http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 41(1), 8-15 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.1.02

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Comparative otolith morphology in two species of *Salmo* genus from Türkiye

Türkiye'den Salmo cinsine ait iki türün karşılaştırmalı otolit morfolojisi

Melek Özpiçak* ● **Semra Saygın** ● **Savaş Yılmaz**

Department of Biology, Faculty of Science, Ondokuz Mayıs University, Samsun, Türkiye

*Corresponding author: melek.zengin@omu.edu.tr

Received date: 26.10.2023 *Accepted date: 25.12.2023*

How to cite this paper:
Özpiçak, M., Saygın, S., & Savaş, Y. (2024). Comparative otolith morphology in two species of *Salmo* genus from Türkiye. *Ege Journal of Fisheries and Aquatic Sciences, 41*(1), 8-15. https://doi.org/10.12714/egejfas.41.1.02

Abstract: In this study, the morphology of the sagittal otolith of *Salmo coruhensis* Turan, Kottelat & Engin, 2010 and *Salmo fahrettini* Turan, Kalayci, Bektaş, Kaya & Bayçelebi, 2020 from Çam Stream (Artvin) and Terme Stream (Samsun) was described by images of scanning electron microscopy. Its shape and contour were also analyzed with shape indices, elliptic Fourier coefficients and wavelet transforms. As the study material, a total of 30 *S. coruhensis* sample and 20 *S. fahrettini* sample were obtained. Interspecies differences in otolith shape and morphometry were evaluated by principal components analysis, canonical discrimination analysis, and permutational multivariate analysis of variance. The two salmonid species studied were distinguished by both morphometric and shape analysis methods. However, wavelet transform was found to be more effective than shape indices and elliptic Fourier coefficients in species discrimination, with an overall classification success rate of 80%. Our results showed that saccular otolith morphology could be an additional diagnostic character for trout species differentiation.

Keywords: Otolith, shape indices, elliptic Fourier coefficients, wavelet transform, Salmonid

Öz: Bu çalışmada, Terme Çayı (Samsun) ve Çam Deresi (Artvin)'nde yaşayan *Salmo coruhensis* Turan, Kottelat & Engin, 2010 ve *Salmo fahrettini* Turan, Kalayci, Bektaş, Kaya & Bayçelebi, 2020 türlerinin sagittal otolit morfolojileri taramalı elektron mikroskobu görüntüleri ile tanımlanmıştır. Otolit şekli ve dış hatları ayrıca, şekil indeksleri, eliptik Fourier katsayıları ve dalgacık dönüşümü ile analiz edilmiştir. Çalışma materyali olarak toplamda 30 *S. coruhensis* sample ve 20 *S. fahrettini* örneği elde edilmiştir. Otolit şekli ve morfometrisindeki türler arası farklılıklar, temel bileşenler analizi, kanonik ayrım analizi ve çok değişkenli varyans analizi ile değerlendirilmiştir. Çalışılan iki salmonid türü hem morfometrik hem de şekil analizi yöntemleriyle ayırt edilmiştir. Bununla birlikte, dalgacık dönüşümünün tür ayrımında şekil indeksleri ve Fourier katsayılarından daha etkili olduğu ve genel sınıflandırma başarı oranının %80 olduğu bulunmuştur. Sonuçlarımız sakkular otolit morfolojisinin alabalık türlerinin farklılaşmasında ek bir tanısal karakter olabileceğini göstermiştir.

Anahtar Kelime: Otolit, şekil indeksleri, eliptik Fourier katsayısı, dalgacık dönüşümü, alabalık

INTRODUCTION

Taxonomy and systematics are the cornerstone of all biological sciences. Many morphological traits, for example hard elements like otoliths, scales, and bones, are used by taxonomic investigations in ichthyology to identify species (Kontaş et al., 2020; Kikuchi et al., 2021; Akbay et al., 2022; Mejri et al., 2022; Jawad et al., 2022). These hard structures are one of the most useful anatomical features for various research of fish, leading to many practical applications (Schulz-Mirbach et al., 2019; D'Iglio et al., 2022). These studies range from ichthyology to paleontology, geology, archeology, zoogeography, and ecological analyses of predator fish.

Globally, the family Salmonidae is divided into three subfamilies: Coregoninae, Thymallinae, and Salmoninae. It is well known that species of salmonids (family Salmonidae), including those from genus *Salmo*, *Parasalmo*, *Oncorhynchus*, and *Salvelinus*, exhibit a variety of anadromous behaviors and habitat preferences (Savvaitova, 1989; Thorpe, 1994; Pavlov et al., 1999; Pavlov and Savvaitova, 2008). Genus *Salmo* is found throughout Europe, extending southeast into Africa to Morocco and eastwards into upper Amu Darya drainage of Afghanistan (Kottelat, 1997) and widespread in the almost all cold streams and rivers of (Turan et al., 2021). Trouts are economically important, therefore overfishing has a severe impact on *Salmo* populations. There are 16 species of trout described as living in Türkiye in the literature (Turan et al., 2009; Turan et al., 2014 a, b; Turan and Aksu, 2021; Turan et al., 2021). *Salmo coruhensis* Turan, Kottelat & Engin, 2010 is described from the lower and the middle part of the streams and rivers of south and southeastern Black Sea. Additionally, it is well-known in the region that is between the Çoruh drainage in the east and the Yeşilırmak drainage in the west. According to Turan et al. (2009), *S. coruhensis* is known from streams flowing to the southeastern Black Sea coast in Türkiye. For this reason, the trout species in the relevant area should be *S. coruhensis*. However, Yılmaz et al. (2021) reported *Salmo fahrettini* Turan, Kalayci, Bektaş, Kaya & Bayçelebi, 2020, an endemic fish, is distributed in the northern tributaries of the Euphrates River and from Samsun, too. Numerous taxonomic research on *Salmo* species have been realized because of Anatolia's geographic, geological, and geomorphological significance as a center of speciation.

