MORPHOMETRIC AND GENETIC ANALYSES ON FRESHWATER FISH, *PSEUDOPHOXINUS* (TELEOSTEI: CYPRINIDAE) POPULATIONS IN TURKEY

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MORPHOMETRIC AND GENETIC ANALYSES ON FRESHWATER FISH, *PSEUDOPHOXINUS* (TELEOSTEI: CYPRINIDAE) POPULATIONS IN TURKEY

submitted by **AYHAN ALTUN** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Biology Department, Middle East Technical University** by,

Prof. Dr. Gülbin DURAL ÜNVER _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Orhan ADALI _____
Head of Department, **Biology Department**

Prof. Dr. Aykut Kence _____
Supervisor, **Biology Dept., METU**

Assoc. Prof. Dr. Meral Kence _____
Co-Supervisor, **Biology Dept., METU, Retired**

Examining Committee Members:

Prof. Dr. Musa DOĞAN _____
Biology Dept., METU

Prof. Dr. Orhan ADALI _____
Biology Dept., METU

Assoc. Prof. Dr. Can BİLGİN _____
Biology Dept., METU

Assoc. Prof. Dr. Şule BARAN _____
Biology Dept., Sakarya University.

Assoc. Prof. Dr. Gülnaz ÖZCAN _____
Faculty of Marine Sci. and Tech., İSTE.

Date: *February 06, 2017*

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Ayhan ALTUN

Signature:

ABSTRACT

MORPHOMETRIC AND GENETIC ANALYSES ON FRESHWATER FISH, *PSEUDOPHOXINUS (TELEOSTEI: CYPRINIDAE*) POPULATIONS IN TURKEY

Altun, Ayhan

Ph.D., Department of Biology

Supervisor: Prof. Dr. Aykut Kence

Co-Supervisor: Doç. Dr. Meral Kence

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With exceptional species richness and high level of endemism the Mediterranean region is recognized as one of the most important freshwater biodiversity hotspots in the world. One of the main temperate Mediterranean refugia is Anatolia with high biodiversity and strong genetic imprints. Complex patterns of population structure within the Mediterranean refugia are the results of the existence of fragmented distribution, dispersion process and gene flow occurred during range expansion in the interglacial periods.

Pseudophoxinus is a highly diverse and endemic genus among freshwater fish belonging to the family Cyprinidae. The number of species of the genus is 29 of which 21 inhabits the freshwaters of Turkey. Despite the presence of the species in the territories of countries such as Algeria, Azerbaijan, Israel, Jordan, Lebanon, Syria and Tunisia almost 70% of *Pseudophoxinus* (20 species) is endemic to Turkey. High endemism and diversity of the genus and vulnerability of aquatic ecosystems necessitate thorough studies to be conducted for taxonomic investigation and conservation of the species of the genus.

In this study geometric morphometrics and molecular markers, allozymes and microsatellites, were used to determine phylogenetic relationship among 17 populations comprising six species and five populations with undetermined taxonomic status.

Pseudophoxinus battalgili represented with four populations, Çavuşçu Lake, Oymapınar Dam Lake, Taşağıl Stream and Suğla Lake was clustered together in the same species complex in all three methods used. While allozyme and microsatellite analyses revealed close relationship between *P. hittitorum* inhabiting Eflatun Spring and *P. battalgili*, landmark based geometric morphometrics placed *P. hittitorum* in a group geographically close to each other.

Three populations of *P. burduricus* were usually grouped together. However, the species complex of *P. burduricus* obtained from morphometric data included *P. fahrettini and P. crassus* from İnsuyu Stream as well as Kuğulu Park and Kırkpınar populations.

P. egridiri morphologically placed close to *P. battalgili*. However, both allozyme and microsatellite results showed that this species genetically distinctly singled out from the rest of 16 populations.

P. crassus was represented with two populations, Gök Lake and İnsuyu Stream. Although they were genetically found to be closely related, Gök Lake population was the most divergent taxon morphologically. This population recently was revised as a new species, *P. iconii*.

Keywords: Population Genetics, Geometric Morphometry, *Pseudophoxinus*, Allozymes, Microsatellites.

ÖZ

TATLI SU BALIĞI *PSEUDOPHOXİNUS* (*TELEOSTEİ: CYPRİNİDAE*)'UN TÜRKİYE'DEKİ TOPLUMLARI ÜZERİNDE MORFOMETRİK VE GENETİK ANALİZLER

Altun, Ayhan
Doktora, Biyoloji Bölümü
Tez Yöneticisi: Prof. Dr. Aykut Kence
Ortak Tez Yöneticisi: Doç. Dr. Meral Kence

Şubat 2017, 139 sayfa

Olağanüstü tür zenginliği ve yüksek endemizmi ile Akdeniz bölgesi yeryüzündeki en önemli tatlısu biyo-çeşitlilik merkezlerinden biri olarak kabul edilmektedir. Ana ılıman Akdeniz korunaklarından biri, yüksek biyoçeşitliliği ve güçlü genetik izleriyle Anadolu'dur. Akdeniz korunağında gözlemlenen karmaşık toplum yapıları, parçalanmış dağılımın varlığı, yayılma süreci ve buzul çağları arasındaki dönemlerdeki alan genişletme sırasında gerçekleşen gen akışı sonucunda ortaya çıkmıştır.

Pseudophoxinus, Cyprinidae ailesinde yer alan tatlısu balıklarının arasında çeşitliliği yüksek ve endemik bir cinstir. Bu cinse ait tür sayısı 29 olup bunlardan 21 tanesi Türkiye'nin tatlısularında bulunmaktadır. Azerbaycan, Cezayir, İsrail, Lübnan, Suriye, Tunus ve Ürdün gibi ülkelerin topraklarında da bulunmasına karşın bu cinsin hemen hemen %70 (20 tür) Türkiye'ye endemiktir. Bu cinsin yüksek endemizmi ve çeşitliliği ve sucul ekosistemlerin kolay bozulabilirliği, bu cinsin türlerinin sistematiğinin araştırılması ve korunması konularındaki titiz çalışmaların gerçekleştirilmesini gerektirmektedir.

Bu çalışmada, altı tür ve beş tür durumu kesin olmayan toplumu kapsayan 17 *Pseudophoxinus* toplumunun filogenetik ilişkilerini belirlemek üzere geometrik morfometri ve iki moleküler belirteç, allozim ve mikrosatelit kullanılmıştır.

Dört toplum (Çavuşçu Gölü, Oymapınar Baraj Gölü, Taşağıl Nehri ve Suğla Gölü) ile temsil edilen *P. battalgili* kullanılan üç yöntemde de beraber bir grup oluşturarak aynı tür kopleksi içerisinde yer almaktadır. Allozim ve mikrosatelit analizleri Eflatun Pınarında yaşayan *P. hittitorum* ile *P. battalgili* arasında yakın akrabalık olduğunu gösterse de nirengi noktalarına dayalı geometrik morfometri analizleri bu türü coğrafi olarak yakın bulunan diğer toplumlarla birlikte göstermektedir.

Üç *P. burduricus* toplumu genellikle bir grup oluşturmaktadırlar. Fakat morfometrik veriler sonucu elde edilen *P. burduricus* tür kompleksi, Kuğulu Park ve Kırkpınar toplumlarıyla birlikte *P. fahrettini ve P. crassus*'un İnsuyu toplumunu da içermektedir.

P. egridiri şekilsel olarak *P. battalgili*'ye yakındır. Fakat hem allozim hem de mikrosatelit sonuçları bu türün genetik olarak diğer 16 toplumdan belirgin şekilde ayrıldığını göstermektedir.

P. crassus, Gök Göl ve İnsuyu Çayı olmak üzere iki toplumla temsil edilmektedir. Bu iki toplum genetik olarak yakın akrabalık gösterseler de Gök Göl toplumu şekilsel olarak en fazla ayrışmış gruptur. Bu toplum yakın zamanda yeni bir tür, *P. iconii,* olarak yeniden tanımlanmıştır.

Anahtar Kelimeler: Toplum Genetiği, Geometrik Morfometri, *Pseudophoxinus*, Allozim, Mikrosatelit.

To my family,

my beloved wife, Beyza ERSOY ALTUN

my parents, Emine and Ali ALTUN

and

my sisters, Serap ALTUN and Mehtap KARASU

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CHAPTER 1

INTRODUCTION

Speciation and extinction is an on-going event in the nature. Existing populations are the starting point of new species when the conditions are favorable for speciation. Both processes are naturally very slow. Nevertheless, organisms living on the earth are under the pressure of rapid extinction largely due to human activities. Alterations of natural habitats due to factors such as global climate change, anthropogenic and industrial pollution, and usage of land or water for the needs of man-kind as well as introduction of exotic species adversely affect biological diversity. Deterioration of environments forces the inhabitants to be exist in small fragmented populations (Smith and Wayne, 1996; Thomas *et al*. 2004).

Reduction of genetic diversity as a result of random genetic drift is an expected outcome in fragmented populations where outbreeding becomes impossible and gene flow is restricted. Hence, the process of fixation of alleles work faster in small inbreeding populations. The reduction in the fitness and the rate of extinction is enhanced in the case of presence of deleterious alleles within fixed alleles (Lynch and Gabriel, 1990). The term “extinction vortex” (Mills *et al*. 1993; Soule and Mills 1998) is used to explain this cycle of interactive reduction between population size and genetic drift. Population stability can be maintained with continues gene flow between populations and this is very important for isolated small size populations.

Estimations indicate that about half of the species of existing plants and animals are expected to become extinct in less than a century (Primack 1998; (Crivelli et al., 2000). The necessity for undertaking two types of studies, Taxonomy and Conservation Biology, is obvious considering the fact that species are under the threat of rapid extinction. Taxonomic studies are necessary to establish a catalogue of existing species in order to create a certain description of what is available in the context of biological diversity. This is important for determining genetic resources, increasing sustainability

of the ecosystems, protecting available resources, improving stocks and stock qualities, and handling desired traits such as disease resistance, increased productivity, etc. In the countries such as Turkey where the list of species is not well-defined the extinction of species may even take place before the description of many species has been completed (Altun, 2003). Therefore taxonomic study is an absolute necessity in order to take further steps in the field of conservation biology.

Molecular markers such microsatellite, allozymes, and DNA sequences are used for the identification of the pattern of genetic diversity within and among lineages at population level. Furthermore, they are helpful to figure out evolutionary interaction among species at ecosystem level (Caballero and Toro 2002). Combining information obtained using advanced techniques such as molecular markers with biological data i.e., morphology and ecological information is effectively used for the conservation and management of endangered species (Moran 2002).

1.1. Freshwater Fish in Turkey

Fish is a highly diverse taxon amongst the living organisms and their evolutionary history goes back to at least 500 million years (Moyle and Cech, 2004). Eschmeyer (1998) enlisted 23,250 fish species about two decades ago. Since then, the number of recorded fish species has increased to 33,200 (Froese and Pauly, 2016), and Moyle and Cech (2004) claims that 200 new species have been added to the number each year as the result of detailed studies and/or description of new species.

Oceanic waters make up 97% of all water on earth and the contribution of freshwaters to the total is less than 0.3%. Despite this little contribution freshwaters are like the islands on the earth, and hence promote speciation. As a result freshwaters shelter almost half of the fish species exist on earth (Moyle and Cech 2004). Around 2.5% of freshwater fishes inhabit the freshwaters of Turkey. The number has been given as 368 (Çiçek, et al., 2015) which is an indication of noticeable species richness. Besides, 153 species is recognized as endemic. Therefore there is a high level of endemism with a ratio of 41.58% within the ichthyofauna of Turkey. The total number includes eight extinct species, of which three is globally extinct while remaining five is locally exhausted. The

represented with 31 families belonging to 16 orders. The most prominent taxon at the family level is Cyprinidae represented with 188 species comprising 51.1% of the total species. The other families represented with more than 10 species are Nemacheilidae (39), Salmonidae (21 species), Cobitidae (20 species), Gobidae (18 species) and Cyprinodontidae (14 species) (Çiçek, et al., 2015).

With exceptional species richness and high level of endemism the Mediterranean Region is recognized as one of the most important freshwater biodiversity hotspots in the world, (Myers et al., 2000; Froufe et.al., 2016). According to IUCN-International Union for Conservation of Nature (IUCN) reports, a third of all freshwater fish species in lakes and streams on the Earth have become extinct or endangered and only around 3 to 5% of those are listed on the IUCN list of endangered fauna (Crivelli *et al.*, 2000). Unfortunately 56% of total number of freshwater fish species in Mediterranean region are threatened (IUCN, http://www.iucn.org/). Major threats to freshwater fish are habitat destruction, irrigational water use, dam constructions, industrial waste discharge, fragmentation and introduction or translocation of species. Harrison and Stiassny (1999) claims that global warming is also a factor affecting freshwater fish populations. Today, 131 species and 98 subspecies of fish endemic to the northern Mediterranean were identified and 31 % of these species were found in Turkey. However, the fish distribution, taxonomy and the conservation status of southern Europe and Mediterranean countries were not well studied and documented in the literature, although, this is not the case for northern Europe. Furthermore, around 10 % of freshwater fish species are listed as endangered or vulnerable in the southern Europe (Geldiay and Balık, 1998). Southern Europe especially Mediterranean basin had provided refuge areas for many species including freshwater fish populations in the post glacial expansion during the four main ice ages of the Pleistocene, so that the large number of species with large number of endemism has been found in this region (Kosswig 1955). As environmental conditions in Mediterranean region are mostly semi-arid or arid, aquatic habitats are very fragmented and isolated. That condition enhances speciation processes that result in presence of number of species within single or few hydrographic basins (Kottelat and Barbieri 2004).

1.1.1. Classification of *Pseudophoxinus* Bleeker, 1860

Phylum: Chordata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Subfamily: Leuciscinae

Genus: *Pseudophoxinus* Bleeker, 1860

Altun (2003) indicates that (1) many species in Turkey have been poorly defined, (2) their systematic are questionable and in need of systematic revision, (3) their distribution are mainly unknown, (4) their abundance is not well determined, and (5) there is a lack of knowledge and research. Cyprinidae are the richest and most important family of freshwater fish group, and its members are distributed world-wide. Classification of the most of genera belonging to European cyprinids is poorly defined. There used to be conflictions relating the taxa within cyprinids and genus *Pseudophoxinus* takes its share from these conflictions as well. Spring minnows, locally known as "yağ balığı" or "çiçek", are classified under the genus *Pseudophoxinus* Bleeker 1860 which is one of the bony fish genera belonging to the family *Cyprinidae.* The genus is a taxon of *Leuciscinae* which is widely distributed in freshwaters running to Mediterranean Sea. Although the recognition of the species dated back to 1843 (Heckel, 1843), detailed studies were initiated by Kosswig around mid-1950s. In the past the classification of the genus was quite questionable and it has been considered under a few different genera for many years. Systematic status of taxa is left untouched since early 19^{th} century. Therefore there was quite a few misnaming over the years. Battalgil (1944), Balık (1995) and Karaman (1972) contributed greatly in the description of fish in Turkey. Consequently, the number of described fish as well as *Pseudophoxinus* is increasing in Turkey. A revision by Bogutskaya (1992) and a phylogenic study (Hrbek et al., (2004) on the biogeography of the genus have clarified the necessity for revision of the genus. As a result of focused studies nearly half of existing *Pseudophoxinus* species was identified in the last decade (Bogutskaya et al., 2007; Freyhof & Özuluğ, 2006; Freyhof

& Özuluğ (2010a, 2010b and 2010c); Küçük et al., (2013), Küçük & Güçlü 2014; Ekmekçi et al., 2015; Küçük et al., 2016).

Table 1. *Pseudophoxinus* of the world.

No	Species	Country	State	CS
1	*Pseudophoxinus alii* Küçük, 2007	Tr	Endemic	EN
2	*Pseudophoxinus anatolicus* (Hankó, 1925)	Tr	Endemic	EN
3	*Pseudophoxinus antalyae* Bogutskaya, 1992	Tr	Endemic	VU
4	*Pseudophoxinus atropatenus* (Derjavin, 1937)	Az	Native	CR
5	*Pseudophoxinus battalgili* Bogutskaya, 1997	Tr	Endemic	LC
6	*Pseudophoxinus burduricus* Küçük, Gülle, Güçlü, Çiftçi & Erdoğan, 2013	Tr	Endemic	EN
7	*Pseudophoxinus callensis* (Guichenot, 1850)	Al, Tu	Native	DD
8	*Pseudophoxinus crassus* (Ladiges, 1960)	Tr	Endemic	EN
9	*Pseudophoxinus drusensis* (Pellegrin, 1933)	I, S	Endemic	EN
10	*Pseudophoxinus egridiri* (Karaman, 1972)	Tr	Endemic	EN
11	*Pseudophoxinus elizavetae* Bogutskaya, Küçük & Atalay, 2006	Tr	Endemic	CR
12	*Pseudophoxinus evliyae* Freyhof & Özuluğ, 2010	Tr	Endemic	EN
13	*Pseudophoxinus fahrettini* Freyhof & Özuluğ, 2010	Tr	Endemic	EN
14	*Pseudophoxinus firati* Bogutskaya, Küçük & Atalay, 2006	Tr	Endemic	EN
15	*Pseudophoxinus handlirschi* (Pietschmann, 1933)	Tr	Endemic	EX
16	*Pseudophoxinus hasani* Krupp, 1992	S	Endemic	CR
17	*Pseudophoxinus hittitorum* Freyhof & Özuluğ, 2010	Tu	Endemic	EN
18	*Pseudophoxinus iconii* Küçük, Gülle & Güçlü, 2016	Tr	Endemic	NE
19	*Pseudophoxinus libani* (Lortet, 1883)	L, J, S, I	Native	EN
20	*Pseudophoxinus maeandri* (Ladiges, 1960)	Tr	Endemic	EN
21	*Pseudophoxinus maeandricus* (Ladiges, 1960)	Tr	Endemic	CR
22	*Pseudophoxinus mehmeti* Ekmekçi, Atalay, Yoğurtçuoğlu, Turan & Küçük, 2015	Tr	Endemic	NE
23	*Pseudophoxinus ninae* Freyhof & Özuluğ, 2006	Tr	Endemic	CR
24	*Pseudophoxinus punicus* (Pellegrin, 1920)	Al, Tu	Native	EN
25	*Pseudophoxinus sojuchbulagi* (Abdurakhmanov, 1950)	Az	Endemic	CR
26	*Pseudophoxinus syriacus* (Lortet, 1883)	S	Endemic	CR
27	*Pseudophoxinus turani* Küçük & Güçlü, 2014	Tr	Endemic	NE
28	Pseu*dophoxinus zekayi* Bogutskaya, Küçük & Atalay, 2006	Tr	Endemic	NE
29	*Pseudophoxinus zeregi* (Heckel, 1843)	Tr, S	Native	LC

Al, Algeria; **Az**, Azerbaijan; **L**, Lebanon; **J**, Jordan; **I**, Israel; **Tu**; Tunisia; **Tr**, Turkey; **S,** Syria; **CS**, Conservation Status according to IUCN (2015). **CR**, critically endangered; **DD**, data deficient **EN**, endangered; **EX**, extinct; **LC**, Least concerned; **NE**, not evaluated; **VU**, vulnerable.

Although the species of this genus has been eventually classified as *Pseudophoxinus*, they were initially placed in genus *Phoxinellus* (Karaman, 1972) and considered to inhabit commonly in the freshwaters of Syria and Palestine (Geldiay and Balık, 1988). The genus has been confused with genera *Acanthobrama, Acanthorutilus, Alburnus, Leucaspius* (Vinciguerra, 1921), *Leuciscus* (Stephanidis, 1974; Valenciennes, 1844), *Oreoleuciscus, Pachychilon* (Karaman, 1972), *Paraphoxinellus, Pararhodeus* (Fang, 1942), *Rhodeus, Phoxinellus* (Karaman, 1972; Stephanidis, 1974), *Rutilus, Telestes* (N G

Bogutskaya, Küçük, & Atalay, 2007) until relatively recent revisions (Banarescu, 1992; Bianco, 1990; Bogutskaya, 1997; Economidis & Banarescu, 1991; Economidis, 1991).

In addition to misplacement in other genera, *Pseudophoxinus* possessed unrelated species in the past as well. *P. beoticus, P.epiroticus, P. marathonicus, P.minutes, P.prespensis, P. stymphalicus, P. thesproticus* were considered as the species of the genus inhabiting European freshwaters. Recent evaluation of them resulted in the replacement of these Balkan species under the genus *Pelasgus* (Kottelat, 1997). One previous species, *P. fahirae*, revised as belonging to the genus Telestes now.

There is an extinct species, *Pseudophoxinus handlirschi* (Pietschmann, 1933) in the list of the genus. Although the species was an inhabitant of the Lake Eğirdir, predation caused by an introduced species, *Sander lucioperca* forced the species into extinction. Another species, *Pseudophoxinus sojuchbulagi*, is also reported as possibly extinct due to agricultural use of the spring water and cattle grazing (Freyhof, 2014i).

1.1.2. Geographic History of the Habitat of Genus *Pseudophoxinus*

The world pattern of fish distribution can be explained by bringing information from various disciplines of science together. Pattern of fish distribution, especially over large areas may be problematic due to the lack of complete information. While undertaking taxonomic researches on fish, possible involvement of geology, biogeography, ichthyology, ecology, physiology, systematic and paleontology may be required. However, development of plate tectonics and the rise of phylogenetic systematics have provided new tools for exploring zoogeographic hypotheses (Moyle & Cech, 2004).

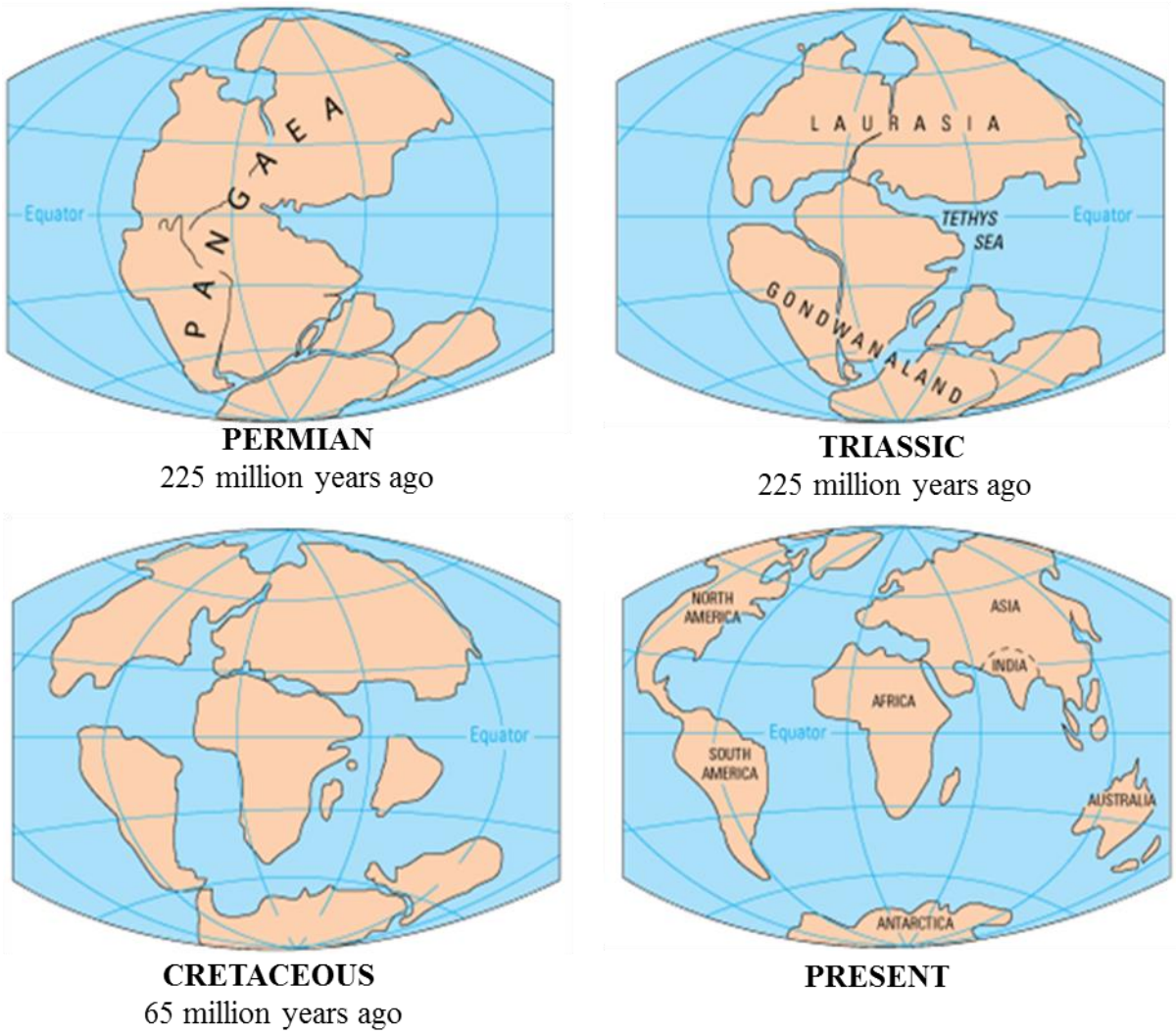

Figure 1. The earth 65 million years ago and now (modified from USGS)

It can be claimed that such a diverse speciation is the result of geographic isolation during the formation of the earth. Mediterranean Basin has formed about 65 million years ago (WEB1, 2017). While it was a part Tethys sea at Triassic period, the closing of the Tethys Sea at the boundary of Oligocene/Miocene brought Gondwanan (African) and Laurasian (Eurasian) elements into contact (Brown and Lomolito, 1998) and Mediterranean sea has become a closed sea surrounded by Africa and Eurasia (Fig.1.). This was the first encounter of the region since the break of Pangaea 200 million year ago (MYA) (Smith et al.,1995). This formation resulted in both mixing of the elements of Africa, Europe and Asia first and, then, increasing in the number of species through local geographical isolations.

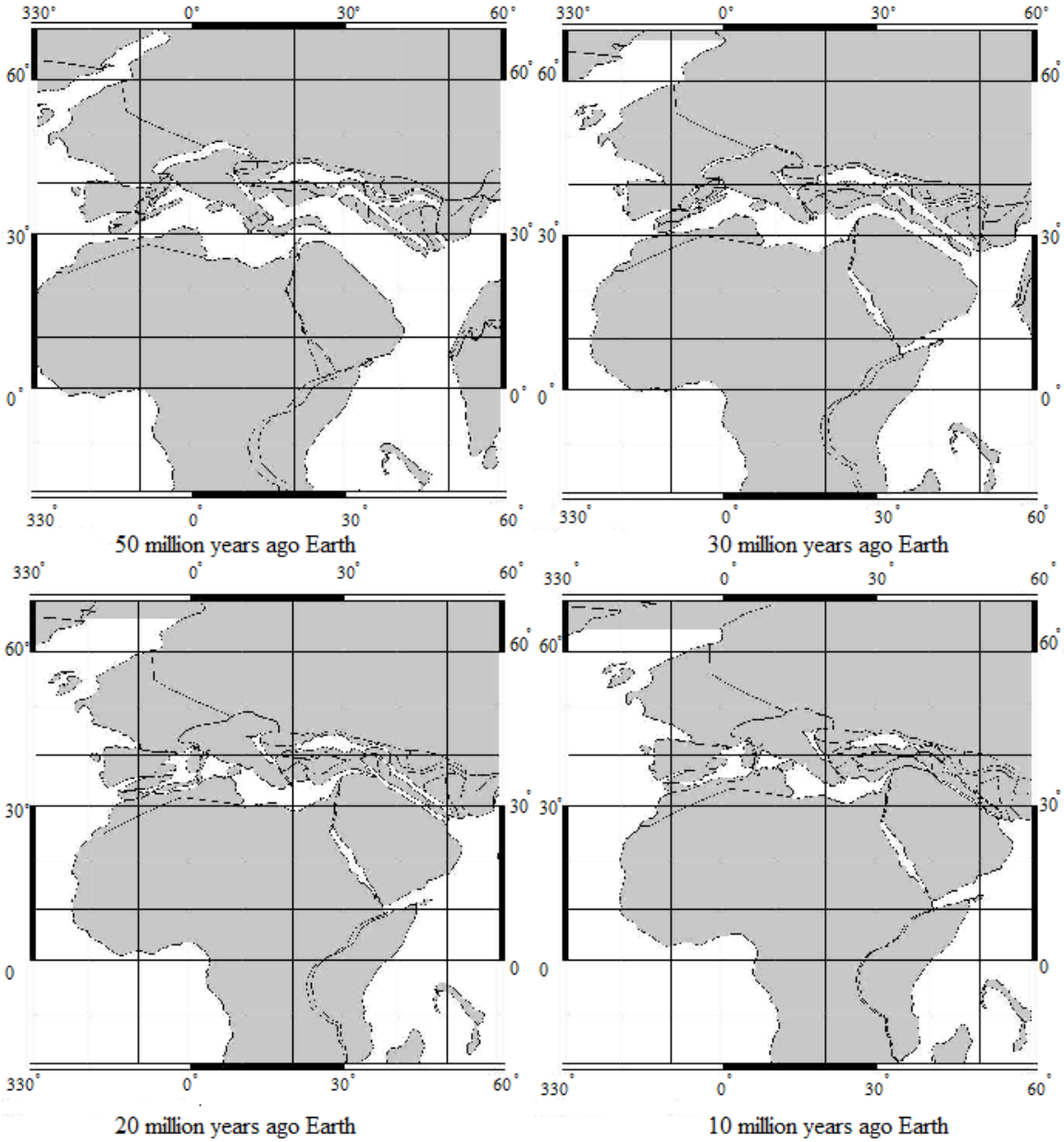

Figure 2. The formation of Eurasian with African Elements (retrieved from ODSN).

The area covering Turkey has taken its recent geological shape 5-10 MYA (Fig.2.). As the result of on-going geological events Turkey has taken its geological shape (WEB2, 2017) with the formation of five tectonic plates (Fig.3.). Black sea plate has been divided into two eastern and western blocks in the picture. Eventually Anatolia (Asia Minor) became the center of a vast variety of biological diversity although the data for testing this hypothesis is not enough and limited within the geological areas (Caccone et

al., 1997; Macey et al, 1997; Penzoet al., 1998; Veith et al., 1998; Weisrock et al., 2001; Zardoya & Meyer, 1996).

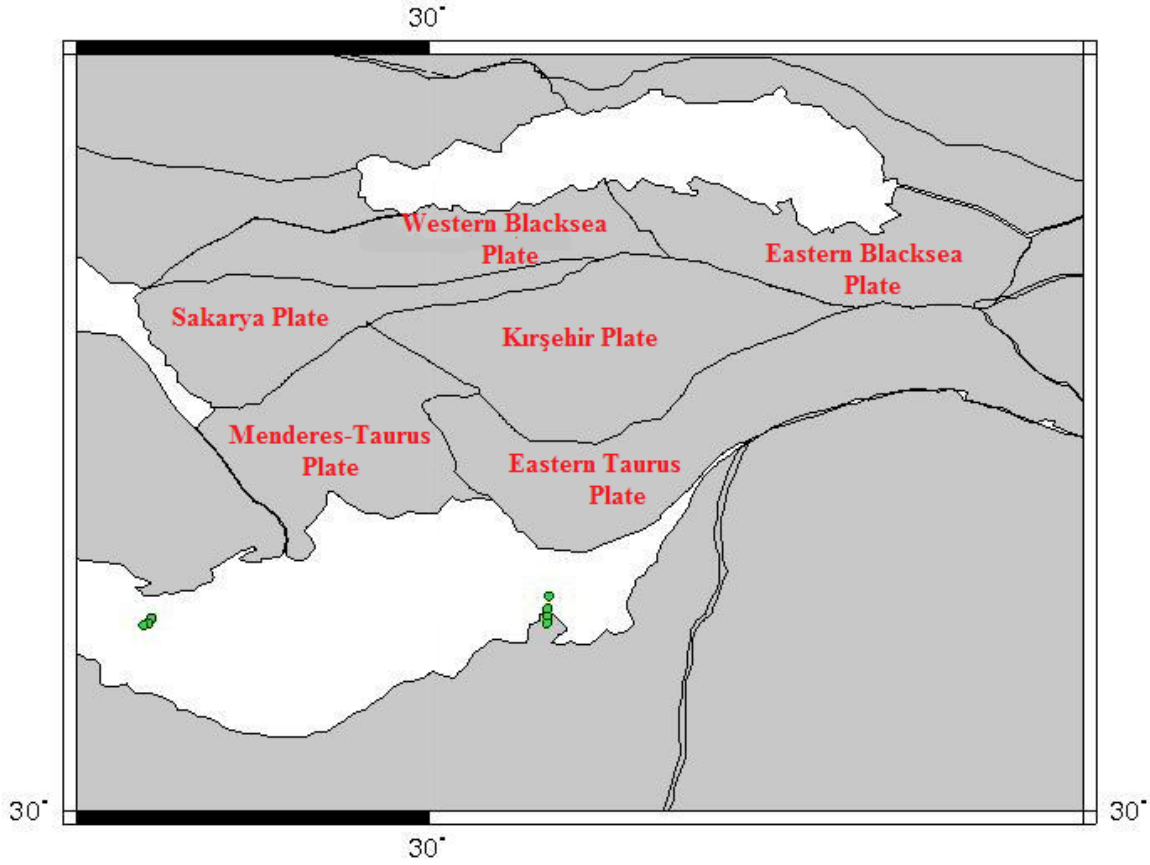

Figure 3. Plates forming Asia Minor (modified from ODSN).

