DE GRUYTER

DOI 10.1515/helmin-2016-0034

HELMINTHOLOGIA, 53, 4: 354 – 362, 2016

Hexabothriid monogeneans from the gills of deep-sea sharks off Algeria, with the description of *Squalonchocotyle euzeti* n. sp. (Hexabothriidae) from the kitefin shark *Dalatias licha* (Euselachii, Dalatiidae)

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Article info

Received June 13, 2016
Accepted September 20, 2016

Summary

Sharks (765 specimens from ten species) from the Mediterranean Sea off Algiers, Algeria, were examined for the presence of gill monogeneans. The following deep-sea sharks were investigated from 2009 to 2015: *Centrophorus granulosus* (27 specimens); *Centrophorus uyato* (39); *Etmopterus spinax* (67); *Somniosus rostratus* (19); *Galeus melanostomus* (189); *Scyliorhinus canicula* (261), *Hexanchus griseus* 3), and *Dalatias licha* (100). In addition, two pelagic shark species were examined: *Alopias vulpinus* (7), and *Prionace glauca* (53). Only two species of gill monogeneans were found. *Protocotyle grisea* (Cerfontaine, 1899) Euzet et Maillard, 1974 was found on its type-host *Hexanchus griseus*; comparative measurements are provided, and Algeria is a new geographic record. *Squalonchocotyle euzeti* n. sp. from *Dalatias licha* is described here. We found that the species of *Squalonchocotyle* Cerfontaine, 1899 can be separated into two groups, according to body size. Small-bodied species include 7 species. Large-bodied species (body > 20mm) include *S. borealis* (Van Beneden, 1853), *S. laymani* Yamaguti, 1958 and *S. euzeti* n. sp; the latter is distinguished from the two other species by a characteristically slender body. A sequence of Cytochrome Oxidase Type I (COI) gene, potentially useful for barcoding, was obtained for *S. euzeti* n. sp. and is the first for the family Hexabothriidae.

Keywords: Monogenea; *Protocotyle grisea*; parasitological survey; barcoding

Introduction

From 2009 to 2015, we examined sharks from off Algeria, mainly deep-sea species, for gill monogeneans. Only two species of gill monogeneans were collected, although ten species of sharks and 765 shark specimens were investigated. One was *Protocotyle grisea* (Cerfontaine, 1899) Euzet et Maillard, 1974 from *Hexanchus griseus*, for which we provide measurements; the other is a species of *Squalonchocotyle* Cerfontaine, 1899 which we describe herein as a new species.

Material and Methods

Sharks

Sharks were obtained from fishermen in Dellys (36° 55' N; 3° 53' E), Cap Djenet (36° 43' N; 3° 36' E), Bou Haroun (36° 40' N; 4° 40' E), and Cherchell (36° 37' N; 2° 11' E). All four localities are on the Mediterranean coast within 100 km near Algiers, Algeria and thus results are not detailed according to the localities. The following deep-sea shark species were examined for gill monogeneans from 2009 to 2015: gulper shark, *Centrophorus granulosus* (Bloch et

