepsy, Kurchum, Zharma, Solonovka, Ulken-Boken, Sarkand, and probably Ayagoz). The Pischevoy population was similar to that from Ayagoz, probably also with respect to the remaining vectors (the MST tree connected it with Ayagoz). The distance between Pischevoy and Ayagoz was similar to that between Ayagoz and the remaining central Asian populations. The choice of a cut-off point of the central Asian group for high values of vector I was thus arbitrary. All remaining populations were, to varying degrees, intermediate between these three main clusters. Considering the information conveyed by the MST tree (Fig. 4B), which corresponded not only to the first three but also to the remaining canonical vectors, it was possible to interpret the status of the intermediate populations of Bystry, Belaya, and Oskemen. They did not form a single cluster as suggested by the first vectors, since each was closest to a different population from outside the three. The MST tree suggested that: (1) Brzozówka was closest to Oskemen; (2) Belaya to Ulken Boken; and (3) despite the great distance in the MST tree, Bystry was closest to Birobidzhan. The Oskemen population was, thus, morphometrically close to the typical populations of P. phoxinus (Brzozówka, Czadeczka).

 Table 2. Classification of specimens from particular populations based on the CVA model.

Population	Percentage of correct classification
Ayagoz	96.55
Belaya	81.82
Birobidzhan	100.00
Brzozówka	84.00
Bystry	83.33
Czadeczka	100.00
Kurchum	66.67
Lepsy	71.43
Oskemen	100.00
Pischevoy	100.00
Sarkand	81.82
Solonovka	100.00
Ulken Boken	85.71
Zharma	100.00
Mean	89.38

Results from PCA (not shown) were essentially congruent with those of CVA regarding the pattern of morphometric variation of the populations, though it showed, as expected, a lower "resolution". The first two principal components distinguished only three clusters with partly overlapping ranges of variation. These clusters included Czadeczka, Birobidzhan, and a large cluster including all the remaining populations. The remaining populations in that complex cluster maintained positions similar to those resulting from CVA. The first three principal components explained ca. 58% of the total variation.

Participation of characters in the interpopulational variation

Factor structure coefficients (Table 3) indicate that vector I (35% of the variation) is mainly due to individual differences in characters c5, c12, c13, c14, c18, c19, c21, c22 and c25 (absolute values of individual correlations ranged between 0.30 and 0.49). Differences explained by the first vector were responsible for the complex change of body shape (Fig. 5A). The increasing value of vector I was accompanied by: (1) a relative increase in the head size, with the shape changing toward a trapezoid, increasingly resembling a rectangle (due to the elongation of distances c13 and c14 relative to the remaining cephalic distances); and (2) a decrease in height and length of the posterior part of the body, excluding the length of the base of the dorsal fin, which increased. Specimens from populations on the right-hand side of the diagram (Fig. 4A, central Asian populations, in particular Ayagoz and Ulken Boken, as well as Pischevoy from the Kolyma basin), i.e., those with greater values of vector I, had larger heads and smaller posterior body parts compared with those populations on the left-hand side of the diagram (Czadeczka). In order to illustrate its effect on morphology of the studied minnows, we can call vector I the 'cephalisation factor'.

Vector II (22% of the variation) was mainly due to differences in characters c5, c6, c7, c17, c18, c20, c21, and c25 (absolute values of individual correlations ranged between 0.32–0.64). Differences explained by vector II corresponded to the following changes that took place with





increasing value of this vector: (1) an increase in the height of the whole body, especially its posterior part and the posterior part of the caudal peduncle (base of the caudal fin); and (2) a shortening of the whole body, especially the caudal peduncle, and to a lesser extent the anterior part of the thorax (Fig. 5B). Specimens in the bottom part of the diagram (Fig. 4A, Birobidzhan) were,

with respect to the effect of this vector, more slender compared with those in the top part of the diagram (most populations). To illustrate its effect on morphology, we can term vector II the 'slendering factor'.

Vector III (12% of the variation) was due mainly to differences in characters c7, c16, and c21 (absolute values of individual correlations ranged between 0.30–0.35). With an increasing value of vector III, the following morphological changes were observed: (1) an increase in the length of the caudal peduncle but, in contrast to vector II, the height of its posterior part remained essentially unchanged; (2) a shortening of the anterior part of the thorax (from the end of the head to the beginning of the dorsal and caudal fins); and (3) a shortening of the bases of dorsal and anal fins, and a complex change of shape of the whole posterior region of the thorax, which was difficult to interpret (Fig. 5C). Specimens

from populations in the bottom part of the diagram (Fig. 4B, Zharma and Birobidzhan) tended to have a reduced length of the caudal peduncle in relation to the rest of the body compared with specimens in the top part of the diagram (most populations). To illustrate its effect the morphology, we can call vector III the 'peduncle factor'.

