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Research Article

# Genetic and Morphologic Structure of *Liza abu* (Heckel, 1843) Populations from the Rivers Orontes, Euphrates and Tigris

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**Abstract:** *Liza abu* stocks from the rivers Orontes, Euphrates and Tigris were investigated using genetic and morphometric data. Allozyme electrophoresis for genetic comparison and the truss network system for morphometric comparison were simultaneously applied to the same sample set. Highly significant morphological differences were observed between the 3 *Liza abu* stocks. The correct classification of individuals into their original samples for morphological characters was very high (100% of individuals). In discriminant function analyses, plotting discriminant functions revealed high isolation of the 3 stocks; the Tigris stock was very isolated from the Euphrates and Orontes stocks, and the Euphrates stock was located between the Tigris and Orontes stocks, but was closer to the Orontes stock. The pattern of phenotypic discreteness suggests a direct relationship between the extent of phenotypic divergence and geographic separation. However, genetic data do not support the detected morphometric variations. A 5 enzyme system (ICD, PGM, ME, MDH, G3PDH) composed of 6 loci was used to determine genetic comparison. All the loci were monomorphic and therefore there was no genetic divergence among the stocks.

Key Words: *Liza abu*, stock identification, Orontes, Euphrates, Tigris, genetic, morphologic, variation

## Asi, Fırat ve Dicle Nehirlerinde Bulunan *Liza abu* (Heckel, 1843) Populasyonlarının Genetik ve Morfolojik Yapısı

**Özet:** Asi, Fırat ve Dicle nehirleri *Liza abu* populasyonları toplanan örneklerle morfolojik ve genetik olarak incelendi. Genetik karşılaştırma yapmak üzere kullanılan allo-enzim elektroforezi ve morfolojik karşılaştırma yapmak için kullanılan Truss ağı sistemi aynı örnekler üzerinde eşzamanlı olarak kullanıldı. *L. abu* populasyonları arasında çok önemli derecede morfolojik farklılıklar gözlendi. Morfolojik karakterler bakımından balıkların kendi orijinal grubuna doğru olarak sınıflandırılması % 100 olarak yüksek bulundu. Kümelerarası korelasyon analizinde, varyasyonların kümeleştirilmesi sonucu; Dicle stoğunun Asi ve Fırat stoklarından çok farklı olduğu gözlenmiş, Fırat stoğunun Asi ve Dicle stokları arasında bir yer aldığı, fakat Asi stoğuna daha yakın olduğu gözlenmiştir. Gözlenen fenotipik yapı, stokların coğrafik uzaklıkları ile morfolojik farklılığı arasında yakın bir ilişki olduğunu işaret etmektedir. Fakat elde edilen genetik veriler gözlenen morfolojik farklılığı desteklememektedir. Genetik analiz için 5 enzim (ICD, PGM, ME, MDH, G3PDH) sistemi (6 losi) kullanılmıştır. Kullanılan bütün losiler monomorfik çıktığı için populasyonlar arasında herhangi bir genetik farklılık gözlenememiştir.

Anahtar Sözcükler: *Liza abu*, stok farklılığı, Asi, Fırat, Dicle, genetik, morfolojik, varyasyon

## Introduction

*Liza abu* is a mugilid species and inhabits Asia: Iraq, Syria, Pakistan (1-3) and Turkey (4). *L. abu* is found in the Tigris and Euphrates and was recently reported in the Orontes (5) river system in Turkey. The species remains in fresh waters (2,6), but has also been recorded at Kishni in Iraqi brackish waters and in the north-west of the Arabian Gulf (7). There are numerous studies on the meristic characters (7), biological aspects (8,9), and parasites (10) of *L. abu*. However, there are no studies showing the present status of *L. abu* stocks in the Orontes, Euphrates and Tigris river systems. The capacity of fish populations or stocks to adapt and evolve as independent biological entities is limited by the exchange of genes among populations. A sufficient degree of isolation may result in notable morphological, meristic

and genetic differentiation among stocks within a species, which may be recognisable as a basis for the management of distinct stocks. Meristic (11-15) and morphometric (16-19) characters and allozyme electrophoresis (18, 20-23) have been commonly used as a marker in fisheries biology for stock identification.

