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Development of the method for identification of selected populations of torpedo scad, *Megalaspis cordyla* (Linnaeus, 1758), using microsatellite DNA analyses. CELFISH project – Part 4

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ABSTRACT

Catch and consumption of torpedo scad (*Megalaspis cordyla*) over the western Indian Ocean, but also the western Pacific from Japan to Australia is constantly increasing. Taking into account the degree of exploitation and missing information on the population structure of torpedo scad stocks it is crucial to provide population data. The analysis included individuals obtained in 2012 and 2013 from local markets in Madagascar, Tanzania, Vietnam and Cambodia and after successful DNA extraction fragment of the nuclear rhodopsin gene (RH1) and 9 microsatellite regions (SSRs) were amplified and analysed. Based on the obtained results it was found that there was no 100% overlap between the compared RH1 sequences and those from GenBank. In the case of the studied SSRs, the results allowed the initial characterisation and assessment of the genetic diversity of populations. Moreover, population assignment test distinguished the studied populations into two geographically distant subpopulations.

1. Introduction

Torpedo scad (*Megalaspis cordyla*) is a pelagic species caught on the continental shelf, where it preys on other fish. The range of occurrence of the species covers the western Indian Ocean, but also the western Pacific from Japan to Australia. As the population of the species is large, torpedo scad is of high economic importance, particularly in Thailand and Malaysia where approximately 20.000 tonnes are caught annually. It is also important as a pelagic species in India (Kasim, 2003). The average landings in 2009–2010 were 32.000 tonnes, constituting 18% of the catches of Carangidae and 1% of the total catches of sea fish in India (CMFRI, 2011). In waters around India, the species is caught mainly using trawls, drift nets, surrounding nets and longlines (Kasim, 2003; Sivakami, 1995; Thomas & Hridayanathan, 2003). It is prevalent among the Carangidae species caught using gillnets in India, constituting 37.3% (Kasim, 2003). According to FAO data, the catches of this species in 2011–2012 decreased significantly in countries such as Indonesia (from 41,000–38.000 tonnes) and Malaysia (from approx. 32.000–23.000 tonnes). In the Philippines and Thailand, the observed catches are at a similar level and amount to approx. 17.100 tonnes per year (Fischer & Bianchi, 1984). The consumption of this species in countries such as Malaysia, Indonesia, Thailand, Vietnam and Cambodia is high due to its availability and a relatively low price. The meat of torpedo scad is characterized by a high protein content and a low fat content (Harivaindaran & Tajul, 2014). Usually the fish are sold fresh, but are also subjected to drying and salting. This form of conservation extends the shelf life in countries with a climate unfavourable for longer storage of fresh fish when continuous refrigeration is not possible. In low-income countries in which other sources of animal proteins are unavailable or expensive, torpedo scad is an essential ingredient of local diet. Torpedo scad has not been evaluated according to IUCN criteria which is unfavourable, taking into account the degree of its exploitation. No data on the genetic stability of its populations and on the extent of their isolation and classification is available. Very few papers describe the attempts to use morphometric and meristic analyses to identify morphologically distinct populations of *M. cordyla* (Sajina, Chakraborty, Jaiswar, Pazhayamadam, & Sudheesan, 2011; Sajina, Chakraborty, Jaiswar, & Sudheesan, 2013). Currently, population studies are essential for the correct management of fishing economy,

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particularly in countries in which local fishermen are not bound by regulations, and fishing methods are sometimes very invasive, as evidenced by, e.g., degradation of coral reefs on the coasts of the Philippines. Plunder policy and overfishing of marine species force researchers to conduct studies that would allow a successful management of fishing economy. *Megalaspis cordyla* belongs to the Carangidae family which includes 130 species according to Nelson (2006) or 149 valid species according to Eschmeyer (2010). Carangidae are extremely variable, with body shape ranging from the torpedo-shaped *Decapterus* and *Elagatis* genera to the deep-bodied and narrowed forms such as the *Selene* genus (Nelson, 2006). The *Megalaspis* genus belongs to the Caranginae subfamily, the only one with scales, which also includes 21 other genera that encompass 96 species. Torpedo scad was previously classified to the genera *Scomber*, *Caranx* and *Citula* (Eschmeyer, 2010; Fricke, 1999; Paxton, Hoese, Allen, & Hanley, 1989). In the fish markets of Southeast Asian countries, the species occurs along with other Carangidae: *Decapterus kuroides*, *Decapterus macrosoma*, *Selar crumenophthalmus*, *Scomberoides lysan* (Agusa et al., 2007). Torpedo scad can potentially be confused with some of these species, especially of the *Decapterus* and *Selar* genera. It is possible since in India alone approx. 35 fish species belonging to 21 genera of Carangidae are constantly present in the catches (Kasim, 2003). Currently, population genetics is based on state-of-the-art methods allowing analysis of fragments of the nuclear and mitochondrial genome. One of the methods is the identification of microsatellite sequences (SSRs) that allow researchers to confirm or exclude whether an individual belongs to a particular species or even a particular population.