Otoliths can be utilized to identify the species of trout, according to previous investigations (L'Abée-Lund and Jensen, 1993). However, there are limited studies in Türkiye that reveal the otolith morphology of trout species (Yıldız and Yılmaz, 2021) and examine the effectiveness of this morphology in species differentiation. Studies on the otolith morphology of trout will contribute to the issue of whether they are an additional taxonomic character in solving the problems related to the taxonomy of existing species. In this study, it was aimed to (i) describe otolith morphology of *S. coruhensis* and *S. fahrettini* (ii) contribute to the realization of species distinctions by determining the difference between the otolith morphologies of two salmonid species using otolith morphometric descriptions, Fourier and wavelet analyzes.

MATERIAL AND METHODS

Sampling

S. coruhensis (*n*= 30, mean TL±SD, 15.47 ± 3.93 cm TL) and *S. fahrettini* (*n* = 20, mean TL±SD, 13.67 ± 4.09 cm TL) individuals were collected from Çam Stream (Artvin) and Terme Stream (Samsun) using SAMUS 725 MP electroshocker, respectively. The care and use of experimental animals, sampling and analysis techniques used in this work are approved by Ondokuz Mayıs University Animal Experiments Local Ethics Committee with decree no "2017/38". For each sample, a total length (TL, nearest to 0.1 cm) was recorded. A pair of sagittal otoliths (sagitta) were extracted and cleaned with 70% ethanol to remove any additional membranes or surface residues and stored in labelled eppendorf tubes.

Otolith preparation and imaging

Because of there were no statistical differences between left and right otolith pairs (*P*>0.05), left sagittal otolith was chosen for analysis. Each left sagitta was positioned with the *sulcus acusticus* facing upward and the rostrum facing right. Two-dimensional digital images of the otoliths were captured with Leica DFC295 camera. High-contrast digital photos were produced using reflected light. Otoliths were captured on camera as a white silhouette against a dark background (Çöl and Yılmaz, 2022). Also, sagittal otoliths were photographed from their proximal surfaces under a scanning electron microscope for morphological identification (SEM-JEOL JSM 7001 F) (Figure 1). The morphological terminology used for the sagittal otoliths is based on Tuset et al. (2008), and Lin and Chang (2012). SEM photographs were conducted at KITAM, Ondokuz Mayıs University.

Morphometric analysis

Leica Application Suit ver. 3.8 Imaging Software was used to calculate the sagittal otolith length (*OL*), otolith height (*OH*), otolith perimeter (*OP*), and otolith area (*OA*) (±0.001 mm). Due to allometric correlations, the otolith shape indices, which are utilized as dimensionless markers of otolith form, can still be influenced by fish size. To remove the effects of fish size on otolith parameters, the following formula was used to standardize all otolith measurements:

$$Y_i^* = Y_i \times (X_0 / X_i)^b,$$

where, Y_i^* is the standardized parameter; Y_i is the original parameter; X_0 is the mean total length for all specimen (14.75 cm); X_i is the total length of each specimen; *b* is the slope of the regression between log Y_i and log X_i , respectively (Elliott et al., 1995; Lleonart et al., 2000).

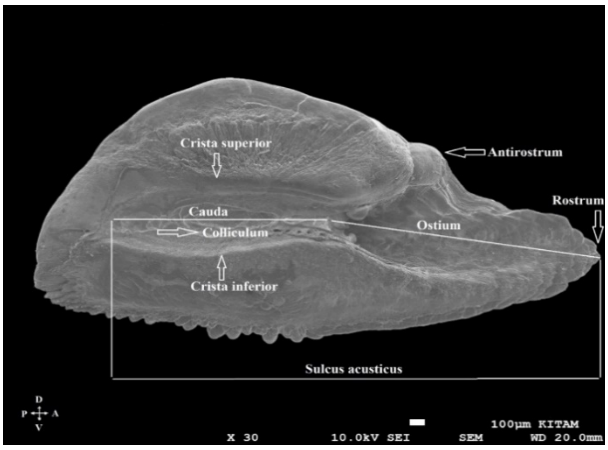

Figure 1. The proximal surface of left sagittal otolith of *S. coruhensis* (24.0 cm TL of fish), illustrates various features described in the text. D: Dorsal, V: Ventral, A: Anterior and P: Posterior

These standardized measurements were then used to calculate the following shape index parameters (SIs): aspect ratio (*AR*), form factor (*FF*), circularity (*C*), rectangularity (*REC*), roundness (*RO*) and ellipticity (*E*) according to Tuset et al. (2003) and Ponton (2006).

First of all, normality and homogeneity of variance were determined for each data set using Shapiro-Wilk and Levene's tests. An independent two-sample t-test was used to compare the otolith shape indices of *S. coruhensis* and *S. fahrettini*. Since the multicollinearity problem was detected between SIs, a principal component analysis (PCA) based on the variance-covariance matrix was performed to reduce the dimensionality of data (Sadighzadeh et al., 2014; Çöl and Yılmaz, 2022). Principal component scores (PCs) were used in a canonical discriminant analysis (CDA, Box's M test, *P* = 0.183) to distinguish species (Song et al., 2018). One-way PERMANOVA (Anderson, 2001) based on Euclidean distance was used for SIs comparisons between *S. coruhensis* and *S. fahrettini*.

Shape analyses

Both the wavelet transform (*WT*) and the elliptic Fourier analysis were used to assess the otolith's shape. The software Shape 1.3 (Iwata and Ukai, 2002) was operated to calculate the elliptic Fourier coefficients (EFCs) from two-dimensional otolith images. The EFCs were made to be invariant to variations in otolith size, orientation, and starting point by normalizing them in accordance with the first harmonic. The Fourier power spectrum was also employed to assess the number of harmonics necessary to effectively represent the otolith shape (Crampton, 1995). 32 Fourier coefficients were used to represent otolith shape of *S. coruhensis* and *S. fahrettini*, with the first eight harmonics accounting for 99.99% of the cumulative power. However, the first three coefficients were degenerated during the normalization procedure. Thus, the total number of EFCs was determined as 29 (4 × 8 − 3). An analysis of covariance (ANCOVA) was used to determine the effect of fish length on the EFCs. Because two EFCs (b2 and d2) were significantly different between species (ANCOVA, $P < 0.05$), these coefficients were not used in the further analysis. As two (d1 and c2) of the remaining EFCs exhibited significant linear correlation with fish size, they were standardized according to the following formula (Song et al., 2018):

$$Y_i^* = Y_i + b\,(X_0 - X_i).$$

Also, a PCA analysis was carried out to minimize the dimensionality of the data and identify the effective EFCs because multicollinearity problems were not found among the EFCs (Sadighzadeh et al., 2014; Çöl and Yılmaz, 2022). The CDA (Box's M test, $P = 0.063$) was performed using raw data to compare otolith shape variations between two salmonid species. Also, the one-way PERMANOVA was used to assess inter-species differences.