In the present study, morphometric and meristic characters as morphological data and allozyme electrophoresis as genetic data were used to determine the morphological and genetic differentiation of *L. abu* originating from the rivers Orontes, Euphrates and Tigris.

## Materials and Methods

Approximately 20 specimens were collected from 3 locations throughout the species distribution range consisting of the rivers Orontes, Euphrates and Tigris in Turkey (Figure 1). Samples were caught by cast net, placed on ice and kept frozen at -20 ºC. In the laboratory, after morphometric measurement, samples of liver, eye and muscle tissues were dissected quickly and stored at –20 ºC until examined for allozymes.

### Morphometric and Meristics

The truss network system described for fish body morphometrics (24) was used to construct a network on *L. abu*. Thirteen landmarks determining 24 distances were chosen and measured on the body, as illustrated in Figure 2. The fish were thawed, placed on their right side on acetate sheets, and the body posture and fins were teased into a natural position. Each landmark was taken by piercing the acetate sheet with a dissecting needle, defining 13 landmarks (Figure 2). Additional data, such as eye diameter and head width, were also recorded. Only undamaged fish were included in these measurements.

There were significant correlations between standard length and all morphometric measurements. Therefore it was necessary to remove the size effect from the data. Thus principal component analysis (PCA) was used to remove the size effect from the shape measures (25). This method extracts the first component as the isometric size factor, allowing the subsequent components to be interpreted as summarising shape variation independent of size and random variation among the sampled individuals. The subsequent principal components were

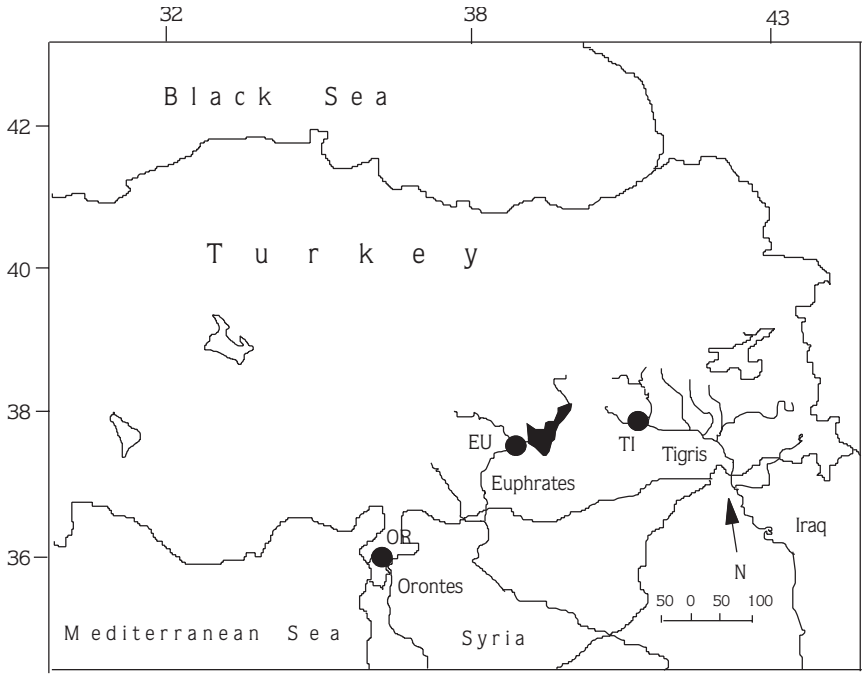


Figure 1. The map of the sampling area. • sampling locations. OR, Orontes; EU, Euphrates; TI, Tigris samples.

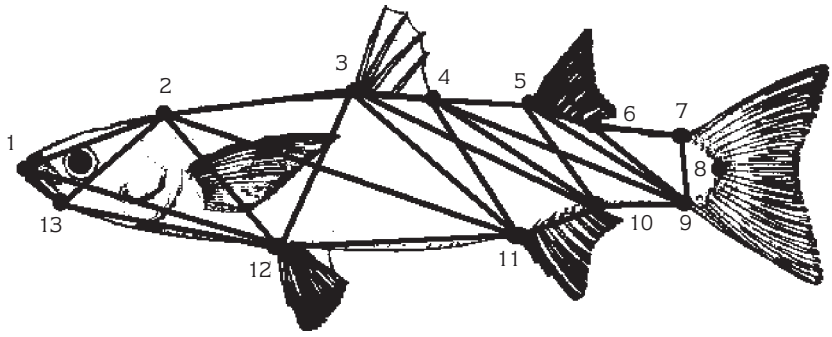


Figure 2. Locations of the 13 landmarks used for constructing the truss network on *Liza abu*.

