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Taxonomic research on *Squalus megalops* (Macleay, 1881) and *Squalus blainvillei* (Risso, 1827) (Chondrichthyes: Squalidae) in Tunisian waters (central Mediterranean Sea)

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SUMMARY: Two species of spurdog of the genus *Squalus* occur in the Gulf of Gabès (southern Tunisia, central Mediterranean): the longnose spurdog *Squalus blainvillei* (Risso, 1827) and a short-snout spurdog of the *Squalus megalops-cubensis* group. Morphometric and meristic data as well as a genetic analyses (DNA inter-simple sequence repeat markers and molecular barcoding methods) support the assignation of this short-snout spurdog to *Squalus megalops* (Macleay, 1881). *Squalus megalops* occurs commonly in temperate and tropical Australian waters, and is also thought to occur in the eastern Atlantic, southern Indian Ocean and western North Pacific although these records need to be confirmed. Our study confirms that it occurs in the Mediterranean Sea. Populations of both *S. blainvillei* and *S. megalops* are described based on Tunisian material.

Keywords: *Squalus megalops, Squalus blainvillei*, taxonomy, Gulf of Gabès, Central Mediterranean.

RESUMEN: Estudio taxonómico de *Squalus megalops* (Macleay, 1881) y *Squalus blainvillei* (Risso, 1827) (Chondrichthyes: Squalidae) en aguas de Túnez (Mediterráneo central). – En el golfo de Gabès (sur de Túnez, Mediterráneo central) se encuentran dos especies del género *Squalus*: el galludo *Squalus blainvillei* (Risso, 1827) y otra de morro más corto perteneciente al grupo *Squalus megalops-cubensis*. Los datos morfométricos y merísticos junto con el análisis genético (ADN *Inter Simple Sequence Repeats markers* y métodos moleculares *Barcoding*) apoyan la identificación de esta última especie como *Squalus megalops* (Macleay, 1881). *Squalus megalops* se encuentra en aguas australianas templadas y tropicales, y se piensa que habita también en el Atlántico este, en el océano Índico sur y en el Pacífico noroccidental, aunque estos registros deben ser confirmados. Nuestro estudio confirma su presencia en el mar Mediterráneo. La descripción tanto de *S. blainvillei* como de *S. megalops* está basada en especímenes capturados en aguas tunecinas.

Palabras clave: *Squalus megalops, Squalus blainvillei*, taxonomía, golfo de Gabès, Mediterráneo central.

INTRODUCTION

The genus *Squalus* includes 25 species, 11 of which have been described recently from the Indo-West Pacific (Last *et al*. 2007a). In the Mediterranean Sea, two species occur commonly (Bradai *et al.* 2004, Serena *et al.* 2009): *Squalus acanthias* (Linnaeus, 1758) and *Squalus blainvillei* (Risso, 1827). However, a third species has been recorded as *Squalus megalops* by Muñoz-Chápuli *et al.* (1984) and Muñoz-Chápuli and Ramos (1989). *Squalus megalops* (Macleay, 1881) has been recorded from many localities of the eastern Atlantic and Indo-West Pacific (Compagno 2005), but Duffy and Last (2007) and Last and Stevens (1994) suggested that it may belong to a species complex, and stated that records of *Squalus megalops* outside Australia need to be confirmed.

Outside its main distributional area (Mediterranean Sea and eastern Atlantic), *Squalus blainvillei* has also been recorded erroneously off Australia and New Zealand (Garrick 1960). It was thought to be widespread in the Atlantic, Indian and Pacific Oceans (Bigelow and Schroeder 1948, 1957), as well as off Japan (Chen *et al*. 1979). Compagno *et al.* (2005) restricted the distribution of *Squalus blainvillei* to the Mediterranean Sea and eastern Atlantic, and questioned the Pacific records. The confusion is due largely to the poor original description given by Risso (1827) and the lack of type material.

The doubtful taxonomic status of the Tunisian dogfish led us to make a taxonomic study of these sharks, including both morphological and genetic analyses. The detailed descriptions provided here should allow the species to be clearly differentiated and contribute to a better understanding of the geographical distribution of *Squalus megalops.*

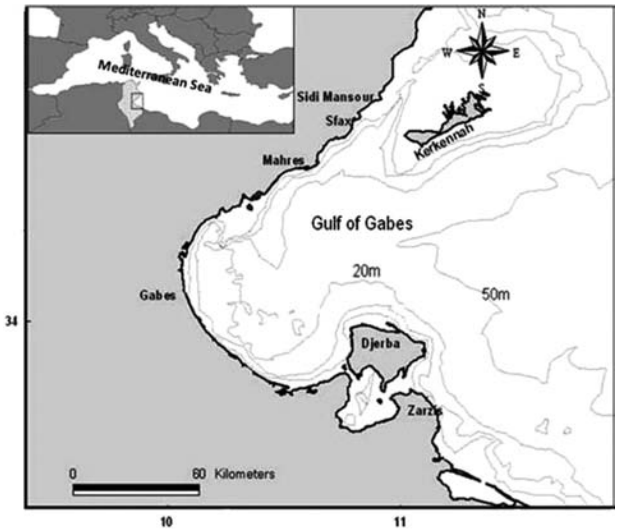

FIG. 1. – Map of the sampling area, Gulf of Gabès, Tunisia, Central Mediterranean Sea.

MATERIALS AND METHODS

Morphological studies

The studied material consisted in 32 specimens of *Squalus blainvillei* (18 females ranging from 27 to 96 cm TL, and 14 males from 34 to 72 cm TL) and 26 specimens of *Squalus megalops* (14 females ranging from 33 to 70 cm TL, and 12 males from 35 to 53.5 cm TL). They were caught by commercial trawlers in the Gulf of Gabès (Fig. 1) between January 2007 and May 2009.

A subsample of 9 specimens of each species (*S. blainvillei*: 5 females ranging from 63.0 to 82.0 cm TL and 2 males from 66.5 to 68.5 cm TL as well as a 96.0 cm TL female (INSTM /SQUAL 02) and a 69.6 cm TL (INSTM /SQUAL 09) male; *S. megalops*: 6 females (INSTM /SQUAL 03-08) ranging from 33.0 to 69.5 cm TL and 3 males (INSTM /SQUAL 10-12) from 46.8 to 51.1 cm TL) were used for the morphometry study. All encoded samples were preserved whole in 10% formalin at the National Institute of Marine Sciences and Technology (Center of Sfax, Tunisia). The morphometric methodology followed Compagno (1984) and Last *et al.* (2007a), and measurements were expressed in a percentage of the total length (TL). Some measurements were provided as proportions to make comparison with previous studies possible.

The skeletal anatomy (neurocranium, clasper components) was studied using the rest of the samples (23 specimens of *S. blainvillei* ranging from 27 to 71 cm TL, and 17 specimens of *S. megalops* ranging from 35 to 70 cm TL). They were dissected to reveal the structure of the chondrocrania and claspers and to count meristic characters. Chondrocranial measurements follow Muñoz-Chápuli and Ramos (1989) and were expressed as a percentage of the total length of the chondrocranium (TLC). Meristic characters were obtained separately for the trunk (monospondylous centra), precaudal (monospondylous + diplospondylous centra to origin of the caudal-fin upper lobe) and caudal (centra of the caudal fin) vertebrae. Tooth rows were counted directly on specimens by making incisions at the jaw angles to expose the teeth, and the tooth shape was noted. The terminal cartilages of claspers, called spurs and claws by Leigh-Sharpe (1920), were described and used to discriminate between the two species. Skin samples, observed by optic microscopy for characterising the dermal denticles, were obtained from the laterodorsal area, anterior to the first dorsal spine.

Genetic studies

ISSR technique

The ISSR genetic analysis was performed on tissue samples of 7 specimens of *S. blainvillei* (4 females ranging from 36 to 64 cm TL and 3 males from 28.5 to 34.5 cm TL) and 7 specimens of *S. megalops* (4 females ranging from 50.1 to 68 cm TL and 3 males from 35 to 49.1 cm TL) from the dissected samples.

DNA inter-simple sequence repeat (ISSR) markers were introduced by Gupta *et al.* (1994) and Zietkiewics *et al.* (1994). The ISSR technique is based on amplifying anonymous nuclear DNA sequences delimited by two inverted microsatellites. Using a single primer, composed of a short microsatellite sequence with one

Table 1. – Primer sequences used in the ISSR analysis, number of polymorphic loci per primer and range of band molecular weights in base pairs (bp) amplified by PCR-ISSR for the 14 individuals of *Squalus* analysed.

Primer	Sequence (5'-3')	No. of polymorphic loci	Size range of DNA fragments (*bp*)
PT1	$(GT)_8C$	9	425-1500
IT1	$(CA)_8GT$	14	290-1370
IT2	$(CA)_8AC$	5	360-1420
IT3	$(GAG)_4AG$	10	250-1230
SAS1	$(GTG)_4GC$	9	300-1650
SAS3	$(GAG)_4G$	11	380-1450
UBC809	$(AG)_8G$	9	310-1920
UBC811	$(GA)_8C$	4	460-850

to four degenerate nucleotides anchored at the 5' or 3' end, allows several DNA regions to be amplified, which are treated as loci. In the present work, ISSRs were used to verify the presence of two species of the genus *Squalus* in the Gulf of Gabès.

Portions of the muscle were removed from each individual and stored in 95% ethanol at 4°C. For the DNA extraction, highly pure genomic DNA was isolated from approximately 25 mg of muscle tissue using the Speedtools tissue DNA extraction kit (Biotools B and M Lab. S.A. Spain) according to the manufacturer's instructions. Once the DNA had been extracted it was stored at 4°C until amplification through polymerase chain reaction (PCR).

