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RESEARCH ARTICLE **Open access**

Comparison of the body shape of *Aphanius vladykovi* populations (Teleostei: Aphaniidae) using geometric morphometric method

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Abstract
The main purpose of this study was to investigate the morphological changes and body shape variations of the Zagros Tooth-carp *Aphanius vladykovi* populations in six water bodies located in Chaharmahal and Bakhtiari province, Iran. For this purpose, 240 specimens were collected from the Choghakhor and Gandoman wetlands, the Shalamzar, Balagholi, and Brovi springs, and the Beheshtabad River. Twelve landmark points were digitized on the right side of the fish. Statistical analyses including Principal Component Analysis (PCA), Discriminant Function Analysis (DFA), and Cluster Analysis (CA) were performed using TPS package, SYSTAT 9, and PAST Software. Discriminant function analysis revealed that female fish in different localities show more pronounced body shape differences compared to males from different localities (P<0.001). In addition, in clustering analysis performed among males and among females, two clusters were resolved, which were fairly similar for males and females. These differences might be attributed to the morphological adaptation of the Zagros Tooth-carp to their habitats but a clear inference on the observed morphological divergence will be possible with more genetic, ecological, and experimental evidence.

Key words: *Zagros Tooth-carp, Morphology, Landmark, Ecological conditions.*

INTRODUCTION

Killifishes (Cyprinodontiformes) are a large group of secondary freshwater fishes that tolerate a wide range of temperatures and salinities Teimori *et al.,* (2014). They have evolved special adaptations enabling them to survive in shallow fresh-to-hyperhaline waters, coping with marked spatio-temporal fluctuations of environmental parameters (Kroll, 1984). Killifishes have been widely used in environmental monitoring programs and in studies concerning the adaptations to environmental pressures that characterize these aquatic systems (Burnett *et al*., 2007).

The family Aphaniidae is composed of one genus (*Aphanius*) and 39 described species Esmaeili *et al*. (2018). The greatest species diversity is found in the Near East, particularly in Iran and Turkey Teimori *et al*. (2018). Species belonging to killifishes are characterized by small size, short life span, and the ability to adapt and counteract with fluctuations of environmental

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parameters Cavraro *et al*. (2017). The endemic Tooth-carp, *Aphanius vladykovi* is found in the Central Zagros Mountains of Iran and its type locality is the Choghakhor Wetland in the upper reaches of the Karun River of the Tigris River drainage (Coad, 2017). The male *Aphanius vladykovi* has vertical stripes on the flanks and the female has dark spots on both sides of the body (Coad, 2017). Also, the unique feature of the female is absence of black spot in the base of caudal fin.

Morphological features of organisms can adapt to different environmental conditions and their evolution and diversity are due to competition, hunting, or other biological interactions Santos *et al*. (2011). Because fishes are more sensitive to changes in the environment than other vertebrates, they are more likely to experience intra- and inter-species changes (Turan, 2008). The environmental changes can lead to the separation of fish populations from each other Yamamotoa *et al*. (2006). Different tools have been used to assess morphological variations of different fish populations. Geometric morphometrics is a fairly new approach that extracts biological forms using landmark coordinates and statistical analyses for the purpose of a better evaluation of morphological characteristics of fish (Eagderi *et al*., 2014; Tabatabei *et al.,* 2014). In fact, the differences in the shape of individuals and parts of the body of fish can be shown using a geometric morphometric technique with greater accuracy and it is a time saving method (Zelditch, 2004; Sfakianakis *et al*., 2011).

In the present study, the body shape variation of *A. vladykovi* inhabiting different habitat types including spring, wetland, and riverine habitats, was studied using a geometric morphometric approach.