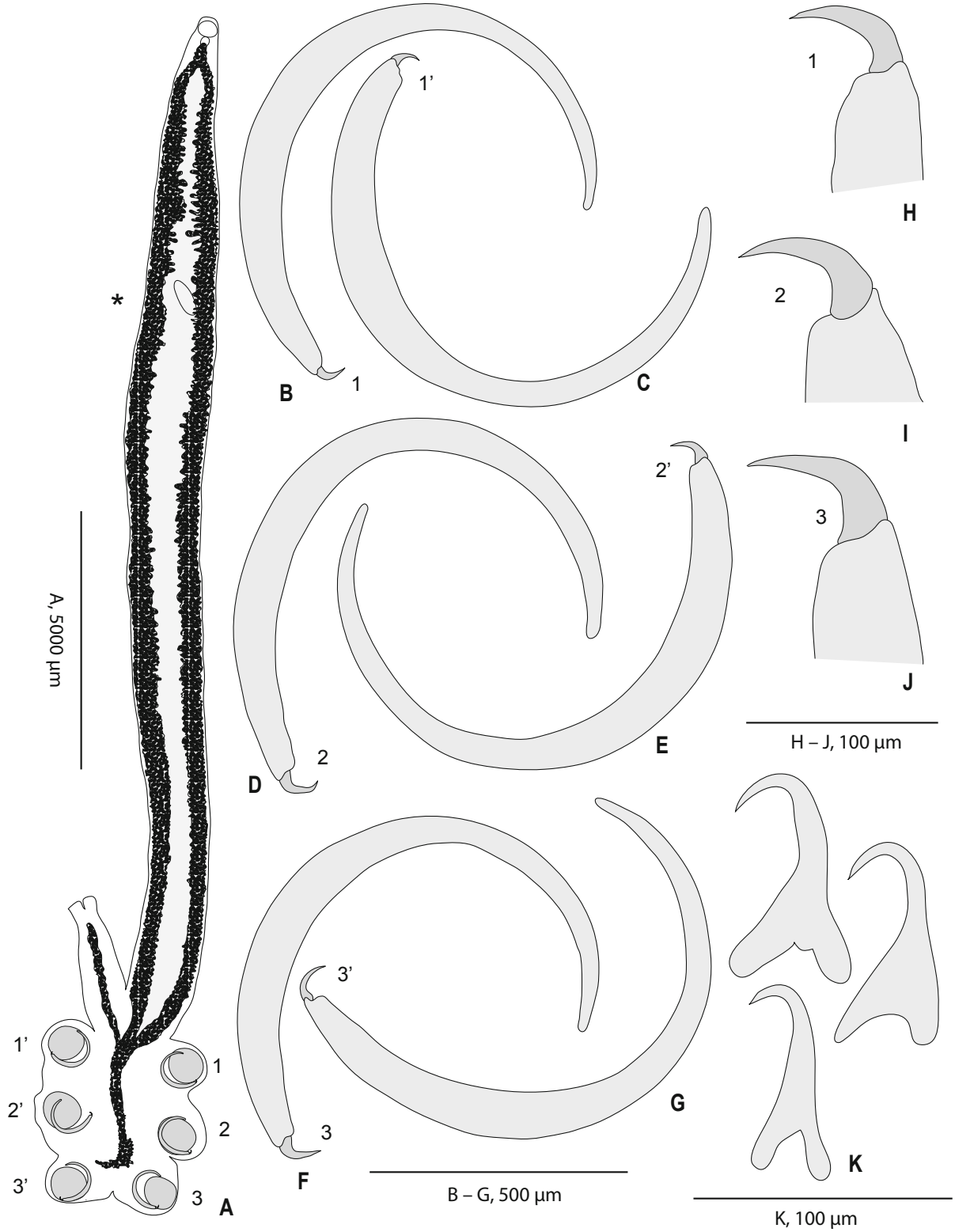

Fig. 1. *Squalonchocotyle euzeti* n. sp. from *Dalatias licha* off Algeria. A, holotype, whole body. Due to the slender body, only limited anatomy is represented. Asterisk, level of seminal receptacle (outline of seminal receptacle drawn) and ovary. B – G, sclerites. H – J, extremities of sclerites. For A – J, numbers of sclerites are indicated. K, hamuli of various specimens. A – J, holotype; K, paratypes.

Table 1. "Small" species of *Squalonchocotyle*. Measurements in various publications