We used eight primers provided by Operon Molecules for Life (Table 1). The PCR reaction mixture contained up to 30 mg of genomic DNA, 2.5 mM Mg Cl2, 0.2 µM primer, 200 µM of each dNTP, 0.5 U of Taq DNA polymerase and 10X reaction buffer in a final volume of 25 µl. The amplification program was 3 min at 94°C, with the following cycle repeated 45 times: 40 s at 94°C, 45 s at 55°C and 1 min 40 s at 72°C; finally 5 min at 72°C. In order to exclude PCR artefacts and verify the repeatability of the results, a negative control and replicates were included. After separation by electrophoresis on a 2% agarose gel, the PCR products were stained with ethidium bromide. One hundred base pair ladders (100 bp DNA ladder, Promega) were used for reference with each primer.

For the ISSR analysis, based on the total band presence/absence, we obtained a triangular matrix of Nei (1978) inter-individual genetic distance. UPGMA cluster analysis was carried out using the program TFPGA (Miller, 1997). The nodes of the dendrogram were tested by bootstrapping with 10000 replicates.

Molecular barcoding methods

For this study we used a specimen of each species (a 96 cm TL female *S. blainvillei* and a 69.5 cm TL female *S. megalops*) from the conserved individuals used for morphometric analysis. White tissue samples were preserved in 95% ethanol. DNA was extracted using a method outlined by Ward *et al.* (2007) for barcoding Australian chondrichthyans. The Barcoding analysis was mainly used to compare the Tunisian specimen of *S. megalops* with specimens from Australia. The analysis was performed with CSIRO and the sequences of the two Tunisian specimens were inserted in the results already obtained by Ward *et al.* (2007) for discriminating spurdogs of the genus *Squalus*.

Table 2. – Proportional dimensions as percentages (±SD) of total length for specimens of *Squalus blainvillei* from southern Tunisia, eastern Atlantic-Mediterranean, New Zealand, equatorial, western Indian Ocean and Mediterranean coasts of Spain.

Reference / area	Present study Gulf of Gabès	Muñoz-Chápuli and Ramos (1989) Eastern Atlantic Mediterranean	Garrick (1960) New Zealand	Merrett (1973) equatorial western Indian Ocean	Muñoz-Chápuli *et al.* (1984) Mediterranean coasts of Spain
Number of specimens	N =9	N =15	N =3	N =4	N =6
TL range in cm	63-96.0	40.2-89.0	54.5-100.8	46.0-67.9	56.0-73.0
PCL – Precaudal length	79.06±0.62	78.93±0.98	79.57±1.83	77.95±1.32	-
PD2 – Pre-second dorsal length	63.82±3.27	-	-	62.77±0.08	-
PD1 – Pre-first dorsal length	28.24±1.12	28.53±0.97	32.57±0.81	30±1.19	-
IDS – Interdorsal space	27.05±0.79	25.82±1.11	28.34±0.66	27.05±0.56	-
DCS – Dorsal-caudal space	10.32±0.54	11.03±0.47	9.77±0.65	10.87±0.5	-
HDL– Head length	20.98±1.12	20.5±0.9	20.8±0.40	22.9±0.2	-
PG1 – Prebranchial length	17.02±0.59	16.68±0.78	17.16±0.25	19.5±0.4	-
POB – Preorbital length	6.19±0.53	5.55±0.78	6.3±0.46	5.4±0.17	-
INO – Interorbital space	7.43±0.37	-	-	5.3±0.25	-
EYH – Eye height	1.81±0.25	-	-	2±0.20	-
EYL – Eye length	3.86±0.23	4.03±0.39	4.37±0.21	5.22±0.12	-
PSP – Prespiracular length	10.78±0.76	-	-	12.2±0.67	-
PRN – Prenarial length	4.02±0.32	-	-	-	-
INW – Internarial space	4.16±0.26	-	-	-	4.48±0.29
PP2 – Prepelvic length	48.86±3.62	-	-	36.39±1.4	-
PPS – Pectoral-pelvic space	21.75±0.86	22.89±1.55	-		-
SVL – Pre-vent length	50.4±3.13	50.57±1.37	51±2.64	47.65±1.10	-
POR – Preoral length	8.22±0.16	8.4±0.44		10.52±0.15	-
MOW – Mouth width	7.29±0.53	7.49±0.89	5.83±0.11	6.72±1.07	-
ULA – Labial furrow length	1.93±0.37	-	-	-	-
PINL – Pre-inner nostril length	4.3±0.15	3.41±0.65	-	4.22±0.45	-
INLF – Inner nostril-labial furrow space	4.32±0.3	-	-	-	-

TABLE 2 (Cont.). – Proportional dimensions as percentages (±SD) of total length for specimens of *Squalus blainvillei* from southern Tunisia, eastern Atlantic-Mediterranean, New Zealand, equatorial, western Indian Ocean and Mediterranean coasts of Spain.

Reference / area	Present study Gulf of Gabès	Muñoz-Chápuli and Ramos (1989) Eastern Atlantic Mediterranean	Garrick (1960) New Zealand	Merrett (1973) equatorial western Indian Ocean	Muñoz-Chápuli *et al.* (1984) Mediterranean coasts of Spain
Number of specimens	N =9	N =15	N =3	N =4	N =6
TL range in cm	63-96.0	40.2-89.0	54.5-100.8	46.0-67.9	56.0-73.0
First dorsal fin					
D1L – First dorsal length	13.32±0.76	-	-	-	-
D1B –First dorsal base length	8.03±0.25	8.44±1.54	6.07±0.68	7.22±0.54	-
D1I – First dorsal inner margin	5.4±0.28	6.1±0.53	-	-	-
D1P – First dorsal posterior margin	9.24±0.79	-	-	5.92±0.23	-
D1A – First dorsal anterior margin	11.4±0.85	-	-	-	-
D1H –First dorsal height	7.07±0.7	8.09±0.61	8.03±0.15	8.6±0.91	-
D1ES –First dorsal spine length	5.06±0.3	4.32±0.71	-	4.15±1.30	-
D1BS – First dorsal spine base width	0.83±0.29				
Second dorsal fin		-	-	-	-
D2L – Second dorsal length	9.45±0.61	-	-	-	-
D2B – Second dorsal base length	5.13±0.41	6.42±1.27	4.8±0.78	4.55±0.25	-
D2I – Second dorsal inner margin	4.29±0.21	-	-	-	-
D2P – Second dorsal posterior margin	4.93±0.46	4.79±0.46	-	6±0.62	-
D2A – Second dorsal anterior margin	7.19±0.59	-	-	-	-
D2ES – Second dorsal spine length	5.22±0.41	4.92±0.94	-	4.2±1.01	4.69±0.45
D2BS –Second dorsal spine base width	0.87±0.2	-	-	-	-
Pectoral fin					
P1L – Pectoral length	11.46±0.52	-	-	-	-
P1B – Pectoral base length	5.85±0.41	6.77±0.70	-	5.7±0.46	-
P1I — Pectoral inner margin	6.24 ±0.36	7.18±0.51	-	-	7.08±0.39
P1P – Pectoral posterior margin	11. 4±0.9	11.1±0.8	-	-	-
P1A – Pectoral anterior margin	13.31±0.95	13.99±1.02	14.43±0.91	15.05±0.91	13.63±0.85
P1H – Pectoral height	12.62 ±0.98	-	-	-	-
Pelvic fin					
P2L – Pelvic length	9.05±1.49	9.69 ±0.68	-	-	9.82±0.84
P2B – Pelvic base length	5.39±0.39	-	-	4.67±0.25	-
P2I – Pelvic inner margin	5.2±0.79	-	-	-	-
P2A – pelvic anterior margin	4.76±0.9	5.86±0.72	-	5.75±0.36	-
P2H – Pelvic height	4.03±1.87	-	-	-	-
PDI-Pelvic midpoint-2nd dorsal origin	13.32±0.62	-	-	-	-
PDO-Pelvic midpoint-first dorsal insertion	13.66±0.53	-	-	-	-
Gills					
ING- Intergill length	4.66±0.67	4.18±0.63	-	-	-
GS1 – first gill-slit height	1.85±0.26	1.95±0.23	1.9±0.42	1.77±0.27	-
GS3 – third gill-slit height	2.2±0.17	2.23±0.26	-	1.85±0.31	-
GS5 – Fifth gill-slit height	2.07±0.27	2.49±0.42	2.33±0.21	2.04±0.12	-
Caudal fin					
CPH – Caudal peduncle height	2.24±0.12	-	-	-	-
CDM – Dorsal caudal margin	20.74±0.9	-		-	-
CPV – Preventral caudal margin	10.15±0.99	-	-	-	-
CFL – Caudal fork length	9.16±0.52	-	-	-	-
CPU – Upper postventral caudal margin	11.86±1.07	-	-	-	-
CPL – Lower postventral caudal margin	4.85±1.25	-	-	-	-
CFW – Caudal fork width	6.18±0.74	-	-	-	-
HANW – Head width at nostrils	7.28±0.55	-	-	-	-
HAMW – Head width at mouth	10.14±0.49	-	-	-	-
HDW – Head width	12.6±0.32	-	-	-	-
TRW – Trunk width	10.00±0.94	-	-	-	-
ABW – Abdomen width	10.19±0.8	-	-	-	-
TAW – Tail width	6.4±0.15	-	-	-	-
CPW – Caudal peduncle width	2.53±0.29	-	-	-	-
HDH – Head height	8.76±2	-	-	-	-
TRH – Trunk height	9.12 ±0.68	-	-	-	-
ABH – Abdomen height	8.98 ±0.48	-	-	-	-
TAH – Tail height	5.38±0.9	-	-	-	-
Clasper					
CLO – Clasper outer length	3.96±0.28	-	-	-	-
CLI – Clasper inner length	9.40±0.11	-	-	-	-
CLB – Clasper base width	2.18±0.84	-	-	-	-

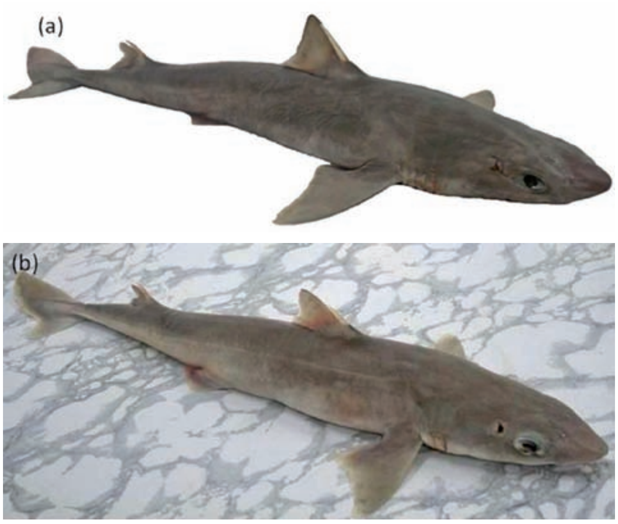

Fig. 2. – (a) Squalus *blainvillei*, adult female 960 mm TL and (b) *S. megalops*, adult female 760 mm TL from Tunisia.