Evidential geographic events have given rise to many isolated freshwater bodies. Hence the organism remained in these freshwaters have taken their own evolutionary path and undergone speciation accordingly. Due to the geographical isolation and difficulty to cross the barriers organisms became highly variant in the region. Many species of the genus *Pseudophoxinus* are endemic to Turkey showing a great biodiversity. Although most of the *Pseudophoxinus* species are reported from Turkey, there are reports of presence of the species form other countries such as Algeria and Tunisia from North Africa, and Azerbaijan, Israel, Jordan, Lebanon and Syria from Arabian Peninsula and Middle East

1.1.3. Distribution and Conservation Status of *Pseudophoxinus* Bleeker, 1860.

Pseudophoxinus is a species rich genus and represented with 29 species in the world (Table 1.). However, spring minnows are restricted to an area covering Asia Minor up to Azerbaijan in the east and down to Israel in the south. Besides there are two species present in the North Africa. The presence of the genus is reported from Algeria, Azerbaijan, Israel, Jordan, Lebanon, Tunisia, Turkey and Syria, (Fig.1.).

The genus is highly endemic to Turkey. Out of 29, the number of the species of the genus reported from Turkey is 21 (Table 2.). Twenty of them are endemic to Turkey and only one species occupies the territories of both Turkey and Syria. Remaining eight species are distributed in Algeria and Tunisia (2), Azerbaijan (2), Syria (2), Israel and Syria (1) and Jordan, Israel, Lebanon and Syria (1).

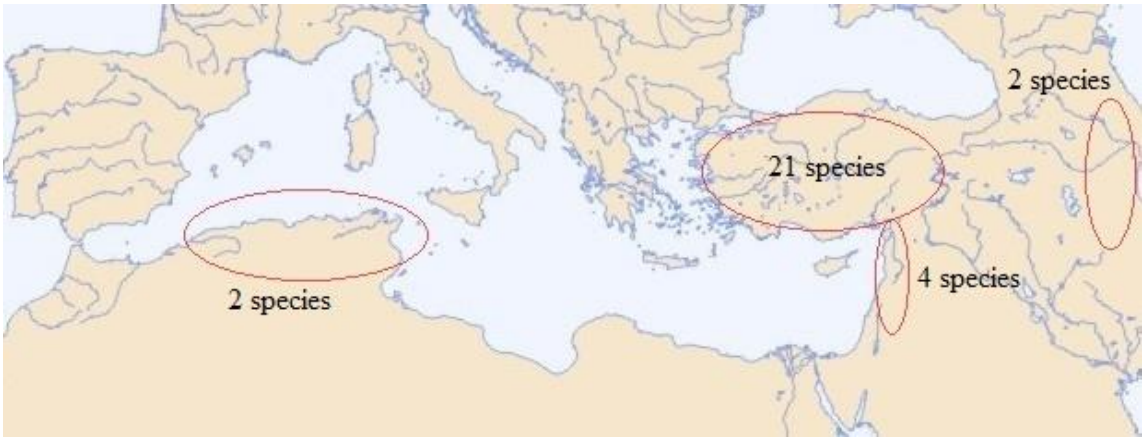

Figure 4. World-wide distribution of *Pseudophoxinus*.

Members of the genus in Turkey is geographically restricted and their temporal and spatial diversity in Anatolia are driven by the geological events (Hrbek *et al*. 2004). Therefore the distribution pattern of the genus is vicariance where individuals or populations are carried to new places by plate tectonics and formed new species there (Moyle & Cech, 2004). In this case, the land moves more than the fish, and hence, populations of species are separated into new evolutionary lineages (Mooi & Gill, 2002).

The genus *Pseudophoxinus* is the most endangered group of Cyprinid because of the continuous damage of freshwater ecosystems in Mediterranean region (Balık 1995; Geldiay and Balık, 1988). Conservation status (Table.1 and Table 2.) of only two species is considered as least concerned (LC) in the red list of IUCN (2015). Two

species is not evaluated (NE) yet and the others are considered as vulnerable (VU), endangered (EN) and critically endangered (CR).

Table 2. Type location and conservation status of *Pseudophoxinus* of Turkey.

No	**Species**	**Location**	**State**	**1**	**2**	**3**
1	*Pseudophoxinus alii* Küçük, 2007	Aksu river	Endemic	EN		
2	*Pseudophoxinus anatolicus* (Hankó, 1925)	Lake Beyşehir and Suğla, Beyşehir	Endemic	EN	CR	EN
3	*Pseudophoxinus antalyae* Bogutskaya, 1992	Kırkgöz and Düden springs, Antalya	Endemic	VU	CR	VU
4	*Pseudophoxinus battalgilae* Bogutskaya, 1997	Lake Beyşehir	Endemic	LC	CR	VU
5	*Pseudophoxinus burduricus* Küçük, Gülle, Güçlü, Çiftçi & Erdoğan, 2013	Lake Burdur and Salda, Değirmendere, Karamanlı stream, Düger sp Sazak spring	Endemic	EN		
6	*Pseudophoxinus crassus* (Ladiges, 1960)	Tuz Lake, Aksaray, Niğde, Central Anatolia	Endemic	EN	EN	EN
7	*Pseudophoxinus egridiri* (Karaman, 1972)	Eğirdir Lake	Endemic	EN	CR	CR
8	*Pseudophoxinus elizavetae* Bogutskaya, Küçük & Atalay, 2006	Kayseri Province	Endemic	CR		
9	*Pseudophoxinus evliyae* Freyhof & Özuluğ, 2010	Western Anatolia, Finike lake Avlan and Lake Söğüt	Endemic	EN		
10	*Pseudophoxinus fahrettini* Freyhof & Özuluğ, 2010	Köprü River drainage in central Anatolia	Endemic	EN		
11	*Pseudophoxinus firati* Bogutskaya, Küçük & Atalay, 2006	Gögdeli thermal spring in upper Tohma Stream in Euphrates drainage	Endemic	EN	VU	
12	*Pseudophoxinus handlirschi* (Pietschmann, 1933)	Lake Eğirdir, Isparta	Endemic	EX	CR	EX
13	*Pseudophoxinus hittitorum* Freyhof & Özuluğ, 2010	Lake Beyşehir, Eflatun spring	Endemic	EN		
14	*Pseudophoxinus iconii* Küçük, Gülle & Güçlü, 2016	Gök Lake, Kozanlı, Kulu, Konya	Endemic	NE		
15	*Pseudophoxinus maeandri* (Ladiges, 1960)	Işıklı and Düden, Dinar, Büyük Menderes	Endemic	EN	EN	CR
16	*Pseudophoxinus maeandricus* (Ladiges, 1960)	Karadirek stream, Sandıklı	Endemic	CR		VU
17	*Pseudophoxinus mehmeti* Ekmekçi, Atalay, Yoğurtçuoğlu, Turan & Küçük, 2015	Alanköy basin, Burdur, Southwestern Turkey	Endemic	NE		
18	*Pseudophoxinus ninae* Freyhof & Özuluğ, 2006	Onaç drainage	Endemic	CR		
19	*Pseudophoxinus turani* Küçük & Güçlü, 2014	İncesu Spring, Asi River	Endemic	NE		
20	*Pseudophoxinus zekayi* Bogutskaya, Küçük & Atalay, 2006	Ceyhan river, Tekir, Aksu, Zeytin, Körsulu, Sabun, Hamus, Yarpuz	Endemic	VU		
21	*Pseudophoxinus zeregi* (Heckel, 1843)	Orontes and Quwayq river Turkey	Native	LC		

Conservation status are [1]IUCN (2015**),** [2]Fricke et al (2007) and [3]Küçük, (2006)

Unfortunately water ecosystems can readily be affected from a variety of adverse sources. Main threats to *Pseudophoxinus* are water extraction, irrigational use of water, dam construction as well as the discharge of anthropogenic and industrial pollutants. *P. sojuchbulagi* is reported to be CR (possibly extinct) due to the effect of heavy agriculture practice. As in the case of *P. handlirschi* introduction of exotic species is also a major threat for the populations inhabiting restricted ecosystems.

1.1.4. Biology of *Pseudophoxinus* Bleeker, 1860.

Spring minnows inhabit freshwaters of temperate and subtropical regions usually surrounding Mediterranean Sea. Spring minnows occupy freshwaters close to springs where the water is considerably clean and relatively cold. However, the occurrence of the species is not limited to springs. Some species are found in running waters while a few species adapted successfully to the lakes. They inhabit upper layers and bottom of clear waters of rivers and lakes. Nevertheless, the distribution of single species is not wide and restricted to one or a few water bodies. Large species number, 21 out of 29, of *Pseudophoxinus* in unconnected freshwaters of Turkey supports the vicariance hypothesis rather than dispersal for distribution of the species.

The members of the taxon usually prefer living around stones and vegetation in slow-moving or still areas of the water. It is also claimed (Küçük and İkiz 2004; Özel *et al*. 2006) that *Pseudophoxinus* can survive drought periods sank in the mud. Adults feed on zooplankton, small insects, larvae and plants. The spawning period is from April to July depending on species and region (Küçük and İkiz 2004; Özel *et al*. 2006). Temperature tolerance is between 5-20 °C. High, minimum population doubling time is less than 15 months. Most of the members of the genus are threatened due to water abstraction and habitat destruction (Froese and Pauly, 2016).

Biodiversity deals with the variety among the organisms. Different species co-exist in the same habitat to form communities. Genus *Pseudophoxinus* tends to co-exist with genera *Aphanius, Nemacheilus* and *Cobitis*. As well as geological factors historical and environmental factor are also influence the biodiversity and species richness.

1.1.5. Pseudophoxinus of Turkey

P. alii is endemic to Ilıca and Kömürcüler streams Aksu River (Küçük, 2007) and reported as endangered species in International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species, 2015. Water abstraction, water retention by dams, pollution and climate change induced reduction in rainfall are all major threats for these two streams which are about 40 km long in total. They are situated in an area densely populated by domestic waste to due increasing human population. Rapid conservation action plans have to be implemented for this species since there is none at all at the moment (Freyhof, 2014a).

Endemic *P. anatolicus* (Hanko 1924) inhabits lakes and streams at Ereğli as well as Lake Beyşehir, Lake Suğla and reported as critically endangered species by Fricke et al., 2007. The species is also reported as endangered species in the Red List of Threatened Species of International Union for Conservation of Nature and Natural Resources (IUCN. 2015 and Küçük, 2006). The species has no predator in its native environment, therefore it is very vulnerable to introduction of exotic species. The population in the lake Beyşehir used to be an economically important species until 1970's where its decline has started. Recent threats for this species are pollution, water abstraction and fishing pressure. Although there aren't any conservation plans for the species, its habitat should be protected urgently (Freyhof, 2014b).

P. battalgilae is endemic species for Lake Beyşehir, dam lake northeast of Gümüşler, Niğde province and small pool in former Lake Büget 2 km north of Eşmekaya, Aksaray province. There are conflicting reports about the conservation status of this species. Küçük (2006) has been reported the species as vulnerable, Fricke et al. (2007) claims that it is critically endangered. Nevertheless it has recently been categorized under the least concern species by IUCN (2015). The introduction of exotic *Sander lucioperca* created predation pressure over the species. Besides water extraction, reduced rainfall seems to cause a decline in populations of *P. battalgili.* There might not be a need for implementing any conservation plan for this species for the time being. However, over population and reduced rainfall causes a decline of populations. Therefore habitat of

this species closely monitored for protection and increasing demand for water use for human consumption IUCN (2015).

P. burduricus is an endemic species inhabiting freshwaters running in to the Lake Burdur and the Lake Salda. Occurrence of the species in Değirmendere, Karamanlı stream, Düger spring and Sazak spring near Lake Yarışlı has also been reported. It is listed as an endangered species by IUCN (2015). Inhabiting lake, stream and springs this species faces threats of habitat loss due to water abstraction, pollution and climate change (Freyhof, 2014c). Therefore it is advisable to take precautions for habitat conservation for this species.

P. crassus, endemic to Turkey, has been reported as an endangered species (IUCN, 2015; Küçük, 2006; and Fricke et al., 2007). Distributed in central Anatolia the occurrence of species has been reported from the Lake Tuz basin including freshwaters of Cihanbeyli stream, İnsuyu, Güneşli, Haymana and Eşmekaya in central Anatolia. *P. crassus* populations are threatened by water abstraction, climate change and habitat destruction (Freyhof, 2014d) due to heavy agricultural activity, and water pollution.

P. egridiri is endemic to Eğirdir Lake (Anatolia). Although it has been reported as critically endangered species by Küçük (2006) and Fricke et al. (2007), IUCN (2015) Red List of Threatened Species puts it under endangered species category. Major threat for the species has become the introduction of exotic species. Existing populations of the species inhabits two streams 5 km in total and Karaot location which provides about 1 km shelter for the species. Low rainfall and water abstraction threatens the species due to dry-out in the streams. Habitat conservation of this rare occurring species is at upmost importance (Freyhof, 2014e). Hrbek *et al* (. 2004) reports that *P. egridiri* has got a small population that survive in few surrounding streams

.*P. hittitorum* is endemic to Eflatun spring and Bakaran stream (Beyşehir Lake). It is enlisted as an endangered species in the Red List of Threatened Species (IUCN, 2015). It was indicated that the species distribution range is about 70 km^2. Water abstraction, water retention by dams, pollution and climate change induced reduction in rainfall are

major threats to this species. Habitat preservation is required for this rare species (Freyhof, 2014f).

P. maeandri is endemic to two small springs, Işıklı and Düden at Dinar, both in the upper Büyük Menderes drainage. This species prefers water resources with dense vegetation. Conservation status of the species reported as endangered (IUCN, 2015). It has been estimated that the estimated distribution range of the species is 4 km^2. Threats affecting the species which prefers water resources with dense vegetation are water abstraction, water retention by dams, pollution and habitat loss due to reduced rainfall. Habitat conservation for the existence of the species is necessary (Freyhof, 2014g).

P. maeandricus is endemic to Karadirek stream near Sandıklı, an isolated basin which flows underground to Işıklı spring and Hotamış Lake basin. It is distributed to an area of 50 km^2. It is reported as an endangered species (IUCN, 2015). Water abstraction, water retention by dams, pollution and climate change induced reduction in rainfall are major threats to this species in Karadirek stream. Being restricted to a single water resource this species requires habitat preservation action (Freyhof, 2014h).

1.2. Geometric Morphometrics for the Analysis of Shape Variation

Morphometry is the basic tool of classification of species as well as any of other objects to be identified. Observation of the variation in morphology has always been starting point for the identification and classification of organisms. Biological shape is the most important aspects of an organism's phenotype providing a link between the genotype and the environment (Ricklefs and Miles 1994). Shape differences can be a result of evolutionary diversification, ontogenic development, disease and/or adaptation (Rohlf and Marcus, 1993; Richtsmeier et al., 2002). Within taxa patterns of intraspecific phenotypic variation have great importance in the study of evolutionary history of a species since this morphometric variation is acknowledged to be the raw material of evolutionary change (D'anatro & Loureiro, 2005). Morphometric variation data of organisms have successfully been employed in aquaculture studies, in assessing fish health, estimation of biomass, conservation driven biogeographical studies and population discrimination (Haas & McPhail, 1991; von Cramon-Taubadel et al, 2005).

The development of morphometric features of organisms is partly influenced by genetics and partly by the environment (von Cramon-Taubadel et al, 2005). The main argument in morphometric studies is the relative importance of genetic and environmental factor in the determination of morphology. It has been shown that a pattern of morphometric variation within a species is consistent with differences in genetic variation among freshwater fish (Corti *et al.*, 1988). Cadrin (2000) also claimed that some morphometric traits seem to be under the control of genetic structure of the organism. It is known that organisms have a developmental stability in their shape as long as they have adapted to their environment. Therefore endangered populations having low genetic diversity are expected to have an increase in their morphometric variance. The comparative study on the morphology of hatchery and wild salmonids showed that different optimum morphologies exist for the same species reared in different environment. Moreover, salmonids from different habitats show morphological convergence when they are reared in similar environment (Hedenskog *et al.*, 1997; Swain *et al.*, 1991) Thus, fish species can show great phenotypic plasticity in their body shape that provides big advantage to respond different environmental conditions (Thompson, 1991). Scientists have inferred from these studies that selection pressure on heritable traits governing shape would be expected to differ among the same fish species growing in different environments, leading to greater survival of some genotypes in different habitats (Haas & McPhail, 1991; Swain et al., 1991; von Cramon-Taubadel et al., 2005).

A controversy has arisen over which method is the "best" for quantifying the morphological difference within and between species. Traditionally, classification process involves measuring distances, counting numbers and scale colors to figure out resemblance. Traditional morphometry uses the ratios in order to reveal the shape variation. However there has never been a sound theory for what to count and what to measure. Therefore every assessment has its own measurement approach as far as the traditional measurement of characters is concerned (Zelditch *et al.*, 2004). Traditional morphometric analysis of the shape generally involves multivariate statistical analysis of discontinuous data combined with linear distance measurements such as length, width, angles and ratios to carry out statistical analyses (Marcus, 1990). Nevertheless, variables of this kind of data sets are often not independent and subject to errors since

they are dependent on the measurement of distances from a single point on organism (Monteiro et al., 2002). This approach may be misleading when comparing shape differences of closely related taxa and populations of the same species. Such an approach may lead to overlapping data as well as obtaining irrelevant data.

Traditional multivariate data are not sufficient to capture the actual shape of an organism (Zelditch et al, 1995, Adams, *et al*., 2004). For instance, results of the measurements of maximum length and width could be highly similar between two organisms that may not even be remotely related. Size dependent shape variation is the most important aspect in traditional morphometrics as the variables are highly correlated with the size. Therefore obtaining size free shape variation requires crucial correction methods (Jolicoeur 1963; Sundberg 1989; Jungers *et al*. 1995). Main set back is that it is difficult to choose which methods to apply for a certain data set since, among many, different methods give different (Adams, *et al*. 2004).

Geometric morphometrics came into account as a revolution in the analysis of shape variation (Rohlf and Marcus, 1993) since the method provides data free of non-shape elements to evaluate the shape of a wide range of organisms from soil mites (Baran et al, 2011) to fish (Corti and Crosetti 1996; Loy *et al*. 1996; Loy *et al*., 1999a and 1999b; Loy *et al*. 2000a and 2000b; Douglas *et* al. 2001; Gallo *et al*. 2002; Cavalcanti 2004, Altun et al., 2015). Kendall (1984) described the shape as "all geometric information that remains when location, scale and rotation effects are filtered out from an object".

Geometric morphometrics investigates spatial and temporal morphometric variation in shape of organisms and also its relationship between genetic structures of fish populations (von Cramon-Taubadel et al, 2005). Morphometric differences between hybrid and subspecies, which cannot be detected by traditional morphometric measurements, are easily identified by geometric morphometry techniques.

The method is based on the landmark configurations (Bookstein, 1986). Landmarks provide 2 (x, y) or 3 (x, y, z) dimensional coordinates of homologous points depicting the shape of a given morphological structure, whether it be an organism as a biological entity or else. It provides 2 or 3 dimensional coordinates from the previously defined

morphological landmarks on the images of biological specimens. Zelditch *et al.* (2004) defined the landmark as a point of correspondence on each object that matches between and within populations. Landmarks should be (1) homologous anatomical points, (2) reside in the same topological position, (3) cover the shape of the organism adequately and (4) be repeatable.

Landmarks allows a detailed analysis of how the former shift relative to one another when shapes of organisms are compared, so that geometric morphometry permits body size and shape to be analyzed separately (Zelditch *et al.*, 2004). Landmark data comprises both shape and non-shape variation. Rotation (orientation around the axis), translation (displacement along the axis) and scale (size) are non-shape information of the biological morphometry. Non-shape variation should be eliminated in order to analyze the shape itself (Bookstein, 1991; Bookstein, 1996).

Analysis of landmark data is done by using procrustes distances obtained from a matrix including x, y coordinates of a 2 dimensional landmark configuration as variables (K) and the number of specimens (M) as operational taxonomic units (OTUs). Hence, a matrix defined as K x M is obtained. For example, a shape configured by 13 landmarks in a population of 50 specimens enables a matrix of 26 x 50.

First of all, a mean called consensus is obtained for the matrix of landmarks coordinates. The consensus represents the average shape of the organism. Then, the translation effect is removed from the shape by centering each specimen onto consensus so that the specimens differ only in the position of centroid. As the result of this action the shape has not changed but freed from translation effect (Bookstein 1991).

Next action is the elimination size variation from the shape. This is done by calculating Centroid size which is the square root of the sum of the squared distances of the landmarks from the centroid (Bookstein 1986). All the centered configurations of landmarks are scaled to unit centroid size in this operation. Centroid size is not the correlation between size and shape. Therefore, all the centroid sizes can be rescaled to be one without altering the shape (Bookstein 1996).

Finally, the removal of the effect of orientation is realized by the selection of a convenient references configuration and orientation of the target configurations onto the nearest references configuration to minimize the distances corresponding to target and the reference. This action results in the removal of all of non-shape information form the actual shape (Bookstein 1996) and the shape is removed to a new space called as Kendall's Shape Space (Rohlf, 1999). All the landmark configurations are represented as a single point on the Kendall's Shape Space and special statistical techniques are required for the analysis of shape variations (Kendall 1984; Dryden and Mardia 1998; Rohlf 1999). However, Kendall's Shape Space can be linearized by the projection, and then, linear multivariate statistics can be applied as well (Kendall, 1984; Rohlf, 1999; Slice, 2001).

These series of mathematical operation is called superimposition (Bookstein 1996). Generalized Procrustes Superimposition (GPS) is a superimposition method using sum of the least squared distances and it is applied to reveal shape variation where the landmark number is large enough to represent the shape. (Rohlf and Slice, 1990).

Visual presentation of the shape variation is important since geometric morphometry computes the variation in shape. Transformation grid called thin plate splines *(TPS)* are used for the visualization of shape differentiation present among specimens, populations, and species (Bookstein, 1991). Corresponding GPS partial warp scores, tangent space measure of distances (procrustes distances) as a metric, are used to visualize shape variation (Rohlf 1993). TPS provides a visually interpretable description of shape differences (Zelditch *et al.*, 2004; Rohlf 1999; Bookstein 1996).

Relative Warp Analysis uses partial warps scores as variables in conventional multivariate statistical analyses such as Principle Component Analyses (PCA) or Canonical Variate Analyses (CVA) (Rohlf 1993). Shape variation determined by geometric morphometrics can be correlated with independent data such as genetic, ecological or demographic variation. Tps-Reg software was developed for multivariate multiple regression analysis between shape and independent variability (Rohlf 2003, tpsRegr, Department of Ecology and Evolution, State University of New York at Stony Brook).

1.3. Variations at Molecular Level

1.3.1. Allozymes

Genetic variability in the natural populations can be examined at protein level using the advantage of different forms of proteins. A protein can have either isozymes or allozymes or both. Isozymes are functionally same proteins produced by different loci. However, allozymes are co-dominant variants (alleles) of proteins governed by the same loci and has been being used widely in systematics, evolution, ecology and conservation genetics for the determination of genetic variability since 1960's (Harris 1966; Lewontin and Hubby, 1966). Isozymes are also equally important structures for molecular studies (Smith and Wayne 1996). Despite the fact that new approaches for molecular genetics, i.e. microsatellite, allozymes techniques are widely used because of relatively low cost, and easy and rapid application (Leary *et al*. 1993).

Allozyme electrophoresis technique relies on the separation of alleles of an enzyme or a protein in an electrophoretic media, usually starch gel. Migration of allozyme alleles through such media is mostly different due to the differences in their electrical charges. Shape and size of the tissue or experimental material are also important factors for the migration due to electrical charge applied. Visualization of the product is realized by a staining technique known as enzyme specific staining. Allozyme analysis reveals the genetic variations of gene products. Amino acid composition is the reason of difference in alleles, and hence, the difference in the sequence of the DNA encoding the protein (Smith and Wayne 1996). A number of tissue as well as blood and secretions of organisms can be used for allozyme analysis. However, tissue specific enzyme expression should be taken into account when an experiment is designed (Pasteur *et al*., 1988).

The electrical charge of a protein is determined by the total charge resulting from radicals of amino acids forming the protein. Hydrophobic amino acids such as alanine, valine, etc., have non-polar radicals that are ionisable. Non-charged amino acids with a polar radical like glycine, serine, etc., are more soluble than hydrophobic amino acids. Amino acids, i.e. aspartic acid and glutamic acid, with negatively charged radical are

acidic at intra-cellular pH whereas amino acids with a negatively charged radical are alkaline at intra-cellular pH. Point mutations can change the form of amino acids in polypeptide chain and creates alloproteins (allozymes) with different electrical charges. Despite fact that such mutations may cause the replacement of one amino acid with another, net charge of the protein may not change if new amino acid is in the same group according to their radicals. This means that some mutations may remain undetected in the allozyme electrophoresis technique, which result in under-estimation of the level of polymorphism (Pasteur *et al*., 1988; Thelen and Allendorf, 2001).

Allozyme electrophoresis has a wide range of application in conservation biology. The technique allows evaluation of genetic variation, polymorphism, heterozygosity, population history like bottlenecks (Wayne *et al*. 1991; Hartl and Pucek 1994). Allozyme electrophoresis has also dealt with assessment of level of hybridization (Leary *et al*. 1993), breeding systems, gene flow (Dole and Sun 1992), population viability, effective population size (Briscoe *et al*. 1992), paternity, species determination and phylogenetics (Erwin 1991). It has been suggested that there is strong correlation between allozyme heterozygosity and individual fitness (Allendorf and Leary 1986; Leberg 1990; Leberg, 1993).

However allozyme technique might be a valuable technique to address quite a number of questions in biology, it has got its weakness as well. Large sample size is required to be able to increase the precision in the estimation of heterozygosity and polymorphism. The number of loci under examination should also be large to enough to detect most of the available alleles. Sjorgen and Wyoni (1994) claims that a majority of loci examined within most population is monomorphic, and hence, certain genetic information relating to populations are often unrecognized even when sampling size and loci are kept high.

Sometimes estimation of genetic diversity using allozyme techniques could be problematic, too. Presence of the silence and undetected mutations is one of the problems since this situation reduces the estimation of the level of heterozygosity and polymorphism. Koehn *et al*. (1988) claims that another problem is the reality that some of the allozymes are under the effect of natural selection. However, most of the models

in population genetics assumes that the loci in consideration is not influenced by the natural selection.

1.3.2. Microsatellites

Species management programs mainly focuses on the conservation of genetic variation after all genetic variation is what let species adapt to changing environmental conditions. Microsatellites also known as simple sequence repeats (SSPs) are repetitive short (1-6 bp) DNA sequences (Tautz and Renz, 1984) and highly polymorphic, especially, when they are long and uninterrupted. They seem to be equally distributed across eukaryotic genome (Goldstein and Schlötterer, 1999) and claimed to occur approximately once every 10 kb in fish species (O'Connell and Wright, 1997). Most common satellites are the repeats of Poly (A)/Poly (T). Most of the microsatellites are found in the non-coding regions of genome. However, it been claimed that mutational changes in microsatellites leads to phenotypic changes (Grünewald et al., 2015; Gymrek et al., 2016*).* Ellegren (2004) also claims the functional role of microsatellites as coding and regulatory elements. Moreover, microsatellite fragment variation has also have phenotypic effects on physiology and development of organisms. A strong correlation between microsatellite repeat length and age of onset and severity of human diseases has also been claimed (Ashley and Warren 1995; Goldstein and Schlötterer 1999)

Microsatellites are useful neutral markers and extensively used in conservation biology, evolutionary genetics and systematics because of very high levels of observed. Microsatellites offer a powerful alternative to other marker systems where a large number of loci are required, such as genome mapping or identification of quantitative trait loci (QTL). They have also find place in practical applications such as forensics and disease diagnostic (Di Rienzo *et al*. 1998; Goldstein and Schlötterer. 1999; Sainudiin *et al*. 2004) since they can easily be isolated (Schlötterer *et al*., 1991). Microsatellites allow PCR amplification of sequences using minute quantities of tissue for analysis. Investigation of material obtained non-invasibly is another advantage of the technique (Goldstein and Schlötterer 1999). Moreover, there is potential for significant increases in the number of samples that can be genotyped in a short time (O'Connell and Wright, 1997).

Despite the advantages there are some difficulties associated with microsatellite applications. Null alleles is one of them caused by the change in the microsatellite loci and results with no amplification (Goldstein and Schlötterer 1999; Coltman and Slate 2003). Polymerase enzyme digestion may cause slippage and interferes with the scoring of the microsatellite.

Microsatellite polymorphism at the genomic level is enhanced by mutations (Weber and Wong 1993). Goldstein and Schlötterer (1999) indicated that rate of mutation at most microsatellite loci is much higher compare to that of other loci in the same genome. However, flanking regions of microsatellites are subjected to mutations less (Angers and Bernatchez 1997). Schlötterer (2000) emphasized that this high rate mutation and variability could be affected by DNA slippage, length constraints, repeat numbers, repeat types, flanking regions, recombination rates, sex and age.

Slatkin (1995) suggests that stepwise mutation model (SMM) which relies the size difference between alleles is a more appropriate statistic for analysis of microsatellite data. However, the application of the model should be considered carefully since it is inadequate to explain variation cause by differences in the size of the alleles when long microsatellite alleles are present (Huang et al., 2002). Goldstein *et al*. (1995) suggests that stepwise mutations are useful to obtain historical information about the decent of an organism.

Feldman *et al*. (1997) favors two phase stepwise mutation model (TPM) where the number of repeat unit is not limited. Deletions and insertions in microsatellite loci are always expected (Paetkau *et al*., 1995). Colson and Goldstein (1999) claims that allele frequency distribution of many microsatellites fits in TPM.

Proportional Slippage Model suggested (Point Mutation Model) by Kruglyak *et al*. (1998) emphasizes on the microsatellite length. The model suggests that the longer the length of microsatellites, the more the mutation occurs within the loci (Watkins, 2006).

1.3.2.1. Microsatellites in Fish Populations

The very high levels of variability associated with microsatellites, the speed of processing and the potential to isolate large numbers of loci provides a marker system capable of detecting differences among closely related populations of fish. Therefore microsatellites have high potential for evaluating small and endangered species (Moxon and Wills 1999).

Comparison of mtDNA and microsatellite has shown that the single and combined microsatellite loci data revealed significantly higher levels of differentiation although the number of mtDNA haplotypes is similar to the number of alleles of microsatellite loci (O'Connell and Wright, 1997). Microsatellites are useful tools for the characterization of genetic stocks, broodstock selection, constructing dense linkage maps, mapping economically important quantitative traits, identifying genes responsible for these traits and application to marker-assisted breeding programs in the field of aquaculture (Chistiakov et.al, 2006)

O'Connell and Wright (1997) compared two types of microsatellites, dinucleotide simple sequence repeats and tri-, tetra-nucleotide simple sequence repeats, with other molecular techniques such as allozyme, mitochondrial DNA (mtDNA), randomly amplified polymorphic DNAs (RAPDs), single-copy nuclear DNA restriction fragment length polymorphism (ScnDNA), expressed sequence tags (ESTs), multilocus fingerprinting (MLF) and single-locus minisatellite probes (SNPs) used in fisheries. They reported that technical requirements for development of the technique is moderate to high for dinucleotide microsatellites and high for tri-, tetra-nucleotide microsatellites. Technical requirements for screening is claimed to be high for dinucleotide microsatellites whereas it is low for tri-, tetra-nucleotide microsatellites compare to other techniques. Comparison of cost revealed that it is high for the development and screening of dinucleotide microsatellites although it is high to moderate for the development and low for screening of tri-, tetra-nucleotide microsatellites. Storage requirements for microsatellites, as well as mtDNA are low. Potential of use of microsatellites in genome mapping, parentage assessment and population genetics is high.

Investigations of microsatellite variability compare to allozyme, mtDNA and minisatellite techniques revealed significant allele frequency differences between populations of Atlantic salmon (*Salmo salar*) separated due to preference of feeding area (McConnell et al, 1995; McConnell et al, 1997). Tessier et al., (1995) have also reported significant microsatellite differentiation for adjacent populations of the same species. Significant microsatellite variation among populations of Atlantic cod (*Gadus morhua*) was also demonstrated by Brooker et al. (1994) and Bentzen et al. (1996).

O'Connell and Wright (1997) suggested allelic diversity is more informative than estimates of heterozygosity in the case of severe population bottlenecks.

Investigation of differentiation index (FST) has shown that microsatellites revealed at least twice as much higher value for Atlantic cod (*Gadus morhua*), Pacific herring (*Clupea harengus*) and Brook charr (*Salvelinus fontinalis*) than allozyme technique. However, FST revealed by allozyme was Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) (O'Connell and Wright, 1997).

Inbreeding is an important issue in conservation genetics. Lack gene flow in inbreed populations causes rapid loss genetic variation due to random genetic drift. Therefore inbreeding depression causes reduction in the fitness in populations. Outbred populations, on the contrary, have better fitness (heterosis) because the genetic variation is kept high in such populations due to constant gene flow between populations. However, outbreeding may also cause depression resulting in the loss of fitness at the species boundary of adjacent populations (Goldstein and Schlötterer 1999). Co-dominant markers such as allozymes and microsatellites are used to analyze the level of inbreeding. However, Britten (1996) claims that microsatellites are more appropriate markers since the number of alleles are higher than that of the allozymes which may not give as much polymorphic as the microsatellites.

