

IMPORTANCE OF CHARACTERS IN FISH TAXONOMY AND PROBLEMS ENCOUNTERED IN SPECIES DESCRIPTIONS

Fahrettin KÜÇÜK*, Salim Serkan GÜÇLÜ

Süleyman Demirel University, Eğirdir Fisheries Faculty, East Campus, Isparta, Turkey

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*Corresponding Author: Tel.: +902462118677; E-mail: fahrettinkucuk@sdu.edu.tr

ABSTRACT

In this review, the importance of taxonomic characters used as key or delimiters in fish taxonomy and the encountered problems were discussed. The taxonomical functions of morphological and molecular data forming the basis of these problems were discussed in the study using cyprinid genus *Pseudophoxinus* samples as an example.

Key words: Taxonomy, character, phylogeny, *Pseudophoxinus*

BALIK TAKSONOMİSİNDE KARAKTERLERİN ÖNEMİ VE TANIMLAMALARDA KARŞILAŞILAN SORUNLAR

ÖZET

Bu araştırmada balık taksonomisinde anahtar veya ayraç olarak kullanılan taksonomik karakterlerin önemi ve bu konudaki karşılaşılan sorunlar irdelenmiştir. Çalışmada bu sorunların temelini oluşturan morfolojik ve moleküler verilerin taksonomideki işlevi bir sazangil cinsi *Pseudophoxinus* örneği üzerinde tartışılmıştır.

Anahtar kelimeler: Taksonomi, karakter, filogeni, *Pseudophoxinus*

INTRODUCTION

Concepts: Taxonomy and Systematics

Taxonomy is a branch of science that is based on classification of organisms according to their similarities, relationships and common ancestry. This branch, therefore, explains the theory and application of an organization reflecting the diversity and evolutionary relationships of organisms in a system. Systematics on the other hand categorizes the biodiversity according to the evolutionary relationships, which is used to group organisms from the primitive to the advanced (Wagele 2004).

What are Characters (Delimiters)?

Morphological, ethological, molecular and any other objective feature that can be used to determine and define phylogenetic relationships among organisms is called as a character (delimiter) (Wagele 2004). The concept of homology used as the basis for phylogenetic systematics is accepted as complicated by many, hence the necessity of cautiousness during justification of hereditary traits and selected characters as verifiable and testable is often underlined (Wagele 2004).

What is Phylogenetic Systematics?

Evolutionary history of a certain organism group is

termed as phylogeny. Using several subdisciplines of biology, it is possible to determine when and which taxon shared a certain organism with the last common ancestor. Significant achievements have been made in comparative morphology and molecular methods in discrimination of the degree of kinship (Bardakçı and Karataş 2005).

Species Concept and Origin of Species

Delineation of the term species is among the hardest questions of biology. Although still there are substantial divergences in character selection and methodology among researchers, it can be said that an established

consensus around main principles has been formed. The idea of binomial nomenclature, scientific method of naming species, was first suggested by Swedish naturalist Gaspard Bauhin in "Pinax" (1623) for plants and applied by another Swedish naturalist Carl von Linnaeus (1707-1778) initially for plants in *Species Plantarum* (1753), afterwards for animals in tenth edition of his *Systema Naturae* (1758) (Demirsoy 1997). This practice gained worldwide acceptance since then and essence of the species concept has not been changed greatly in the mean time, although several descriptions of it have been introduced (Wagele 2004) (Table 1).

Table 1. The species concepts and problems since Linnaeus (1758) (Wagele 2004).

Term and its definition	Encountered problems	Author
<i>Morphological or typological species</i> : a morphologically identical organism group.	No objective discrimination between varieties and species.	Linnaeus 1758
<i>Biological species</i> : a lineage forming reproductively isolated natural populations.	Time limitation ignored. Klonal populations are excluded by definition.	Mayr 1942, 1969
<i>Ecological species</i> : evolutionary distinct generation descending from a common ancestor populating a peculiar territory	Generations do not evolve. Description cannot be applicable to synoptic sister species.	Van Valen 1976
<i>Phylogenetic species</i> : Any of reproductively isolated natural populations.	Clones excluded. Explains formation of the new species.	Hennig 1982
<i>Evolutionary species</i> : Each of lineages descending from a common ancestor	Space, time and environment do not evolve. Species disappear with the next generation. Evolution appears in local population.	Wiley 1978, 1980
<i>Diagnostic species</i> : irreducible organism cluster diagnostically distinct from other clusters sharing the same ancestry.	Diagnostic features may be shared with different races and species groups. No discrimination between races and species. Definition of diagnostic features subjective and not belong to the organism.	Cracraft 1987, Mishler and Brandon 1987
<i>Cohesive species</i> : organism groups that have cohesion potential through genetic or demographic cohesion mechanisms	Applicable only to sexually reproducing organisms.	Templeton 1989