Species	***S. abbreviata***	***S. centrophori***	***S. cerfontaini***	***S. mitsukurii***	***S. cerfontaini***	***S. spinacis***	***S. squali***	***S. squali***	***S. squali***	***S. squali***	***S. tropai***	***S. tropai***
Source	Cerfontaine, 1899	Maillard, 1970	Maillard, 1970	Kitamura *et al.*, 2006	This paper	Goto, 1894	MacCallum, 1931	Price, 1942	Dillon & Hargis, 1968	Martorelli *et al.*, 2008	Tendeiro & Valdez, 1955	Maillard, 1966
Name in source	*Onchocotyle abbreviata*	*Squalonchocotyle centrophori*	*Squalonchocotyle cerfontaini*	*Squalonchocotyle mitsukurii*	*S. cerfontaini*	*Onchocotyle spinacis*	*Squalonchocotyle squali*	*Erpocotyle squali*	*Erpocotyle squali*	*Squalonchocotyle squali*	*Erpocotyle tropai*	*Squalonchocotyle tropai*
Host name in source	*Acanthias vulgaris*	*Centrophorus granulosus*	*Dalatias licha*	*Squalus mitsukurii*	*Dalatias licha*	*Spinax sp*	*Squalus acanthias*	*Squalus acanthias*	*Squalus lebruni*	*Squalus acanthias*	*Squalus fernandinus*	*Squalus fernandinus*
Host valid name	*Squalus acanthias* Linnaeus, 1758	*Centrophorus granulosus* (Bloch et Schneider, 1801)	*Dalatias licha* (Bonnaterre, 1788)	*Squalus mitsukurii* Jordan et Snyder, 1903	*Dalatias licha* (Bonnaterre, 1788)		*Squalus acanthias* Linnaeus, 1758	*Squalus acanthias* Linnaeus, 1758	*Squalus acanthias* Linnaeus, 1758	*Squalus acanthias* Linnaeus, 1758	*Squalus acanthias* Linnaeus, 1758	*Squalus acanthias* Linnaeus, 1758
Locality	East Atlantic (Roscoff, France)	Mediterranean (Sète, France)	Mediterranean (Sète, France)	Pacific (Japan)	Mediterranean (Sète, France) MNHN 711 H, Ti 52	Pacific (Japan)	Several	Atlantic (USA)	Pacific (New Zealand)	Atlantic (Argentina)	Indo-Pacific (Angola)	Mediterranean (Sète, France)
n		7	2	13	1		several	13	56	11	2	4
Total body length	7,000 – 8,000	3,300 – 7,500	3,000	3,200 – 7,500	3,533	8,000 – 9,000	7,000 – 10,000	3,400 – 7,000	4,310 – 6,650	4,760 – 6,960	1,420 – 1,690	1,700 – 2,300
Body proper width		1,000 – 1,500	350	500 – 1,200	407	–	1,500	765 – 935	620 – 1,060	62 – 87	680 – 690	330 – 450
Anterior sucker diameter		340 – 420	200					220 – 228		190 – 270		200 – 250
Length				190 – 350	170							
Width				250 – 430	218							
Pharynx diameter			120 – 130					75		50 – 120		
Pharynx length		120		70 – 120	130				84 – 117			57 – 67
Pharynx width		60		60 – 120	122				61 – 69			40 – 60
Haptor length		900 – 1,400		1,000 – 2,800	1,147			1,300		1,120 – 1,260	400 – 510	546
Haptor width		700 – 1,000		800 – 1,700	1240					830 – 1,250	600 – 660	336
Anterior sclerite length		650 – 770	560 – 650	230 – 300	654		280	500 – 600	325 – 480	570 – 910		300 – 350
Median sclerite length		680 – 800	580 – 690	230 – 300	683		280	500 – 600	366 – 480	570 – 910		290 – 360
Posterior sclerite length		660 – 770	560 – 630	190 – 238	650		280	430 – 600	337 – 494	570 – 910		310 – 330
Appendix length			400	800 – 1,900	537			765 – 935	641 – 925	740 – 1,350	440 – 450	300 – 370
Appendix width			200	250 – 500	185			255 – 340		100 – 210	200 – 220	200 – 230
Hamulus length		47 – 61	63 – 70	55 – 65	85	40	72	72	61 – 72	50 – 70		37.5 – 42.5
Hamulus outer length				8 – 16	59					14 – 18		
Hamulus inner length				15 – 23	61					12 – 14		
Testes number		30 – 40	100 – 150	25 – 40			25	60	40 – 60	30 – 69	numerous	few
Cirrus bulb												
Cirrus length		650	350	200 – 370						75 – 100		
Cirrus width		40		60 – 110						40 – 50		
Egg, proper length	100			125 – 360			320	285 – 340	210 – 247	250 – 350	230	220
Egg, filament number & length	2			2			2	2		2; 50 – 60	2	
Seminal receptacle length												
Seminal receptacle length				210 – 350	241					400 – 550		
Seminal receptacle width				75 – 160	155					100 – 150		

Schneider, 1801): 27 specimens; little gulper shark, *Centrophorus uyato* (Rafinesque, 1810): 39; velvet belly, *Etmopterus spinax* (Linnaeus, 1758): 67; little sleeper shark, *Somniosus rostratus* (Risso, 1827): 19; blackmouth catshark, *Galeus melanostomus* Rafinesque, 1810: 189; lesser spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758): 261; bluntnose sixgill shark, *Hexanchus griseus* (Bonnaterre, 1788): 3; and kitefin shark, *Dalatias licha* (Bonnaterre, 1788): 100. In addition, two pelagic shark species were examined: thresher, *Alopias vulpinus* (Bonnaterre, 1788), 7 specimens, and blue shark, *Prionace glauca* (Linnaeus, 1758), 53. Sharks were collected as fresh as possible, photographed and immediately brought back to the laboratory for examination. Identification was done according to usual keys (Fischer *et al.*, 1987). The parasitological survey and fish identifications were done by HK.

Monogeneans

The gills were removed and observed in filtered seawater for monogeneans. Monogeneans, located using a stereo-microscope were removed alive (dead for the few specimens from *H. griseus*) from between the gill lamellae and were studied either directly or fixed, slightly flattened, between a slide and cover slip. Monogeneans were fixed either with ethanol or Bouin's fixative. Specimens were stained with carmine, cleared in clove oil and mounted in Canada balsam. Specimens for molecular analysis were collected in 95 % ethanol. All drawings were made with the help of an Olympus BH-2 microscope drawing tube. Drawings were scanned and redrawn on a computer with Adobe Illustrator. Measurements are in micrometres.

Molecular sequences

We used a QIAmp DNA Micro Kit (Qiagen) to extract DNA. Elution was performed in 60µL. The specific primers JB3 (=COI-ASmit1) (forward 5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4.5 (=COI-ASmit2) (reverse 5'-TAAAGAAAGAACATAATGAAAATG-3') were used to amplify a fragment of the COI gene (Bowles *et al.*, 1995; Littlewood *et al.*, 1997). The PCR reaction was performed in 20 µl, containing 1 ng of DNA, 1× CoralLoad PCR buffer, 3 mM MgCl2, 66 µM of each dNTP, 0.15 µM of each primer, and 0.5 units of Taq DNA polymerase (Qiagen). The amplification protocol was: 4' at 94 °C, followed by 40 cycles of 94 °C for 30'', 48 °C for 40'', 72 °C for 50'', with a final extension at 72 °C for 7'. Sequences were edited with CodonCode Aligner software version 3.7.1 (CodonCode Corporation, Dedham, MA, USA), compared to the GenBank database content with BLAST, and deposited in GenBank under accession numbers KX389260 – KX389262. Trials to obtain 28S partial sequences with the routine method previously used for other polyopisthocotylean monogeneans (Justine *et al.*, 2013) were unsuccessful.