used in discriminant function analysis (DFA) using SPSS (*v*9.0). Discriminant function analysis combines a selection of body measures in a linear fashion to produce a mathematical function that can be used to classify individuals into groups. Individuals were assigned to groups using the discriminate functions, and the percentage of correctly assigned fish was an additional measure of differentiation among stocks.

Five meristic characters commonly used to describe mullets (first and second dorsal fin rays (DFRs I and DFRs II), ventral fin rays (VFRs), anal fin rays (AFRs), pectoral fin rays (PFRs), gill rakers (GRs) and pyloric caeca (PYC)) were examined under a binocular microscope and used together with morphometric characters in the multivariate analyses.

### Allozyme

Standard methods of horizontal starch gel electrophoresis (26,27) using 13% hydrolysed starch were applied to screen allozymic variation. The nomenclature for enzyme loci and allele designation followed the recommendations of Shaklee et al. (28). Seventeen enzyme systems were screened, and, after screening, 5 enzymes composed of 6 putative loci producing well-resolved staining patterns consistent with known enzyme sub-unit structures were routinely examined. The enzymes used were glycerol-3-phosphate dehydrogenase (EC 1.1.1.8; G3PDH-1* and G3PDH-2*), isocitrate dehydrogenase (EC 1.1.1.42; IDHP*), malate dehydrogenase (EC 1.1.1.37; MDH*), malic enzyme (EC 1.1.1.40; ME*) and phosphoglucose mutase (EC 5.4.2.2; PGM*). Mussel samples were used for all enzyme systems, which were run using Tris citrate buffer (pH 8). Alleles were scored according to their mobility relative to the most commonly observed allele, which was designated as 100. Allele frequencies and measures of genetic variability were estimated by the BIOSYS-1 computer package (release 1.7).

## Results

All the loci were found to be monomorphic in all samples. Therefore there were no allele frequency differences between the samples, showing genetic homogeneity among the samples taken from the Orontes, Euphrates and Tigris stocks.

In PCA, 32 principal components (PCs), which contain the percentage of total variance of all variables, were produced, and 77% of the total variation was presented in the first PC, which presents allometric size factor and was excluded from the analyses. The subsequent components (31 PCs) represented 23% of the variation and these were used in DFA. The first 2 discriminant functions (DFs) explained 100% of between-group variability and plotting DF1 (71%) and DF2 (29%) showed a clear between-stock differentiation (Figure 3). The stocks seemed to be clearly distinct from each other. The Tigris stock was very isolated from Euphrates and Orontes stocks. On the other hand, the Euphrates stock was closer to the Orontes one.

The overall random assignment of individuals into their original stocks was 100%, showing a clear differentiation of samples from each other.

The range of meristic characters for each river sample is given in Table. Only PFR and GR showed clear differences between the stocks. The GRs were highest in the Tigris stock and lowest in the Orontes stock; this may attributed to the feeding regimes of *L. abu* in different river systems. PFRs were low in the Orontes population and similar in the Euphrates and Tigris populations.

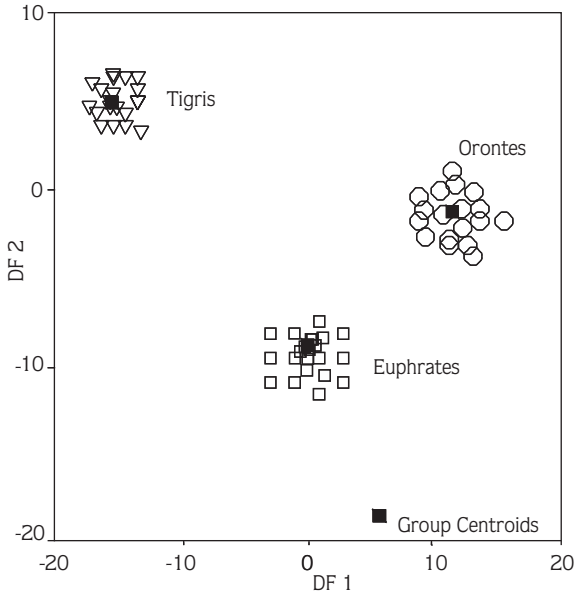


Figure 3. DFA scores of morphometric characters. The first discriminant function (DF1) accounts for 71%, and the second (DF2) accounts for 29% of the between-group variability.

