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Research Article

Identification complexity of critically endangered *Squatina squatina* (Linnaeus, 1758) and *Squatina aculeata* Cuvier, 1829 in the Mediterranean Sea (Turkey)

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Abstract

Sharks and rays, besides their economic importance, are ecologically important organisms with a diverse group. After a pregnant *Squatina* sp. was captured as by-catch, Akyol et al. (2015) published this species as *Squatina squatina* while the COI and 16S rDNA analysis demonstrated that the species is *S. aculeata*. This complexity revealed that utilizing morphologic identification solely might not be enough to distinguish these two *Squatina* species accurately. We aim to compare morphologic and molecular techniques during species identification of critically endangered *S. squatina* and *S. aculeata*. Two different gene regions were used for molecular identification of 3 *Squatina* specimens obtained from the Aegean coasts of Turkey. Sequence analysis of two gene regions was conducted after PCR analysis. An aligned data set was used for creating phylogenetic trees. The results demonstrated that the previously identified *S. squatina* specimen was revealed as *S. aculeata* after molecular analysis. Two other specimens which were morphologically identified as *S. squatina* demonstrated the same results both with molecular and morphological analysis. Our results suggest that adopting morphological identification as the only tool is not enough to accurately determine the *Squatina* species; both morphological and molecular tools should be used for taxonomical identification of shark species, especially the endangered ones, to assure their conservation status.

Keywords: Angel Shark, COI, 16S rDNA, Eastern Mediterranean Sea

Introduction

Accurate identification of species is a fundamental step in biodiversity and monitoring studies (Dayrat, 2005). Fish species are mainly identified by their morphological characteristics which might differ according to organisms' age, life cycle, gender (Strauss & Bond, 1990; Moftah et al., 2011), and morphological similarities. Therefore, DNA-based methods are suggested to be used along with a morphological approach while identifying an organism (Ward et al., 2009). Squatinidae Bonaparte, 1838 is a family with a single genus *Squatina* (Dumeril 1806) known as angel sharks. According to the World Register of Marine Species (WoRMs), there are more than 25 species belonging to this genus. All species are benthic and their distribution starts from inshore to 1300 meters in-depth, with several species inhabiting warm temperate waters (Ebert and Stehmann 2013). Angel sharks are represented with 3 species (*Squatina squatina* (Linnaeus, 1758), *S. aculeata* Cuvier, 1829, *S. oculata* Bonaparte, 1840) in the Mediterranean Sea, simply distinguished by leveling the first dorsal fin to the pelvic fin end (Serena, 2005). In the case of *S. squatina*, the origin of 1st dorsal fin is in line with the pelvic fin rear extremity, whereas; in the case of *S. aculeate*, the origin of 1st dorsal fin is before the pelvic fin rear extremity (Serena, 2005). Although such features are good identifiers, it is still difficult to distinguish different species. Therefore; misidentifications can be observed (Ferretti et al., 2015; Soldo & Bariche, 2015). According to the IUCN Red List of Threatened Species, all angle shark species are listed as Critically Endangered (CR). Feretti et al. (2015) stated that in the first half of the century, angel sharks were reported as frequent but in the second half, their population declined. For instance, *S. squatina* populations have almost disappeared since 1985, and only one specimen has been sampled (Cavallaro et al., 2015) in the area through the Sicily Strait, close to Malta. As for *S. aculeata*, it was recorded in the Mediterranean in restricted areas such as Turkey (Kabasakal 2002; 2003), Greece (Ekonomidis 1973), and Israel (Golani 1996) but has not been recorded in Israel since then (Capape et al., 2005). Intensive trawling and demersal fisheries have resulted in a decline in the angel shark population and even caused extinction in some areas (Cavallaro et al., 2015).

The sawback angel shark, *S. aculeata* is found in the northeast Atlantic from Morocco to Angola and the Mediterranean Sea but not in the Black Sea (Serena, 2005). The common angel shark *S. squatina* is found in the Mediterranean Sea, the Black Sea, and the Atlantic from the North Sea to Mauritania. Its maximum total length is 250 cm, while the sawback angel shark can reach up to 180 cm. These two species are distinguished from each other by some morphological features such as the shape of the nasal barbels, the shape of the dermal denticles, position of the dorsal fin to the pelvic fin, color, etc. (Serena, 2005). Carvalho et al. (2010), also suggested considering neurocranial features besides other characteristics for identification.

Nowadays many systematic studies have focused on molecular-based methodologies besides species' morphological identifications. The use of a DNA-based approach for the correct identification of species that are morphologically difficult to distinguish from each other is not a new technique but is now becoming a popular technique for cartilaginous fish, too. Both morphometric and DNA-based identification of these species, which are predominantly in the high-risk group, has great importance for the sustainability of the species population. The information produced on such species has been reliably increased by comparing all the sequences registered and morphometrically determined and consequently supporting each other in both determinations. Especially mitochondrial DNA (mtDNA) is considered a suitable tool for phylogenetic studies among several fish groups. Therewith, 16S rDNA gene markers are widely adopted to differentiate fish species by molecular tools (Turan, 2007). In this study, we examined if the morphological features are enough to distinguish two *Squatina* species; *S. aculeata* and *S. squatina* by comparing 16S rDNA and cytochrome c oxidase subunit I (COI) genes of 3 samples from the Aegean Sea. The aim of this work is to evaluate the accuracy of morphological identification of the species regarding our DNA-based results and deposited sequences in GenBank.

Materials and methods

Sample collection and Morphological Identification

Sample #1 sample was obtained from Gökova Bay after it was captured bycatch by a trammel net. Other samples (Sample #2 and #3) (Fig. 1-2) were obtained from a fisherman who caught the specimens as by-catch from the central Aegean Sea. After the samples were brought to the laboratory, their morphological measurements were done and species identification was completed in accordance with the characteristics suggested by Serana (2005) and Compagno et al. (2005). Length and weight were measured; length measurements were made with a measuring board with a sensitivity of 1 mm, and weight measurements were made by an electronic scale with a sensitivity of 0.01 g. After the morphological measurements, samples were dissected and tissue samples were collected.

Figure 1. Specimen of *Squatina squatina* (# 2) by-cached from the central Aegean Sea.

Figure 2. Specimen of *Squatina squatina* (# 3) by-cached from the central Aegean Sea.

DNA Isolation and PCR analysis

Muscle tissue samples (Sample #1, Sample #2, and #3) were stored at -20˚C until the DNA extraction. DNA isolation was performed with a Tissue and Cell DNA Purification kit (GeneMark, Taiwan) according to the manufacturer's instructions. DNA quality and quantity were measured

with a NanoDrop spectrophotometer (Nanodrop, Thermo Fisher Scientific, USA). All the DNAs were found suitable for PCR (Polymerase Chain Reaction) analysis after quality and quantity measurements.

