Application of functional tree classification (FTs) for detection of body shape variation within and among two species of *Aphanius* (Teleostei, Cyprinodontidae)

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Received: June 2024 Accepted: September 2024

Abstract

Morphometric investigation was conducted on two species of Aphanius (*Aphanius dispar* (Rüppell, 1828), and *A. ginaonis* (Holly, 1929). *A. ginaonis* was sampled from Geno hot spring in Hormozgan province, Iran while *Aphanius dispar*, was from two locations in Bushehr proviance (Boneh Jabery drainage and Mir Ahmad Hot spring). Fourteen characters were measured based on the conventional morphometric concept. In this paper, we introduce an innovative data mining technique (functional trees classification) to assess the pattern of variation within and among the population of cyprinodontidae and to classify them in the northern part of Persian Gulf coast. In the first model, *A. dispar* population was classified as one class and *A. ginaonsis* population as another class using different parameters. To do so, +1 was set as class of fishes from *A. dispar* and -1 as class of fishes from *A. ginaonis*. The speices of *A. ginaosis* from Geno hot spring were discriminated from *A. disparfrom* from Mirahmad hot spring and Boneh Jaberi driage. The result also showed that two populations of *A. dispar* from Bushehr province were well discriminated from each other using functional tree technique. The derived model of the two populations of *A. dispar* and one population of *A. ginaonsis* in this study could be explained as the result of the isolation of the populations and species.

Keywords: Aphanius, conventional morphometric, functional tree modeling, data mining, Iran

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[DOI: DOI: 10.22034/INJBIR.3.1.35]

Introduction

Morphometric is the quantitative study of patterns of covariance with shape (Bookstein, 1991). Morphometric characters are continuous characters describing the body shape, which have provided signal for stock discreteness as documented by Corti *et al*. (1988) and Murta (2000). Meristic characters are the number of discrete serially countable structures, and frequently being used for species identification because they are consierably less affected by environmental changes. A number of studies have dealt with the morphological variation of teleost populations(González, et al, 2016). Many of these studies are based on the analysis of morphometric and meristic characters. Such characters are useful in defining external morphology and show that population-specific morphological traits often differentiate populations from distinct drainage or marine regions (Wilde and Echelle, 1997; Jerry and Cairns, 1998; Mamuris *et al*., 1998; Murta, 2000; Cakic´ *et al*., 2002; O'Reilly and Horn, 2004; Jaferian *et al.*, 2010). According to Bookstein *et al* (1985) and Thorpe, R. (1976), morphological features and their pattern of geographic variation can be influenced both by phylogenies and by current ecology. It is known that both environmental and genetic components are contributed to phenotypic variation in organisms. The genus Aphanius is the only genus of Cyprinodontidae available in Iran which is represented by six species. Among them *A. ginaonis* and *A. dispar* is distributed in southern Iran in endorheic basin (coastal provinces) (Coad, 1998). A morphological analysis based on several selected morphological measurements might be the best method to distinguish the different stocks of Aphanius.

In this research, we used functional trees as data mining technique to classify two tooth–carp. Data mining is a rising field of machine learning which aims to extract or "mine" knowledge from large amounts of data (Han and Kamber, 2006). Data mining techniques are categorized to supervised learning and unsupervised learning. In supervised learning techniques, we build a model using expermental data (training data) , the training data consist of pairs of input objects and desired outputs. Then using built model, we predict the value of the function for any valid input object. If the output of the function is continues, we call the method regression and otherwise we call it classification. This paper represents the result of examination of the pattern of morphological variation among the two cyprinodontidae species population using functional trees as a data mining technique for assessing pattern of variation within and among populations in south of Iran.. Thus, morphometric analyses of *A. ginaonis* and *A dispar* can be a first step in investigation to use those parameters for discriminating two species of Aphanius and discrimination of *A dispar* population fro Bushehr Province.