MATERIAL AND METHODS

A total of 240 specimens were collected from six localities including the Choghakhor and Gandoman wetlands, the Shalmazar, Brovi, and Balagholi springs, and the Beheshtabad River using scope net and a SAMUS backpack electrofisher. Geographic coordinates of the sampling sites are presented in table 1 and Fig. 1. After collection, fish were fixed in 10% formalin solution. To facilitate determination of landmark points, fish were fixed using colored needles on a Styrofoam panel for photography (Hashemzadeh Segherloo *et al*., 2012). The right sides of fish were photographed with a digital camera from a fixed height and with a vertical angle to fish side. Twelve landmarks were digitized on the 2D images of male and female fish (Fig. 2). A Generalized Procrustes Analysis (GPA) was used in order to eliminate non-form features (including scale, orientation and position of the images). Principal Components Analysis (PCA) and Discriminant Function Analysis (DFA) were used to make intra-sex comparisons among individuals of the same sex (males or females) from the studied habitats. To select variables in DFA analysis a Backward Stepwise approach with F-to-Enter: 4.0 and F-to-Remove: 3.9 was used. The multivariate analyses such as Principal Component Analysis (PCA), Discriminant function analysis (DFA), and cluster analysis were performed using TPS package (Version 1.20), SYSTAT V9 and PAST (Version 2.17).

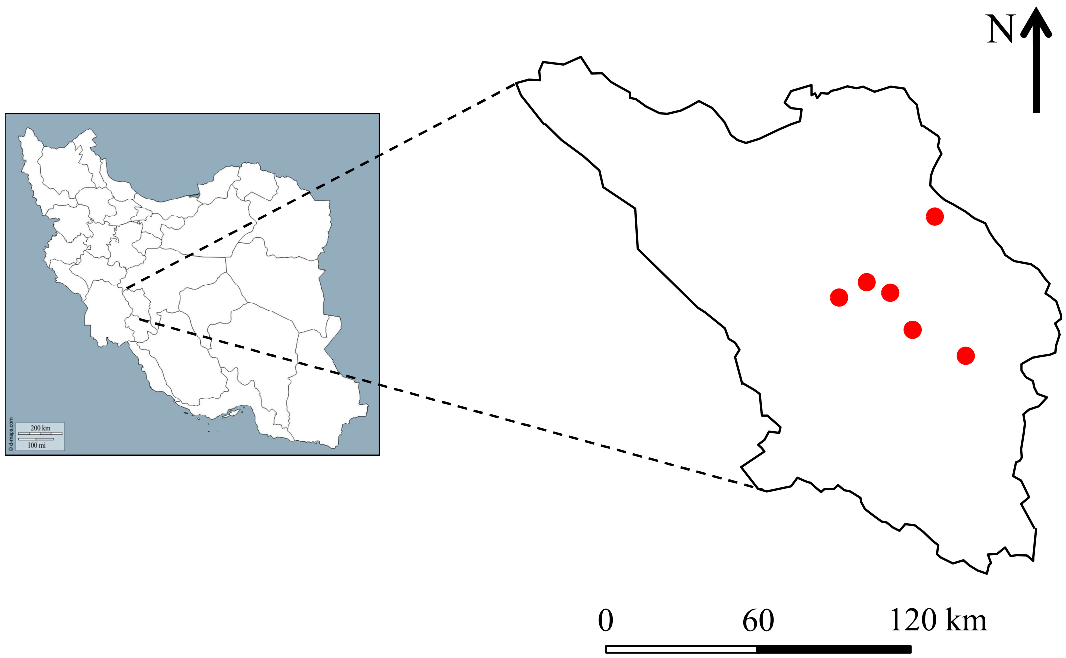

FIGURE 1. Sampling localities (red circles).

FIGURE 2. Position of landmarks on male (A) and female (B) *A. vladykovi*. 1- snout; 2- anterior end of the dorsal fin base; 3- posterior end of the dorsal fin base; 4- postero-dorsal end of the caudal peduncle at its connection to the caudal fin; 5- the most posterior part of the caudal peduncle; 6- postero-ventral end of the caudal peduncle at its connection to the caudal fin; 7- posterior end of anal fin base; 8- anterior end of anal fin base; 9- pelvic fin base; 10- lower edge of pectoral fin base; 11- posterior end of the operculum; 12- eye center.

TABLE 1. Geographical coordinates of the sampled areas.