16S rDNA and cytochrome c oxidase subunit I (COI) genes were amplified by PCR analysis. Two primer pairs were used for amplification of the regions. 16S rDNA gene region (~600 bp) was amplified by using primers L2510: 5'-CGC CTG TTT ATC AAA AAC AT-3' and H3080: 5'-CCG GTC TGA ACT CAG ATC ACG T-3' (Palumbi 1991). The COI gene fragment (~700 bp) was amplified with primers LCO1490: 5'-GGT CAA CCAA ATC ATA AAG ATA TTG G-3' and HCO2198: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer, 1994).

Both PCR reactions were performed in 25 μL total volume of reaction buffer containing 2.5 mM dNTPs (Thermo Scientific), 20 μM of each primer, 0.4 μL Dream Taq DNA polymerase, (Thermo Scientific), 10 × Buffer, and 5 ng template DNA. PCR analysis was performed by Biorad Thermal Cycler (USA). PCR conditions for both primer pairs were started with an initial denaturation step at 95°C for 2 minutes, which was followed by 30 cycles of DNA denaturation at 95°C for 30 seconds, primer annealing for 1 min. at 48°C, DNA strand extension at 72°C for 1 min., and a final extension step at 72°C for 10 min. The PCR products were separated on 1.5% agarose gel electrophoresis, stained with SafeView Stain (ABM), and visualized by an image analyzer.

Sequence and phylogenetic analysis

Sequence analysis of PCR amplicons was performed at Ankara University Evolutionary Genetics Laboratory (eGL). Forward and Reverse sequencing was performed, and results were edited by Sequencher 5.0.1 DNA sequence analysis software. Consensus sequences were aligned using ClustalW algorithm in MEGA 7 (Kumar et al., 2015). BLAST algorithm was used to compare our sequences with the sequences available in the GenBank database. Afterward, the sequences produced in our study were combined with the previously published COI and 16S rDNA sequences. Best DNA models were determined for both gene region sequences in MEGA 7 software. Models with the lowest Bayesian Information Criterion scores (BIC) were considered as the best substitution pattern. T92+G+I and HKY+G models were found to be the best sequence evolution models for 16S rDNA and COI gene regions, respectively. An aligned data set was used to create phylogenetic trees in MEGA 7 software (Kumar et al., 2015). Neighbor-Joining (NJ) and Maximum Likelihood (ML) algorithm was used to infer the phylogenetic relationships.

There were 163 COI gene sequences; 36 from *S. californica*; 12 from *S. dumeril*; 2 from *S. occulta*; 6 from *S. guggenheim*; 3 from *Squatina* sp.; 3 from *S. armata*; 19 from *S. africana*; 8 from *S. formosa*; 4 from *S. legnota*; 1 from *S. tergocellatoides*; 1 from *S. japonica*; 1 from *S. oculata*; 9 from S. *australis*; 9 from *S. albipunctata*; 5 from *S. pseudocellata*; 6 from *S. tergocellata*; 3 from *S.*

aculeata and 27 from *S. squatina*, and 90 16S rDNA gene, sequences; 29 from *S. californica*; 7 from *S. dumeril*; 2 from *S. occulta*; 4 from *S. guggenheim*; 3 from *S. armata*; 17 from *S. africana*; 6 from *S. formosa*; 1 from *S. tergocellatoides*; 1 from *S. japonica*; 9 from *S. oculata*; 3 from S. *australis*; 5 from *S. albipunctata*; 3 from *S. pseudocellata*; 3 from *S. tergocellata*; 2 from *S. aculeata* and 3 from *S. squatina* deposited in GenBank databases. To avoid the complex appearance of the phylogenetic trees, representative sequences for each species from the GenBank database were chosen for tree construction (Table 1). Haplotypes for both gene regions were identified using MEGA 7 software. Haplotype analysis was conducted on *Squatina* sequences on Network 5.5 (Bandelt et al., 1995) to eliminate the sequences with the same haplotypes within the species to epitomize the trees with representative haplotypes.

Table 1. Materials and Accession numbers examined in this study (Accession numbers indicated with (*) are from this study.

species	Accession # (16S rDNA)	Accession # (COI)
S. aculeata	KR493424*, FN431790, FN431791	KR610532*, FN431671, FN431672, KJ709642
S. squatina	KY216163*, KY216164*, FN431879, FN431880, FN431881	KY216165*, KY216166*, KC501660, KC501661, KC501665, KC501666, KC501668, KC501669, FN431762
S. oculata	FN431873	KY909582, KY176652, FN431754
S. lengota	-	FN431751, KF590400
S. formosa	FN431863, FN431864, FN431870	EU399040, EU399041
S. japonica	-	FN431750
S. tergocellatoides	-	FN431766
S. armata	FN431815, FN431816	FN431694, FN431696
A. occulta	FN431871, FN431872	FN431752, FN431753
S. guggenheim	FN431868	FN431749
S. californica	FN431824, FN431828, FN431832, FN431845, FN431848, FN431851, FN431852	FN431717, FN431728, FN431733, GU440531
S. dumeril	FN431858, FN431859	FN431737, FN431738, FN431739, FJ519598, FJ519599
S. africana	FN431808	HQ945896, HQ945905
S. australis	FN431818, FN431819	EU339038, FN431699
S. albipunctata	FN431813	FN431759

S. pseudocellata	FN431875, FN431876	FN431756, FN431757
S. tergocellata	FN431883, FN431884	FN431763, FN431764, FN431765

Results

Species identification of the three specimens was carried out by morphological examination and DNA analysis.

Morphological measurements and identification

Sample #1 which was obtained from Gökova Bay was identified by Akyol et al. (2015) as a pregnant female *S. squatina*. The specimen was identified by the authors according to its broad trunk, smaller spiracle length than eye diameter, external nasal flap with two barrels, dermal folds on the sides of the head with a single triangular frontal lobe, high pectoral fins broad with rounded rear tips, small spines on the back midline, rough dorsal surface and greenish-brown color with a beige belly (Akyol et al., 2015). The measurement of Sample #1 was published by Akyol et al. (2015) as 1560 mm TL and 402 as DL and 32600 g. as total weight.

Samples #2 and #3 were identified as *S. squatina* according to the position of their first dorsal fin, nasal lobs, dorsal spines distribution, and other characteristics mentioned by Serana (2005) and Compagno et al. (2005). Both samples were females and their total length, disk length and weight measurements were 835 mm TL, 474 mm DL, 5140 gW for the larger individual (Fig. 1), and 695 mm TL, 294 mm DL, 3190 gW for the small individual (Fig. 2).

