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Investigation on the genus *Squalus* in the Sardinian waters (Central-Western Mediterranean) with implications on its management

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Abstract

In the Mediterranean Sea, in addition to the two historically known species belonging to the *Squalus* genus, a third species, *Squalus megalops*, has been reported. Considering the high level of morphologic similarity of this species with the native species *S. blainville*, this study aims to evaluate the Central-Western Mediterranean spurdog population in order to test the hypothesis of the presence of two distinct species *S. blainville* and *S. megalops*. A total of 137 spurdogs, caught in the Sardinian waters, were analyzed morphologically and genetically after their subdivision into two groups depending on the number of the lateral processes in the chondrocranium basal plate. The CAP analysis, employing all body and chondrocranial measurements, revealed no clear segregation among the *a priori* assigned groups with a high misclassification percentage. Besides, no evident dissimilarities in teeth and dermal denticle morphology between the two groups were observed. All the 18 specimens which were genetically analyzed, by sequencing of the mtDNA marker COI, clustered together resulting to be *S. blainville*. All the obtained results indicated the presence, in the study area, of only one species, ascribable to *S. blainville*.

Keywords: *Squalus blainville*; *Squalus megalops*; Mediterranean Sea; taxonomy; mtDNA sequencing; morphology.

Introduction

The correct taxonomic identification of species provides a critical baseline that supports the rest of biological research (Last *et al*., 2007). Generally, Elasmobranchs have suffered major taxonomic constraints that have led to misidentification issues related to by-catch and fisheries, which were usually solved by grouping data at higher taxonomic levels, such as genus or family (*e.g*. Zeeberg *et al.*, 2006; Coelho & Erzini, 2008).

Squalidae represent one of the most commercially targeted families among Elasmobranchs (Ebert *et al*., 2013). Indeed, several species belonging to this family are landed by up to 50 countries in direct fisheries or as bycatch (Ebert *et al*. 2013). Their relatively high commercial value, in addition to *K*-selected life strategy that commonly characterizes Elasmobranchs, identifies this taxonomic group as exceptionally susceptible to fishing mortality. This particular situation, despite the considerable abundance and the wide habitat range of some species, could easily lead them to stock depletion (Ebert *et al*., 2013).

Squalids belonging to the genus *Squalus* (Blainville, 1816), otherwise known as spurdogs, dogsharks and dogfishes, are among the most taxonomically problematic shark groups due to their strong morphological similarities. Until 2013, 25 species were known (Ebert *et al*., 2013) including 14 species recognized as valid by Compagno *et al*. (2005) and 11 species added later from the Western Indo-Pacific Ocean by Last *et al*. (2007). In addition, considering the resurrection of *S. acutipinnis* (Regan 1908) by Viana & Carvalho (2016) from South Africa and the description of four new species (*S. albicaudatus*, *S. bahiensis*, *S. lobularis* and *S. quasimodo*) from the South-West Atlantic (Viana *et al*., 2016), this number has recently been increased.

Squalus species have been divided into three main species groups, based on morphological features such as the relative position of the pectoral fins, the anterior nasal flap shape and skin colour (Bigelow & Schroeder 1957; Ebert *et al*., 2010): 1) the '*acanthias* group'; 2) the '*mitsukurii* group' historically known as the '*blainville-fernandinus* group', and 3) the '*megalops* group', also known as 'the *brevirostris-cubensis* group'. However, a correct identification of several widespread species still remains doubtful. Besides, this particular condition has also been reinforced for the *Squalus* genus due to their high overlapping level of morphological features (Last *et al*., 2007). Such classification uncertainties constituted an impediment to stakeholders, scientists and managers, somehow retarding the development of management measures because of the difficulties in evaluating the population status of several *Squalus* species.

In the Mediterranean Sea, two *Squalus* species commonly occur (Serena *et al*., 2005; Serena *et al*., 2009): the spiny spurdog *S. acanthias* (Linneaus, 1758) belonging to the '*acanthias* group' and the longnose spurdog *S. blainville* (Risso, 1827) belonging to the '*mitsukurii* group'. In this Basin, in the 1980s, Muñoz-Chápuli *et al.* (1984) and Muñoz-Chápuli & Ramos (1989) also recorded a third species, the piked spurdog *S. megalops* (Macleay, 1881), commonly distributed in the Eastern Atlantic and Indo-Pacific Oceans (Ebert *et al*., 2013).

Despite the fact that *S. acanthias* shows diagnostic characters, such as the presence of white spots on the back or narrowly round to acutely angular rear tips and inner margins of the pectoral fins, which permit an easier identification and discrimination from the other two species (Bonello *et al*., 2016), *S. blainville* and *S. megalops*, do show a very similar morphology. According to Muñoz-Chápuli *et al.* (1984) and Muñoz-Chápuli & Ramos (1989), *S. blainville* and *S. megalops* can be discriminated principally based on the number of chondrocranial lateral processes, in addition to other morphological features such as teeth and dermic denticles morphology. These findings have been confirmed by Marouanì *et al*. (2012) in the Gulf of Gabès (southern Tunisia, central western Mediterranean Sea) through morphometric, meristic and genetic analyses, suggesting that *S. megalops* could be even more common than *S. blainville* in these waters. On the other hand, in a recent study, *S. blainville* was the only *Squalus* species identified in the Maltese waters (Bonello *et al*., 2016). Indeed, the authors asserted that the species identification based only on morphological characteristics can easily lead to taxonomic misidentifications, especially when multiple anatomical characters (e.g. skull and teeth morphology) are used (Bonello *et al*., 2016). Moreover, Veríssimo *et al*. (2017) reported that *S. blainville* and *S. megalops* are two names used almost interchangeably along the Eastern Atlantic and the Mediterranean Sea to identify the same species with the former mostly employed in the Mediterranean area while the latter in the Eastern Atlantic. Nevertheless, the results provided by those authors suggest that the 'true' *S. megalops* from Australia is not present in the eastern Atlantic and Mediterranean waters, but a different species that remains unidentified can occur (Veríssimo *et al*., 2017).

Considering these last studies, the present paper aims to investigate the presence of the two species around Sardinian Sea through genetic and morphometric analyses, providing new evidences in order to solve the spurdogs taxonomic confusion in the investigated region.

Materials and Methods

A total of 137 spurdogs were sampled during experimental trawl surveys (MEDITS, Mediterranean International Trawl Survey, Bertrand *et al*., 2000) and commercial hauls performed from 2010 to 2011 in Sardinian waters (Central Western Mediterranean Sea) at depths from 123 to 682 m (Fig. 1).

Once in the laboratory, specimens were measured (Total Length, TL) and weighed (Total Mass, TM). For the morphometric analysis, specimens were photographed with a digital camera (Nikon D90) in order to take 45 somatic measurements (expressed in millimetres). All measurements, including names and abbreviations, were defined according to Compagno (2001) and Last *et al*. (2007) and expressed in % of TL.

Each shark chondrocranium, after being extracted through a boiling process, was photographed in both dorsal and ventral view in order to obtain 16 measurements following Muñoz-Chápuli & Ramos (1989). Measurements were expressed in millimetres and in % of Total Length of Chondrocranium (TLC). The total number of vertebrae was counted after dissection. Teeth samples from both dental arches were extracted from each individual. Moreover, following Muñoz-Chápuli & Ramos (1989) and Marouani *et al.*, (2012), a skin portion was extracted from the lateral-dorsal area (anterior to the first dorsal spine) for the observation of dermal denticles.

According to Muñoz-Chápuli & Ramos (1989) the number of lateral processes of the chondrocranium basal plate allows the two spurdogs species *S. blainville* and *S. megalops* to be subdivided. For this reason, in the present study, specimens were subdivided into two groups: S1, hypothetically belonging to *S. blainville* (presenting a single lateral process) and S2, hypothetically belonging to *S. megalops* (presenting two lateral processes) (Fig. 2). This characteristic was preferred considering the uncertainty level typical of the other specific features suggested, subjected to corrosion, such as the teeth morphology, or characterized by a relatively high morphological variability degree due to the simultaneous presence of different development stages of the fast replacing rated structures, such as dermal denticles (Kemp, 1999).