Material and methods

Fish sampling were conducted in three different locations in Northern part of

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the Persian Gulf as shown in Figure 1, namely Mirahmad and Boneh Jabery in Bushehr province and Geno (27°14́ N and 55°46́ E, Alt. 197 m) in Hormozgan province. Specimens *A. dispar* from Bushehr province were divided into two groups, based on their place of origin: Mirahmad (27°83′10N; 56°82′80E, Alt. 154 m) and Boneh Jabery (27°83′10N; 56°82′80E, Alt. 154 m) populations. Furthermore, the analysis included data from population of *A. ginaunis* from Geno hot spring in Hormoszgan Province.

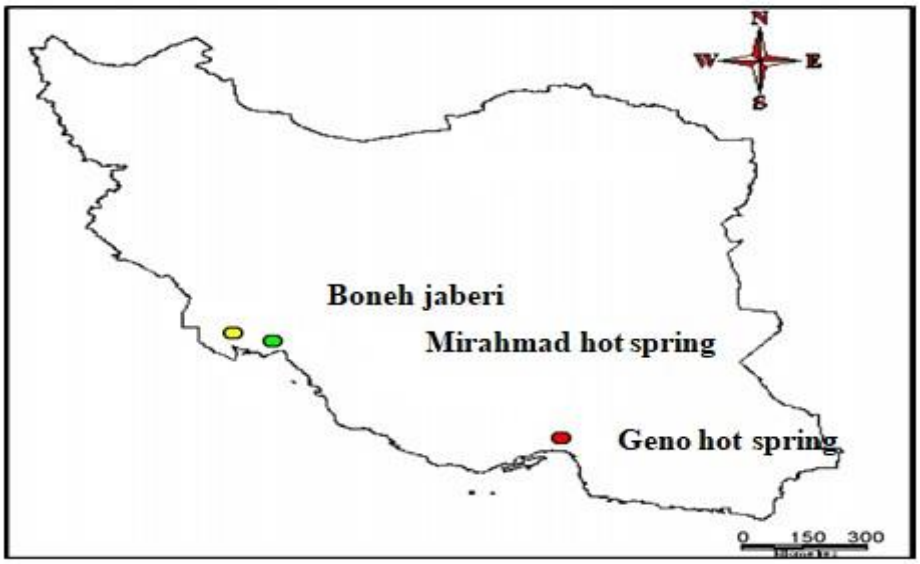


Figure 1: Sampling locations of tooth-carp in various locations in southern Iran.

A total of 99 specimens of *A. dispar* (49 male and 44 female species) and 47 specimens of *A. ginaonis* were collected from Bushehr and Hormozgan provinces by hook and line and castings net . In addition some was being collected by hand and scope net and anaesthetized with 3-aminobenzoic acid ethyl ester (methanesulphonate salt) 0/1% and then fixed in 5% formalin and preserved in 90% ethanol alcohol. Counts, measurements, and descriptions of general body shape were carried out following the method of Coad (1980). All measurements were straight line distances made with digital calipers and recorded to the nearest 0.1mm (Rohlf, 1990; Bronte *et al*., 1999; Daud *et al*., 2005). The body characteristics measured is listed in Table 1.

Fourteen selected conventional morphometric characters were measured on individual sample (Fig. 2). The following morphometric characters were measured: total length (TL), standard length (SL), head length (HL), head width (HW), head depth (HD), snout length (SNL), predorsal length (PDL), eye diameter (ED), body depth (BD), first dorsal fin length (D1L), pectoral fin length (PFL), anal fin length (AFL), caudal fin length (CFL), and caudal peduncle length (CPL) . To reduce the allometric effects and make the results more comparable, each measurement was expressed as a ratio to the standard length or head length.