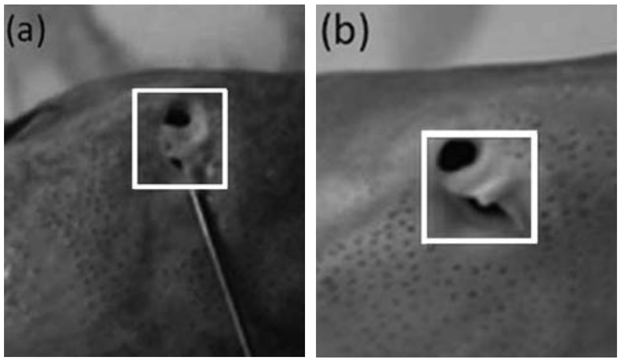

Fig. 3. – Left nasal flap of (a) *S. blainvillei*, adult female 960 mm TL and (b) *S. megalops*, adult female 760 mm TL.

RESULTS

Description of the Tunisian specimens of *Squalus blainvillei*

Table 2 shows the proportional measurements of the Tunisian specimens of *S. blainvillei* and Table 3 the ratios between some of these measurements. Table 4 provides the chondrocranial measurements of these specimens.

S. blainvillei is a moderate-sized species of *Squalus* of the "*mitsukurii*" group: the maximum TL of the specimens observed in Tunisia were 96.0 and 82.0 cm for females and males respectively. Body elongate fusiform to slightly compressed (Fig. 2a). According to the values given in Table 2, we concluded the following proportions: head long 20.98 (19.04-22.55) %TL; head length fits 2.57 (2.02-4.18) times in prevent length. Snout moderately long, triangular in lateral view, narrowly rounded in dorsal view; horizontal prenarial length fits 2.02 (1.76-2.29) times in preoral length. Eye broadly oval, relatively large, length fits 5.45 (4.81-6.25) times in head, 2.17 (1.69-3) times its height. Spiracle small, broadly crescentic; diameter of spiracle fits 2.65 (1.4-3.85) times in eye length. Gill openings small, oblique, subequal in size, height of

Table 3. – Distinctive characters of *Squalus blainvillei* and *Squalus megalops* from the Gulf of Gabès (Tunisia).

Characters	*Squalus blainvillei*	*Squalus megalops*
Flank denticles	Tricuspid	Unicuspid
Cluspers (mature males):		
Claws	Hook-like	Bent
spurs	Slender	Massive
Number of cartilaginous process on each side of the basal plate of the chondrocranium	One	Two
Palatoquadrate	Rounded	Sharpen
Vertebrae:		
Precaudal centra	85-90	109-113
Total centra	71-81	91-101
First dorsal fin height to rear tip	Half length from origin to rear tip	Less than half length from origin
First dorsal spine	More than 1/2 fin base 1/2 fin anterior margin	0.5-0.7 dorsal fin base less than 1/2 fin anterior margin
Diagonal distance from tip to inner nostril	0.9-1 times distance from nostril to upper labial furrow	0.8-1 times distance from nostril to upper labial furrow
Preoral snout	about 1-1.1 times mouth width	about 1 to 1.4 times mouth width
Anterior nasal flap	with small posterior secondary lobe	with a developed posterior secondary lobe
Pre-second dorsal length	4.3-5.0 times pectoral-fin anterior margin	4.1-5.0 times pectoral-fin anterior margin
Pelvic midbases	About equidistant between first and second dorsal bases	Closer to first dorsal base than second dorsal bases
Pectoral free rear tip	Rounded	Angular
Posterior pectoral margin	slightly concave	moderately concave
Preventral caudal margin	1.7-2.7 times inner margin of pelvic fin	1.3-2.5 times inner margin of pelvic fin
Caudal fin	Dusky posterior margin	Pale posterior margin

TABLE 4. – Chondrocranial measurements of *Squalus blainvillei* and *Squalus megalops* from the Gulf of Gabès, and comparison through a t-Student test (p<0.05; bold values: statistically different).

Total length of chondrocranuim (TLC)	*Squalus blainvillei* 4.85-8.85 cm N	Mean	SD	*Squalus megalops* 4.00-8.70 cm N	Mean	SD	*t*- Student
Posterior tip-precerebral fenestra	23	65.46	3.02	17	67.03	3.25	1.57
Length precerebral fenestra	23	33.41	2.93	17	31.95	1.61	1.85
Width precerebral fenestra	23	19.12	3.05	17	20.33	1.90	1.44
Width across nasal capsules	23	54.35	2.36	16	51.92	3.59	**2.55**
Interorbital width	23	31.77	2.32	16	28.72	1.79	**4.40**
Postorbital width	23	57.02	2.76	16	58.19	2.38	1.38
Distance between orbital processes	23	36.03	2.77	16	36.31	2.55	0.32
Width between pterotic processes	22	39.52	2.33	16	39.80	2.16	0.38
Width between hyomandibular facets	22	45.73	3.39	16	47.06	2.04	1.39
Posterior tip-rostral keel	22	68.10	2.76	16	68.43	3.21	0.34
Length rostral keel	22	19.96	2.13	16	21.02	3.16	1.24
Subthmoidean width	22	14.37	2.12	16	13.60	1.54	1.21
Width basal angle	22	19.10	1.85	16	20.01	1.56	1.58
length basal plate	22	46.61	2.53	16	46.24	1.75	0.49
Width anterior basal plate	22	22.02	3.73	17	20.58	1.72	1.47
Width between processes of basal plate	22	31.37	2.25	17	33.39	3.61	**2.14**
Maximum sagital height	22	37.54	3.24	16	37.13	3.85	0.35
Width of foramen magnum	22	9.88	1.70	16	9.68	1.50	0.38

first gill slit 1.85 (1.32-2.3)%TL; fifth gill slit wrapping around pectoral fin origin. Nostrils small, anterior nasal flap with posterior secondary lobe rather large (Fig. 3a); internarial space fits 1.98 (1.86-2.27) times in preoral length. Mouth arched, width fits 1.13 (1.00-1.36) times in preoral length; upper labial furrows 1.93 (1.26-2.56) %TL, continued as prominent grooves extending posterior-laterally from angle of jaws. Teeth similar in both jaws, small, compressed, and with a single cusp deeply notched and outward end strongly oblique (Fig. 4a); 27 teeth in upper jaw, 24 in the lower jaw. Dermal denticles of flank below first dorsal fin

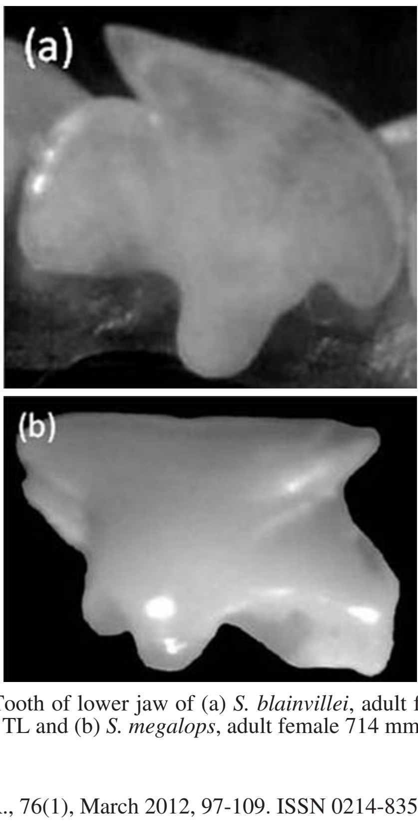

FIG. 4. – Tooth of lower jaw of (a) *S. blainvillei*, adult female 960 mm TL and (b) *S. megalops*, adult female 714 mm TL.