Most important driving factors in speciation are geographical isolation and mutational changes formed during evolutionary processes (Demirsoy 1997, Wagele 2004). Mutational changes initially do not affect all the individuals of a population, but can spread to all individuals within a few generations period in reproductive populations. Afterwards, an isolated population can form new species (Wagele 2004), splitting time of which can be calculated from the differences in the DNA composition. Allopatry or natural segregation is another important factor behind speciation. In areas subjected to multiple vicariant events due to active geology as in the case of Anatolia, high biodiversity is observed. However, due to changes not reflected by phenotypic characters, classical taxonomy methods fail to discriminate taxa in many cases. In phylogenetic systematic, whether morphological or molecular data are more informative is still under debate; for instance while some researchers find molecular characters are weak, others consider morphological characters are confusing (Frelin and Vuilleumier 1979, Bardakçı and Karataş 2005). Some

comparative studies show that morphological and molecular differentiation is highly independent. Molecular approaches are potentially strong enough to develop and test phylogenetic hypotheses, however morphologists argue that still they do not differ much from the phylogenetic hypotheses based on morphological traits.

FUNCTIONING OF MORPHOLOGICAL AND MOLECULAR DATA IN TAXONOMY:

Pseudophoxinus (Cyprinidae) Example

In this part, the usage of morphological and molecular features in fish taxonomy and systematics and encountered problems are discussed with an example of genus *Pseudophoxinus* (Cyprinidae).

Morphological Characters

The measurable (metric) and countable (meristic) characters used by researchers should be in accordance with the international standard terminology. The international standard morphological characters for bony fishes are given in Figure 1.

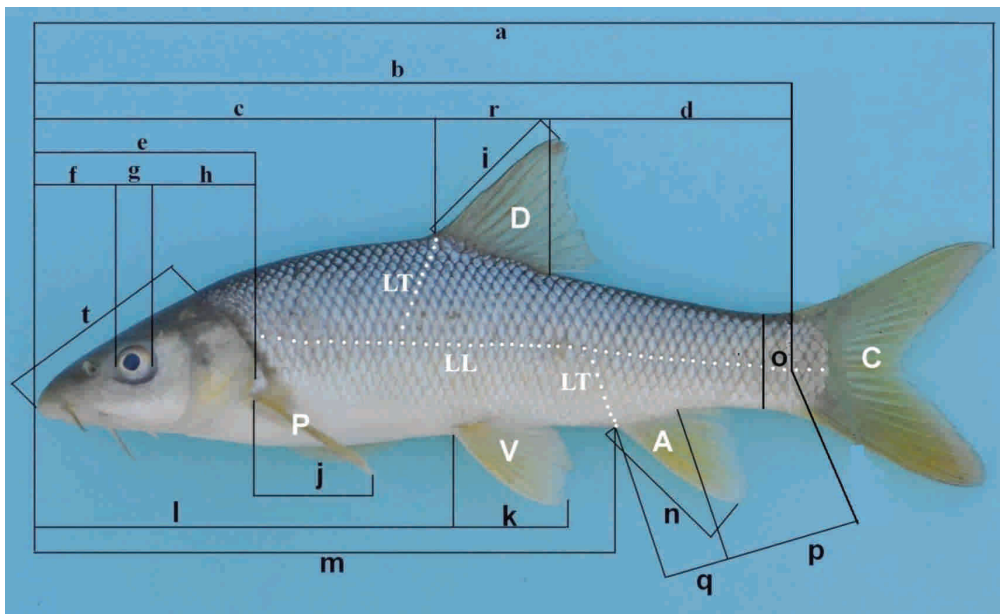


Figure 1. The international standard morphological characters for bony fishes (Modified from Kottelat and Freyhof (2007))