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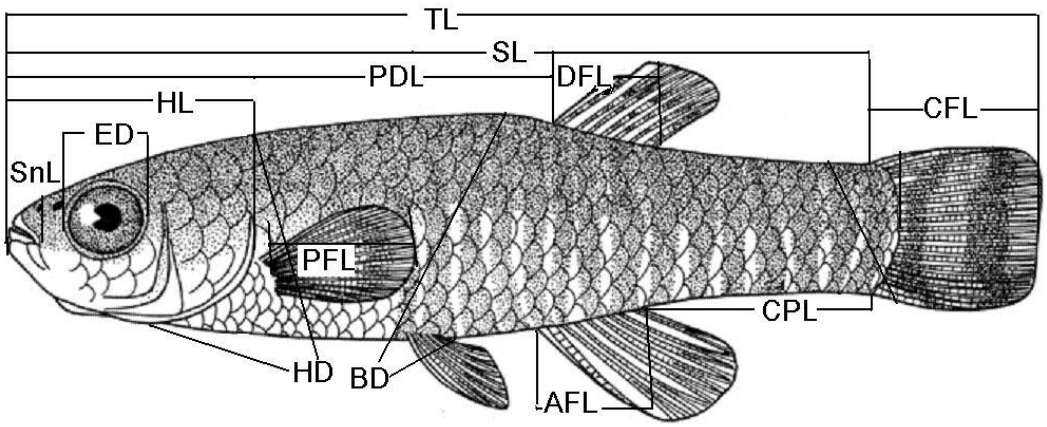


Figure 2: Location of conventional morphometric character measued in tooth-carp.

In this paper, we used functional tree as a data mining technique to recognize the pattern of morphological variations. Data mining techniques are categorized to supervised and unsupervised learning. In supervised learning techniques, we build a model using expermental data (training data). The training data consist of pairs of input objects and desired outputs. Then using built model, we predict the value of the function for any valid input object. If the output of the function is continues, we call the method regression and otherwise we call it classification. In unsupervised learning, we seek to determine how the data are organized by categorizing input data set, so we have no value for prediction. Therefore, it is clear that our task, in this paper, is in category of supervised learning (classification). Therefore, we should have a data set as training data. Using the training data set, we build a functional tree model (Gama 2004; Landwehr *et al*., 2005; Daud *et al*., 2005). Also, to valid the model and estimate its accuracy we use stratified cross validation (Han and Kamber, 2006). Functional trees (FTs) are a generalization of multivariate trees. FTs are able to explore to do decision tests based on a combination of attributes, in addition to single attributes.

The general algorithm to build trees (i.e. decision trees) has two phases. In the first phase a large tree is constructed and then, in the second phase this tree is pruned back to reach an optimal tree in terms of size and generality. The growing tree algorithm follows the standard divide-and-conquer approach. The most relevant aspects are the splitting rule, the termination criterion, and the leaf assignment criterion. If we have two different classes A and B (as the current work), the growing process of the tree continues until a node contains only instances of one type (A or B) or number of instances positioned in a node becomes less than a predefined threshold. If the growing continues until no two distinct values of x for the cases in the training samples belong to the same node, the tree may be overfitting the training samples and not be a good classifier of future unseen cases.

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In the growing process, the model (tree) accuracy typically improves as the tree grows, but usually at some point, the misclassification rate for unseen cases will start to get worse as the tree becomes more complex. In this case, it is said that the model is overfitted. An overfitted model has good accuracy for training samples but bad prediction accuracy for unseen cases. Therefore, overfitted models are not generalizable and have high generalization error. After completion of the tree growth, we initiate the pruning process. The aim is that the model becomes more generalizable. In the pruning process, some subtrees of the initial tree are lumped into their root nodes. The most important part of the pruning process is pruning rule. The rule determines which subtrees should be lumped into their root. shows a tree that classifies samples from two classes A and B using attributes B6-8, B8-10, B1-4, and B6-8.

Different strategies for the splitting rule, the termination criterion, and the leaf assignment criterion in tree growing phase and pruning rule in pruning phase lead to different tree models. In fact functional tree presented by Gama (2004) is a framework that can be used to develop different tree shows the general algorithm for building a functional tree from a data set and shows the pruning algorithm.