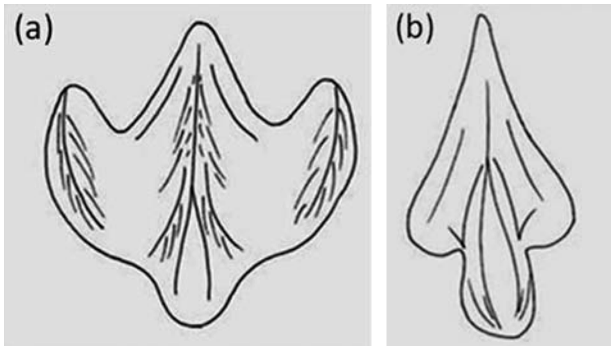

FIG. 5. – Cusps of flank denticle of (a) *S. blainvillei*, adult female 960 mm LT and (b) *S. megalops*, adult female 760 mm TL.

tricuspid (Fig. 5a). First dorsal fin origin posterior of pectoral insertions and spine origin over pectoral inner margins and well in front of their rear tip; pre-first dorsal fin length fits 3.54 (3.26-3.74) times in TL; first dorsal-fin length 1.9 (1.44-2.45) times its height, 1.54 (1.09-2.48) times second dorsal-fin length; first dorsal-fin height 1.85 (1.56-2.15) times second dorsal fin height; first dorsal spine 0.7 (0.6-0.79) times fin height, 0.63 (0.58-0.7) times fin base. Second dorsal fin smaller than first, inner margin length 1.13 (1.0-1.37) times fin height; second dorsal-fin length 2.38 (1.37-3.0) times its height; second dorsal spine length 1.35 (1.2-1.60) times fin height; fin-spine origin well behind free rear tip of pelvic fin, exposed below level of junction with spine and fin; second spine with broad base, slender distally. Interdorsal space fits 1.04 (0.97-1.11) times in pre-first dorsal fin length. Pectoral fin large, anterior margin weakly convex, apex rounded; posterior margin concave to nearly straight; inner margin length 6.24 (5.71-6.88)%TL, free-rear tip narrowly rounded; base fits 2.28 (2.02-2.78) times in anterior margin length. Pelvic fins small, anterior and posterior margins almost straight, apex broadly rounded, free

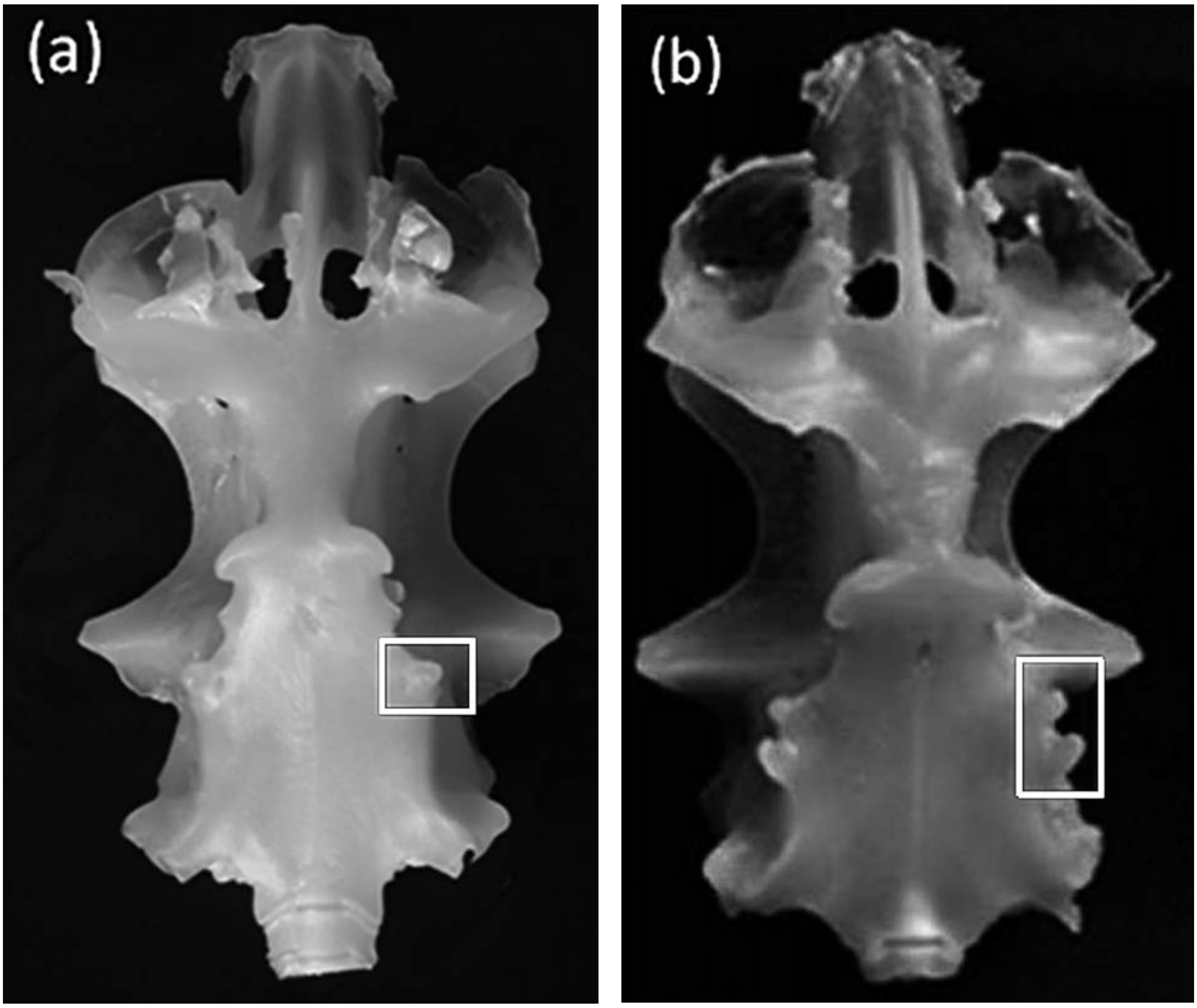

FIG. 6. – Dissected chondrocranium of (a) *S. blainvillei*, adult male 780 mm TL, showing the single lateral process of the basal plate and (b) *S. megalops*, adult female 714 mm TL, showing the two lateral processes of the basal plate.

rear tip narrow, acute. Precaudal tail tapering to caudal fin, broadly semicircular posteriorly, ventral groove well developed; prominent lateral keels extending posteriorly from below insertion of second dorsal fin past caudal fin insertion; dorsal-caudal space fits 2.62 (2.45-2.85) times in interdorsal space; upper and lower precaudal pits present, upper pit more pronounced than lower pit. Caudal fin well developed, dorsal caudal margin 20.74 (19.69-22.76)%TL, fits 0.01 (0.8-1.14) times in head length, without a subterminal notch. Chondrocranial basal plate with a single lateral process (Fig. 6a); posterior tip-precerebral fenestra 65.64 (59.52-72.22)%TLC; width across nasal capsules 54.35 (50.97-60.0)%TLC; interorbital width 31.77 (25.71-36)%TLC; width between processes of basal plate 31.37 (24-34.92)%TLC (Table 4). Claspers (in mature males): hook-like spurs and slender claws (Fig. 7a). Rounded palatoquadrate cartilage (Fig. 8a).

Coloration: When fresh, uniform grey brown dorsally, white below; dorsal and caudal fins grey, first dorsal-fin anterior base and free rear tip paler than rest

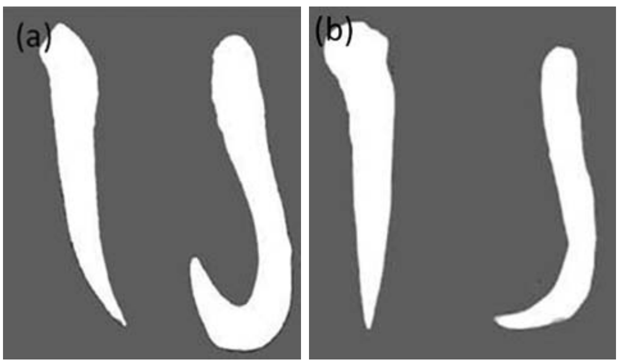

FIG. 7. – Claw (left) and spur (right) of clasper of (a) *Squalus blainvillei*, adult male 780 mm TL and (b) *S. megalops*, adult male 680 mm TL.

of fin, second dorsal-fin anterior base not darker than rest of fin, dorsal-fin tips and upper posterior margin with narrow black margin; pectoral, pelvic and caudal fins grey with white posterior margins and tips; naked axils of fins and pectoral origin dusky; eyes bright green in life.

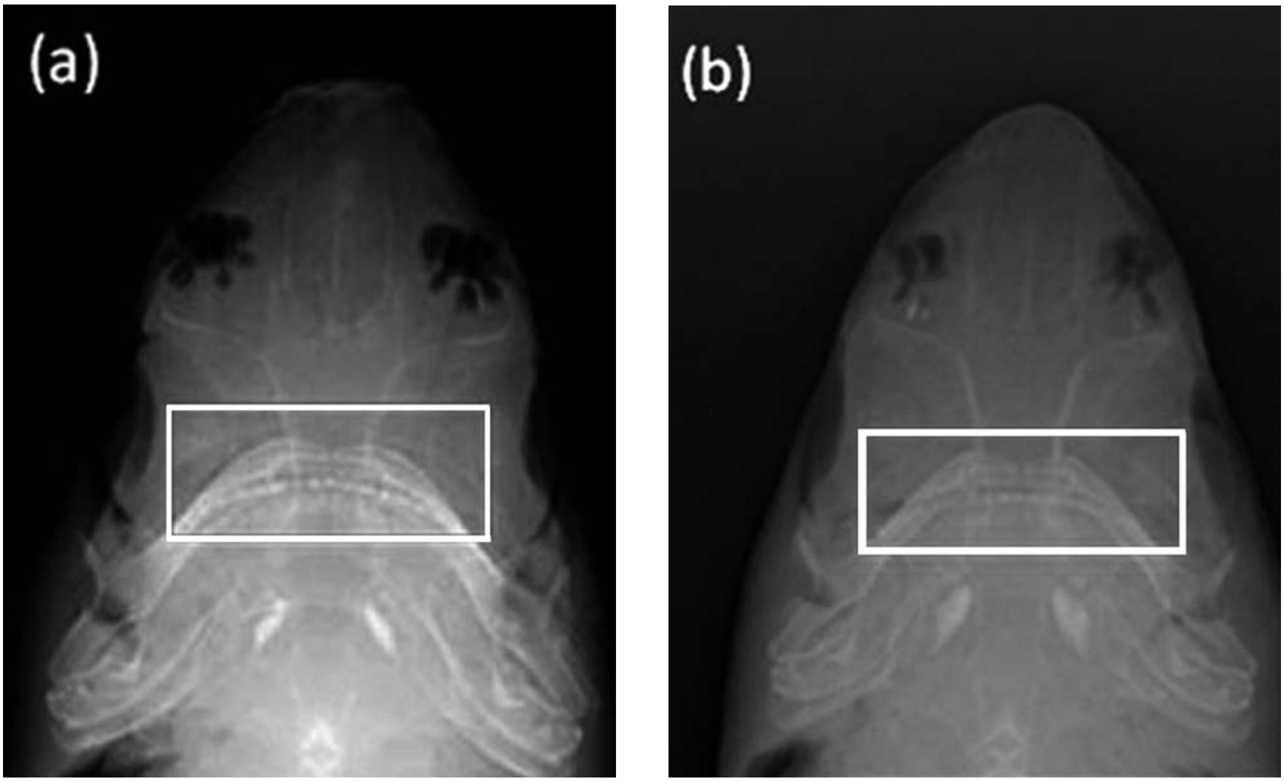

Fig. 8. – Radiograph of the head of (a) *S. blainvillei*, adult female 960 mm TL, showing the rounded palatoquadrate cartilage and (b) *S. megalops*, adult female 760 mm TL, showing the angular palatoquadrate cartilage.