(a: total length, b: Standard length, c: predorsal length, d: postdorsal length, e: head length, f: snout length, g: eye diameter, h: postorbital length, i: length of dorsal fin, j: length of pectoral fin, k: length of pelvic fin, l: prepelvic length, m: preanal length, n: length of anal fin, o: depth of caudal peduncle, p: length of caudal peduncle, q: length of base of anal fin, r: length of base of dorsal fin, t: dorsal head length, A: anal fin, D: dorsal fin, P: pectoral fin, V: pelvic fin, C: caudal fin, LL: lateral line, LT: ligne transversal)

Metric (Measurable) Characters

For all metric characters, measurements are taken as a straight line from a certain point to the other in fish body and longitudinal curvature or the natural position of the body are not taken into account. Except total (TL) and standard length (SL), all measurements are expressed as a percentage or a proportional value (Kottelat and Freyhof 2007). Explanations to international standard morphological characters are given below:

Standard length (SL): It is the distance between snout tip or tip of upper lip and the center of rays supporting caudal fin (hypural complex) at the caudal notch. A measurement using the last scale on the caudal base is incorrect as in some species the scales can extend into the fin. As it is the most commonly used character by fish taxonomists, standard length can be really confusing and therefore ideally it should be done by a single and experienced researcher to minimize the differences between measurements and measurement errors.

Total length (TL): It is the distance between anterior tip of the body and the tip of the longer lobe of the caudal fin. Although standard length usage is more common, total length is used for fishes with unforked caudal fins like eels.

Head length (HL): It is the distance between snout or tip of upper lip and operculum or opercular membrane.

Caudal peduncle length: It is the distance between the base of the last anal fin ray to posterior end of SL.

Minimum body depth: It is the height of the least distance between two points on the body, which in general can be found on caudal peduncle.

Countable (Meristic) Characters

Fin rays: Anatomically the rays supporting fins are unbranched or not segmented. These are normally bony (spinous) and punctated, the rays right and left sides to these are soft and branched (segmented) in general. In some taxa from groups including Cyprinidae, last spinous rays of the dorsal and anal fins are segmented but not flaccid and may be notched posteriorly. Spinous and soft fin rays counts may be given separately or together. Since the last two rays of the dorsal and anal fins are supported by a single pterygiophor (fin supporting bone), they are counted as $1 \frac{1}{2}$. For example, count of a dorsal fin having 13-

14 branched rays will be $12-13 \frac{1}{2}$. However, there are some other counting methods (Kottelat and Freyhof 2007).

Scales: Except sturgeons, all inland fishes in Turkey have elasmoid scales. Only perforated scales on the lateral line which may be either complete or incomplete are used as a key character in taxonomy. Number of a single row of scales from nape to caudal fin base is also considered as an important taxonomic feature. Even though the scales on the caudal fin are fully developed and perforated, they are added into the count, but their number is shown after plus (+) sign in addition. The counts may slightly vary within populations; however greater variations originate from the small scales partly covered by the skin or irregular arrangement of scale roots. In this case, extreme and aberrant values should be excluded. Furthermore, the number of scale rows from the lateral line to the beginning of dorsal fin (if more than one, the first dorsal fin) and to the beginning of ventral or anal fin is termed as transversal scale number. Circular count of scales at the narrowest point of the caudal peduncle is another countable character.

Gill rakers: Gill rakers are extrusions found in the inner side of the gill arch and due to their direct relationship with the feeding system of the fish, number of the gill rakers is used as a taxonomic tool that can be used to identify fishes to genus level, sometimes even to species level. In taxonomic studies, only gill rakers of the gill arch I on the left are utilized (Figure 2).



Figure 2. A gill arch and gill rakers in a cyprinid fish

Pharyngeal teeth: It is an important character especially in the cyprinid taxonomy. Along with number and arrangement of the pharyngeal teeth, shape of the teeth has taxonomical importance. These teeth, determined by x-ray or removed (Figure 3).

Number of vertebrae: It is used in genus level identification of cyprinid fishes (Figure 4).

Sensory channels: Some studies suggest that the number of pores on sensory channels and some features like connection shape can be used as taxonomic characters especially in some cyprinid genera. The number of pores on supraorbital (CSO), infraorbital (CIO), preopercula-mandibular (CPM) and supratemporal channels (CST) are reported to be of taxonomic importance in some cyprinid genera (Figure 5) (Bogutskaya 1996).