Otolith shape analysis depending the WT is based on enlarging the contour into a family of functions derived as the translations and elongations of a specific function called as a mother wavelet (Mallat, 1991). A total of 512 equidistant Cartesian coordinates of the otolith was extracted, being the rostrum the origin of the contour (Parisi-Baradad et al., 2005, 2010). Each contour generated nine wavelets depending on the degree of otolith detail. We selected the wavelet 5 as an intermediate function (Sadighzadeh et al., 2014). Wavelets were produced online using AFORO (Shape Analysis of Fish Otoliths) website (Lombarte et al., 2006). A PCA based was performed to reduce the dimensionality of the 512 data of the wavelet 5 function for each otolith without loss of information. Since it was determined that the linear correlations between the effective PCs and the total length of the fish were not significant, no standardization was applied. The CDA (Box's M test, $P = 0.021$) was performed with the building new PCA matrix and the accuracy of species identification was determined. Otolith shape variations were compared between species by non-parametric PERMANOVA. The Microsoft Excel package, Minitab 17.0, PAST 3.0 (Hammer et al., 2001), and SPSS 21.0 were used for all statistical analyses.

RESULTS

Otolith morphology

Morphological characters of sagittal otoliths of *S. coruhensis* and *S. fahrettini* are presented in Table 1. The medial surface of otoliths is fusiform to elongate convex. Antirostrum is not prominent in both species. The *sulcus acusticus* is described as median type and it opens both anteriorly and posteriorly representing a biostial sulcus type. The shape of ostium type is funnel-like and cauda type is tubular. Rostrum is extended, sharply peaked. Dorsal margin is entire for both species and ventral margin is crenate and sinuate for *S. coruhensis* (Figure 2) and *S. fahrettini* (Figure 3), respectively (Table 1).

Morphometric analysis

Standardized values of the saccular otolith shape indices for two trout species were given in Table 2. All shape indices, except rectangularity ($F = 10.79$, $P = 0.002$), were not significantly different between two species (t-test, $P > 0.05$). In the PCA, only one PC was obtained. This PC discriminate the species based on circularity ($R = 0.99$). Only one canonical discriminant function was used in the CDA ($\lambda = 0.982$, $P = 0.346$). The function 1 explained 100% of the total variance (Eigenvalue=0.019). A 58% overall categorization success rate was produced by the CDA. The percentages of classified individuals obtained with the CDA were 65% for *S. fahrettini* and 53.3% for *S. coruhensis* (Table 3). The PERMANOVA did not show significant difference between the species studied ($F = 0.887$; $P = 0.346$).

Table 1. Morphological otolith characteristics of *S.coruhensis* and *S. fahrettini*

Otolith characteristics	***S. coruhensis***	***S. fahrettini***
Otolith shape	Fusiform/Elongate convex	Fusiform/Elongate convex
Distal region	Concave	Concave
Proximal region	Convex	Convex
Anterior region	Peaked	Peaked
Posterior region	Round/oblique	Round/oblique
Dorsal margin	Entire	Entire
Ventral margin	Crenate	Sinuate
Sulcus acusticus	Median	Median
Ostium	Funnel-like	Funnel-like
Cauda	Tubular	Tubular
Antirostum	Absent/Not well expressed or small/narrow	Absent/ Not well expressed
Rostrum	Extended, broad, peaked	Extended, broad, sharply peaked
Crista superior	Well developed	Well developed
Crista inferior	Well developed	Well developed
Excisura	Moderately wide	Moderately wide

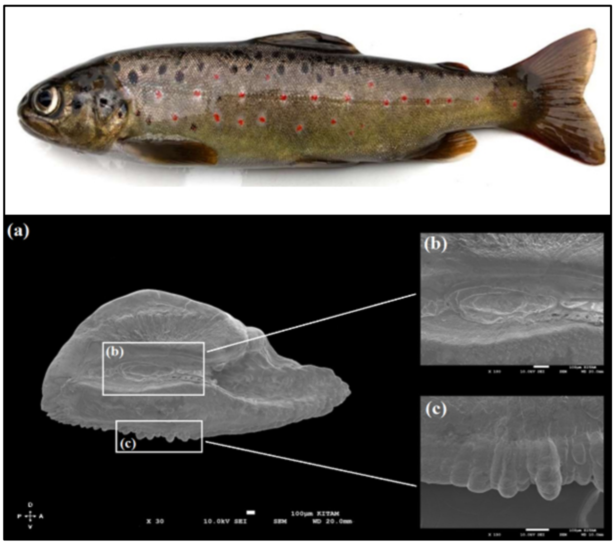

Figure 2. Original photo and otolith SEM images of *S. coruhensis* (24.0 cm TL of fish). (a) General view (SEM-30X, (b) Colliculum (SEM-100X), (c) Ventral edge (SEM-150X)

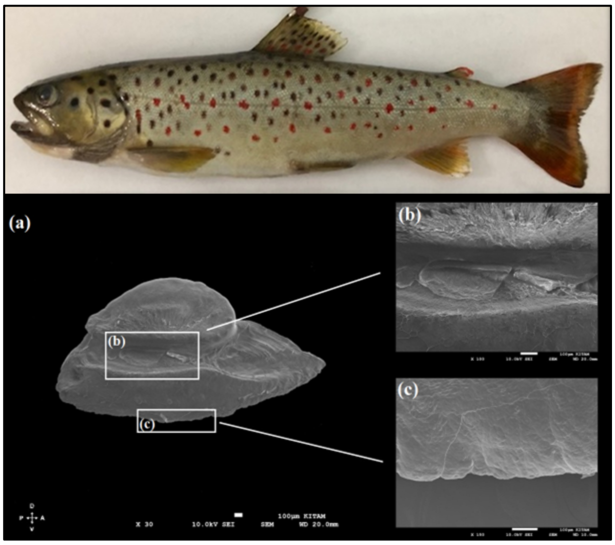

Figure 3. Original photo and otolith SEM images *S. fahrettini* (19.2 cm TL of fish). (a) General view (SEM-30X, (b) Colliculum (SEM-100X), (c) Ventral edge (SEM-150X)