Statistical analyses

Through a similarity matrix based on Euclidean distance, *a priori* multivariate differences in the morphological features of the species have been illustrated using the bi-plot produced after Canonical Analysis of Principal Coordinates CAP (Anderson & Willis, 2003) obtained through PRIMER (ver. PRIMER Permanova +) CAP routine. This analysis was chosen as a flexible method for constrained ordination on the basis of any distance or dissimilarity measure, which displays a cloud of multivariate points by reference to a specific *a priori* hypothesis; in our case the hypothesis was that different species of the genus *Squalus* are characterized by different morphological parameters. The routine was conducted on two

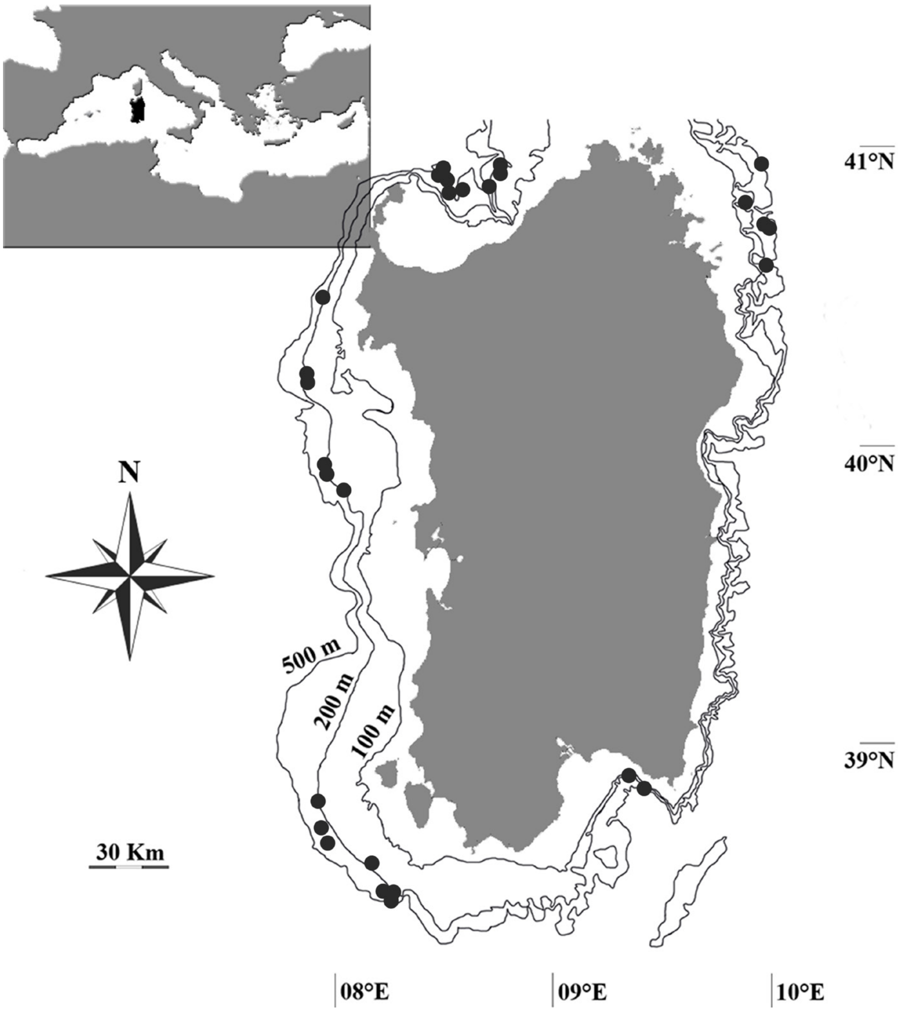

Fig. 1: Study area. The black dots represent the hauls in which specimens were caught.

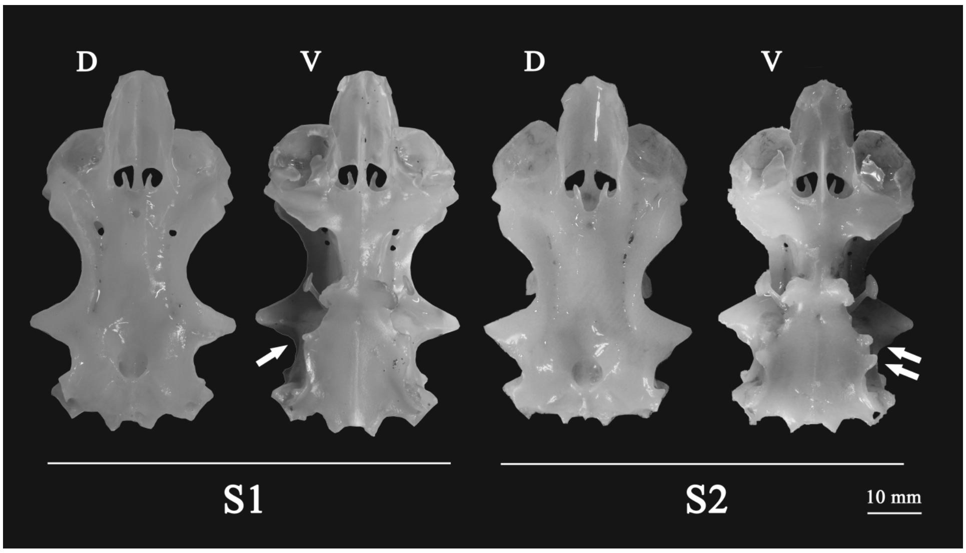

Fig. 2: Dorsal (D) and ventral (V) view of dissected chondrocrania of specimens caught in the Sardinian waters, belonging to S1 group (male, TL= 594 mm) and S2 group (male, TL= 583 mm). White arrows indicate the processes of the chondrocranium basal plate.

data matrixes (and relative similarity matrixes) describing body parameters and chondrocranium. The cross-validation, given by the same routine, was used to further confirm (or reject) the *a priori* assignment of the species.

Moreover, a *t*-Student test (Zar, 1999) was conducted in order to test for differences in chondrocranial measurements between the two groups.

Genetic analysis

A subsample of 18 individuals were selected, based on the characteristics of their chondrocranium, and genetically analysed: 13 individuals (8 males and 5 females) presented two lateral processes and 5 individuals (all males) presented a single lateral process. Total genomic DNA was extracted from the tissues using a salting-out protocol (Miller *et al*., 1988).

The primers (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'; HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') for the amplifications of mitochondrial COI gene were obtained from Folmer *et al*. (1994). The amplification was based on the following cycling parameters: 3 min at 94°C for the initial denaturation, followed by 37 cycles of 30 sec at 94°C, 45 sec at 50°C for the annealing of primers, and 60 sec at 72°C for extension, and then 4 min at 72°C for the final extension. The sequences were sequenced on both directions, aligned in MEGA v. 6 (Tamura *et al*., 2013) and translated into aminoacidic sequences using the vertebrate genetic code to exclude the occurrence of codon stop and nuclear pseudogenes. Number of haplotypes, haplotype diversity [hd], and nucleotide diversity [π] were retrieved using DnaSP v. 5.1 (Librado & Rozas, 2009). Graphically, the haplotypes were arranged in a network with PopART (http://popart.otago.ac.nz) using the Median Joining method (Bandelt *et al*., 1999).

The sequences obtained in this study were compared to COI sequences published for the three species of the genus *Squalus* reported to be present in the Mediterranean Sea (*S. acanthias*, *S. blainville*, and *S. megalops*) (Table S1). Moreover, the analyses also included sequences of the species included in Group I (*S. suckleyi*) and Group II (*S. cubensis, S. raoulensis, S. brevirostris*) in the *Squalus* phylogeny by Veríssimo *et al*. (2017). Sequences were retrieved from GenBank (https://www.ncbi.nlm.nih.gov/genbank). *Cirrhigaleus australis* was used as outgroup (Veríssimo *et al*., 2017). The list and details of the sequences used in the analyses are provided as supplementary table (Table S1).