Figure 3. Pharyngeal teeth in Cyprinidae

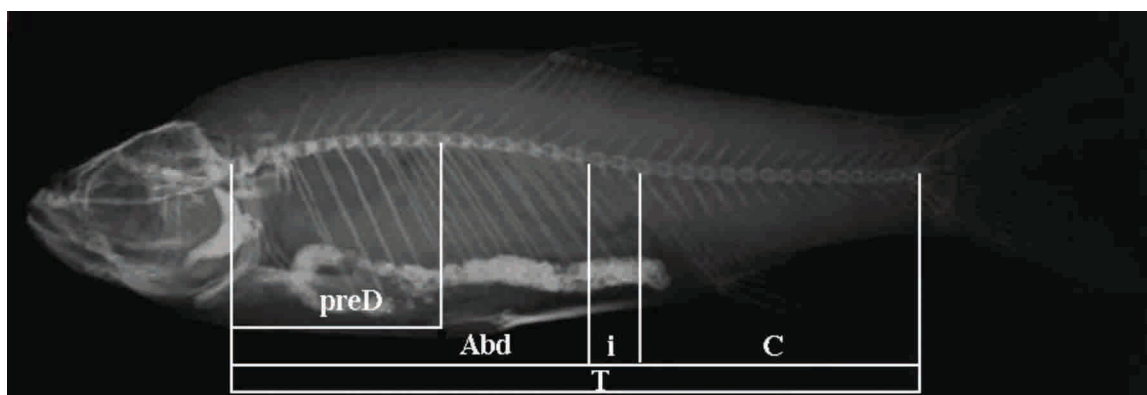


Figure 4. X-ray photo of *Pseudophoxinus turani* and its parts

(T: Total vertebrae, C: Caudal vertebrae, preD: Predorsal vertebrae, Abd: Abdominal vertebrae, İ: Interstitial vertebrae)

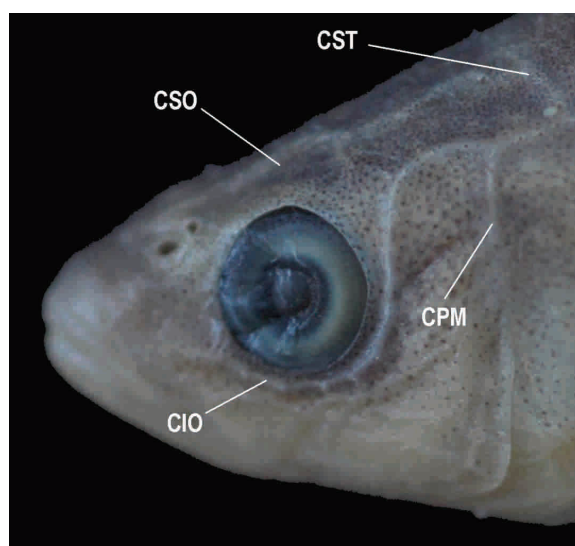


Figure 5. Sensory channels on the head of *Pseudophoxinus*

(CSO: canali supraorbitalis, CIO: canalis infraorbitalis, CPM: canalis preoperculomandibularis, CST: canalis supratemporalis)

Evaluation of the Morphological Characters Using the Principal Component Analysis (PCA)

Principal components analysis (PCA) is a statistical technique for determining the key variables in a multidimensional data set that explain the differences in the observations, and can be used to simplify the analysis and visualization of multidimensional data sets (Rachaudhuri et al. 2000). In recent years, this method is often used to fish taxonomy study (Turan et al. 2011). We observed the morphological changes between species of this analysis are thoroughly distinguished. It is observed that an application of principal component analysis in comparison of two morphologically similar *Pseudophoxinus* species, *P. kervillei* and *P. turani*, can easily discriminate species (Figure 6).