PCR and sequence analysis

PCR products were separated on 1.5% agarose gel electrophoresis and photographed by gel documentation system (Axygen, USA). COI gene region of Sample #1 was submitted to GenBank with the accession number KR610532 and Sample #2 and #3 with KY216165 and KY216166, respectively. 16S rDNA sequence Sample #1 was submitted to GenBank with the accession number KR493424 and Sample #2 and #3 with KY216163 and KY216164, respectively.

The 16S rDNA and COI gene sequences of the Sample #1 showed high-level similarity (99%-100%) with *S. aculeata* sequences in GenBank data (Table 2) which was contradicting Akyol et al. (2015)'s morphological identification. Blast results supported our morphological determination of Samples #2 and #3 as *S. squatina* (Table 3).

Table 2. Comparison of 16S rDNA and COI gene sequences of our sample Sample #1 with GenBank data.

Gene Region	Sample #1	GenBank Accession #	Similarity (%)
16S rDNA	KR493424	FN431790	100
		FN431791	99
COI	KR610532	KJ709642	100

	FN431671	99
	FN431672	99

Table 3. Comparison of 16S rDNA and COI gene sequences of our Sample #2 and #3 with GenBank data.

Gene Region	Sample #2 and #3	GenBank Accession #	Similarity (%)
16S rDNA	KY216163 KY216164	FN431879	100
		FN431880	99
		FN431881	99
		KU577284	100
		AY462192	100
COI	KY216165 KY216166	JN641253	100
		JN641254	99
		JN641255	99
		FN431760	99
		FN431761	100
		FN431762	99
		KC501653	99
		KC501662	99
		KC501667	99
		KC501670	99
		JQ624004	99

Phylogenetic analysis

All available COI and 16S rDNA sequences of *S. squatina* and *S. aculeata* species in GenBank were used to create phylogenetic trees with our samples in MEGA 7 software (Kumar et al., 2015). Both ML and NJ phylogenetic trees were constructed by MEGA 7 software (Fig. 3-6).

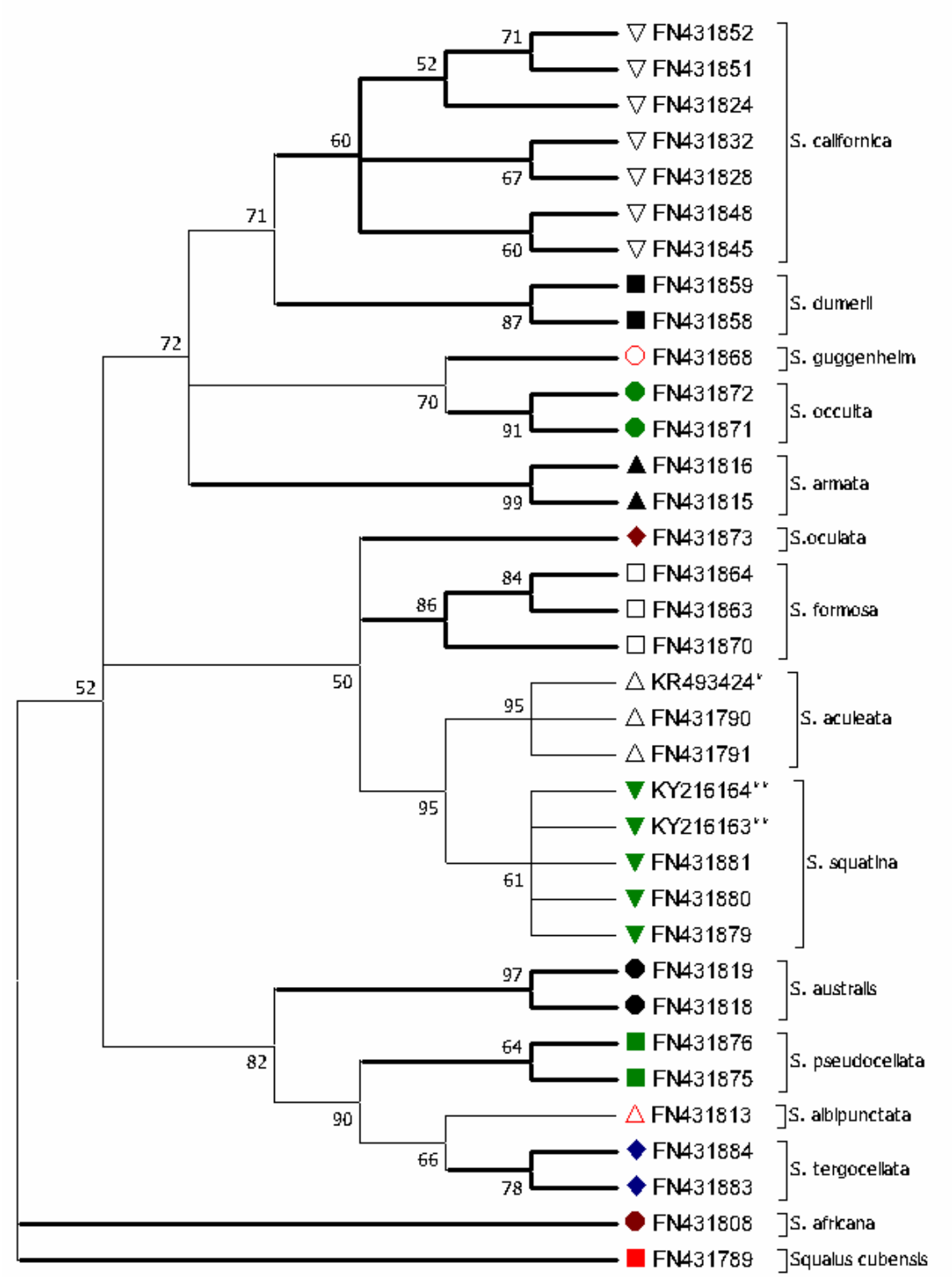

Figure 3. Neighbour Joining (NJ) tree of *Squatina* species based on the partial sequence of the 16S rDNA gene constructed by Tamura-Nei model with 1000 bootstrap. The values on the branches indicate the bootstrap percentage. FN431789 (*Squalus cubensis*) is the outgroup, KR493424 is our *S. aculeata* sample from Gökova indicated with asterix (*), and KY216163 and KY216164 our *S. squatina* samples both indicated with double asterix (**).