The relationships among haplotypes were investigated with the Bayesian approach using MrBayes v. 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). In MrBayes the analyses were performed using two parallel runs of 2 million generations each, using four chains, sampling every 100 generations, burnin 0.25, and saving branch lengths. The performance of the analyses was evaluated using the software Tracer v. 1.6 (Rambaut *et al*., 2014). The tree was visualized with MEGA.

Results

According to the number of processes in the chondrocranium, out of the total 137 spurdogs, 19 were pooled in the S1 group (one process, 15 males and 4 females) and 118 were pooled in the S2 group (two processes, 55 males and 63 females) (Table 1).

Chondrocranium description

The chondrocranium measurements obtained are reported in Table 2 for S1 and Table 3 for S2. The distance between the posterior tip and the precerebral fenestra (PPF) was 62.93 and 63.33 in %TLC in S1 and S2 group respectively. In S1, the width across nasal capsules and the interorbital width were 54.74 and 28.25 in %TLC, while in S2 the same measurements were 55.34 and 28.34 in %TLC. Finally, the distance between the basal plate processes was equal to 31.32 in %TLC in S1 and 31.48 in %TLC in S2.

No significant differences in all chondrocranial measurements were found between the two groups (*t*-test $p>0.05$) (Table 4).

Morphological description

Biometric data from S1 and S2 is reported in Tables 5 and 6, respectively. All studied specimens (S1 and S2) showed a fusiform and elongated body (Fig. 4). In both groups the head appeared slightly triangular from lateral view with a moderately long and sharp snout. The mouth,

Table 1. Number (N) and Total length (TL) range and mean (±SD, standard deviation) of the samples used in this study, for each sex and group (S1 and S2).

Sex	S1			S2		
	N	Range TL (mm)	Mean TL (±SD)	N	Range TL (mm)	Mean TL (±SD)
Males	15	249-594	396.7±90.0	55	272-595	399.8±83.2
Females	4	361-792	523.3±234.35	63	207-834	433.3±140.9
Total	**19**	249-792	416.7±122.9	**118**	207-834	415.4±114.5

Table 2. Proportional cranial dimensions expressed as percentages of TLC (±SD) for specimens belonging to S1 group, compared with what reported for *S. blainville* by other authors in other world regions.

References		Muñoz-Chápuli & Ramos (1989)		Marouani *et al.* (2012)		Bonello *et al.* (2016)		Present study	
Study area		Eastern Atlantic, Mediterranean		Tunisian waters (Central Mediterranean)		Maltese waters (Central Mediterranean)		Central Western Mediterranean	
Measurements	Codex	*S. blainville*		*S. blainville*		One-lobed chondrocranial		**S1**	
Total length of chondrocranium range (mm)	TLC	57.9-115.7		48.5-88.5				47.3-104.5	
		N	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**
Posterior tip-precerebral fenestra	PPF	9	63.85±1.36	23	65.46±3.02	23	61.58±3.34	16	62.93±2.84
Length precerebral fenestra	LPF	9	36.18±1.77	23	33.41±2.93	23	35.52±5.74	16	28.11±1.87
Width precerebral fenestra	WPF	9	14.95±1.45	23	19.12±3.05	23	23.08±5.49	16	19.62±1.79
Width across nasal capsules	WNC	9	54.39±1.73	23	54.35±2.36	23	54.47.4.83	16	54.74±1.93
Interorbital width	IOW	9	31.18±1.17	23	31.77±2.32	23	33.10±5.48	16	28.25±1.33
Postorbital width	PsOW	9	56.49±1.36	23	57.02±2.76	23	58.98±6.99	16	54.55±1.54
Distance between orbital processes	OPD	9	42.60±1.72	23	36.03±2.77	23	35.33±1.87	16	36.48±1.02
Width between pterotic processes	PtPW	9	37.52±1.53	22	39.52±2.33	23	-	16	37.65±0.96
Width between hyomandibular facets	HFW	9	45.61±1.16	22	45.73±3.39	23	43.85±2.56	16	44.69±0.99
Posterior tip-rostral keel	PtRK	9	64.79±1.35	22	68.10±2.76	23	70.65±6.35	16	63.83±1.97
Length rostral keel	RKL	9	20.05±2.94	22	19.96±2.13	23	14.32±2.16	16	22.58±1.64
Subethmoidean width	SEtW	9	17.22±1.12	22	14.37±2.12	23	17.01±2.16	16	14.70±1.11
Width basal angle	BAW	9	21.20±1.77	22	19.10±1.85	23	22.84±4.03	16	19.12±1.74
Length basal plate	BpL	9	39.53±1.62	22	46.61±2.53	23	-	16	40.12±1.36
Width between processes of basal plate	BBpW	9	30.39±1.01	22	31.37±2.25	23	-	16	31.32±0.68

Table 3. Proportional cranial dimensions expressed as percentages of TLC (±SD) for specimens belonging to S2 group, compared with what reported for *S. megalops* by other authors in other world regions.

References		Muñoz-Chápuli & Ramos (1989)		Marouani *et al.* (2012)		Bonello *et al.* (2016)		Present study	
Study area		Eastern Atlantic, Mediterranean		Tunisian waters (Central Mediterranean)		Maltese waters (Central Mediterranean)		Central Western Mediterranean	
Measurements	Codex	*S. megalops*		*S. megalops*		Two-lobed chondrocranial		**S2**	
Total length of chondrocranium range (mm)	TLC	32.0-83.8		40.0-87.0				34.3-109.2	
		N	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**
Posterior tip-precerebral fenestra	PPF	22	65.08±1.14	17	67.03±3.25	146	62.78±8.42	102	63.33±3.13
Length precerebral fenestra	LPF	22	35.7±1.02	17	31.95±1.61	146	35.75±4.25	102	28.13±1.72
Width precerebral fenestra	WPF	22	16.99±1.84	17	20.33±1.90	146	21.73±5.15	102	19.53±1.61
Width across nasal capsules	WNC	21	50.93±2.44	16	51.92±3.59	146	53.63±13.50	102	55.34±1.93
Interorbital width	IOW	22	28.57±1.26	16	28.72-1.79	146	31.85±5.75	102	28.34±1.19

(continued)

Table 3 continued

References		Muñoz-Chápuli and Ramos (1989)		Marouani *et al.* (2012)		Bonello *et al.* (2016)		Present study	
Study area		Eastern Atlantic, Mediterranean		Tunisian waters (Central Mediterranean)		Maltese waters (Central Mediterranean)		Central Western Mediterranean	
Measurements	Codex	*S. megalops*		*S. megalops*		Two-lobed chondrocranial		**S2**	
Total length of chondrocranium range (mm)	TLC	32.0-83.8		40.0-87.0				34.3-109.2	
Postorbital width	PsOW	22	55.38±2.00	16	58.19±2.38	146	57.77±7.64	102	54.87±1.70
Distance between orbital processes	OPD	19	32.85±2.58	16	36.31±2.55	146	36.04±4.44	102	36.73±2.72
Width between pterotic processes	PtPW	22	37.3±1.24	16	39.80±2.16	146	-	102	38.06±1.58
Width between hyomandibular facets	HFW	22	45.62±1.16	16	47.06±2.04	146	43.74±3.65	102	44.96±1.27
Posterior tip-rostral keel	PtRK	22	63.6±3.12	16	68.43±3.21	146	68.79±14.58	102	64.21±2.03
Length rostral keel	RKL	22	22.82±2.69	16	21.02±3.16	146	14.97±5.89	102	21.89±1.66
Subethmoidean width	SEtW	22	15.57±1.31	16	13.60±1.54	146	16.33±3.53	102	15.08±1.20
Width basal angle	BAW	22	17.82±1.36	16	20.01±1.56	146	21.86±5.39	102	19.43±1.99
Length basal plate	BpL	22	40.56±1.09	16	46.24±1.75	146	-	102	40.41±1.78
Width between processes of basal plate	BBpW	22	31.08±0.85	17	33.39±3.61	146	-	102	31.48±1.12

Table 4. Comparison between chondrocranial measurements of spurdogs belonging to S1 and S2 groups from the Sardinian waters.