Table 5. – Proportional dimensions as percentages of total length for specimens of *Squalus megalops* from southern Tunisia, southern Australia, Qeensland, Western Australia, eastern Atlantic-Mediterranean and Spanish waters.

Reference / area	Present study Gulf of Gabès			Last *et al*. (2007) southeastern Australia			Last *et al*. (2007) Queensland			Last *et al*. (2007) western Australia			Chápuli and Ramos (1989) eastern-Atlantic Mediterranean	Chápuli *et al*. (1984) Mediterranean coasts of Morroco
Number of specimens	N = 9			N = 6			N = 3			N = 4			N = 34	N = 24
TL range in cm	33-69.5			37.3-52.7			32.8-38.4			41.4-54.1			31.8-74.2	48.5-68.0
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean ±SD	Mean±SD
PCL-Precaudal length	78.69	76.25	80.34	77.7	76.1	79.3	78.5	77.8	78.9	78.4	77.7	79.2	78.92±1.08	-
PD2-Pre-second dorsal length	62.41	59.74	64.30	61.6	60.6	62.3	61.2	60.5	62.4	61.4	60.2	62.1	-	-
PD1-Pre-first dorsal length	29.48	28.41	30.70	30.2	29.1	31.6	30.6	29.9	31.6	29.6	29.1	30.2	28.99±0.89	-
SVL-Pre-vent length	48.32	46.29	49.85	48.5	47.6	50.1	46.5	46.1	47.2	47.9	45.9	50.4	49.18±2.06	-
PP2-Prepelvic length	46.73	44.87	48.53	47.4	46.4	49.9	45	44.8	45.4	46.9	45.5	49.1	-	-
PP1-Prepectoral length	20.88	18.16	22.12	21.5	20.4	23.2	22.6	22.2	22.9	21.9	20.9	23	-	-
PG1-Prebranchial length	17.88	16.76	19.79	18.5	17.7	19.8	18.9	18.6	19.2	18.3	17.8	19.1	16.62±0.88	-
PSP-Prespiracular length	12.82	11.36	16.06	12.5	11.5	13.5	12.9	12.8	13	12.3	12.1	12.7	-	-
POB-Preorbital length	6.83	6.18	7.27	7	6.4	7.5	7.2	7	7.4	7	6.4	7.4	6.01±0.51	-
PRN-Prenarial length	4.23	3.88	4.48	3.9	3.7	4.1	4.3	4.2	4.4	4.2	3.9	4.4	3.49±0.57	-
POR-Preoral length	8.84	7.98	9.39	9.1	8.6	9.9	9.7	9.3	9.9	9.2	8.9	9.7	8.65±0.83	-
MOW-Mouth width	7.86	7.48	8.53	8.1	7.8	8.6	8.3	8	8.5	8.2	7.8	8.6	-	-
ULA-Labial furrow length	2.33	2.02	2.72	2.3	2.1	2.4	2.4	2.3	2.5	2.4	2.2	2.7	-	-
INW-Internarial space	3.98	3.71	4.19	4.5	4.3	4.7	4.7	4.6	1.9	1.5	4.2	4.8	3.83±0.26	3.82±0.20
INO-Interorbital space	7.28	6.97	7.73	8.8	8.4	9.8	9.1	8.8	9.3	8.4	7.6	9	-	-
EYL-Eye length	4.19	3.59	4.84	4.8	4.4	5.4	5	4.9	5	4.8	4.3	5.3	4.27±0.53	-
EYH-Eye height	2.10	1.69	2.4	2.2	1.9	2.6	2.3	2.1	2.5	2.5	2.3	2.9	-	-
SPL-Spiracle length	1.24	1.00	1.46	1.4	1.0	1.7	1.4	1.2	1.5	1.6	1.5	1.7	-	-
GS1-first gill-slit height	1.99	1.51	2.60	2.3	2	2.4	1.9	1.8	1.9	2.2	1.9	2.4	2.00±0.30	-
GS5-Fifth gill-slit height	2.09	1.76	2.53	2.4	2.1	2.5	2.5	2.3	2.6	2.2	1.8	2.4	2.46±0.39	-
IDS-Interdorsal space	25.42	22.77	27.59	24.8	24	25.3	24.6	23.2	25.8	25.3	23.7	26	24.49±1.9	-
DCS-Dorsal-caudal space	10.93	9.49	11.81	10.4	9.5	10.9	12.2	11.5	12.7	10.7	9.9	12	11.16±0.60	-
PPS-Pectoral-pelvic space	21.72	20.29	23.46	22.3	20.9	26.1	19.1	18	20.3	22.6	20.5	24.6	23.10±2.64	-
PINL-Pre-inner nostril length	4.34	3.95	4.54	-	-	-	-	-	-	-	-	-	-	-
INLF-Inner nostril-labial furrow space	4.55	4.17	4.74	-	-	-	-	-	-	-	-	-	-	-

TABLE 5 (Cont.). – Proportional dimensions as percentages of total length for specimens of *Squalus megalops* from southern Tunisia, southern Australia, Queensland, Western Australia, eastern Atlantic-Mediterranean and Spanish waters.