Results

Parasitological survey

Among the 765 sharks examined over six years, belonging to ten species, only two species had monogeneans on their gills. *Hexanchus griseus* had *Protocotyle grisea*, and *Dalatias licha* had a new species of *Squalonchocotyle*.

Protocotyle grisea (Cerfontaine, 1899) Euzet et Maillard, 1974

Brief description of the material from Algeria

Our specimens were not in optimal state of conservation because these sharks were not fresh; however, the sclerotised parts could be observed and measured. Measurements (in parenthesis, measurements in Justine, 2011 for comparison): anterior sclerites

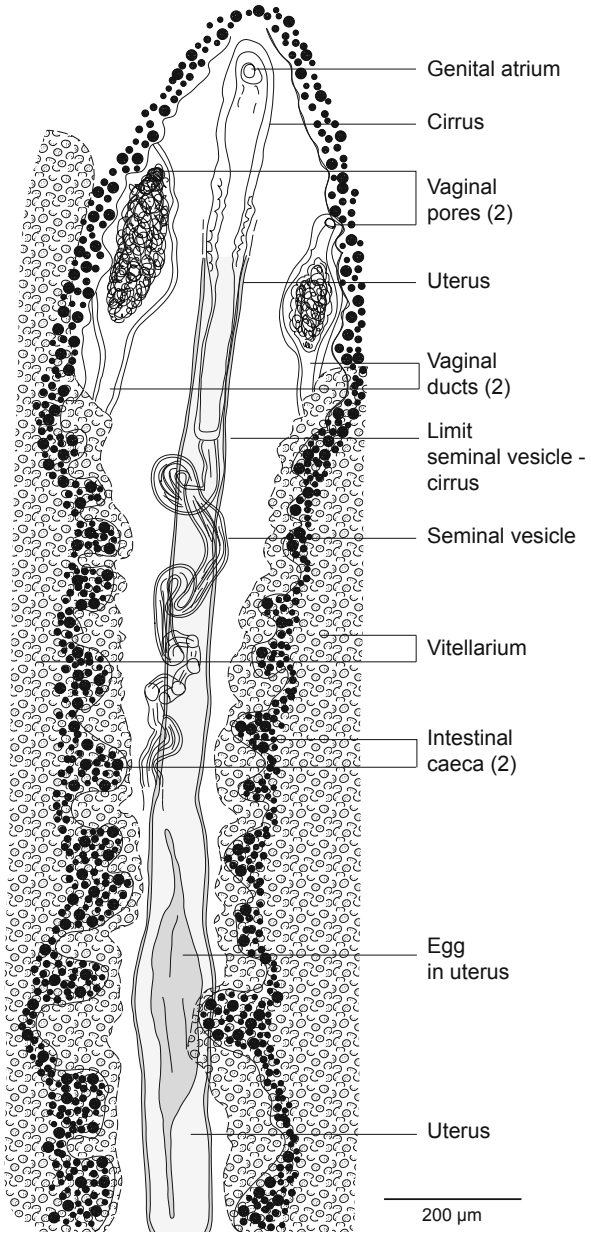

Fig. 2. *Squalonchocotyle euzeti* n. sp.
Anatomy of anterior part of reproductive system

1,480 – 2,054 (1,680 – 1,720); median sclerites 1,850 – 2,498 (1,950 – 2,550); posterior sclerites 1,795 – 2,331 (1,820 – 2,330); hamulus outer length 89 – 96 (66 – 88); hamulus inner length 74 – 85 (70 – 85).

Taxonomic summary
Type host: *Hexanchus griseus* Bonnaterre, 1788
Type locality: Naples, Italy (Cerfontaine, 1899)
Additional localities: Trieste (Italy) (Cerfontaine, 1899); Sète (France) (Maillard & Oliver, 1966; Euzet & Maillard, 1974); near Algiers (Algeria) (this paper).
Specimens examined: 7 specimens from 3 host fish.
Prevalence in Algeria: 100 % (3/3).
Material deposited: MNHN, slides HEL558.

Remarks
Measurements of our specimens from Algeria are consistent with an identification with *P. grisea* and allow to differentiate the specimens from the two only other species in the genus, namely *Protocotyle taschenbergi* (Maillard et Oliver,1966) Euzet et Maillard, 1974 and *Protocotyle euzetmaillardi* Justine, 2011 (Maillard & Oliver, 1966; Euzet & Maillard, 1974; Justine, 2011). Algeria is a new geographical record for the species.