Measurements	Codex	*t*-test	p-value
Total length of chondrocranium range (mm)	TLC		
Posterior tip-precerebral fenestra	PPF	-0.48	0.63
Length precerebral fenestra	LPF	-0.03	0.97
Width precerebral fenestra	WPF	0.19	0.85
Width across nasal capsules	WNC	-1.15	0.25
Interorbital width	IOW	-0.30	0.76
Postorbital width	PsOW	-0.69	0.49
Distance between orbital processes	OPD	-0.36	0.72
Width between pterotic processes	PtPW	-1.01	0.31
Width between hyomandibular facets	HFW	-0.83	0.41
Posterior tip-rostral keel	PtRK	-0.71	0.48
Length rostral keel	RKL	1.55	0.12
Subethmoidean width	SEtW	-1.17	0.24
Width basal angle	BAW	-0.58	0.56
Length basal plate	BpL	-0.63	0.53
Width between processes of basal plate	BBpW	-0.59	0.56

deeply convex, was situated on the ventral side and fitted 0.82 times in preoral length (POR) in S1 and 0.85 times in S2. The two groups shared the same teeth morphology (Fig. 3): teeth were similar in both jaws, looking small and compressed; the only sharp cuspid present seemed deeply turned towards the jaw termination, whereas the opposite margin appeared moderately rounded. Both groups showed the same dental formula (12-13 / 12-13 in the upper jaw and 11-13 / 11-13 in the lower jaw).

Nostrils looked narrow, with well-developed nasal flaps. These structures, composed substantially by two lobes, were quite similar in the two groups with the external lobe considerably bigger than the internal one.

In both groups, the eye appeared relatively wide and

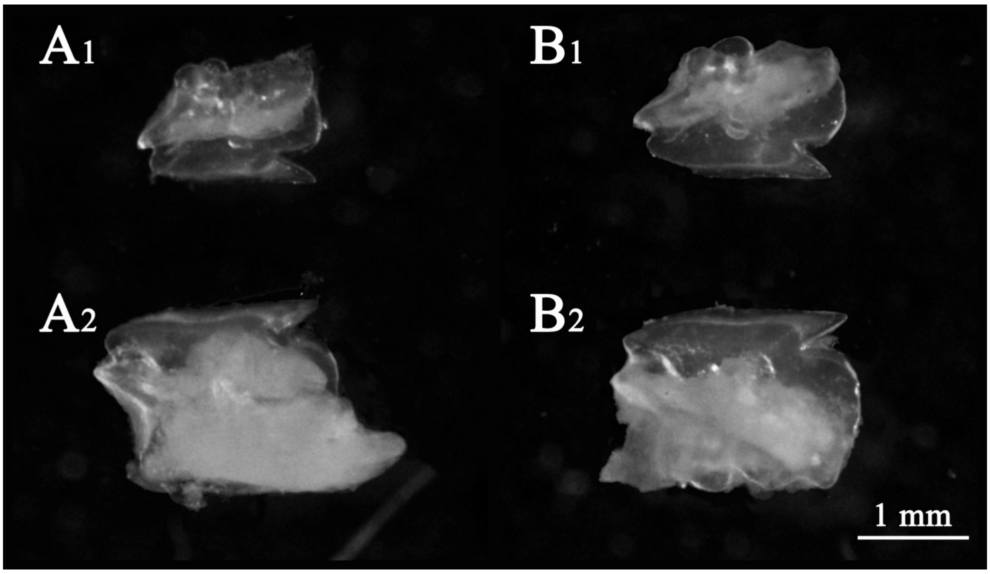

Fig. 3: Teeth of *Squalus sp.* from the Sardinian waters extracted from a S1 group male TL= 446 mm (teeth belonging to the higher and the lower jaw, A1 and A2 respectively) and a S2 group male TL= 470 mm (teeth belonging to the higher and the lower jaw, B1 and B2 respectively).

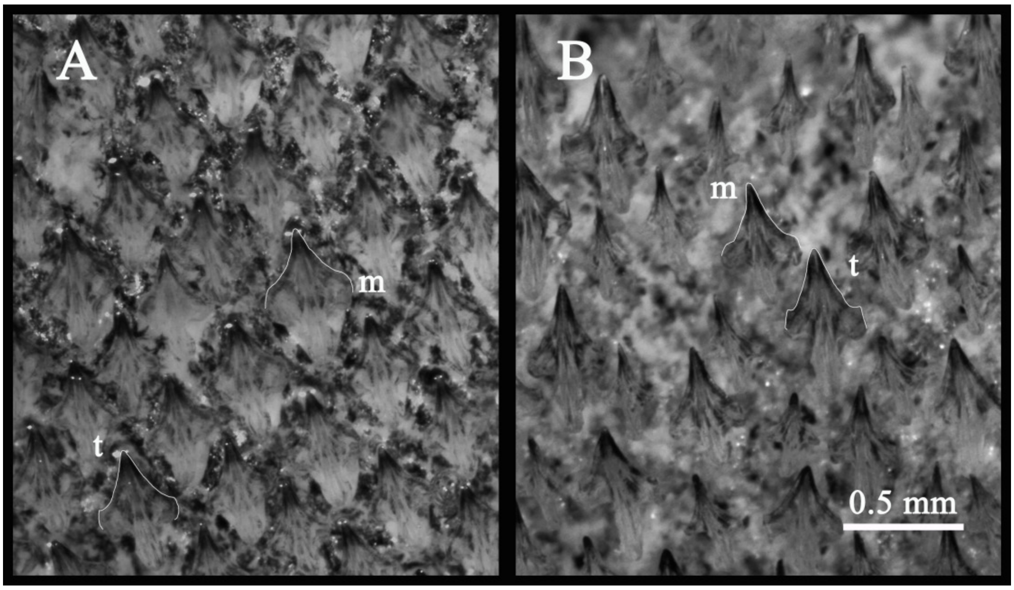

Fig. 4: Dermal denticles of *Squalus sp*. from the Sardinian waters. S1 group male TL= 552 mm (A) and a S2 group male TL= 634 mm (B). In both images an example of monocuspid (m) and tricuspid (t) typed denticle was highlighted.

more developed in length than in height; it fitted 4.70 and 4.54 times in head length (length at the 5th gill opening, PG5) for S1 and S2 group, respectively. The first dorsal fin was situated behind the pectoral fin and the pre-first dorsal length fitted 3.31 times in TL in S1 and 3.32 times in S2. In S1 and S2 groups, the first dorsal fin appeared more developed in length than in height; it fitted in length 1.79 times its height in both shark groups. Moreover, the first dorsal fin looked bigger than the second one, both in length (1.26 times in S1 and 1.23 times in S2) and in height (1.87 times in S1 and 1.80 times in S2). The second dorsal fin length fitted 2.65 and 2.62 times its height in S1 and S2 respectively, looking mainly developed in length than in height, similarly to what was observed for the first dorsal fin. A strong spine with a triangular section was observed at the origin of each dorsal fin. The first dorsal spine length fitted 0.55 times in the fin base in both shark groups, while the second dorsal spine length

Table 5. Proportional dimensions expressed as percentages of TL (±SD) for specimens belonging to **S1** group, compared with what reported for *S. blainville* by other authors in other world regions.