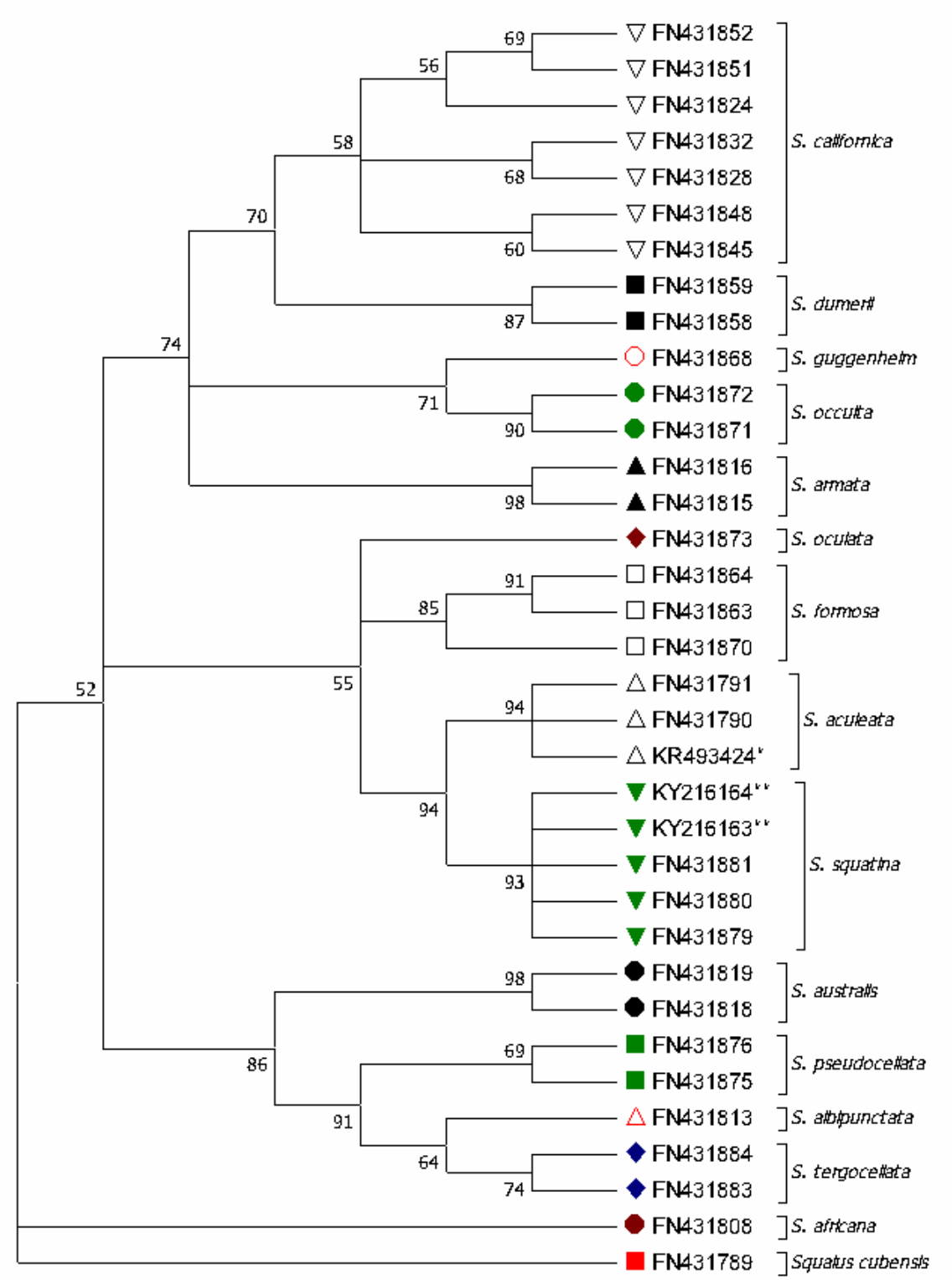

Figure 4. Maximum Likelihood (ML) tree of *Squatina* species based on the partial sequence of the 16S rDNA gene constructed by T92+G+I with 1000 bootstrap. The values on the branches indicate the bootstrap percentage. FN431789 (*Squalus cubensis*) is outgroup, KR493424 is our *S. aculeata* sample from Gökova indicated with asterix (*), KY216163 and KY216164 our *S. squatina* samples both indicated with double asterix (**).