2. Materials and methods

The study material consisted of fin sections from the torpedo scad (*Megalaspis cordyla*) individuals obtained in 2012 and 2013 from local markets in Madagascar (Fenoarivo) (SMG), Tanzania (Dar es Salaam) (STZ), Vietnam (Quy Nhon) (SVN) and Cambodia (Phnom Pehn) (SKH). The collected fin fragments of the SMG (n = 3), STZ (n = 6), SVN (n = 8) and SKH (n = 5) populations were dried for transport and then conserved in 5 ml tubes containing silicone medium. DNA isolation from fin sections was performed using the peqGOLD Tissue DNA Mini Kit (peqlab) following the attached protocol. The qualitative and quantitative assessment of the isolates was carried out by electrophoresis in 1.5% agarose gel followed by spectrophotometric measurements using the NanoDrop 2000 instrument (Thermo Scientific). For all investigated samples, PCR amplification of a fragment of the nuclear rhodopsin gene (RH1) was conducted using the primers Rod-F2W and Rod-R4n (Sevilla et al., 2007) in accordance with the proposed methodology. Subsequently, in the obtained SMG, STZ, SVN and SKH samples, 9 microsatellite regions (SSRs) were analysed: Orla 22–135, Orla 21–231, Orla 20–134, Orla 16–185, Orla 9–204, Orla 8–113, Orla 9–38, Orla 12–160 and Orla 2–91. The amplification of the regions was conducted in accordance with the methodology used by Gotoh, Tamate, Yokoyama, Tamate, and Hanzawa (2013). The only change in the protocol was modification of the standard PCR profile to that of touchdown PCR: 94 °C for 5 min, then 30 cycles of denaturation (94 °C, 45 s), primer annealing (62 °C, 30 s × 7 and 59 °C, 30 s × 27) and elongation (72 °C, 20 s), followed by final elongation at 72 °C for 7 min. The reaction mix was prepared based on the GoTaq®G2 Hot Start polymerase (Promega), Green Master Mix 12.5 µl, F-primer 0.5 µl (10 pmol/µl), R-primer 0.5 µl (10 pmol/µl), H_2O DEPC 10.5 µl, DNA 1 µl. All PCR amplifications were carried out on a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems), and the results were evaluated by amplicon electrophoresis in 2% agarose gel. Sequencing of the obtained RH1 PCR products was ordered from Genomed, Warsaw, Poland. Analysis of thus obtained sequences was performed using the BioEdit software and BLAST (Altschul, Gish, Miller, Myers, & Lipman, 1990; Hall, 1999). The separation of SSR fragments was carried out using the SEQ 8000 sequencer (Beckman Coulter), while the analysis of the obtained SSR data was conducted using the GenAlEx software (Peakall & Smouse, 2012).