		Muñoz-Chápuli and Ramos (1989)		Marouani *et al.* (2012)		Garrick (1960)		Merrett (1973)		Muñoz-Chápuli *et al.* (1984)		Present study	
		Eastern Atlantic, Mediterranean		Tunisian waters (Central Mediterranean)		New Zealand		Equatorial western Indian Ocean		Mediterranean coasts of Spain		Central Western Mediterranean	
		S. blainville		*S. blainville*		*S. blainville*		*S. blainville*		*S. blainville*		**S1**	
N specimens		15		9		3		4		6		19	
Size range (mm, TL)		402-890		630-960		545-1008		460-679		560-730		249-792	
	Codex	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**
Pre-inner nostril length	PNR	15	3.41±0.65	9	4.30±0.15	-	-	4	4.22±0.45	-	-	16	2.31±0.35
Preorbital length	POB	15	5.55±0.78	9	6.19±0.53	-	-	-	-	-	-	16	4.44±0.44
Preoral length	POR	8	8.40±0.44	9	8.22±0.16	-	-	4	10.52±0.15	-	-	16	8.26±0.72
Prebranchial length	PG1	15	16.68±0.78	9	17.02±0.59	3	17.16±0.25	4	19.5±0.4	-	-	16	15.47±1.01
5th gill opening	PG5	15	20.50±0.95	-	-	-	-	-	-	-	-	16	19.97±1.01
Pre-first dorsal length	PD1	14	28.53±0.97	9	28.24±1.12	3	32.57±0.81	4	30.0±1.19	-	-	16	30.23±1.09
Pre ventral length	SVL	14	50.57±1.37	9	50.40±3.13	3	51±2.64	4	47.65±1.10	-	-	16	52.84±2.48
Pre caudal length	PCL	15	78.93±0.89	9	79.06±0.62	3	79.57±1.83	4	77.95±1.32	-	-	16	80.51±2.42
Nostril-Labial furrow	NLF	-	-	-	-	-	-	-	-	-	-	16	4.63±0.68
Interdorsal space	IDS	14	25.82±2.11	9	27.05±0.79	3	28.34±0.66	4	27.05±0.56	-	-	16	25.02±1.30
Dorsal caudal space	DCS	15	11.03±0.47	9	10.32±0.54	3	9.77±0.65	4	10.87±0.5	-	-	16	11.03±0.75
Pectoral-pelvic space	PPS	15	22.89±1.55	9	21.75±0.86	-	-	-	-	-	-	16	24.69±1.47
Pelvic and caudal	PCA	12	27.26±1.13	-	-	-	-	-	-	-	-	16	28.48±3.00
Internarial space	INW	15	4.53±0.48	9	4.16±0.26	-	-	-	-	6	4.48±0.29	16	4.59±0.43
Between outer corners	ONW	15	6.79±0.59	-	-	-	-	-	-	-	-	16	8.28±0.86
Nostril length	NOW	15	1.48±0.28	-	-	-	-	-	-	-	-	16	1.87±0.22
Mouth width	MOW	15	7.49±0.89	9	7.29±0.53		5.83±0.11	4	6.72±0.7	-	-	16	10.08±0.83
Lenght of preoral cleft	MOL	15	2.85±0.92	-	-	-	-	-	-	-	-	16	2.33±0.33
Eye length	EYL	15	4.03±0.39	9	3.86±0.23	3	4.37±0.21	4	5.22±0.12	-	-	16	4.24±0.42
Spiracles													

(continued)

Table 5 continued

		Muñoz-Chápuli and Ramos (1989)		Marouani *et al.* (2012)		Garrick (1960)		Merrett (1973)		Muñoz-Chápuli *et al.* (1984)		Present study	
		Eastern Atlantic, Mediterranean		Tunisian waters (Central Mediterranean)		New Zealand		Equatorial western Indian Ocean		Mediterranean coasts of Spain		Central Western Mediterranean	
		S. blainville		*S. blainville*		*S. blainville*		*S. blainville*		*S. blainville*		**S1**	
N specimens		15		9		3		4		6		19	
Size range (mm, TL)		402-890		630-960		545-1008		460-679		560-730		249-792	
	Codex	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**
Distance between tips	ISP	15	8.11±0.90	-	-	-	-	-	-	-		16	8.45±0.32
Gills													
First gill-slit height	GS1	15	1.95±0.23	9	1.85±0.26	3	1.9±0.42	4	1.77±0.27			16	2.01±0.25
Third gill-slit height	GS3	15	2.23±0.26	9	2.20±0.17				1.85±0.31			16	2.11±0.30
Fifth gill-slit height	GS5	15	2.49±0.42	9	2.07±0.27	3	2.33±0.21	4	2.04±0.12			16	2.26±0.21
Intergill length (1st and 5th)	ING	15	4.18±0.63	9	4.66±0.67							16	4.47±0.39
First dorsal fin													
First dorsal length	D1L	-	-	9	13.32±0.76							16	13.55±0.55
First dorsal base length	D1B	14	8.44±1.54	9	8.03±0.25	3	6.07±0.68	4	7.22±0.54			16	7.63±0.41
First dorsal height	D1H	15	8.09±0.61	9	7.07±0.7	3	8.03±0.15	4	8.6±0.91			16	7.56±0.66
First dorsal inner margin	D1I	15	6.10±0.53	9	5.40±0.28							16	5.87±0.44
First dorsal spine length	D1ES	14	4.32±0.71	9	5.06±0.3				4.15±1.30			16	4.19±0.44
Second dorsal fin													
Second dorsal length	D2L	-	-	9	9.45±0.31							16	10.73±1.27
Second dorsal base length	D2B	14	6.42±1.27	9	5.13±0.41	3	4.8±0.78	4	4.55±0.25			16	5.94±0.63
Second dorsal height	D2H	15	4.46±0.56	-	-							16	4.04±0.58
Second dorsal inner margin	D2I	15	4.79±0.46	9	4.29±0.21			4	4.2±1.01			16	4.57±0.89
Second dorsal spine length	D2ES	13	4.92±0.94	9	5.22±0.41					6	4.69±0.45	16	5.89±0.57
Pectoral fin													
Pectoral length	P1L			9	11.46±0.52							16	14.62±1.61
Pectoral base length	P1B	15	6.77±0.70	9	5.85±0.41			4	5.7±0.46			16	4.94±0.44
Pectoral anterior margin	P1A	15	13.99±1.02	9	13.31±0.95	3	14.43±0.91	4	15.05±0.91	6	13.63±0.85	16	13.20±0.51

(continued)

Table 5 continued

		Muñoz-Chápuli and Ramos (1989)		Marouani *et al.* (2012)		Garrick (1960)		Merrett (1973)		Muñoz-Chápuli *et al.* (1984)		Present study	
		Eastern Atlantic, Mediterranean		Tunisian waters (Central Mediterranean)		New Zealand		Equatorial western Indian Ocean		Mediterranean coasts of Spain		Central Western Mediterranean	
		S. blainville		*S. blainville*		*S. blainville*		*S. blainville*		*S. blainville*		**S1**	
N specimens		15		9		3		4		6		19	
Size range (mm, TL)		402-890		630-960		545-1008		460-679		560-730		249-792	
	Codex	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**	**N**	**Mean±SD**
Pectoral posterior margin	P1P	15	11.10±0.80	9	11.40±0.90							16	11.29±0.82
Pectoral inner margin	P1I	15	7.18±0.51	9	6.24±0.36					6	7.08±0.39	16	10.81±0.97
Pelvic fin													
Pelvic anterior margin	P2A	15	5.86±0.72	9	4.76±0.90			4	5.75±0.36			16	6.42±1.06
Pelvic Lenght	P2L	15	9.69±0.68	9	9.05±1.49					6	9.82±0.84	16	11.66±0.53
Caudal fin													
Dorsal caudal margin	CDM	15	21.10±0.54	9	20.74±0.90							16	19.77±1.55
Preventral caudal margin	CPV	14	11.08±0.70	9	10.15±0.99							16	9.78±0.67
Trunk at pectoral origin:													
Trunk width	TRW	8	11.72±0.94	9	10.00±0.94							16	12.88±0.71

appeared as long as the fin base, fitting 0.99 and 0.98 times the second dorsal base in S1 and S2, respectively. The interdorsal space fitted 1.21 times the pre-first dorsal length in S1 and 1.22 times in S2. The pectoral fins of both groups presented a large and almost straight anterior margin, culminating with a deeply rounded apex and their inner margin ends with a small rounded tip. The pectoral fin base fitted 2.67 and 2.68 times in the anterior margin length in S1 and S2, respectively. The pelvic fins instead were small and triangular with a rounded apex and almost straight anterior and posterior margin. The pelvic fin anterior margin fitted 1.82 times in fin length in S1 and 1.83 times in S2. The caudal peduncle appeared well developed with two solid lateral keels that origins behind the second dorsal base termination and ends below caudal fin insertion. The dorsal-caudal space fitted 2.27 times in the interdorsal space in S1 and 2.23 times in S2. The caudal fin presented an extended dorsal caudal margin (19.77 in %TL in S1; 20.41 in %TL in S2) without sub-terminal notch.