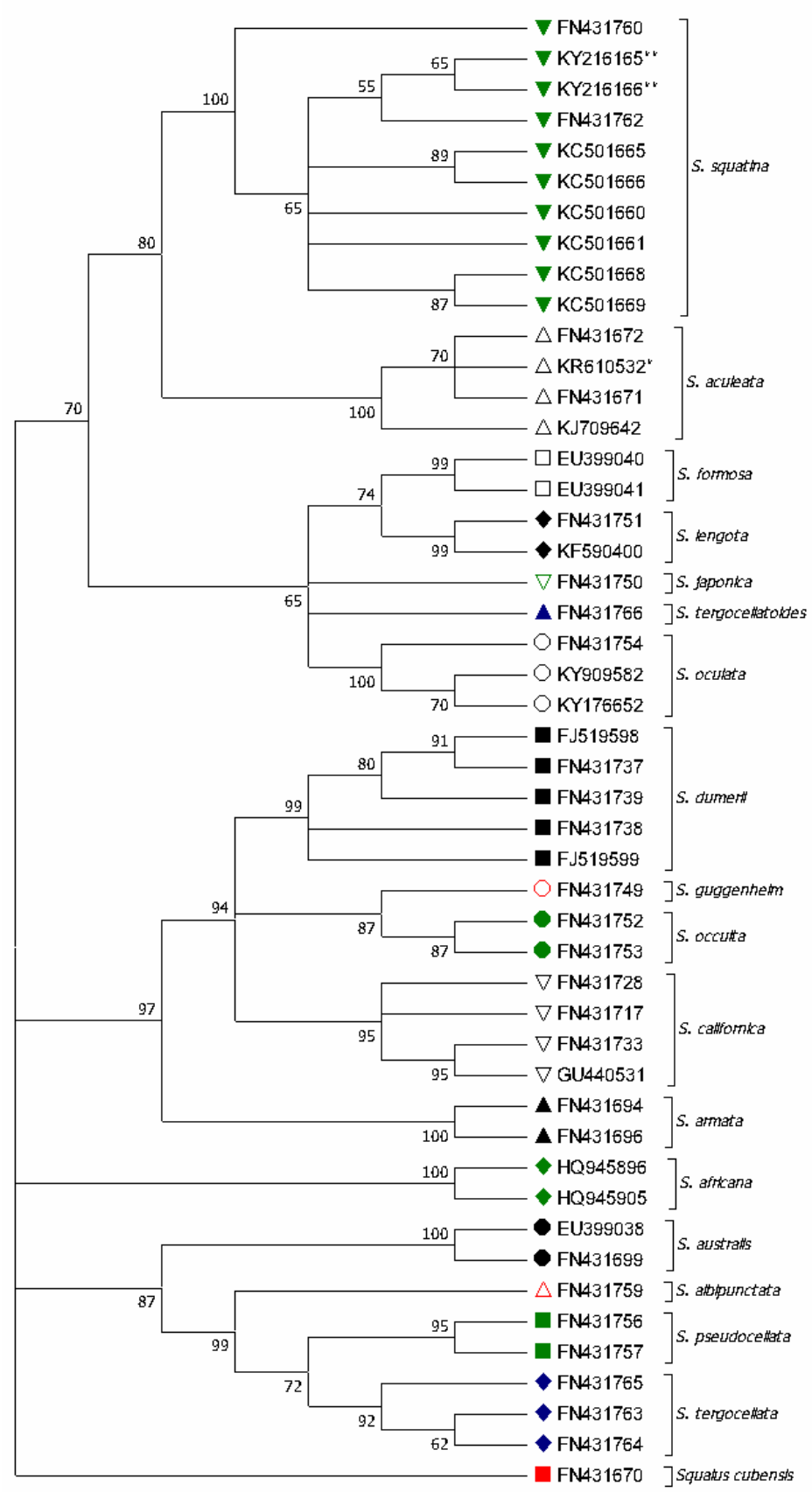

Figure 5. Neighbour Joining (NJ) tree of *Squatina* species based on sequence of the COI gene constracted by Tamura-Nei model with 1000 bootstrap cut off value 50. The values on the branches indicate the bootstrap percentage. FN431670 (*Squalus cubensis*) is outgroup, KR610532 is our *S. aculeata* sample from Gökova

indicated with an asterix (*), KY216165 and KY216166 our *S. squatina* samples collected from South Aegean Sea indicated with double asterix (**).

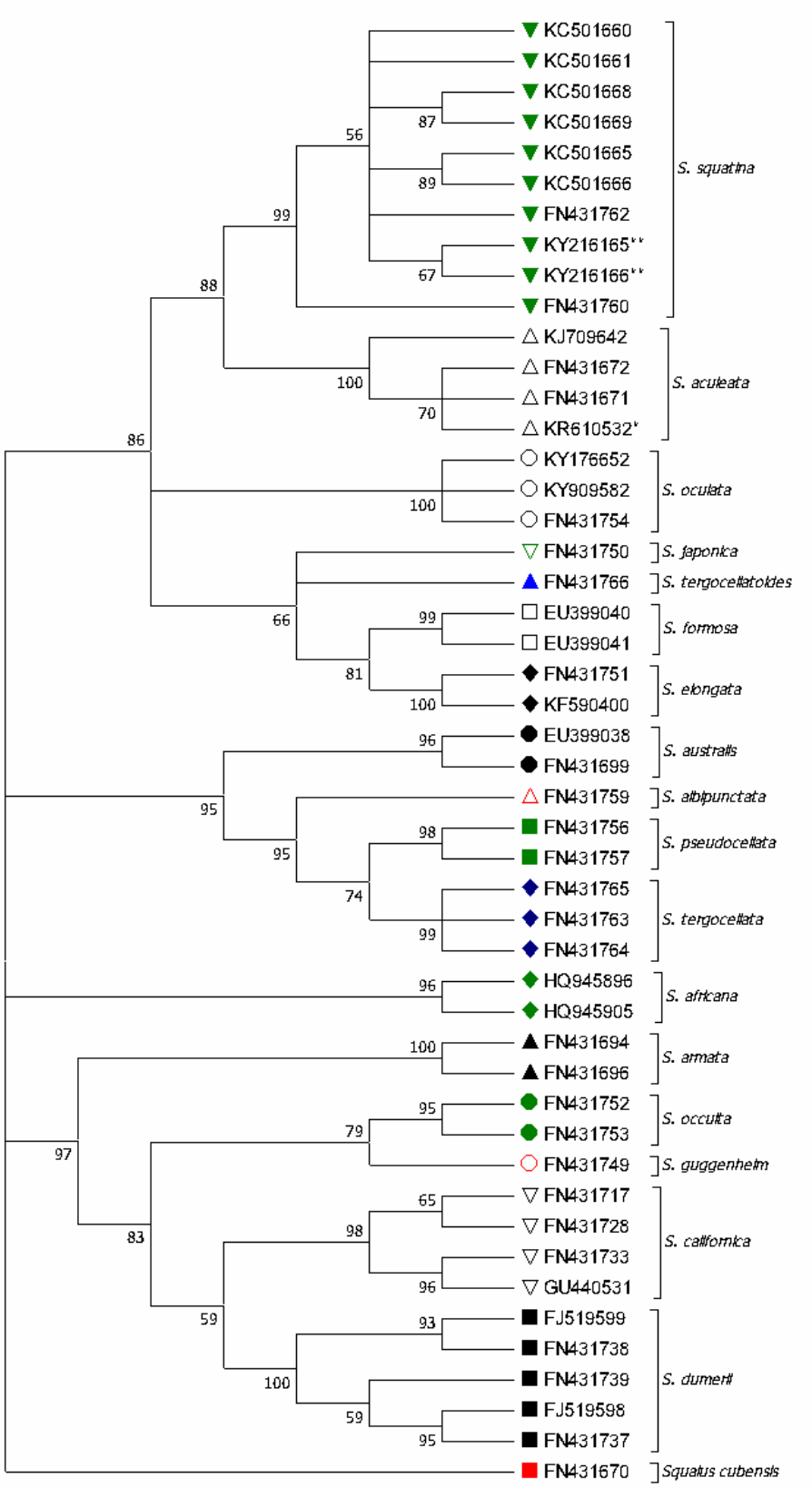

Figure 6. Maximum Likelihood (ML) tree of *Squatina* species based on sequence of the COI gene constracted by HKY+G model with 1000 bootstrap cut off value 50. The values on the branches indicate the

bootstrap percentage. FN431670 (*Squalus cubensis*) is outgroup, KR610532 is our *S. aculeata* sample from Gökova indicated with an asterix (*), KY216165 and KY216166 our *S. squatina* samples collected from South Aegean Sea indicated with double asterix (**).

While constructing phylogenetic trees 35 of 16S rDNA sequences and 48 of COI gene sequences from GenBank were used. According to the NJ tree of 16S rDNA region of *Squatina* species, four major clades were obtained. The first one was with *S. californica, S. dumeril, S. guggenheim, S. occulta, S. armata*; the second clade consisted of *S. formosa, S. oculata, S. aculeata* and *S. squatina* species, while the third clade was with *S. australis, S. pseudocellata, S. albipunctata, S. tergocellata,* and the last one was with only *S. africana* (Fig. 3). In NJ trees of 16S rDNA tree, clade 1 comprehends North & South American species (*S. californica, S. dumeril, S. guggenheim, S. occulta, S. armata*) while the second clade consists of European & North African & Asian species (*S. formosa, S. oculata, S. aculeata* and *S. squatina*). The third clade consists of one group of Australian species (*S. australis, S. pseudocellata, S. albipunctata, S. tergocellata*) and the third one comprises South African species (*S. africana*). Same clades with the same species were obtained with the ML tree (Fig. 4).