Table. Meristic counts of *L. abu* living in the 3 different rivers and mean standard length (STL; cm) of the samples. DFRs I, first dorsal fin rays; DFRs II, second dorsal fin rays; AFRs, anal fin rays; VFRs, ventral fin rays; PFRs, pectoral fin rays; GRs, gill rakers; PYC, pyloric caeca. Standard deviations are given in brackets.

| | Samples | | |
|---|---|---|---|
| Meristic characters | Orontes | Euphrates | Tigris |
| DFRs I | 4 | 4 | 4 |
| DFRs II | 9 | 9 | 8 |
| AFRs | 11 | 11 | 11 |
| VFRs | 6 | 6 | 6 |
| PFRs | 11-12 | 14-15 | 15 |
| GRs | 60-62 | 69-72 | 70-76 |
| PYC | 3 - 5 | 4 - 5 | 4 |
| STL | 10.47 (0.40) | 11.12 (0.32) | 16.86 (0.83) |

## Discussion

In the present study, highly significant morphological variation was detected among *L. abu* stocks comprising the rivers Orontes, Euphrates and Tigris. The detected pattern of phenotypic discreteness also suggests a direct relationship between the extent of phenotypic divergence and geographic separation, indicating that geographic separation is a limiting factor to migration among stocks. However, the pronounced phenotypic differentiation was not supported by genetic data. The major limitation of morphological characters at the intra-specific level is that phenotypic variation is not directly under genetic control but is subjected to environmental modification (29). The phenotypic plasticity of fish allows them to respond adaptively to environmental change by modifications in their physiology and behaviour, which lead to changes in their morphology, reproduction or survival that mitigate the effects of environmental change (30). Such phenotypic adaptations may not result in genetic changes in the stock (31) and thus detection of such phenotypic differences among stocks cannot usually be taken as evidence of genetic differentiation. For example, Swain et al. (32) used the truss system in the identification of hatchery and wild populations of Coho salmon (*Oncorhynchus kisutch*). They found significant morphometric variation, which was attributed to an effect of the rearing environment rather than genetic differences between the hatchery or wild stocks.

Environmentally induced phenotypic variation, however, may have advantages in the stock structure analysis of exploited species, especially when the time is insufficient for significant genetic differentiation to accumulate among populations. Genetic markers may not be sufficient to detect existing genetic variation among populations, and also a small proportion of DNA is analysed by genetic markers. However, phenotypic markers may detect morphological differentiation due to environmental differences in the habitats of partially isolated stocks, which may be a practical level of partitioning among self-recruiting stocks. Such self-recruiting stocks may react independently to exploitation (33). Morphometric and meristic analysis could thus be a first step in investigating the stock structure of species with large population sizes.

It is also likely that the apparent genetic homogeneity in the present study arises from the use of monomorphic loci. If a number of polymorphic loci had been used in the present study significant genetic heterogeneity concordant with morphological data might have been found among the stocks. In the present study, 17 enzyme systems were screened. After screening, 5 enzymes composed of 6 putative loci producing well-resolved

staining patterns consistent with known enzyme sub-unit structures were routinely examined. Therefore, in future studies, screening a high number of enzymes will increase the number of polymorphic loci for *L. abu* and may reveal genetic heterogeneity among the river populations supporting the detected phenotypic differentiation.

In this present study, the findings reveal the potential power of the truss network system for identifying phenotypic stocks of *L. abu*. An unbiased network of morphometric measurements over a 2 dimensional outline of *L. abu* removes the need to find the types of characters and optimal number of characters for stock separation, and provides information over the entire fish form.

