

Fish morphology and mitochondrial phylogeny reveal translocations of a native *Aphanius* Nardo, 1827 (Teleostei: Cyprinodontidae) in Iran

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Abstract: Species of the genus *Aphanius* are known for their attractive color pattern. They are also suitable biological tool for controlling of mosquito. For that reason, they could be potential candidates to be translocated from natural environments to artificial man-made environments such as aquarium, canals and pools. In present paper, morphometric, morphological and molecular evidences as well as fish external morphology suggest the translocation of native *Aphanius* populations (belong to *A. dispar* group) between different drainages in Iran. The results indicate that *A. dispar* found in a man-made pool in Kahnuj city, and in man-made canals in Bandar Abbas city had been introduced. It has already been documented that populations from south, southeastern and southwestern Iran probably are distinct; therefore, translocation of the individuals between these genetically divergent populations probably have adverse effects through the loss of genetic diversity, and also make their taxonomic relationships difficult to understand.

Keywords: Fish movement, Genetic diversity, Human activities, Cyprinodontids.

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Introduction

The movement of fish species beyond their natural range is potentially one of the most ecologically damaging of human activities (Koehn 2004). Consequently, management of translocated species may be one of the biggest challenges that conservation biologists face in future (Harris & Battaglene 1990; Harris 2003; Lintermans 2004). The translocation of native species in particular can have impacts upon indigenous populations of native fish, the general ecosystem into which translocations occur, as well as subsequent social and economic impacts over time (Morgan et al. 2004).

The killifish *Aphanius* Nardo, 1927 are small fishes, which are naturally distributed in Old World region including; costal drainages of Mediterranean Sea, all coastal lines of Red Sea and Persian Gulf as well as the inland waters in Anatolia and Iran

(Wildekamp 1993; Teimori et al. 2012; Gholami et al. 2014; Esmaili et al. 2014). Because of a well-known color pattern seen in the members of genus *Aphanius*, they are good candidate to be translocated between and within drainages. The other characteristic that made them to be a target of translocation is their wide range tolerance to temperatures and salinities in different environments.

Iran is a center of speciation for *Aphanius* species, so that approximately half of the known *Aphanius* species (14 species) living in this country (e.g., Esmaili et al. 2014; Gholami et al. 2014; Teimori et al. 2012, 2014) and of which 10 species are endemic to Iran (see also Esmaili et al. 2010, 2015a, b; Masoudi et al. 2016). Therefore and as considering the background mentioned above, translocation of the members of this taxon within and even between different drainages is likely. Since

Table 1. Details of sampling sites and comparative materials (*) used in this study. Numbers after sites refer to sampling sites in Fig. 1.

Site	Population status	Number	Basin	GPS points
Shur River (1)	native	35	Hormuzgan	E 56°,28',10.2" N 27°,19',37.6"
Band Abbas city (2)	translocated	17	Hormuzgan	E 56°,17',37.5" N 27°,11',77.2"
Kahnuj city (3)	translocated	14	Hormuzgan	E 57°,42',26.1" N 27°,57',18.4"
Kol River* (4)	native	17	Hormuzgan	E 55°,45',31.2" N 27°,07',40.3"
Mirahmad spring* (5)	native	40	Helleh	E 51°,16',50.9" N 28°,47',56.4"
Irandedgan* (6)	native	37	Makran	E 60°,49',52.3" N 27°,26',48.2"
Kahnuj city (7)	native	1, used in molecular analysis	Hormuzgan	E 57°,42',26.1" N 27°,57',18.4"

translocation of the native fish populations between geographically and genetically isolated drainages can promote the mixing of genetic pool; therefore, it would cause adverse effects through the loss of genetic diversity.

In this paper, we aim to use both morphometric and morphological as well as molecular evidences to identify the similarity and differences between populations of *Aphanius dispar* group in different drainages in south, southwestern and southeastern Iran, and provide new information on translocation in this region. Also, this is the first report on translocation of *A. dispar* group in this region. It is important to improve our knowledge concerning this issue because more than 90 percentages of the Iranian *Aphanius* species are endemic and therefore, this huge genetic diversity should be monitored and conserved.