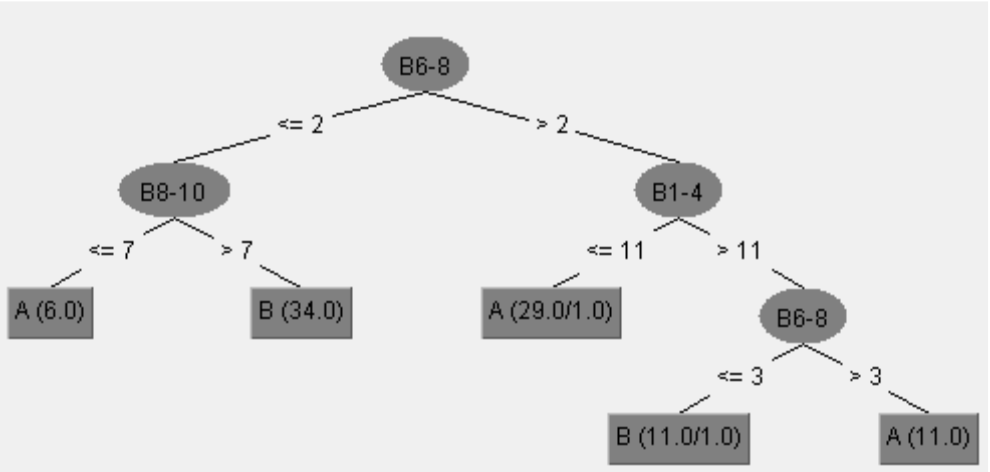


Figure 3: A sample tree for classification of samples from two classes A and B.

The model development

As shows the tree growing process is a recursive algorithm, it means that we start from a root node and assign all training samples to it. Then we partition training samples, using a technique based on an attribute or combination of attributes, into two categories and assign each category to a child node of the root node (we create two children of the initial node and in this way, the tree grows). Recursively we develop subtrees of the children nodes using assigned training samples. The termination criterion for our study is that a node contains instances (samples) of only one class of fishes or the number of assigned instances to the node is less than five (we set 5 as termination threshold).

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Function GrowTree(Dataset, Constructor)

1. If Stop_Criterion(DataSet)
   - Return a Leaf Node with a constant value.
2. Construct a model Φ using Constructor
3. For each example $\vec{x} \in DataSet$
   - Compute $\hat{y} = \Phi(\vec{x})$
   - Extend $\vec{x}$ with new attributes $\hat{y}$.
4. Select the attribute of original as well as of newly constructed attributes that maximizes some merit-function
5. For each partition i of the DataSet using the selected attribute
   - $\text{Tree}_i = \text{GrowTree}(\text{Dataset}_i, \text{Constructor})$
6. Return a *Tree*, as a decision node based on the selected attribute, containing the Φ model, and descendants Tree_i.

End Function

Figure 4: Pruning a functional tree [Gama 2004].

The technique, we use to partition the training sample in each recurrence is logistic regression (Le Cessie and Houwelingen, 1992). To do so, if the termination criterion is not meet for a node, we classify training samples assigned to the node using logistic regression. We use the linear combination of the attributes resulted from logistic regression as a compound attribute. In addition, we partition the assigned training sample using different attributes of the data set. Finally, we compare number of misclassifications in the different partitions (using single attributes and the compound attribute) and select the attribute (single or the compound attribute) with the minimum classification error as the splitting attribute of the current node. The tree growing process continues until all leaves of the tree meet termination criterion. In this case, we initiate the pruning algorithm.

In the pruning process, we extract a sequence of smaller trees by pruning the developed large tree in the previous phase. To do so, splits that were made are removed and a tree having a fewer number of nodes is produced. Then we compare accuracies of the members of this sequence of subtrees using good estimates of their misclassification rates and we chose the best performing tree in the sequence as the final classifier tree. To create the sequence of the subtrees, we prune off all of the nodes that arise from a specific internal (non-leaf) node (leaving that specific node as a leaf node), and the specific node selected is the one for which the corresponding pruned nodes provide the smallest per node decrease in the misclassification rate using training samples. In some cases, just two leaves are pruned from a parent node, making it a leaf. However, in other cases, a larger group of descendant nodes may be pruned all at

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once from an internal node of the tree. For example, if the increase in the misclassification rate caused by pruning four nodes is no more than twice the increase in the misclassification rate caused by pruning two nodes, pruning four nodes will be favored over pruning two nodes.

Results and disscution

Fourteen morphometric measurements were carried out on each sample and the datas were recorded in mm The range and mean±standard deviation values of morphometric characters (male and female) for 2 Aphanius species and two populations of A. *dispar* are presented in Table 1.