1.4. Morphology and Genetics

Morphometry of organisms, hence the shape as one of the major components of phenotype, has the upmost importance since the description of any objects starts with the

validation of resemblance to closely related ones. Adams *et al*. (2007) claims that phylogenetic diversification of populations can be depicted by the evaluation of features of phenotypic traits which are the result of interaction of genetic background and environmental conditions. Morphology of an organism is highly affected by some fitness traits (Ricklefs and Miles 1994) and the variation of phenotype is supported by the genetic variation between and within populations (Boag 1983).

However phenotypic variations in natural populations are determined by the range of genetic variations within the populations, the occurrence of the variation is directed by the pressure of natural selection (Clarke 1979) and, hence, the characteristics of morphology of populations is arranged by the level and direction of natural selection on phenotypic traits (Leary *et al*. 1985; Swain et al., 1991; Corti *et al*. 1988).

Morphology of organisms is under the effect of environmental conditions and genetic variations possessed by the populations. However, considerable stability in the morphology has been considered to be a result of canalized selection of phenotypic variations where the deviations are eliminated by the process of natural selection (Waddington, 1942; Lerner, 1954; Mukai and Nagano, 1983; Boag, 1983; Leary *et al*., 1985).

Developed from Waddington's (1942) theory of "canalization of phenotypes" the theory of "*genetic homeostasis*" (Lerner, 1954) suggests that the level of heterozygosity is correlates with the sustainability of populations. Since the individuals with more heterozygosity have better chance to cope with environmental conditions, heterozygosity helps to find the fittest phenotypic trait. The level morphological differentiation within population is reduced with the increasing level of heterozygosity, and hence, correlation between morphology and heterozygosity is negative. This is caused by developmental buffering effect of heterosis (Mitton and Grant 1984; Mitton and Koehn 1985; Shikanoa *et al*., 2005). However, Zink *et al*. (1985) claims that the relationships between morphometric traits and genetics of an organism is complex and is in need of clarification.

Morphological deformities correlated with high level of inbreeding were reported in farm-reared populations of rainbow trout (Aulstad and Kittelsen, 1971; Kincaid, 1976a and Kincaid, 1976b), in cultured strains of guppy (Shikanoa *et al*., 2005) and in molluscs (Mitton and Koehn, 1985). Allozyme investigations on salmonid fish revealed that morphological traits were more stable in individuals with higher heterozygosity (Leary *et al*., 1983 and Leary et al., 1985). Although it was low Gjerde *et al*. (2005) were also reported negative correlation between deformation and inbreeding as a result of allozyme investigation of Atlantic salmon in capture.

As oppose to negative correlation expected between heterozygosity and morphology, some investigations revealed either no correlation or positive correlation between genetics and morphology of organisms. For example, Baranyi *et al*. (1997) reported that investigation of allozymes and morphology of roach (*Rutilus rutilus*) did not reveal significant correlation since both genetic and morphological variation were remarkably high within samples. Similar results were also reported for birds (Zink *et al*., 1985) and insects (Fowler and Whitelock 1994; Gilligan *et al*. 2000; Carchini *et al*., 2001; Hosken *et al*., 2000).

Positive correlation between morphological variation and allozyme heterozygosity was also reported in freshwater fish genus *Cottus* (Strauss, 1989). However, further investigation of the genus revealed that level of variations in morphology of body parts differs greatly and the morphology of more stable parts i.e. head region are more correlated with the genetic structure of the individual or the population (Strauss, 1991).

In the investigation of freshwater fish *Pseudophoxinus* Telli (2008) reported that there were negative correlation between morphology and microsatellite variation. However, the results of allozyme heterozygosity showed no such correlation.

Aims of the study are

- to investigate morphometric and genetic variations using three methods, (1) geometric morphometrics, (2) allozyme and (3) microsatellite, in 17 populations of *Pseudophoxinus*, comprising six species as well as five populations with questionable species status,

- to investigate evolutionary relationships between these populations in the light of the data obtained using aforementioned three approaches,
- to evaluate the strength of morphometric and molecular data for phylogenetic analyses,
- to find out the occurrence of gene flow levels if they were effective in the differentiation of populations and
- to determine the level of heterozygosity in order to find out population structure and to be able to suggest conservation plans for the species of the genus.

CHAPTER 2

MATERIAL AND METHODS

2.1. Collection of *Pseudophoxinus* Samples

Pseudophoxinus samples were collected from 17 different locations in central and southern Anatolia. The details of locations, number individuals collected, dates and the positional details of the locations are enlisted (Table 3.) and locations are illustrated in Fig.5.

Table 3. *Pseudophoxinus* sampling locations.

Populations	**Locations**	**Latitude**	**Longitude**	**Dates:**	**#**
P. batalgili	Lake Çavuşçu, Ilgın, Konya	37,3041	32,0473	07.08.2006	36
	Oymapınar Dam, Manavgat, Antalya	36,8782	31,52	06.08.2006	33
	Taşağıl Stream, Seydişehir, Konya	38,3269	31,8642	04.08.2006	37
	Lake Suğla, Seydişehir, Konya	37,3783	31,8914	07.06.2006	29
P. burduricus	Düger Spring, Burdur.	37,5747	30,0224	05.08.2006	24
	Salda Stream -Burdur	37,5289	29,6453	05.06.2006	33
	Sazak Spring, Burdur	37,5461	29,9446	05.06.2006	39
P. crassus	İnsuyu Stream, Cihanbeyli, Konya	38,7034	32,7637	08.08.2006	34
	Lake Gök, Kulu, Konya	39,0081	32,8363	19.07.2006	35
P. egridiri	Eğirdir Lake, Isparta	38,1466	30,8642	03.08.2006	33
P. fahrettini	Köprüçay-Aksu -Isparta	37,7957	31,0359	03.05.2006	25
P. hittitorum	Eflatun Spring, Beyşehir, Konya	37,8267	31,6746	03.08.2006	24
P. sp	Bademli, Beyşehir, Konya	37,6458	31,7005	03.06.2006	4
	Deliktaş Location, Yeşildağ, Konya	37,5125	31,4549	06.06.2006	32
	Kırkpınar Köyü; Korkuteli, Antalya	37,1316	29,9167	05.08.2006	30
	Körkuyu Location, Yeşildağ, Konya	37,5278	31,4631	06.06.2006	30
	Kuğulu Park, Seydişehir, Konya	37,3923	31,8417	08.06.2006	23

Specimens were caught with a net from their habitats using electrofishing. Specimens were placed individually in 15 ml or 50 ml Falcon tubes depending on the size of the specimen and stored in dry ice at around - 70 °C in the field. This procedure applied to

prevent the destruction of molecular structure of the samples as well as conserving the morphological structure of the fish. Eventually, all samples were brought to laboratory and store at -80°C in the laboratory until further morphological examination and tissue sampling for molecular investigation.

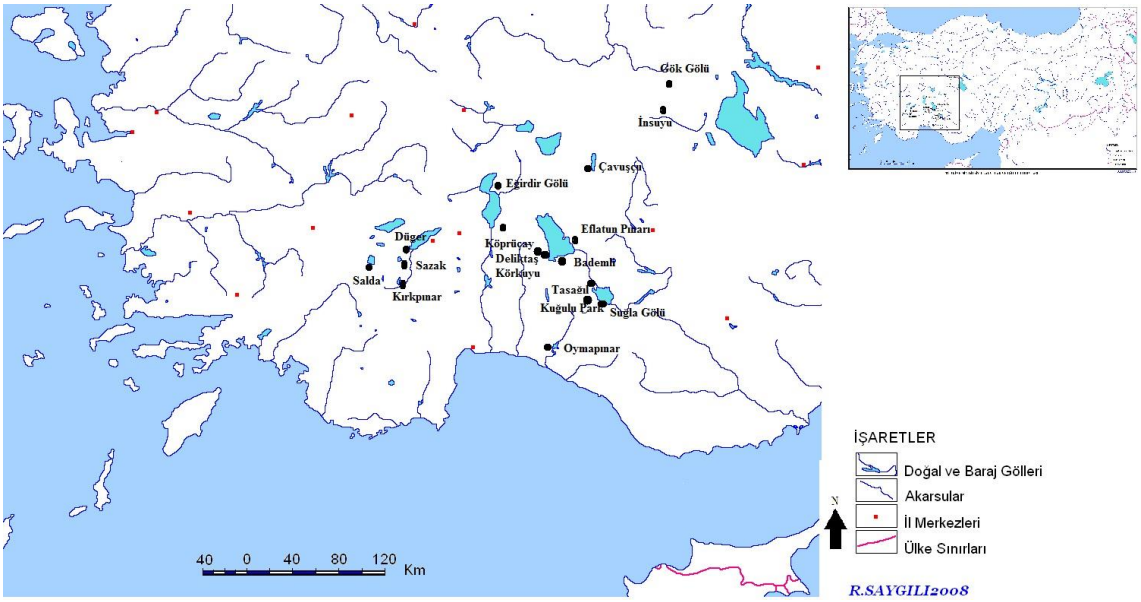

Figure 5. Map of *Pseudophoxinus* sampling locations.

2.2. Geometric Morphometrics Analysis of Shape Variation in *Pseudophoxinus* Populations

Geometric morphometrics analysis of *Pseudophoxinus* were carried out on 17 populations including six species. First of all the pictures of 499 specimens were taken using a Nicon D70s (a high resolution digital SLR camera carrying a Nicon 60 mm macro lens to produce high quality specimen pictures for digitizing. The camera was stabilized using a tripod over a table, where the specimens were placed. Light reflected on the specimens was standardized using circular macro flash (Vivitar Macro Flash 5000) which also resulted in the production of sharp and clear images.

Secondly, the images were digitized using TPS series softwares. TPSUtil (Rohlf, 2004) is a file utility software including file handling options. The software enables building data file, converting the file to be handled in other programs, appending or splitting the files, ordering and deleting specimens, etc.

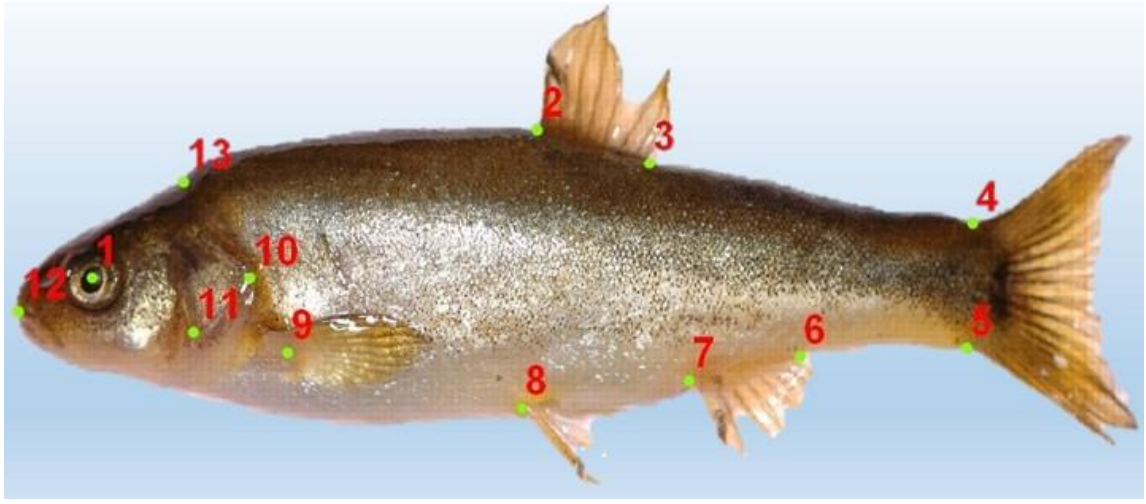

Figure 6. Landmark configuration on Pseudophoxinus specimens.

Upon the building of a tps file TPSDig2 (Rohlf, 2005) was used to produce landmark based data from images. Shape variation of the populations of *Pseudophoxinus* determined on the base of 13 homologues landmarks (Table.4) depicting whole shape of the specimens (Fig.1.). X-y coordinate data of the landmarks stored in tps files to be used for geometric morphometrics analysis.

Table 4. Homologous landmarks on Pseudophoxinus specimens.

Landmark	Description
1	Central point eye.
2	Anterior point of dorsal fin base
3	Posterior point of dorsal fin base
4	Dorsal point of peduncle-caudal fin conjunction
5	Ventral point of peduncle-caudal fin conjunction
6	Posterior point of anal fin base
7	Anterior point of anal fin base
8	Anterior point of pelvic fin base
9	Pectoral fin base
10	Posterior tip of operculum
11	Ventral tip of operculum
12	Tip of mouth
13	Head-body conjunction at dorsal

Third step in the analysis of shape variation is to eliminate non-shape information such as size, rotation and translation. Freeing the shape from non-shape information is done by superimposing the specimens onto a consensus (average) shape. The consensus is

obtained by generalized procrustes analysis (GPA) which is based on the generalized least-squares superimposition (GLS). Consensus is obtained by selecting a specimen at random and superimposing others onto that until an average is obtained from all of the specimens (Rohlf and Slice, 1990). Further to obtaining consensus shape variation within and among groups is calculated using the scores of partial warps and uniform components. NtSys 2.21q (Rohlf, 2009), as well as Morpheus et al. (Slice, 1998) was used for the calculation of consensus shape. NtSys 2.21q (Rohlf, 2009) was also used for the calculation of within and among groups variations, clustering of populations and plotting the results when plots i.e. CVA plots, PCA plots, or dendograms were available. Dis/similarity matrices, and scores of principal component analysis (PCA) and canonical variates analysis (CVA) were built in NtSys 2.2 (Rohlf, 2009) for statistical analysis of the shape variation.

PCA is a highly important method of ordination analysis since it creates such a new set of orthogonal coordinate axes that maximum variance is reached up on the projection of points onto them. While defined in terms of variances and covariances, PCA results are sensitive to the arbitrary choices of units of measurement. Therefore the data were standardized prior to PCA computation.

CVA determines the variation among groups of specimens relative to the average variation found within the groups. It is performed after means and the average within-group covariance matrix computed by POOLVC module in NtSys2.21. To run this module the data of the groups should be placed in the same file, have the same number of variables and be in the same direction, for example, columns as variables. The overall significance test for CVA is also the appropriate significance test for a single classification multivariate analysis of variances, MANOVA (Rohlf, 2009).

Morpheus et al. (Slice, 1998) was also used for pairwise comparison of the groups and plotting of the partial warps (shape variations) of the groups onto thin plate spline (tps) graphics.

2.3. Allozyme Analyses

Allozyme analyses at 10 loci (Table 5.) were carried out using 50 mg muscle tissue. The tissues were homogenized 100 µl distilled water and centrifuged at 5 000 rpm at 4 °C for 5 minutes. The supernatant was stored in 500 µl Eppendorf tubes at –80 °C for further use. Genotypes of individuals were determined by enzyme specific staining technique following starch gel electrophoresis of the samples.

For the electrophoresis, 9% (weight:volume) starch gel was prepared melting 18 g starch in 200 ml of Tris-Citrate pH 7.5 gel buffer solution prepared following the protocol of Pasteur *et al*. (1988). Nitrogenous gasses of the gel was vacuumed out prior to transfer of the gel onto a glass plate used in electrophoresis. The framed gel, then, cooled down for about 20 minutes to complete polymerization of the gel. The upper layer of the gel removed with an electrical wire to obtain a smooth and easily penetrable surface for placing total homogenized protein extracts previously prepared. The frame placed onto ice to prevent denaturation of the proteins prior to electrophoresis. Watmann 3 filter paper cut into small rectangular pieces dipped in the homogenates first and, then, into the gel in a line. After the loading of the samples the gel subjected to electrophoresis at a constant temperature of 4 °C (Smith and Wayne, 1996) at 60 mamp, 200 V for 3 hours.

Table 5. Allozymes studied in *Pseudophoxinus* populations.

Enzymes	IUBNC Number	Locus	Tissue	Buffer
Isocitrate Dehydrogenase (ICD)	1.1.1.42	ICD	Muscle	TC7.5
Glucose-6- phosphate Dehydrogenase (GPDH)	1.1.1.49	GPDH	Muscle	TC6.7
Glucose-6-phosphate Isomerase (GPI)	5.3.1.9	GPI-I	Muscle	TC6.7
		GPI-II	Muscle	TC6.7
Malate Dehydrogenase (MDH)	1.1.1.37	MDH-I	Muscle	TC7.5
		MDH-II	Muscle	TC7.5
L-Lactate Dehydrogenase (LDH)	1.1.1.27	LDH-I	Muscle	TC6.7
		LDH-II	Muscle	TC6.7
Phosphoglucomutase (PGM)	5.4.2.2	PGM	Muscle	TC6.7
Phosphogluconate Dehydrogenase (PGD)	1.1.1.44	PGDH	Muscle	TC6.7

TC, Tris-citrate gel and electrode buffer solution at pH 6.7 and 7.5.

Enzyme activity specific staining protocols (Pasteur *et al*., 1998) were used for the visualization of the allozyme variation on gel following the electrophoresis. Each of seven enzymes consist of 10 loci (Table 5.) was stained accordingly for about 30 minutes. Allelic designations were made according to (Pasteur *et al*., 1988).

Statistical analysis of allozyme data was carried out using the software POPGENE32 (Yeh, et al., 2000) computes the allelic frequencies, heterozygosity, inbreeding coefficient (fixation indices - Fis), genetic identity and distance matrix and UPGMA according to Nei, (1973a). For each population, observed heterozygosity (Ho) was calculated as the number of heterozygous loci divided by the total number of loci.

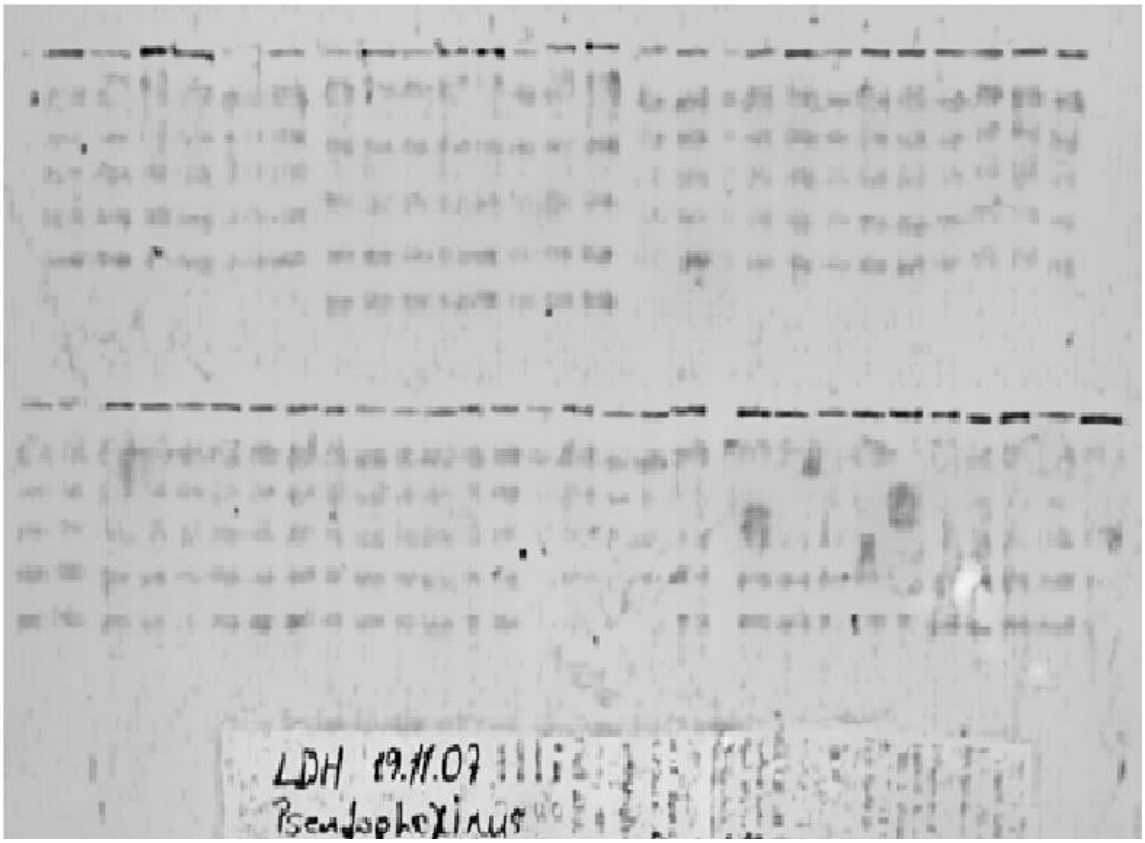

Figure 7. LDH in *Pseudophoxinus* populations.

2.4. Microsatellite Analyses

2.3.1. DNA Isolation

Genomic DNA isolation was carried out using Fermentas DNA purification kid. Around 50 mg muscle tissue from fish was dissected and transferred to sterile Eppendorf tubes including 200 µl TBE buffer (Fermentas). Following homogenization 600 µl Lysis solutions was added into the tubes. Tubes were incubated at 65 °C for 20 minutes with occasional shaking by inverting the tubes. Equal volume of chloroform was added and gently emulsified by inversion (3-5 times) prior to centrifuge at 10.000 rpm for 5 minutes. After centrifugation, supernatant containing DNA was transferred to a new sterile tube and 800 µl of precipitation solution prepared according to the protocol of Fermentas (720 µl of sterile distilled water and 80 µl of precipitation solution for each individual) was added and gently mixing by several inversion at room temperature. Samples were centrifuged again at 10.000 rpm for 5 min to collect a pellet of DNA at the bottom of the tube. Following the removal of supernatant DNA pellet was washed with 200 µl 1.2M NaCl solution without moving the DNA pellet. Then 600 µl of 70% cold ethanol was added to the tubes to precipitate to DNA at -20 °C for 3 hour. After the precipitation ethanol was completely removed by pouring and placing the tubes on tissue paper for a while. Finally, DNA was dissolved in 100 µl sterile distilled water by gently vortexing. Quality and quantification of DNA was determined putting 1 µl of the DNA in a spectrophotometer for absorption 260-280 readings. DNA was also confirmed with 0.8% agarose gel electrophoresis.

2.3.2. PCR Amplification of Microsatellites

Microsatellite investigation of *Pseudophoxinus* populations carried out using six primers used for cyprinids (Crooijmans et al., 1997 and Mesquita et al., 2003). The details relating to the primers given in Table.6. HVD Life Science CG1-96 gradient thermal cycler was used for PCR optimization and for amplification of the microsatellites.

The amount of DNA used for all PCR reactions was 50 ng. The primers were labelled with an appropriate fluorescent dye put in the PCR mixture. Three florescent dye e.g.

HEX, 6-FAM, TET were used in this study in order to separate the products to according to their sizes. This also enabled the reading of more than one products at the same time for time and cost reduction efficiency.

Reaction buffer for MFW-1 was prepared according to Crooijmans et al. (1997) by mixing 50 ng DNA, 20 pmole of both forward and reverse primers, 0,2 nM dNTPs, 0.5 units Taq DNA polymerase (Fermentas) and 1.75 mM $MgCl_2$ in a total 25 µl of 10x Taq buffer containing KCl. PCR cycle for MFW-1 was optimized as initial denaturation of double stranded DNA at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 47 °C for 40 s and elongation at 72 °C for 40 s and a final elongation at 72 °C for 5 minutes.

PCR mixture for Sar series microsatellites (N2F11a, N2F11b, N7F8, N7G5 and N7K4 (Mesquita et al., 2003) included 50 ng DNA, 12,5 pmole of forward and 12.5 pmole of reverse primers, 0.2 dNTPs, 1.5 mM $MgCl_2$ and 0.5 unit Taq polymerase (Fermentas) in 10x Taq buffer containing (NH4)2SO4. Total volume was adjusted to 25 µl. PCR cycle was the same used for MWF-1 except final elongation for 1 minute instead of 5 minutes. The presence of PCR products were first checked against a marker ladder (100 bp ladder) by running a minute sample of the products on 0.8 % agarose gel, staining with ethidium bromide and visualization by UV illumination.

Table 6. Microsatellite loci used in genetic analysis of *Pseudophoxinus* populations.

Locus **Size Range**	**Label**	**Forward and reverse primer**	**Repeat Motif**
MFW-1 112-128	HEX	F:GAGCTTCAGCACCGAGGAC R:GTCCAGACTGTCATCAGGAG	CA
SarN2F11a 140-220	6-FAM	F:GACCACGACACACTGAAA R: CCAGCGTTCCTCTACATCA	$(CA)_{23}N_{41}(AC)_{10}$
SarN2F11b 110-120	TET	F: GAACAAACATCACTGAAGCACTCT R:ACGTCAGACTTCAGGCATCC	$(AC)_{10}$
SarN7F8 143-165	HEX	F: ACATTCCCTCTCTCACTTTCTGTC R: AGTTCATCAACTGACCGAGTTC	$(AG)_4$CAAGTGAGAGG$(AG)_3$G GTG $(AG)_3$TGAGTG$(AG)_4$AAAGCA($AG)_2$
SarN7G5 111-127	6-FAM	F:GAGCTTCAGCACCGAGGAC R:CTACATGACAAGCATCTGCAGTAA	$(TG)_{15}CG(TG)_2CGTGCG(TG)_3$
SarN7K4 137-153	TET	F:CATGTTTCCACATCTGAGCTAAAA R:ACGAGCATCAGTATCCAGAGACAC	$(TG)_{16}$

References are Crooijmans et al. (1997) for MFW-1 and Mesquita et al. (2003) for the rest of the microsatellites

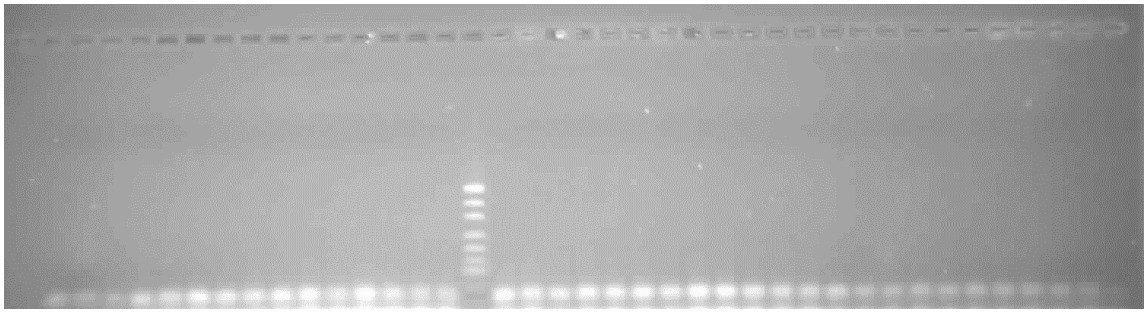

Figure 8. PCR products of microsatellite locus SarN2F11a on agarose gel coupled with Massrular Low Range DNA Ladder.

The length of microsatellite sequences were determined by fluorescence techniques and Sprecher *et al*. (1996) indicated that correct fragment peaks are the ones that reveals more than 200 relative fluorescence units (RFU). The maximum size difference between heterozygote alleles was evaluated as 10 base pairs (Telli, 2008). Peaks out of the expected allele size more than 10 bases are evaluated as non-specific bands. Although Taq polymerase sometimes causes size differences of 1 bp this kind of peaks are eliminated according to their height and frequency. Appropriate allele size for a population for given microsatellite locus is the size of the most frequent allele (Ginot *et al*. 1996; Telli, 2008).

Statistical analyses of microsatellite loci was carried out using two softwares; FSTAT 2.9.3.2 developed by Goudet (2001) and GENEPOP developed by Raymond and Rousset (1995) and updated to version 4.2 by Rousset (2008). Both softwares are capable giving results on allele number and frequency per locus and per population, allelic diversity, heterozygosity and fixation index (Fis). Pairwise FST calculations were carried out using GENEPOP 4.2.

GenAlEx 6.1 is another useful excel based program developed by Peakall and Smouse (2006).

CHAPTER 3

RESULTS

3.1. Geometric Morphometrics Analysis.

Shape variation in *Pseudophoxinus* populations examined using three softwares; TPS series (TPSUtil (Rohlf, 2004) and TPSDig2 (Rohlf, 2005)), NtSys 2.21q (Rohlf, 2009) and Morpheus et al. (Slice, 1998). Fig.9. shows a plot of the specimens whose shape data is scattered over 13 landmarks. This figure, produced by Morpheus et al. (Slice, 1998), represent grouped data before any transformation.

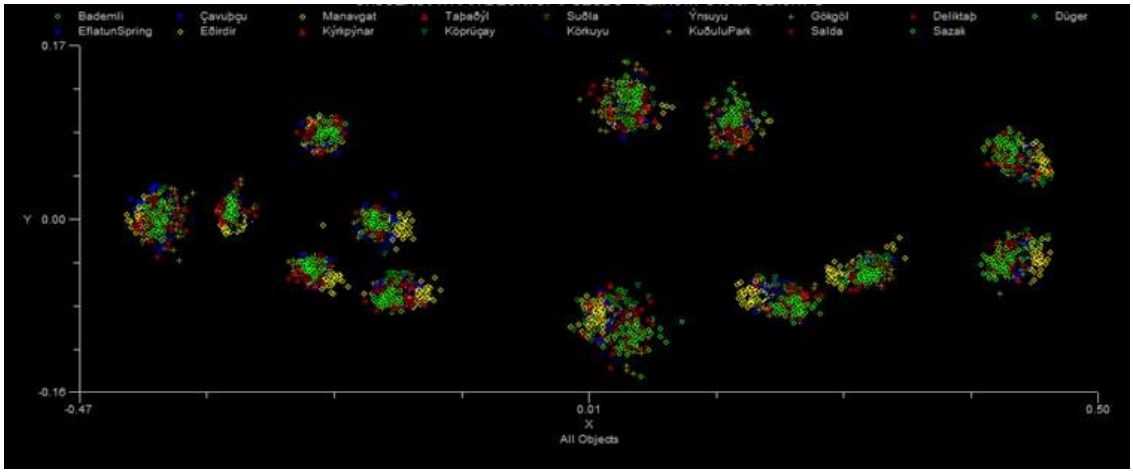

Figure 9. Aligned specimens using 13 landmarks.

First transformation of landmark data was superimposition of the shape of specimens onto each other starting from an arbitrary specimen. A consensus shape (red circles in Fig.10.) was reached at the end of this alignment procedure.

Pairwise comparison (Table.7.) of 17 populations comprised of 136 pairs were carried out applying ptest using the software Morpheus et al (Slice, 1998). Significance level of the test was set as α=0.050. Although the results showed that most of the populations were highly significant, four populations, Gök Lake, Eflatun Spring, Eğirdir Lake and Kuğulu Park, were highly significantly different from the rest of the populations.

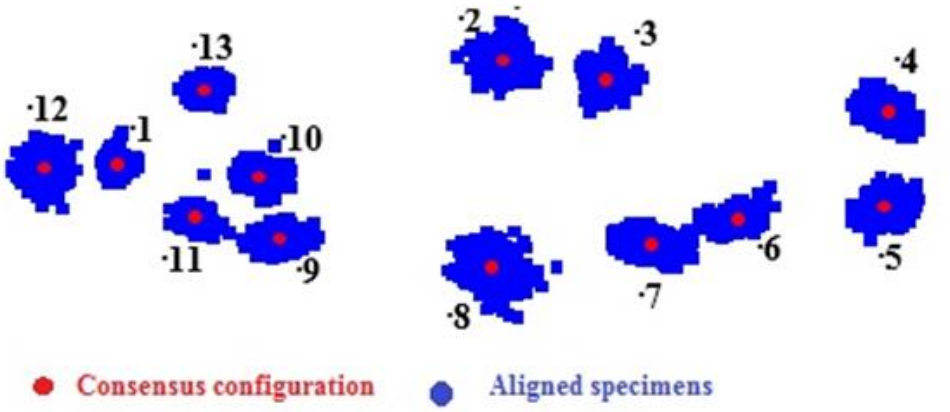

Figure 10. Consensus shape of Pseudophoxinus revealed by 13 landmarks and aligned specimens.

Obvious shape variation for Bademli population was observed between six populations, Gök Lake, Eflatun Spring, Eğirdir Lake, Kırkpınar, Körkuyu and Kuğulu Park. Çavuşçu population differed slightly over (0.066) the p value from this population.

There was no observed significance between Çavuşçu and Manavgat populations and Çavuşçu and Bademli paired just above the threshold level. Çavuşçu population was highly different from the rest of the populations.

Non-significant variations from Manavgat population were observed in Bademli, Çavuşçu, İnsuyu, Kırkpınar and Köprüçay populations.

Taşağıl population was highly significantly different from most of the populations except that of Bademli and Suğla.

The shape variation in Suğla population was non-significant when compared to Bademli and Taşağıl populations.

The differences between İnsuyu population and two populations, Bademli and Manavgat, were not statistically important.

Dissimilarity was not significant between Deliktaş population and Bademli, Salda and Sazak populations. It was also true for Körkuyu population although the significance level (0.059) was close to the threshold level.

Table 7. Pairwise comparison (P=0.05) of landmark data in *Pseudophoxinus* populations.

	Bademli	Çavuşcu	Manavgat	Taşağıl	Suğla	İnsuyu	Gökgöl	Deliktaş	Düger	Eflatun	Eğirdir	Kırkpınar	Köprüçay	Körkuyu	Kuğulu	Salda
Bademli																
Çavuşcu	0.066*															
Manavgat	0.721*	0.513*														
Taşağıl	0.635*	0.001	0.020													
Suğla	0.356*	0.001	0.008	0.327*												
İnsuyu	0.139*	0.005	0.202*	0.001	0.001											
Gökgöl	0.001	0.001	0.001	0.001	0.001	0.001										
Deliktaş	0.094*	0.001	0.001	0.001	0.001	0.001	0.001									
Düger	0.087*	0.001	0.004	0.001	0.001	0.001	0.001	0.008								
Eflatun	0.005	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001							
Eğirdir	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001						
Kırkpınar	0.009	0.007	0.168*	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001					
Köprüçay	0.131*	0.003	0.347*	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.014				
Körkuyu	0.032	0.001	0.001	0.001	0.001	0.001	0.001	0.059*	0.032	0.001	0.001	0.001	0.001			
Kuğulu	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001		
Salda	0.267*	0.001	0.002	0.002	0.001	0.001	0.001	0.430*	0.090*	0.001	0.001	0.001	0.001	0.415*	0.001	
Sazak	0.269*	0.001	0.007	0.001	0.001	0.001	0.001	0.081*	0.086*	0.001	0.001	0.001	0.007	0.003	0.001	0.067*

Düger populations was similar to three populations namely Bademli, Salda and Sazak. However, the similarity was at around 0.090 significance level.