Materials and Methods

Sampling and studied taxon: The fish specimens were collected by using a hand net. The sample sizes, and geographic coordinates of the sampling sites are listed in Table 1. For this study, only adult fish were considered (standard length >25mm) to have a correct morphological comparison of their color pattern. A small muscle tissue of the caudal peduncle (few mm³) of a single specimen from each sites were

taken for genetic analysis.

The studied populations belong to *A. dispar* group. The study is based on three new collected populations from southern Iran; a single wild population from Shur brackish waters River (number 1 in Fig. 1), and one population from a man-made canal within Bandar Abbas city (number 2 in Fig. 1), Hormuzgan province, and a population from a small man-made pool, Kahnuj city in Kerman province (number 3 in Fig. 1). The studied specimens are deposited in the Zoological Museum of Shahid Bahonar University of Kerman (ZM-SBUK).

Comparative materials: The comparative material are as follow; a single populations from Kol River (number 4 in Fig. 1 as typical population of Hormuzgan Basin), a single population from Mirahmad spring (number 5 in Fig. 1 as typical population of Helleh Basin in southwestern part), and a single population from Irandedgan (number 6 in Fig. 1 as typical population from southeastern part) as well as a single specimen native to Kahnuj, which is used only for molecular analysis (number 7 in Fig. 1).

According to Teimori et al. (2012), *A. dispar* in drainages of south (Hormuzgan, number I in Fig. 1), southeastern (Irandedgan, number II in Fig. 1), and southwestern Iran (Helleh, number III in Fig. 1) probably form separate taxonomic units. By considering the external color pattern of the new