***Squalonchocotyle euzeti* n. sp.**

Description
Based on 32 specimens; measurements in Table 2, including separate measurements for holotype and means for all specimens.
Body elongate, slender, haptor wider than body. Haptor symmetrical, armed with six suckers, each provided with hook-shaped sclerite, and appendix bearing single pair of terminal suckers and single pair of hamuli, each with one sclerite. Haptoral sclerites in 3 pairs arranged symmetrically, each with same shape and with point at right-angles to distal end of sclerite shaft; median sclerites slightly longer than those of anterior and posterior pairs. Appendix elongate, directed anteriorly in flattened specimens. Pair of hamuli with V-shaped root situated near distal end of appendix. Pair of terminal suckers oblong.
Anterior sucker terminal. Pharynx subspherical. Oesophagus short. Caeca internally moderately diverticulate, confluent in posterior part of body, end as two short caeca, one which extends into haptor and one into appendix.
Testes numerous, occupy intercaecal area of posterior part of body, end posteriorly before confluence of caeca. Single sperm duct (vasa efferentia) well visible from testes to seminal vesicle. Seminal vesicle, begins just anterior to oötype, convoluted, thin-walled, contains spermatozoa, continues anteriorly and connects with cirrus; no posterior lobe. Cirrus elongate, unarmed, connects with genital atrium. Prostatic glands not seen. Genital atrium ventral, median, just posterior to bifurcation of caeca.
Ovary located at mid-length of body proper; proximal part of ovary slightly branched; descending and ascending ovarian parts straight; ovary terminates as slender canal superposed to seminal receptacle. Connections of terminal ovary, anterior part of seminal receptacle, posterior part of ovovitelloduct, posterior part of median vitelloduct and genitointestinal canal apparently all located just anteriorly to seminal vesicle. Ovovitelloduct convoluted, without diverticulum, connects anteriorly with oötype. Seminal receptacle cylindrical, oblique with anterior connection. Two lateral vitelloducts unite to form posteriorly directed median vitelloduct, with coil. Oötype wall with longitudinal rows of large cells (‘oötype côtelé’ of Euzet and Maillard 1974). Mehlis’ glands surround oötype. Oötype anteriorly joins uterus. Uterus straight, contains few eggs, ends anteriorly in genital atrium. Two vaginal openings, located just posteriorly to genital atrium or at the same level; anterior portion of vaginae often widened, filled with spermatozoa; posterior portion not well visible.
Eggs fusiform, elongate, operculum not seen, with two polar filaments.

Molecular information
We obtained COI sequences, 396 bp in length, for three specimens; the sequences differed between them by 4 and 6 nucleotide (1 – 1.5 %). A GenBank BLAST of the sequences showed that the closest species were polystomatid polyopisthocotylean monogeneans. These sequences were widely different (20 – 30 %) as polystomatids and hexabothriids are not closely related family. COI sequences are generally appropriate for distinguishing species; in the absence of any other sequence of hexabothriid monogeneans in databases, further comments are useless. Our sequences of *S. euzeti* n. sp. might be useful only when other hexabothriid sequences are available.

Taxonomic summary
Type-host: *Dalatias licha* (Bonnaterre, 1788) (Dalatiidae).
Type-Locality: Off Dellys (36° 55’ N; 3° 53’ E), Algeria.
Additional localities: Off Cap Djenet (36° 43’ N; 3° 36’ E), off Bou Haroun (36° 40’ N; 4° 40’ E), off Cherchell (36° 37’ N; 2° 11’ E), Algeria; all these localities are within 100 km of Algiers.
Site of infection: gills

Type-specimens: Holotype MNHN HEL556, Paratypes MNHN HEL557.
Comparative material observed: One slide of *Squalonchocotyle cerfontaini* collected by Claude Maillard and deposited in the MNHN collections, MNHN 711H-Ti 52 (measurements in Table 1).
Prevalence: 85/100 (85 %).
Etymology: named in honour of Professor Louis Euzet, famous parasitologist and author of major works on hexabothriids, who examined the specimens and confirmed their interest.

Remarks

Species included in *Squalonchocotyle*

Species attributed to *Squalonchocotyle* Cerfontaine, 1899 include: *S. borealis* (Van Beneden, 1853), the type-species, and *S. abbreviata* (Olsson, 1876) Cerfontaine, 1899, *S. cerfontaini* Maillard, 1970, *S. centrophori* Maillard, 1970, *S. laymani* Yamaguti, 1958, *S. mitsukurii* Kitamura, Ogawa, Taniuchi et Hirose, 2006, *S. rajae* Brinkmann, 1971, *S. spinacis* (Goto, 1894), *S. squali* MacCallum, 1931, and *S. tropai* (Tendeiro et Valdez, 1955) (Van Beneden, 1853; Olsson, 1876; Goto, 1894; Cerfontaine, 1899; MacCallum, 1931; Tendeiro & Valdez, 1955; Yamaguti, 1958; Maillard, 1970; Brinkmann, 1971; Kitamura *et al.*, 2006)

Boeger & Kritsky (1989) included only four species in the genus: *S. borealis*, *S. cerfontaini*, *S. centrophori*, and *S. squali*. They considered that *S. somniosi* (Causey, 1926) was a synonym of *S. borealis*, but did not comment on the other species they considered as "unconfirmed".