The NJ tree of the COI gene region also gave four major clades. The first clade consists of *S. oculata, S. tengocellatoides, S. elongata, S. formosa, S. japonica, S. aculeata, S. squatina*; the second clade has *S. dumeril, S. guggenheim, S. occulta, S. californica* and *S. armata*, third clade has only *S. africana* and the fourth clade has *S. australis, S. albipunctata, S. pseudocellata* and *S. tergocellata* species (Fig. 5). The first clade is formed of 2 subclades; 1- European (*S. aculeata* and *S. squatina*), 2-Asian (*S. oculata, S. formosa, S. legnota, S. tengocellatoides, S. japonica*). The second clade involves North & South American species (*S. dumeril, S. guggenheim, S. occulta, S. californica* and *S. armata*). While the third group consists of only African species and finally the last clade includes Australian species (*S. australis, S. albipunctata, S. pseudocellata,* and *S. tergocellata*). Same clades with the same species were obtained with the ML tree (Fig. 6).

Both gene regions gave similar results in the phylogenetic trees; both trees consisted of 4 main clades with the same region groups; 1-European & North African & Asian, 2- South African, 3-Australian, 4- North & South American. In all trees, our two *S. squatina* specimens made a clade with other *S. squatina* specimens from GenBank. Likewise, the *S. aculeata* specimen appeared in a clade among other *S. aculeata* species from GenBank.

Discussion

Although there are various studies on these species from the coasts of Turkey, they are mainly focused on the presence-absence, by-catch reports and distribution pattern of these species (Başusta,

2002; Kabasakal, 2002; Kabasakal, 2003; Tekinay et al., 2003; Filiz et al., 2005; Öğretmen et al., 2005; Karakulak et al., 2006; Akyol & Ceyhan, 2007; Sağlam et al., 2008; Ismen et al., 2009; Keskin, 2010; Unal et al., 2010; Turan, 2012; Bulguroğlu et al., 2014; Çoker & Akyol, 2014; Kabasakal and Kabasakal, 2014; Erguden, 2015; Kabasakal & Karhan, 2015; Akyol et al., 2015; Başusta, 2016). In other parts of the world, there are numerous studies about these two species' biology and ecology (Capape et al., 1990; Capape et al., 2005; Carvalho et al., 2010; Alioto, 2012; Cavallaro et al., 2015; Fortibuoni & Borme, 2016). The studies conducted in the Mediterranean Sea are mainly concentrated on conservation status, reproduction, morphological characteristics and presence/absence of the genus (Capape et al., 1990; Corsini & Zava 2007; Moftah et al., 2011; Cavallaro et al., 2015; Fortibuoni et al., 2016; Zava & Serena, 2016; Holcer & Lazar, 2017; Narváez & Osaer, 2017). The studies on the presence/absence records of the genus are generally on morphological features themselves without molecular identification. This lack of information increases the importance of correct identification since morphological identification can be incorrect due to several reasons such as damage on certain descriptive features or changes depending on life cycle. Reporting of accurately identified species becomes crucial for the species' conservation studies as the members of the genus are becoming rare throughout the Mediterranean Sea. Therefore, the importance of these types of studies has increased due to the vital need to follow the species' current state and global conservation efforts. Since *Squatina* species are difficult to distinguish from each other and they are on the verge of extinction in the Mediterranean Sea, it is more important to identify an individual correctly; which can only be possible by supporting morphological identification with genetic tools. This study shows and points out that *S. squatina* and *S. aculeata* species are difficult to distinguish from one another solely based on morphological identification but with the support of genetic markers. Providing accurate identification of shark and ray species, the DNA sequence-based approach is not a new technique that is used but has become more widespread in recent years (Shirai, 1992; Turan, 2007; Stelbrink et al., 2009; Moftah et al., 2011; Velez-Zuazo & Agnarsson, 2011; Garcia-Vazquez et al., 2012; Naylor et al., 2012; Ramirez-Amaro et al., 2017). Trustworthy identification of species, especially the exploited ones has crucial importance for conservation purposes since wrong identification can lead to accidental extinction in regard to sustainability.

Mitochondrial gene sequences are widely used to reveal the diversity of the species and the relationship between the populations (Fitzpatrick et al. 2017). Stelbrink et al. (2009) revealed four major clades corresponding to geographic regions according to 16S rRNA and COI gene regions in their phylogenetic analysis. Velez-Zuazo & Agnarsson (2011) also confirmed Stelbrink et al. (2009)'s findings. These groups comprise 1) the European and North African species, 2) the South

African species, 3) Australian species, and 4) North and South African species. When we compared our results, we also obtained four major clades within our 16S rDNA and COI gene regions. Our subject species appeared in European and North African species in NJ and ML trees with both gene regions like previous studies (Stelbrink et al., 2009).

Haplotype analysis based on the 16S rDNA gene region gives only one haplotype for *S. squatina* specimens (KY216164, KY216163, FN431881, FN431880, FN431879). Two 16S rDNA haplotypes were found in *S. aculeata*, one of which was registered from Senegal (FN431791, FN431790) and the other one is our sample from Gökova Bay (KR493424). In consideration of COI gene region, we obtained two haplotypes for *S. aculeata* like in the 16S rDNA gene. On the other hand, *S. squatina* gave 6 haplotypes for COI gene regions (10 out of 8 sequences were from Turkey (KY216165, KY216166, KC501660, KC501661, KC501665, KC501666, KC501668, KC501669); one from Spain (FN431760) and the other one from Ireland (FN431762)). The haplotype results were supported by the sister clades in NJ and ML trees for both gene regions.

In the 16S rDNA tree, the Asian species and European & North African species formed one clade as in the NJ tree of the COI gene region. The difference between the two gene region trees are the lack of sequences of three Asian species (*S. lenota*, *S. japonia* and *S. tergocellatoides*). The reason that the gene sequences of these 3 species were not included in the 16S rDNA tree was the missing nucleotides in the sequences. To avoid any error, these three sequences were excluded from the analysis. Similar results were obtained with the ML tree for both gene regions.

In all trees, our two *S. squatina* specimens made a clade with the other *S. squatina* specimens from GenBank. Likewise, our *S. aculeata* specimen made a clade with other *S. aculeata* species from GenBank. These results demonstrated that the morphological species identification correlates with molecular identification analysis for our *S. squatina* specimen. Although the molecular analysis showed that Sample #1 was *S. aculeata*, the same sample was published by Akyol et al. (2015) as *S. squatina* solely based on its morphological characteristics. In consideration of our findings, we would like to draw attention to the necessity of molecular-based species identification for *S. aculeata* and *S. squatina* species.