Speciece name	Locality	N(♀/♂)	Date	Geographical position
A. vladycovi	BAL	35 (18 /17)	2017-06-08	N 32° 03′ 22.81″ E 50° 46′ 12.88″
A. vladycovi	BEH	33 (19 /14)	2017-01-03	N 32° 03′ 17″ E 50° 39′ 28″
A. vladycovi	BER	48 (30 /18)	2017-01-02	N 32° 16′ 56.7″ E 50° 59′ 22.9″
A. vladycovi	CHO	34 (19 /15)	2016-11-28	N 31° 55′ 24″ E 50° 54′ 29″
A. vladycovi	GAN	40 (19 /21)	2017-06-08	N 31° 50′ 08″ E 51° 06′ 04″
A. vladycovi	SHA	50 (20 /30)	2016-11-28	N 32° 02′ 49″ E 50° 49′ 03″

* BAL: Balagholi Spring, BEH: Behesht-abad River, BER: Berovi Spring, CHO: Choghakhor Wetland, GAN: Gandoman Wetland, SHA: Shalamzar Spring.

RESULTS

Body shape: In PCA analysis the first three principal components described 49.95% of the total morphological variation among the female fish (PC1=28.81%, PC2=12.40%, PC3=9.73%) and 62.67% of the total morphological variation among the male fish (PC1=23.83%, PC2=12.22%, PC3=11.57%) (Fig. 3A and B). Within-sex (among males and among females) discriminant function analysis showed that the populations from different localities can be discriminated differentially (Fig. 4A and B). For example, female fish from Balagholi Spring, Gandoman Wetland, and Beheshtabad River clustered very closely along the first and second discriminant functions, and females from Shalamzar Spring and Berovi Spring showed close relationship along the first DF axis (Fig. 4 A). Males from Choghakhor Wetland and Shalamzar Spring clustered closely along the first and second DF axes (Fig. 4B). Male fish from Beheshtabad River, Gandoman Wetland, and Balagholi Spring clustered closely along the first and second DF axes (Fig. 4B). The distribution patterns of both male and female fish from different localities in DF graphs were nearly similar. Both males and females from the Berovi Spring were separated from other populations along the second DF axis.

Assignment based on discriminant functions, showed that female fish from the Shalamzar Spring and the Choghakhor Wetland with correct assignment of 89 and 84 percent were highly diverged compared to females from other localities with correct assignment of 70-78 percent. Female fish from the Gandoman Wetland with 57 percent correct assignment were the least discriminated female fish group (Table 2; Wilks's Lambda: 0.057, P<0.001). The average correct assignment for female fish was 74 percent. The average correct assignment of male fish was 52 percent and males from Balagholi Spring showed the highest correct assignment (71 percent) (Table 2; Wilks's Lambda: 0.090, P<0.001). The correct assignment of male fish from other localities were 32-62 percent.

TABLE 2. Jackknifed classification matrix for female and male *A. vladykovi*

Populations	Females							Males						
	BAL	BEH	BER	CHO	GAN	SHA	%C	BAL	BEH	BER	CHO	GAN	SHA	%C
BAL	14	0	0	0	4	0	78	1	0	0	3	12	1	71
BEH	0	14	2	0	4	0	70	8	0	0	4	1	0	62
BER	0	4	21	1	2	2	70	1	10	1	0	3	3	56
CHO	0	1	1	16	1	0	84	2	1	8	0	0	4	53
GAN	2	4	1	0	12	2	57	7	2	1	6	2	1	32
SHA	0	0	0	1	1	17	89	2	4	9	0	1	15	48
Total	16	23	25	18	24	21	74	21	17	19	13	19	24	52

* %C: Percentage of correct assignment, BAL: Balagholi Spring, BEH: Behesht-abad River, BER: Berovi Spring, CHO: Choghakhor Wetland, GAN: Gandoman Wetland, SHA: Shalamzar Spring.