Reference / area	Present study Gulf of Gabès			Last *et al.* (2007) southeastern Australia			Last *et al.* (2007) Queensland			Last *et al.* (2007) western Australia			Chápuli and Ramos (1989) eastern-Atlantic Mediterranean	Chápuli *et al.* (1984) Mediterranean coasts of Morroco
Number of specimens	N = 9			N = 6			N = 3			N = 4			N = 34	N = 24
TL range in cm	33-69.5			37.3-52.7			32.8-38.4			41.4-54.1			31.8-74.2	48.5-68.0
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean ±SD	Mean±SD
First dorsal fin														
D1L-First dorsal length	13.56	13.09	14.14	14.4	13.8	15.1	13.3	12.7	13.7	14	13.3	14.9	-	-
D1A-First dorsal anterior margin	11.36	10.56	13.73	11.5	11.1	12.4	11.1	10.3	12.2	11.5	10.8	12.2	-	-
D1B-First dorsal base length	7.63	7.27	8.02	8.2	7.9	8.9	7.6	7.2	8	8.3	7.7	8.9	8.06±0.75	-
D1H-First dorsal height	6.06	5.60	6.56	7	6.1	7.4	6.4	6.2	6.6	7.2	7	7.5	8.48±0.81	-
D1I-First dorsal inner margin	5.68	5.12	6.25	6.3	6.1	6.6	5.7	5.7	5.7	5.9	5.4	6.3	-	-
D1P-First dorsal poster or margin	7.71	6.83	8.41	8.3	6.6	9	7.9	7.6	8.1	7.9	7.5	8.1	6.72±0.54	-
D1ES-First dorsal spine length	4.58	4.09	5.08	3	2.4	3.3	3	2.9	3.2	3.3	3	3.4	4.63±0.49	-
D1BS-First dorsal spine base width	0.92	0.78	1.06	0.8	0.7	0.8	0.7	0.6	0.7	0.7	0.6	0.8	-	-
Second dorsal fin														
D2L-Second dorsal length	10.26	9.10	11.60	12	11	12.7	12.1	11.6	12.8	12.2	11.8	12.8	-	-
D2A-Second dorsal anterior margin	8.25	7.48	9.33	10.1	9.4	10.6	9.8	9.6	10.3	10.5	10.1	11	-	-
D2B-Second dorsal base length	5.28	4.64	6.13	7.1	6.4	7.5	7.2	6.9	7.6	7.5	7.1	8.2	6.98±1.40	-
D2H-Second dorsal height	3.49	3.03	4.23	4	3.6	4.6	3.7	3.2	4	3.9	3.7	4.3	5.20±1.1	-
D2I-Second dorsal inner margin	4.97	4.47	5.50	4.9	4.5	5.3	4.9	4.5	5.1	4.9	4.7	5	-	-
D2P-Second dorsal posterior margin	4.65	3.83	5.29	4.5	3.7	5.3	4.5	4.2	4.9	4.1	3.9	4.4	5.74±0.38	-
D2ES-Second dorsal spine length	5.98	5.33	5.56	4.3	3.6	5	4.6	4	5	4.5	4.2	4.6	5.37±0.92	5.61±0.67
D2BS-Second dorsal spine base width	0.91	0.71	1.06	0.8	0.8	0.9	0.8	0.7	0.8	0.8	0.8	0.9	-	-
Pectoral fin														
P1L-Pectoral length	12.95	11.94	13.63	-	-	-	-	-	-	-	-	-	-	-
P1A-Pectoral anterior margin	14.12	13.03	15.25	14.3	13.6	14.9	12.5	12.3	12.6	14.3	13.7	15.1	15.3±0.83	15.26±0.96
P1I-Pectoral inner margin	7.40	6.61	8.26	8.2	7.4	6.2	8.4	7.7	8.8	9	8.4	9.7	9.34±0.69	9.29±0.66
P1B-Pectoral base length	5.61	5.15	6.22	5.3	4.4	5.7	5.3	4.9	5.8	4.9	4.4	5.3	6.51±0.76	-
P1P-Pectoral posterior margin	11.31	10.17	12.32	11.6	10.8	12.7	10.4	9.6	10.9	11.2	10.3	12.3	12.20±0.97	-
P1H-Pectoral height	12.82	11.51	13.63	-	-	-	-	-	-	-	-	-	-	-
Pelvic fin														
P2L-Pelvic length	10.38	9.56	11.32	10.5	9.9	11.5	10.6	9.9	11.2	10.4	9.9	10.8	11.21±1.15	11.55±0.95
P2A-Pelvic anterior margin	5.24	5.02	5.72	-	-	-	-	-	-	-	-	-	-	-
P2I-Pelvic inner margin	5.01	3.82	6.84	5.5	4.2	6.8	5.8	5.1	6.8	5.9	4.8	6.6	-	-
P2B-Pelvic base length	5.05	4.06	5.92	-	-	-	-	-	-	-	-	-	-	-
P2P-Pelvic posterior margin	6.37	5.26	7.27	-	-	-	-	-	-	-	-	-	-	-
P2H-Pelvic height	2.99	2.40	3.52	4.8	4.3	5.2	4.7	4.5	5.1	4.9	4.7	5.2	-	-
PDI-Pelvic midpoint-2nd dorsal origin	12.14	10.18	13.69	-	-	-	-	-	-	-	-	-	-	-
PDO-Pelvic midpoint-1s dorsal insertion	13.39	12.07	14.59	-	-	-	-	-	-	-	-	-	-	-
Caudal fin														
CDM-Dorsal caudal margin	19.43	16.38	21.82	20.9	20	21.4	20.1	19.3	20.9	20.6	20.2	21.1	21.36±0.76	-
CPV-Preventral caudal margin	9.54	8.45	10.80	11	10.5	11.3	10.6	10.4	10.7	10.9	10.7	11	-	-
CPU-Upper postventral caudal margin	13.30	11.53	15.57	15.9	15.0	16.6	14.6	14.1	15.3	15.2	14.5	15.9	11.39±1.14	-
CPL-Lower postventral caudal margin	4.07	4.64	3.48	4.3	4.0	4.6	3.6	3.4	3.7	4.6	3.8	5.6	-	-
CFW-Caudal fork width	6.65	6.06	7.05	7.1	6.6	7.5	7.0	6.9	7.1	7.0	6.6	7.3	-	-
CFL-Caudal fork length	8.41	7.20	9.32	9.5	8.9	10.2	9.5	9.3	9.8	9.4	9.2	9.6	-	-
HANW-Head width at nostrils	7.22	6.25	7.87	6.7	6.3	7.6	6.9	6.7	7.2	6.7	6.3	7.1	-	-
HAMW-Head width at mouth	10.21	8.91	11.21	11.6	11.1	12.8	12.5	12.3	12.7	11.6 1	10.9	12.6	-	-
TRW-Trunk width	10.36	8.55	11.84	12.1	11.2	13.2	10.8	10.3	11.7	12.2	11.3	14.5	11.29±0.59	-
ABW-Abdomen width	10.57	9.82	11.65	10.9	10.1	12.2	10.0	9.1	11.0	10.8	10.0	11.5	-	-
TAW-Tail width	6.90	5.75	8.68	6.8	6.4	7.2	7.2	6.9	7.4	7.0	6.4	7.6	-	-
CPW-Caudal peduncle width	2.78	2.33	3.63	3.0	2.4	3.6	3.0	2.9	3.1	3.0	2.8	3.3	-	-
HDH-Head height	8.48	7.53	9.97	10.5	9.2	11.5	10.6	10.1	11.4	10.2	9.3	10.9	-	-
TRH-Trunk height	7.84	6.46	10.26	11.3	9.5	13.4	11.0	10.4	12.0	11.8	10.5	13.1	-	-
ABH-Abdomen height	7.99	6.66	9.82	11.3	9.1	14.4	11.7	10.4	13.4	11.5	9.5	9.5	-	-
TAH-Tail height	6.79	5.65	8.20	7.1	6.0	7.6	7.1	6.0	7.6	7.2	6.3	7.7	-	-
CPH-Caudal peduncle height	2.18	1.91	2.35	2.4	2.3	2.5	2.2	2.2	2.3	2.4	2.2	2.5	-	-
Clasper														
CLO-Clasper outer length	3.86	3.83	3.91	3.9	3.9	3.9	3.4	2.6	4.1	2.8	3.6	3.9	-	-
CLI-Clasper inner length	9.98	9.69	10.47	9.6	9.6	9.6	9.6	9.2	10	7.4	9.6	10	-	-
CLB-Clasper base width	1.49	1.31	1.70	1.8	1.8	1.8	1.6	1.5	1.6	1.1	1.4	1.6	-	-

Description of the Tunisian specimens of *Squalus megalops*

The proportional measurements of the Tunisian specimens of *S. megalops* and the ratios between some of these measurements are given in Tables 5 and 3 respectively. Chondrocranial measurements are shown in Table 4.

S. megalops is a moderate-sized species of *Squalus* of the "*megalops-cubensis"* group: the maximum TLs of the specimens observed in Tunisia were 74.2 and 72.0 cm for females and males respectively.

Body elongate fusiform to slightly compressed (Fig. 2b). Head long 20.88 (18.16-22.12)%TL; head length fits 2.26 (1.88-2.6) times in pre-vent length. Snout moderately long, triangular in lateral view, narrowly rounded in dorsal view, horizontal prenarial length fits 2.08 (2.0-2.18) times in preoral length. Eye broadly oval, relatively large, length fits 5.02 (4.25-5.69) times in head, 2.01 (1.72-2.62) times height. Spiracle small, broadly crescentic; greatest diameter fits 3.40 (2.66-4.20) times in eye length. Gill openings small; subequal in size, height of first gill slit 1.99 (1.51-2.60)%TL; fifth gill slit wrapping around pectoral fin origin. Nostrils small, anterior nasal flap with a small posterior secondary lobe (Fig. 3b); internarial space fits 2.22 (2.05-2.34) times in preoral length. Mouth arched, width fits 1.12 (1.06-1.17) times in preoral length; upper labial furrows 2.33 (2.02-2.72) %TL, continued as prominent grooves extending posterior-laterally from angle of jaws. Teeth similar in upper and lower jaws; lower teeth unicuspid, elongated, interlocking; cusps directed strongly laterally (Fig. 4b); 27 teeth in upper jaw, 24 in the lower jaw. Dermal denticles on flank below first dorsal fin unicuspid (Fig. 5b). First dorsal fin origin posterior to pectoral insertions and spine origin over pectoral inner margins and well in front of their rear tip; pre-first dorsal fin length fits 3.39 (3.25-3.51) times in TL; first dorsal-fin length 2.24 (2.09-2.38) times its height, 1.32 (1.14-1.52) times second dorsal-fin length; first dorsal-fin height 1.63 (1.5-2.1) times second dorsal fin height; first dorsal spine 0.75 (0.54-0.86) times fin height, 0.59 (0.53-0.68) times fin base. Second dorsal fin smaller than first, inner margin length 1.33 (0.72-1.7) times fin height; second dorsal-fin length 2.76 (2.55-3.40) times its height; second dorsal spine length 1.59 (0.80-2.03) times the fin height; fin-spine origin well behind free rear tip of pelvic fin; second spine with broad base, slender distally. Interdorsal space fits 1.16 (1.04-1.29) times in pre-first dorsal fin length. Pectoral fin large, anterior margin weakly convex, apex rounded; posterior margin concave; inner margin length 7.4 (6.61-8.28) %TL, free-rear tip narrowly angular; base fits 2.52 (2.17-2.88) times in anterior margin length. Pelvic fins small, anterior and posterior margins almost straight, apex broadly rounded, free rear tip narrow, acute. Precaudal tail tapering to caudal fin, broadly semicircular posteriorly, ventral groove developed; dorsal-caudal space fits 2.33 (2.1-2.72) times in interdorsal space; upper and lower precaudal pits present, upper pit better defined than lower pit. Caudal fin well developed, dorsal caudal margin 19.63 (18.38-21.82) %TL, fits 1.8 (0.91-1.29) times in head length, without a subterminal notch. Chondrocranial basal plate with two lateral processes on each side (Fig. 6b); posterior tip-precerebral fenestra 67.03 (61.93-73.52)%TLC; width across nasal capsules 51.92 (45.0-55.69)%TLC; interorbital width 28.72 (25.31-31.74)%TLC; width between processes of basal plate 33.39 (20.61-36.84)%TLC (Table 4). Cluspers (in mature males): bent spurs and massive claws (Fig. 7b), angular palatoquadrate cartilage (Fig. 8b).

Coloration: When fresh, uniform grey brown dorsally, white below; dorsal and caudal fins grey, first dorsal-fin anterior base and free rear tip paler than rest of fin; dorsal fins apex dusky; pectoral, pelvic and caudal fins grey with white posterior margins and tips; naked axils of fins and pectoral origin dusky; eyes bright green in life.

Table 3 summarises the main differences between the two *Squalus* species occurring in Tunisian waters. The external characters listed in this table are sufficient for field identification of these commercial species.