Most Frequently Used Molecular Methods in Understanding Phylogenetic Relationships

Phylogenetic systematic is based on ancestral relationships, therefore used characters should be hereditary and the most suitable tool for this purpose is DNA, which itself is the genetic molecule. According to the targeted study, both nuclear and organelle DNA can be used to investigate evolutionary relationships among related organisms. The markers to be used, which can be either proteins or DNA segments, should be chosen by the researcher (Bardakçı and Karataş 2005). Genetic characters are essential in determination

of true homologies and constructing phylogenies (Bardakçı and Karataş 2005). Speciation and phylogenetic relationships within the genus *Pseudophoxinus* in Anatolia were first studied by Hrbek et al. (2004), who argued that the Tohma Stream population (Fırat River drainage) (originally published as *Pseudophoxinus* new species, now *P. firati*) and *P. kervillei* from the Asi River form a separate lineage distinct from all other congeners in Central Anatolia, the Lakes Region and Büyük Menderes basins. A more comprehensive and detailed study based on cytochrome b, COxI and RAG1+S7 genes data corroborates the hypothesis that the genus *Pseudophoxinus* is represented in Anatolia by two monophyletic lineages (Central Anatolian and Eastern Mediterranean Region clades) and noted uncertainty in Anatolian *Pseudophoxinus* species boundaries (Perea et al. 2010).

CONCLUSION

In the light of morphological and molecular data, the morphological, phylogenetic and zoogeographic relationships *Pseudophoxinus* species distributed in the inland waters of Turkey are summarized below:

Classification of Genus *Pseudophoxinus* According to the Morphological Features

Development of lateral line (complete or incomplete) and number of perforated scales on lateral line, body shape and number of vertebrae are the

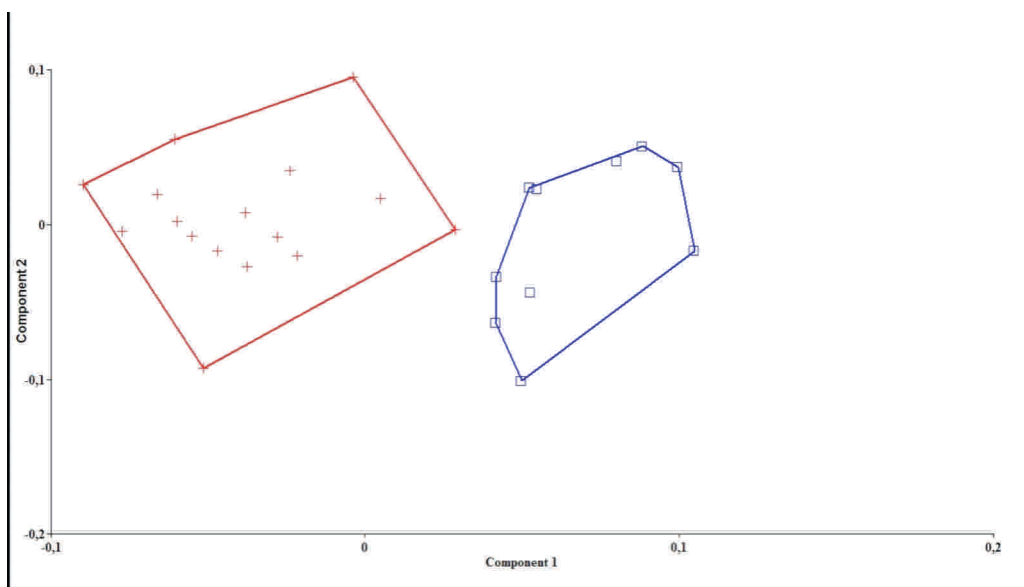


Figure 6. PCA graphs of two *Pseudophoxinus* species, *P. turani* (+) and *P. kervillei* (□) drawn using 27 morphometric characters (Küçük and Güçlü 2014).

morphological characters that were considered. Two species groups are formed according to the morphological features. The first group is formed by relatively short and stout bodied taxa with incomplete lateral line and large scales. The species in this group is *Pseudophoxinus alii*, *P. antalyae*, *P. burduricus*, *P. egridiri*, *P. elizavetae*, *P. evliya*, *P. firati*, *P. kervillei*, *P. maeandri*, *P. ninae*, *P. turani*, *P. zekayi* and *P. zeregi* (type species of the genus). The second group, which in contrast are formed by rather slender and elongated bodied taxa with complete lateral line and small scales, includes *P. anatolicus*, *P. battalgilae*, *P. crassus*, *P. fahrettini*, *P. handlirschi*, *P. hittitorum* and *P. meandricus* (Figure 7).