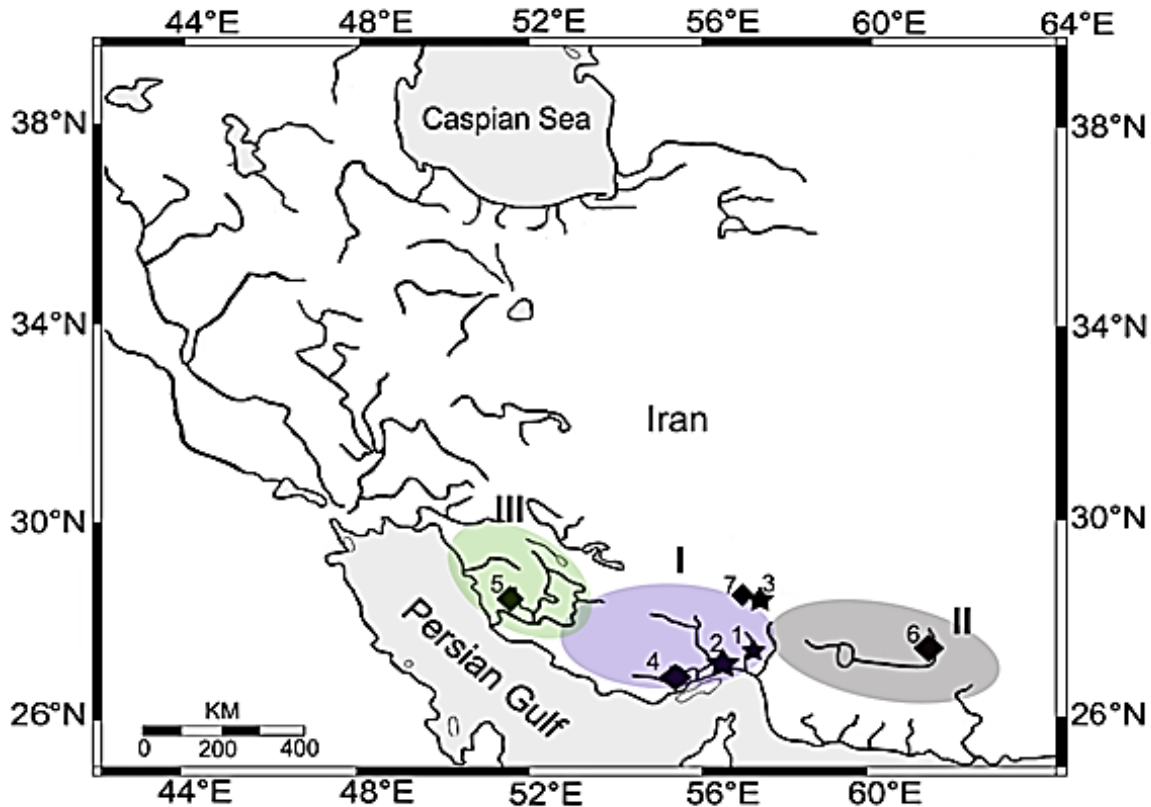


Fig.1. Geographic locations and details of sampling sites in this study. I, II, and III refer to Hormuzgan, Irandegan and Helleh basins respectively. Star symbols show new sampling sites, in which numbers 1-3 are Shur River, Bandar Abbas and Kahnuj sites respectively. Rectangle symbols show comparative sites, in which numbers 4-7 are Kol River, Mirahmad spring, Irandegan, and Kahnuj respectively. All comparative sites and number 1 are native populations. The numbers correspond to the collection sites in Table 1.

collected specimens, we expected that they are translocated to these new sites in Hormuzgan Basin, and that their origin is probably the drainages in southwestern and southeastern Iran. Therefore, we selected our comparative materials from these drainages to have a comparison with the new samples, and to see if a possible translocation has occurred.

Morphometric and molecular analysis: Eighteen linear morphometric characters were measured using a Vernier calliper and recorded to the nearest 0.5mm. Measurements were standardized using standard length (SL), head length (HL) and preanal distance (Prad). Eventually, 31 morphometric variables were calculated and analyzed using PASW 21.00 (SPSS Inc, 2015). The multivariate analysis as canonical discriminant analysis (CDA) was used to show the classification success of the studied populations from

different sites.

To do molecular analysis, a single specimen from each individual location (including the comparative populations) was selected and total DNA was extracted using a commercial DNA extraction kit (DNeasy Tissue Kit, Qiagen) following the manufacturer's protocol.

The complete mitochondrial cytochrome b gene was amplified by polymerase chain reaction (PCR) using the primers GluF (5' AACCCACGTTG TATTCAACTACAA 3') and ThrR (5' ACCTCCGA TCTTCGGATTACAAGACCG 3', Machordom & Doadrio 2001), and then forward and reverse strand were sequenced. Sequences were trimmed and assembled in Geneious 7.1 (Biomatters), and were subsequently aligned using the Muscle 3.6 (Edgar 2004) as incorporated in Geneious. Maximum likelihood reconstructions searches of the partitioned