Conservation of organisms is a complicated task. For a better conservation action, good taxonomic knowledge and a wide range of information is necessary (Harsan, 2008). As seen in our Blast (Tables 2-3) and phylogenetic tree results (Fig.s 3-6), Sample #1, which was morphologically identified as *S. squatina* by Akyol et al. (2015), was revealed as *S. aculeata* after 16S rDNA and COI gene region analysis. With the other two individuals, Sample #2 and Sample #3, morphological identification was supported with DNA sequences. In this case, DNA-based identification becomes a necessity along with morphological identifications. To prevent misidentification, both

morphological characteristics and molecular tools should be used for species identification purposes.

Conclusion

In conclusion, the results of this study highlight the importance of the use of molecular data as well as morphological characteristics in species identification. We believe that the results we achieved will contribute to conservation biology studies in which awareness efforts towards the conservation of shark species are accelerating. We hope that our work will be practical data for further works on phylogenetics of these important and highly threatened organisms since it is important to have a high number of gene sequence data worldwide for accurate identification.

References

Akyol, O. & Ceyhan, T. (2007). Datca-Bozburun Yarimadasi (Ege Denizi) kiyi balikciligi ve sorunlari uzerine bir arastirma. E.U. Journal of Fisheries and Aquatic Sciences, 24 (1-2), 121-126.

Akyol, O., Ünal, V., Capape, C. (2015). Occurrence and Biological Observation on Angel Shark *Squatina squatina* (Chondrichthyes: Squatinidae) from the Turkish waters (Eastern Mediterranean). Turkish Journal of Fisheries and Aquatic Science, 15, 931-935.

Alioto, D. (2012). A Review of the systematics of Angel Sharks emphasizes the species of the Eastern Pacific region with a modified set of morphometrics for order Squatiniformes. Ph.D., University of California, Los Angeles, USA.

Bandelt, H.J., Forster, P., Sykes, B.C., Richards, M.B. (1995). Mitochondrial portraits of human populations, Genetics, 141, 743-753.

Basusta, N. (2016). New records of neonate and juvenile sharks (*Heptranchias perlo*, Squatina aculeata, *Etmopterus spinax*) from the North-eastern Mediterranean Sea. Marine Biodiversity, 46, 525-527.

Basusta, N. (2002). Occurrence of Sawback Angelshark (*Squatina aculeata* Cuvier, 1829) off the Eastern Mediterranean Coast of Turkey. Turkish Journal of Veterinary and Animal Science, 26, 1177-1179.

Bulguroglu, S.Y., Korun, J., Gokoglu, M. & Ozvarol, Y. (2014). The marine leech *Stibarobdella moorei* (Oka, 1910) (Hirudinea, Piscicolidae) parasitic on the thornback ray Raja clavata Linnaeus, (1758) 1758 and angel shark *Squatina squatina* in Antalya Bay, Mediterranean Sea of Turkey. Helminthologia 51, 3, 250-252.

Capapé, C., Diatta, Y., Seck, A.A., Guelorget, O. & Ben, S. J. (ed.) (2005). Reproduction of the sawback angel shark *Squatina aculeata* (Chondrichthyes: Squatinidae) of Senegal and Tunisia. Cybium, 29, 2, 147-157.

Capapé, C., Quignard, J.P. & Mellinger, J. (1990). Reproduction and development of two angel sharks, *Squatina squatina* and *S. oculata* (Pisces: Squatinidae), off Tunisian coasts: Semi-delayed vitellogenesis, lack of egg capsules, and lecithotrophy. Journal of Fish Biology, 37, 3, 347-356.

Carvalho, M.R., Faro, C. & Gomes, U.L. (2010). Comparative neurocranial morphology of angel sharks from the south-western Atlantic Ocean (Chondrichthyes, Elasmobranchii, Squatinidae): implications for taxonomy and phylogeny. Acta Zoologica, 93, 171-183.

Cavallaro, M., Danze, A., Ammendolia, G. & Navarra, E. (2015). Finding of a rare Squatina squatina (Linnaeus, 1758) (Chondrichthyes: Squatinidae) along the Tyrrhenian coast of Strait of Messina and its maintenance in an aquarium. Marine Biodiversity Records, 8, 1-4. doi:10.1017/S1755267215000226. [accessed 10 February 2020]

Coker, T. & Akyol, O. (2014) .An evaluation of the fish diversity of Gökova Bay (Aegean Sea). E.U Journal of Fisheries and Aquatic Science, 31, 3, 161-166.

Compagno L. Dando, M. & Fowler, S. (2005). Sharks of the World. Princeton Field Guides.

Corsini, M. & Zava, B. (2007). Recent capture of Squatina oculata and Squatina aculeata from Dodecanese Islands (SE Aegean Sea, Eastern Mediterranean). Biologia Marina Mediterranea, 14, 2, 352-353.

Dayrat, B. (2005). Towards integrative taxonomy. Biological Journal of Linnean Society, 85, 407–415.

Ebert, D.A., Stehmann, M.F.W. (2013) Sharks, batoids, and chimeras of the North Atlantic FAO Species Catalogue for Fishery Purposes. Food and Agriculture Organization of the United Nations, 7, pp. 523.

Economidis, P.S. (1973). Catalogue des passions de la Grece. Oceanology Limnology, 11, 421-600.

Erguden, D. & Bayhan,. YK. (2015). Three Fish Species Known to be Rare for Turkey, Captured from the Northeastern Mediterranean Coast of Turkey, Mersin Bay, Sudis hyalina Rafinesque, Chlopsis bicolor Rafinesque, Squatina aculeata Cuvier. International Journal of Scientific and Technological Research, 1,4, 1-8.

Ferretti, F., Morey, G., Serena, F., Mancusi, C. & Fowler, S.L. (ed) (2015). Squatina squatina. The IUCN Red List of Threatened Species 2015. e.T39332A48933059. http://dx.doi.org/10.2305/IUCN.UK.2015-1.RLTS.T39332A48933059.en. [accessed 11 November 2019]

Filiz, H., Irmak, E. & Mater, S. (2005). Occurrence of Squatina aculeata Cuvier, 1829 (Elasmobranchii, Squatinidae) from the Aegean Sea, Turkey. E.U Journal of Fisheries and Aquatic Sciences, 22, 2-3, 451-452.

Fitzpatrick, C.K., Finnegan, K.A., Osaer, F., Nervaez, K. & Shivji, M.S. (2017). The complete mitochondrial genome of the Critically Endangered Angelshark, Squatina squatina. Mitochondrial DNA Part B, 2, 1, 212-213.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome coxidase subunit I from diverse metazoan invertebrates. Molecular and Marine Biology and Biotechnology, 3, 5, 294-299.

Fortibuoni, T., Borme, D., Franceschini, G., Giovanardi, O. & Raicevich, S. (2016). Common, rare or extirpated? Shifting baselines for common angelshark, Squatina squatina (Elasmobranchii: Squatinidae), in the Northern Adriatic Sea (Mediterranean Sea). Hydrobiologia, 772, 1, 247-259.