Classification of Genus *Pseudophoxinus* According to the Molecular Markers (*Cyt B* Gene Region)

According to the phylogenetic tree model formed

by Bayesian Analysis data using mitochondrial *cyt b* gene Anatolian *Pseudophoxinus* are represented by two different clades, which are also congruent with the zoogeography (Figure 8). First of these clades are formed by species distributed in Eastern Mediterranean inland waters (Ceyhan, Firat, Asi and Kuveik rivers): *Pseudophoxinus firati*, *P. kervillei*, *P. turani*, *P. zekayi* and *P. zeregi*. The second on the other hand is composed of Central and Southwest Anatolian species. While the first clade is morphologically more homogenous, the clade taxa is rather complex and subdivided into two subclades: The first is formed by *P. battalgilae* (Beyşehir and Çavuşcu lakes, Niğde environs) and *P. elizavetae* (Sultansazlığı), while the second includes 14 *Pseudophoxinus* distributed across a large area from Tuz Lake to Büyük Menderes River. (Hrbek et al. 2004, Perea et al. 2010).

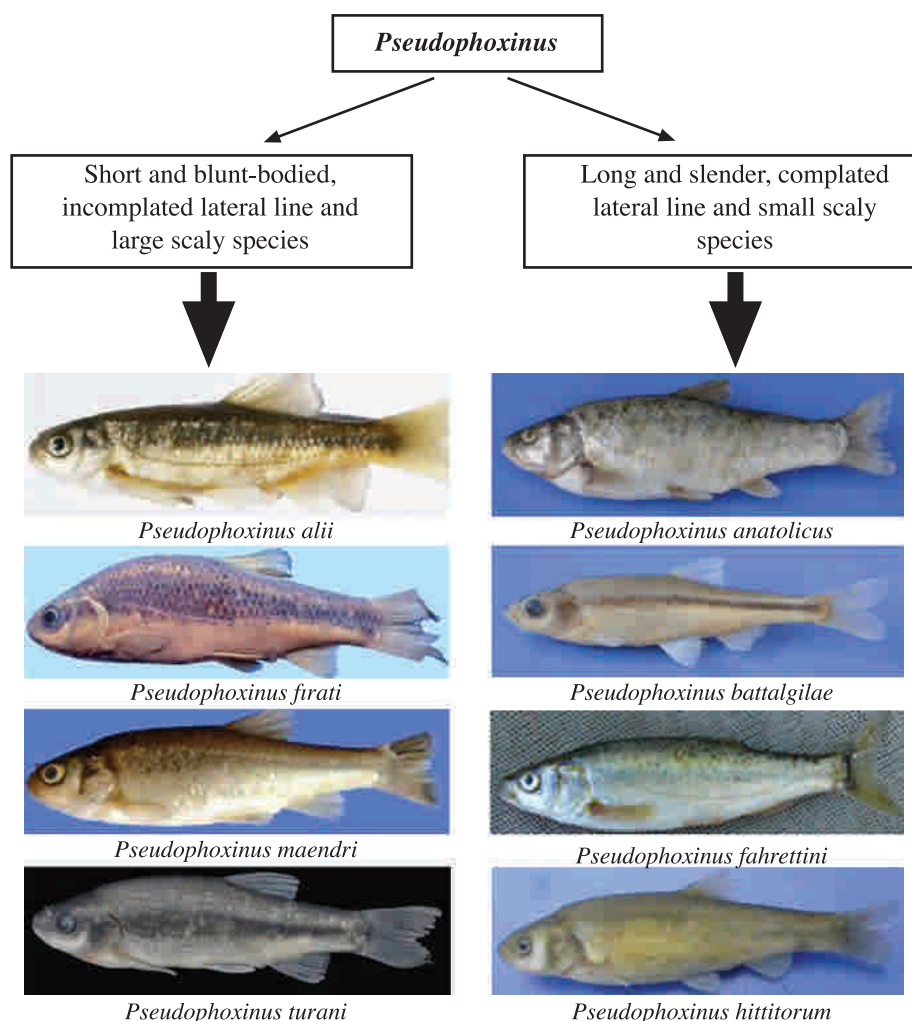


Figure 7. Classification of *Pseudophoxinus* species according to their morphological features