Population of Kırkpınar varied significantly from the rest of the population except Manavgat.

Köprüçay population was similar to Bademli and Manavgat populations, and clearly differed from the rest.

Although Körkuyu population was not significantly different from Salda and Deliktaş populations the level of non-significance was rather low (0.059) with Deliktaş population.

Salda population showed similarities with five populations, Bademli, Deliktaş, Düger, Körkuyu and Sazak. However, the level of similarity was low for Düger and Sazak where the values were 0.090 and 0.067, respectively.

The pairing with Sazak population revealed non-significant results for the populations of Bademli, Deliktaş (0.081), Düger (0.086) and Salda (0.067.

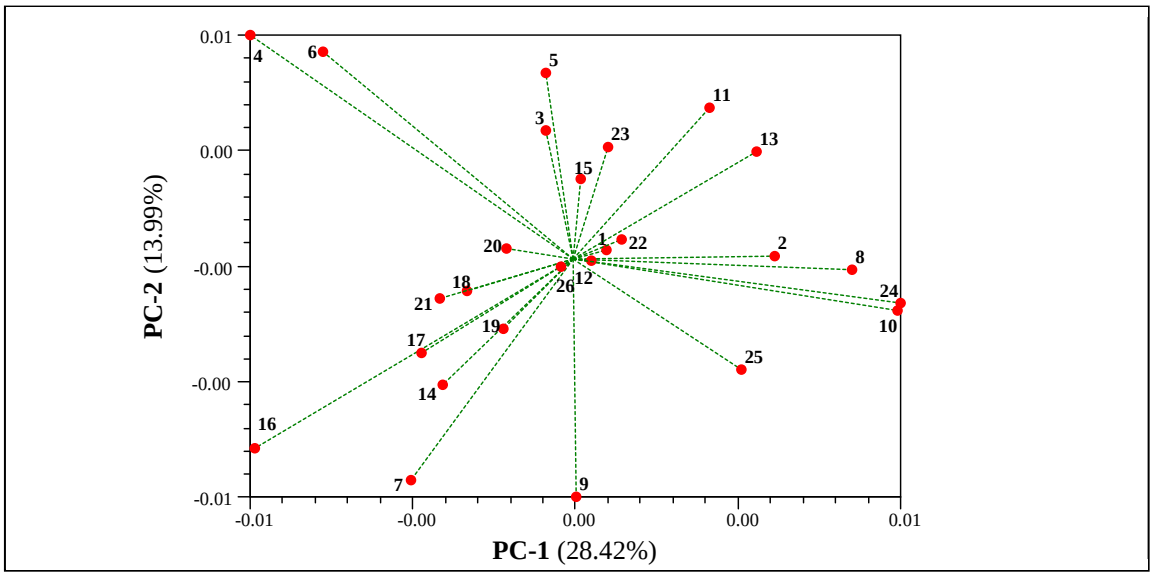

Figure 11. 2D PCA plot of variables depicting shape of *Pseudophoxinus* populations.

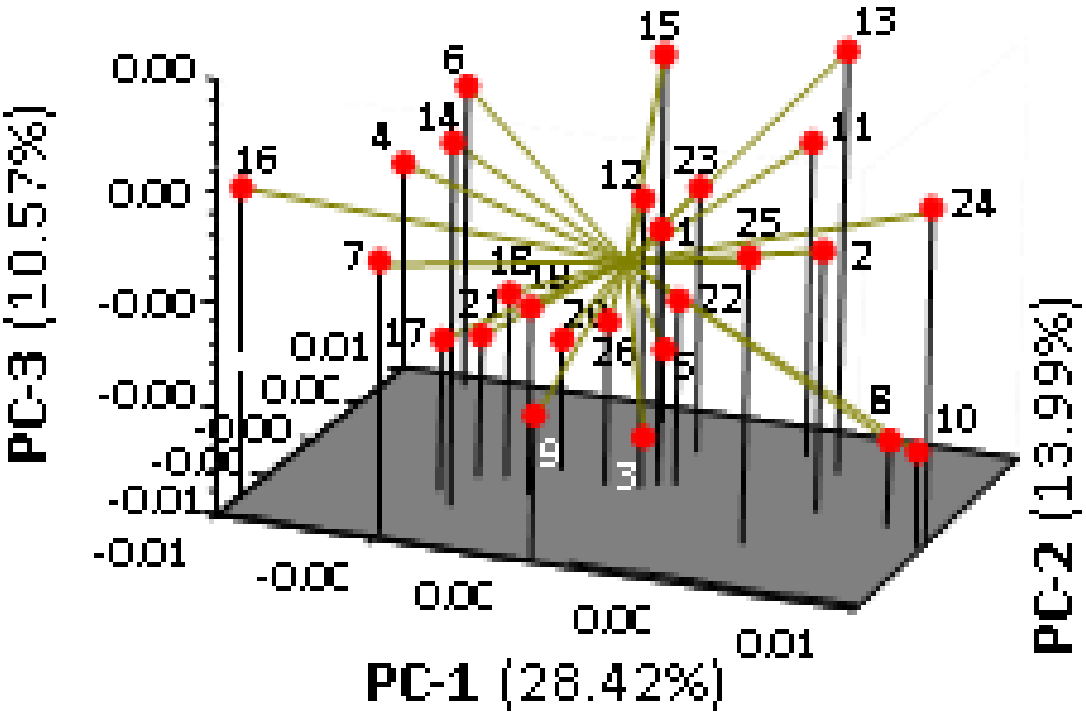

Figure 12. 3D PCA plot of variables depicting shape of *Pseudophoxinus* populations.

The statistical analysis of landmark data was continued by applying principal component analysis (PCA) to calculate variation within populations to determine the way variables affected the shape. X-y coordinates of 13 landmark resulted with 26 variables to figure out the variation in the shape of *Pseudophoxinus*. PCA results (Fig.11. and Fig.12.) showed that 28.42% of the variation was explained in the first PC axis and 13.99% in the second PC axis. More than 50% of the variation was explained in the first three axes. Explanation of more than 90% of the variation within populations was realized by the 12th axis. Correlation between the variables shown in Fig.4. Positive/none/negative correlations between variables were observed in the first PC axis. The strength of the correlation was determined by the angle between the variables, for example variables 14 and 19, 16 and 17, and 18 and 21 had strong negative correlations while 10 and 24 correlated strongly positive on x axis and negative on y axis. Variable 19 had no correlation on x axis, however, its correlation on y axis was negative and weak as well.

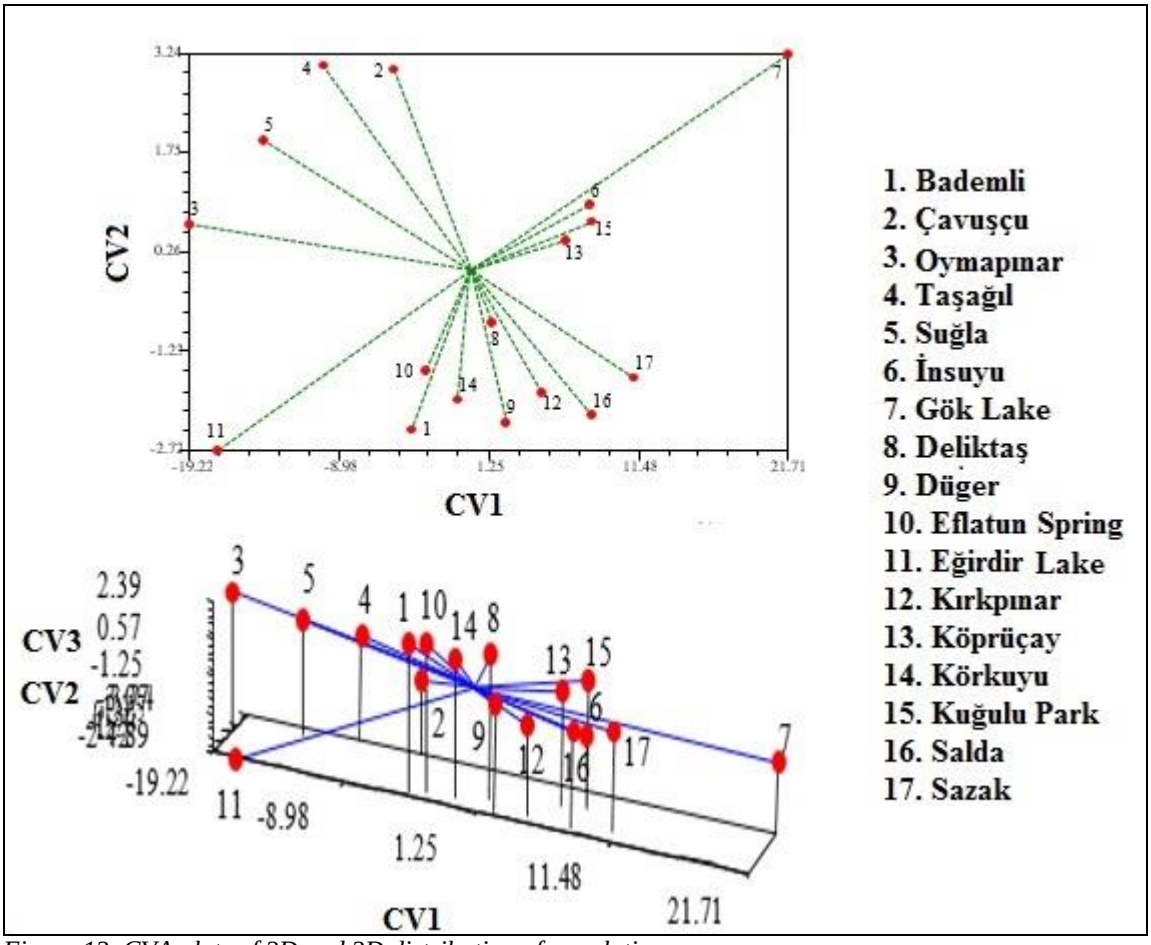

Figure 13. CVA plots of 2D and 3D distribution of populations.

Variations among populations were assessed by the application of canonical variates analysis (CVA). CVA, using partial warps and uniform components, revealed position of the populations. Interpreting 3D CVA plot is much informative than 2D CVA although both are the plots of the same results and included in Fig.13. For example, populations of Gök Lake and Eğirdir Lake are the outliners of both graphics. However, close relatedness of Oymapınar, Taşağıl and Suğla populations are not so distinct in 2D plot despite the fact that they still cluster together in 2D CVA plot. Eigen values relating to among group variation revealed that 90.72% of the variation was explained in the first canonical variate (CV1) of the CVA. Almost all of the variation was clearly determined in the first CV axis. CV2 contributed to the explanation of 3.05% of the shape variation among populations. Consequentially a scatter plot to explain the relation between populations was also produced by CVA (Fig.13.)

Scatter plot scores of CVA scores (Fig.14.) indicated that Gök Lake population, known as *Pseudophoxinus crassus,* singled out from the rest of the populations as well as the other population of the same species, İnsuyu population. This population is in habits the upper most north of the sampling area. Geographical barriers and the geographic location of this population may have caused it to be close population this may have effected its divergence in the evolutionary time scale.

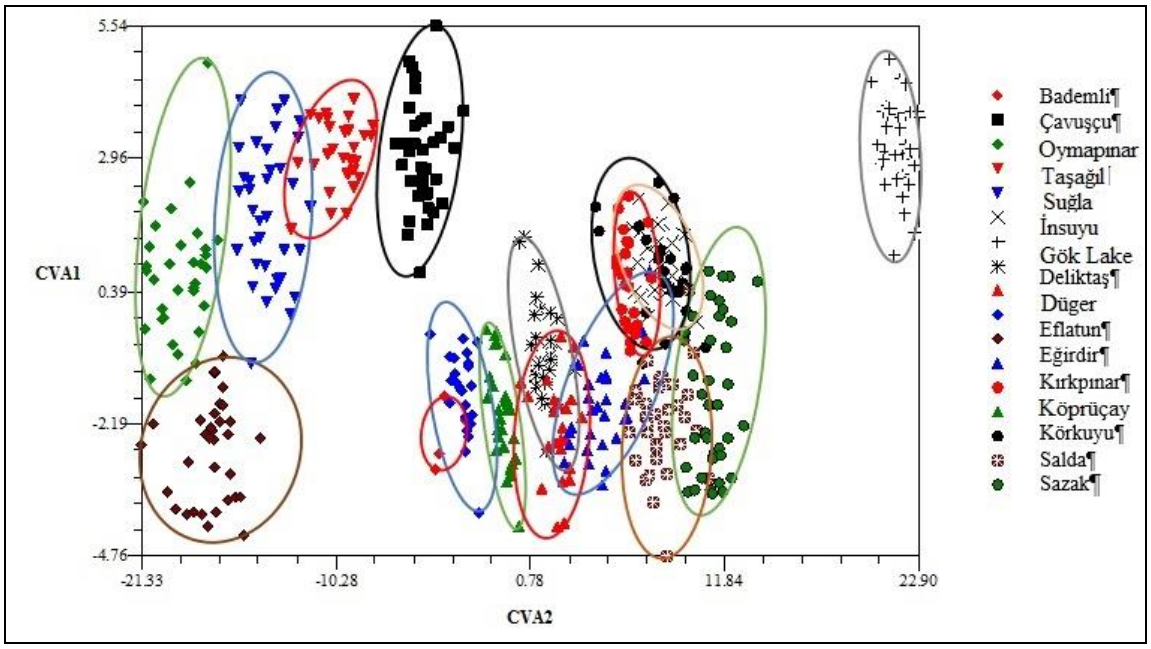

Figure 14. CVA generated scatter plot of 17 Pseudophoxinus populations.

Similarly, the Lake Eğirdir population, described as *P. egirdiri,* came out to be a distinct group. Nevertheless, *P. egirdiri* still had some kind of relatedness to the group at the upper left part of the scatter plot. This group has been classified as *P. battalgili* and comprised of four populations, Çavuşçu Lake, Oymapınar Dam Lake (Manavgat), Suğla Lake and Taşağıl Stream (Seydişehir, Konya).

The cluster in the middle encompassed two groups connected with Kırkpınar (Korkuteli, Antalya) drainage population of *Pseudophoxinus sp.* The group at the left of Kırkpınar population included five populations with two known species, *P. burduricus* from Düger, Burdur and *P. hittitorum* from Eflatun spring, Beyşehir, Konya. The other three were Bademli, Deliktaş and Körkuyu populations located close to each other at the bank of Beyşehir Lake in Konya.

Remaining five population included two population of *P. burduricus,* Salda spring and Sazak stream in the province Burdur, at the lower part of the group. Upper part had three closely scattered populations, İnsuyu (Cihanbeyli, Konya), Köprüçay) Aksu, Isparta) and Kuğulu Park (Seydişehir, Konya). The species representing these populations are *P. crassus, P. hittitorum and Pseudophoxinus sp,* respectively.

3.1.1. Visualization of Shape Variation

Superimposed consensus shape obtained as a function of partial warps and uniform components were visualized on thin plate spline (TPS) graphics following Cartesian Transformation (Fig.15). Shape variation on the base of 13 landmarks in populations were plotted against the consensus to be able to visualize actual morphological differentiation. Green circles on the TPS indicated consensus shape and blue stars indicated the shape obtained for each population.

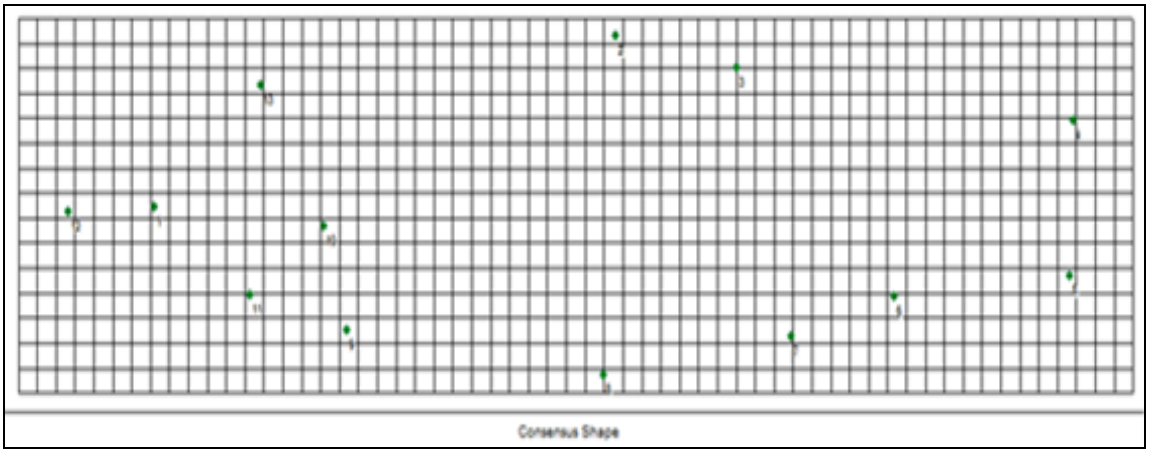

Figure 15. TPS configuration of consensus shape for *Pseudophoxinus*.

Figure 16. A specimen from Bademli popluation

Pseudophoxinus population in Bademli, Beyşehir (Konya) has not been identified as any known species of the genus (Fig.16). Four specimens were caught from a fast running part of the stream where the visibility was high.

In general the shape differentiation in the population was characterized by the compression of the head and the enlargement of the body. TPS visualization of the population against the consensus showed that the body was enlarged outward while the head was narrowed down inward (Fig.17). Landmark showing anterior pelvic fin base clearly extended ventrally. Posterio-ventral replacement of the mouth is also obvious in Bademli population.

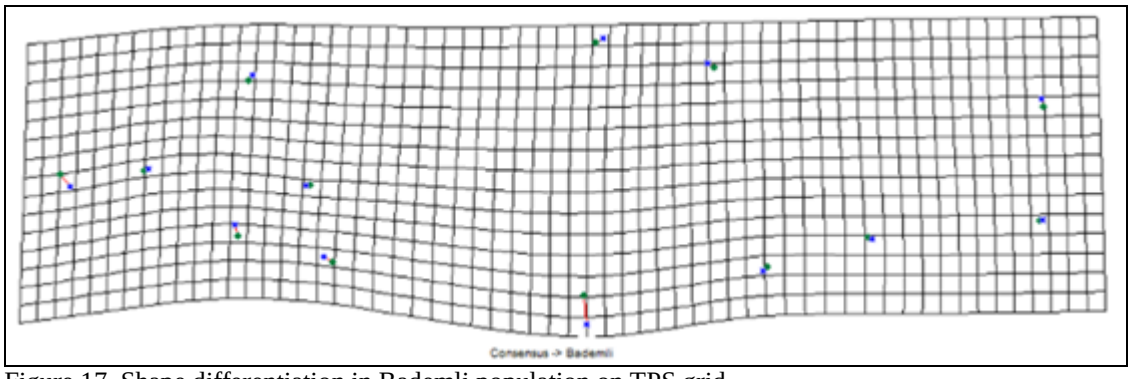

Figure 17. Shape differentiation in Bademli population on TPS grid.

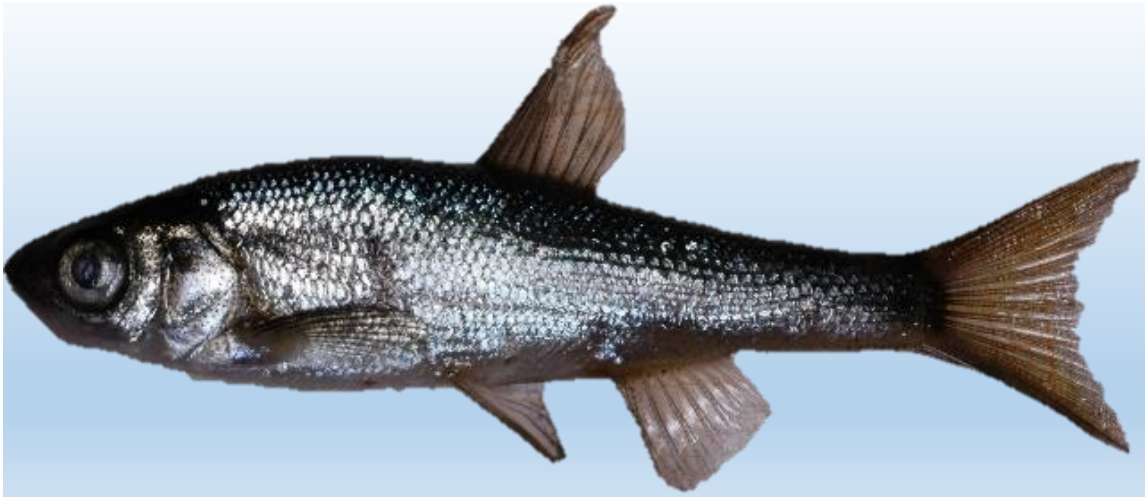

Figure 18. A specimen representing Çavuşçu population of *Pseudophoxinus*

First population (Fig 18) of *Pseudophoxinus battalgili* is a lacustrine population inhabiting Çavuşçu Lake, Ilgın-Konya. Geometric morphometric analysis of the population revealed an obvious shape differentiation from the consensus. Anterior extension of mouth and posterior extension of landmarks of operculum (lm 10 and 11) as well as pectoral fin base indicated an elongated and downward enlarged head shape (Fig.19). The head is also compressed dorsally. The body is compressed between head and virtual line between anterior dorsal fin and anal fin bases. Caudal part of the fish elongate slightly posteriorly.

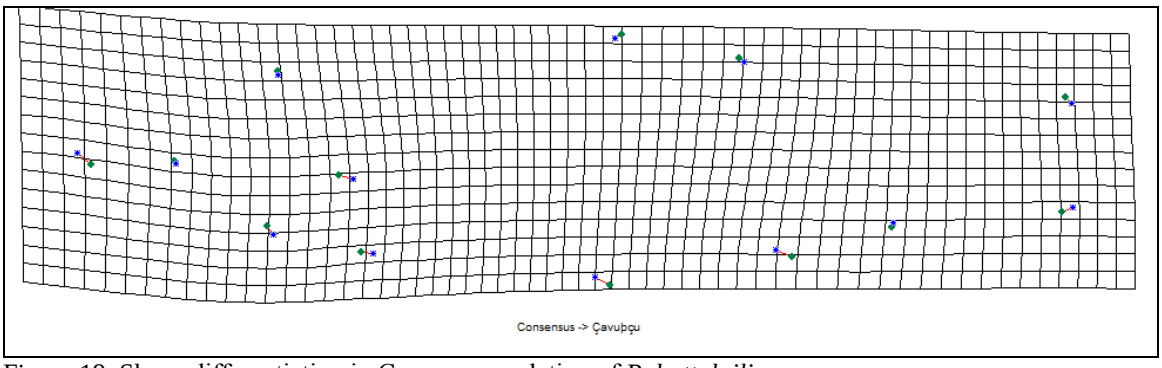

Figure 19. Shape differentiation in Çavuşçu population of *P. battalgili.*

Figure 20. A specimen of *P. battalgili* from Oymapınar Dam Lake.

Second population of *P. battalgili* was collected from Oymapınar Dam Lake built on Manvgat River, Antalya (Fig.20.). Although the study area is in lacustrine nature it is difficult to assign this population as a lacustrine population since the dam providing a lacustrine environment was built on a natural river system.

Shape differentiation of Oymapınar dam lake population showed that head and body was combusted inwards and the extension of landmarks on peduncle showed that the body is more slender and elongated in this population (Fig.21.) compare to the consensus.

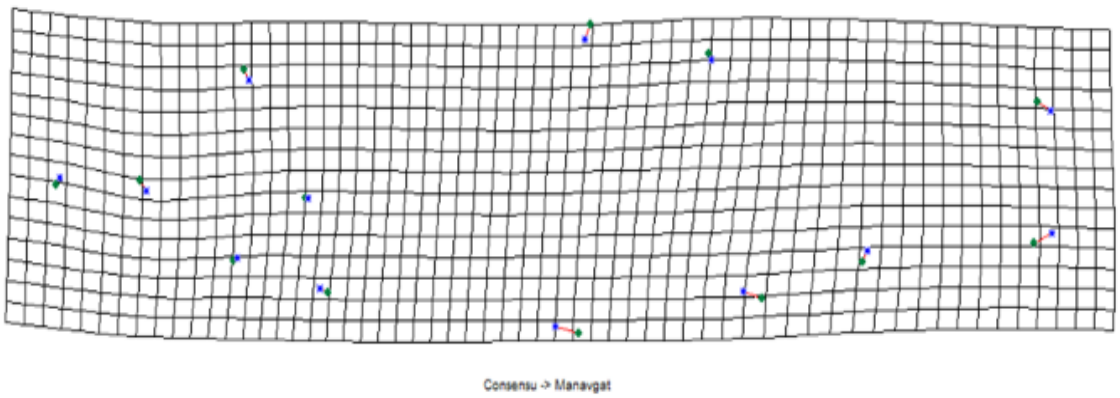

Figure 21. Shape diferentiaition in Oymapınar Dam Lake population of *P. battalgili.*

Figure 22. A specimen of *P. battalgili* from Taşağıl Stream.

The third population of *P. battalgili* is a riverine population inhabiting Taşağıl Stream, Beyşehir, Konya (Fig.22). In this population population head region had a downward replacement except the mouth which moved upwards. A slender compressed body was reached by downward movement of dorsal landmarks and upward movement of ventral landmarks. The length of the body was elongated with posterior replacement of landmarks on peduncle (Fig.23.). It was also evidencial from the plot that outer replacement of landmarks of dorsal and anal fins indicated the enlargement of the base of these fins.

Shape differentiation from the consensus was less in head region than in body region. This is most possible due to bony nature of the head region which is a more stable compare to rest of the body and more correlated with the genetics of the organisms as it was implied by Strauss (1991).

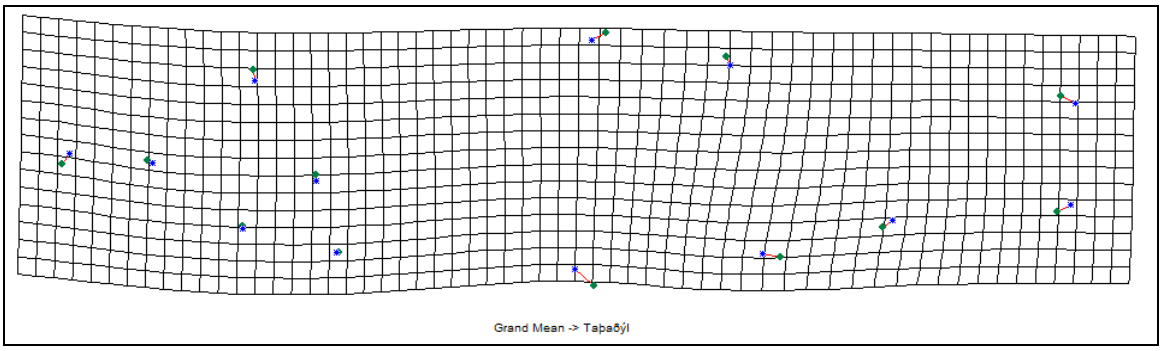

Figure 23. Shape differentiiation in Taşağıl Stream population of *P. battalgili*.

Figure 24. A specimen of *P. battalgili* from the Lake Suğla

The last population of *P. battalgili* inhabits Suğla Lake in Seydişehir, Konya (Fig.24.). Although the population is a lacustrine population the shape was similar to that of Taşağıl population though the variation was in a smaller scale (Fig.24.). This may be due to geographical closeness of these populations and highly likely gene flow between them.

Different from the population in Taşağıl Strream the head in this population was compressed with the additional upward movement of landmark 9. Slender body shape and posterior elongation of the body was also observed as well as the elongation of dorsal fin and anal fin bases (Fig.25.).

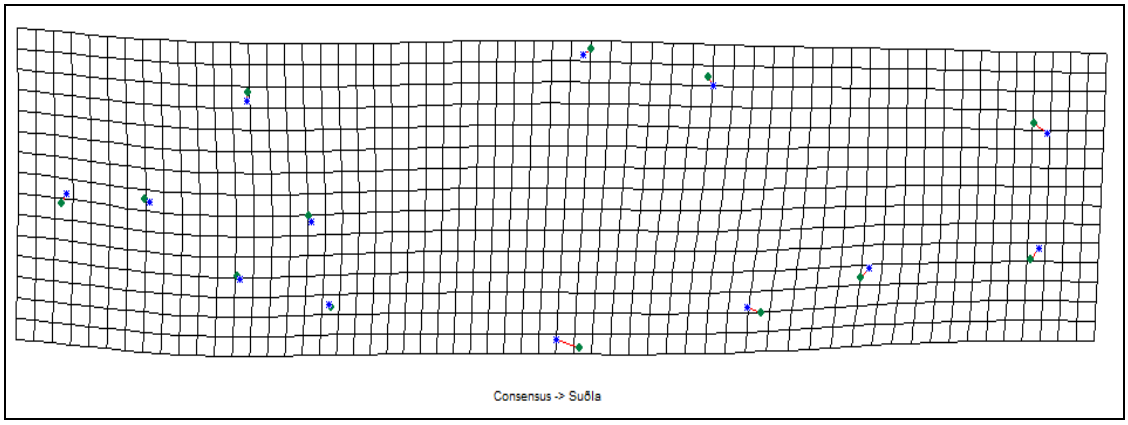

Figure 25. Shape differentiaiton in Suğla Lake Population of *P. battalgili.*

Figure 26. A specimen of *P. crassus* from İnsuyu Stream.

Insuyu population is a revirine population of *P. crassus* (Fig.26). The body shape in this population tended to expand with outward replacement of both the landmarks of head and body region (Fig.27.). Therefore, the body depth was higher compare to the consensus. On the other hand anterior movement of peducle landmarks (landmarks 4 and 5) resulted in the shortening of the body length. The higher depth for the head was also observed in this population. ,

Variation in the head region in *P. crassus* of Insuyu Stream was more than that of its body region. This was a controversy to the claim of Staruss (1991) that morphometric variation in head reagion in fish is less since this region is more stable compare to the rest of the body.

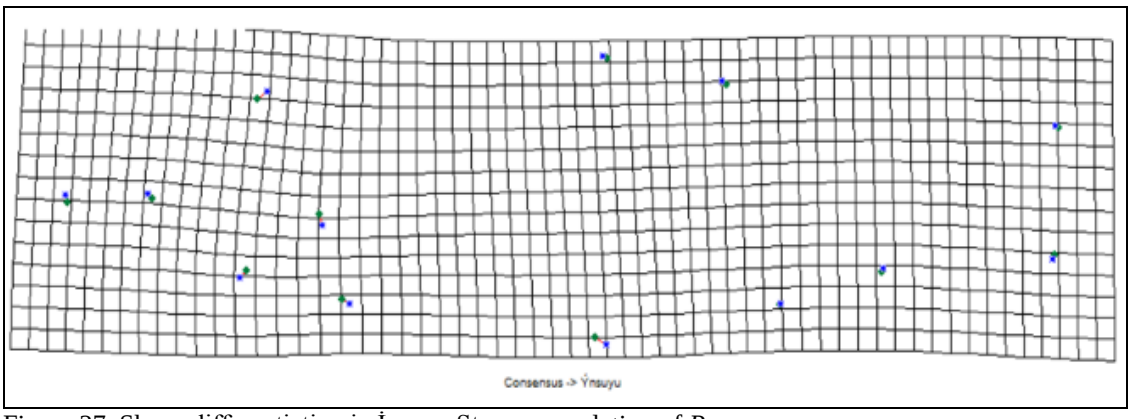

Figure 27. Shape differentiation in İnsuyu Stream population of *P. crassus.*

Figure 28. A specimen of *P. crassus* from Gök Lake.

Second population of *P. crassus* is a lacustrine population and inhabits Gök Lake in Kulu, Konya (Fig.28). Geometric morphometric investigation of the population revealed that the variation in the body region was more than that of the head region. However, inward movement of the mouth resulted in shortening of the head.

The body depth was increased by both upward movement of dorsal landmarks and downward movement of ventral landmarks. Although the body length did not changed a lot except the head region dorsal fin was moved anteriorly. On the contrary, pectoral and pelvic fins showed a posterior replacement (Fig. 29.).