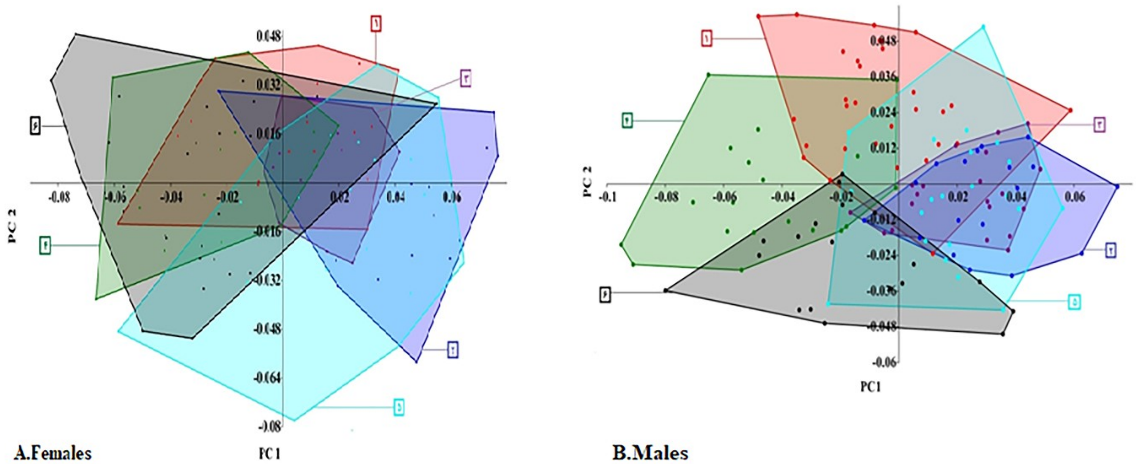

FIGURE 3. PCA graph showing distribution of *A. vladykovi* (A. Female and B. Male) from different localities (1- Brovi 2- Balagholi 3- Behesht-abad 4- Choghakhor 5- Gandoman 6- Shalamzar) along the first and second PC axes.

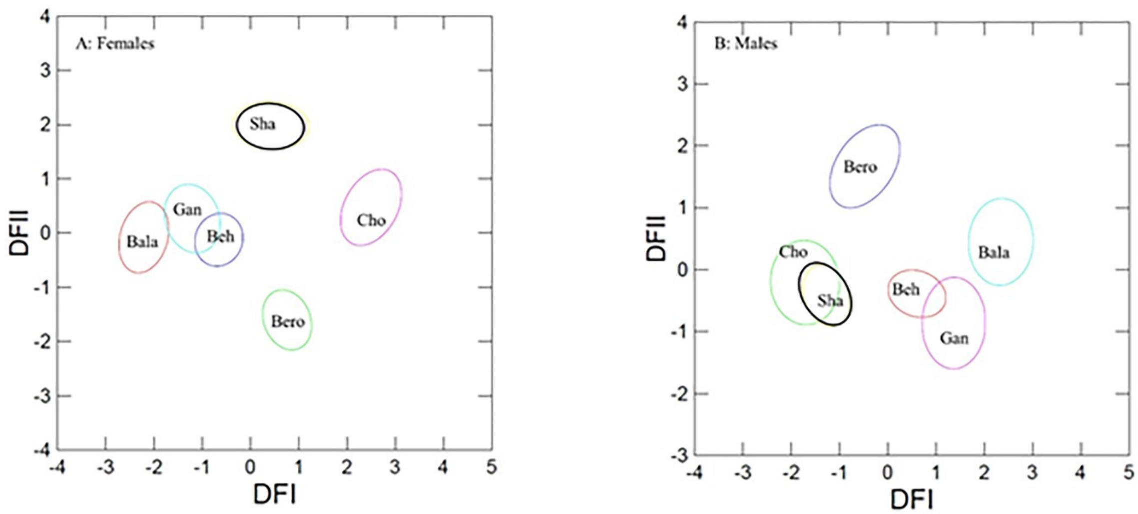

FIGURE 4. Distribution of 95% centroids of A: female and B: male individuals from different localities along the first and second discriminant function axes. Abbreviations are: Bero: Berovi Spring, Cho: Choghakhor Wetland, Sha: Shalamzar Spring, Beh: Beheshtabad River, Gan: Gandoman Wetland, and Bala: Balagholo Spring.

The changes of each landmark in male and female populations are shown in Fig. 5 (A & B). Landmarks 2 (anterior end of dorsal fin base), 8 (anterior base of anal fin), 9 (posterior base of pelvic fin) and Landmark 11 (posterior end of the operculum) showed considerable changes compared to other landmarks among the female fish populations (Fig. 5A). Landmarks 8 (anterior base of anal fin) and 9 (posterior base of pelvic fin) had the most variations compared to other landmarks among the male fish populations (Fig. 5B).

According to cluster analysis of female fish (Fig. 6A), the female fish populations were composed of two main clusters. The first cluster included populations of the Shalamzar Spring, the Choghakhor Wetland, and the Brovi Spring with a 77% bootstrap support. Populations from the Gandoman Wetland, the Balagholi Spring, and the Beheshtabad River were nested in the second group with 83% bootstrap support (Fig. 6A). Male fish nested in two main clusters. The first cluster included populations from the Shalamzar Spring and the Choghakhor Wetland with a 45% bootstrap support. Populations from the Gandoman Wetland, the Brovi Spring, the Balagholi Spring, and the Beheshtabad River nested in the second cluster with a bootstrap support value of 63% (Fig. 6B).