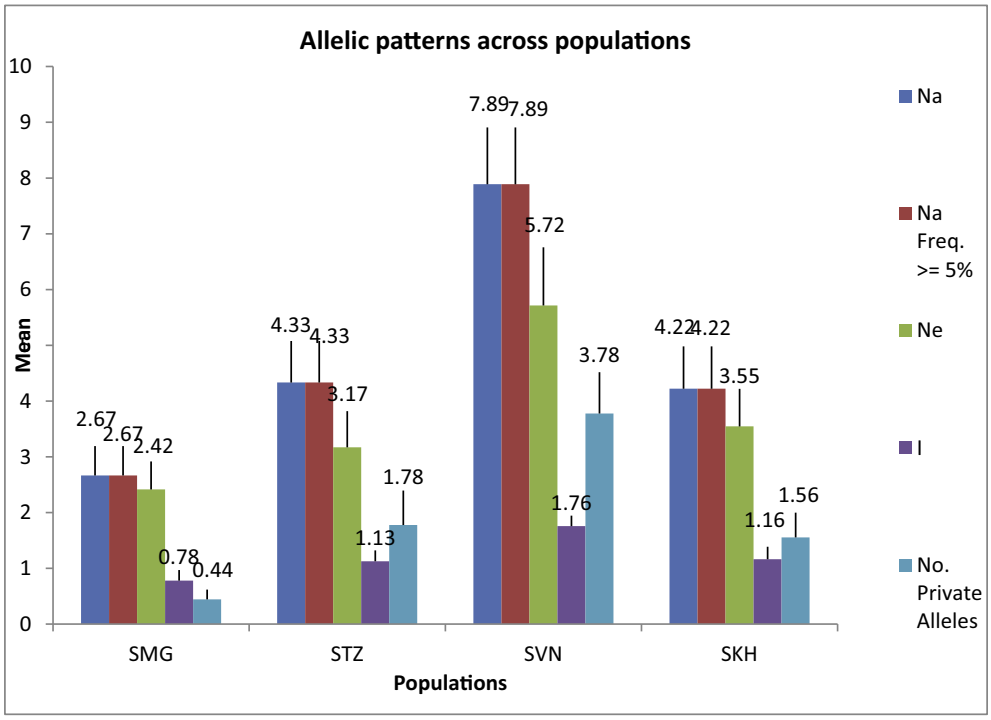

Fig. 1. Distribution of alleles in 9 SSR loci for the SMG, STZ, SVN and SKH populations.

Table 1
Average genetic diversity of the SMG, STZ, SVN and SKH populations based on the analysis of 9 SSR loci.

Pop		Na	Ne	I	Ho	He	uHe	F
SMG (n = 3)	Mean	2.667	2.416	0.780	0.296	0.457	0.548	0.435
	SE	0.527	0.502	0.190	0.117	0.095	0.114	0.152
STZ (n = 6)	Mean	4.333	3.171	1.128	0.500	0.569	0.621	0.145
	SE	0.745	0.650	0.195	0.104	0.081	0.088	0.144
SVN (n = 8)	Mean	7.889	5.716	1.757	0.528	0.752	0.802	0.325
	SE	1.020	1.043	0.189	0.097	0.057	0.060	0.111
SKH (n = 5)	Mean	4.222	3.546	1.164	0.444	0.587	0.652	0.232
	SE	0.760	0.675	0.224	0.114	0.100	0.111	0.124

Number of different alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I), Observed (Ho) and Expected (He) Heterozygosity, Unbiased Expected Heterozygosity (uHe), Fixation Index (F), Standard Error (SE).

Table 2
Summary of Chi-square test for the assessment of the Hardy–Weinberg equilibrium in the SMG, STZ, SVN and SKH populations.

Locus	Population	DF	ChiSq	p	Population	DF	ChiSq	p
Orla 22–135	SMG	Monomorphic			SVN	21	18.880	0.593
Orla 21–231	SMG	15	15.000	0.451	SVN	55	72.000	0.062
Orla 20–134	SMG	Monomorphic			SVN	10	16.222	0.093
Orla 16–185	SMG	3	3.333	0.343	SVN	3	16.000	0.001**
Orla 9–204	SMG	1	3.000	0.083	SVN	66	76.000	0.187
Orla 8–113	SMG	6	6.333	0.387	SVN	55	56.000	0.437
Orla 9–38	SMG	1	3.000	0.083	SVN	15	26.320	0.035*
Orla 12–160	SMG	3	3.333	0.343	SVN	21	48.000	0.001***
Orla 2–91	SMG	1	0.333	0.564	SVN	36	35.333	0.500
Orla 22–135	STZ	6	0.667	0.995	SKH	6	3.889	0.692
Orla 21–231	STZ	36	36.000	0.469	SKH	3	10.000	0.019*
Orla 20–134	STZ	3	1.227	0.747	SKH	Monomorphic		
Orla 16–185	STZ	3	8.667	0.034*	SKH	3	1.667	0.644
Orla 9–204	STZ	10	24.000	0.008**	SKH	21	20.556	0.486
Orla 8–113	STZ	15	13.500	0.564	SKH	10	15.556	0.113
Orla 9–38	STZ	1	0.050	0.824	SKH	10	11.389	0.328
Orla 12–160	STZ	10	10.000	0.440	SKH	1	0.062	0.804
Orla 2–91	STZ	1	6.000	0.014*	SKH	28	25.000	0.628

Degrees of freedom (DF), Chi-squared value (ChiSq), Probability (P), *P < 0.05, **P < 0.01, ***P < 0.001.