Table 2. Summary of descriptive statistics of otolith shape indices and TL of *S. coruhensis* and *S. fahrettini* from Türkiye

Character	***S. coruhensis*** **(*n*=30) (Çam Stream)**		***S. fahrettini*** **(*n*=20) (Terme Stream)**	
	Min-Max	Mean±SD	Min-Max	Mean±SD
TL (cm)	9.90-24	15.47±3.93	9.60-24.10	13.67±4.09
Aspect Ratio	1.60-1.98	1.75±0.09	1.61-1.88	1.76±0.07
Form Factor	0.68-081	0.77±0.03	0.71-0.79	0.75±0.02
Circularity	15.46-18.43	16.63±0.68	15.92-17.41	16.79±0.51
Roundness	0.41-0.56	0.49±0.03	0.44-0.53	0.47±0.02
Rectangularity	0.63-0.71	0.67±0.02	0.64-0.67	0.65±0.01
Ellipticity	0.23-0.33	0.27±0.02	0.23-0.30	0.27±0.02

Otolith shape analyses

In the PCA using EFCs, only one PC was obtained, and it differentiated two species based on the coefficients d1 (*R* = -0.19), a2 (*R* = 0.49), c2 (*R* = 0.36), a3 (*R* = 0.49), b3 (*R* = 0.47), d3 (*R* = 0.19) and b5 (*R* = 0.19). Only one canonical discriminant function was used in the CDA (λ = 0.777, *P* = 0.129). This function explained 100% of the total variance (Eigenvalue = 0.287). Overall classification success for the CDA was 58%. The percentages of classified individuals based on the CDA results were 60% for *S. coruhensis* and 55% for *S. fahrettini*, respectively (Table 3). The PERMANOVA test did not indicate significant difference between two salmonid fish (*F* = 0.589; *P* = 0.613).

The PCA using wavelet 5 coefficients was created only one PC, which was employed in the CDA. Only one canonical discriminant function was used in the CDA (λ = 0.602, *P* = 0.000). The function 1 described 100% of the total variance (Eigenvalue=0.662). The CDA produced an overall classification success rate of 80%. The percentages of classified individuals obtained with the CDA were 90% for *S. fahrettini* and 73.3% for *S. coruhensis* (Table 3).

The PERMANOVA analysis yielded significant difference between two trout species (*F* = 31.76; *P* = 0.0001). Average decomposition of otolith contour of two salmonid species using wavelet 5 is shown in Figure 4.

Table 3. Classification matrix results of the CDA based on otolith morphometrics and different shape analyzing methods of *S. coruhensis* and *S. fahrettini* (The correct classification percentages are in bold; the number of individuals is given in parentheses)

	Predicted group memberships		
Species	**Shape Indices**	**Fourier Transform**	**Wavelet Analyses**
S. coruhensis	**53.3** (16)	**60.0** (18)	**73.3** (22)
S. fahrettini	**65.0** (13)	**55.0** (11)	**90.0** (18)

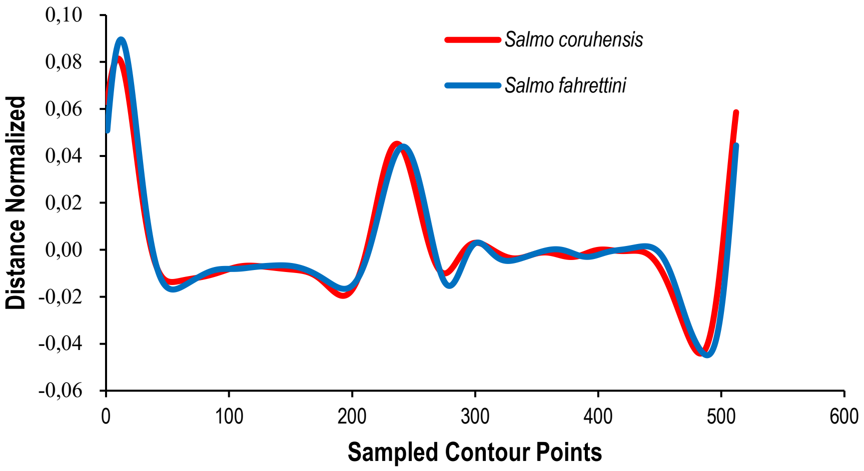

Figure 4. Decomposition of otolith contour of two salmonid species using WT 5

DISCUSSION

According to recent research, teleost fish's saccular otoliths exhibit significant inter- and intraspecies shape diversity and can be used to distinguish different fish species as well as between different stocks and populations, sexes, age groups, and reproductive variants (Mille et al., 2015; Mejri et al., 2018; Wiff et al., 2020; Sadeghi et al., 2020; Yedier, 2021; Çöl and Yılmaz, 2022; Mejri et al., 2022). In this study, we used otolith shape analysis and morphometry for the differentiation of *S. coruhensis* and *S. fahrettini*. This study offers details on the analysis of the variation in sagittal otolith shape between *S. fahrettini* and *S. coruhensis* using a variety of techniques (SIs, EFCs and WT). The attempts for the identification of salmonids have been conducted before based on several meristic, morphometric, and genetic characters (Karakousis et al., 1991; Bardakci et al., 1994; Bernatchez, 2001; Bardakçı et al., 2006; Turan et al., 2009; Berrebi et al., 2019; Delling et al., 2020; Turan et al., 2020; Guinand et al., 2021; Yılmaz et al., 2021). Phenotypic plasticity is a trait shared by individuals of several salmonid species that facilitates evolutionary adaptation. Such phenotypic adaptations, however, are not brought about by variations in the population's gene frequencies (Hutchings, 2004), though some genotypes may better endure environmental changes and leave more progeny. The adaptation based on phenotypic plasticity and genetic modifications under the influence of selection occurs at varying rates. Generic and phenotypic adaptations appear over the course of several generations. When it comes to the first scenario, adaptations should be seen as an individual's tactical response to the effects of the environment, and when it comes to the second scenario, as a population-level (gene pool) strategic response (Pavlov and Savvaitova, 2008). When evaluated in this context, otolith features are an additional very important and cheap taxonomic markers used in intra- and inter-species distinctions.

L'abèe-lund and Jensen (1993) reported otoliths as a natural tag for *Salmo* species. In addition, they evaluated the precision of intraspecific, interspecific, and intergeneric identification using the morphology of the otoliths in two species of *Salmo* and two species of *Salvelinus*. Shape indices, one of the morphometrically and morphologically important otolith characters, were reported in previous studies on *Salmo* species. Yıldız and Yılmaz (2021) were calculated shape indices (*FF* = 0.69 ± 0.05; *AR* = 1.71 ± 0.10; *C* = 18.08 ± 1.58; *RO* = 0.49 ± 0.03; *REC* = 0.65 ± 0.02; *E* = 0.26 ± 0.02) of *S. coruhensis* from Çam Stream. Also, Basçınar (2020) was reported *AR* (1.67 ± 0.15), *FF* (0.62 ± 0.07), and *RO* (1.53 ± 0.22) for *S. trutta*. Morat et al. (2008) investigated shape indices of *Salmo trutta*, too. These findings concur with those of the current investigation. In terms of shape indices, the current study shows that rectangularity is more useful for differentiating between *S. coruhensis* and *S. fahrettini*. In addition, CDA results produced 58% total successful classification rate. A series of studies confirmed that otolith shape indices could be used for inter and intraspecific discrimination of species (Tuset et al., 2003; Morat et al., 2008; Ozpiçak et al., 2018; Yedier, 2021; Akbay et al., 2022; Pavlov, 2022).

More comprehensive information on the variability of otolith shape is provided by contour analysis techniques (Tuset et al., 2021). Fourier analysis, which characterizes the general shape of the otolith, and wavelet analysis, which is useful for estimating the otolith edge, were both used in this study (Parisi-Baradad et al., 2005). The results of the elliptic Fourier analysis demonstrates that sagittal otoliths of *S. coruhensis* and *S. fahrettini* can be described with limited numbers of harmonics (total of 8 harmonics explain 99.99% of the cumulative Fourier power). Especially in the recent studies, Fourier and wavelet analyzes are mostly preferred to examine the otolith shape in different fish species (Bourehail et al., 2015; Libungan et al., 2015; Pavlov, 2022; Pavlov and Osinov, 2023). Morat et al. (2008) compared the morphological and chemical characteristics of otoliths of *S. trutta* and *Salvelinus fontinalis* with elliptic Fourier analysis and shape indices. The discrimination between the two species has a Wilks' λ of 0.18 ($P < 0.05$) and Cohen-kappa test revealed that 89% of fish were correctly classified.

Friedland and Reddin (1994) used Fourier analysis of otolith morphology in stock discriminations of *Salmo salar* population and used to calculate a complex Fourier transform and two shape indices, rectangularity and circularity. Many physiological (Mille et al., 2015; Assis et al., 2020), and environmental factors (Mahé et al., 2021; Çöl and Yılmaz, 2022) affect otolith morphology. Therefore, it is an expected result that high or low discrimination rates will occur depending on these factors in intraspecific and interspecies discrimination studies using otolith shape. Although elliptic Fourier analysis is the most popular technique (Campana and Casselman, 1993), it only approximates outline variability globally because each harmonic coefficients have no morphological significance on its own and cannot distinguish between local singularities (Tuset et al., 2021). At this stage, it has been established that wavelet analysis is a highly effective technique for highlighting morphological singularities (Lombarte and Tuset, 2015).

The discrimination between the species is higher based on wavelet transform then Fourier analysis and shape indices. Similarly, wavelet analysis (80%), one of the methods used in this study, gave a higher discrimination rate between species compared to the other two methods. Tuset et al. (2021) pointed that wavelets were a more adequate option and excellent method for the classification of species. The values of Wilks' λ range from zero to one, the closer the Wilks' λ is to zero, the better is the discriminating power of the CDA. In this study, scores of Wilks' λ calculated as 0.982 > 0.777 > 0.602 for SIs, EFCs and WT, respectively. The discrimination between the groups is higher based on wavelet transform than based on Fourier analysis and shape indices for sagittal otoliths. In the literature, there is no study using wavelet analyzes for the differentiation of *S. coruhensis* and *S. fahrettini* species. Wavelet analyses in otolith shape were employed by Koeberle et al. (2020) to distinguish *Oncorhynchus tshawytscha* population. Analysis of otolith shape (shape indices, elliptic Fourier and wavelet transform analysis) in the present study showed significant phenotypic heterogeneity between *S. coruhensis* and *S. fahrettini* from Türkiye. And also WT of otolith shape (80%) revealed a much more successful discrimination rate than the other two methods. This study was the first approach to elaborate the otolith shape of *S. coruhensis* and *S. fahrettini* using different morphological analyzing methods.

ACKNOWLEDGEMENTS

The authors are thankful to Recep YILDIZ for sampling *S. coruhensis* individuals.

AUTHORSHIP CONTRIBUTIONS

Melek Özpiçak: Conceptualization, investigation, methodology, software, validation, writing – original draft, writing – review and editing. Semra Saygın: Methodology; resources; software; validation; writing – original draft; review and editing. Savaş Yılmaz: Conceptualization; investigation; methodology; validation; writing – original draft.

CONFLICT OF INTEREST

The authors state that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICS APPROVAL

The care and use of experimental animals, sampling and analysis techniques used in this work are approved by Ondokuz Mayıs University Animal Experiments Local Ethics Committee with decree no "2017/38".

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Akbay, R., Yilmaz, S., Ozpicak, M., Saygin, S., & Polat, N. (2022). Lagenar otolith morphometry of gibel carp, *Carassius gibelio* (Cyprinidae): Comparisons among four populations in Samsun Province (Turkey). *Journal of Ichthyology*, *62*, 770-776. https://doi.org/10.1134/S0032945222050022

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*, 32-46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Assis, I.O., da Silva, V.E., Souto-Vieira, D., Lozano, A.P., Volpedo, A. V., & Fabré, N.N. (2020). Ecomorphological patterns in otoliths of tropical fishes: assessing trophic groups and depth strata preference by shape. *Environmental Biology of Fishes*, *103*(4), 349-361. https://doi.org/10.1007/s10641-020-00961-0

Bardakci, F., Degerli, N., Ozdemir, O., & Basibuyuk, H.H. (2006). Phylogeography of the Turkish brown trout *Salmo trutta* L.: mitochondrial DNA PCR-RFLP variation. *Journal of Fish Biology*, *68*(A), 36-55. https://doi.org/10.1111/j.0022-1112.2006.00948.x

Bardakci, F., Tanyolac, J., Akpinar, M.A., & Erdem, U. (1994). Morphological comparison of trout (*Salmo trutta* L., 1766) populations caught from streams in Sivas. *Turkish Journal of Zoology*, *18*, 1-6.

Bernatchez, L. (2001). The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution*, *55*, 351-379. https://doi.org/10.1111/j.0014-3820.2001.tb01300.x

Berrebi, P., Barucchi, V.C., Splendiani, A., Muracciole, S., Sabatini, A., Palmas, F., Tougard, C., Arculeo, M., & Marić, S. (2019). Brown trout (*Salmo trutta* L.) high genetic diversity around the Tyrrhenian Sea as revealed by nuclear and mitochondrial markers. *Hydrobiologia*, *826*(1), 209-231. https://doi.org/10.1007/s10750-018-3734-5

Bourehail, N., Morat, F., Lecomte-Finiger, R., & Kara, M. H. (2015). Using otolith shape analysis to distinguish barracudas *Sphyraena sphyraena* and *Sphyraena viridensis* from the Algerian coast. *Cybium*, *39*(4), 271-278.

Campana, S.E., & Casselman, J.M. (1993). Stock discrimination using otolith shape analysis, *Canadian Journal of Fisheries and Aquatic Science*, *50*(5), 1062–1083. https://doi.org/10.1139/f93-123

Çöl, O., & Yilmaz, S. (2022). The effect of ontogenetic diet shifts on sagittal otolith shape of European perch, *Perca fluviatilis* (Actinopterygii: Percidae) from Lake Ladik, Turkey. *Turkish Journal of Zoology*, *46*(4), 385-396. https://doi.org/10.55730/1300-0179.3090

Crampton, J.S. (1995). Elliptic Fourier shape analysis of fossil bivalves, practical consideration. *Lethaia*, *28*, 179-186. https://doi.org/10.1111/j.1502-3931.1995.tb01611.x

D'Iglio, C., Natale, S., Albano, M., Savoca, S., Famulari, S., Gervasi, C., Lanteri, G., Panarello, G., Spanò, N., & Capillo, G. (2022). Otolith analyses highlight morpho-functional differences of three species of mullet (Mugilidae) from transitional water. *Sustainability*, *14*, 398. https://doi.org/10.3390/su140103988

Delling, B., Sabatini, A., Muracciole, S., Tougard, C., & Berrebi, P. (2020). Morphologic and genetic characterization of Corsican and Sardinian trout with comments on *Salmo* taxonomy. *Knowledge and Management of Aquatic Ecosystems*, *421*(21). https://doi.org/10.1051/kmae/2020013

Elliott, N.G., Haskard, K., & Koslow, J.A. (1995). Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. *Journal of Fish Biology*, *46*(2), 202–220. https://doi.org/10.1111/j.1095-8649.1995.tb05962.x

Friedland, K. D., & Reddin, D. G. (1994). Use of otolith morphology in stock discriminations of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, *51*(1), 91-98. https://doi.org/10.1139/f94-011

Guinand, B., Oral, M., & Tougard, C. (2021). Brown trout phylogenetics: A persistent mirage towards (too) many species. *Journal of Fish Biology*, *99*(2), 298-307. https://doi.org/10.1111/jfb.14686

Hammer, Ø., Harper, D.A., & Ryan, P.D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*, 1-9.

Hutchings, J.A. (2004). Norm of Reaction and Phenotypic Plasticity in Salmonid Life Histories. In: A. Hendry, S. Stearns (Eds.), *Evolution Illuminated: Salmon and Their Relatives*, Oxford University Press, Oxford, pp 154–174.

Iwata, H., & Ukai, Y. (2002). SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity*, *93*, 384-385. https://doi.org/10.1093/jhered/93.5.384

Jawad, L. A., Shamsan, E. F., Aguilar, G., & Hoedemakers, K. (2022). Scanning electron microscopy and morphological analysis reveal differences in the otolith morphology of three species of the family Lethrinidae (Teleostei: Perciformes) from Yemen. *The Anatomical Record*. https://doi.org/10.1002/ar.25115

Karakousis, Y., Triantaphyllidis, C., & Economidis, P. S. (1991). Morphological variability among seven populations of brown trout, *Salmo trutta* L., in Greece. *Journal of Fish Biology*, *38*(6), 807-817. https://doi.org/10.1111/j.1095-8649.1991.tb03620.x

Kikuchi, E., Cardoso, L.G., Canel, D., Timi, J.T., & Haimovici, M. (2021). Using growth rates and otolith shape to identify the population structure of *Umbrina canosai* (Sciaenidae) from the Southwestern Atlantic. *Marine Biology Research*, *17*(3), 272-285. https://doi.org/10.1080/17451000.2021.1938131

Koeberle, A. L., Arismendi, I., Crittenden, W., Di Prinzio, C., Gomez-Uchida, D., Noakes, D. L., & Richardson, S. (2020). Otolith shape as a classification tool for Chinook salmon (*Oncorhynchus tshawytscha*) discrimination in native and introduced systems. *Canadian Journal of Fisheries and Aquatic Sciences*, *77*(7), 1172-1188. https://doi.org/10.1139/cjfas-2019-0280

Kontaş, S., Yedier, S., & Bostancı, D. (2020). Otolith and scale morphology of endemic fish *Cyprinion macrostomum* in Tigris–Euphrates Basin. *Journal of Ichthyology*, *60*(4), 562-569. https://doi.org/10.1134/S0032945220040086

Kottelat, M. (1997). European freshwater fishes: a heuristic checklist of the freshwater fishes of Europe (exclusive of former USSR), with an introduction for non-systematists and comments on nomenclature and conservation. *Biologia*, *52*, 1-271.

L'Abée-Lund, J. H., & Jensen, A. J. (1993). Otoliths as natural tags in the systematics of salmonids. *Environmental Biology of Fishes*, *36*(4), 389-393. https://doi.org/10.1007/BF00012418

Libungan, L.A., Óskarsson, G.J., Slotte, A., Jacobsen, J.A., & Pálsson, S. (2015). Otolith shape: A population marker for Atlantic herring *Clupea harengus*. *Journal of Fish Biology*, *86*(4), 1377-1395. https://doi.org/10.1111/jfb.12647

Lin, C.H., & Chang, C. W. (2012). *Otolith Atlas of Taiwan Fishes*. National Museum of Marine Biology & Aquarium.

Lleonart, J., Salat, J., & Torres, G.J. (2000). Removing allometric effects of body size in morphological analysis. *Journal of Theoretical Biology*, *205*, 85–93. https://doi.org/10.1006/jtbi.2000.2043

Lombarte, A., Chic, O., Parisi-Baradad, V., Olivella, R., Piera, J., &. García-Ladona, E. (2006). A web-based environment from shape analysis of fish otoliths. The AFORO database. *Scientia Marina*, *70*, 147-152. https://doi.org/10.3989/scimar.2006.70n1147

Lombarte, A., & Tuset, V. (2015). Morfometria de otólitos. *Métodos de estudo com otólitos: princípios e aplicações*. Buenos Aires: CAFP-BA-PIESCI, 269-302.

Mahé, K., MacKenzie, K., Ider, D., Massaro, A., Hamed, O., Jurado-Ruzafa, A., Gonçalves., O., Anastasopoulou, A., Jadaud, A., Mytilineou, C., Randon, M., Elleboode, R., Morell, A., Ramdane, Z., Simith, J., Bekaert, K., Amara, R., de Pantual, H., & Ernande, B. (2021). Directional bilateral asymmetry in fish otolith: A potential tool to evaluate stock boundaries? *Symmetry*, *13*(6), 987. https://doi.org/10.3390/sym13060987

Mallat, S. (1991). Zero crossings of a wavelet transform. *IEEE Transactions on Information Theory, 37*, 1019-1033. https://doi.org/10.1109/18.86995

Mejri, M., Bakkari, W., Tazarki, M., Mili, S., Chalh, A., Shahin, A.A.B., Quignard, J.P., Trabelsi, M., & Ben Faleh, A. R. (2022). Discriminant geographic variation of saccular otolith shape and size in the common pandora, *Pagellus erythrinus* (Sparidae) across the Gulf of Gabes, Tunisia. *Journal of Ichthyology*, *62*(6), 1053-1066. https://doi.org/10.1134/S0032945222060169

Mejri, M., Trojette, M., Allaya, H., Faleh, A. B., Jmil, I., Chalh, A., & Trabelsi, M. (2018). Use of otolith shape to differentiate two lagoon populations of *Pagellus erythrinus* (Actinopterygii: Perciformes: Sparidae) in Tunisian waters. *Acta Ichthyologica et Piscatoria*, *48*(2), 153-161. https://doi.org/10.3750/AIEP/2376

Mille, T., Mahe, K., Villanueva, M.C., De Pontual, H., & Ernande, B. (2015). Sagittal otolith morphogenesis asymmetry in marine fishes. *Journal of Fish Biology*, *87*(3), 646-663. https://doi.org/10.1111./jfb.12746

Morat, F., Betoulle, S., Robert, M., Thailly, A. F., Biagianti-Risbourg, S., & Lecomte-Finiger, R. (2008). What can otolith examination tell us about the level of perturbations of Salmonid fish from the Kerguelen Islands? *Ecology of Freshwater Fish*, *17*(4), 617-627. https://doi.org/10.1111/j.1600-0633.2008.00313.x

Ozpiçak, M., Saygın, S., Aydın, A., Hançer, E., Yılmaz, S., & Polat, N. (2018). Otolith shape analyses of *Squalius cephalus* (Linnaeus, 1758) (Actinopterygii: Cyprinidae) inhabiting four inland water bodies of the middle Black Sea region, Turkey. *Iranian Journal of Ichthyology*, *5*(4), 293-302. https://doi.org/10.22034/iji.v5i4.311

Parisi-Baradad, V., Lombarte, A., Garcia-Ladona, E., Cabestany, J., Piera, J., & Chic, Ò. (2005). Otolith shape contour analysis using affine transformation invariant wavelet transforms and curvature scale space representation. *Marine and Freshwater Research*, *56*, 795–804. https://doi.org/10.1071/MF04162

Parisi-Baradad, V., Manjabacas, A., Lombarte, A., Olivella, R., Chic, Ő., Piera, J., & Garcia-Ladona E. (2010). Automated Taxon Identification of Teleost fishes using an otolith online database- AFORO. *Fisheries Research*, *105*, 13–20. https://doi.org/10.1016/j.fishres.2010.02.005

Pavlov, D.A. (2022). Otolith morphology in gibel carp *Carassius gibelio* and crucian carp *C. carassius* (Cyprinidae). *Journal of Ichthyology*, *62*, 1067–1080. https://doi.org/10.1134/S0032945222060200

Pavlov, D.A., & Osinov, A.G. (2023). Differentiation of Arctic Charr *Salvelinus alpinus* complex (Salmonidae) in lakes Lama and Kapchuk (Taimyr) based on genetic analysis, external morphology, and otolith shape. *Journal of Ichthyology*, 1-19. https://doi.org/10.1134/S0032945223010101

Pavlov, D.S., & Savvaitova, K.A. (2008). On the problem of ratio of anadromy and residence in salmonids (Salmonidae). *Journal of Ichthyology*, *48*(9), 778-791. https://doi.org/10.1134/S0032945208090099

Pavlov, D.S., Savvaitova, K.A., & Kuzishchin, K.V. (1999). On the problem of formation of epigenetic variations of the life strategy in the species of the Red Data Book—Kamchatka Mykiss *Parasalmo mykiss*. Dokl. Ross. Akad. Nauk. Obshch. *Biol. 367* (5), 709–713.

Ponton, D. (2006). Is geometric morphometrics efficient for comparing otolith shape of different fish species? *Journal of Morphology*, *267*(6), 750-757. https://doi.org/10.1002/jmor.10439

Sadeghi, R., Esmaeili, H. R., Zarei, F., & Reichenbacher, B. (2020). Population structure of the ornate goby, *Istigobius ornatus* (Teleostei: Gobiidae), in the Persian Gulf and Oman Sea as determined by otolith shape variation using ShapeR. *Environmental Biology of Fishes*, *103*(10), 1217-1230. https://doi.org/10.1007/s10641-020-01015-1

Sadighzadeh, Z., Valinassab, T., Vosugi, G., Motallebi, A. A., Fatemi, M. R., Lombarte, A., & Tuset, V. M. (2014). Use of otolith shape for stock identification of John's snapper, *Lutjanus johnii* (Pisces: Lutjanidae), from the Persian Gulf and the Oman Sea. *Fisheries Research*, *155*, 59-63. https://doi.org/10.1016/j.fishres.2014.02.024

Savvaitova, K. A. (1989). Arctic chars (structure of population systems and perspectives of commercial use) (Agropromizdat, Moscow, 1989) [in Russian].

Schulz-Mirbach, T., Ladich, F., Plath, M., & Heß, M. (2019). Enigmatic ear stones: What we know about the functional role and evolution of fish otoliths. *Biological Reviews, 94*, 457-482. https://doi.org/10.1111/brv.12463

Song, J., Zhao, B., Liu, J., Cao, L., & Dou, S. (2018). Comparison of otolith shape descriptors and morphometrics for stock discrimination of yellow croaker along the Chinese coast. *Journal of Oceanology and Limnology*, *36*(5), 1870-1879. https://doi.org/10.1007/s00343-018-7228-0

Thorpe, J. E. (1994). Reproductive Strategies in Atlantic Salmon *Salmo salar*. *Aquaculture Research, 25*, 77–87. https://doi.org/10.1111/j.1365-2109.1994.tb00668.x

Turan, D., & Aksu, S. (2021). A new trout species from southern Marmara Sea drainages (Teleostei: Salmonidae). *Journal of Anatolian Environmental and Animal Sciences*, *6*(2), 232-239. https://doi.org/10.35229/jaes.903810

Turan, D., Aksu, İ., Oral, M., Kaya, C., & Bayçelebi, E. (2021). Contribution to the trout of Euphrates River, with description of a new species, and range extension of *Salmo munzuricus* (Salmoniformes, Salmonidae). *Zoosystematics and Evolution*, *97*, 471. https://doi.org/10.3897/zse.97.72181

Turan, D., Dogan, E., Kaya, C. & Kanyılmaz, M. (2014a). *Salmo kottelati*, a new species of trout from Alakır stream, draining to the Mediterranean in Southern Anatolia, Turkey (Teleostei, Salmonidae). *Zookeys, 462*, 135-151. https://doi.org/10.3897/zookeys.462.8177

Turan, D., Kalaycı, G., Bektaş, Y., Kaya, C., Baycelebi, E. (2020). A new species of trout from the northern drainages of Euphrates River, Turkey (Salmoniformes: Salmonidae). *Journal of Fish Biology*, *96*, 1454-1462. https://doi.org/10.1111/jfb.14321

Turan, D., Kottelat, M., & Engin, S. (2009). Two new species of trouts, resident and migratory, sympatric in streams of northern Anatolia (Salmoniformes: Salmonidae). Ichthyological Exploration of Freshwaters, 20(4), 333-364.

Turan, D., Kottelat, M. & Engin, S. (2014b). Two new species of trouts from the Euphrates drainage, Turkey (Teleostei: Salmonidae). *Ichthyological Exploration of Freshwaters*, *24*(3), 275-287.

Tuset, V.M., Lombarte, A., & Assis, C.A. (2008). Otolith atlas for the western Mediterranean, north and central eastern Atlantic. *Scientia Marina*, *72,* 7–198. https://doi.org/10.3989/scimar.2008.72s17

Tuset, V.M., Lozano, I.J., González, J.A., Pertusa, J. F., & García-Díaz, M. M. (2003). Shape indices to identify regional differences in otolith morphology of comber, *Serranus cabrilla* (L., 1758). *Journal of Applied Ichthyology*, *19*(2), 88-93. https://doi.org/10.1046/j.1439-0426.2003.00344.x

Tuset, V.M., Otero-Ferrer, J. L., Siliprandi, C., Manjabacas, A., Marti-Puig, P., & Lombarte, A. (2021). Paradox of otolith shape indices: routine but overestimated use. *Canadian Journal of Fisheries and Aquatic Sciences*, *78*(6), 681-692. https://doi.org/10.1139/cjfas-2020-0369

Wiff, R., Flores, A., Segura, A. M., Barrientos, M. A., & Ojeda, V. (2020). Otolith shape as a stock discrimination tool for ling (*Genypterus blacodes*) in the fjords of Chilean Patagonia. *New Zealand Journal of Marine and Freshwater Research*, *54*(2), 218-232. https://doi.org/10.1080/00288330.2019.1701047

Yedier, S. (2021). Otolith shape analysis and relationships between total length and otolith dimensions of European barracuda, *Sphyraena sphyraena* in the Mediterranean Sea. *Iranian Journal of Fisheries Sciences*, *20*(4), 1080-1096. https://doi.org/10.22092/ijfs.2021.124429

Yıldız, R., & Yılmaz, S. (2021). Morphometric Analysis of Sagittal Otoliths in Coruh Trout (*Salmo coruhensis* Turan, Kottelat & Engin, 2010). *Journal of Anatolian Environmental and Animal Sciences*, *6*(2), 270-277. https://doi.org/10.35229/jaes.913183

Yilmaz, S., Ozpicak, M., Saygin, S. & Polat, N. (2021). Determination of morphometric and genetic structure in *Salmo* Populations inhabiting Samsun province: A new record for Black Sea region. *Journal of Anatolian Environment and Animal Sciences*, *6*(4), 765-773. https://doi.org/10.35229/jaes.1008194