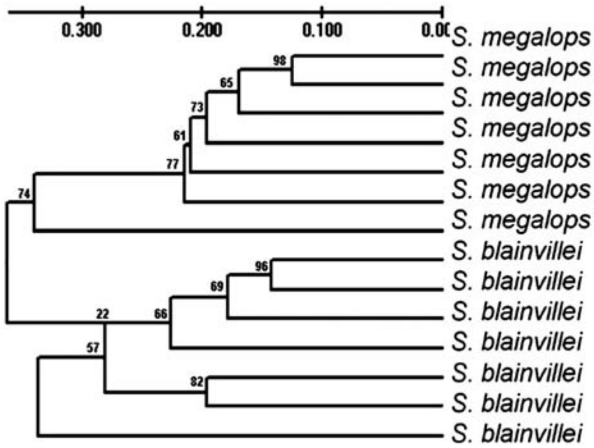

FIG. 9. – UPGMA consensus dendrogram of Rogers and Tanimoto's (1960) dissimilarity among individuals analysed. Bootstrap values on branching points represent the number of times (in percentage) a particular cluster group was formed out of 10000 iterations.

Genetic studies

ISSR method

The ISSR molecular analysis separated the Tunisian specimens into two clusters (species). The ISSR profiles were characterised by 71 polymorphic loci. The amplified DNA fragment lengths (for all primers) were approximately from 247 to 1922 pb. Maltagliati *et al.* (2005) defined a locus as ''fully diagnostic'' when it produced bands in all individuals of one species and not in the other, and ''nearly diagnostic'' when it pro-

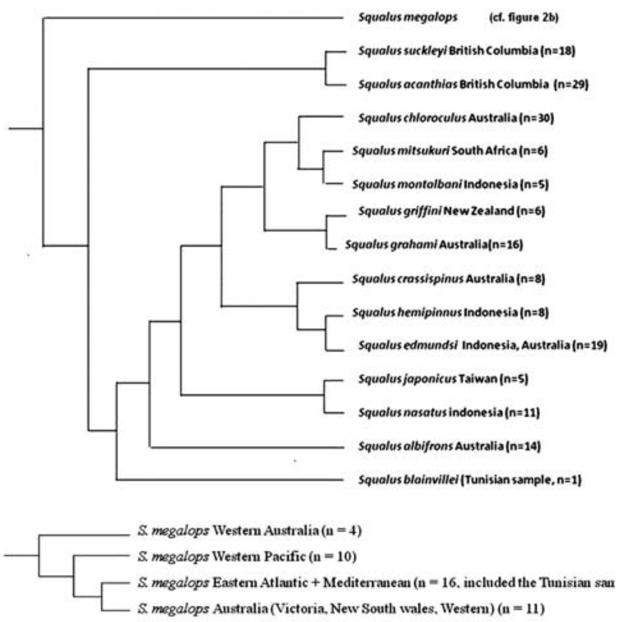

FIG. 10. – A, simplified tree of *Squalus* spp. analysis (on this simplified tree, the branches are not proportional to the distance between groups). B, *Squalus megalops* grouping extract from the general analysis of *Squalus* spp. (on this simplified tree, the branches are not proportional to the distance between groups).

duced species-specific bands in some individuals (but not all) of one species and not in the other species. We detected 2 fully diagnostic and 9 nearly diagnostic loci in the presumed species *S. blainvillei* and only 6 nearly diagnostic loci in the second species *S. megalops.* The UPGMA dendrogram of Rogers and Tanimoto's (1960) dissimilarities was characterised by high bootstrap support and clearly separated the studied individuals into two clusters (Fig. 9).

Molecular barcoding method

Figures 10a and 10b provide the topology of the neighbour-joining K2P tree for the sequences analysed by Ward *et al.* (2007), among which we inserted the sequences of the Tunisian samples. The sequence of the Tunisian specimen of *S. blainvillei* appears as a separate cluster set apart from the Australasian *Squalus* species (Fig.10a), whereas the Tunisian specimen of *S. megalops* is included in the *S. megalops* cluster (Fig.10b), which indicates that it has similarities with the Australian *S. megalops.*

DISCUSSION

Morphological and biological similarities among squalids have led to considerable confusion over their taxonomy (Myakov and Kondyurin, 1986). The taxonomic status of *S. blainvillei* is problematical as there are no extant types and the description and figures of Risso (1827) do not correspond to any known species of *Squalus* (Chen *et al.* 1979, Muñoz-Chápuli and Ramos 1989). This led Garrick (1960), in a review of the Australian species of *Squalus*, to incorrectly synonymise *S. griffini* and *S. fernandinus* (Molina, 1782) with *S. blainvillei*. However, in a review of Japanese *Squalus*, Chen *et al.* (1979) defined *S. blainvillei* as a species with high dorsal fins and long dorsal-fin spines based on their examination of Japanese material and descriptions of *S. blainvillei* from its type locality, the northern Mediterranean. They observed that *Squalus*, referring to *S. fernandinus* and *S. blainvillei* by Bigelow and Schroeder (1948) and Garrick (1960), had short dorsal-fin spines and were more similar to *S. mitsukurii* from Japan, and suggested that nominal *S. blainvillei* from New Zealand could be identical to *S. mitsukuri*. Compagno (1984) also noted that dogfish resembling *S. mitsukurii* occurred off Australia and New Zealand, and he did not recognise *S. blainvillei* from the Southern Hemisphere.

Actually, both *S. blainvillei* and *S. megalops* were identified from the Mediterranean Sea based on morphometry in a previous study (Muñoz-Chápuli and Ramos 1989), but the occurrence of *S. megalops* in the region has been questioned by other authors (Last and Stevens 1994). Thus, we expanded this study by including other morphometric characters and a molecular study to confirm that *S. megalops* occurs as a valid species in the Mediterranean Sea.

Our results show that the longnose spurdog is not characterised by its high first dorsal fin and spine as considered by Compagno (1984) following Chen *et al.* (1979), but rather it is a short-spine species. We compared our data for *S. blainvillei* with the measurements given by other authors for this species in different regions. While these data generally agree, there are some differences in the morphometrics between populations. *S. blainvillei* from the equatorial western Indian Ocean has a longer prebranchial length (19.5±0.4 versus 17.02±0.59%TL), a smaller interorbital space (5.3±0.3 versus 7.43±0.37%TL), a shorter prepelvic length (36.4±1.4 versus 48.86 ±3.62%TL), a longer preoral length (10.5±0.2 versus 8.22±0.16%TL) and a shorter first dorsal-fin posterior margin (5.9±0.2 versus 9.24±0.79%TL). *S. blainvillei* from New Zealand has a longer pre-first dorsal length (32.5±0.8 versus 28.24±1.12%TL), and a smaller mouth (5.8±0.1% versus 7.29±0.53%TL). *Squalus blainvillei* specimens examined herein and those specimens studied by Springer and Garrick (1964), Bass *et al.* (1976), Merrett (1973), Muñoz-Chápuli *et al.* (1984) and Muñoz-Chápuli and Ramos (1989) have similar vertebral counts.

The Tunisian *Squalus megalops* species are consistent for characters typifying the "*megalops-cubensis*" group and fit the description of *S. megalops* from Australian waters (Last *et al.* 2007b), as well as the eastern Atlantic-Mediterranean (Muñoz-Chápuli and Ramos 1989) and Mediterranean waters (Muñoz-Chápuli *et al.* 1984). Its presence in the Mediterranean Sea has been considered doubtful by many authors. In addition, Last and Stevens (1994) suggested that the southern

TABLE 6. – Vertebral counts and dental formula in *Squalus blainvillei* and *S. megalops.* References: 1, present study, Gulf of Gabès; 2, Springer and Garrick (1964), Italy; 3, Merrett (1973), Equatorial western Indian Ocean; 4, Bass *et al.* (1976), East coast of southern Africa; 5, Muñoz-Chápuli *et al.* (1984), Mediterranean coasts of Morocco; 6, Muñoz-Chápuli and Ramos (1989), Eastern-Atlantic - Mediterranean; 7, Present Study, Gulf of Gabès; 8, Springer and Garrick (1964), Japan; 9, Bass *et al.* (1976), East coast of southern Africa; 10, Muñoz-Chápuli *et al.* (1984), Mediterranean coasts of Morocco; 11, Muñoz-Chápuli and Ramos (1989), Eastern-Atlantic - Mediterranean; 12, Last *et al.* (2007), Australian waters.

	Squalus blainvillei						*Squalus megalops*					
References	1	2	3	4	5	6	7	8	9	10	11	12
Monospondylous	45	-	38		44	44				38		37
trunkal vertebrae			46	-	45	46	37	-	-	41	40	40
Precaudal vertebrae	85	78	70	80		88	71	79		77		78
(mono. + diplos. Vertebrae)	90	79	92	90	87	90	81	80	81	82	81	84
Total number of vertebrae	109	106	95				91					102
	113	108	120	-	-	-	101	106	-	-	-	110
Dental formula-number												
Upper jaw	14-13		11-14				14-13					
Lower jaw	12-12	-	11-12	-	-	-	12-12	-	-	-	-	-

Australian *S. megalops* could be distinct from *megalops* like spurdogs in other parts of the world, and that *S. megalops* might be endemic to Australia. However, the story may be even more complicated. Recent morphological studies have shown that more than a single form of this species exists in Australian seas (Last, unpublished data).