Both spurdog groups showed a uniform grey-brown coloration on the dorsal side while the ventral one and all the fins rear margins appeared paler. The eyes were bright green when observed in live specimens. In Figure 4 dermal denticles obtained from S1 (Fig.4a) and S2 (Fig. 4b) specimens are showed. These structures appeared mostly monocuspid typed but, in every group it was possible to simultaneously discriminate some tricuspid denticles.

Analysis of Principal Coordinates CAP

The bi-plot produced after CAP analysis emphasized no clear segregation among the *a priori* assigned groups (S1 and S2), with a higher overlapping for the chondrocranium parameters compared to the somatic ones (Fig. 5b and Fig. 5a respectively). The cross-validation also showed an elevated percentage of misclassification (i.e., 41.03% for chondrocranium and 37.5 for somatic), further confirming that a considerable portion of samples did not follow the *a priori* grouping.

Genetic analysis

A 609 bp fragment of COI gene was obtained for the 18 individuals revealing a total of 7 haplotypes (Hd: 0.765), differing in 6 nucleotide positions (π: 0.00245).

Table 6. Proportional dimensions expressed as percentages of TL (±SD) for specimens belonging to **S2** group, compared with what reported for *S. megalops* by other authors in other world regions.

		Marouani *et al.* (2012)		Muñoz-Chápuli & Ramos (1989)	Last *et al.* (2007)		Last *et al.* (2007)		Last *et al.* (2007)		Chápuli *et al.* (1984)	Present study
		Tunisian waters (Central Mediterra-nean)		Eastern Atlantic, Mediterranean	Southeastern Aus-tralia		Queensland		Western Aus-tralia		Mediterranean, coasts of Mo-rocco	Central Western Mediterranean
		S. megalops		*S. megalops*	*S. megalops*		*S. megalops*		*S. megalops*		*S. megalops*	**S2**
N specimens		9		34	6		3		4		24	118
Size range (mm, TL)		330-695		318-742	373-527		328-384		414-541		485-680	207-834
	Codex	**Mean**	**Min-Max**	**Mean±SD**	**Mean**	**Min-Max**	**Mean**	**Min-Max**	**Mean**	**Min-Max**	**Mean±SD**	**Mean±SD**
Pre-inner nostril length	PNR	4.23	3.88-4.48	3.49±0.57	3.9	3.7-4.1	4.3	4.2-4.4	4.2	3.9-4.4	-	2.47±0.46
Preorbital length	POB	6.83	6.18-7.27	6.01±0.51	7	6.4-7.5	7.2	7-7.4	7	6.4-7.4		4.53±0.73
Preoral length	POR	8.84	7.98-9.39	8.65±0.83	9.1	8.6-9.9	9.7	9.3-9.9	9.2	8.9-9.7	-	8.84±1.77
Prebranchial length	PG1	17.88	16.76-19.79	16.62±0.88	18.5	17.7-19.8	18.9	18.6-19.2	18.3	17.8-19.1	-	15.89±1.68
5th gill opening	PG5	-	-	19.97±1.03	-	-	-	-	-	-	-	20.19±1.61
Pre-first dorsal length	PD1	29.48	28.41-30.70	28.99±0.89	30.2	29.1-31.6	30.6	29.9-31.6	29.6	29.1-30.2	-	30.07±2.26
Pre ventral length	SVL	48.32	46.29-49.85	49.18±2.06	48.5	47.6-50.1	46.5	46.1-47.2	47.9	45.9-50.4	-	53.33±3.30
Pre caudal length	PCL	78.69	76.95-80.34	78.92±1.08	77.7	76.1-79.3	78.5	77.8-78.9	78.4	77.7-79.2	-	80.12±3.69
Nostril-Labial furrow	NLF											
Interdorsal space	IDS	25.42	22.77-27.59	24.49±1.9	24.8	24-25.3	24.6	23.2.-25.8	25.3	23.7-26	-	24.73±1.89
Dorsal-caudal space	DCS	10.93	9.49-11.81	11.16±0.60	10.4	9.5-10.9	12.2	11.5-12.7	10.7	9.9-12	-	11.09±1.08
Pectoral-pelvic space	PPS	21.72	20.29-23.46	23.10±2.64	22.3	20.9-26.1	19.1	18-20.3	22.6	20.5-24.6		24.42±2.29
Pelvic-caudal space	PCA			27.73±1.44								27.91±2.49
Internarial space	INW	3.98	3.71-4.19	3.83±0.26	4.5	4.3-4.7	4.7	4.6-4.9	4.5	4.2-4.8	3.82±0.20	4.66±0.44
Between outer corners	ONW			6.68±0.57								8.39±0.81
Nostril length	NOW			1.60±0.20								1.89±0.26

(continued)

Table 6 continued

		Marouani *et al.* (2012)		Muñoz-Chápuli & Ramos (1989)	Last *et al.* (2007)		Last *et al.* (2007)		Last *et al.* (2007)		Chápuli *et al.* (1984)	Present study
		Tunisian waters (Central Mediterranean)		Eastern Atlantic, Mediterranean	Southeastern Australia		Queensland		Western Australia		Mediterranean, coasts of Morocco	Central Western Mediterranean
		S. megalops		*S. megalops*	*S. megalops*		*S. megalops*		*S. megalops*		*S. megalops*	**S2**
N specimens		9		34	6		3		4		24	118
Size range (mm, TL)		330-695		318-742	373-527		328-384		414-541		485-680	207-834
	Codex	**Mean**	**Min-Max**	**Mean±SD**	**Mean**	**Min-Max**	**Mean**	**Min-Max**	**Mean**	**Min-Max**	**Mean±SD**	**Mean±SD**
Mouth width	MOW	7.86	7.48-8.53	7.69-0.45	8.1	7.8-8.6	8.3	8-8.5	8.2	7.8-8.6	-	10.36±1.11
Lenght of preoral cleft	MOL	-	-	3.30±1.10	-	-	-	-	-	-	-	2.50±0.49
Eye length	EYL	4.19	3.59-4.84	4.27±0.53	4.8	4.4-5.4	5	4.9-5	4.8	4.3-5.3	-	4.44±0.68
Spiracles												
Distance between tips	ISP			8.10±0.60								8.55±0.50
Gills												
First gill-slit height	GS1	1.99	1.51-2.60	2.00±0.30	2.3	2-2.4	1.9	1.8-1.9	2.2	1.9-2.4	-	2.10±0.32
Third gill-slit height	GS3			2.09±0.24								2.16±0.31
Fifth gill-slit height	GS5	2.09	1.76-2.53	2.46±0.39	2.4	2.1-2.5	2.5	2.3-2.6	2.2	1.8-2.4	-	2.32±0.29
Intergill length (1st and 5th)	ING			4.48±0.92								4.39±0.57
First dorsal fin												
First dorsal length	D1L	13.56	13.09-14.14	-	14.4	13.8-15.1	13.3	12.7-13.7	14	13.3-14.9	-	13.56±1.10
First dorsal base length	D1B	7.63	7.27-8.02	8.06±0.75	8.2	7.9-8.9	7.6	7.2-8	8.3	7.7-8.9		7.63±0.76
First dorsal height	D1H	6.06	5.60-6.56	8.48±0.81	7	6.1-7.4	6.4	6.2-6.6	7.2	7-7.5	-	7.57±0.88
First dorsal inner margin	D1I	5.68	5.12-6.25	6.72±0.52	6.3	6.1-6.6	5.7	5.7-5.7	5.9	5.4-6.3	-	5.85±0.74
First dorsal spine length	D1ES	4.58	4.09-5.08	4.63±0.49	3	2.4-3.3	3	2.9-3.2	3.3	3-3.4	-	4.20±0.65
Second dorsal fin												
Second dorsal length	D2L	10.26	9.10-11.60	-	12	11-12.7	12.1	11.6-12.8	12.2	11.8-12.8		11.01±0.97
Second dorsal base length	D2B	5.28	4.64-6.13	6.98±1.40	7.1	6.4-7.5	7.2	6.9-7.6	7.5	7.1-8.2		6.07±0.73
Second dorsal height	D2H	3.49	3.03-4.23	5.20±1.1	4	3.6-4.6	3.7	3.2-4	3.9	3.7-4.3		4.19±0.55
Second dorsal inner margin	D2I	4.97	4.47-5.50	5.47±0.38	4.9	4.5-5.3	4.9	4.5-5.1	4.9	4.7-5	-	4.70±0.70
Second dorsal spine length	D2ES	5.98	5.33-5.56	5.37±0.92	4.3	3.6-5	4.6	4-5	4.5	4.2-4.6	5.61±0.67	6.00±0.67