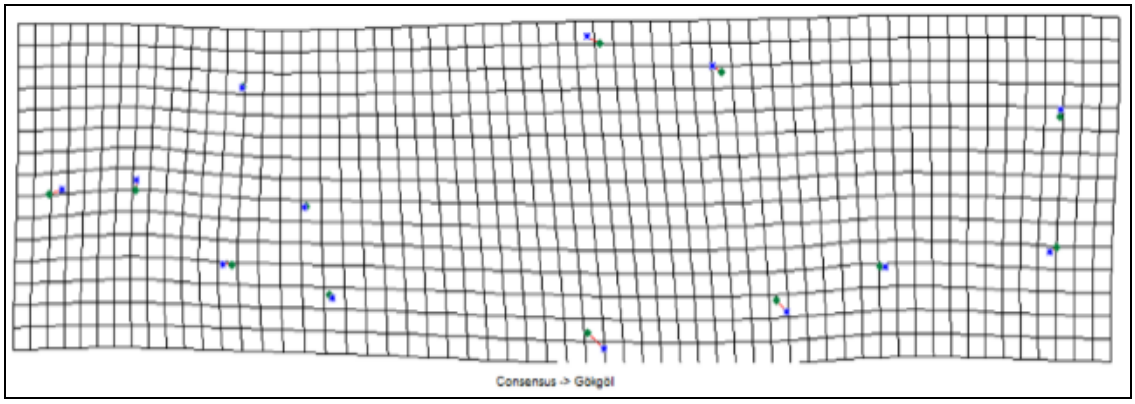

Figure 29. Shape differentiation in Gök Lake population of *P. crassus.*

Figure 30. A specimen from the population of Deliktaş, Yeşildağ, Beyşehir, Konya.

Deliktaş population of the genus *Pseudophoxinus* is one of the populations that has not been properly classified yet (Fig.30.). Shape variation in this population was more obvious in the head region and the shape of Deliktaş, Yeşildağ (Beyşehir) population was compact compare to the consensus.

All landmarks representing homologous points on the head showed an inward replacement (anterio-dorsal movement of landmarks 9, 10 and 11, posterior-ventral movement of landmark 12 and ventral movement of landmark 13) causing shortening in length and depth of the head.

Similarly slight inward replacement was also obvious for the body region. Nevertheless, only peduncle enlarged and moved slightly posteriorly (Figure 31.). The

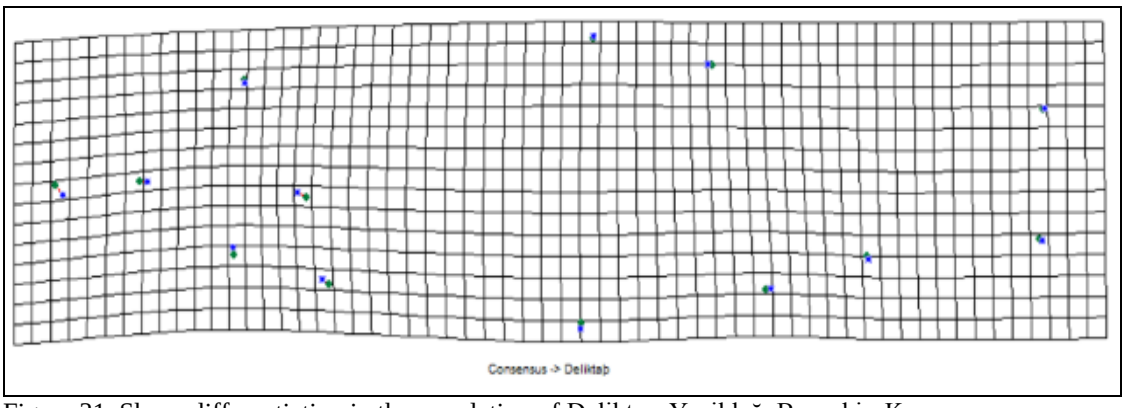

Figure 31. Shape differentiation in the population of Deliktaş, Yeşildağ, Beyşehir, Konya.

Figure 32. A specimen from Düger population of *P. burduricus.*

P. burduricus represented with three populations in this study. The first population of the species was from Düger Spring in Burdur (Fig.32.). The shape of the population was differed more at the body region than at the head region compare to the consensus.

The head was enlarged with the outward replacement of landmarks 9 at ventral, 12 at the tip of the mouth and 13 at dorsal. Hence the length and the depth (height) of the head was larger compare to the consensus.

The body was compressed anteriorly at the peduncle and enlarged both dorsally and ventrally. Dorsal and anal fins moved posteriorly and shortened caudal region of the fish (Fig. 33.).

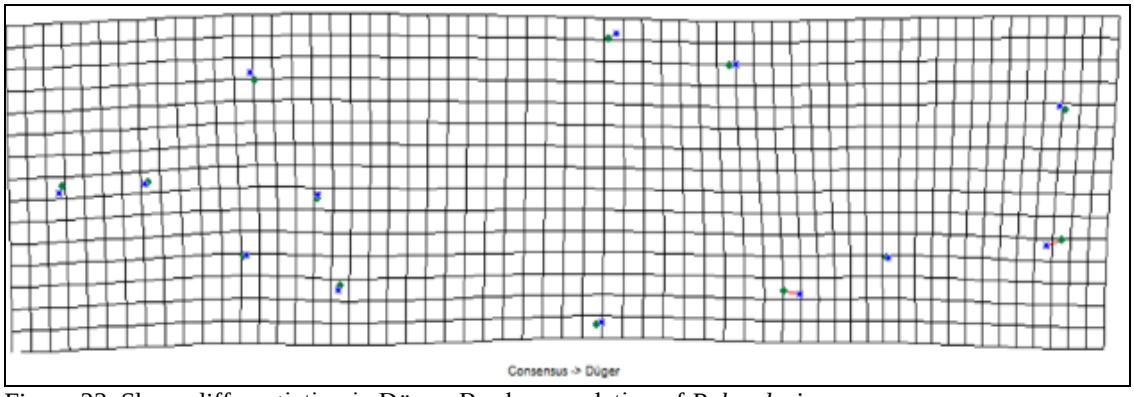

Figure 33. Shape differentiation in Düger, Burdur population of *P. burduricus.*

Figure 34. A specimen from Salda population of *P. burduricus.*

The second population of *P. burduricus* was collected from Salda Stream, Burdur (Fig.34.). Similar to Düger population of the same species shape variation was more obvious in the body part of this population, too. Caudal region of fish of this population shortened with anterior replacement of the landmarks on the peduncle (Fig.35.).

The head length was enlarged with the anterior replacement of the tip of the mouth. The increase in the depth of the head was supported with outward movement of the landmarks 9 at ventral and 13 at dorsal.

The body was compressed anteriorly by replacement of the landmarks marking peduncle. Beside horizontal compression the body enlarged vertically by the replacement of both dorsal and ventral landmark. Dorsal and anal fins moved posteriorly and shortened caudal region of the fish (Fig. 35.).

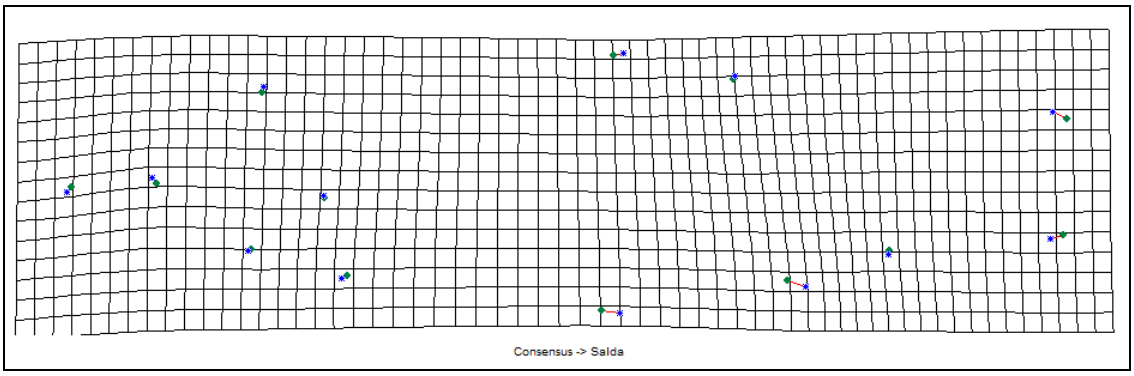

Figure 35. Shape differentiation in Salda Stream, Burdur population of *P. burduricus.*

Figure 36. A specimen from Sazak population of P. burduricus

A vertical head enlargement was also observed in the third *P. burduricus* population (Fig.36.) inhabiting Sazak Spring, Burdur. Body shape variation in this population was strong at pelvic fin base and anterior base of the anal fin. Upward movement of the landmarks representing dorsal fin enhanced the elongation along the body depth

Although the caudal region was horizontally shortened and vertically enlarged with anterior and outward replacement of the landmarks, mid-body was enlarged and elongated with posteriorly outward movement of the landmarks (Figure 37.).

Similar to all other *P. burduricus* populations shape variation Sazak was clearly more at body region than it was at head region as well.

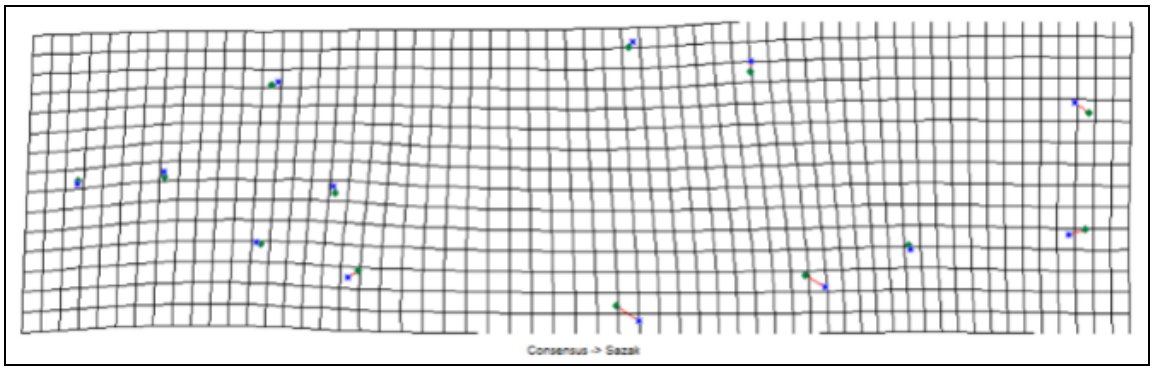

Figure 37. Shape differentiation in Sazak Spring, Burdur population of P. burduricus..

Figure 38. A specimen of Eflatun Spring population of *P. hittitorum.*

P. hittitorum (Fig.38) is one of single population species of the genus. The species has been reported only from Eflatun Spring in Beyşehir, Konya.

Individuals of this species have got smaller head resulted from inward compression of all the landmarks of the head. Shape variation seem to be higher in the head region as well.

Slight body enlargement is aslo obvious from the replacement landmaks representing the body (landmarks 2-8).

Landmarks 4 and 5 indicated that the peduncle in this population elongated vertically (Fig.39.). Whole body of the population was compressed due to posterior replacement of the mouth.

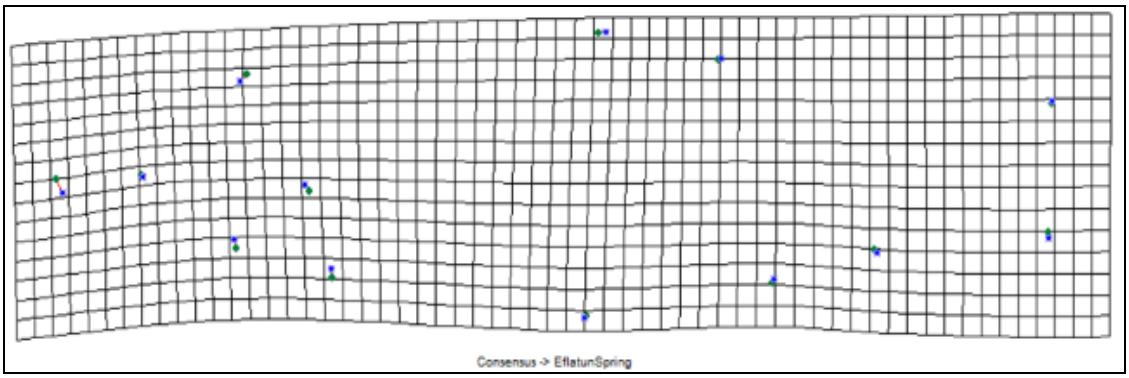

Figure 39. Shape differentiation in *P. hittitorum* from Eflatun Spring.

Figure 40. A specimen of *P. egirdiri* represented with a single population inhabiting Eğirdir Lake.

P. egirdiri (Fig.40.) is another species of the genus *Pseudophoxinus* with a single known population. *P. egirdiri* inhabits a spring in Karaot region of Eğirdir Lake, Isparta.

Considerable difference in morphometric variation of this species was observed for both head and body shape. Eyes moved down in this species. Head was considerably bigger compare to the consensus and enlargement was observed at all directions e.i., anteriorly extended mouth tip, dorsally extended head-body conjunction, posteriorly extended edge of operculum and posterio-ventral extension of lower tip of the operculum. While dorsal body stayed relatively stable, abdomen moved anteriorly. Clear posterior movement of landmarks on peduncle indicated a posterior elongation in the body length (Figure 41.). As a result the species obtained an elongated slender boby shape.

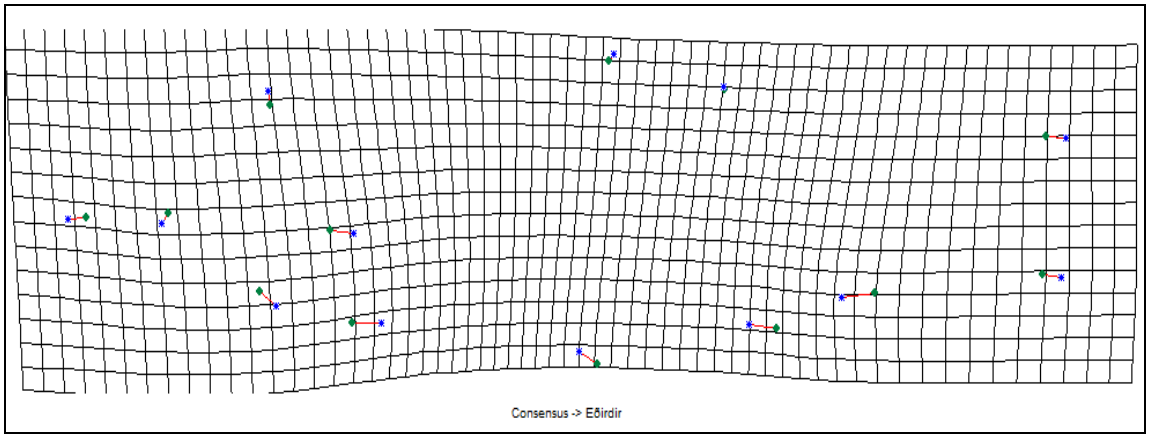

Figure 41. Shape differentiation in single population of *P. egirdiri* inhabiting Eğirdir Lake, Isparta.

Figure 42. A specimen of *P. sp.* from the population of Kırkpınar drainage channel.

Kırkpınar population (Fig.42) of the genus *Pseudophoxinus* is one of five populations whose species status has not been evaluated yet. Geometric morphometric analysis of the population revealed that the shape variation was higher in the body than it was in the head region. Head was slightly bigger due to anterior replacement of the tip of the mouth and it was also vertically enlarged. Body was considerably slender with posteriorly inward displacement of corresponding landmarks. However, the body length was shortened with the anterior movement of the landmarks on the peduncle. This shape variation was also an indication of reduced length of the caudal region. The replacement of landmarks relating to fins has shown that dorsal fin base and anal fin base moved posteriorly as well as pelvic fin (Figure 43.).

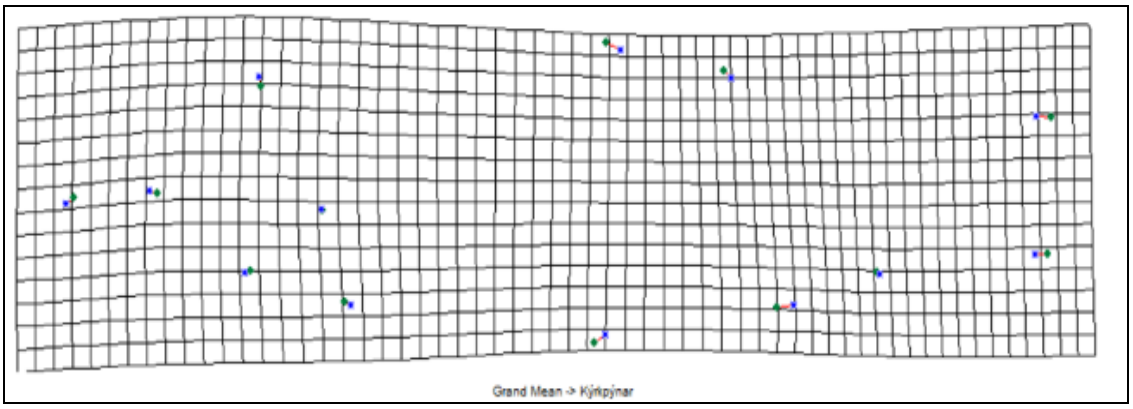

Figure 43. Shape differentiation in Kırkpınar population.

Figure 44. A specimen of P. fahrettini from the population of Köprüçay.

Köprüçay population of *P. fahrettini* is a riverine population collected from Köprüçay, Aksu, Isparta (Fig.44). Variation in the body shape of the population was more than the variation in the shape of head region in this population.

The population has revealed a very slender body shape compare to the consensus. The head was elongated with anterio-ventral movement of mouth tip. Clear compression of the body was also observed in this population. Vertical replacement of dorsal fin and dorsal movement of pelvic and pectoral fins resulted in the reduction of the body depth and in the formation of a highly slender body. Body was also compressed considerably with ventral movement of dorsal landmarks and dorsal movement of pelvic fin (Fig. 45.). Base lengths of dorsal fin and anal fin were reduced as it can be concluded from the TPS plot of shape variation in *P. fahrettini.*

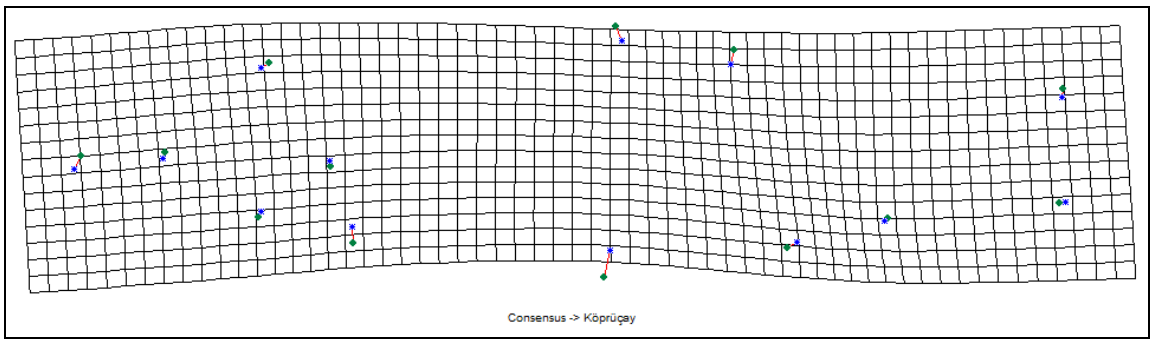

Figure 45. Shape variation in Köprüçay population of *P. fahrettini.*

Figure 46. A specimen of *Pseudophoxinus* population inhabiting Körkuyu location.

This population of *Pseudophoxinus* (Fig.46.) is collected from Körkuyu location in Yeşildağ (Beyşehir-Konya). The species status of the population has not been established yet. This population is located highly close to Bademli and Deliktaş populations.

The head shape of this population was varied from the consensus with laterally compression and upward replacement causing recudtion in the length and the depth of the head region.

An increase in the body depth in this population was caused by the opposite movement of dorsal and ventral landmarks. The variation also indicated that the length of the base of dorsal fin was reduced (Fig.47.).

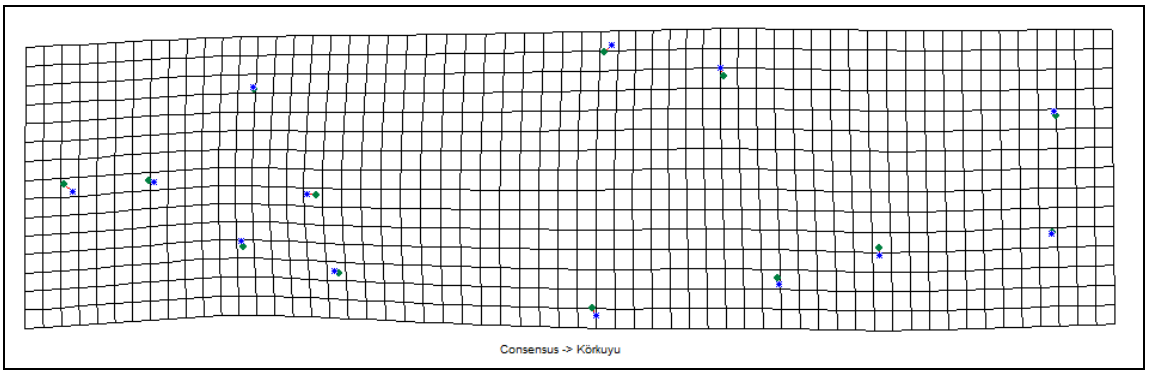

Figure 47. Shape differentiation in Körkuyu population of *Pseudophoxinus.*

Figure 48. A specimen of *Pseudophoxinus* population inhabiting Kuğulu Park.

Kuğulu Park (Seydişehir, Konya) population (Fig.48) of the genus *Pseudophoxinus* is another population of the taxon which is in need of proper consideration for classification.

The head in this population became smaller depicted from the inward replacement of all landmarks governing the head region. Althogh the depth of body was increased due to opposite movement of dorsal and ventral landmarks of the body region, anterior movement of the peduncle landmarks (4 and 5) caused the shortening of the body. Both the space between the edges of dorsal and anal fin bases were also increased in this population (Figure 49.).

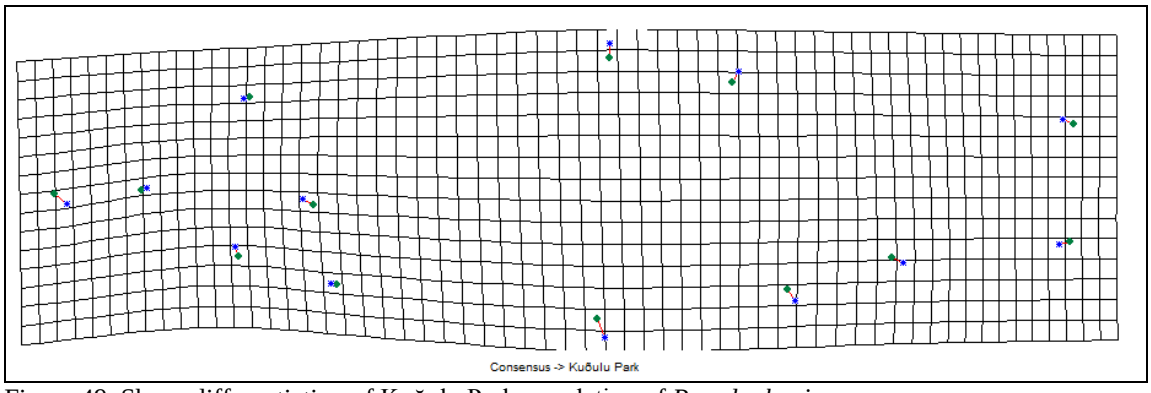

Figure 49. Shape differentiation of Kuğulu Park population of *Pseudophoxinus.*

3.1.2. Geometric Morphometric Phylogeny of the Genus *Pseudophoxinus*

Geometric morphometrics analysis revealed that *Pseudophoxinus crassus* inhabiting Gök Lake separated from the rest of the populations as far as the shape is concerned. Remaining 16 populations gathered in two main braches. In the first, *P. battalgili* consisted of four populations were clustered together with *P. egirdiri*. The cluster included two main groups (Fig.50). Upper part of the dendogram consisted of four populations from close geographical locations in Beyşehir, Konya. In this group, three populations with undefined species, Bademli, Deliktaş and Körkuyu clustered together with *P. hittitorum* from Eflatun Spring. Second group of this cluster comprised three species, *P. burduricus, P. crassus* and *P. fahrettini* and two populations with undefined species.

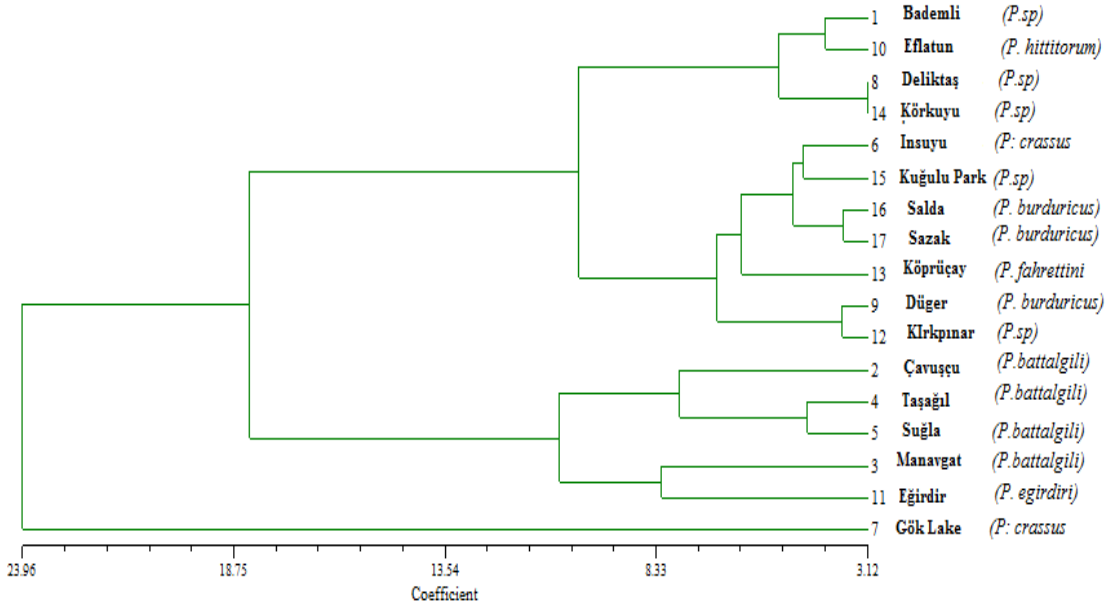

Figure 50. Dendogram of *Pseudophoxinus* populations resulted from geometric morphometrics analysis.

3.2. Allozymes

Polymorphism is a measure of genetic variation in populations (Halliburton, 2004). Analysis of 10 allozyme loci revealed that all of the loci were indeed polymorphic in general (Table.8.). Total number of alleles observed was 41. MDH-I and GPDH were represented with two alleles. ICH and LDH-I had three alleles in total whereas LDH-II had four alleles. MDH-II, GPI-I and GPI-II consist of five alleles and, finally, PGM and PGD were found to be consisted of six alleles. Overall observed allele frequencies

belonging to these alleles were given in Table.8. Nevertheless, not all forms of the allozymes were present in all populations. Evolutionary process might have help populations to retain the most appropriate allele(s) for the populations.

Table 8. Allozymes loci of *Pseudophoxinus* populations and their overall frequencies.

Allele \ Locus	ICH	MDH-I	MDH-II	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-I	LDH-II
Allele A	0.7485	0.9970	0.1457	0.1557	0.3483	0.0559	0.0060	0.8313	0.9242	0.1607
Allele B	0.2475	0.0030	0.0868	0.0160	0.1337	0.2126	0.4192	0.1687	0.0100	0.5200
Allele C	0.0040		0.6996	0.2575	0.4840	0.3174	0.1707		0.0659	0.2655
Allele D			0.0659	0.5609	0.0279	0.1248	0.3912			0.0539
Allele E			0.0020	0.0100	0.0060	0.2585	0.0090			
Allele F						0.0309	0.0040			

Allelic polymorphism (Table.9.) results indicated that Gök Lake population was polymorphic for a single locus, PGD. Bademli, Çavuşçu and İnsuyu populations were polymorphic at three loci. Polymorphism in Suğla population was 40% with four loci. It was 5 loci for Oymapınar population and the highest polymorphism (80%) was observed in Körkuyu population. Details of allele frequencies within populations and polymorphism can be examined in Appendix A.

Table 9. Allozyme *Polymorphism* in Pseudophoxinus populations.

Population	**Species**	**Locus**	**Polymorphic Locus**	**Polymorphism %**
Bademli Stream, Beyşehir	*P. sp*	10	3	30
Çavuşçu Lake, Ilgın, Konya	*P. battalgili*	10	3	30
Oymapınar Dam Lake, Manavgat, Antalya	*P. battalgili*	10	5	50
Taşağıl Stream, Seydişehir, Konya	*P. battalgili*	10	6	60
Suğla Lake, Seydişehir	*P. battalgili*	10	4	40
İnsuyu Stream, Cihanbeyli	*P. crassus*	10	3	30
Gök Lake, Kulu	*P. crassus*	10	1	10
Deliktaş Locality, Yeşildağ	*P. sp*	10	7	70
Düger, Spring, Burdur	*P. burduricus*	10	6	60
Eflatun Spring, Beyşehir	*P. hittitorum*	10	7	70
Eğirdir Lake, Isparta	*P. egirdiri*	10	3	30
Kırkpınar Drainage, Korkuteli, Antalya	*P. sp*	10	6	60
Köprüçay, Aksu, Isparta	*P. fahrettini*	10	7	70
Körkuyu Locality, Beyşehir	*P. sp*	10	8	80
Kuğulu Park, Seydişehir, Konya	*P. sp*	10	7	70
Salda, Stream, Burdur	*P. burduricus*	10	7	70
Sazak Spring, Burdur	*P. burduricus*	10	8	70

Calculation of Hardy-Weinberg equilibrium for polymorphic loci showed that some loci are still under the strong effect of evolutionary forces and, hence, is not in equilibrium yet. The results of Chi-square test for the populations which were not in HW equilibrium was given in Table.10. MDH-II was the most unstable locus and it was not in accord with HW equilibrium in six populations. Likewise, Taşağıl population of *Pseudophoxinus battalgili* had four loci considerably different from 0.05 significance level.

Another way of determining genetic variation is the heterozygosity present in the population (Nei, 1973a). Detailed results on heterozygosity in populations were presented in Appendix C. Observed heterozygosities were smaller than expected heterozygosity in most of the polymorphic loci. The same observation was made for overall heterozygosity. This was an indication of inbreeding within *Pseudophoxinus* populations (Table.10.) where mean observed heterozygosity was 0.0513 and mean expected heterozygosity was 0.4549.

Table 10. Allozyme deviations from HW equilibrium in Pseudophoxinus populations.

Locus	**Population**	**Chi-square**	**df**	**P**
GPI-I	Eflatun	0.5890	1	0.4428
GPI-I	İnsuyu	0.0000	1	1.0000
GPI-I	Taşağıl	0.0000	1	1.0000
GPI-II	Taşağıl	0.6400	1	0.4237
ICD	Kuğulu Park	1.0688	1	0.3012
LDH-II	Bademli	0.0833	1	0.7728
MDH-I	Eflatun	0.0697	1	0.7918
MDH-II	Deliktaş	0.2813	1	0.5958
MDH-II	Düger	0.0697	1	0.7918
MDH-II	Kırkpınar	3.0758	1	0.0795
MDH-II	Köprüçay	2.2532	1	0.1333
MDH-II	Körkuyu	0.1130	1	0.7368
MDH-II	Kuğulu Park	0.1533	1	0.6954
PGD	Eflatun	0.1459	1	0.7025
PGD	Suğla	0.0000	1	1.0000
PGD	Taşağıl	0.0000	1	1.0000
PGM	Eğirdir	0.0000	1	1.0000
PGM	Suğla	0.0922	1	0.9928
PGM	Taşağıl	7.2551	1	0.0642

df, degree of freedom; **P**, probability, significance level 0.50

Fixation index (F_{ST}) values for all populations have also pointed the presence of inbreeding in *Pseudophoxinus* populations (Table.11). A negative FST means excess of

heterozygosity where the expected value is lower than observed value. In the contrary there is heterozygous deficiency when the opposite is the case. The population is in equilibrium when F_{ST} is 0. LDH was a fixed locus apparent from the fixation value of 1 and had no observed heterozygous individual in all populations. The lowest fixation value was observed for MDH-II locus (0.6446). A similar value (0.6657) was also observed for the locus MDH-I. However, the fixation value was close to 1 and varied from 0.8363 to 0.9530. F_{ST} for populations was provided in Appendix D.

Table 11. Overall heterozygosity in *Pseudophoxinus* Populations.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	1002	0.9681	0.0319	0.6212	0.3788	0.3785	0.1932
MDH-1	1002	0.9980	0.0020	0.9940	0.0060	0.0060	0.0245
MDH-2	1002	0.8303	0.1697	0.5221	0.4779	0.4774	0.1336
GPI-I	1002	0.9721	0.0279	0.4049	0.5951	0.5945	0.2728
GPI-II	1002	0.9441	0.0559	0.3737	0.6263	0.6257	0.1499
PGM	1002	0.9401	0.0599	0.2316	0.7684	0.7676	0.2369
PGD	1002	0.9421	0.0579	0.3574	0.6426	0.6420	0.2818
GPDH	1002	0.9541	0.0459	0.7193	0.2807	0.2804	0.1292
LDH-1	1002	1.0000	0.0000	0.8584	0.1416	0.1415	0.0151
LDH-2	1002	0.9381	0.0619	0.3689	0.6311	0.6304	0.1952
Mean	1002	0.9487	0.0513	0.5451	0.4549	0.4544	0.1632
St. Dev		0.0475	0.0475	0.2472	0.2472	0.2469	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Table 12. Fixation index (Wright, 1978) for overall *Pseudophoxinus* populations.