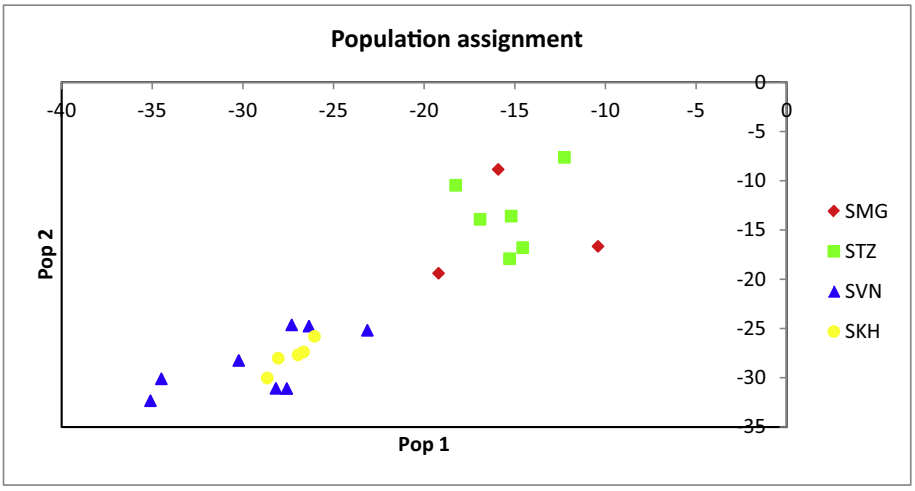

Fig. 2. Analysis of the main components based on the genetic distances between individuals from the SMG, STZ, SVN and SKH populations.

3. Results

Comparison of the nucleotide sequences of the rhodopsin gene from the SMG, STZ, SVN and SKH samples in the BioEdit software revealed no differences between SMG and STZ, while there were differences between SMG and SVN, SMG and SKH, STZ and SVN, STZ and SKH, SVN and SKH, respectively. The RH1 sequences were then compared with those submitted to GenBank using BLAST. It was found that there was no 100% overlap between the compared sequences and those from GenBank. No sequences originating from this species were also found. A preliminary analysis of the SSR markers demonstrated that all investigated loci in the four

populations were polymorphic and, therefore, qualified for further analysis. The total number of alleles for the SMG, STZ, SVN and SKH populations was 24, 39, 71 and 38, respectively, while the total number of alleles of the investigated loci was 1–6 (mean 2.67) for the SMG population, 2–9 (mean 4.33) for the STZ population, 3–12 (mean: 7.89) for the SVN population, and 1–8 (mean 4.22) for the SKH population. The average values of allele distribution with the expected heterozygosity (He) marked for all populations are shown in Fig. 1. The observed heterozygosity (Ho) which determines the ratio between the heterozygous genotypes and the total number of genotypes per locus was between 0.00 and 1.00 (mean 0.30) for the SMG population, between 0.00 and 0.83 (mean 0.5) for the STZ population, between 0.25 and 0.88 (mean 0.53) for the SVN population, and between 0.00 and 1.00 (mean 0.44) for the SKH population. Genetic diversity (He) was between 0.00 and 0.83 (mean 0.46) for the SMG population, between 0.15 and 0.86 (mean 0.57) for the STZ population, between 0.42 and 0.89 (mean 0.75) for the SVN population, and between 0.00 and 0.86 (average 0.59) for the SKH population (Table 1). There were statistically significant deviations from the Hardy–Weinberg equilibrium: in the STZ population for loci Orla 16–185, Orla 9–204 and Orla 2–91 at $p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively; in the SVN population for loci Orla 16–185 and Orla 12–160 at $p < 0.01$ and $p < 0.001$, respectively; in the SKH population for loci Orla 21–231 at $p < 0.05$ (Table 2). The process of assigning randomly selected individuals to the baseline populations, based on the results of