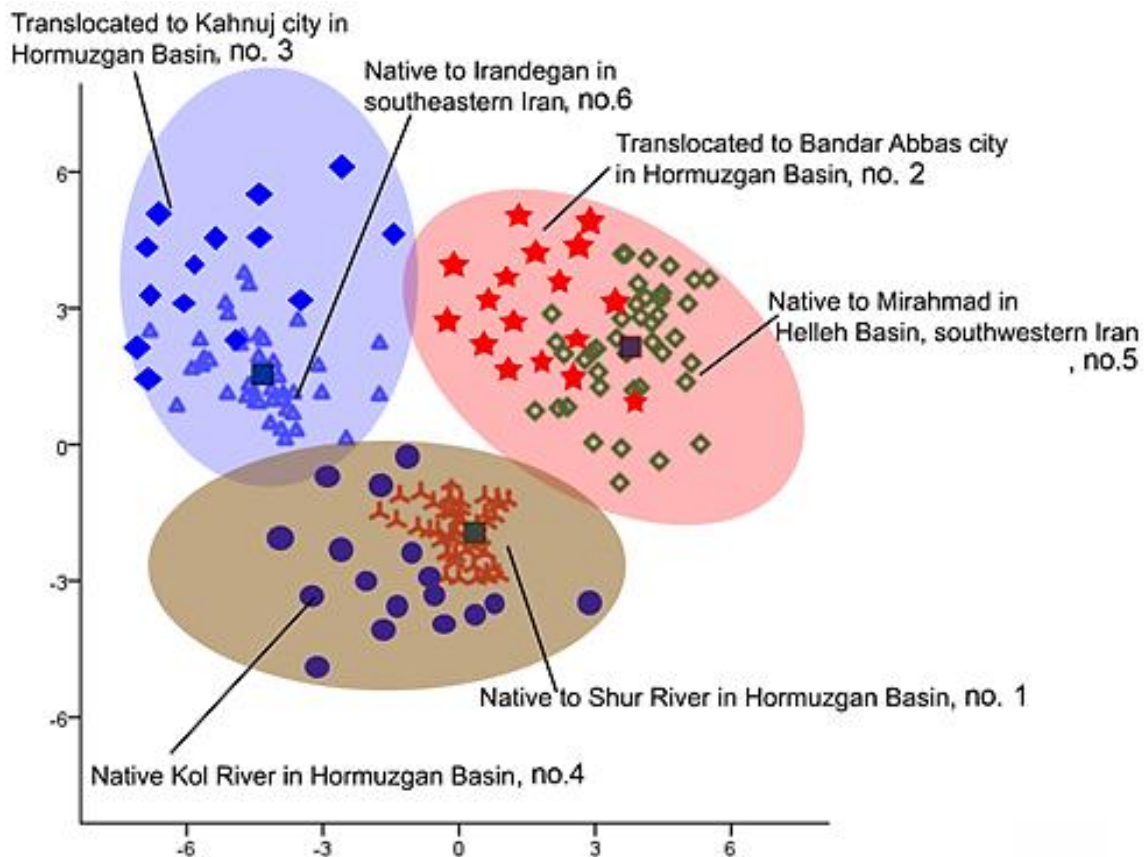


Fig.2. Discriminant function scores with 95% ellipses based on 31 studied morphological variables. The numbers after each site refer to sampling sites in Fig. 1.

dataset were conducted with RAxML v. 7.2.5 (Stamatakis 2006) using a GTR model of nucleotide substitution, with a-shape parameters, GTR-rates, with CAT approximation of rate heterogeneity and fast bootstrap (1000 bootstrap replicates).

Results

Morphological analysis: To do a multivariate analysis, we included the data of morphometric variables of fish specimens from both new sites and comparative materials together to see the pattern of their classification. The results of CDA analysis based on 31 morphometric variables showed a 97.99% overall classification success for the studied populations. Based on the CDA analysis, the translocated specimens (numbers 2-3 in Fig. 1) to Hormuzgan Basin are clearly clustered with those specimens that we expected to be their origin. As results, the translocated specimens to Kahnuj city

(no. 3) are clustered with those from Irandegan (no. 6) in southeastern Iran; the translocated specimens to Bandar Abbas city (number 2) are clustered with those from Mirahmad (number 5) in southwestern Iran. However, both native populations from Hormuzgan Basin (Shur, number 1 and Kol, number 1, Rivers) clustered together (Fig. 2).

In addition, comparison of the external morphology shows similarity between the translocated specimens with those from their original drainages so that, population collected from canals within Bandar Abbas city is similar to those in southwestern Iran (Fig. 3a-b) while, the population collected from a pool within Kahnuj city is morphologically similar to those in southeastern Iran (Fig. 3c-d).