The present study demonstrates significant variation within and among two species of moderately differentiated *A. dispar* and *A. ginaosis*. Rohlf and Marcus (1993) and Struass stated that morphometrics essentially deal with methodology for the statistical study of shape variation and the co-variation of shape with intrinsic (morphogenetic) and extrinsic (ecological and evolutionary) causes. Coad (1980) were found significant differences in all morphometric characters between male and famele of A. ginaonis except body depth in total length, interorbital distance in head length and snout length in head length (p>0.05). While in present study only five important morphometric characters (Hw, HD, SnL, BD, D1B) were used to charactraize male of *A. ginaonis*. In female fish 4 characters were used (ED, AB, CFL, PFL) to charactraize body shape of female fish of A. ginaonis with acceptable precision of the model (97.91 and 97.67). Coad (1980) showed no significant, difference between female A. ginaonis and female A. dispar but male of A. ginaonis had shorter ventral fins than male A. dispar. Whereas, those models (1 and 2) used 9 different characters to difference between female A. ginaonis and female A. dispar. Differences among the species fall into three broad categories: meristic characters, pigmentation and morphometric parameter. Our analyses showed significant body shape differences among the two species beyond what would be expected, based on the variation among populations within those species.

In this research a model was developed to classify two populations of tooth-carp fish from Bushehr province (two populations of *A. dispar*) and one population from Hormozgan province (*A. ginaonis*). In the first model, Mirahmad and Boneh Jaberi populations was classified as one class and Geno population as another class using parameters of Sex, HL, HW, HD, SnL, ED, TL, SL, BD, D1B, AB, PFL, CFL,CPL, PreDL, PFL.

To do so, +1 was set as class of fishes from *A. dispar* and -1 as class of fishes from *A. ginaonis* species. After building the larger initial tree in the first phase, the tree was pruned according to the mentioned algorithm.

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Table 1: Mean and standard deviation of morphometric characters in *Aphanius ginaonis* and *Aphanius dispar* populations.

Location	*Cyprinodontidae*					
	Mirahmad (Bushehr)		**Boneh Jaberi (Bushehr)**		**Geno (Hormozgan)**	
Species	***A. dispar***		***A.dispar***		***A. Ginaosis***	
	(58) Male	**Famale**	**(10) Male**	**Famale**	**(14) Male**	**Famale**
MC	**Mean±sd (cm)**	**Mean±sd**	**Mean±sd (cm)**	**Mean±sd**	**Range (cm)**	**Mean±sd**
Weight	0.88±0.49	1.1±0.53	0.43±0.14	?0.57±0.25	0.72±0.39	0.78±0.30
TL	37.6±0.52	38.44±7.01	32.69±5.44	34.38±5.05	36.2±6.41	36.9±6.52
SL	31.7±0.47	32.49±4.82	26.28±2.96	29.38±4.86	29.18±5.72	30.04±5.64
HL	0.9.66±1.39	9.7±1.89	7.82±0.80	8.54±1.14	9.18±0.39	9.87±1.95
HW	6.14±1.13	6.52±1.69	5.11±0.56	5.89±0.99	6.58±1.30	7.1±1.55
HD	6.76±1.42	7.07±1.42	5.71±0.65	6.4±1.02	5.91±1.28	6.31±1.33
SNL	2.85±1.17	32.49±4.82	2.51±0.4	2.68±0.44	3.32±1.05	3.44±0.82
PDL	20.1±2.94	6.24±1.39	16.78± 2.05	18.87±2.96	18.3±5.92	20.6±4.10
ED	2.13±0.93	2.2±0.87	2.37±0.32	2.5±0.32	2.45±0.34	2.47±0.35
BD	8.51±2.09	9.33±2.11	6.96±0.91	8.11±1.35	6.93±1.92	7.52±1.85
DL	3.3±0.75	3.43±1.36	3.28±0.73	3.34±0.43	2.61±0.68	2.41±0.52
PFL	6.77±1.3	6.24±1.39	4.8±0.85	5.58±0.94	5.49±1.35	5.42±0.87
AFL	3.2±1.34	3.2±1.33	3.27±0.79	3.39±0.7	3.12±0.91	2.88±0.56
CFL	5.74±1.03	6.67±1.88	4.81±0.45	4.8±1.4	6.01±1.34	6.04±1.12
CPL	5.97±4.47	6.25±4.06	3.98±0.47	4.18±0.66	5.82±1.74	4.59±0.81
Meris						
D	6.9±7.04	7.04±2.22	7.7±1.25	8±0.66	9.3±1.37	9.30±1.88
P_1	16.08±0.59	16.15±0.48	15.1±0.32	16.1±0.7	16.07±0.64	15.61±1.19
A	8.61±1.72	9±1.91	10±1.2	11±0.7	8.76±0.92	9.07±2.4
LLS	26.61±1.36	26.76±1.8	26±1.4	28±1.2	26.76±1.36	26.61±1.8
PDS	12±0.92	13±0.87	10.67±81	10.8±0.83	11.23±0.59	10..84±0.68