Vector IV (9% of the variation) was mainly due to combined differences in characters c3, c4, c10, c16, c17, and c26 (absolute values of individual correlations ranged between 0.30–0.35). Differences explained by this vector were rather difficult to interpret. With increasing values of vector IV, the following morphological changes occurred: (1) a decrease in lengths of the snout and the mandible, accompanied by an increase in the size of the remaining parts of the head; (2) a shortening of the anterior part of the thorax; and (3) an elongation of the posterior part of the thorax including the base of the dorsal fin

Table 3. Factor structure coefficients of the first six canonical vectors. Cum. prop = cumulative percentage of explained variance.

Character	Vector 1	Vector 2	Vector 3	Vector 4	Vector 5	Vector 6
c1	0.25	0.07	0.26	-0.24	0.06	0.10
c2	0.26	-0.01	-0.04	0.22	-0.23	0.08
c3	0.10	-0.17	-0.22	-0.32	0.04	-0.03
c4	0.16	0	-0.17	0.30	0.08	0.16
c5	-0.32	-0.64	0.24	0.21	-0.09	-0.13
c6	-0.05	0.40	0.10	0.13	0.02	0.27
c7	-0.20	-0.40	0.34	-0.05	0.01	0.02
c8	0.07	-0.08	-0.24	-0.02	0.28	0.07
c9	-0.21	-0.07	0.15	0.24	0.17	-0.09
c10	0.09	-0.17	-0.27	-0.33	-0.15	0.01
c11	0.12	-0.15	0.17	0.26	-0.29	0.09
c12	0.30	0.18	0.01	-0.11	0.30	-0.12
c13	0.43	0.22	0.03	-0.20	-0.07	0.08
c14	0.42	0.05	-0.09	0.12	-0.23	0.11
c15	-0.03	0.16	0.01	0.07	-0.18	-0.16
c16	-0.08	-0.08	-0.30	-0.48	0.03	-0.20
c17	-0.24	0.38	0.14	0.30	-0.20	-0.04
c18	-0.49	0.32	0.05	-0.07	-0.02	0.26
c19	-0.44	0.13	-0.13	0.28	-0.24	0.07
c20	-0.24	-0.48	0.26	0.04	0.02	0.03
c21	-0.32	-0.47	0.35	0.14	-0.12	-0.04
c22	-0.42	0.17	-0.11	0.07	0.39	-0.11
c23	0.13	-0.11	-0.25	-0.26	-0.19	-0.05
c24	0.18	-0.01	0.14	0.21	-0.30	0.07
c25	-0.33	0.32	-0.12	-0.04	0.19	-0.38
c26	-0.09	0.10	-0.18	0.47	-0.07	-0.02
Eigenvalue	5.28	3.29	1.78	1.34	1.17	0.69
Cum. prop.	0.35	0.57	0.69	0.78	0.86	0.90



Fig. 5. Morphological interpretation of the first four canonical vectors.

(Fig. 5D). The other changes were not easily interpreted in geometric terms. Considering the effect of vector IV, individuals with the smallest values of this vector (Pischevoy and Zharma) tended to display a greater proportion of the preorbital part of the head in the total size of the head compared with the specimens with the greatest values of the vector (Lepsy and Sarkand). Morphological tendencies associated with the values of individual vectors often depended on relatively small correlations, with absolute values ranging between 0 and 0.64.

Discussion

Infraspecific systematics

Our results indicate the existence of three groups: populations from the Amur basin (1); the central Asian group (2); and a typical group (3). Differences primarily concerned the relative size of the head and the length of the posterior part of the thorax (1), the height of the body (2), and the relative length of the caudal peduncle (3). Assuming that the described morphometric vari-



Fig. 6. Taxonomic interpretation of the morphometric diversity of *Phoxinus phoxinus*. Numbers correspond to the possible taxa postulated in this paper, the dotted line indicates ranges of the taxa, and "?" indicates uncertain status.

ation is genetically based, it may also provide the basis for reasonable hypotheses on the infraspecific systematics of *P. phoxinus*, and make it possible to address suggestions in the literature of the existence of forms deserving a formal distinction within the taxon (Kottelat 2006, Bogutskaya *et al.* 2008).