Phylogenetic relationships: new collection vs. original populations: The phylogenetic tree based on Maximum likelihood estimation reveals a clear

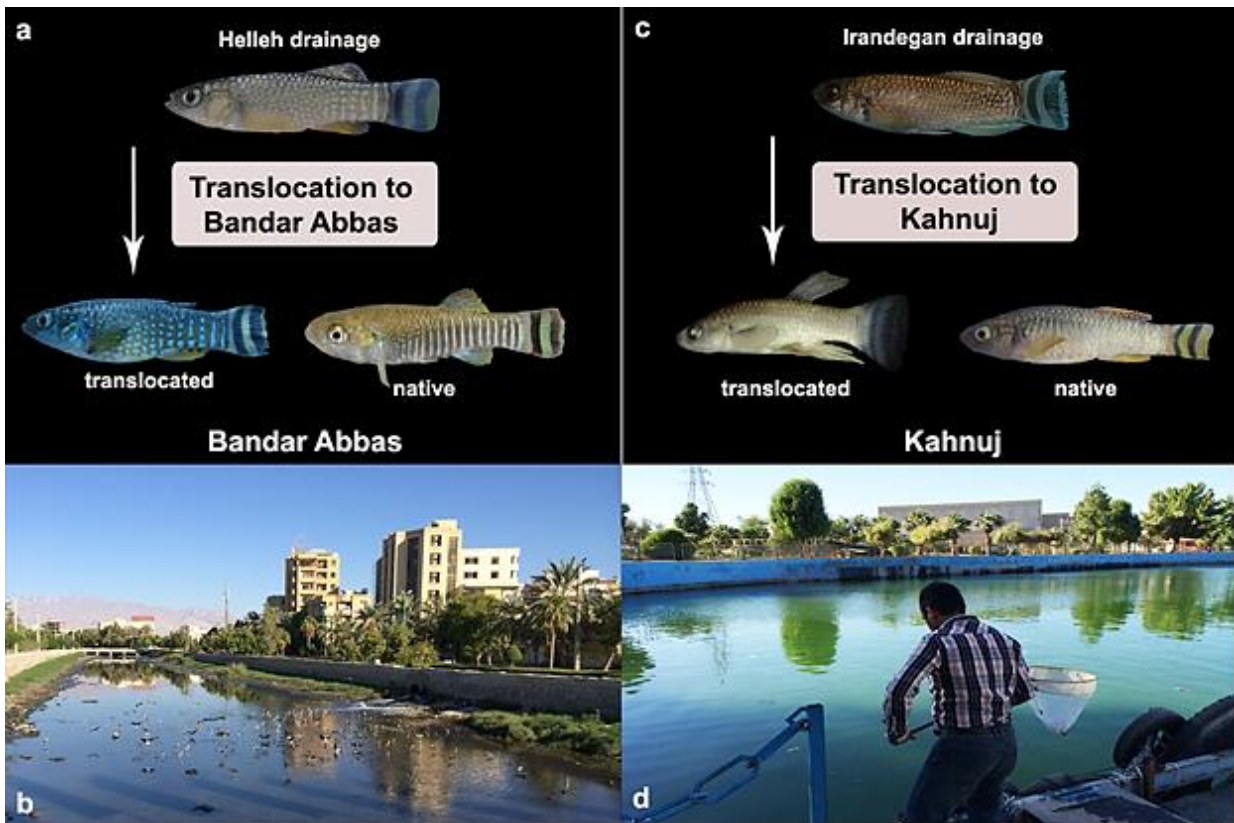


Fig.3. Comparative external morphology shows translocation of populations between drainages. (a) translocation to Bandar Abbas, (b) a man-made canal in Bandar Abbas city where specimens were collected, (c) translocation to Kahnuj, (d) a man-made pool in Kahnuj city where specimens were collected.

differentiation among new collected populations and comparative materials. According to the phylogenetic estimation, without considering their location, all the native populations from Hormuzgan Basin (numbers 1, 4 and 7 in Fig. 4) clustered together. In addition, the studied sequences which considered being translocated are clustered with the sequences of their original specimens. Accordingly, translocated specimens to Kahnuj (number 3 in Fig. 4) is clustered with *A. dispar* from Irandegan, which is native to southeastern Iran (number 6 in Fig. 4) while, translocated specimens to Bandar Abbas city (number 2 in Fig. 4) is clustered with *A. dispar* from Mirahmad, which is native to southwestern Iran (number 5 in Fig. 4).