Specimens described from other areas clearly agree with our Tunisian samples of *S. megalops* for most of the morphometric characters (Table 5). However, there are some morphometric differences: the Australian *S. megalops* studied by Last *et al.* (2007b) has a larger internarial space 4.5 (4.3-4.7) versus 3.9 (3.7-4.1)%TL, a larger eye 5.0 (4.9-5.0) versus 4.1 (3.5-4.8)%TL, a longer second dorsal fin 12.0 (11.0-12.7) versus 10.2 (9.1-11.6)%TL; a longer anterior margin and base second dorsal fin 10.1 (9.4-10.6) versus 8.2 (7.4-9.3)% TL and 7.1 (6.4-5.2) versus 5.2 (4.6-6.1)%TL respectively, and a higher pelvic fin 4.8 (4.3-5.2) versus 2.9 (2.4-3.5)%TL. Eastern Atlantic-Mediterranean specimens have a higher first dorsal fin 8.5±0.8 versus 6.0 (±0.2 / 5.6-6.5)%TL and a longer pectoral-fin inner margin 9.3±0.7 versus 7.4 (6.6-8.2 / ±0.2)%TL. The Mediterranean *S. megalops* studied by Muñoz-Chápuli *et al.* (1984) also had a longer pectoral-fin inner margin 9.2±0.6 versus 7.4 (6.6-8.2 / ±0.2)%TL. Tunisian specimens of *Squalus megalops* had similar vertebral counts to those studied by Springer and Garrick (1964) (Indo-Pacific), Bass *et al.* (1976) (South Africa), Muñoz-Chápuli *et al.* (1984) (Mediterranean coasts of Spain), Muñoz-Chápuli and Ramos (1989) (east Atlantic) and Last *et al.* (2007b) (south Western Australia, Queensland). They had a low number of monospondylous centra (37), typical of the *Squalus megalops-cubensis* group (Muñoz-Chápuli and Ramos, 1989), and a relatively high number of precaudal centra (81), which is somewhat fewer than in *S. blainvillei* (Table 6).

Ledoux (1970) and Merret (1973) pointed out a close similarity between *S. blainvillei* and *S. megalops*, when compared with *S. acanthias* and *S. asper*. Moreover, the relationships between the snout tip and nostril distance and the distance from the nostril to the preoral clefs, which were proposed by Bass *et al.* (1976) as the best features for discriminating between species of the genus *Squalus*, proved to be of little use for discriminating our material. However, the differences in morphometrics observed in our study allow these two species to be differentiated. In addition, the presence of two lateral processes on each side of the basal plate in *S. megalops* (only one in *S. blainvillei*) also allows the two species to be discriminated. The chondrocranium of *S. megalops* has a narrower interorbital distance and smaller olfactory capsules than that of *S. blainvillei*.

Molecular analysis (ISSR and barcoding methods) evidenced the non-conspecificity of these species and the similarity between the Tunisian *Squalus megalops* and the “true” Australian *S. megalops*.

The external characters of *S. blainvillei* and *S. megalops* can be used for their field identification. However, these results should be completed by an analysis of the ontological stages to determine variability. The main traits of their life history (reproduction, diet, distribution), which are currently under study, should also provide more information on their respective biologies and help to differentiate them.

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REFERENCES

Bass A.J., D'Aubrey J.D., Kistnasamy N. 1976. Sharks of the east coast of southern Africa. VI. The families Oxynotidae, Squalidae, Dalatiidae and Echinorhinidae. *Invest. Rep. Oceanogr. Res. Inst. Durban* 45: 1-103.

Bigelow H.B., Schroeder W.C. 1948. Fishes of the Western North Atlantic. Part One. Lancelets, Cyclostomes, Sharks. *Mem. Sears Found. Mar. Res.* 1(1): 1-576.

Bigelow H.B., Schroeder W.C. 1957. A study of the sharks of the suborder Squaloidea. *Bull. Mus. Comp. Zool.* 117: 1-150.

Bradai M.N., Quignard J.P., Bouain A., Jarboui O., Ouanes-Ghorbel A., Ben Abdallah L., Zaouli J., Ben Salem S. 2004. Ichtyofaune autochtone et exotique des côtes tunisiennes: Recensement et Biogéographie. *Cybium* 28(24): 315-328.

Chen C., Taniuchi T., Nose Y. 1979. Blainville's dogfish, *Squalus blainville*, from Japan, with notes on *S. mitsukurii* and *S. japonicus*. *Jpn. J. Ichthyol*. 26: 26-44.

Compagno L.J.V. 1984. FAO species catalogue: Sharks of the World. An annotated and illustrated catalogue of shark species known to date. Part 1. Hexanchiformes to Lamniformes. *F.A.O. Fish. Synop.* 125(4)1: 1-250.

Compagno L.J.V., Dando M., Fowler S. 2005. *A Field Guide to the Sharks of the World*. Harper Collins Publishing Ltd., London, 368 pp.

Duffy J.A.C., Last P.R. 2007. Redescription of the Northern Spiny Dogfish *Squalus griffini* Phillipps, 1931 from New Zealand. In: Last P.R., White W.T., J.J. Pogonoski (eds.), *Descriptions of new dogfishes of the genus* Squalus *(Squaloidea: Squalidae). C.M.A.R.* pp. 91-101.

Garrick J.A.F. 1960. Studies on New Zealand Elasmobranchii. Part XII. The species of *Squalus* from New Zealand and Australia; and a general account and key to the New Zealand Squaloidea. *Trans. R. Soc. N Z* 88: 519-557.

Gupta M., Sinha S., Chandra P. 1994. Uptake and toxicity of metals in *Scirpus lacustris L.* and *Bacopa monniferi L. J. Environ. Sci. Health. A* 29(10): 2185-2202.

Last P.R., Stevens J.D. 1994. *Sharks and Rays of Australia*. CSIRO 513 pp.

Last P.R., White W.T., Pogonoski J.J., Gledhill D.C., Yearsley G.K., Ward D.C. 2007a. Application of a rapid taxonomic approach to the genus *Squalus*. In: Last P.R., White W.T., Pogonoski J.J. (eds.), *Descriptions of new dogfishes of the genus Squalus (Squaloidea: Squalidae). C.M.A.R.* pp. 1-10.

Last P.R., Edmunds M., Yearsley G.K. 2007b. *Squalus crassispinus* sp. nov., a new spurdog of the 'megalopscubensis group' from the eastern Indian Ocean. In: Last P.R., White W.T., Pogonoski J.J. (eds.), *Descriptions of new dogfishes of the genus* Squalus *(Squaloidea: Squalidae). C.M.A.R.* pp. 11-22.

Ledoux J.C. 1970. Affinités et origines du *Squalus blainvillei* de Méditerranée. *J. Ichthyol.* 3: 65-69.

Leigh-Sharpe W.H. 1920. The comparative morphology of the secondary sexual characters of elasmobranch fishes. *Mem. I. J. Morphol*., 34(1): 245-265.

Maltagliati F., Casu M., Lai T., Iraci Sareri D., Casu D., Curini Galletti M., Cantone G., Castelli A. 2005. Taxonomic distinction of *Ophelia barquii* and *O. bicornis* (Annelida, Polychaeta) in the Mediterranean as revealed by ISSR markers and the number of nephridiopores. *J. Mar. Biol. Ass. UK* 85: 835-841.

Merrett N.R. 1973. A new shark of the genus *Squalus* (Squalidae: Squaloidea) from the equatorial western Indian Ocean; with notes on *Squalus blainvillei*. *J. Zool.* 171: 93-110.

Miller M.P. 1997. *Tools for population genetic analyses (TFPGA 1.3): a windows program for the analysis of allozyme and molecular population genetic data.* Computer software distributed by the author at http://bioweb.usu.edu/mpmbio/index.htm.

Molina G.I. 1782. Saggio sulla storia naturale del Chile, del Signor Abate Giovanni Ignazio Molina. Bologna. *Saggio Chile* i-v + 306 pp.

Muñoz-Chápuli R., Ramos F., Garcia Garrido C.M. 1984. *Squalus megalops* (Mac Leay, 1882) en el Mediterráneo. Notas sobre su diagnosis, sistematica y distribucion. *Bull. Soc. Cat. Ictio. Herp*. 9: 16-21.

Muñoz-Chápuli R., Ramos F. 1989. Morphological comparison of *Squalus blainvillei* and *S. megalops* in the eastern Atlantic, with notes on the genus. *Jpn. J. Ichthyol.* 36: 6-21.

Myagkov N.A., Kondyurin V.V. 1986. Dogfishes *Squalus* (Squalidae), of the Atlantic Ocean and comparative notes on the species of this genus from other regions. *Vopr. Ihtiol.* 26: 560-575 (in Russian).

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.

Risso A. 1827. *Histoire naturelle des principales productions de l'Europe méridionale, et particulièrement de celles des environs de Nice et des Alpes maritimes.* F.G. Levrault, Paris et Strasbourg xvi +480 pp.

Serena F., Papaconstantinou C., Relini G., De Sola L.G., Bertrand J.A. 2009. Distribution and abundance of spiny dogfish in the Mediterranean Sea based on the Mediterranean International Trawl Survey Program. In: Gallucci V.F., McFarlane G.A., Bargmann G.G. (eds.), *Biology and Management of Dogfish Sharks*. *Am. Fish. Soc.* pp. 139-149.

Springer V.G., Garrick J.A.F. 1964. A survey of vertebral numbers in Sharks. *Proc. U.S. natl. Mus.* 116: 73-96.

Rogers D.J., Tanimoto T.T. 1960. A computer program for classifying plants. *Science* 132: 1115-1118.

Ward R.D., Holmes B.H., Zemlak T.S., Smith P.J. 2007. DNA barcoding discriminates spurdogs of the genus *Squalus*. In: Last P.R., White W.T., Pogonoski J.J. (eds.), *Descriptions of new dogfishes of the genus* Squalus *(Squaloidea: Squalidae). C.M.A.R.* pp. 117-130.

Zietckiewicz E., Rafalsky A., Labuda D. 1994. Genome fingerprinting by Inter-Simple Sequence Repeat (ISSR)-anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.

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