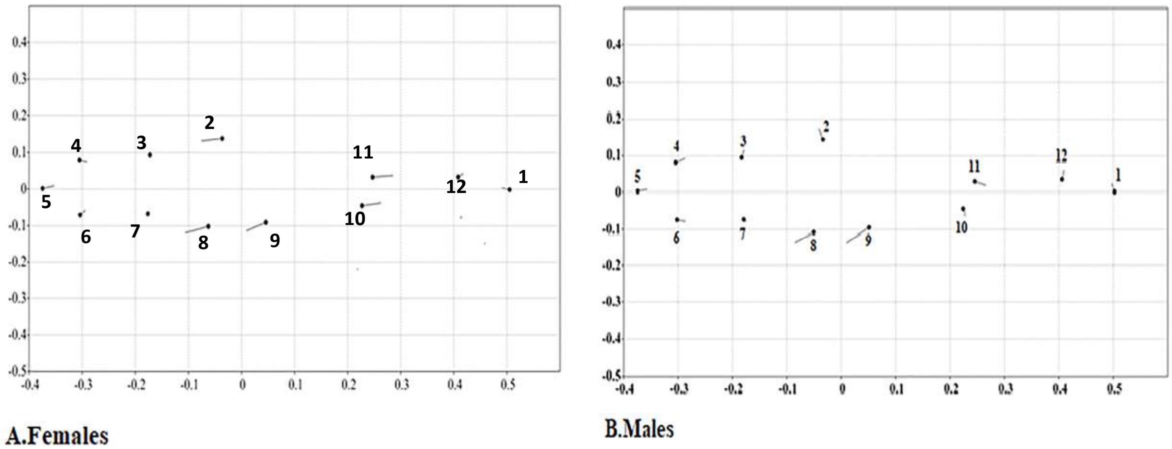

FIGURE 5. Changes in landmarks and effective landmarks (2, 8, 9, and 11).

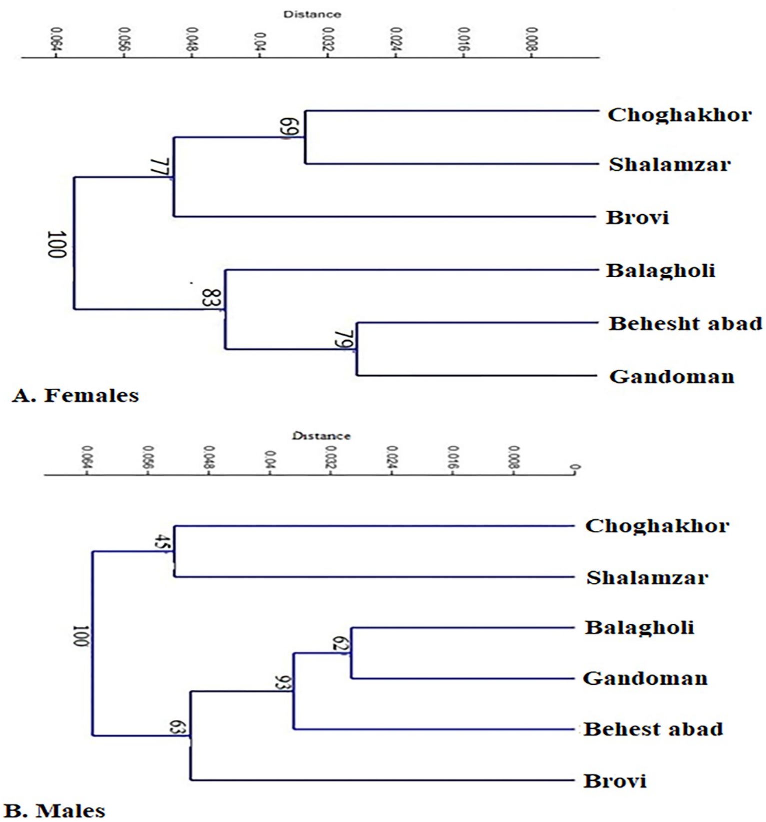

FIGURE 6. Dendrograms reconstructed via cluster analysis of the body shape of *A.vladykovi* populations in the studied areas. Values along the branches are bootstrap supports.