Discussion

Presence of fish outside their natural range can affect indigenous fish populations via predator-prey interactions as well as direct or indirect competition

for food, habitat and resources. Moreover, the introduction of disease and parasites is also possible via translocated species from other regions. The important issue of fish translocation in view of evolutionary study is the hybridization potential if non-indigenous and indigenous species interbreed. This is important because interbreeding can compromise the genetic reliability of native fish (Barlow et al. 1987). The later can also be important when a taxonomist try to identify fish populations and even species.

Translocation of native *Aphanius* in Iran: This is a first report on translocation of *Aphanius dispar* group between drainages in Iran. Gholami et al. (2015) has already reported translocation in *A. sophiae* group in southern Iran. Based on the total approach (i) morphological analysis (ii) external morphology, and (iii) molecular phylogeny given here, translocation of native *Aphanius* populations from drainages of southeastern and southwestern to drainages of

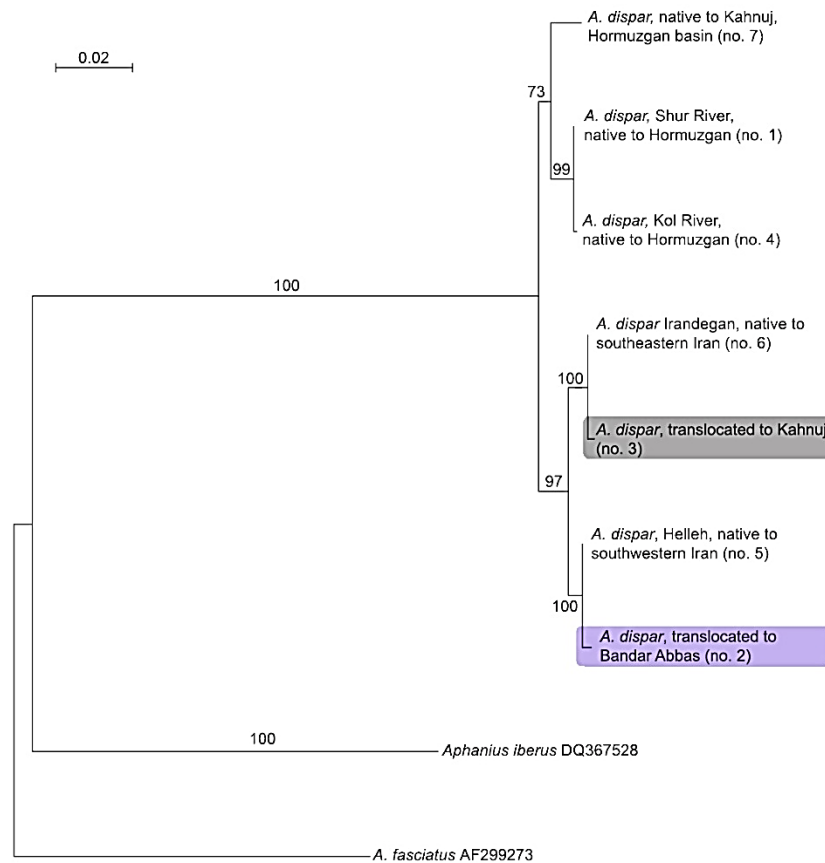


Fig.4. Maximum likelihood estimate of the phylogenetic relationships of the studied populations based on the cytb sequences. Values above branches indicate bootstrap values based on 1000 replicates. Numbers after specific names refer to sites listed in Fig. 1.

southern Iran (i.e. Kahnuj and Bandar Abbas) has occurred.

By considering these evidences, we concluded that the specimens collected from a man-made pool in Kahnuj city have been translocated from drainages in southeastern Iran to this artificial habitat, and that the specimens collected from a man-made canal in Bandar Abbas city have been translocated from drainages in southwestern Iran to this artificial habitat.