Any attempt at interpreting the morphometric variation within P. phoxinus in terms of systematics requires reference to the nominal form of the species. According to Linnaeus (1758), terra typica of the species includes the whole of Europe. Unfortunately, the type locality is unknown and the holotype no longer exists. Thus, it was necessary to designate a neotype. Kottelat (2007) designated Cyprinus phoxinus Linnaeus, 1758, specimen NRM 55108 (Naturhistoriska Riksmuseet, Stockholm) as the neotype, and the River Agger (Rhine drainage basin, western Germany) as the type locality. It seems reasonable to assume that other European populations should be closer to the neotype (typical population) and that, in the absence of a sample from this population, they should be regarded as representatives of the typical form of P. phoxinus. It is difficult to decide which

of the European populations used in this study is closest to the neotype population from the Rhine basin, but since results of the analysis place them close to each other (Fig 4A and B) it is not important or decisive for taxonomic interpretations. Assuming that one of the European populations analysed in our paper (Vistula basin: Brzozówka, Danube basin: Czadeczka) is closest to the neotype of *P. phoxinus* and based on results of our morphometric analyses, the following hypotheses can be formulated and conclusions drawn pertaining to the taxonomic differentiation of the species (Fig. 6):

- The population Birobidzhan from the Amur basin may be a distinct taxon. It shows the greatest morphometric departure from the typical populations with respect to the first three vectors.
- The central Asian populations, both from the upper Irtysh basin (Kurchum, Solonovka, Ulken Boken, Zharma) and from the undrained basin of Lake Balkhash (Lepsy, Sarkand), may form one taxon that is distinct from typical populations; the position of the Ayagoz population is ambiguous.

- 3. The population from the Kolyma basin (Pischevoy) is morphometrically close to the Ayagoz population, and the one from the Irtysh river (Oskemen) is close to populations from central Europe (Czadeczka and Brzozówka). In both cases, conspecificity of these forms is unlikely (the distance between them is more than 4500 km and they are separated by several river systems). Similarity of the two remote forms, combined with their considerable departure from the typical form, may indicate convergent evolution and repeated acquisition of a relatively small spectrum of body shapes by different populations of *P. phoxinus*. In a species with a rather simple body shape like the Eurasian minnow, independent adaptive changes, often under similar environmental conditions may lead to similar changes in a few crucial body regions (caudal peduncle length and height as well as the following thorax sections: head size; mouth size; and length of fin bases). This may result in morphologically similar but genetically different forms. The possibility of independent formation of a similar body shape, conditioned by similar but also independent genetic changes, was shown by Collin and Fumagalli (2011) for river and lake populations of P. phoxinus in the Swiss Alps. The effects of purely plastic variation also cannot be excluded. We should be cautious when attempting to interpret the taxonomic position of the Pischevoy population because this is the only sample in our analysis where a part of the data for some specimens were missing and were replaced with mean values for purposes of analysis. This could have an effect on the position of this population in the analyses.
- 4. Populations from the remaining localities in Eurasia (Bystry, Belaya) have an unclear status. They differ only slightly from the remaining populations (central position in diagrams in Figs. 4A and B, 6) and do not form a cluster. It must be noted that we did not sample the entirety of the Eurasian minnow distribution and there may be additional morphotypes that could either be a completely distinct from, similar to, or intermediate between the distinct morphotypes found in the present study.

Body shape variation

Individual populations/clusters within *P. phoxi*nus tend to adopt different body shapes that can be characterised based on the values of the first four canonical vectors combined with the Factor Structure Matrix (Figs. 4–6 and Table 3), and the values of the mean population characters after removing the effect of body size (Fig. 3). Characters with values close to the extremes for the whole inter-population variation of *P. phoxinus* are potentially good diagnostic characters for individual taxa.

Specimens from the Czadeczka population (Fig. 7A), which occupied a more extreme position in the diagram than the second typical population (Brzozówka), had: (1) a very high head/anterior part of the thorax (c15); (2) a very high posterior part of the thorax (c25, c18); (3) anterior part of the thorax (from the end of the head to the beginning of the dorsal and ventral fins [c3, c10]) of average length; (4) a very short base of the dorsal fin (c4); (5) an extremely short base of the anal fin (c8); (6) a very long pV-pA distance (c9); (7) a long (c5, c7) caudal peduncle extremely high in its anterior part (c19) and of average height in its posterior part (c6, base of pC); (8) an extremely short (c1) and extremely low (c13) snout; (9) an extremely short mandible (c12); and (10) a head very short in its upper part (c2), of average length in its lower part (c11), and very high (c15). On the whole, the head was thus very high and rather short and with a very short snout. The head shape, combined with the very high posterior part of the thorax, very small snout, short pA and long pV-pA distance, can be regarded as diagnostic for typical populations (close to typical) of the Eurasian minnow.