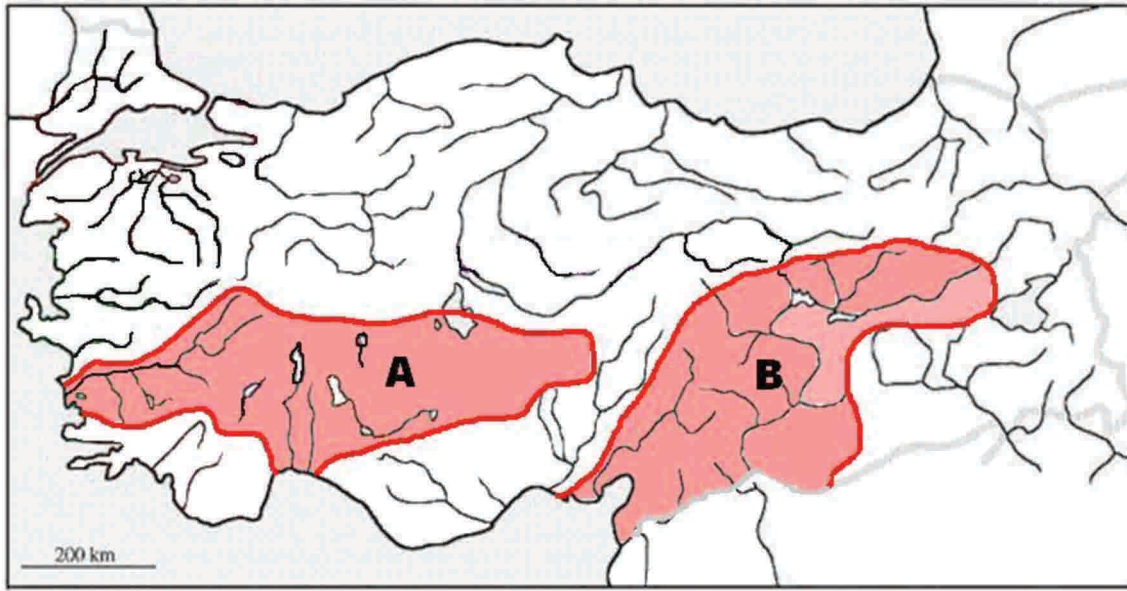


Figure 8. Separation of *Pseudophoxinus* species according to *cyt b* gene region and the distribution are in Anatolia (A: Central and West Anatolian species, B: Eastern Mediterranean species)

DISCUSSION

The populations very well isolated of *Pseudophoxinus* species are encountered in rivers, lakes and springs in Central and Southwest Anatolia (Lakes Region). Therefore, it is assumed that the speciations center of *Pseudophoxinus* genus are Central and Western Anatolia (Hrbek et al. 2004, Küçük et al. 2012). The other area of intense speciations are rivers in the Eastern Mediterranean Region. Up to date, no ichthyological research record has been reported from Marmara and Black Sea Regions. It has been understood that phylogenetic relationships of the genus *Pseudophoxinus* cannot be resolved solely by the morphological features sufficiently and definitely. The most important discrepancies are in the development of the lateral line, scales on the lateral line and the variability of the vertebrae numbers. However, taxonomic importance of bones of the mandible and pharynx region of skull as indicated by Bogutskaya et al. (2006) must not be rule out. In the first molecular study on the genus by Hrbek et al. (2004), which was based on the *cyt b* data, it was argued that the radiation of the Central Anatolian *Pseudophoxinus* species occurred during Miocene (15 mya) due to splitting of 4 Anatolian and Eastern Mediterranean clades due to orogenesis. In a study of Perea et al. (2010), who used mitochondrial and nuclear markers, the separation of

the Anatolian *Pseudophoxinus* clades were more clearly identified as morphologically rather complex Anatolian species and morphologically homogenous Eastern Mediterranean species. Ceyhan, Fırat, Asi and Kuveik rivers form the distribution area of Eastern Mediterranean species, while Sultansazlığı and Tuz Lake basins, Lakes Region, and Büyük Menderes River are populated by the Anatolian species. In conclusion, molecularly identified “Eastern Mediterranean species” form a morphologically close subgroup, with the exception of *P. zekayi* having a complete lateral line as opposed to the other taxa from the group. The Anatolian species having complex morphology can be grouped morphologically into two groups formed by slender bodied species with small scales and complete or nearly complete lateral lines and short and stout bodied species with large scales and incomplete lateral lines.

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