Kitamura et al. (2006) apparently followed Boeger & Kritsky (1989) when they considered their new species *S. mitsukurii* as the fifth species of the genus. They commented that the taxonomic position of *S. spinacis* was uncertain because the type-specimens were lost.

The list of species of *Squalonchocotyle* in WoRMS (Bray, 2004) includes nine species, i.e. the ten listed above minus *S. rajae*.

We provide here a few remarks about *S. tropai*. The species was described as *Erpocotyle tropai* by Tendeiro and Valdez in 1955, from *Squalus acanthias* (designated as *S. fernandinus*, now considered a synonym (Froese & Pauly, 2016)) off Luanda, Angola, and never mentioned or redescribed again in the scientific literature. However, we found that Maillard (1966) in his unpublished thesis, described new specimens from the same host, collected off Sète, Mediterranean Coast, France (Maillard, 1966); his measurements are included in Table 1. Maillard did not examine the type-specimens and wrote that he could only compare with photographs (the origin and whereabouts of these photographs is unknown; the original description by Tendeiro and Valdez includes only drawings). Euzet and Maillard (1974) claimed that the types of species described by Tendeiro and Valdez were lost. Unfortunately, the slides prepared and described by Claude Maillard were not located in the Euzet collection (13,000 slides, now in MNHN, Paris) and should probably be considered lost. Maillard's thesis (1966) should be considered unpublished for nomenclatural purposes. Euzet and Maillard (1974) used the binomial *Squalonchocotyle tropai* but did not formally indicate that they made a new combination for this species, but it is likely that they were the authors of the current combination, as *S. tropai* (Tendeiro et Valdez, 1955) Euzet & Maillard, 1974; we did not find it in earlier published works.

Generic diagnosis of our specimens

The characteristic oötype with longitudinal rows of cells ('oötype côtelé' of Euzet and Maillard, 1974) is found only in three hexabothriid genera, including *Protocotyle* Euzet et Maillard, 1974, *Rajonchocotyle* Cerfontaine, 1899 and *Squalonchocotyle*. This was considered a synapomorphy uniting these three genera (Boeger & Kritsky, 1989). Our specimens have the characteristic oötype and all characters listed for *Squalonchocotyle* (Boeger & Kritsky, 1989, and in Justine, 2011) i.e. distal cirrus unarmed, ovary branched in its proximal part, two egg filaments, vaginal ducts parallel, seminal receptacle present, and thus belong to the genus.

Species diagnosis

In the following discussion, we do not consider *S. rajae*; whether the species is valid and is really a member of *Squalonchocotyle* is an interesting question, since it is the only member of the genus described from rays (*Raja smirnovi*, *R. rosispinis* and *Breviraja isotrachys*) (Brinkmann, 1971); this is outside of the scope of this paper, but we are confident that the new species described here is distinct from *S. rajae*, on the basis of very different hosts (Rays vs Sharks) and widely separate localities (North Western Pacific vs Mediterranean).

We found that species of *Squalonchocotyle* can be separated into two groups according to body length: a group of seven relatively small species includes *S. abbreviata, S. centrophori*, *S. cerfontaini*, S. *mitsukurii, S. spinacis*, *S. squali*, and *S. tropai* (Table 1); a group of relatively large species includes *S. borealis*, *S. laymani* and *S. euzeti* n. sp. (Table 2). It cannot be excluded, however, that some of the small species were described from immature specimens, as it was the case for *Mobulicola dubium* (Euzet & Maillard, 1974) Patella & Bullard, 2013 (Euzet & Maillard, 1967; Patella & Bullard, 2013).

Squalonchocotyle euzeti is differentiated from *S. laymani* by much longer sclerites (ca 2,000 vs ca 600), different hosts (*Dalatias licha* vs *Mustelus manazo*) and widely separated localities (Mediterranean vs Japan). We measured sclerites on the figures of *S. borealis* by Cerfontaine, and found that they were of similar size to *S. euzeti*. Differential characters include body length (7 – 21 mm vs 25 – 30 in *S. borealis*) and, more importantly, body width (777 – 1,813 vs 3,000 – 4,000 in *S. borealis*) which gives to *S. euzeti* n. sp. a characteristic slender body. Since our specimens were flattened, we consider that their slender body is a genuine condition and not a consequence of insufficient flattening. Therefore, we consider that the slender body separates *S. euzeti* from *S. borealis*. In addition, the hosts are different (*D. licha* vs *Somniosus microcephalus*) and the localities are separate (Mediterranean vs Northern Atlantic).