Wright's (1978) fixation index (Fis) as a measure of heterozygote deficiency or excess

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.9152	0.6657	1.0000	1.0000	0.9340	0.9243	1.0000	0.8363	1.0000	0.8890
Allele B	0.9143	0.6657	-0.0699	1.0000	0.8622	0.9940	0.8934	0.8363	1.0000	0.9400
Allele C	1.0000	****	0.5964	0.9269	0.9481	0.9355	0.9788	****	1.0000	0.9181
Allele D	****	****	1.0000	0.9433	0.5591	0.9909	0.8911	****	****	0.6868
Allele E	****	****	1.0000	1.0000	1.0000	0.8698	0.6636	****	****	****
Allele F	****	****	****	****	****	0.5007	1.0000	****	****	****
Total	0.9156	0.6657	0.6446	0.9530	0.9107	0.9220	0.9098	0.8363	1.0000	0.9019

Evolutionary relationship based on allozyme data, between *Pseudophoxinus* populations, hence species, was determined according to Nei (1972). Dendogram (Fig.51) shows that *Pseudophoxinus egirdiri* clearly separated from the rest of 16 population. Remaining populations made up two groups. The first one had two main braches *P. crassus* and *P. battalgili*. *P. crassus* was represented with two populations, Insuyu and Gök Lake whereas *P. battalgili* had four populations namely Çavuşçu Lake, Oymapınar Dam Lake, Taşağıl Stream and Suğla Lake populations. Second main group was consisted of

nine populations and *P. sp* in Bademli stream was a distinct taxon within the group. Kuğulu Park and Kırkpınar populations of *P. sp* diverged from the other populations within the second group. Although Salda stream and Sazak spring populations of *P. burduricus* closely related Düger population of the same taxon was grouped with Köprüçay population of *P. fahrettini* and Deliktaş and Körkuyu populations of *P. sp.*

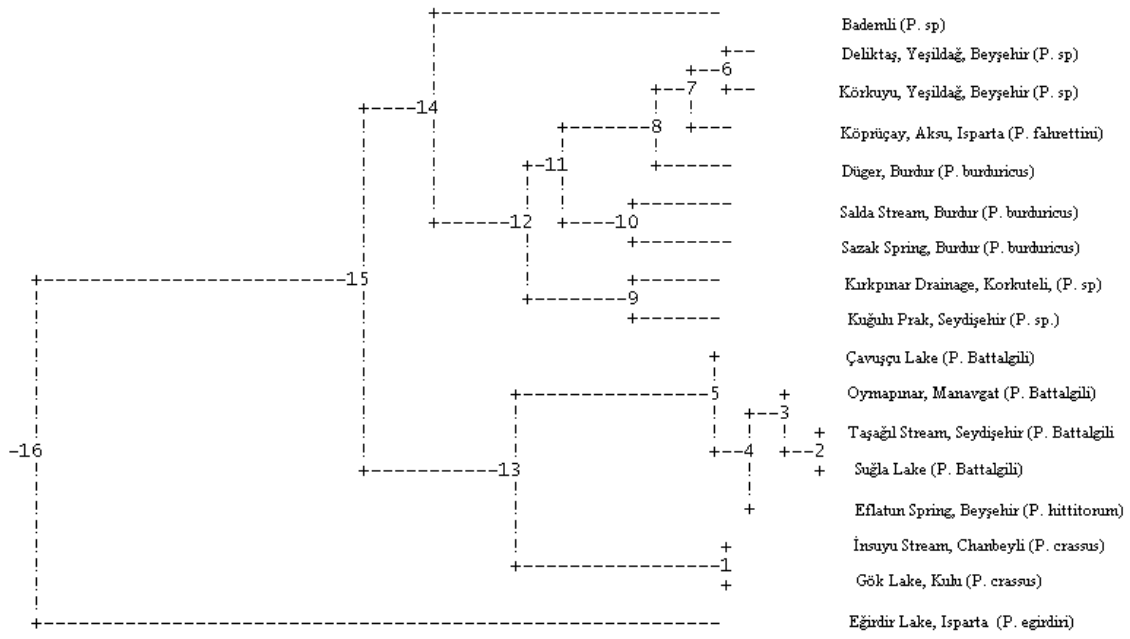

Figure 51. Dendogram obtained from allozyme variation in Pseudophoxinus..

3.3. Microsatellite Analysis

Variations within and among *Pseudophoxinus* populations were analyzed using six microsatellite markers at genomic level using the software FSTAT 2.9.3 (Goudet, 2001). A total of 126 alleles was observed in six microsatellite loci. The most variable locus was Sar N7G5 with 33 alleles (Table.13.) ranging from 80 to 123 bp. While the highest number of alleles (45) was present in *Pseudophoxinus hittitorum* population inhabiting Eflatun Spring, the lowest level of allele was recorded in Bademli population as 13 alleles. Raw data of allele frequencies provided in Appendix E.

As far as the microsatellite markers are concerned gene diversity was relatively high (Table.14). However, it was less than 0.50 for Bademli population except the locus Sar

N2F11b which was 0.705. The lowest value among populations was observed in this population and it was zero for the locus Sar N7F8.

Low diversity was also observed in Çavuşçu lake population of *P. battalgili* for Sar N7F8 (0.24) and Sar N2F11a (0.293) loci. *P. battalgili* of Oymapınar dam lake showed diversity over 0.50 for all loci. The lowest diversity for this species was found to be 0.108 in locus Sar N2F11a in Taşağıl stream population. The last population of this species, Suğla lake population, has also shown low diversity at the locus Sar N2F11a (0.134) as well as Sar N7G5 (0.25).

Table 13. Microsatellite loci and allelic variation in *Pseudophoxinus* populations.

	Sar N7F8	**Sar N7G5**	**Sar N7K4**	**Sar N2F11b**	**Sar N2F11a**	**WMF1**	**Total**
Size Range(bp)	**158-190**	**80-123**	**145-175**	**97-127**	**130-179**	**112-128**	
Bademli	1	2	2	4	2	2	13
Çavuşçu	2	9	8	8	3	8	38
Oymapınar	4	6	9	8	5	10	42
Taşağıl	5	4	8	5	3	8	33
Suğla	4	3	10	7	3	6	33
İnsuyu	3	6	3	2	2	3	19
Gök Lake	2	6	4	3	3	3	21
Deliktaş	6	2	7	7	7	5	34
Düger	3	5	7	5	3	6	29
Eflatun Spring	5	11	9	8	6	6	45
Eğirdir Lake	3	4	4	3	5	4	23
Kırkpınar	4	6	5	6	3	6	30
Köprüçay	4	4	9	6	4	6	33
Körkuyu	4	6	4	8	6	5	33
Kuğulu Park	5	6	10	5	7	6	39
Salda	5	11	6	5	3	6	36
Sazak	6	10	5	4	3	7	35
Total allele	**18**	**33**	**23**	**18**	**23**	**11**	**126**

P. crassus, represented with two populations, generally had low gene diversity. Among six microsatellite loci only two loci i.e., Sar N7G5 and Sar N2F11b were higher than 0.50 in İnsuyu stream population. Similarly, Sar N7G5 and Sar N7K4 loci were above 0.50 in Gök lake population of this species.

High gene diversity was obvious in three populations - Düger Spring, Salda Stream and Sazak Spring- representing *P. burduricus.* The lowest value was calculated as 0.404 for Sazak Spring population of this species.

Restricted to Eğirdir Lake *P. egirdiri* has shown gene diversity varying from 0.093 to 0.75. It was more than 0.50 for only two loci namely Sar N7G5 and Sar N7K4.

Table 14. Gene diversity of microsatellite markers.

	SarN2F11a	SarN2F11b	Sar N7F8	Sar N7G5	Sar N7K4	WMF1
Bademli (*P. sp.)*	0,409	0,705	0	0,409	0,500	0,409
Çavuşçu (*P. battalgili)*	0,293	0,716	0,240	0,743	0,823	0,678
Oymapınar (*P. battalgili)*	0,550	0,698	0,575	0,539	0,860	0,834
Taşağıl (*P. battalgili)*	0,108	0,636	0,479	0,467	0,842	0,791
Suğla (*P. battalgili)*	0,134	0,669	0,580	0,250	0,863	0,697
İnsuyu (*P. crassus)*	0,030	0,061	0,246	0,700	0,290	0,198
Gök Lake (*P. crassus)*	0,165	0,259	0,494	0,681	0,682	0,139
Deliktaş (*P. sp.)*	0,817	0,794	0,720	0,472	0,769	0,726
Düger (*P. burduricus)*	0,552	0,785	0,664	0,631	0,810	0,807
Eflatun Spring (*P. hittitorum)*	0,570	0,758	0,580	0,861	0,807	0,712
Eğirdir Lake (*P. egirdiri)*	0,454	0,093	0,315	0,584	0,750	0,178
Kırkpınar (*P.sp.)*	0,419	0,786	0,633	0,701	0,762	0,797
Köprüçay (*P. fahrettini)*	0,710	0,815	0,592	0,662	0,860	0,808
Körkuyu (*P. sp)*	0,794	0,803	0,680	0,686	0,692	0,755
Kuğulu Park (*P. sp.)*	0,809	0,724	0,773	0,794	0,878	0,765
Salda (*P. burduricus)*	0,515	0,761	0,786	0,854	0,777	0,764
Sazak (*P. burduricus)*	0,404	0,737	0,820	0,860	0,764	0,806

Populations collected from Deliktaş Locality, Körkuyu Spring, Kırkpınar Drainage and Kuğulu Park had high gene diversity. The lowest gene diversity among these populations was 0.419 in the locus Sar N2F11a in Kırkpınar population. However it was extended to 0.878 in for the locus Sar N7K4 in Kuğulu Park population.

P. fahrettini sampled from Köprüçay was also another species with high gene diversity between 0.592 at the locus Sar N7F8 and 0.860 at the locus Sar N7K4.

Finally, *P. hittitorum* inhabiting Eflatun Spring was also showed high gene diversity ranging from 0.580 at the locus Sar N7F8 and 0.861 at the locus Sar N7G5.

3.2.1. Allele Frequency of Microsatellite Loci and Allelic Richness

Microsatellite locus SarN2F11a was represented with 23 alleles ranging from 130 bp to 179 bp (Table 13 Table E.1.) within the 17 populations of *Pseudophoxinus*. The most divergent populations for the locus were Deliktaş and Kuğulu Park populations with seven alleles ranging from 165 bp and 175 bp and 155 bp and 166 bp, respectively (Table E.1.). The most frequent allele of the locus was 166 bp microsatellite fragment and constituted 26% of total allele frequency. Second in frequency was 176 bp allele. However, this allele was almost fixed within *Pseudophoxinus crassus* represented with two populations i.e. İnsuyu and Gök Lake. Similarly, 130 bp allele was only present in *P. burduricus.* This species had the lowest allele band of the SarN2F11a locus ranging 130 to 155 bp. Although 168 bp allele was the most common with its occurrence in nine population, its contribution was only 5%. The least occurring alleles of SarN2F11a locus were 159 bp, 167 bp, 177 bp, 178 bp and 179 bp fragments each of which was encountered once in one population only.

Microsatellite locus SarN2F11b was represented with 18 alleles ranging from 97 bp to 127 bp within 17 populations of *Pseudophoxinus* (Table E.2.). There were four populations; Çavuşçu Lake and Oymapınar dam lake populations of *P. battalgili*, Eflatun Spring population of *P. hittitorum* and Körkuyu population of *P. sp.*, highly polymorphic for the locus and possessed eight of 18 alleles (Table 13 and Table E.2.). The most frequent allele of the locus was 103 bp microsatellite fragment and constituted 19% of total allele frequency. This allele seemed to be fixed in İnsuyu population of *P. crassus* with a constitution of 97%., and hence, this population is considered as monomorphic for the locus. Although 101 bp allele frequency was the second, 111 bp allele making up 13% of all of the alleles was the most common allele occurring 14 of 17 populations investigated.

Microsatellite locus Sar N7F8 was represented with 18 alleles ranging from 158 to 190 bp. Bademli population is monomorphic for this locus and only 164 bp allele is present in the population. The most common allele of this locus is 169 bp allele and is present

in 11 population. The most divergent groups for the locus are *P sp* of Deliktaş spring and *P burduricus* of Sazak spring with the presence of six alleles in both populations Eflatun spring

The most polymorphic microsatellite locus was found to be SarN7G5 with 33 alleles present in the populations of *Pseudophoxinus.* The size range of the alleles were between 72 bp and 123 bp. The most frequent allele was 84 bp microsatellite fragment and was present in six populations. Fragment length in *P. battalgili* populations was at the lower range of the locus and alleles at size range of 72 bp to 90 were observed in this population. On the contrary alleles of the *P. crassus* were at the upper range of the microsatellite fragments and the range covered alleles between 111 bp and 123 bp.

SarN7K4 represented with 23 alleles in *Pseudophoxinus* populations. The allele size range was varied between 145 bp and 175 bp, and the most frequent allele, and the most common one with occurrence in 12 populations as well, of the locus was 161 bp allele with 13% followed by 165 bp allele with 11%.

WMF1 was the most conserved locus within microsatellite loci. The locus represented with 11 alleles ranging from 112 bp to 128 bp. Although 118 bp allele was the commonest with the occurrence in 16 of 17 populations, the highest frequency was observed for 116 bp allele.

Allelic richness (mean number of alleles per locus) estimates recent bottleneck in populations (Leberg, 2002). Table 15 indicates that allelic richness was considerably low in Bademli population. Although this might be a result of recent bottleneck in the population, low sample size should be taken into account for the interpretation of the result.

Mean allele number was the highest for the locus SarN7K4 (7.22) and the lowest for the locus WMF1 (5.106).

Table 15. Allelic Richness found in *Pseudophoxinus* populations.

	Sar N2F11a	Sar N2F11b	Sar N7F8	Sar N7G5	Sar N7K4	WMF1
Bademli (*P. sp.)*	1.990	3.620	1.000	1.980	1.998	1.99
Çavuşçu (*P. battalgili)*	2.191	4.260	1.790	4.800	5.086	4.132
Oymapınar (*P. battalgili)*	3.163	4.538	3.080	3.333	5.600	5.378
Taşağıl (*P. battalgili)*	1.524	3.454	2.662	2.59	5.318	4.683
Suğla (*P. battalgili)*	1.626	3.818	2.837	2.032	5.754	3.866
İnsuyu (*P. crassus)*	1.152	1.282	1.904	3.703	2.077	1.794
Gök Lake (*P. crassus)*	1.735	1.996	1.996	3.710	3.469	1.642
Deliktaş (*P. sp.)*	4.747	4.763	3.672	1.992	4.234	3.802
Düger (*P. burduricus)*	2.420	4.157	2.908	3.738	4.860	4.520
Eflatun Spring (*P. hittitorum)*	3.529	5.081	3.302	6.017	5.113	4.144
Eğirdir Lake (*P. egirdiri)*	3.006	1.446	2.267	2.820	4.000	1.815
Kırkpınar (*P. sp.)*	2.361	4.44	3.047	4.049	3.948	4.531
Köprüçay (*P. fahrettini)*	3.305	4.608	3.115	3.457	5.607	4.637
Körkuyu (*P. sp)*	4.412	4.899	3.191	3.464	3.25	4.048
Kuğulu Park (*P. sp.)*	4.602	3.556	4.100	4.559	5.974	4.088
Salda (*P. burduricus)*	2.718	3.902	4.196	5.797	4.101	4.215
Sazak (*P. burduricus)*	2.182	3.646	4.659	5.572	3.923	4.653
Total	6.473	6.346	5.774	7.152	7.22	5.106

3.2.2. Heterozygosity in Microsatellite Loci and Population Structure

Table 16. Nei's (1972) estimation of heterozygosity.

Locus	Ho	Hs	Ht	Dst	Dst'	Ht'	Gst	Gst'	Gis
Sar N2F11a	0.055	0.454	0.89	0.436	0.463	0.917	0.49	0.505	0.879
Sar N2F11b	0.291	0.634	0.894	0.259	0.275	0.91	0.29	0.303	0.542
Sar N7F8	0.131	0.541	0.845	0.304	0.323	0.864	0.36	0.374	0.759
Sar N7G5	0.336	0.641	0.917	0.276	0.293	0.934	0.301	0.314	0.476
Sar N7K4	0.618	0.749	0.921	0.172	0.183	0.932	0.187	0.196	0.174
Sar WMF1	0.271	0.639	0.825	0.187	0.198	0.837	0.226	0.237	0.576
Overall	0.283	0.61	0.882	0.272	0.289	0.899	0.309	0.322	0.535

Expected heterozygosity (Hs) was lower than that of observed heterozygosity (Ho) in all microsatellite loci. This is an indication of heterozygote deficiency for all loci. Therefore gene flow among *Pseudophoxinus* population was considerably low.

Table 17. Fis in *Pseudophoxinus* populations

	SarN2F11a	SarN2F11b	SarN7F8	SarN7G5	SarN7K4	WMF1	All
Bademli (*P. sp.)*	1	0.645	NA	1	0.5	1	0.794
Çavuşçu (*P. battalgili)*	1	0.358	1	0.527	0.113	0.322	0.427
Oymapınar (*P. battalgili)*	0.61	0.63	0.473	0.944	0.048	0.401	0.476
Taşağıl (*P. battalgili)*	-0.026	0.185	-0.083	0.762	0.032	0.297	0.209
Suğla (*P. battalgili)*	0.379	0.211	0.282	-0.109	0.195	0.344	0.231
İnsuyu (*P. crassus)*	0	1	0.877	0.264	-0.15	0.847	0.384
Gök Lake (*P. crassus)*	1	1	1	0.161	-0.027	0.794	0.463
Deliktaş (*P. sp.)*	0.958	0.566	0.713	0.764	0.396	0.715	0.682
Düger (*P. burduricus)*	1	0.612	0.935	0.656	0.463	0.677	0.703
Eflatun Spring (*P. hittitorum)*	0.582	0.56	1	-0.017	-0.131	0.532	0.372
Eğirdir Lake (*P. egirdiri)*	0.931	0.663	0.385	-0.05	0.2	0.475	0.342
Kırkpınar (*P. sp.)*	0.918	0.605	0.837	0.508	0.25	0.567	0.583
Köprüçay (*P. fahrettini)*	1	0.607	0.797	0.456	0.209	0.653	0.604
Körkuyu (*P. sp)*	0.916	0.502	0.951	0.465	0.181	0.691	0.622
Kuğulu Park (*P. sp.)*	0.893	0.52	0.775	0.672	0.307	0.602	0.624
Salda (*P. burduricus)*	1	0.602	1	0.539	0.141	0.683	0.64

Table 18. Pairwise comparison of similarity among *Pseudophoxinus* populations resulting from microsatellite data

	Çavuşçu	Oymapınar	Taşağıl	Suğla	İnsuyu	Gök Lake	Deliktaş	Düger	Eflatun S.	Eğirdir	Kırkpınar	Köprüçay	Körkuyu	Kuğulu P.	Salda	Sazak
Bademli	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00074*	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Çavuşçu		0.04301*	0.00515*	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Oymapınar			0.01765*	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Taşağıl				0.01324*	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Suğla					0.00037	0.00037	0.00037	0.00037	0.00037	0.00074*	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
İnsuyu						0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Gök Lake							0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Deliktaş								0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Düger									0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Eflatun S										0.00368*	0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Eğirdir Lake											0.00037	0.00037	0.00037	0.00037	0.00037	0.00037
Kırkpınar												0.00037	0.00037	0.00037	0.00037	0.00037
Köprüçay													0.00037	0.00037	0.00037	0.00037
Körkuyu														0.00037	0.00037	0.00037
Kuğulu Park															0.00037	0.00037
Salda																0.12794*

*, non-significant variation; P-values were obtained after: 2720 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is: 0.000368

Pairwise significance test (Table 18) following 2720 permutation has shown that most of the populations were significantly separated from each other. However, genetic variation was not found significant within between *P. battalgili* populations. Although Çavuşçu lake population of this species was not significantly different from Oymapınar Dam Lake and Taşağıl stream populations, it was significantly different from Suğla lake population of the same species.

The difference between *P. egridiri* and Suğla population of *P. battalgili* and the population inhabiting Bademli stream was not significant although it was found to be near significant after 2720 permutation. Nevertheless, the variation between *P. egridiri* and *P. hittitorum* was significant.

The highest relatedness (lack of significant variation) was observed between two populations, Salda and Sazak, of *P. burduricus*.

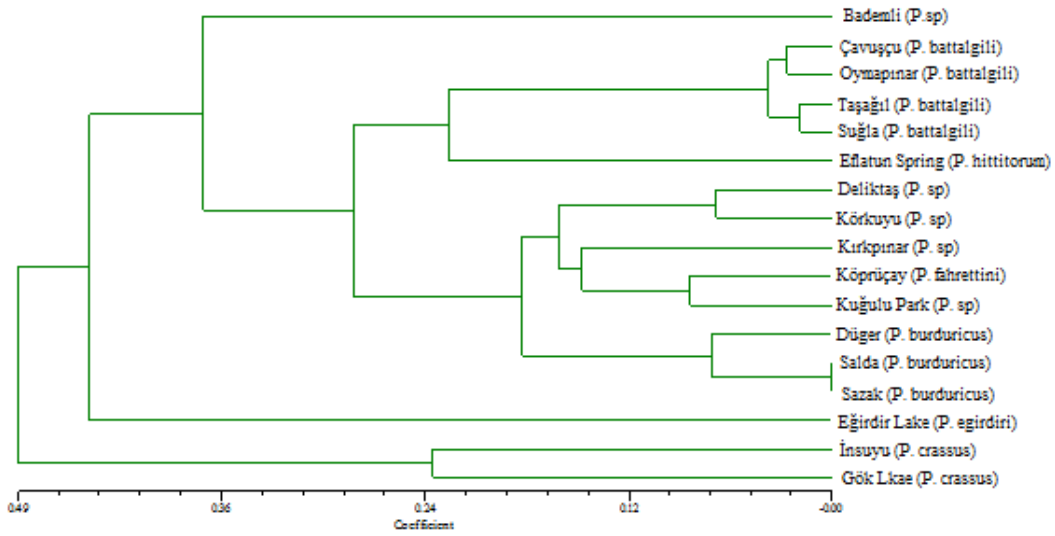

Figure 52. Dendogram obtained from microsatellite variation in *Pseudophoxinus*.

Phylogenetic relationship of populations of *Pseudophoxinus* driven from microsatellite data showed that *P. crassus* separated from the rest of the population as a unique cluster. Similarly, *P. egirdiri* inhabiting the Lake Eğirdir only was also separated from the rest. Bademli population of *Pseudophoxinus* among remaining 14 populations were the one distinctly separated from the others which were clustered in to groups. While four populations of *P. battalgili* and *P. hittitorum* were in one group, *P. burduricus* and *P. fahrettini* formed the second group together with four populations whose species status has not been clarified yet.

Two populations of *P. burduricus* were identical.

Two populations; Deliktaş and Körkuyu, of *P. sp* are present at very close geographical locations and clustered together.

CHAPTER 4

DISCUSSION

Genus *Pseudophoxinus,* Cyprinidae is a primary freshwater fish genus consisting species highly endemic to Turkey. There are 29 species representing the genus and 21 of them inhabit the freshwaters of Turkey. Presence of only one species of these 21 is also reported from Syria. Remaining eight species distributed around the Mediterranean basin from North Africa to Arabian Peninsula. Although the taxonomic recognition of the genus dates back to mid-1800s, recent detailed studies resulted in the exclusion of some taxa from the genus (Kottelat and Barbieri, 2004; Bogutskaya et al., 2007) as well as in description of new species (Bogutskaya et al., 2007; Freyhof & Özuluğ, 2006; Freyhof & Özuluğ (2010a, 2010b and 2010c); Küçük et al., 2013; Küçük & Güçlü, 2014; Ekmekçi et al., 2015; Küçük et al., 2016).

This remarkable diversity in the heart of one of the world's main biodiversity hotspots (Myers et. al.,2000) is threatened by the vulnerability of freshwaters. A variety of factors can adversely affect freshwater ecosystems and cause a decline in number of species in aquatic habitats. Anthropogenic activities such as discharge of industrial and domestic pollutants, habitat destruction, water abstraction, and irrigation are main threats for the inhabitants of freshwaters. Global warming and resulting climate change is also a major threat for aquatic ecosystems (Moyle and Cech, 2004). The introduction of exotic species is another threat due to competition or direct predation. It may even cause extinction of the species as in the case of the extinction of *Pseudophoxinus handlirschi* due to predation by *Sander lucioperca* introduced into the Lake Eğirdir (Küçük, 2006).

Aforementioned threats necessitate thorough evaluation of organisms of aquatic ecosystems. Investigations to clarify taxonomic status of such organisms, their population structures are necessary to take further steps for planning and implementing conservation actions in order to sustain their existence.

Evaluation of morphological variation have successfully been used in a variety of field in fish studies such as fish health, estimation of biomass, population discrimination and conservation (Haas & McPhail, 1991; von Cramon-Taubadel et al, 2005) as well as sexual dimorphism (Altun et al., 2015). Phenotypic variation is highly important for the determination of evolutionary changes since the development of morphological features is influenced by both genetics of the organisms and their environment (Cadrin, 2000; von Cramon-Taubadel et al, 2005). Therefore evolutionary history of a species can be depicted by the evaluation of phenotypic variation within the populations of a species (D'anatro & Loureiro, 2005). Studies on fish has shown that morphological variations within a species is consistent with differences in genetic variation of that species (Corti *et al.*, 1988). High phenotypic plasticity encountered in fish is a great advantage for their response to different environmental conditions (Thompson, 1991).

Freshwaters are considered as islands of a land and the speciation in freshwaters through dispersal is not a common occurrence except in the lakes. Freshwaters are usually separated from each other either with geographical barriers or with salt waters such as seas and oceans. Hence, the dispersal for fish is usually restricted to the length of the freshwater. Restriction in the dispersal of the fish causes them to live in fragmented populations with a reduced or prevented gene flow between populations. Therefore freshwater fish populations are expected to have low genetic diversity and faster rate of differentiation which would also be reflected in the morphology of the fish.

Brown and Lomolito (1998) claim that Mediterranean basin formed 65 million year ago and includes both African and European elements. The region was one of the important temperate refugia in glacial periods. High biodiversity in Anatolian freshwaters may be attributed to the geographical history of the region.

None of the species of *Pseudophoxinus* co-exists with another species of the same genus *Pseudophoxinus*. Moreover, half of the known species consisted of a single population. For example, *P. egridiri, P. hittitorum,* and *P. fahrettini* were species restricted to a single population. Whereas, *P. battalgili* had the largest distribution with four populations studied. *P. burduricus* was represented with three populations. Although

P. crassus was studied in two populations, *P. crassus* of Gök Lake has recently been revised as *P. iconii (*Küçük et al, 2016). Remaining five populations have not been correctly identified yet.

4.1. Geometric Morphometrics

Strauss (1991) claimed that the body parts of the fish affect the morphometric variation. It has been emphasized that head region composed of bony structures is less variable in morphology compared to body region built up off soft tissues. Muscles, gonads, and stomach influence the body shape seasonally and according to feeding conditions. Analysis of geometric morphometrics in populations of the species of *Pseudophoxinus* revealed similar results to that of Strauss (1991). Although head shape has revealed significant variation compared to consensus, it is in general more stable than body shape. However, in four populations; Bademli, Deliktaş, Eflatun Spring, and Körkuyu, head shape variation was relatively close to or more than variation in the body shape. In remaning 13 populations the body shape was more variable than the head shape compared to consensus

Investigation of shape variation in *Pseudophoxinus* genus has shown that fragmented small populations became distinctly separated from each other. Phylogenetic dendogram obtained as the result of analysis of geometric morphometrics data has revealed five species complexes. First of all, *P. crassus* (recently classified as *P. iconii*) singled out from rest of the populations. Next cluster can be considered as two species complexes of *P. battalgili.* In the first one three populations, Çavuşçu Lake, Taşağıl Stream and Suğla Lake, has shown close phylogenetic relationship. In the second *P. battalgili* species complex Oymapınar dam lake population found to be closely related to *P. egirdiri.*

Phylogenetic tree has shown more recent diversification of two species coplexes. One of them included *P. hittitorum* of Eflatun Spring and three *P. spp.* populations, namely Bademli, Deliktaş and Körkuyu. These four populations were geographically close to

each other. Moreover, significantly high head shape variation was observed compared to body shape variation in these populations. In this species complex Deliktaş and Körkuyu populations were found to be identical.

The fifth one can be considered as *P. burduricus* species comlex. This cluster includes three *P. burduricus* populations and one population of *P. fahrettini* in Köprüçay, one population of *P. crassus* found in Insuyu Stream and, two populations of *P. spp.* Inhabiting Kuğulu Park and Kırkpınar.

4.2. Allozyme Variation

Allozyme technique allows evaluation of genetic variation, population structure, polymorphism, and heterozygosity, population history like bottlenecks, gene flow and inbreeding (Wayne *et al*. 1991; Hartl and Pucek 1994).

Investigation of population structure of the species of *Pseudophoxinus* using 10 allozyme loci revealed that these loci were polymorphic among the populations (Table 8.). However, polymorphism and the level of heterozygosity within populations were not very high. The lowest polymorphism (10%) was observed in Gök Lake population of *P. crassus* while the highest polymorphism was found to be 80% in two populations, *P. sp.* of Körkuyu and *P. burduricus* of Sazak Spring. Low heterozygosity may suggest low gene flow between the populations that could be the result of allopatric speciation where the populations were separated by barriers and speciation was progressed in fragmented small inbred populations.

The presence of rare alleles could be an indication of a recent bottleneck. Each of the loci except LDH-II had at least one rare allele whose overall frequency is less than 5% (Table 8.). For example, MDH-I was represented with two alleles in the populations studied. However, overall frequency of MDH-I allele B was found to be less than 1% and it was observed in only two populations, Bademli and Eflatun Spring. This allele was either lost in other populations or occurred in these two populations as a result of recent mutations. Nevertheless, PGD locus was represented with six alleles and three of them were rare alleles.

Pseudophoxinus populations were tested for Hardy-Weinberg equilibrium and found that only two loci, GPDH and LDH-I, were in equilibrium in general (Table 10). This may indicate that evolutionary forces such as mutation, random genetic drift, gene flow and/or natural selection are still in operation.

Inbreeding was also revealed by use of allozyme technique in *Pseudophoxinus* populations. The observed heterozygosity levels (Table 11) calculated according to Nei (1973) were lower than the expected heterozygosities at all loci in all populations Fixation index, F_{ST} (Wright, 1978) confirmed the lack of gene flow for almost all allozyme loci (Table 12). An overall F_{ST} value over 83.63% for all loci expect MDH-I (66.57%) and MDH-II (64.46%) indicated the heterozygote deficiency and lack of gene flow.

Allozyme analysis has shown that *P. egirdiri* was separated from the rest of the populations. *P. battalgili* species complex were similar to morphometric phylogeny. However, *this* complex included *P. hittitorum* of Eflatun Spring.

Two populations of *P. crassus* were found to be identical to each other and closely related to *P. battalgili* species complex. However, they were less related to *P. burduricus.*

Although remaining populations were clustered together, Bademli population seems to be separated from the large group including *P. burduricus* species complex. Nevetheles, it has to be taken in to account that the specimen number of this population is very low and, hence, the result should be considered carefully while commenting on Bademli population.

P. spp of Deliktaş and Körkuyu, and *P. fahrettini* of Köprüçay were clustered within *P. burduricus* complex. Kırkpınar and Kuğulu Park populations were separated from this complex and formed another group with two populations of *P. spp.*

4.3. Microsatellite Variation

O'Connell and Wright (1997) indicated that the variability in microsatellite loci are higher than that of the allozyme loci in fishes. Analysis of six microsatellite loci in *Pseudophoxinus* populations has supported that genetic variation was considerably higher in microsatellite loci than in allozyme loci. In order to investigate the genetic variation in 17 populations of the genus six microsatellite were used. Observed total number of microsatellite alleles was 126 at six loci. The least variable microsatellite locus was WMF-1 with 11 alleles. The most variable locus was SarN7G5 with 33 alleles (Table 13). Allele number within population varied from 13 in Bademli population to 45 in *P. hittitorum* of Eflatun Spring.

Although there were considerably high level of allelic richness in microsatellite loci, heterozygosity deficiency was also observed as the result of the analysis. Hence, inbreeding and the lack of gene flow were also confirmed by the investigation of microsatellite data.

Pylogeny of microsatellite data resulted with separation of *P. crassus* from the rest the population as a unique cluster. Similarly, *P. egirdiri* inhabiting only the Lake Eğirdir was also separated from the rest as single group. Bademli population of *Pseudophoxinus* among remaining 14 populations were the one distinctly separated from the others which were clustered in to groups. While four populations of *P. battalgili* constituted a species complex with *P. hittitorum, P. burduricus* populations were observed in the same species complex where Salda and Sazak populations were found to be genetically identicaland. *P. fahrettini* were in another species complex with the rest of the populations.

Two populations; Deliktaş and Körkuyu, of *P. sp* are present at very close geographical locations and clustered together

4.4. Comparison of Morphometric and Genetic Phylogeny

Although *P. crassus* populations were morphologically clustered into different species complex, allozyme data showed them as identical populations. Microsatellite results

also agreed that these populations are closely related and constitutes a distinct species group.

P. battalgili was represented with four populations which were clustered in the same species complex by all three methods used to analyze the variation and differentiation among populations. However, *P. battalgili* species complex morphologically included *P. egirdiri* where it was close to Oymapınar dam lake population of *P. battalgili*.