Abbreviations: MC = Morphometric character, Meris= Meristic, TL = total length, SL = standard length, HL = head length, HW = head width, HD = hed depth, SNL = snout length, PDL = predorsal length, ED = eye diameter, BD = body depth, DL = dorsal fin length, PFL = pectoral fin length, AFL = anal fin length, CFL = caudal fin length, CPL = caudal peduncle length, D = dorsal fin ray, P_1 = pectoral fin ray, A = anal fin ray, LLS = lateral line scale, PDS = predorsal scale.

The result of pruning was interesting; all nodes except the root node were pruned. In addition, the splitting attribute of the root was a compound attribute for female fishes:

$$ca = 1.82 + [Hw] * -1.66 + [HD] * 0.52 + [Snl] * -0.64 + [BD] * 0.57 + [D1B] * 1.22 \quad (1)$$

From fourteen morphometric characters measured, the model used five characters (Head width, Head Depht, snout length, Body depth, Dorsal based)

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to classify female and male fish of *A. dispar* from Bushehr populations and *A. ginaonsis* from Hormozgan population. Table 2 shows confusion matrix of the classification of female fishes using equation 1. This table shows that there is one misclassification out of 48 instances. Therefore, precision of the model is 97.91 percent. It can be seen that the 97.91 percent of the studied female fishes are linearly distinguishable.

Table 2: Confusion matrix of classification of female fishes using Equation 1.

Classified As	*A. dispar*	*A. ginaonis*
A. dispar	25	0
A. ginaonis	1	22

By repeating the same process for male fishes, again the result is a single node tree with the following compound attribute (equation 2):

$$ca = 1.45 + [HW] * -1.61 + [HD] * 1.17 + [Snl] * -0.73 + [BD] * 0.17 + [D1B] * 0.66 \quad (2)$$

Therefore, to identify class of a case, we put the involved attributes in the above linear equation and get sign of the result (sign (ca): +1 means class *A. dispar* and -1 means *A. ginaonis*). It should be noted that in the tree growing and pruning processes we have an automatic attribute selection. It means that the algorithm automatically ignores the attributes with less impact on characterization of the fish class.

Table 2 shows confusion matrix of the classification of male fishes using equation 2. It can be seen that the 97.67 percent of the studied female fishes are linearly distinguishable. The results of equations 1 and 2 show that almost all studied fishes are linearly distinguishable. The results of present conventional morphometric investigation using functional tree methods confirms that populations of *A. dispar* are well differentiated from population of *A. ginaosis*. In fact, this study showed that these two populations are linearly distinguishable with very high precision. Esmaeili *et al.* (2008) pointed out karyological study of 5 tooth carp fish of Iran, they were found the karyotypes differentiation formula of *A. dispar* and *A. ginaonis*, which is support species differentiation using mtDNA control in this study. The results reported here indicated that there is a significant differences among the tooth-carp populations from Bushehr in term of the monomorphic characteristics. A significant difference were observed in body size of the fishes from the two locations. Coad (1980) were comprised male and female of *A. dispar* and *A. ginaonis.* He found that A. ginaonis exhibits many meristic, morphometrie and other characters which separate it from *A. dispar*. (Table 1).It is because *A. ginaonis* lives in a unique, isolated environment, a hot spring "These factors are considered sufficient to re-affirm *A. ginaonis* as a species distinct from *A. dispar*" (Coad, 1980).A higher

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morphological values for the Mirahmad samples were also clear (linearly distinguashable) (Table 3).