DISSCUSSION

The present study was conducted to compare the body shape of *Aphanius vladykovi* populations in the Chaharmahal and Bakhtiari province by geometric morphometrics approach. The results obtained from PCA and DFA analyses, revealed some extents of morphological differentiation among the studied populations. The most pronounced variation in body shape in males was related to the landmarks 8 (the origin of the anal fin) and 9 (the base of pelvic fin) and in females it was related to the landmarks 2 (the origin of the dorsal fin), 8, 9, 10 (base of the pectoral fin), and 11 (rear end of the operculum). Khosravi *et al*. (2017) compared three populations of *Aphanius dispar* using morphometric geometrics and reported the dorsal fin as an important source of body shape variation in both male and female fish, which is only concordant with our observation for female *A. vladykovi*. The observed morphological diversity may be created by genetic and environmental variation among the studied populations (Langerhans, 2008). Abolhasani *et al*. (2020) did not report any pronounced mtDNA differentiation among different *A. vladykovi* populations in Chaharmahal and Bakhtiari province. They used COI sequences to explore possible cryptic diversity among different populations. However, at population level the gene they used, does not have enough resolution to provide answers to ecological questions (Hallerman, 2003). Hence, their observation cannot rule out genetic causes for the morphological variation observed here, since in other fishes the use of nDNA markers including microsatellites and genome-wide data had been useful to answer intra-specific morphological diversity or fine-scale differences among fish (Hashemzadeh Segherloo *et al*., 2012; Hashemzadeh Segherloo *et al*., 2018). Hosseini *et al*. (2013, 2016) using microsatellite loci compared *A. vladykovi* populations in the Shalamzar Spring and the Gandoman Wetland and in two springs. They reported an insignificant population differentiation between each pair of populations they studied. It appears that different populations of *A. vladykovi* are not highly differentiated in their population genetic structure that is concordant with our morphological data. Additionally, Pazooki *et al*. (2008) compared four populations of *A. vladykovi* using morphometric and meristic features. They reported that the studied populations were significantly different in 33 out of 38 morphological variables they had used. Their results appear to be contradictory to our results. Bibak *et al*. (2016) compared two *A. dispar* populations in two sulphurous springs in south of Iran and reported an overlapping distribution for the two analyzed populations.

Anyhow, as indicated before the morphological variability can be created also via environmental effects. The habitat types we studied were the river, wetland, and spring habitats. These habitats can be different in respect to flow rate and the observed levels of morphological divergence might be related to the differences in flow rate or other features of the habitats. For example, the Beheshtabad River should have higher flow rate compared to other habitats that are mostly stagnant or slow flowing water bodies. Hence, if the differentiation in body shape was flow rate dependent, the Beheshtabad River population had to show much pronounced differences compared to populations from other non-riverine habitats. Accordingly, we think the flow rate might not bear considerable effect on the observed morphological differences. Furthermore, in the riverine habitat analyzed here, fish were collected from stagnant habitat patches on river banks with aquatic plant growth that decrease the flow rate. In our clustering analyses for males and females also there was no clear relationship between habitat type and the clustering patterns. More robust inferences on the relationship between body shape variation and environmental or genetic factors in the studied populations awaits more data and we may not be able to provide robust inferences on the observed morphological variation using only data produced in this study.

Individual assignment test revealed that females in the studied habitats show higher morphological divergence compared to their male counterparts, since the average correct assignment rate for females was considerably higher compared to males. This may reflect a pronounced morphological plasticity of females compared to males, which should be tested via *in-situ* and *in-vitro* observations on the effects of different environmental factors on phenotypic attributes of female and male *A. vladykovi*.

Overall, our study showed some sort of overlapping shape variability among *A. vladykovi* populations in different habitat types, but we could not find any clear relationship between habitat type and the observed shape variation. One of the reasons for such inconclusive results may be the fact that the studied populations are from very closely located habitats with high degrees of ecological and genetic composition and probably very short isolation background. A robust inference on relationships of these populations would be possible via collection of high-quality population genetic and ecological data along with different experiments and observations on the response of the species to different environmental variables.

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