Discussion

The family Hexabothriidae has been the focus of several revisionary works, including a revision with historical account (Euzet & Maillard, 1974) and a revision associated with a cladistic analysis (Boeger & Kritsky, 1989). The number of genera included in the family has slowly increased from eleven (Euzet & Maillard, 1974) and thirteen (Boeger & Kritsky, 1989) to a total of fifteen in most

Table 2. "Large" species of *Squalonchocotyle*. Measurements in various publications and comparison with the new species *S. euzeti*. *** From measurements on drawings

Species	***S. borealis***	***S. borealis***	***S. laymani***	***S. euzeti* n. sp.**	***S. euzeti* n. sp.**
Source	Van Beneden, 1853	Cerfontaine, 1899	Yamaguti, 1958	This paper	This paper
Name in original description	*Onchocotyle borealis*	*Squalonchocotyle borealis*	*Squalonchocotyle laymani*		
Host name in original description	*Scimnus glacialis*	*Scimnus glacialis*	*Mustelus manazo*	*Dalatias licha*	*Dalatias licha*
Host valid name	*Somniosus microcephalus* (Bloch et Schneider, 1801)	*Somniosus microcephalus* (Bloch et Schneider, 1801)	*Mustelus manazo* Bleeker, 1855	*Dalatias licha* (Bonnaterre, 1788)	*Dalatias licha* (Bonnaterre, 1788)
Locality	Atlantic, North Sea (Belgium)	Atlantic, North Sea (Belgium)	Pacific (Japan)	Mediterranean (Algeria)	Mediterranean (Algeria)
n		6	5	Holotype	31 paratypes
Total body length	25,000 – 30,000	20,000 (unflattened)	8,500 – 14,000	18,463	12,921±3,289 (7,326 – 21,830, n=32)
Body proper width	3,000 – 4,000		1,200 – 1,400	1,628	1,078±239 (777 – 1,813, n = 32)
Anterior sucker diameter			310 – 390		
Anterior sucker length				306	286 (201 – 410, n = 20)
Anterior sucker width				366	343 (261 – 448, n = 20)
Pharynx diameter			70 – 110		
Pharynx length				194	179 (112 – 246, n = 21)
Pharynx width				149,2	164 (119 – 216, n = 21)
Haptor length				4,051	2,428 (1,758 – 4,051, n = 12)
Haptor width				3,552	2,450 (1,610 – 3,552, n = 12)
Anterior sclerite length		2,228 ***	750 – 920	1,638	1,763 (1,130 – 2,906, n = 20)
Median sclerite length			600 – 620	1,675	1,851 (1,171 – 2,909, n = 20)
Posterior sclerite length			600 – 620	1,586	1,749 (1,089 – 2,738, n = 20)
Appendix length			800 – 1,100	1,029	872 (522 – 1,186, n = 10)
Appendix width			400 – 530	306	207 (142 – 336, n = 10)
Hamulus length			40 – 50		
Hamulus outer length		83 ***		87	70 (59 – 87, n= 13)
Hamulus inner length		66 ***		74	63 (44 – 74, n = 13)
Testes number	numerous		100 or more	82	82 (69 – 96, n = 10)
Cirrus length			130 – 150	307	421 (307 – 551, n = 7)
Cirrus width			120 – 150		
Eggs proper length		250	450		539 (463 – 644, n = 5)
Egg filament number and length			2; 100 – 150		2
Seminal receptacle length			250 – 420	410	667 (522 – 858, n = 11)

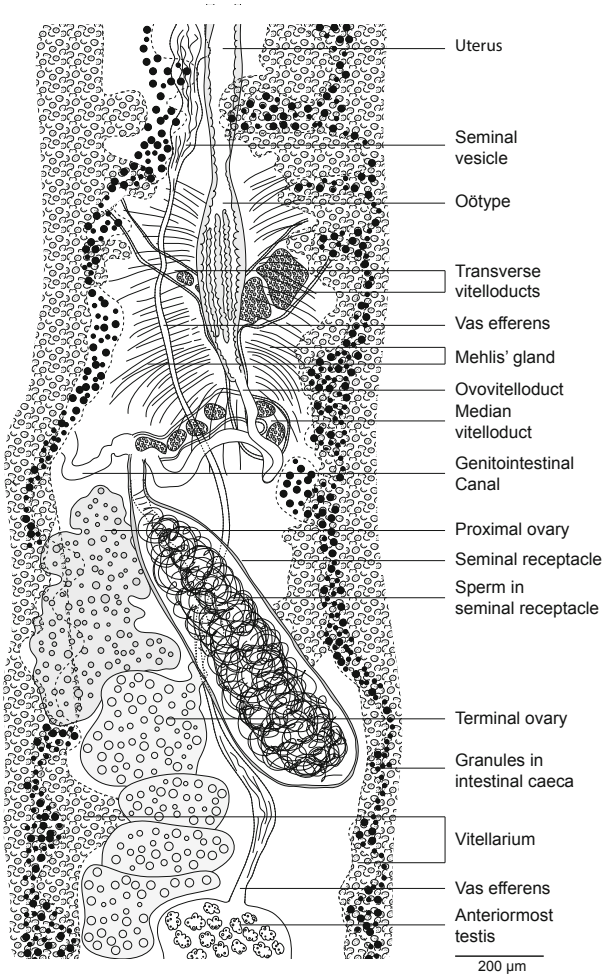

Fig. 3. *Squalonchocotyle euzeti* n. sp.
Anatomy of median part of reproductive system