The possible cause of translocation and its affects:

The movement of fish between drainages has a long history in Iran with both alien and native species moved since a long time ago. There are several reasons and/or mechanisms for moving fish between the drainages. Coad (1980) has highlighted several natural and human-based factors affecting distribution of fishes in Iran from about 8000 BC, which have been known to be responsible for moving

fish fauna in this country. Example of the natural factor is climate conditions such as last pluvial conditions in Iran, which is ceased before the end of the Pleistocene about 8000BC (Butzer 1972). These conditions which are associated with glacial and post glacial events have caused moving fish populations among different drainages, and provided diversification of fish fauna in Iran (e.g., Teimori et al. 2012; Esmaili et al. 2014).

Natural water level fluctuations are the other known reason, which is suggested to cause the moving fishes between the drainages in southern Iran. For example, Sistan basin in southeastern Iran is a region that receives much of its water from large catchment areas from other regions with a different topography and even climate. However, water level fluctuation could not play role for translocation of populations from drainages of southeastern to south (here Kahnuj) because (i) a long geographic distance

occur between the two locations, and (ii) all the water networks in southeastern Iran running to the Gulf of Oman not to the western part, where Kahnuj city is located (see also Fig. 1).

Irrigation is a kind of human activity which are known to be undertaken in many parts of Iran (Coad 1980), and could act as potential for movement of fishes between the different drainages. However, since in the studied areas of Iran there is little surface water for irrigation, therefore, the essential mechanism for irrigation is “Qanat”. Since, geographic distance between the drainages of southeastern and Kahnuj city is significant therefore; fish populations could not be dispersed naturally by the qanat systems. The other reason is that almost all the qanat systems in these regions have been drought during the recent years and therefore no permanent water network exists between them.

In the case of a possible translocation from the Helleh Basin in southwestern Iran to Bandar Abbas city, none of the abovementioned factors can be considered because of a long geographic distance between the two drainage systems (Fig. 1). However, the members of the genus *Aphanius* demonstrate an attractive color pattern, and in addition, they are very suitable biological tools for controlling of mosquito. Therefore they are potential candidate to be translocated from natural environments to the artificial man-made habitats such as aquarium, canals and pools within the cities and homes (observation of first author).

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جابجایی یک گونه بومی از جنس *Aphanius Nardo, 1827* (ماهیان استخوانی عالی، کپورماهیان دندان‌دار) در ایران بر پایه ریخت‌شناسی ماهی و فیلوژنی ژن میتوکندریایی

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چکیده: اعضای جنس *Aphanius* یا گورماهی به‌خاطر داشتن الگوی رنگی زیبا مورد توجه‌اند. به همین دلیل، اعضای این جنس کاندیدی مناسب برای جابجایی از زیستگاه‌های طبیعی به محیط‌های مصنوعی و دست‌ساز از قبیل اکواریوم، کانال‌های شهری و استخرها می‌باشند. در مطالعه حاضر، برای اولین بار با استفاده از شواهد ریختی و مولکولی اطلاعاتی از جابجایی جمعیت‌های آفانیوس (متعلق به گروه کپورماهی دندان‌دار معمولی *A. dispar*) در بین حوضه‌های ایران گزارش شده است. مقایسه ریختی به همراه آنالیز فیلوژنتیکی جمعیت‌ها نشان می‌دهد که جابجایی جمعیتی از جنوب شرق به حوضه‌ی جنوب (استخری در شهر کهنوج) و از جنوب غرب به حوضه‌ی جنوب (کانال‌های شهری در بندرعباس) در گذشته صورت گرفته است. از آنجایی که بر اساس مطالعات قبلی، جمعیت‌های حوضه‌های جنوب، جنوب شرق و جنوب غرب ایران احتمالاً از نظر ژنتیکی تاکسون‌های جدایی را تشکیل می‌دهند، بنابراین، جابجایی افراد و جمعیت‌ها بین این سه حوضه می‌تواند منجر به کاهش تنوع ژنتیکی شود. همچنین این نوع جابجایی‌ها باعث پیچیده‌تر شدن مشکلات تاکسونومیکی جمعیت‌های مختلف این گروه خواهد شد.

کلمات کلیدی: جابجایی ماهیان، تنوع ژنتیکی، فعالیت‌های انسانی، کپورماهیان دندان‌دار.