Table 3: Confusion matrix of classification of male fishes using Equation 2. The table shows that there is one misclassification out of 43 instances. Therefore, precision of the model is 97.67 percent.

Classified As	*A. dispar*	*A. ginaonis*
A. dispar	22	0
A. ginaonis	1	20

In addition, based on morphological attributes used to classify fishes, +1 was set as class of population of A. dispar from Mirahmad and -1 as class of population from Boneh jaberi.

Using the following compound attribute, female fishes can be classified:

$$ca = 2.09 + [ED] * 0.72 + [AB] * 1.18 + [CFL] * -0.39 + [PFL] * -1.66$$

Table 4 showa that female fishes from Mirahmad are linearly distinguishable from fishes from Boneh jaberi with precision of 96 percent. The table shows that there is one misclassification out of 25 instances. Therefore, precision of the model is 96 percent. According to possible reasons, sympatric morphotypes in Mirahmad habitats are could be related to hot spring morphology. Factors such as physico-chemical condition of spring and the type of substrate available to populations in Mirahmad may affect the development of multiple morphotypes (Coad, 1980). While drainage has special condition in which salinity was higher and temperature was lower than Mirahmad which affects on food abundance.

Table 4: Confusion matrix of classification of female fishes using Equation 3.

Classified As	Mirahmad	Boneh Jaberi
Mirahmad	12	0
Boneh Jaberi	1	12

However, Behnke (1980) was of the opinion that sympatric stocks may have been subject to multiple invasions of that have developed in geographical isolation or they may be the result of differentiation in response to ecological conditions within a particular water body.

Thus, the results of Behnke (1980) could be imply for Aphanius species in Iran which inhabits in different ecological condition such as different hot spring and drainage.

In addition, the following compound attribute classifies male fishes:

$$ca = 11.03 + [HL] * -1.28 + [ED] * 0.98 + [AB] * 0.93 + [PFL] * -1.04 \quad (4)$$

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Table 5 shows that male fishes from Mirahmad are linearly distinguishable from fishes from Boneh jaberi with precision of 95 percent. The table shows that there is one misclassification out of 20 instances. Therefore, precision of the model is 95 percent.

Table 5: Confusion matrix of classification of male fishes using Equation 4.

Classified As	Mirahmad	Boneh jaberi
Mirahmad	8	0
Boneh Jaberi	1	11

From Fourteen morphometric characters examined, the model used four characters to classify populations of female and male *A. dispar* from Bushehr (Head lenght, Eye dymeter, Anal based and Pectoral Fin lenght). The two geographically isolated populations (Mirahmad Hotspring and Boneh Jaberi) were well differentiated from each other (Equations 3,4). Populations of *A. dispar* from Bushehr were also well differentiated from *A. ginaosis* species from Hormozgan (Equations 1,2).

In conclusion, this study applied functional tree classification technique as a method of fish populations differentiation, for the first time. The observed functional tree of the two populations of A. dispar and one population of A. ginaonsis in this study could be explained as the result of the isolation of the populations and species. There is a possibility that the morphological differentiation of these populations is a result of differences between the environmental conditions where the populations are from, which has been reported for other cyprinodontidae. Species have been chosen for study, it would probably have been sufficiently representative to draw conclusions about differences at the species level.The results found here, while interesting, are to some extent preliminary, considering the wide distribution of the species in all coastal drainage, brackish water and hot spring of the northern parts of Persian Gulf indicate the need for more extensive sampling and comparison of populations.

Acknowledgement

The authors would like to thank by Persian Gulf Research and Studies Center, Boushehr, Iran for financial support. We would also like to thank the following technicians for help in collection of the samples :Sakineh Avizhgan and Ali Fakhri.

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[DOI: DOI: 10.22034/INJBIR.3.1.35]

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