Specimens from the Birobidzhan population (Fig. 7B) had: (1) an extremely low head/ anterior part of the thorax (c15); (2) a very low posterior part of the thorax (c25, c18); (3) an extremely long anterior part of the thorax (from the end of the head to the beginning of the dorsal and ventral fins [c3, c10]); (4) a long base of the dorsal fin (c4); (5) an extremely long base of the anal fin (c8); (6) pV–pA (c9) distance of average length or long; (7) a very long or long (c5 and c7) caudal peduncle of average or low height in its anterior part (c19) and extremely



Fig. 7. Typical body shapes of *Phoxinus phoxinus* specimens from the **(A)** Czadeczka, **(B)** Birobidzhan and **(C)** Ulken Boken populations.

low in its posterior part (c6, base of pC); (8) a snout of average length or short (c1) and of average height or low (c13); (9) a short mandible (c12); and (10) a specific shape of the head, long/very long in its upper part (c2), of average length in its lower part (c11), and extremely low (c15). We can regard the very low head and the whole thorax, extremely long anterior part of the thorax, extremely long pA, and long and low caudal peduncle as diagnostic characters of the population from the Amur basin (Birobidzhan).

The very low caudal peduncle as a character distinguishing the population from the Amur basin from typical *P. phoxinus* populations has been pointed out by Kottelat (2007). The hypothesis that this population represents a distinct taxon is shared by Bogutskaya *et al.* (2008). Based on their preliminary, unpublished results of genetic studies, Bogutskaya *et al.* reported that *P. phoxinus* from the Amur basin differed from populations from the basin of the Arctic Sea, and from *Phoxinus tumensis* from rivers of southern Primorye and northern Korea.

Specimens from the Ulken Boken population (Fig. 7C), which we regard as representative of the central Asian cluster, had: (1) a very high head/anterior part of the thorax (c15); (2) a very low posterior part of the thorax (c25, c18); (3) the anterior part of the thorax (from the end of the head to the beginning of the dorsal and ventral fins - c3, c10) of average length/short; (4) base of the dorsal fin of average length (c4); (5) a short/very short base of the anal fin (c8); (6) pV-pA (c9) distance of average length; (7) a caudal peduncle of average length/short (c5 and c7), of average/low height in its anterior (c19) and posterior parts (c6, base of pC); (8) a snout of average length (c1) and an extremely high (c13); (9) an extremely short mandible (c12); and (10) a head of specific shape, of average length or long in its upper part (c2), of average length in its lower part (c11), and very high (c15). The very high head and very low posterior part of the thorax made the middle part of the fish body resemble a rectangle (small difference in height). We can regard the shape of the thorax and the extremely small mouth in the extremely high snout as diagnostic characters of the central Asian population from Ulken Boken.

At present, it would be premature to analyse morphometric and potentially diagnostic characters of the Pischevoy population because of the partially missing data in the initial matrix. Among the three above-mentioned populations, it was most similar to the Ulken Boken population.

The hypothesis on the distinct taxonomic status of populations from the upper Irtysh basin (Kurchum, Solonovka, Ulken Boken, Zharma) and the undrained basin of Lake Balkhash (Lepsy, Sarkand, Ayagoz) is compatible with Kottelat's (2006, 2007) view, according to which differences between forms from the upper Irtysh basin and those from the Ob and Yenisey are so great that a more detailed analysis should classify them into at least a few distinct species.

The issue regarding body shape and its variation with habitat has long been discussed (Haas et al. 2010, Pfennig et al. 2010). The analysis conducted by Langerhans (2008) revealed that phenotypic plasticity and genetic diversity played important roles in the differentiation of fish body shapes, but the relative importance of these two sources was not unambiguously determined. Collin and Fumagalli (2011) demonstrated that lake populations of P. phoxinus in the Swiss Alps had a higher body and caudal peduncle compared with stream populations. Those authors ascribed differences in body shape to genetic factors rather than plastic variation. Some of the AFLP loci examined by these authors (2.9%) showed deviations from neutrality and a significant relationship with a particular type of habitat - lacustrian or fluvial. Therefore, we can reasonably assume that morphological variation, which in our study on P. phoxinus concerned, inter alia, height of the body and the caudal peduncle (slendering factor), is at least partly related to genetic factors. It can thus be assumed that the observed differences are of distinctly taxonomic character.

Perspectives

General morphological differences between closely related populations do not provide a sufficient basis for the recognition of monophyletic groups and the reconstruction of their divergence history. Ongoing genetic studies sampling mitochondrial and nuclear loci will reveal the actual significance of the described morphometric differentiation. Mapping variation on an independent and reliable molecular phylogeny should make it possible to determine the significance of supposed frequent homoplasy in the evolution of body shape of *P. phoxinus* and the role of habitat-induced plasticity in total morphometric variation. One should also remember that it is difficult (or impossible) to interpret the ecological or evolutionary relevance of genetic phylogenies that do not include a phenotypic component.

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