recent works (Patella & Bullard, 2013). However, the hexabothriid literature is plagued with confusion and discrepancies (Vaughan & Christison, 2012) but probably no more than any large family of monogeneans. The Hexabothriidae are considered a basal group within the Polyopisthocotylea in phylogenies based on morphology (Boeger & Kritsky, 1993) and molecules (Mollaret *et al.*, 2000; Jovelin & Justine, 2001; Olson & Littlewood, 2002). Our survey of deep-sea sharks, with many negative results, emphasizes one of the major problems with hexabothriids, which is that specimens are rare. For *Squalonchocotyle*, our Tables show that most species have been described from a very small number of specimens. Whittington and Chisholm (2003) commented upon the low number of monogeneans in sharks, remarked that only 15 species of hexabothriids had been described from sharks, and proposed several biases which could explain these low numbers. One of the biases is the lack of sampling (Whittington & Chisholm, 2003); after more than 700 sharks investigated, we believe, however, that even large samplings provide only a limited number of hexabothriid species.

Our study also emphasizes the very small number of molecular sequences available for members of this family – so far, our COI sequence of *Squalonchocotyle euzeti* n. sp. is the first for the family, and a research on Hexabothriidae in GenBank (date: June 9, 2016) returns only 17 sequences, from a very small total number of three species; this, however, might improve in the future.

Acknowledgements

Delphine Gey (Service de Systématique Moléculaire, UMS 2700 CNRS, MNHN, Paris) produced the new sequences presented in this paper. This work was supported by MNHN grants "ATM Barcode" and "ATM PARSUDMED" and the Mistrals ENVI-MED project BIOPARMED (Ministère des Affaires Étrangères, France).

References

Boeger, W.A., Kritsky, D.C. (1989): Phylogeny, coevolution, and revision of the Hexabothriidae Price, 1942 (Monogenea). *Int. J. Parasitol.*, 19: 425 – 440. DOI: 10.1016/0020-7519(89)90099-4

Boeger, W.A., Kritsky, D.C. (1993): Phylogeny and a revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). *Syst. Parasitol.*, 26: 1 – 32. DOI: 10.1007/BF00009644

Bowles, J., Blair, D., McManus, D.P. (1995): A molecular phylogeny of the human schistosomes. *Mol. Phylogenet. Evol.*, 4(2): 103 – 109. DOI: 10.1006/mpev.1995.1011

Bray, R.A. (2004): *Squalonchocotyle* Cerfontaine, 1899. Accessed through: World Register of Marine Species at *http://www.marinespecies.org/aphia.php?p=taxdetails&id=119365* on 2016-06-07.

Brinkmann, A.J. (1971): Hexabothriids (Monogeneoidea) from far eastern skates. *Parazitologiya*, 5: 532 – 538 (In Russian)

Cerfontaine, P. (1899): Contribution à l'étude des Octocotylidés. V. Les Onchocotylinae. [Contribution to the study of octocotylids. V. Onchocotylinae]. *Arch. Biol.*, 16: 345 – 478 (+ Plates XVIII – XXI) (In French)

Dillon, W.A., Hargis, W.J., Jr. (1968): Monogenetic Trematodes from the Southern Pacific Ocean. Part IV. Polyopisthocotyleids from New Zealand Fishes: The families Mazocraeidae, Diclidophoridae dnd Hexabothriidae. *Proc. Biol. Soc. Wash.*, 81: 351 – 366

Euzet, L., Maillard, C. (1967): Parasites de Poissons de mer ouest-africains, récoltés par J. Cadenat. VI. Monogènes de Sélaciens. [Parasites of West-African marine sea fish, collected by J. Cadenat. VI. Monogeneans of Selacians]. *Bull. Inst. Fondam. Afr. Noire Ser. A Sci. Nat.*, 24(4): 1435 – 1493 (In French)

Euzet, L., Maillard, C. (1974): Les Monogènes Hexabothriidae Price, 1942. Historique, systématique, phylogenèse. [The monogenean Hexabothriidae Price, 1942. History, systematic, phylogeny]. *Bull. Mus. Natl. Hist. Nat. 3° série, 206, Zoologie*, 136: 113 – 141 (In French)

Fischer, W., Bauchot, M.-L.Schneider, M. (1987): *Fiches FAO d'identification des espèces pour les besoins de la pêche. (Révision 1). Méditerranée et mer Noire. