Folia biologica (Kraków), vol. **57** (2009), No 1-2
doi:10.3409/fb57_1-2.219-226

Systematics of Scorpaeniformes Species in the Mediterranean Sea Inferred from Mitochondrial 16S rDNA Sequence and Morphological Data

Cemal TURAN, Islam GUNDUZ, Mevlüt GURLEK, Deniz YAGLIOGLU and Deniz ERGUDEN

Accepted September 15, 2008

TURAN C., GUNDUZ I., GURLEK M., YAGLIOGLU D., ERGUDEN D. 2009. Systematics of Scorpaeniformes species in the Mediterranean Sea inferred from mitochondrial 16S rDNA sequence and morphological data. Folia biol. (Kraków) **57**: 219-226.

Genetic and morphological divergence and phylogenetic relationships of Scorpaeniformes fish including two genera and six species, *Helicolenus dactylopterus*, *Scorpaena maderensis*, *Scorpaena porcus*, *Scorpaena elongata*, *Scorpaena scrofa*, *Scorpaena notata*, living in the Mediterranean Sea, were investigated with morphological and mitochondrial 16S rDNA sequence data. The mean nucleotide diversity was found to be 0.0792. Average sequence divergence between species of Sebastidae and Scorpaenidae was 8.4%, and 6.4%. between species of the genus Scorpaena. For congeneric comparisions, the lowest genetic divergence (0.7%) was observed between *S. porcus* and *S. notata*, and the highest divergence (10.8%) was detected between *S. maderensis* and *S. notata*. High levels of nucleotide divergence were detected between species of two families, and the maximum value was found to be 14.5% between *H. dactylopterus* and *S. elongata*. The two phylogenetic methods (NJ and MP) identified two major lineages. In the NJ tree *S. elongata* was the sister group to *S. scrofa*. *S. maderensis* was more divergent from these groups. Another lineage contained *S. porcus* and *S. notata*. The topology of the MP tree is similar to that of the NJ tree. The pattern and degree of morphological differentiation was not congruent with the genetic differentiation. The Euclidiean distances of morphological data revealed very high morphological divergence between the two families. The highest morphological divergence was observed between *H. dactylopterus* and *S. porcus*, and the lowest was detected between *S. elongata* and *S. notata*. The present genetic and morphological data support the present monophyletic status of the *Scorpaena* genus.

Key words: Mediterranean Sea, Scorpaeniformes, systematics, mtDNA, morphology.

Cemal TURAN, Mevlüt GURLEK, Deniz YAGLIOGLU and Deniz ERGUDEN, Fisheries Genetics Laboratory, Faculty of Fisheries, Mustafa Kemal University, 31200 Iskenderun, Hatay, Turkey.
E-mail: cturan@mku.edu.tr; cturan@ymail.com
Islam GUNDUZ, Department of Biology, Faculty of Arts and Sciences, University of Ondokuz Mayis, Samsun, Turkey.

Scorpaeniformes include almost 1000 teleost species widely distributed in the temperate and tropical seas. This order represents a natural monophyletic group (NELSON 1994; JOHNSON 1993; SHINOHARA 1994) in which the taxonomical position of the higher categories, i.e. families and genera, is still a matter of debate. The Scorpaeniformes fishes are generally represented by two families in the Mediterranean Sea: Scorpaenidae and Sebastidae. The family Scorpaenidae is a commercially important demersal fish group distributed throughout the Mediterranean Sea, including four genera and nine species (NELSON 1994). The five species of the genus *Scorpaena* are phenotypically very similar (HUREAU & LITVINENKO 1986). The Madeira rockfish, *Scorpaena maderensis* Valenciennes (1833), is distributed along the coasts of northwestern Africa and around some islands of the eastern Atlantic (HUREAU & LITVINENKO 1986; MORATO *et al*. 2001). In the Mediterranean, its occurrence has been reported for several localities, such as southeastern Spain, Sicily, Greece (Ionian and south Aegean seas), Croatia, Lebanon and Cyprus (HUREAU & LITVINENKO 1986; FIORENTINO *et al*. 2004; FOLLESA *et al*. 2004). The lack of knowledge o the actual distribution and population density of *S. maderensis* in the Mediterranean might be partially explained by misidentification with other scorpaenids. For instance, FISCHER *et al*. (1987) stated that *S. maderensis* has been frequently confused with the black scorpionfish, *Scorpaena porcus* (Linnaeus, 1758), which is a non-migratory species and occurs in the Mediterranean Sea and the Black Sea, British Isles to the Azores, and the Canary Islands. The slender rockfish, *Scorpaena elongata,* is a

sedentary species which occurs in the Mediterranean Sea and eastern Atlantic: Morocco to northern Namibia. The largescaled scorpionfish, *Scorpaena scrofa*, is a demersal non-migratory fish, and occurs in the Mediterranean Sea (but not in the Black Sea), eastern Atlantic: British Isles (rare) to Senegal including Madeira, the Canary Islands, and Cape Verde. The small red scorpionfish, *Scorpaena notata*, is a demersal species and inhabits the Mediterranean (rare in the northern Adriatic) and the Black Sea and eastern Atlantic: Bay of Biscay to Senegal, Madeira, Azores and the Canary Islands (HUREAU & LITVINENKO 1986). The family Sebastidae is represented by one genera and one species, *Helicolenus dactylopterus* (Delaroche 1809), in the Mediterranean Sea. The bluemouth, *Helicolenus dactylopterus*, is a deep-sea scorpionfish widely distributed in the eastern Atlantic, from the Norwegian coasts to the southwestern coast of Africa (HUREAU & LITVINENKO 1986). In the Mediterranean it is found along the continental shelf edge and slope (BAUCHOT 1987).

Several studies have brought detailed information on the biology, distribution and the diagnostic characteristics of Scorpaeniformes species (FISCHER *et al*. 1987; HUREAU & LITVINENKO 1986; MORATO *et al*. 2001). Despite limited morphological and karyological based taxonomy (CAPUTO *et al*. 1998; CAPUTO & SORICE 1999; MESA 2005), the phylogenetic relationships and amount of genetic divergence among these species is still in need of investigation. A central challenge with respect to marine species is to understand patterns of genetic differentiation. Fishes inhabiting the marine pelagic environment generally have broad distribution ranges. The absence of physical barriers and high potential for dispersal provide little opportunity for allopatric speciation. There are a small number of known examples of cryptic diversity among species in the pelagic environment (KNOWLTON 1993; TARJUELO *et al*. 2001), but the use of molecular genetic markers has provided powerful tools to resolve ambiguous taxonomic classification in marine species (MIYA & NISHIDA 1997; KNOWLTON 2000; WIRTH & BERNATCHEZ 2001; COLBORN *et al*. 2001; BORSA *et al*. 2004).

Rapidly evolving mitochondrial DNA regions are very useful tools for molecular systematic studies (MEYER *et al*. 1990; NORMARK *et al*. 1991; MEYER 1992). The pattern of maternal inheritance and rapid rate of evolutionary change of mtDNA compared to nuclear DNA makes it a suitable tool to accomplish genetic studies among fishes at multiple taxonomic levels (KOCHER & STEPIEN 1997; TURAN *et al*. 1998; DURAND *et al*. 2002).

In the present study the systematic relationships of five Mediterranean species of the genus Scorpaena (Family Scorpaenidae; *Scorpaena maderensis*, *Scorpaena porcus*, *Scorpaena elongata*, *Scorpaena scrofa*, *Scorpaena notata)* were investigated with mitochondrial 16S rDNA sequence and morphological data. *Helicolemus dactylopterus*, a species belonging to the closely related family Sebastidae, was used as an outgroup.

Material and Methods

Samples

Sampling of Scorpaenidae species was accomplished through the years of 2005-2006 from Iskenderun Bay in the northeastern Mediterranean Sea, using trap, trawl, or during scuba diving. The number of samples used in the molecular and morphological analyses are given in Table 1. The samples were placed individually in plastic bags, and kept frozen at -40°C until transportation to the laboratory. In the laboratory fin clips and muscle tissue were collected and preserved in 95% ethanol for DNA extraction.

Sequences

Total genomic DNA was extracted from a piece of fin tissue (approximately 2 mm^2) using AGOWA mag Midi DNA isolation Kits (AGOWA, Berlin, Germany). The amplification of the mitochondrial 16S rDNA gene was performed using PCR with a profile of 94^{o}C for 4 min, followed by 35 cycles of 94^{o}C/30 s strand denaturation, 52 ^{o}C/20 s annealing and 72 ^{o}C/1 min 30 sec primer extension, and a final 7 min elongation at 72^{o}C. The 16S rDNA amplification conditions were: 1.5 μl 10 x polymerase buffer, 0.5 μl dNTP (10 mM), 0.3 μl Teg DNA polymerase (3 U/ μl) equivalent to Taq DNA polymerase, 0.05 μl 16Fi140 primer (100 μM) (5′-CG(CT)AAGGGAA(ACT)GCTGAAA-3′), 0.05 μl 16Fi1524 primer (100 μM) (5′-CCGGTCTGAACTCAGATCACGTAG-3′), 3-5 μl DNA from AGOWA purification, and water for a total reaction volume of 15 μl. Amplified DNA was purified with Exo/Sap enzymes (Cleveland, Ohio, USA) following the manufacturer's instructions. Finally, all the samples were sequenced in both directions using 16Fiseq1463 (5′-TGCACCATTAGGATGTCCRGATCCAAC-3′) and 16sarL (5′-CGCCTGTTTAACAAAAACAT-3′) primers. The sequencing products were loaded onto an ABI3730 (Applied Biosystems) automated sequencer.

Sequences were aligned and ambiguous bases resolved by eye using Sequencer v.4.5 (Gene Codes Corp.). Nucleotide composition was ana-

Table 1

Sampling corrdinates and GenBank accession numbers for the 16S rDNA segment equenced in this study. N-H – number of haplotypes observed, n-S – number of specimens used in the sequencing; n-M – number of specimens used in morphological analyses

Species	Sampling location	n-H	n-S	n-M	GenBank
Helicolenus dactylopterus	36° 05'N 36° 65'E	1	3	30	EU747064 EU747065 EU747066
Scorpaena elongata	36° 05'N 36° 65'E	2	2	30	EU747067 EU747068
Scorpaena scrofa	36° 05'N 36° 65'E	1	3	30	EU747069 EU747070 EU747071
Scorpaena notata	36° 05'N 36° 65'E	1	2	30	EU747072 EU747073
Scorpaena porcus	36° 05'N 36° 65'E	1	3	30	EU747074 EU747075 EU747076
Scorpaena maderensis	26° 85'N 38° 35'E	1	3	30	EU747077 EU747078 EU747079

lyzed using MacClade v.4.08 (MADDISON & MADDISON 2000).

Phylogenetic reconstruction was performed using PAUP* v. 4.0 (SWOFFORD 2002). Neighbor-Joining (NJ) (SAITOU & NEI 1987) and Maximum Parsimony (MP) methods were employed to evaluate phylogenetic relationships among haplotypes. The hierarchical likelihood ratio test (hLRT) and the Akaike information criterion (AIC), implemented in the computer program MODELTEST v. 3.7 (POSADA & CRANDALL 1998), were used to establish the most appropriate model of DNA substitution for our data. For the data including the outgroup, the HKY model (HASEGAWA *et al.* 1985) with gamma correction of 0.1258, and the TVM model with gamma correction of 0.1583 were selected by hLRT and AIC, respectively.

For data without the outgroup, the HKY model with gamma correction of 0.0147, and the K81uf model (KIMURA 1981) with the frequency of invariable sites set at 0.7746 were selected by hLRT and AIC, respectively. Bootstrap resampling (10 000 replicates) was applied to assess the relative stability of NJ trees produced with different substitution models.

The MP analyses were carried out using the branch-and-bound option. The MP run generated one MP tree (119 steps, CI: 0.815). To assess support for the tree, bootstrap analysis was performed with 10 000 replications with the branch-and-bound search option. *H. dactylopterus* was used as an outgroup for all the phylogenetic analyses.

Nucleotide diversity and total DNA divergence (Dxy) were estimated using MEGA v. 4. (TAMURA *et al*. 2007).

Obtained sequences have been deposited in GenBank with accession numbers given in Table 1.

Morphology

Meristic characters commonly used to distinguish Scorpaeniformes species were used for morphological analysis (TORTONESE 1986; FISCHER *et al*. 1987; AKSIRAY 1987). Numbers of unbranched and branched rays in first dorsal fin (DFR), ventral fin (VFR), anal fin (AFR), pectoral fin (PFR), caudal fin (CFR), gill rakers (GR) and scales in lateral line (LS) under a binocular microscope were recorded. Vertebrate numbers (VN) were counted after taking X-ray films of fish.

No significant correlations between standard length and meristic counts were observed, and, therefore, it was not necessary to remove the size effect from the data. Principal component analysis (PCA) was used to extract shape variation among the sampled individuals. Hierarchical cluster analysis using the Euclidean distances with the average linkage method was performed, and the Neighbor-Joining method was used to estimate phenotypic relationships between species. The reliability of the inferred phylogenies was evaluated using the bootstrap method (FELSENSTEIN 1985) with 1000 replicates. Morphological analyses

Table 2

Total genetic (below the diagonal) and morphologic (above the diagonal) distance between the species

Species	*H. dactylopterus*	*S. elongata*	*S. scrofa*	*S. notata*	*S. porcus*	*S. maderensis*
H. dactylopterus	–	11.426	11.196	11.460	12.295	12.156
S. elongata	0.145	–	10.649	10.325	10.580	10.736
S. scrofa	0.141	0.016	–	10.876	11.027	11.809
S. notata	0.134	0.083	0.076	–	11.205	11.277
S. porcus	0.133	0.079	0.073	0.007	–	10.916
S. maderensis	0.141	0.079	0.080	0.108	0.104	–

were performed using SPSS*v*13, SYSTAT *v*11 and PHYLIP (FELSENSTEIN 1993) program packages.

Results

Molecular data

There were 6 haplotypes in the five species of the genus *Scorpaena*, whereas the three *H. dactylopterus* specimens revealed a single haplotype (Table 1). These haplotypes displayed 164 variable nucleotides of which 79 were parsimony informative over 873 bp. Examination of the gene fragment revealed a bias against cytosine (C; 22.1%) and abundance of thymine (T; 30.9%). The mean nucleotide diversity (*Pi*) was found to be 7.92%. Total genetic distances between the species are given in Table 2. Average divergence between comparisons of all species was 8.4 %, and between pairwise comparisons of congeneric species 6.4%. For congeneric comparisons, the lowest genetic divergence (0.7%) was observed between *S. porcus* and *S. notata*, and the highest value (10.8%) was detected between *S. maderensis* and *S. notata.* High levels of genetic divergences were detected between the two genera, and the greatest divergence was found to be 14.5 % between *H. dactylopterus* and *S. elongata*.

NJ analyses using the substitution models determined by MODELTEST yielded the same tree topology. However, NJ analysis applying the HKY model with gamma correction of 0.1258 generated the highest bootstrap values. Thus, we used the NJ tree constructed under the assumption of the HKY model to evaluate the phylogenetic relationships among haplotypes. The MP analyses of the 79 parsimony informative sites generated a single tree that had a length of 119 steps and a consistency index of 0.815.

The two phylogenetic methods (NJ and MP) yielded similar topologies (Figs 1 & 2). Both methods identified two main lineages: one including *S. notata* and *S.porcus* (100% bootstrap support for both methods) and another comprising the remaining Scorpaena species (66% and 58% boostrap support for NJ and MP respectively). Within the clade including the remaining species, the two haplotypes of *S. elongata* clustered together (100% bootstrap support for both methods) and were the sister group to *S. scrofa* (100% bootstrap support for both methods). *S. maderensis* was the sister lineage of this clade.

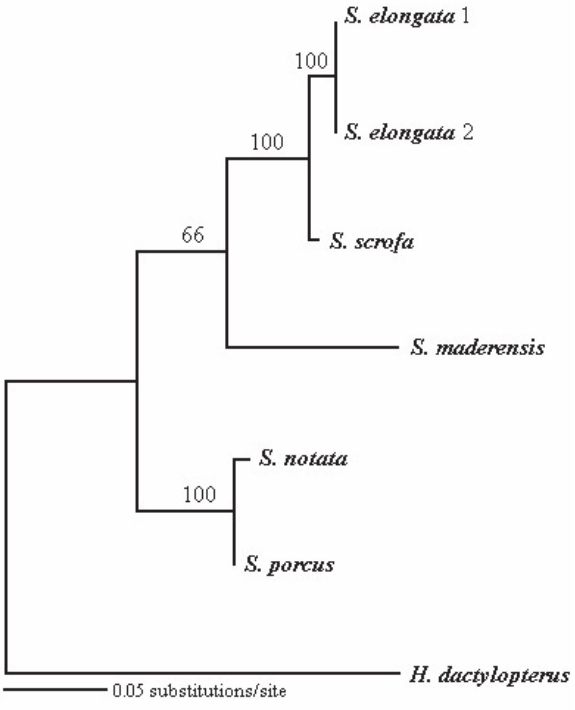

Fig. 1. Neighbour joining phylogenetic tree for 16S rDNA. Bootstrap values over 50% are shown on the tree. *H. dactylopterus* was used as an outgroup.

Table 3

Observed meristic characters of the analyzed samples for each species. Roman letters show spine rays. Numbers of: dorsal fin rays (DFR); ventral fin rays (VFR); anal fin rays (AFR); pectoral fin rays (PFR); gill rakers (GR); vertebra (VN); caudal fin rays (CFR); scales in lateral line (LS)

Species	DFR	VFR	AFR	PFR	GR	VN	CFR	LS
H.dactylopterus	XII 12-13	I 5	III 5	18-19	19-22	25	20-21	56-57
S. elongata	XII 10	I 5	III 5	18-19	13-15	24	18-20	44-46
S. scrofa	XII 9	I 5	III 5	18-19	15-17	24	17-20	43-47
S. notata	XII 9	I 5	III 5	17-18	15-17	23	18-19	38-40
S. porcus	XII 9	I 5	III 5	17-18	15-17	24	19-21	53-55
S. maderensis	XII 9	I 5	III 5	15	15-18	24	16-18	52-57

Morphology

The range of observed meristic characters of the species were in agreement with the description of TORTONESE (1986) and FISCHER *et al*. (1987) (Table 3). Univariate statistics (ANOVA) revealed highly significant ($P<0.001$) differences between species for all meristic characters. AFR and VFR were not considered in the analysis because these variables were constant among species. In the principal components analysis, first and second principal components represented 57% and 25% of the total variation, respectively, which were used in Hierarchical cluster analysis. Euclidean distances of the morphological data revealed very high morphological divergence between the two families (Table 2). The highest morphological divergence was observed between *H. dactylopterus* and *S. porcus*, and the lowest was detected between *S. elongata* and *S. notata*. In the Neighbor-Joining analysis based on the Euclidean distances (Fig. 3), two main clusters were recovered: one including *S. porcus* and *S. maderensis* (100% bootstrap support) and another including the three remaining species (100% bootstrap support). *S. elongata* and *S. notata* were closely related in one node (100% bootsrap support) while *S. scrofa* was in the neighboring node.

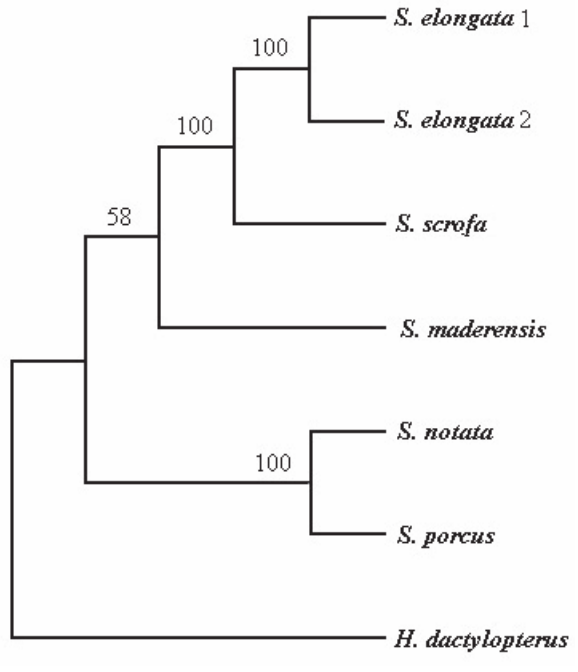

Fig. 2. A parsimony phylogenetic tree for 16S rDNA. It is a single shortest tree obtained by the branch-and-bound method. The tree shows all bootstrap values over 50%. *H. dactylopterus* was used as an outgroup.

Examination of the contribution of each variable to the principal components showed high contributions from pectoral fin rays, lateral scale numbers, vertebrate numbers and caudal fin rays, indicating that these characters are important to species differentiation (Fig. 4).

Discussion

The degree of molecular divergence between the species of the Scorpaenidae supports their present taxonomy and also provides substantial evidence to maintain their separate species status. In the congeneric comparisons, low sequence divergence was detected only between *S. porcus* and *S. notata*. However, the percentage of sequence divergence based on 16S rDNA (0.7%) fell within the values reported for some other marine species (DOUKAKIS *et al*. 1999; TINTI & PICCINETTI 2000; FARIA *et al*. 2006). For example, TINTI & PICCINETTI (2000) investigated the molecular systematics of the *Solea* species, and found that sequence divergences of 16S rDNA between species ranged from 0.7% to 11%. ORRELL & CARPENTER (2004) studied the phylogeny of the fish family

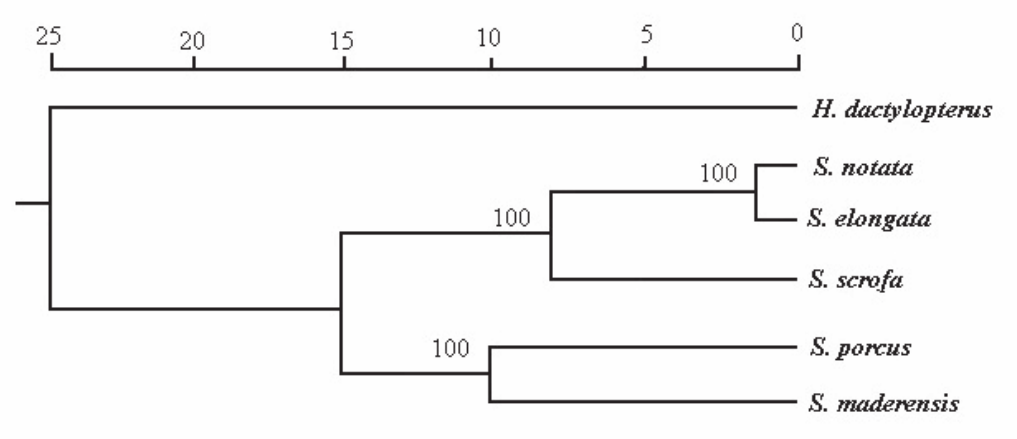

Fig. 3. Neighbor-joining tree based on Euclidean distances of morphologic data. Numbers on nodes indicate the bootstrap values.

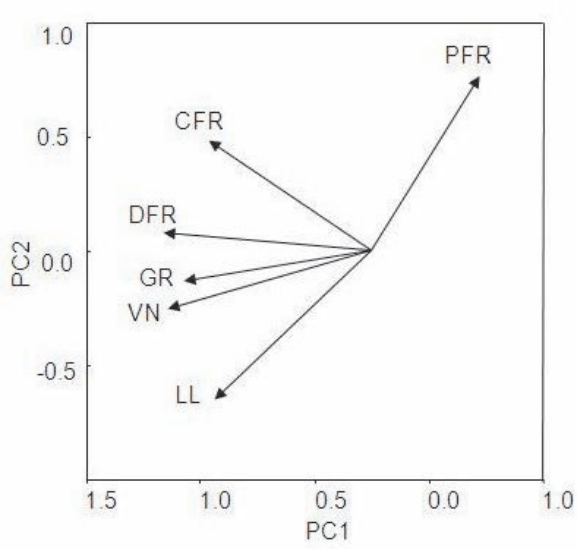

Fig. 4. Contribution of meristic variables to the principal components. Vectors indicate the loadings of the scores for each variable on principal components for species differentiation (see Table 3 for variables labels).

Sparidae using 16S ribosomal RNA and cytochrome *b* genes and found that average divergence between comparisons of ingroup taxa was 6.21%.

Taxonomic description of a species has commonly relied on description of unique sets of morphological characters. The morphological identification of the five Scorpaenidae species relies, mainly, on the scales on the lateral line (NELSON 1994). However, similarities of specific traits and overlapping of their respective habitats make species identification difficult, especially when dealing with young juveniles, in which morphological features cannot be easily distinguished. MESA (2005) examined diagnostic elements to facilitate the identification of *S. maderensis* during field and laboratory studies, and to eliminate the risk of misidentification with *S. porcus*, and reported that the most useful diagnostic characters of *S. maderensis* were the presence of cycloid scales on the chest and the pectoral fin rays. In the present taxonomic classification, meristic characters such as dorsal and pectoral fin rays, gill rakers, vertebrate numbers and caudal fin rays were the most discriminative characters. These morphological characters are informative for distinguishing among species of Scorpaenidae and Sebastidae.

The pattern and degree of morphological differentiation was not congruent with genetic differentiation. *S. notata* showed morphological similarity to *S. elongata*, but the degree of genetic differentiation between these species was high. Moreover *S. maderensis* showed morphological similarity to *S. porcus* which is congruent with other studies (FISCHER *et al*. 1987; MESA 2005), but genetic distances between the two species were also high. NUMACHI (1972) demonstrated the replacement of alleles in allozyme analyses of two local populations of *Sebastes inermis*, separated by only 30 km distance, but did not list the morphological characters of these two genetically isolated populations. Diagnostic discrimination of the species of the Scorpaenidae family is difficult because of their morphological similarity and variable extent of overlap in criteria used to define them. Indeed, some species of Scorpaenidae exhibit little or no phenotypic differences. Therefore, morphological classification based on phenotypic characters of Scorpaenidae may lead to misclassification. For this reason, studies using other molecular ORMARKers (in particular nuclear genes) are needed, especially because of the contradictory results obtained with genetic and morphological markers.

In conclusion, pairwise sequence divergences between species of the genus *Scorpaena* were similar to those reported for 16S in other teleosts, and the amount of molecular and morphological divergence between the species of Scorpaenidae and Sebastidae support their present taxonomic

description at the family level. The pattern and degree of morphological differentiation between species of the genus *Scorpaena* was not congruent with genetic differentiation. Additional molecular genetic analyses based on different parts of the mitochondrial and nuclear genomes could improve the findings presented here.

Acknowledgements

Thanks to the Turkish Academy of Sciences in the framework of the young scientist award program (TUBA-GEBIP-2005) and Mustafa Kemal University for financial support.

References

AKSIRAY F. 1987. Türkiye deniz balıkları ve tayin anahtarı. İ.Ü Rektörlüğü Yayınları, No: 3490. Istanbul, 426-430.

BAUCHOT M. L. 1987. Serranidae. (In: Fiches FAO d'Identification des Espèces pour les Besoins de la Pêche. (Révision 1), Méditerranée et Mer Noire. Zones de Pèche 37, II (Vertébrés). Fischer, W. Bauchot, M. L. Schneider M. eds. FAO-CEE, Rome): 1301-1319.

BORSA P., COLLET A., DURAND J. D. 2004. Nuclear-DNA markers confirm the presence of two anchovy species in the Mediterranean. Compt. Rend. Biol. **327**: 1113-1123.

CAPUTO V., SORICE M. 1999. Electrophoretic studies on some species of Scorpaeniformes (Teleostei, Percomorpha). Italian J. Zool. **66:** 79-85.

CAPUTO V., SORICE M., VITTURI R., MAGISTRELLI R., OLMO E. 1998. Cytogenetic studies in some species of Scorpaeniformes (Teleostei: Percomorpha). Chrom. Res. **6**: 255-262.

COLBORN J., CRABTREE R. E., SHAKLEE J. B., PFEILER E., BOWEN B. W. 2001. The evolutionary enigma of bonefishes (*Albula* spp.): cryptic species and ancient separations in a globally distributed shorefish. Evolution **55**: 807-820.

DOUKAKIS P., BIRSTEIN V. J., RUBAN G. I., DESALLE, R. 1999. Molecular genetic analysis among subspecies of two Eurasian sturgeon species, *Acipenser baerii* and *A. stellatus*. Mol. Ecol. **8**: 117-128.

DURAND P., POINTIER J. P., ESCOUBEYROU K., ARENAS J., YONG M., AMARISTA M., BARGUES M. D., MAS-COMA S., RENAUD F. 2002. Occurrence of a sibling species complex within neotropical lymnaeids, snail intermediate hosts of fascioliasis. Acta Trop. **83**: 233-240.

FARIA R., WEISS S., ALEXANDRINO P. 2006. A molecular phylogenetic perspective on the evolutionary history of *Alosa* spp. (Clupeidae). Mol. Phyl. Evol. **40**: 298-304.

FELSENSTEIN J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39**: 783-791.

FELSENSTEIN J. 1993. PHYLIP 3.5 (Phylogeny Inference Package). Seattle, Washington, USA, University of Washington.

FIORENTINO F., VASSALLO C., MASSARI F. 2004. Preliminary list of the fish assemblage of the coast between Isola dele Femmine and Punta Barcarello (North-western Sicily) according to underwater photos. Biol. Mar. Medit. **11**: 70-72.

FISCHER W., BAUCHOT M. L. SCHNEIDER M. 1987. Fiches FAO d'identification des espèces pour les besoins de la peche Mediterranée et Mer Noire. Zone de Peche **37**: 761-1530.

FOLLESA M. C., CABÝDDU S., CUCCU D. CAU A. 2004. First observations on *Scorpaena maderensis* (Valenciennes, 1833) (Osteichthyes, Scorpaeniformes) in the Sardinian Seas. Biol. Mar. Medit. **11**: 504-507.

HASEGAWA M., KISHINO H., YANO T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. **22**: 160-174.