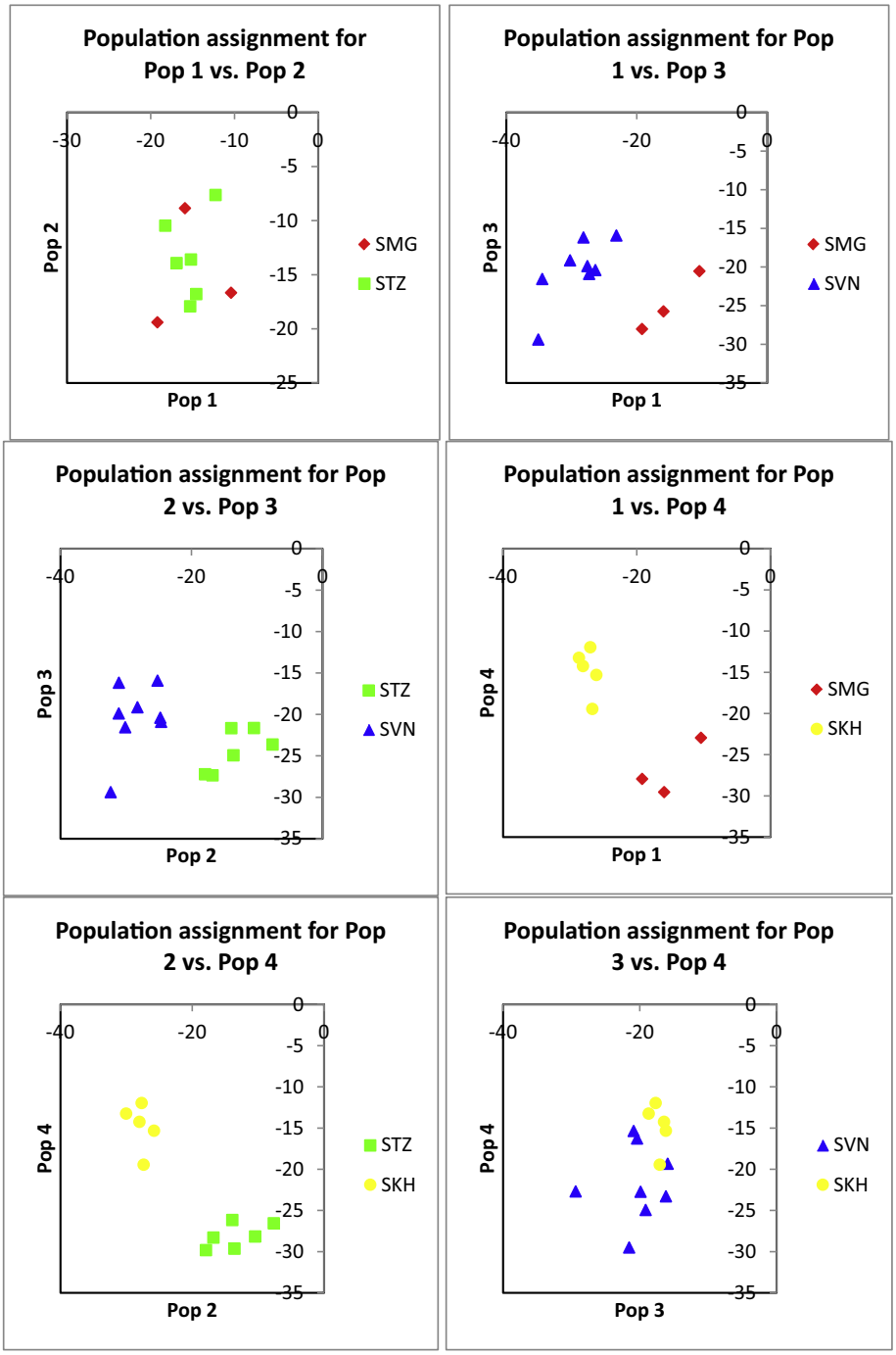

Fig. 3. Analysis of the main components based on the genetic distances between individuals from each population.

the species identification test, correctly indicated the origin of 68% of the analysed samples. The test was carried out using the significance level of 0.05 (Fig. 2).