Three populations of *P. burduricus* were found to be forming another distinct species complex according to analyses of molecular data both microsatellite and allozyme loci. This group was found to be closely related to four populations of non-identified species and *P. fahrettini.* Similar situation was observed in morphological investigation except that the species complex include one of the populations of *P. crassus;* İnsuyu population.

CHAPTER 5

CONCLUSION

Pseudophoxinus is a species rich genus of Cyprinidae and most of the species of genus inhabits the freshwaters, especially the springs, of Turkey. The number of species being reported is increasing with the increasing number of detailed studies.

In this study, three approaches; geometric morphometrics, allozyme and microsatellite analyses were used in order to determine the differentiation of 17 populations representing six species and five non-identified population of the genus.

The results have shown that all of the three methods were useful for the classification of species within the genus. Phylogenies obtained from different approaches were highly similar to each other with slight differences.

Number of specimens could be caught was small in some locations. Keeping sample size high would give better differentiation of the populations in question. However, it is also important that except a few species with more than one population the species of this genus is highly fragmented and requires special concern even when sampling.

Especially the analyses of allozyme loci revealed that heterozygosity, polymorphism and gene flow, hence the genetic variation, is low within and between the populations. This situation is an indication of inbreeding and the fragility of the species of the genus.

Within the 17 populations six species out of 21 exist in Turkey was covered in this study. The extension of the study to whole range of the genus would provide more information that may give raise to better understanding of the population structures and ecosystems that they live in and accordingly may result in better conservation strategies. Easily destructible nature of freshwater ecosystems necessitates the considerations for the conservation of the genus as well as their habitats.

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APPENDICES

APPENDIX A

Allozyme Allele Frequencies in *Pseudophoxinus* Populations

Table A 1. Allele frequencies of 10 loci in Bademli population of *P. sp.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	1.0000	0.7500	1.0000		1.0000				1.0000	0.5000
Allele B		0.2500		0.2500		1.0000		1.0000		0.5000
Allele C				0.7500						
Allele D							1.0000			

Table A 2. Allele frequencies of 10 loci in Çavuşçu Lake population of *P. battalgili.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	1.0000	1.0000			0.2162			1.0000	1.0000	
Allele B					0.1622		0.7297			1.0000
Allele C			1.0000		0.6216					
Allele D				1.0000			0.2162			
Allele E						0.7973				
Allele F						0.2027	0.0541			

Table A 3. Allele frequencies of 10 loci in Oymapınar dam lake population of *P. battalgili.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	0.9697	1.0000		0.0909		0.0152	0.0303	1.0000	0.8485	
Allele B							0.8030		0.1515	1.0000
Allele C	0.0303		1.0000		1.0000	0.2121				
Allele D				0.7576			0.1667			
Allele E				0.1515		0.7727				

Table A 4. Allele frequencies of 10 loci in Taşağıl stream population of *P. battalgili.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	0.9655	1.0000						1.0000	1.0000	
Allele B	0.0345		0.0345				0.9828			1.0000
Allele C			0.9655	0.0172	0.8621	0.1379				
Allele D				0.9828	0.1379		0.0172			
Allele E						0.8448				
Allele F						0.0172				

Table A 5. Allele frequencies of 10 loci in Suğla Lake population of *P. battalgili.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	1.0000	1.0000		0.0278				1.0000	1.0000	
Allele B							0.9028			1.0000
Allele C			1.0000		0.9861	0.0417				
Allele D				0.9722	0.0139		0.0972			
Allele E						0.9444				
Allele F						0.0139				

Table A 6. Allele frequencies of 10 loci in İnsuyu Stream population of *P. crassus.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	1.0000	1.0000	1.0000			0.0735	0.0294	1.0000	1.0000	
Allele B							0.9412			
Allele C				0.0147	1.0000	0.9265				1.0000
Allele D				0.9853			0.0294			

Table A 7. Allele frequencies of 10 loci in Gök Lake population of *P. crassus.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	1.0000	1.0000	1.0000					1.0000	1.0000	
Allele B							0.8714			
Allele C					1.0000	1.0000				1.0000
Allele D				1.0000			0.1286			

Table A 8. Allele frequencies of 10 loci in Deliktaş Spring population of *P. sp.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	0.7344	1.0000			0.7500	0.1250		1.0000	1.0000	0.5312
Allele B	0.2656		0.0938			0.8750				0.4688
Allele C			0.9062	0.5000	0.0625		0.4688			
Allele D				0.5000	0.1875		0.5312			

Table A 9. Allele frequencies of 10 loci in Düger spring population of *P. burduricus.*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	0.7917	1.0000		0.0833	1.0000		0.0417	0.6250	1.0000	
Allele B	0.2083		0.0625	0.0417		1.0000	0.1667	0.3750		
Allele C			0.9375	0.7083			0.0417			0.7708
Allele D				0.1667			0.7500			0.2292

Table A 10. Allele frequencies of 10 loci in Eflatun spring population of *P. hittitorum.*

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.9583	0.9792						1.0000	1.0000	
Allele B		0.0208					0.9167			1.0000
Allele C	0.0417		0.9583	0.1458	0.8125	0.0417				
Allele D				0.8542	0.0625		0.0833			
Allele E			0.0417		0.1250	0.6667				
Allele F						0.2917				

Table A 11. Allele frequencies of 10 loci in Eğirdir Lake population of *P .egirdiri.*

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	1.0000	1.0000		0.4848						1.0000
Allele B				0.1818		0.0152		1.0000		
Allele C				0.0758	1.0000		0.8636		1.0000	
Allele D			1.0000	0.2576		0.9848				
Allele E							0.1364			
Allele F										

Table A 12. Allele frequencies of 10 loci in Kırkpınar drainage population of *P. sp.*

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.3000	1.0000		0.5000				0.8500	1.0000	
Allele B	0.7000		0.2500		1.0000	0.2000	0.2000	0.1500		1.0000
Allele C			0.7500	0.4333		0.8000	0.0667			
Allele D				0.0667			0.7333			
Allele E										
Allele F										

Table A 13. Allele frequencies of 10 loci in Köprüçay population of P. fahrettini.

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.7600	1.0000			0.8400	0.5200		0.8800	1.0000	0.7200
Allele B	0.2400		0.2400		0.1600	0.4800	0.0400	0.1200		0.2800
Allele C			0.7600	1.0000			0.1200			
Allele D							0.8400			
Allele E										
Allele F										

Table A 14. Allele frequencies of 10 loci in Körkuyu population of *P. sp.*

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.5333	1.0000			0.9333			0.9167	1.0000	0.3500
Allele B	0.4667		0.0667			0.5667		0.0833		0.5833
Allele C			0.9333	0.9000		0.4333	0.4000			0.0667
Allele D				0.1000	0.0667		0.6000			
Allele E										
Allele F										

Table A 15. Allele frequencies of 10 loci in Kuğulu Park population of P. sp.

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.5870	1.0000		0.1739	0.0435	0.3478		0.8043	1.0000	
Allele B	0.4130		0.0870		0.8478	0.6522		0.1957		1.0000
Allele C			0.9130		0.1087		0.4348			
Allele D				0.8261			0.5652			
Allele E										
Allele F										

Table A 16. Allele frequencies of 10 loci in Salda stream population of *P. burduricus.*

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.1818	1.0000		0.1818	0.9242			0.8182	1.0000	
Allele B	0.8182		0.3182		0.0758			0.1818		0.2121
Allele C			0.6818	0.6364		1.0000	0.0606			0.4545
Allele D				0.1818			0.9394			0.3333
Allele E										
Allele F										

Table A 17. Allele frequencies of 10 loci in Sazak spring population of *P. burduricus.*

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.1795	1.0000		0.7949	0.8718			0.5385	1.0000	
Allele B	0.8205		0.2564		0.1282			0.4615		
Allele C			0.7436			0.2308	0.3077			0.7308
Allele D				0.2051		0.7692	0.6923			0.2692
Allele E										
Allele F										

APPENDIX B

Allozyme Genic Variation in *Pseudophoxinus* Populations

Table B 1. Allozyme Genic Variation in Bademli population of *P. sp.*

Locus	Sample Size	na*	ne*	I*
ICDH-1	8	1.0000	1.0000	0.0000
MDH-1	8	2.0000	1.6000	0.5623
MBH-2	8	1.0000	1.0000	0.0000
GPI-I	8	2.0000	1.6000	0.5623
GPI-II	8	1.0000	1.0000	0.0000
PGM	8	1.0000	1.0000	0.0000
PGD	8	1.0000	1.0000	0.0000
GPBH	8	1.0000	1.0000	0.0000
LDH-1	8	1.0000	1.0000	0.0000
LDH-2	8	2.0000	2.0000	0.6931
Mean	8	1.3000	1.2200	0.1818
St. Dev		0.4830	0.3706	0.2949

na*, observed number of alleles; **ne***, Effective number of alleles (Kimura and Crow, 1964); I*, Shannon's Information index (Lewontin, 1972)

Table B 2. Allozyme Genic Variation in Çavuşçu lake population of *P. battalgili.*

Locus	Sample Size	na*	ne*	I*
ICDH-1	74	1.0000	1.0000	0.0000
MDH-1	74	1.0000	1.0000	0.0000
MBH-2	74	1.0000	1.0000	0.0000
GPI-I	74	1.0000	1.0000	0.0000
GPI-II	74	3.0000	2.1765	0.9217
PGM	74	2.0000	1.4776	0.5041
PGD	74	3.0000	1.7177	0.7188
GPBH	74	1.0000	1.0000	0.0000
LDH-1	74	1.0000	1.0000	0.0000
LDH-2	74	1.0000	1.0000	0.0000
Mean	74	1.5000	1.2372	0.2145
St. Dev		0.8498	0.4170	0.3591

na*, observed number of alleles; **ne***, Effective number of alleles (Kimura and Crow, 1964); I*, Shannon's Information index (Lewontin, 1972)

Table B 3. Allozyme Genic Variation in Oymapınar dam lake population (*P. battalgili*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	66	2.0000	1.0624	0.1358
MDH-1	66	1.0000	1.0000	0.0000
MBH-2	66	1.0000	1.0000	0.0000
GPI-I	66	3.0000	1.6525	0.7142
GPI-II	66	1.0000	1.0000	0.0000
PGM	66	3.0000	1.5568	0.5916
PGD	66	3.0000	1.4847	0.5807
GPBH	66	1.0000	1.0000	0.0000
LDH-1	66	2.0000	1.3461	0.4253
LDH-2	66	1.0000	1.0000	0.0000
Mean	66	1.8000	1.2103	0.2448
St. Dev		0.9189	0.2692	0.2977

na*, observed number of alleles; **ne***, Effective number of alleles (Kimura and Crow, 1964); I*, Shannon's Information index (Lewontin, 1972)

Table B 4 Allozyme Genic Variation in Taşağıl stream population *(P. battalgili).*

Locus	Sample Size	na*	ne*	I*
ICDH-1	58	2.0000	1.0713	0.1500
MDH-1	58	1.0000	1.0000	0.0000
MBH-2	58	2.0000	1.0713	0.1500
GPI-I	58	2.0000	1.0351	0.0871
GPI-II	58	2.0000	1.3120	0.4012
PGM	58	3.0000	1.3642	0.4857
PGD	58	2.0000	1.0351	0.0871
GPBH	58	1.0000	1.0000	0.0000
LDH-1	58	1.0000	1.0000	0.0000
LDH-2	58	1.0000	1.0000	0.0000
Mean	58	1.7000	1.0889	0.1361
St. Dev		0.6749	0.1348	0.1738

na*, observed number of alleles; **ne***, Effective number of alleles (Kimura and Crow, 1964); I*, Shannon's Information index (Lewontin, 1972)

Table B 5. Allozyme Genic Variation in Suğla lake population *(P. battalgili).*

Locus	Sample Size	na*	ne*	I*
ICDH-1	72	1.0000	1.0000	0.0000
MDH-1	72	1.0000	1.0000	0.0000
MBH-2	72	1.0000	1.0000	0.0000
GPI-I	72	2.0000	1.0571	0.1269
GPI-II	72	2.0000	1.0282	0.0732
PGM	72	3.0000	1.1187	0.2458
PGD	72	2.0000	1.2129	0.3189
GPBH	72	1.0000	1.0000	0.0000
LDH-1	72	1.0000	1.0000	0.0000
LDH-2	72	1.0000	1.0000	0.0000
Mean	72	1.5000	1.0417	0.0765
St. Dev		0.7071	0.0716	0.1179

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 6. Allozyme genic variation in Insuyu stream population (*P .crassus*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	68	1.0000	1.0000	0.0000
MDH-1	68	1.0000	1.0000	0.0000
MBH-2	68	1.0000	1.0000	0.0000
GPI-I	68	2.0000	1.0298	0.0766
GPI-II	68	1.0000	1.0000	0.0000
PGM	68	2.0000	1.1577	0.2627
PGD	68	3.0000	1.1267	0.2645
GPBH	68	1.0000	1.0000	0.0000
LDH-1	68	1.0000	1.0000	0.0000
LDH-2	68	1.0000	1.0000	0.0000
Mean	68	1.4000	1.0314	0.0604
St. Dev		0.6992	0.0596	0.1097

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 7. Allozyme genic variation in Gök lake population *(P. crassus*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	70	1.0000	1.0000	0.0000
MDH-1	70	1.0000	1.0000	0.0000
MBH-2	70	1.0000	1.0000	0.0000
GPI-I	70	1.0000	1.0000	0.0000
GPI-II	70	1.0000	1.0000	0.0000
PGM	70	1.0000	1.0000	0.0000
PGD	70	2.0000	1.2888	0.3837
GPBH	70	1.0000	1.0000	0.0000
LDH-1	70	1.0000	1.0000	0.0000
LDH-2	70	1.0000	1.0000	0.0000
Mean	70	1.1000	1.0289	0.0384
St. Dev		0.3162	0.0913	0.1213

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 8. Allozyme genic variation in Deliktaş spring population *(P. sp.).*

Locus	Sample Size	na*	ne*	I*
ICDH-1	64	2.0000	1.6397	0.5789
MDH-1	64	1.0000	1.0000	0.0000
MBH-2	64	2.0000	1.2047	0.3111
GPI-I	64	2.0000	2.0000	0.6931
GPI-II	64	3.0000	1.6623	0.7029
PGM	64	2.0000	1.2800	0.3768
PGD	64	2.0000	1.9922	0.6912
GPBH	64	1.0000	1.0000	0.0000
LDH-1	64	1.0000	1.0000	0.0000
LDH-2	64	2.0000	1.9922	0.6912
Mean	64	1.8000	1.4771	0.4045
St. Dev		0.6325	0.4296	0.3105

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 9. Allozyme genic variation in Düger spring population (*P. burduricus*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	48	2.0000	1.4922	0.5117
MDH-1	48	1.0000	1.0000	0.0000
MBH-2	48	2.0000	1.1327	0.2338
GPI-I	48	4.0000	1.8581	0.8824
GPI-II	48	1.0000	1.0000	0.0000
PGM	48	1.0000	1.0000	0.0000
PGD	48	4.0000	1.6842	0.7792
GPBH	48	2.0000	1.8824	0.6616
LDH-1	48	1.0000	1.0000	0.0000
LDH-2	48	2.0000	1.5463	0.5383
Mean	48	2.0000	1.3596	0.3607
St. Dev		1.1547	0.3724	0.3541

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 10. Allozyme genic variation in Eflatun spring population (*P. hittitorum*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	48	2.0000	1.0868	0.1732
MDH-1	48	2.0000	1.0425	0.1013
MDH-2	48	2.0000	1.0868	0.1732
GPI-I	48	2.0000	1.3318	0.4154
GPI-II	48	3.0000	1.4713	0.6019
PGM	48	3.0000	1.8824	0.7621
PGD	48	2.0000	1.1803	0.2868
GPDH	48	1.0000	1.0000	0.0000
LDH-1	48	1.0000	1.0000	0.0000
LDH-2	48	1.0000	1.0000	0.0000
Mean	48	1.9000	1.2082	0.2514
St. Dev		0.7379	0.2842	0.2659

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 11. Allozyme genic variation in Eğirdir lake population *(P. egirdiri*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	66	1.0000	1.0000	0.0000
MDH-1	66	1.0000	1.0000	0.0000
MDH-2	66	1.0000	1.0000	0.0000
GPI-I	66	4.0000	2.9393	1.2058
GPI-II	66	1.0000	1.0000	0.0000
PGM	66	2.0000	1.0308	0.0785
PGD	66	2.0000	1.3081	0.3983
GPDH	66	1.0000	1.0000	0.0000
LDH-1	66	1.0000	1.0000	0.0000
LDH-2	66	1.0000	1.0000	0.0000
Mean	66	1.5000	1.2278	0.1683
St. Dev		0.9718	0.6090	0.3852

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 12. Allozyme genic variation in Kırkpınar drainage population (*P. sp.*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	60	2.0000	1.7241	0.6109
MDH-1	60	1.0000	1.0000	0.0000
MDH-2	60	2.0000	1.6000	0.5623
GPI-I	60	3.0000	2.2613	0.8895
GPI-II	60	1.0000	1.0000	0.0000
PGM	60	2.0000	1.4706	0.5004
PGD	60	3.0000	1.7176	0.7299
GPDH	60	2.0000	1.3423	0.4227
LDH-1	60	1.0000	1.0000	0.0000
LDH-2	60	1.0000	1.0000	0.0000
Mean	60	1.8000	1.4116	0.3716
St. Dev		0.7888	0.4262	0.3435

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 13. Allozyme genic variation in Köprüçay population (*P. fahrettini*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	50	2.0000	1.5743	0.5511
MDH-1	50	1.0000	1.0000	0.0000
MDH-2	50	2.0000	1.5743	0.5511
GPI-I	50	1.0000	1.0000	0.0000
GPI-II	50	2.0000	1.3676	0.4397
PGM	50	2.0000	1.9968	0.6923
PGD	50	3.0000	1.3858	0.5296
GPDH	50	2.0000	1.2677	0.3669
LDH-1	50	1.0000	1.0000	0.0000
LDH-2	50	2.0000	1.6756	0.5930
Mean	50	1.8000	1.3842	0.3724
St. Dev		0.6325	0.3315	0.2708

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 14. Allozyme genic variation in Körkuyu population (*P. sp.*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	60	2.0000	1.9912	0.6909
MDH-1	60	1.0000	1.0000	0.0000
MDH-2	60	2.0000	1.1421	0.2449
GPI-I	60	2.0000	1.2195	0.3251
GPI-II	60	2.0000	1.1421	0.2449
PGM	60	2.0000	1.9651	0.6842
PGD	60	2.0000	1.9231	0.6730
GPDH	60	2.0000	1.1803	0.2868
LDH-1	60	1.0000	1.0000	0.0000
LDH-2	60	3.0000	2.1403	0.8624
Mean	60	1.9000	1.4704	0.4012
St. Dev		0.5676	0.4684	0.3056

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 15. Allozyme genic variation in Kuğulu Park population (*P. sp.*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	46	2.0000	1.9413	0.6779
MDH-1	46	1.0000	1.0000	0.0000
MDH-2	46	2.0000	1.1888	0.2954
GPI-I	46	2.0000	1.4032	0.4620
GPI-II	46	3.0000	1.3652	0.5175
PGM	46	2.0000	1.8304	0.6461
PGD	46	2.0000	1.9665	0.6846
GPDH	46	2.0000	1.4593	0.4943
LDH-1	46	1.0000	1.0000	0.0000
LDH-2	46	1.0000	1.0000	0.0000
Mean	46	1.8000	1.4155	0.3778
St. Dev		0.6325	0.3839	0.2851

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 16. Allozyme genic variation in Salda stream population (*P. burduricus*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	66	2.0000	1.4235	0.4741
MDH-1	66	1.0000	1.0000	0.0000
MDH-2	66	2.0000	1.7664	0.6255
GPI-I	66	3.0000	2.1228	0.9075
GPI-II	66	2.0000	1.1628	0.2683
PGM	66	1.0000	1.0000	0.0000
PGD	66	2.0000	1.1285	0.2286
GPDH	66	2.0000	1.4235	0.4741
LDH-1	66	1.0000	1.0000	0.0000
LDH-2	66	3.0000	2.7570	1.0535
Mean	66	1.9000	1.4785	0.4032
St. Dev		0.7379	0.5819	0.3761

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

Table B 17. Allozyme genic variation in Sazak spring population (*P. burduricus*).

Locus	Sample Size	na*	ne*	I*
ICDH-1	78	2.0000	1.4175	0.4706
MDH-1	78	1.0000	1.0000	0.0000
MDH-2	78	2.0000	1.6164	0.5693
GPI-I	78	2.0000	1.4839	0.5074
GPI-II	78	2.0000	1.2879	0.3830
PGM	78	2.0000	1.5505	0.5402
PGD	78	2.0000	1.7423	0.6172
GPDH	78	2.0000	1.9882	0.6902
LDH-1	78	1.0000	1.0000	0.0000
LDH-2	78	2.0000	1.6488	0.5825
Mean	78	1.8000	1.4735	0.4360
St. Dev		0.4216	0.3128	0.2442

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* I = Shannon's Information index [Lewontin (1972)]

APPENDIX C

Allozyme Heterozygosity in *Pseudophoxinus* Populations

Table C 1. Allozyme Heterozygosity in Bademli population (*P. sp.*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	8	1.0000	0.0000	1.0000	0.0000	0.0000	0.1932
MDH-1	8	1.0000	0.0000	0.5714	0.4286	0.3750	0.0245
MBH-2	8	1.0000	0.0000	1.0000	0.0000	0.0000	0.1336
GPI-I	8	1.0000	0.0000	0.5714	0.4286	0.3750	0.2728
GPI-II	8	1.0000	0.0000	1.0000	0.0000	0.0000	0.1499
PGM	8	1.0000	0.0000	1.0000	0.0000	0.0000	0.2369
PGD	8	1.0000	0.0000	1.0000	0.0000	0.0000	0.2818
GPBH	8	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	8	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	8	0.5000	0.5000	0.4286	0.5714	0.5000	0.1952
Mean	8	0.9500	0.0500	0.8571	0.1429	0.1250	0.1632
St. Dev		0.1581	0.1581	0.2333	0.2333	0.2041	0.0924

*, Expected heterozygosity and heterozygosity were calculated using Levene (1949); **, Nei's (1973b) expected heterozygosity;

The number of polymorphic loci is: 3

Percentage of polymorphic loci is: 30%

Table C 2. Allozyme Heterozygosity in Çavuşçu lake population (*P. battalgili*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	74	1.0000	0.0000	1.0000	0.0000	0.0000	0.1932
MDH-1	74	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MBH-2	74	1.0000	0.0000	1.0000	0.0000	0.0000	0.1336
GPI-I	74	1.0000	0.0000	1.0000	0.0000	0.0000	0.2728
GPI-II	74	0.6757	0.3243	0.4521	0.5479	0.5405	0.1499
PGM	74	0.8108	0.1892	0.6723	0.3277	0.3232	0.2369
PGD	74	0.7838	0.2162	0.5765	0.4235	0.4178	0.2818
GPBH	74	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	74	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	74	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	74	0.9270	0.0730	0.8701	0.1299	0.1282	0.1632
St. Dev		0.1222	0.1222	0.2156	0.2156	0.2127	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 3
The percentage of polymorphic loci is : 30.00 %

Table C 3. Allozyme Heterozygosity in Oymapınar dam lake population (*P. battalgili*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	66	1.0000	0.0000	0.9403	0.0597	0.0588	0.1932
MDH-1	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MBH-2	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1336
GPI-I	66	1.0000	0.0000	0.5991	0.4009	0.3949	0.2728
GPI-II	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1499
PGM	66	0.9091	0.0909	0.6368	0.3632	0.3577	0.2369
PGD	66	0.8485	0.1515	0.6685	0.3315	0.3264	0.2818
GPBH	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	66	1.0000	0.0000	0.7389	0.2611	0.2571	0.0151
LDH-2	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	66	0.9758	0.0242	0.8584	0.1416	0.1395	0.1632
St. Dev		0.0531	0.0531	0.1744	0.1744	0.1717	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 5
The percentage of polymorphic loci is : 50.00 %

Table C 4. Allozyme Heterozygosity in Taşağıl stream population (*P. battalgili).*

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	58	1.0000	0.0000	0.9322	0.0678	0.0666	0.1932
MDH-1	58	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MBH-2	58	1.0000	0.0000	0.9322	0.0678	0.0666	0.1336
GPI-I	58	0.9655	0.0345	0.9655	0.0345	0.0339	0.2728
GPI-II	58	0.7241	0.2759	0.7580	0.2420	0.2378	0.1499
PGM	58	0.7931	0.2069	0.7284	0.2716	0.2669	0.2369
PGD	58	0.9655	0.0345	0.9655	0.0345	0.0339	0.2818
GPBH	58	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	58	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	58	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	58	0.9448	0.0552	0.9282	0.0718	0.0706	0.1632
St. Dev		0.1005	0.1005	0.1013	0.1013	0.0995	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 6
The percentage of polymorphic loci is : 60.00 %

Table C 5. Allozyme Heterozygosity in Suğla lake population (*P. crassus*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	72	1.0000	0.0000	1.0000	0.0000	0.0000	0.1932
MDH-1	72	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MBH-2	72	1.0000	0.0000	1.0000	0.0000	0.0000	0.1336
GPI-I	72	1.0000	0.0000	0.9452	0.0548	0.0540	0.2728
GPI-II	72	0.9722	0.0278	0.9722	0.0278	0.0274	0.1499
PGM	72	0.8889	0.1111	0.8924	0.1076	0.1061	0.2369
PGD	72	0.9722	0.0278	0.8220	0.1780	0.1755	0.2818
GPBH	72	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	72	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	72	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	72	0.9833	0.0167	0.9632	0.0368	0.0363	0.1632
St. Dev		0.0351	0.0351	0.0610	0.0610	0.0601	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 4
The percentage of polymorphic loci is : 40.00 %

Table C 6. Allozyme Heterozygosity in İnsuyu stream population (*P. crassus*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	68	1.0000	0.0000	1.0000	0.0000	0.0000	0.1932
MDH-1	68	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MBH-2	68	1.0000	0.0000	1.0000	0.0000	0.0000	0.1336
GPI-I	68	0.9706	0.0294	0.9706	0.0294	0.0290	0.2728
GPI-II	68	1.0000	0.0000	1.0000	0.0000	0.0000	0.1499
PGM	68	0.9118	0.0882	0.8617	0.1383	0.1362	0.2369
PGD	68	0.9412	0.0588	0.8859	0.1141	0.1125	0.2818
GPBH	68	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	68	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	68	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	68	0.9824	0.0176	0.9718	0.0282	0.0278	0.1632
St. Dev		0.0316	0.0316	0.0528	0.0528	0.0520	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 3
The percentage of polymorphic loci is : 30.00 %

Table C 7. Allozyme Heterozygosity in Gök lake population (*P. sp.).*

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.1932
MDH-1	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MBH-2	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.1336
GPI-I	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.2728
GPI-II	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.1499
PGM	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.2369
PGD	70	0.8571	0.1429	0.7727	0.2273	0.2241	0.2818
GPBH	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	70	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	70	0.9857	0.0143	0.9773	0.0227	0.0224	0.1632
St. Dev		0.0452	0.0452	0.0719	0.0719	0.0709	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 1
The percentage of polymorphic loci is : 10.00 %

Table C 8. Allozyme Heterozygosity in Deliktaş population (*P. sp.).*

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	64	0.9062	0.0938	0.6037	0.3963	0.3901	0.1932
MDH-1	64	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MBH-2	64	0.8125	0.1875	0.8274	0.1726	0.1699	0.1336
GPI-I	64	1.0000	0.0000	0.4921	0.5079	0.5000	0.2728
GPI-II	64	1.0000	0.0000	0.5952	0.4048	0.3984	0.1499
PGM	64	1.0000	0.0000	0.7778	0.2222	0.2188	0.2369
PGD	64	1.0000	0.0000	0.4940	0.5060	0.4980	0.2818
GPBH	64	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	64	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	64	0.7500	0.2500	0.4940	0.5060	0.4980	0.1952
Mean	64	0.9469	0.0531	0.7284	0.2716	0.2673	0.1632
St. Dev		0.0932	0.0932	0.2192	0.2192	0.2158	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 7
The percentage of polymorphic loci is : 70.00 %

Table C 9. Allozyme Heterozygosity in Düger spring population (*P. burduricus*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	48	1.0000	0.0000	0.6631	0.3369	0.3299	0.1932
MDH-1	48	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MBH-2	48	0.8750	0.1250	0.8803	0.1197	0.1172	0.1336
GPI-I	48	1.0000	0.0000	0.5284	0.4716	0.4618	0.2728
GPI-II	48	1.0000	0.0000	1.0000	0.0000	0.0000	0.1499
PGM	48	1.0000	0.0000	1.0000	0.0000	0.0000	0.2369
PGD	48	1.0000	0.0000	0.5851	0.4149	0.4062	0.2818
GPBH	48	0.7500	0.2500	0.5213	0.4787	0.4688	0.1292
LDH-1	48	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	48	0.8750	0.1250	0.6392	0.3608	0.3533	0.1952
Mean	48	0.9500	0.0500	0.7817	0.2183	0.2137	0.1632
St. Dev		0.0874	0.0874	0.2122	0.2122	0.2078	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 6
The percentage of polymorphic loci is : 60.00 %

Table C 10. Allozyme Heterozygosity in Eflatun spring population *(P. hittitorum).*

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	48	1.0000	0.0000	0.9184	0.0816	0.0799	0.1932
MDH-1	48	0.9583	0.0417	0.9583	0.0417	0.0408	0.0245
MDH-2	48	1.0000	0.0000	0.9184	0.0816	0.0799	0.1336
GPI-I	48	0.7083	0.2917	0.7456	0.2544	0.2491	0.2728
GPI-II	48	0.8750	0.1250	0.6729	0.3271	0.3203	0.1499
PGM	48	0.7500	0.2500	0.5213	0.4787	0.4688	0.2369
PGD	48	0.8333	0.1667	0.8440	0.1560	0.1528	0.2818
GPDH	48	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	48	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	48	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	48	0.9125	0.0875	0.8579	0.1421	0.1391	0.1632
St. Dev		0.1136	0.1136	0.1627	0.1627	0.1593	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 7
The percentage of polymorphic loci is : 70.00 %

Table C 11. Allozyme Heterozygosity in Eğirdir lake population *(P. egirdiri).*

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1932
MDH-1	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MDH-2	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1336
GPI-I	66	0.8485	0.1515	0.3301	0.6699	0.6598	0.2728
GPI-II	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1499
PGM	66	0.9697	0.0303	0.9697	0.0303	0.0298	0.2369
PGD	66	0.9091	0.0909	0.7608	0.2392	0.2355	0.2818
GPDH	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1292
LDH-1	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	66	0.9727	0.0273	0.9061	0.0939	0.0925	0.1632
St. Dev		0.0524	0.0524	0.2157	0.2157	0.2124	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 3
The percentage of polymorphic loci is : 30.00 %

Table C 12. Allozyme Heterozygosity in Kırkpınar population *(P. sp.)*.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	60	1.0000	0.0000	0.5729	0.4271	0.4200	0.1932
MDH-1	60	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MDH-2	60	0.5000	0.5000	0.6186	0.3814	0.3750	0.1336
GPI-I	60	1.0000	0.0000	0.4328	0.5672	0.5578	0.2728
GPI-II	60	1.0000	0.0000	1.0000	0.0000	0.0000	0.1499
PGM	60	1.0000	0.0000	0.6746	0.3254	0.3200	0.2369
PGD	60	1.0000	0.0000	0.5751	0.4249	0.4178	0.2818
GPDH	60	0.9667	0.0333	0.7407	0.2593	0.2550	0.1292
LDH-1	60	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	60	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	60	0.9467	0.0533	0.7615	0.2385	0.2346	0.1632
St. Dev		0.1573	0.1573	0.2197	0.2197	0.2160	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 6
The percentage of polymorphic loci is : 60.00 %

Table C 13. Allozyme Heterozygosity in Köprüçay population (*P. fahrettini*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	50	1.0000	0.0000	0.6278	0.3722	0.3648	0.1932
MDH-1	50	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MDH-2	50	0.5200	0.4800	0.6278	0.3722	0.3648	0.1336
GPI-I	50	1.0000	0.0000	1.0000	0.0000	0.0000	0.2728
GPI-II	50	0.9200	0.0800	0.7257	0.2743	0.2688	0.1499
PGM	50	1.0000	0.0000	0.4906	0.5094	0.4992	0.2369
PGD	50	1.0000	0.0000	0.7159	0.2841	0.2784	0.2818
GPDH	50	1.0000	0.0000	0.7845	0.2155	0.2112	0.1292
LDH-1	50	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	50	0.9200	0.0800	0.5886	0.4114	0.4032	0.1952
Mean	50	0.9360	0.0640	0.7561	0.2439	0.2390	0.1632
St. Dev		0.1499	0.1499	0.1866	0.1866	0.1828	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 7
The percentage of polymorphic loci is : 70.00 %

Table C 14. Allozyme Heterozygosity in Körkuyu population *(P. sp.)*.