Consequently morphological characters suggest high phenotypic differentiation among the *L. abu* stocks from the rivers Orontes, Euphrates and Tigris. Differences between stocks coincide with geographic proximity. The strong association of different morphological variants with geographic proximity provides a biological basis for the evolution of morphometric and meristic differentiation, since differences in water temperature or food availability, for example, may lead to variation in growth rates, size at maturity and spawning activity. However, it should be emphasised that the application of more powerful genetic techniques (34) or the use of a number of polymorphic loci (35) would be very beneficial to support the detected phenotypic variation.

## References


1. Mahdi, N.: Fishes of Iraq. Ministry of Education. 82 p. Baghdad (Iraq) 1967.
2. Beckman, W.C.: The Freshwater Fishes of Syria and their General Biology and Management. FAO, Fisheries Biology Technical.1962; 297 p. Paper, 8. Rome:.FAO.
3. Coad, B.W.: A Provisional Annotated check-list of the Freshwater Fishes of Iran. J. Bombay Nat. Hist. Soc. 1980; 76: 86-105.
4. Kuru, M.: The Freshwater fish of South-Eastern Turkey-2 (Euphrates-Tigris System). Hacettepe Bull. Nat. Sci. Eng., 1979; 7: 105-114.
5. Yalçın, Ş.: Occurunce of the abu mullet, *Liza abu* (Heckel, 1843) (Pisces, Mugilidae), in Orontes River. Zool. Mid. East. 2004; 30: 111-113.
6. Naama, A.K., Ahmed, H.A., Al-Adhub, A.H.Y.: Aspect of Reproduction of the Mullet *Liza abu* (Heckel) (Pisces, Mugilidae) in Al-Hammar Marsh, Iraq. Cybium. 1986; 10: 47-55.
7. Nasir, N.K., Naama, A.K.: *Liza abu* (Heckel, 1843) (Pisces, Mugilidae) A New record From Khor Al-Zubair, North-West of the Arabian Gulf. Mahasagar.1988; 21: 113-115.
8. Mhaisen, F.T., Al-Jaffery, A.R.: Determination of age and growth of the Mugilid Fish *Liza abu* (Heckel) Inhabiting Babylon Fish Farm, Hilla. Iraq. J. Biol. Sci. Res. 1989; 20: 547-554.
9. Unlu, E., Balcı, K., Meriç, N: Aspects of the biology of *Liza abu* (Mugildae) in the Tigris river (Turkey). Cybium, 2003; 24: 27-43.
10. Mhaisen , F.T., Al-Salım, N.K., Khamees, N.R. : Occurrence of Parasites of the Freshwater Mugilid Fish *Liza abu* (Heckel) from Basrah, Southern Iraq. J. Fish Biol. 1988; 32: 525-532.
11. Mann, G. J., McChart, P. J.: Comparison of sympatric dwarf and normal populations of least cisco (*Coregonus sardinella*) inhabiting Trout Lake, Yukon Territory. Can. J. Fish. Aquat. Sci.,1981; 38: 240-244.
12. Bookstein, F. L.: Foundation of morphometrics. Annu. Rev. Ecol. Syst., 1982; 13: 451-470.
13. Creech, S.: A Multivariate Morphometric Investigation of *Atherina boyeri* Risso, 1810 and a Presbyter Cuvier, 1829 (Teleostei, Atherinidae)- Morphometric Evidence in Support of the two Species. J. Fish Biol. 1992; 41: 341 353.
14. Turan, C .: Otolith Shape and Meristic Analysis of Herring (*Clupea harengus*) in the Northeast Atlantic. Arch. Fish. Mar. Res., 2000; 48: 283-295.
15. Ferreri, F., Nicolais, C., Boglione, B., Bertolini, C.: Skeletal characterization of wild and reared zebrafish: anomalies and meristic characters. J. Fish Biol., 2000; 56: 1115-1128.
16. Shepherd, G.: Meristic and morphometric variation in Black Sea Bass North of Cape hatteras, North Carolina. Am. J. Fish. Man., 1991; 11: 139-149.
17. Haddon, M., Willis, T. J.: Morphometric and Meristic Comparison of Orange Roughy (*Hoplostethus atlanticus*, Trachichthyidae) from the Puysegur Bank and Lord-Howe- Rise, New-Zealand, and Its Implications for Stock Structure. Mar. Biol., 1995; 123: 19-27.
18. Bembo, D.G., Carvalho, G. R., Cingolani, N., Arneri E., Gianetti, G., Pitcher, T.J.: Allozymic and morphometric evidence for two stocks of the European anchovy *Engraulis encrasicolus* in Adriatic waters. Mar. Biol., 1996; 126: 529-538.
19. Turan, C., Basusta, N.: Comparison of Morphometric Characters of Twaite Shad (*Alosa fallax nilotica*, Geoffroy Saint-Hilaire, 1808*)* among three areas in Turkish Seas. Bull. Fr. Peche Piscic., 2001 ; 362/363 : 1027-1035.
20. Smith, P. J. : Protein Electrophoresis for Identification of Australian Fish Stocks. Aus. J. Mar. Fresh. Res., 1990; 41: 823-833.