4. Discussion

Along with the globally increasing trade on the fish and seafood market, problems related with commercial substitution of species are observed. The substitution includes not only reporting a different species name, but also selling fish species that are less economically valuable under the name of highly valued and much more expensive fish species. An analysis of the market substitutions of fish and seafood (Jacquet & Pauly, 2008) helped to establish that approximately 1/3 of all fish on the market are not correctly labelled. The species analysed in this study demonstrated much greater interpopulation diversity, especially between the fisheries in Madagascar and Vietnam, as well as Madagascar and Cambodia. Taking into account the low effectiveness of coast guard and fishing guard in countries in which local fishermen are not subject to penalties and their catches are not recorded, protection of genetically isolated populations should be considered. Torpedo scad is a species heavily exploited in the local population because of its availability and nutritional value. Sajina et al. (2011) identified two distinct populations of torpedo scad, one of which one inhabits the area of the Bay of Bengal, while the other inhabits the Arabian Sea. Moreover, the authors of the study suggest that due to significant differences between populations, gene transfer might occur only to a limited extent. Such information, together with the He, I or F indexes, has a substantial value when fish are caught to rebuild some severely over-exploited fisheries by restocking and stock enhancement interventions (Bell, Bartley, Lorenzen, & Loneragan, 2006). As shown in our study, the *M. cordyla* populations from Vietnam and Tanzania waters had the highest degree of heterozygosity which might result from the still large effective size of the population and reflect a positive association with developmental homeostasis (population stability). Therefore, all relevant Asian countries consider torpedo scad as economically important, although exceeded concentrations of heavy metals (Fathi et al., 2013) or parasites (Ju-Shey & Il-Hoi, 2004; Liu et al., 2010) have been reported in this species. The issue of improving the fishing capacity of *M. cordyla* with a concurrent implementation of sustainable management plan is a key element of the fishing economy in Asian countries (Jadhav & Mohite, 2014).

The microsatellite markers used in this study, due to their high polymorphism and codominant inheritance, allowed the evaluation of the genetic structure of *M. cordyla* samples. The analysis of Fixation index (F) values for each population revealed the excess of homozygotic allele combinations, which is probably a result of the small number of individuals included in the analysis (Table 1). Torpedo scad populations are characterised by a highly migratory life style and a pelagic larval stage in the life-cycle which allows formation of an unlimited number of allele combinations. This relationship is shown in Fig. 1 in which an increase in the number of individuals in each population is associated with a growing number of alleles (Na), the largest in the SVN population and the smallest in the SMG population. An important part of the study was the population assignment test which distinguished the studied populations into two geographically distant subpopulations, that of the Gulf of Thailand and that of South Africa (Figs. 2 and 3). Microsatellite markers have been used multiple times to assign individuals to indigenous populations and to monitor their genetic diversity which can be used to assess population stability (Hansen, Kenchington, & Nielsen, 2001). In the case of Patagonian toothfish (*Dissostichus eleginoides*) which inhabits the waters of the Patagonian Shelf, as well as the waters of the Atlantic Ocean and the western Indian Ocean sectors (Southern Ocean), it was found that the analysis of variations in the SSR regions, allowed the elucidation of the genetic structure of the studied populations (Rogers, Morley, Fitzcharles, Jarvis, & Belchier, 2006). However, in order to take appropriate decisions regarding the protection and management of the populations, the number of evolutionarily significant units (ESUs; Waples, 1991) needs to be established for the studied species, along with smaller management or conservation units within ESUs. The Pacific salmon species (Oncorhynchus spp.) is an example of fish for which a plan was based on the ESU identification (Moran, Teel, Lahood, Drake, & Kalinowski, 2006). Intensive ESU monitoring and implementation of management plans regarding salt-water fish populations are conducted in the Indo-Pacific region (Von Der Heyden et al., 2014). In the case of the studied *M. cordyla* populations, the obtained results allowed the initial isolation and assessment of the genetic diversity of both populations (SVN/SKH and STZ/SMG). However, in order to develop a robust management plan for torpedo scad populations, this study should be expanded with respect to the number of individuals and the analysed loci.

From the consumer's point of view, appropriate labelling helps to avoid counterfeiting of products, taking into account the different consumption and market value of the species (Hubalkova, Kralik, Tremlova, & Rencova, 2007). Incorrect labelling together with high morphological similarities of fish species, such as those reported for horse mackerel and false scad (*Caranx rhonchus*), act as a serious drawback in the estimation of consumer health risk associated with the consumption of fish, especially those caught in more contaminated waters (Bekdas & Belduz, 2009). In the case of *M. cordyla*, Naidu, Rao, and Ramaneswari (2008) reported that the content of heavy metals exceeded acceptable limits and consumption of the species should be avoided in these regions. Even modest consumption of moderately contaminated but commonly eaten fish can put consumers at risk very quickly, therefore labelling practices supported with molecular methods open a way to the reduction of counterfeiting cases and protecting seafood consumers.

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