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	60	1.0000	0.0000	0.4938	0.5062	0.4978	0.1932
MDH-1	60	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MDH-2	60	0.8667	0.1333	0.8734	0.1266	0.1244	0.1336
GPI-I	60	1.0000	0.0000	0.8169	0.1831	0.1800	0.2728
GPI-II	60	1.0000	0.0000	0.8734	0.1266	0.1244	0.1499
PGM	60	1.0000	0.0000	0.5006	0.4994	0.4911	0.2369
PGD	60	1.0000	0.0000	0.5119	0.4881	0.4800	0.2818
GPDH	60	0.9667	0.0333	0.8446	0.1554	0.1528	0.1292
LDH-1	60	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	60	0.9000	0.1000	0.4582	0.5418	0.5328	0.1952
Mean	60	0.9733	0.0267	0.7373	0.2627	0.2583	0.1632
St. Dev		0.0492	0.0492	0.2204	0.2204	0.2167	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

Table C 15. Allozyme Heterozygosity in Kuğulu Park population (*P. sp.*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	46	0.6087	0.3913	0.5043	0.4957	0.4849	0.1932
MDH-1	46	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MDH-2	46	0.8261	0.1739	0.8377	0.1623	0.1588	0.1336
GPI-I	46	1.0000	0.0000	0.7063	0.2937	0.2873	0.2728
GPI-II	46	0.9565	0.0435	0.7266	0.2734	0.2675	0.1499
PGM	46	1.0000	0.0000	0.5362	0.4638	0.4537	0.2369
PGD	46	1.0000	0.0000	0.4976	0.5024	0.4915	0.2818
GPDH	46	0.9565	0.0435	0.6783	0.3217	0.3147	0.1292
LDH-1	46	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	46	1.0000	0.0000	1.0000	0.0000	0.0000	0.1952
Mean	46	0.9348	0.0652	0.7487	0.2513	0.2458	0.1632
St. Dev		0.1268	0.1268	0.2035	0.2035	0.1990	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 7
The percentage of polymorphic loci is : 70.00 %

Table C 16. Allozyme Heterozygosity in Salda stream population *(P. burduricus*).

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	66	1.0000	0.0000	0.6979	0.3021	0.2975	0.1932
MDH-1	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MDH-2	66	0.3636	0.6364	0.5594	0.4406	0.4339	0.1336
GPI-I	66	1.0000	0.0000	0.4629	0.5371	0.5289	0.2728
GPI-II	66	0.9697	0.0303	0.8578	0.1422	0.1400	0.1499
PGM	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.2369
PGD	66	1.0000	0.0000	0.8844	0.1156	0.1139	0.2818
GPDH	66	0.9394	0.0606	0.6979	0.3021	0.2975	0.1292
LDH-1	66	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	66	0.8788	0.1212	0.3529	0.6471	0.6373	0.1952
Mean	66	0.9152	0.0848	0.7513	0.2487	0.2449	0.1632
St. Dev		0.1978	0.1978	0.2355	0.2355	0.2319	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 7
The percentage of polymorphic loci is : 70.00 %

Table C 17. Allozyme Heterozygosity in Sazak spring population (*P. burduricus)*

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het
ICDH-1	78	0.8974	0.1026	0.7016	0.2984	0.2945	0.1932
MDH-1	78	1.0000	0.0000	1.0000	0.0000	0.0000	0.0245
MDH-2	78	0.4872	0.5128	0.6137	0.3863	0.3813	0.1336
GPI-I	78	1.0000	0.0000	0.6697	0.3303	0.3261	0.2728
GPI-II	78	1.0000	0.0000	0.7736	0.2264	0.2235	0.1499
PGM	78	1.0000	0.0000	0.6404	0.3596	0.3550	0.2369
PGD	78	1.0000	0.0000	0.5684	0.4316	0.4260	0.2818
GPDH	78	0.6923	0.3077	0.4965	0.5035	0.4970	0.1292
LDH-1	78	1.0000	0.0000	1.0000	0.0000	0.0000	0.0151
LDH-2	78	0.7692	0.2308	0.6014	0.3986	0.3935	0.1952
Mean	78	0.8846	0.1154	0.7065	0.2935	0.2897	0.1632
St. Dev		0.1790	0.1790	0.1717	0.1717	0.1695	0.0924

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

The number of polymorphic loci is : 8
The percentage of polymorphic loci is : 80.00 %

APPENDIX D

Fixation Indices in *Pseudophoxinus* Populations

Wright's (1978) fixation index (Fis) for populations has been given in this appendix.

Table D 1. Fixation Index of Bademli population (*P. sp.).*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	****	1.0000	****	****	****	****	****	****	****	0.0000
Allele B	****	1.0000	****	1.0000	****	****	****	****	****	0.0000
Allele C	****	****	****	1.0000	****	****	****	****	****	****
Total	****	1.0000	****	1.0000	****	****	****	****	****	0.0000

Table D 2. Fixation Index of Çavuşçu population (*P. battalgili*).

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	****	****	****	****	0.0431	****	****	****	****	****
Allele B	****	****	****	****	-0.1935	****	0.4519	****	****	****
Allele C	****	****	****	****	1.0000	****	****	****	****	****
Allele D	****	****	****	****	****	****	0.3621	****	****	****
Allele E	****	****	****	****	****	0.4147	****	****	****	****
Allele F	****	****	****	****	****	0.4147	1.0000	****	****	****
Total	****	****	****	****	0.4000	0.4147	0.4825	****	****	****

Table D 3. Fixation Index of Oymapınar population *(P. battalgili).*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	1.0000	****	****	1.0000	****	-0.0154	1.0000	****	1.0000	****
Allele B	****	****	****	****	****	****	0.5210	****	1.0000	****
Allele C	1.0000	****	****	****	****	0.8187	****	****	****	****
Allele D	****	****	****	1.0000	****	****	0.4545	****	****	****
Allele E	****	****	****	1.0000	****	0.7412	****	****	****	****
Total	1.0000	****	****	1.0000	****	0.7458	0.5359	****	1.0000	****

Table D 4. Fixation Index of Taşağıl population *(P. battalgili).*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	1.0000	****	****	****	****	****	****	****	****	****
Allele B	1.0000	****	1.0000	****	****	****	-0.0175	****	****	****
Allele C	****	****	1.0000	-0.0175	-0.1600	0.1300	****	****	****	****
Allele D	****	****	****	-0.0175	-0.1600	****	-0.0175	****	****	****
Allele E	****	****	****	****	****	0.3424	****	****	****	****
Allele F	****	****	****	****	****	-0.0175	****	****	****	****
Total	1.0000	****	1.0000	-0.0175	-0.1600	0.2249	-0.0175	****	****	****

Table D 5. Fixation Index of Suğla population *(P. battalgili).*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	****	****	****	1.0000	****	****	****	****	****	****
Allele B	****	****	****	****	****	****	0.8418	****	****	****
Allele C	****	****	****	****	-0.0141	-0.0435	****	****	****	****
Allele D	****	****	****	1.0000	-0.0141	****	0.8418	****	****	****
Allele E	****	****	****	****	****	-0.0588	****	****	****	****
Allele F	****	****	****	****	****	-0.0141	****	****	****	****
Total	****	****	****	1.0000	-0.0141	-0.0473	0.8418	****	****	****

Table D 6. Fixation Index of İnsuyu population *(P. crassus).*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	****	****	****	****	****	0.3524	1.0000	****	****	****
Allele B	****	****	****	****	****	****	0.4688	****	****	****
Allele C	****	****	****	-0.0149	****	0.3524	****	****	****	****
Allele D	****	****	****	-0.0149	****	****	-0.0303	****	****	****
Total	****	****	****	-0.0149	****	0.3524	0.4769	****	****	****

Table D 7. Fixation Index of Gök lake population *(P. crassus*).

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	****	****	****	****	****	****	****	****	****	****
Allele B	****	****	****	****	****	****	0.3625	****	****	****
Allele C	****	****	****	****	****	****	****	****	****	****
Allele D	****	****	****	****	****	****	0.3625	****	****	****
Total	****	****	****	****	****	****	0.3625	****	****	****

Table D 8. Fixation Index of Deliktaş population (*P. sp.).*

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	0.7597	****	****	****	1.0000	1.0000	****	****	****	0.4980
Allele B	0.7597	****	-0.1034	****	****	1.0000	****	****	****	0.4980
Allele C	****	****	-0.1034	1.0000	1.0000	****	1.0000	****	****	****
Allele D	****	****	****	1.0000	1.0000	****	1.0000	****	****	****
Total	0.7597	****	-0.1034	1.0000	1.0000	1.0000	1.0000	****	****	0.4980

Table D 9. Fixation Index of Düger spring population (*P. burduricus*).

Allele \ Locus	ICDH-1	MDH-1	MBH-2	GPI-I	GPI-II	PGM	PGD	GPBH	LDH-1	LDH-2
Allele A	1.0000	****	****	1.0000	****	****	1.0000	0.4667	****	****
Allele B	1.0000	****	-0.0667	1.0000	****	****	1.0000	0.4667	****	****
Allele C	****	****	-0.0667	1.0000	****	****	1.0000	****	****	0.6462
Allele D	****	****	****	1.0000	****	****	1.0000	****	****	0.6462
Total	1.0000	****	-0.0667	1.0000	****	****	1.0000	0.4667	****	0.6462

Table D 10. Fixation Index of Köprüçay population (*P. hittitorum*).

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	1.0000	-0.0213	****	****	****	****	****	****	****	****
Allele B	****	-0.0213	****	****	****	****	-0.0909	****	****	****
Allele C	1.0000	****	1.0000	-0.1707	0.5897	1.0000	****	****	****	****
Allele D	****	****	****	-0.1707	-0.0667	****	-0.0909	****	****	****
Allele E	****	****	1.0000	****	1.0000	0.4375	****	****	****	****
Allele F	****	****	****	****	****	0.3950	****	****	****	****
Total	1.0000	-0.0213	1.0000	-0.1707	0.6098	0.4667	-0.0909	****	****	****

Table D 11. Fixation Index of Eğirdir lake population (*P. egirdiri*).

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	****	****	****	1.0000	****	****	****	****	****	****
Allele B	****	****	****	1.0000	****	-0.0154	****	****	****	****
Allele C	****	****	****	-0.0820	****	****	0.6140	****	****	****
Allele D	****	****	****	0.6038	****	-0.0154	****	****	****	****
Allele E	****	****	****	****	****	****	0.6140	****	****	****
Allele F	****	****	****	****	****	****	****	****	****	****
Total	****	****	****	0.7704	****	-0.0154	0.6140	****	****	****

Table D 12. Fixation Index of Kırkpınar population (*P. sp*).

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	1.0000	****	****	1.0000	****	****	****	0.8693	****	****
Allele B	1.0000	****	-0.3333	****	****	1.0000	1.0000	0.8693	****	****
Allele C	****	****	-0.3333	1.0000	****	1.0000	1.0000	****	****	****
Allele D	****	****	****	1.0000	****	****	1.0000	****	****	****
Total	1.0000	****	-0.3333	1.0000	****	1.0000	1.0000	0.8693	****	****

Table D 13. Fixation Index of Köprüçay population (*P. fahrettini*).

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	1.0000	****	****	****	0.7024	1.0000	****	1.0000	****	0.8016
Allele B	1.0000	****	-0.3158	****	0.7024	1.0000	1.0000	1.0000	****	0.8016
Allele C	****	****	-0.3158	****	****	****	1.0000	****	****	****
Allele D	****	****	****	****	****	****	1.0000	****	****	****
Allele E	****	****	****	****	****	****	****	****	****	****
Allele F	****	****	****	****	****	****	****	****	****	****
Total	1.0000	****	-0.3158	****	0.7024	1.0000	1.0000	1.0000	****	0.8016

Table D 14. Fixation Index of Körkuyu population (*P. sp.).*

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	1.0000	****	****	****	1.0000	****	****	0.7818	****	0.7802
Allele B	1.0000	****	-0.0714	****	****	1.0000	****	0.7818	****	0.7943
Allele C	****	****	-0.0714	1.0000	****	1.0000	1.0000	****	****	1.0000
Allele D	****	****	****	1.0000	1.0000	****	1.0000	****	****	****
Allele E	****	****	****	****	****	****	****	****	****	****
Allele F	****	****	****	****	****	****	****	****	****	****
Total	1.0000	****	-0.0714	1.0000	1.0000	1.0000	1.0000	0.7818	****	0.8123

Table D 15. Fixation Index of Kuğulu Park population (*P. sp.).*

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.1930	****	****	1.0000	1.0000	1.0000	****	0.8619	****	****
Allele B	0.1930	****	-0.0952	****	0.8315	1.0000	****	0.8619	****	****
Allele C	****	****	-0.0952	****	0.7756	****	1.0000	****	****	****
Allele D	****	****	****	1.0000	****	****	1.0000	****	****	****
Allele E	****	****	****	****	****	****	****	****	****	****
Allele F	****	****	****	****	****	****	****	****	****	****
Total	0.1930	****	-0.0952	1.0000	0.8375	1.0000	1.0000	0.8619	****	****

Table D 16. Fixation Index of Salda population (*P. burduricus*).

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	1.0000	****	****	1.0000	0.7836	****	****	0.7963	****	****
Allele B	1.0000	****	-0.4667	****	0.7836	****	****	0.7963	****	1.0000
Allele C	****	****	-0.4667	1.0000	****	****	1.0000	****	****	0.7556
Allele D	****	****	****	1.0000	****	****	1.0000	****	****	0.7273
Allele E	****	****	****	****	****	****	****	****	****	****
Allele F	****	****	****	****	****	****	****	****	****	****
Total	1.0000	****	-0.4667	1.0000	0.7836	****	1.0000	0.7963	****	0.8098

Table D 17. Fixation Index of Sazak population (P. burduricus).

Allele \ Locus	ICDH-1	MDH-1	MDH-2	GPI-I	GPI-II	PGM	PGD	GPDH	LDH-1	LDH-2
Allele A	0.6518	****	****	1.0000	1.0000	****	****	0.3810	****	****
Allele B	0.6518	****	-0.3448	****	1.0000	****	****	0.3810	****	****
Allele C	****	****	-0.3448	****	****	1.0000	1.0000	****	****	0.4135
Allele D	****	****	****	1.0000	****	1.0000	1.0000	****	****	0.4135
Allele E	****	****	****	****	****	****	****	****	****	****
Allele F	****	****	****	****	****	****	****	****	****	****
Total	0.6518	****	-0.3448	1.0000	1.0000	1.0000	1.0000	0.3810	****	0.4135

APPENDIX E

Allele Frequency of Microsatellite Loci in *Pseudophoxinus* Populations

Table E 1. Observed allele frequency of microsatellite locus SarN2F11a.

Population / Allele (bp)	N	130	133	135	155	158	159	160	161	162	163	164	165	166	167	168	170	171	172	175	176	177	178	179	Total
Bademli (*P.sp*)	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,25	0	0	0,75	0	0	0	0	0	1
Çavuşcu (*P.battalgili*)	37	0	0	0	0	0	0	0	0	0	0	0,08	0	0,84	0	0,08	0	0	0	0	0	0	0	0	1
Oymapınar (*P.battalgili*)	28	0	0	0	0	0	0	0	0	0	0	0,21	0,04	0,64	0	0,05	0	0	0,05	0	0	0	0	0	1
Taşağıl (*P.battalgili*)	27	0	0	0	0	0	0	0	0	0	0	0	0	0,94	0	0,04	0	0	0,02	0	0	0	0	0	1
Suğla (*P.battalgili*)	36	0	0	0	0	0	0	0,03	0	0	0	0	0	0,93	0	0,04	0	0	0	0	0	0	0	0	1
İnsuyu (*P.crassus*)	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,99	0,02	0	0	1
Gök Lake (*P.crassus*)	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,06	0,91	0	0	0,03	1
Deliktaş (*P.sp*)	29	0	0	0	0	0	0	0	0	0	0	0	0,07	0,1	0	0,28	0,03	0,03	0,22	0,26	0	0	0	0	1
Düger (*P.burduricus*)	21	0,38	0	0,57	0,05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Eflatun Spring (*P.hititorum*)	21	0	0	0	0	0	0	0	0	0,02	0	0,17	0	0,64	0,05	0,07	0,05	0	0	0	0	0	0	0	1
Eğirdir Lake (*P.egirdiri*)	32	0	0	0	0	0	0	0,05	0,09	0,73	0,03	0,09	0	0	0	0	0	0	0	0	0	0	0	0	1
Kırkpınar (*P.sp*)	29	0	0	0	0	0	0	0,21	0	0	0	0	0,74	0	0	0,05	0	0	0	0	0	0	0	0	1
Köprüçay (*P.fahrettini*)	25	0	0	0	0,36	0,36	0	0	0	0	0,04	0	0,24	0	0	0	0	0	0	0	0	0	0	0	1
Körkuyu (*P.sp*)	30	0	0	0	0	0	0	0	0	0	0	0	0,27	0	0	0,07	0	0,1	0,23	0,3	0	0	0,03	0	1
Kuğulu Park (*P.sp*)	23	0	0	0	0,26	0,3	0,09	0,02	0,09	0	0	0	0,22	0,02	0	0	0	0	0	0	0	0	0	0	1
Salda (*P.burduricus*)	33	0,67	0,15	0,18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sazak (*P.burduricus*)	39	0,74	0,03	0,23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
All_W		0,12	0,01	0,06	0,03	0,03	0	0,02	0,01	0,05	0	0,03	0,09	0,26	0	0,05	0	0,01	0,05	0,04	0,13	0	0	0	1
All_UW		0,11	0,01	0,06	0,04	0,04	0,01	0,02	0,01	0,05	0	0,03	0,09	0,24	0	0,06	0,01	0,01	0,08	0,04	0,11	0	0	0	1

Table E 2. Observed allele frequency of microsatellite locus SarN2F11b.

Population / Allele (bp)	N	97	99	101	103	104	105	106	107	108	109	110	111	113	115	117	119	121	127	Total
Bademli (*P.sp*)	12	0	0	0	0	0	0,5	0	0	0	0	0	0,13	0,25	0,13	0	0	0	0	1
Çavuşçu (*P.battalgili*)	37	0,03	0,03	0,47	0,05	0	0,11	0	0	0	0,23	0	0,07	0,01	0	0	0	0	0	1
Oymapınar (*P.battalgili*)	31	0,03	0	0,53	0,11	0	0,07	0	0,07	0	0,1	0	0,07	0	0,03	0	0	0	0	1
Taşağıl (*P.battalgili*)	27	0	0	0,54	0,07	0	0,07	0	0	0	0,28	0	0,04	0	0	0	0	0	0	1
Suğla (*P.battalgili*)	36	0	0	0,51	0,1	0	0,03	0	0,03	0	0,25	0	0,07	0	0	0	0,01	0	0	1
İnsuyu (*P.crassus*)	33	0	0	0	0,97	0	0,03	0	0	0	0	0	0	0	0	0	0	0	0	1
Gök Lake (*P.crassus*)	35	0	0	0	0,86	0,11	0,03	0	0	0	0	0	0	0	0	0	0	0	0	1
Deliktaş (*P.sp*)	29	0	0	0	0,12	0	0,38	0	0	0,02	0,14	0	0,1	0,19	0,05	0	0	0	0	1
Düger (*P.burduricus*)	23	0	0	0	0	0	0	0	0,04	0	0	0	0,24	0,33	0,17	0,22	0	0	0	1
Eflatun Spring (*P.hittitorum*)	12	0	0	0	0	0,08	0	0	0,08	0	0,5	0,04	0	0	0	0,04	0,13	0,08	0,04	1
Eğirdir Lake (*P.egirdiri*)	32	0	0	0	0	0,02	0	0,95	0	0,03	0	0	0	0	0	0	0	0	0	1
Kırkpınar (*P.sp*)	29	0	0	0	0	0	0	0	0,02	0	0,12	0	0,36	0,19	0,1	0,21	0	0	0	1
Köprüçay (*P.fahrettini*)	25	0	0	0,22	0,26	0	0,18	0	0,24	0	0,06	0	0,04	0	0	0	0	0	0	1
Körkuyu (*P.sp*)	30	0	0	0	0,12	0	0,23	0	0,03	0,02	0,13	0	0,35	0,07	0,05	0	0	0	0	1
Kuğulu Park (*P.sp*)	23	0	0	0,04	0,26	0	0,3	0	0,37	0	0	0	0,02	0	0	0	0	0	0	1
Salda (*P.burduricus*)	33	0	0	0	0	0	0	0	0	0	0,02	0	0,33	0,17	0,26	0,23	0	0	0	1
Sazak (*P.burduricus*)	39	0	0	0	0	0	0	0	0	0	0	0	0,3	0,12	0,24	0,35	0	0	0	1
All_W		0	0	0,15	0,19	0,01	0,1	0,06	0,04	0	0,1	0	0,13	0,07	0,06	0,07	0	0	0	1
All_UW		0	0	0,14	0,17	0,01	0,11	0,06	0,05	0	0,11	0	0,12	0,08	0,06	0,06	0,01	0,01	0	1

Table E 3. Observed allele frequency of microsatellite locus SarN7F8.

Population / Allele (bp)	N	158	160	162	164	165	166	167	168	169	170	171	172	173	175	177	186	188	190	Total
Bademli (*P.sp*)	12	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Çavuşçu (*P.battalgili*)	37	0	0	0	0	0	0	0	0	0.865	0	0.135	0	0	0	0	0	0	0	1
Oymapınar (*P.battalgili*)	33	0	0	0	0	0	0	0.061	0	0.606	0	0.242	0	0.091	0	0	0	0	0	1
Taşağıl (*P.battalgili*)	27	0	0	0	0	0.019	0	0	0	0.685	0	0.241	0	0.037	0.019	0	0	0	0	1
Suğla (*P.battalgili*)	36	0	0	0	0	0	0	0	0	0.569	0.111	0.306	0	0	0	0.014	0	0	0	1
İnsuyu (*P.crassus*)	33	0	0	0.121	0.864	0.015	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Gök Lake (*P.crassus*)	35	0	0	0.400	0.600	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Deliktaş (*P.sp*)	29	0	0	0.017	0.362	0.017	0.362	0.052	0	0.190	0	0	0	0	0	0	0	0	0	1
Düger (*P.burduricus*)	23	0	0	0	0	0.435	0	0	0	0.370	0	0.196	0	0	0	0	0	0	0	1
Eflatun Spring (*P.hittitorum*)	24	0.042	0.042	0.208	0.625	0	0	0	0.083	0	0	0	0	0	0	0	0	0	0	1
Eğirdir Lake (*P.egirdiri*)	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.081	0.823	0.097	1
Kırkpınar (*P.sp*)	29	0	0.017	0	0	0.241	0	0	0	0.534	0	0.207	0	0	0	0	0	0	0	1
Köprüçay (*P.fahrettini*)	25	0	0	0	0	0.600	0	0.040	0	0.220	0	0.140	0	0	0	0	0	0	0	1
Körkuyu (*P.sp*)	30	0	0	0.033	0	0.383	0	0.4	0	0.183	0	0	0	0	0	0	0	0	0	1
Kuğulu Park (*P.sp*)	23	0	0	0.370	0	0.196	0	0.152	0.043	0.239	0	0	0	0	0	0	0	0	0	1
Salda (*P.burduricus*)	33	0	0	0	0	0.303	0	0	0.212	0	0	0.061	0.273	0	0.152	0	0	0	0	1
Sazak (*P.burduricus*)	39	0	0	0	0	0.231	0	0.026	0	0.115	0	0.218	0.231	0	0.179	0	0	0	0	1
All_Weighed		0.002	0.003	0.066	0.174	0.137	0.021	0.042	0.020	0.284	0.008	0.110	0.036	0.008	0.025	0.001	0.005	0.051	0.006	1
All_Unweighed		0.002	0.003	0.068	0.203	0.144	0.021	0.043	0.020	0.269	0.007	0.103	0.030	0.008	0.021	0.001	0.005	0.048	0.006	1

Table E 4. Observed allele frequency of microsatellite locus Sar N7G5

Population / Allele (bp)	N	72	74	78	80	81	82	84	86	87	88	89	90	91	92	93	94	95	97	98	99	100	101	103	105	107	110	111	113	115	117	119	121	123	Total
Bademli (*P.sp*)	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,750	0	0	0	0,250	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Çavuşçu (*P.battalgili*)	37	0,041	0,081	0,054	0,027	0	0,054	0,162	0,473	0	0,081	0	0,027	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Oymapınar (*P.battalgili*)	33	0,015	0	0	0,030	0	0,045	0,121	0,667	0	0,121	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Taşağıl (*P.battalgili*)	27	0	0	0	0	0	0	0,222	0,704	0	0,056	0	0,019	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Suğla (*P.battalgili*)	36	0	0	0	0	0	0	0,042	0,861	0	0,097	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
İnsuyu (*P.crassus*)	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,030	0,015	0,455	0,045	0,242	0,212	1
Gök Lake (*P.crassus*)	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,029	0,043	0,029	0,486	0,257	0,157	0	1
Deliktaş (*P.sp*)	27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,352	0,648	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Düger (*P.burduricus*)	23	0	0	0	0	0	0	0	0	0	0	0	0,587	0	0,065	0	0	0,109	0	0,152	0	0,087	0	0	0	0	0	0	0	0	0	0	0	0	1
Eflatun Spring (*P.hittitor*	24	0	0	0	0	0	0	0	0	0,021	0	0,063	0	0,042	0	0,042	0	0,250	0,250	0	0,104	0	0,063	0,063	0,063	0,042	0,000	0	0	0	0	0	0	0	1
Eğirdir Lake (*P.egirdiri*)	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,016	0	0,097	0,548	0	0,339	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Kırkpınar (*P.sp*)	29	0	0	0	0	0	0	0	0	0	0	0	0,155	0	0,103	0	0	0,500	0,172	0	0	0,034	0,034	0	0	0	0	0	0	0	0	0	0	0	1
Köprüçay (*P.fahrettini*)	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,140	0	0	0	0	0,520	0,240	0,100	0	0	0	0	0	0	0	1
Körkuyu (*P.sp*)	30	0	0	0	0	0	0	0	0	0	0	0	0,017	0	0	0	0	0,383	0,400	0	0,033	0,017	0	0	0	0	0,150	0	0	0	0	0	0	0	1
Kuğulu Park (*P.sp*)	23	0	0	0	0	0	0	0	0	0	0	0,043	0	0	0	0	0	0,370	0,152	0,065	0,196	0	0,174	0	0	0	0	0	0	0	0	0	0	0	1
Salda (*P.burduricus*)	33	0	0	0	0	0	0	0,030	0,030	0,030	0,318	0,030	0,121	0,015	0,106	0	0,121	0,121	0	0,076	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sazak (*P.burduricus*)	39	0	0	0	0	0,026	0	0	0,013	0	0,244	0,013	0,179	0,026	0,090	0	0,128	0,154	0	0,128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
All_Weighed		0,004	0,006	0,004	0,004	0,002	0,007	0,037	0,183	0,003	0,064	0,008	0,062	0,005	0,023	0,003	0,036	0,132	0,123	0,032	0,043	0,007	0,013	0,003	0,029	0,014	0,014	0,002	0,005	0,003	0,064	0,021	0,027	0,014	1
All_Uweighed		0,003	0,005	0,003	0,003	0,002	0,006	0,034	0,162	0,003	0,054	0,009	0,065	0,005	0,021	0,003	0,059	0,137	0,128	0,033	0,054	0,008	0,016	0,004	0,034	0,017	0,015	0,002	0,004	0,003	0,055	0,018	0,024	0,012	1

Table E 5. Observed allele frequency of microsatellite locus Sar N7K4

Population / Allele (bp)	N	145	147	148	150	151	152	153	155	156	157	158	159	160	161	163	165	167	168	169	170	171	173	175	Total
Bademli (*P.sp*)	12	0,63	0	0,38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Çavuşçu (*P.battalgili*)	37	0	0	0	0	0	0	0	0	0	0	0	0,01	0	0,1	0,18	0,07	0,12	0	0,2	0	0,3	0,03	0	1
Oymapınar (*P.battalgili*)	33	0	0	0	0	0	0	0	0	0	0,02	0	0,05	0	0,2	0,17	0,12	0,09	0	0,2	0	0,15	0	0,02	1
Taşağıl (*P.battalgili*)	27	0	0	0	0	0	0	0	0	0	0,02	0	0,06	0	0,26	0,13	0,2	0,15	0	0,15	0	0,04	0	0	1
Suğla (*P.battalgili*)	36	0	0	0	0	0	0	0	0	0	0,01	0	0,08	0	0,24	0,18	0,11	0,11	0	0,17	0,03	0,03	0	0,04	1
İnsuyu (*P.crassus*)	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,14	0,83	0,03	0	0	0	0	0	0	1
Gök Lake (*P.crassus*)	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,27	0,08	0,47	0,18	0	0	0	1
Deliktaş (*P.sp*)	28	0,34	0,02	0,25	0,25	0	0,09	0,02	0,04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Düger (*P.burduricus*)	23	0	0	0	0	0	0	0,2	0,17	0,02	0	0,35	0	0	0,13	0,07	0,07	0	0	0	0	0	0	0	1
Eflatun Spring (*P.hittitorum*)	23	0	0	0	0	0	0	0	0	0	0	0	0,02	0	0,02	0,04	0,17	0,2	0	0,35	0	0,09	0,09	0,02	1
Eğirdir Lake (*P.egirdiri*)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0,5	0	0,2	0,2	0	0,1	0	0	0	0	1
Kırkpınar (*P.sp*)	28	0	0	0	0	0,02	0	0,25	0,34	0	0	0,2	0	0	0,2	0	0	0	0	0	0	0	0	0	1
Köprüçay (*P.fahrettini*)	25	0	0	0,04	0	0,16	0	0,18	0,12	0	0,24	0,02	0,04	0,04	0,16	0	0	0	0	0	0	0	0	0	1
Körkuyu (*P.sp*)	30	0,38	0	0,33	0,25	0	0,03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Kuğulu Park (*P.sp*)	23	0	0,04	0,04	0,02	0,13	0	0,24	0,15	0	0	0,13	0	0	0,15	0	0,07	0	0	0	0	0,02	0	0	1
Salda (*P.burduricus*)	33	0	0	0	0	0	0	0,21	0,26	0	0	0,24	0	0	0,26	0,02	0,02	0	0	0	0	0	0	0	1
Sazak (*P.burduricus*)	39	0	0	0	0	0	0	0,3	0,22	0	0	0,23	0	0	0,24	0	0,01	0	0	0	0	0	0	0	1
All_W		0,06	0	0,05	0,03	0,02	0,01	0,09	0,08	0	0,02	0,07	0,02	0	0,13	0,06	0,11	0,07	0,01	0,1	0,01	0,04	0,01	0,01	1
All_UW		0,08	0	0,06	0,03	0,02	0,01	0,08	0,08	0	0,02	0,07	0,02	0	0,14	0,05	0,11	0,07	0,01	0,1	0,01	0,04	0,01	0,01	1

Table E 6. Observed allele frequency of microsatellite locus WMF1.

Population / Allele (bp)	N	112	114	116	118	120	122	123	124	125	126	128	Total
Bademli (*P.sp)*	12	0	0	0	0,75	0,25	0	0	0	0	0	0	1
Çavuşçu (*P.battalgili)*	37	0,07	0,53	0,07	0,05	0,01	0,2	0	0,05	0	0,01	0	1
Oymapınar (*P.battalgili)*	30	0,2	0,32	0,13	0,12	0,02	0,08	0,02	0,07	0	0,02	0,03	1
Taşağıl (*P.battalgili)*	27	0,09	0,2	0,35	0,02	0,02	0,22	0	0,06	0,04	0	0	1
Suğla (*P.battalgili)*	35	0	0,49	0,17	0	0,03	0,2	0	0,1	0	0,01	0	1
İnsuyu (*P.crassus)*	33	0	0,02	0,89	0,09	0	0	0	0	0	0	0	1
Gök Lake (*P.crassus)*	35	0	0,03	0,93	0,04	0	0	0	0	0	0	0	1
Deliktaş (*P.sp)*	29	0	0	0	0,4	0,33	0,1	0	0,14	0	0	0,03	1
Düger (*P.burduricus)*	23	0	0,04	0,04	0,3	0,17	0,2	0	0,24	0	0	0	1
Eflatun Spring (*P.hittitorum)*	18	0,08	0,19	0,5	0,14	0	0,06	0,03	0	0	0	0	1
Eğirdir Lake (*P.egirdiri)*	32	0,02	0,06	0,91	0,02	0	0	0	0	0	0	0	1
Kırkpınar (*P.sp)*	29	0	0,03	0,12	0,29	0,17	0,09	0	0,29	0	0	0	1
Köprüçay (*P.fahrettini)*	25	0	0,14	0,06	0,32	0,24	0,06	0	0,18	0	0	0	1
Körkuyu (*P.sp)*	30	0	0	0,07	0,37	0,17	0,1	0	0,3	0	0	0	1
Kuğulu Park (*P.sp)*	23	0	0,02	0,02	0,3	0,17	0,13	0	0,35	0	0	0	1
Salda (*P.burduricus)*	33	0	0,06	0,03	0,36	0,08	0,2	0	0,27	0	0	0	1
Sazak (*P.burduricus)*	37	0	0,04	0,07	0,28	0,2	0,14	0	0,26	0	0,01	0	1
All_W		0,03	0,14	0,28	0,2	0,1	0,11	0	0,14	0	0	0	1
All_UW		0,03	0,13	0,26	0,23	0,11	0,1	0	0,14	0	0	0	1

CURRICULUM VITAE

PERSONAL INFORMATION:

Surname, Name: Altun, Ayhan
Nationality: Turkish (TC)
Date and Place of Birth: 07 August 1968, Kayseri
Marital Status: Married
Phone: +90 533 540 36 57
email: altun7@yahoo.com

EDUCATION:

Degree	**Institution**	**Year of Graduation**
PhD	Middle East Technical University	2017
MSc	Çukurova University, Fisheries Faculty	1994
BSc	Black Sea Technical University, Marine Biology Faculty	1989
High School	Çandır High School, Antalya	1984

FOREIGN LANGUAGES:

Advanced English, Basic German, Basic Russian

PUBLICATIONS:

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HOBBIES:

Swimming, Reading, Cultural Trips, Natura Observations