(continued)

Table 6 continued

		Marouani *et al.* (2012)		Muñoz-Chápuli & Ramos (1989)	Last *et al.* (2007)		Last *et al.* (2007)		Last *et al.* (2007)		Chápuli *et al.* (1984)	Present study
		Tunisian waters (Central Mediterranean)		Eastern Atlantic, Mediterranean	Southeastern Australia		Queensland		Western Australia		Mediterranean, coasts of Morocco	Central Western Mediterranean
		S. megalops		*S. megalops*	*S. megalops*		*S. megalops*		*S. megalops*		*S. megalops*	**S2**
N specimens		9		34	6		3		4		24	118
Size range (mm, TL)		330-695		318-742	373-527		328-384		414-541		485-680	207-834
	Codex	**Mean**	**Min-Max**	**Mean±SD**	**Mean**	**Min-Max**	**Mean**	**Min-Max**	**Mean**	**Min-Max**	**Mean±SD**	**Mean±SD**
Pectoral fin												
Pectoral length	P1L	12.95	11.94-13.63									14.59±1.23
Pectoral base length	P1B	5.61	5.15-6.22	6.51±0.76	5.3	4.4-5.7	5.3	4.9-5.8	4.9	4.4-5.3	-	4.94±0.59
Pectoral anterior margin	P1A	14.12	13.03-15.25	15.3±0.83	14.3	13.6-14.9	12.5	12.3-12.6	14.3	13.7-15.1	15.26±0.96	13.28±1.10
Pectoral posterior margin	P1P	11.31	10.17-12.32	12.20±0.97	11.6	10.8-12.7	10.4	9.6-10.9	11.2	10.3-12.3		11.21±0.94
Pectoral inner margin	P1I	7.40	6.61-8.26	9.34±0.69	8.2	7.4-9.2	8.4	7.7-8.8	9	8.4-9.7	9.29±0.66	10.76±0.99
Pelvic fin												
Pelvic anterior margin	P2A	5.24	5.02-5.72	6.36±0.65								6.34±1.39
Pelvic Lenght	P2L	10.38	9.56-11.32	11.21±1.15	10.5	9.9-11.5	10.6	9.9-11.2	10.4	9.9-10.8	11.55±0.95	11.61±0.73
Caudal fin												
Dorsal caudal margin	CDM	19.43	16.38-21.82	21.36±0.76	20.9	20-21.4	20.1	19.3-20.9	20.6	20.2-21.1		20.41±1.52
Preventral caudal margin	CPV	9.54	8.45-10.80	11.39±1.14	11	10.5-11.3	10.6	10.4-10.7	10.9	10.7-11		10.02±0.84
Trunk at pectoral origin:												
Trunk width	TRW	10.36	8.55-11.84	11.29±0.59	12.1	11.2-13.2	10.8	10.3-11.7	12.2	11.3-14.5		12.85±1.09

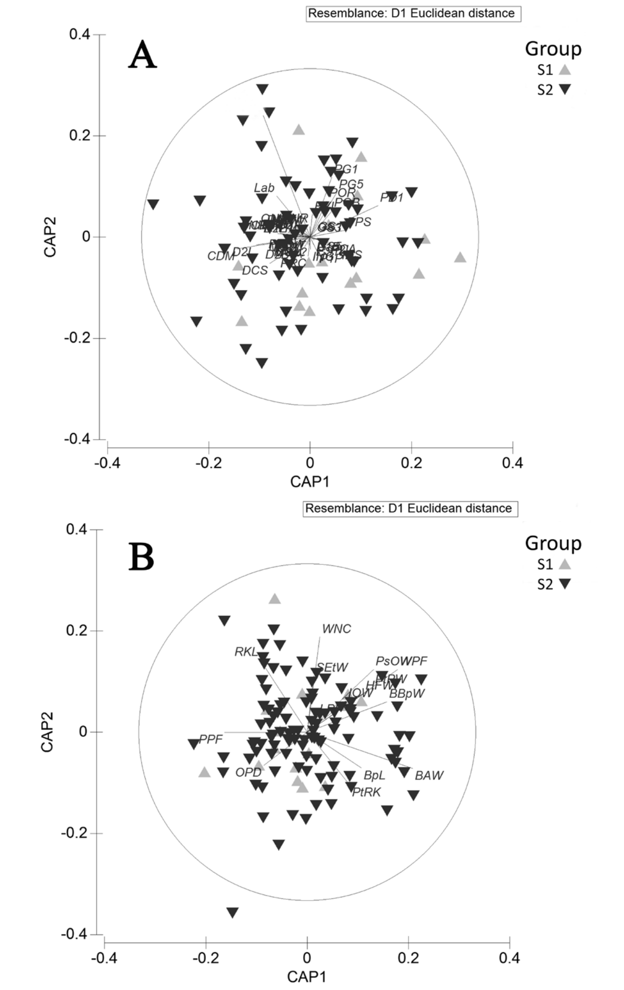

Fig. 5: Biplot produced using CAP analysis for somatic (A) and chondrocranial (B) measurements.

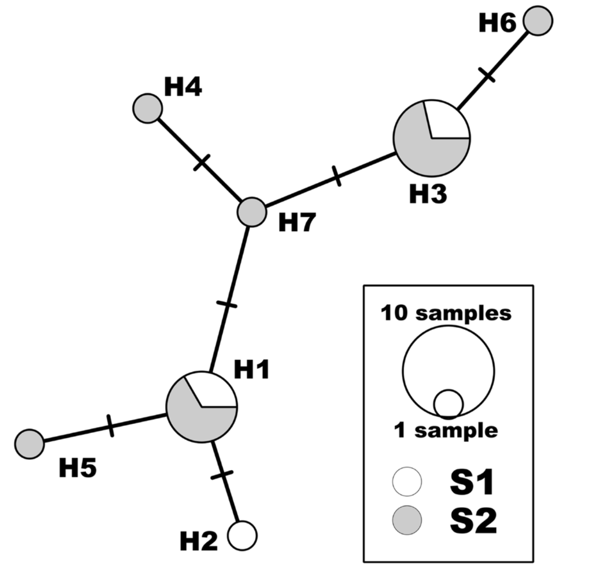

Fig. 6: Median Joining network of the COI haplotypes. Each circle represents a haplotype and the area of the circle is proportional to the haplotype frequency. In white are shown the sequences of individuals with a single lateral process (S1), and in grey the sequences of individuals with double lateral processes (S2). All mutational steps are equal to 1 and represented with a vertical line. Haplotype codes correspond to sequences MF828596-MF828613 (Table S1).

All the newly generated COI sequences were deposited in GenBank (Accession numbers: MF828596-MF828613). The haplotype network (Fig. 6) showed the occurrence of two common haplotypes H1 and H3 (shared by 6 and 7 individuals, respectively), and five private haplotypes (H2, H4-H7). Both haplotypes H1 and H3 were shared by individuals with single and double lateral processes.

In the Bayesian tree (Fig. 7), the sequences of *Squalus* clustered in ten highly supported clades (c1-c10). All the newly obtained sequences of the Sardinian *S. blainville* were in the same clade (c2) with *S. blainville* individuals from the Mediterranean and the Eastern Atlantic. They were clearly different from the sequences of *S. megalops* (c3), *S. raoulensis* (c4), *S. brevirostris* (c5), and *S. cubensis* (c7). Clade c6 comprised a single divergent sequence of a specimen from Libya, originally identified as *S. blainville* (Kousteni *et al*., 2016). Besides, sequences from Sardinia were strongly divergent from sequences in clades c1 (*S. acanthias* and *S. suckleyi*), and c8-c10 (individuals originally identified as S. *megalops* or *S. blainville* but belonging to distinct species still waiting formal description; Veríssimo *et al*., 2017).