Garcia-Vazquez, E., Machado-Schiaffino, G., Campo, D. & Juanes, F. (2012). Species misidentification in mixed hake fisheries may lead to overexploitation and population bottlenecks. Fisheries Research, 114, 52-55.

Golani, D. (1996). The marine ichtyofauna of the Eastern Levant. History, inventory and characterization. Israel Journal of Zoology, 42, 15-55.

Harsan, R. & Petrescu-Mag, I.V. (2008). Endangered fish species of the world. Aquaculture, Aquarium, Conservation and Legislation Journal of Bioflux Society, 1, 2, 193-215.

Holcer, D. & Lazar, B. (2017). New data on the occurrence of the critically endangered common angelshark, Squatina squatina, in the Croatian Adriatic Sea. Natura Croatica: Periodicum Musei Historiae Naturalis Croatici, 26, 2, 313-320.

Ismen, A., Yigin, C.C., Altinagac, U. & Ayaz, A. (2009). Length-weight relationships for ten shark species from Saros Bay (North Aegean Sea). Journal of Applied Ichthyology, 25, 1, 109-112.

Kabasakal, H. (2002). Elasmobranch species of the seas of Turkey. Annales Series Historia Naturalis, 12, 1, 15-2.

Kabasakal, H. (2003). Historical and contemporary records of sharks from the Sea of Marmara, Turkey. Annales Series Historia Naturalis, 13, 1, 4-18.

Kabasakal, H. & Kabasakal, O. (2014). Status of angel shark, *Squatina squatina* (Elasmobranchii: Squatiniformes: Squatinidae) in the Sea of Marmara. Annales Series Historia Naturalis, 24, 41-46.

Kabasakal, H. & Karhan, S.I. (2015). Shark Biodiversity in the Sea of Marmara: departures and arrivals over a century. Marine Biodiversity Records, 8, 1-9.

Karakulak, F.S., Erk, H. & Bilgin, B. (2006). Length-weight relationships for 47 coastal fish species from the northern Aegean Sea, Turkey. Journal of Applied Ichthyology, 22, 274-278.

Keskin, C. (2010). A review of fish fauna in the Turkish Black Sea. Journal of the Black Sea/Mediterranean Environment, 16, 2, 1965-210.

Kumar, S., Stecher, G. & Tamura, K. (2015). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. Molecular Biology and Evolution, 28, 2731-2739.

Moftah, M., Abdel, A.S.H., Elramah, S. & Favereaux, A. (2011). Classification of Sharks in the Egyptian Mediterranean Waters Using Morphological and DNA Barcoding Approaches. Public Library of Science ONE, 6, 11, e27001. doi:10.1371/journal.pone.0027001

Narváez, K. & Osaer, F. (2017). The marine leech *Branchellion torpedinis* parasitic on the angel shark *Squatina squatina* and the marbled electric ray Torpedo marmorata. Marine Biodiversity, 47, 3, 987-990.

Naylor, G.J., Caira, J.N., Jensen, K., Rosana, K.A.M. & White, W.T. (ed.) (2012). A DNA sequence-based approach to the identification of shark and ray species and its implications for global elasmobranch diversity and parasitology. Bulletin of the American Museum of Natural History, pp. 1-262, USA.

Ogretmen, F., Yılmaz, F. & Torcu, K.H. (2005). An investigation on fishes of Gökova Bay (Southern Aegean Sea). BAU Fen Bilimleri Dergisi, 7, 2, 19-36.

Palumbi, S.R., Martin, A.P., Romano, S.L., McMillan, W.O. & Stice, L. (ed.) (1991) The Simple Fool's Guide to PCR. Dept. of Zoology, University of Hawaii, Honolulu

Ramírez-Amaro, S., Ramírez-Macías, D., Vázquez-Juárez, R., Flores-Ramírez, S. & Galván-Magaña, F. (ed.) (2017). Population structure of the Pacific angel shark (Squatina californica) along the northwestern coast of Mexico based on the mitochondrial DNA control region. Ciencias Marinas, 43, 1, 69-80.

Saglam, N.E., Akyol, O. & Ceyhan, T. (2008). İstanbul balık halinde 1998–2007 yılları arasında işlem gören türler üzerine bir değerlendirme. E.U. Journal of Fisheries and Aquatic Sciences, 25, 1-2, 169-172.

Serena, F. (2005). Field identification guide to the sharks and rays of the Mediterranean and the Black Sea. FAO species identification guide for fishery purposes. Food and Agriculture Organization of the United Nations, Rome, pp. 97.

Shirai, S. (1992). Squalean phylogeny: a new framework of "squaloid" sharks and related taxa. Hokkaido University Press, pp. 151.

Soldo, A. & Bariche, M. (2015). *Squatina aculeata*. The IUCN Red List of Threatened Species 2015: e.T61417A48957735. [accessed on 18 November 19]

Stelbrink, B., Von, R.T., Cliff, G. & Kriwet, J. (2009). Molecular systematics and global phylogeography of angel sharks (genus Squatina). Molecular Phylogenetics and Evolution, 54, 2, 395-404.

Strauss, R. & Bond, C. (1990). Taxonomic methods: morphology. In: CB. Schreck, PB. Moyle, eds. Methods for fish biology American Fisheries Society, pp. 109–140.

Tekinay, A.A., Alpaslan, M., Özen, Ö., Akyüz, P.E. & Kahyaoğlu, G. (ed.) (2003) A Comparative Analysis of Istanbul Fish Market Records Between 1998 and 2001. E.U. Journal of Fisheries and Aquatic Sciences, 20, 3-4, 413-418.

Turan, C. (2007). Molecular Systematic Analyses of Mediterranean Skates (Rajiformes). Turkish Journal of Zoology, 32, 437-442.

Turan, C. & Erguden, D. (2012). İskenderun Körfezi'nde nesli tehlike altinda olan kikirdakli balık türleri. Conference paper.

Unal, V., Acarli, D. & Gordoa, A. (2010). Characteristics of Marine Recreational Fishing in the Canakkale Strait (Turkey). Mediterranean Marine Science, 11, 2, 315-330.

Velez-Zuazo, X. & Agnarsson, I. (2011) Shark tales: A molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). Molecular Phylogenetics and Evolution, 58, 207-217.

Ward, R.D., Hanner, R. & Hebert, P.D. (2009). The campaign to DNA barcode all fishes, Journal of Fish Biology, 74, 329–356.

Zava, B., Fiorentino. F. & Serena, F. (2016). Occurrence of juveniles *Squatina oculata* Bonaparte, 1840 (Elasmobranchii: Squatinidae) in the Strait of Sicily (Central Mediterranean), Cybium, 40, 4, 341-343.