HUREAU J. C., LITVINENKO N. I. 1986. Scorpaenidae. (In: Fishes of the North-eastern Atlantic and the Mediterranean, vol 3. P.J.P. Whitehead, M. L., Bauchot, J. C., Hureau, J., Nielsen & Tortonese, E. eds. UNESCO, Paris): 1211-1229.

JOHNSON G. D. 1993. Percomorph phylogeny: progress and problems. Bull. Mar. Sci. **52**: 3-28.

KIMURA M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. Proc. Nat. Acad. Sci. USA **78**: 454-458.

KNOWLTON N. 1993. Sibling species in the sea. Ann. Rev. Ecol. Syst. **24**: 189-216.

KNOWLTON N. 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia **420**: 73-90.

KOCHER T. D., STEPIEN C. A. 1997. Molecular Systematics of Fishes. Academic Press, San Diego.

MADDISON D. R., MADDISON W. P. 2000. MacClade 4: Analysis of phylogeny and character evolution, Version 4.0. Sinauer, Sunderland, MA.

MEYER A., KOCHER T. D., BASASIBWAKI P. 1990. Monophyletic origin of Victoria cichlid fish suggested by mitochondrial DNA sequences. Nature **347**: 550-553.

MEYER A. 1992. Cichlid fishes: Behaviour, ecology and evolution M.H.A. Keenleyside ed. Quart. Rev. Biol. **67**: 221-222.

MESA G. 2005. A revised description of *Scorpaena maderensis* (Scorpaenidae) by means of meristic and morphometric analysis. Mar. Biol. Assoc. UK. **85**: 1263-1270.

MIYA M., NISHIDA M. 1997. Speciation in the open ocean. Nature **389**: 803-804.

MORATO T., AFONSO P., LOURINHO P., BARREIROS J. P., SANTOS R. S., NASH R. D. M. 2001. Length-weight relationships for 21 coastal fish species of the Azores, north-eastern Atlantic. Fish. Res. **50**: 297-302.

NEI M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

NEI M., LI W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nat. Acad. Sci. USA **76**: 5269-5273.

NELSON J. S. 1994. Fishes of the World. Third ed. John Wiley & Sons, Inc., New York. Pp. 600.

NORMARK, B. B., MCCUNE A. R., HARRISON R. G. 1991. Phylogenetic relationships of Neopterygian fishes, inferred from mitochondrial DNA sequences. Mol. Biol. Evol. **8**: 819-534.

NUMACHI K. 1972. Genetic polymorphism of tetrazolium oxidase on black rockfish. Bull. Japan Soc. Sci. Fish. **38**: 789.

ORRELL T. M., CARPENTER K. E. 2004. A phylogeny of the fish family Sparidae (porgies) inferredfrom mitochondrial sequence data. Mol. Phyl. Evol. **32**: 425-434.

POSADA D., CRANDALL K. A. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics **14**: 817-818.

SAITOU N., NEI M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406-425.

SHINOHARA G. 1994. Comparative morphology and phylogeny of the suborder Hexagrammoidei and related taxa. Mem. Fac. Fish., Hokkaido University **41**: 1-97.

SWOFFORD D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4. Sinauer, Sunderland, MA.

TARJUELO I., POSADA D., CRANDALL K. A., PASCUAL M., TURON X. 2001. Cryptic species of *Clavelina* (Ascidiacea)

in two different habitats: harbours and rocky littoral zones in the northwestern Mediterranean. Mar. Biol. **139**: 455-462.

TINTI F., PICCINETTI C. 2000. Molecular systematics of the Atlanto-Mediterranean *Solea* species. J. Fish Biol. **56**: 604-614.

TORTONESE E. 1986. Carangidae. (In: Fishes of the North-Eastern Atlantic and the Mediterranean, (P. M. J.Whitehead, M. L. Bauchot, J. C. Hureau, J. Nielsen, E. Tortonese eds. UNESCO Paris): 908-911.

TAMURA K., DUDLEY J., NEI M., KUMAR S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**: 1596-1599.

TURAN C., CARVALHO G. R., MORK J. 1998. Molecular genetic analysis of Atlanto-Scandian herring (*Clupea harengus)* populations using allozymes and mitochondrial DNA markers. J. Mar. Biol. Assos. UK **78**: 269-283.

WIRTH T., BERNATCHEZ L. 2001. Genetic evidence against panmixia in the European eel. Nature **409**: 1037-1040.