Discussion

In the present study, although the observation of chondrocranial lateral processes initially allowed the investigated specimens to be subdivided into two groups, both morphological and genetic analysis revealed the presence of only one spurdog species in the Sardinian waters, the longnose spurdog (*S. blainville*). Indeed, the comparison of chondrocranial and body morphology of the spurdog specimens examined indicated that none of the considered measurements could discriminate the two squalid groups.

Comparing our results with the available data from literature, chondrocranial morphological measurements recorded in the present study were mostly coherent with others reported for *S. blainville* in other Mediterranean areas. The length of the precerebral fenestra (LPF) represented the only exception, which was smaller for both groups.

As far as the somatic data is concerned, in general, no major differences were found except for few measurements, regarding in particular the head and snout region. Indeed, for the S1 group pre-inner nostril length (PNR) and preorbital length (POB) appeared minor in terms of %TL than what was reported for *S. blainville* in the Mediterranean Sea by Muñoz-Chápuli *et al*. (1984); Muñoz-Chápuli & Ramos (1989) and Marouani *et al*. (2012), in the New Zealand waters (Garrick, 1960) and the

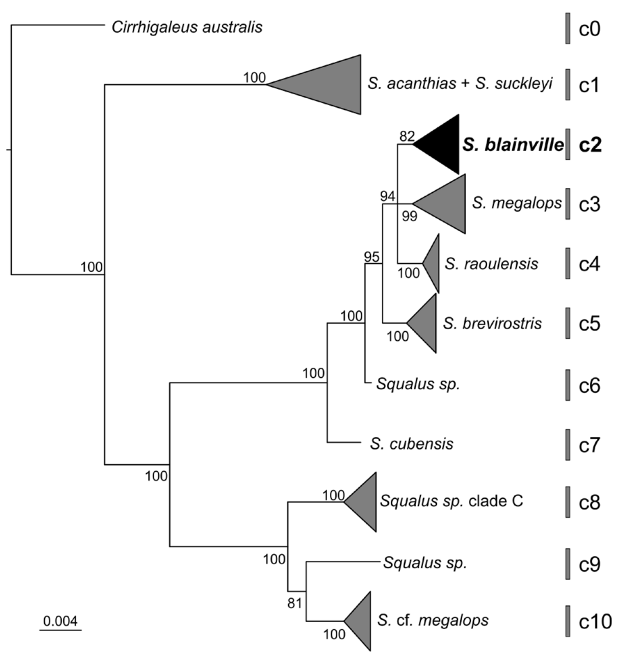

Fig. 7: Bayesian tree based on mitochondrial COI sequences. Bayesian posterior probabilities are next to the nodes. Clade c2, containing all the new Sardinian sequences is highlighted in black. Table S1 contains the complete list of sequences used.

Equatorial Western Indian Ocean (Merret, 1973). On the other hand, mouth width (MOW) of the Sardinian specimens looked larger compared to that reported in previous studies (Garrick, 1960; Merret, 1973; Muñoz-Chápuli & Ramos, 1989; Marouani *et al*., 2012). The same situation occurred for the outer nostril corner width (ONW), even if it was possible to compare our results only with what was described by Marouani *et al*. (2012) because this measurement was not reported in the other cited papers. The only other relevant differences in the body morphology with respect to what is reported in literature for *S. blainville* were found in the pectoral fin measurements, more precisely in pectoral fin length (P1L) and pectoral inner margin (P1I), both achieving higher values than those documented by other authors (Garrick, 1960; Merret, 1973; Muñoz-Chápuli & Ramos, 1989; Marouani *et al*., 2012). Unfortunately, also in this case, it was possible to compare P1L values of Sardinian spurdogs only with Tunisian data (Marouani *et al*., 2012).

Exactly the same situation occurred for the S2 group, in which the only relevant differences between the samples analysed in this study and what was described for *S. megalops* by other authors in Eastern Atlantic and Mediterranean Sea (Muñoz-Chápuli *et al*., 1984; Muñoz-Chápuli & Ramos, 1989; Marouani *et al*., 2012) and in Australian waters (Last *et al*., 2007) coincided precisely with the same measurements previously reported for sharks belonging to the S1 group.

Besides the exact correspondence in the two shark groups of the morphological characters (both somatic and chondrocranial) that have reported disagreeing values from the literature could be a further indication of the presence of only one species.

Moreover, the observation of further characteristics, identified by other authors as different in the two spurdog species, such as teeth and dermal denticles, were not able to clearly discriminate the groups. In particular, S1 and S2 presented very similar teeth in both upper and lower dental arches. Furthermore, regarding the dermal denticles, every specimen analysed in this work presented, at the same time, both denticle shapes described as typical for *S. blainville* (tricuspid) and for *S. megalops* (monocuspid) (Muñoz-Chápuli *et al*., 1984; Muñoz-Chápuli & Ramos, 1989; Marouani *et al*., 2012). Considering the brief half-life and fast replacing rate of these structures (Kemp, 1999), this particular aspect could be due to a different development stage of denticles observed in the analysed skin portion (Kemp, 1999). Moreover, it is reported that some common diagnostic morphological features, such as dermal denticles, teeth and dorsal fin spines could vary in shape with the onthogenetic development (White *et al*., 2013; Veríssimo *et al*., 2014). Consequently, the dermal denticles morphology should be further investigated before it can be properly used as a suitable classification tool, as also suggested by Bonello *et al*. (2016) particularly for the genus *Squalus*.

All the specimens genetically analyzed in Sardinia, despite their morphological variability, clustered together, and resulted to be *S. blainville*. Both present and previous genetic data confirm that this taxon is widely distributed in the Mediterranean (Serena, 2005; Bat *et al*., 2005; Serena *et al*., 2009; Landi *et al*., 2014; Bonello *et al*., 2016; Kousteni *et al*., 2016; Cariani *et al*., 2017; Veríssimo *et al*., 2017).

However, several taxonomic uncertainties still remain in this region with respect to the occurrence and distribution of additional *Squalus* species besides *S. blainville* and *S. acanthias*.

Recently, several studies highlighted the frequent misidentification of *Squalus* taxa in this area, and the inconsistent use of the names *S. blainville* and *S. megalops*, and even of *S. acanthias* (see Cariani *et al*., 2017; Verissimo *et al*., 2017 and Table S1). For instance, the sequence available for a Mediterranean specimen originally identified as *S. megalops* (Marouani *et al*., 2012) proved to be *S. blainville* (Veríssimo *et al*., 2017). However, considering the finding of sporadic divergent sequences (Fig. 9 c6 and c8; Marouani *et al*., 2012; Kousteni *et al*., 2016;Veríssimo *et al*., 2017) different from *S. blainville* (Fig. 9 c2), *S. acanthias* (Fig. 9 c1) but also *S. megalops* from Australia (Fig. 9 c3), the occurrence of a third species in the Mediterranean (apart from *S. acanthias* and *S. blainville*) cannot be ruled out.

In particular, the second sequence by Marouani *et al*. (2012) from a Mediterranean (Tunisian) specimen originally identified as *S. blainville*, clustered in c8 with individuals from Tropical West Africa, originally identified as *S. megalops* (Fig. 9 c8 or clade C *sensu* Veríssimo *et al*., 2017). Nevertheless, as *S. megalops* is to be applied only to Australian spurdogs (Veríssimo *et al*., 2017), which taxon name is to be used for the specimens with eastern Atlantic and Mediterranean origin remains uncertain (Veríssimo *et al*., 2017).

The genetic and morphological analysis carried out in the present paper indicated the presence of only one spurdog species in Sardinian waters, ascribable to *S. blainville*. These results represent an important baseline for future assessment and management studies on Central-Western Mediterranean spurdog populations. However, considering the taxonomical confusion that characterizes the *Squalus* genus and the fact that a classification based only on morphological features can easily lead to misidentifications, as demonstrated in the present paper, additional studies combining genetics and morphology are welcomed and urgent.

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