21. Jørstad, K. E., Dahle, G., Paulsen, O. I.: Genetic Comparison between Pacific Herring (*Clupea pallasi*) and a Norwegian Fjord Stock of Atlantic Herring (*Clupea harengus*). J. Fish. Aquat. Sci., 1994; 51: 233-239.

22. Edmands, S., Moberg, P. E., Burton, R. S.: Allozyme and Mitochondrial DNA Evidence of Population Subdivision in the Purple Sea Urchin in Morphometrics. Sys. Zool., 1996; 31: 113-135.

23. Turan, C., Carvalho, G. R., Mork, J.: Molecular Genetic Analysis of Atlanto Scandian Herring (*Clupea harengus)* Populations using Allozymes and Mitochondrial DNA Markers. J. Mar. Biol. Assoc., 1998; 78: 269-283.

24. Strauss, R.E., Bookstein, F.L.: The Truss: Body Form Reconstruction in Morphometrics. Syst. Zool., 1982; 31: 113-135.

25. Somers, K. M.: Multivariate Allometry and Removal of Size with Principal Component Analysis. Syst. Zool. 1986; 35: 359-368.

26. Harris, H., Hopkinson, D. : Handbook of Enzyme Electrophoresis in Human Genetics. American Elsevier, New York. 1976.

27. Hillis, D.M., Moritz, C.: Molecular Systematic. Sinauer, Sunderland, M.A. 1990.

28. Shaklee, J. B., Allendorf, F. W., Morizot, D.C., Whitt, G.S.: Gene nomenclature for Protein-coding Loci in Fish. Trans. Am. Fish. Soc., 1990; 119: 2-15.

29. Clayton, J. W.: The Stock Concept and the Uncoupling of Organismal and Molecular Evolution. Can. J. Fish. Aquat. Sci., 1986; 38: 1515-1522.

30. Stearns, S.C.: A Natural Experiment in Life-history Evolution: Field data on the introduction of Mosquitofish (*Gambusia affinis*) to Hawaii. Evolution, 1983; 37: 601-617.

31. Ihssen, P. E., Evans, D. O., Christie, W. J., Reckahnand, J. A., Desjardine, R. L.: Life History, Morphology, and Electrophoretic Characteristics of Five Allopatric Stocks of Lake Whitefish (*Coregonus clupeaformis*) in the Great Lake region. Can. J. Fish. Aquat. Sci., 1981; 38: 1790-1807.

32. Swain, D.P., Ridell, B.E., Murray, C.B.: Morphological Differences Between Hatchery and Wild Populations of Coho Salmon (*Oncorhynchus kisutch*): Environmental Versus Genetic Origin. Can. J. Fish. Aquat. Sci. 1991; 48: 1783-1791.

33. Carvalho, G. R., Hauser, L.: Molecular-Genetics and the Stock Concept in Fisheries. Rev. Fish Biol. Fish., 1994; 4: 326-350.

34. Shaw, P., Turan, C., Wrigth, J., O'connell, M., Carvalho, G. R.: Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses, Heredity, 1999; 83: 490-499.

35. Hauser, L., Turan, C., Carvalho, G. R.: Haplotype frequency distribution and discriminatory power of two mtDNA fragments in a marine pelagic teleost (Atlantic herring, *Clupea harengus*). Heredity, 2001; 87: 1-10.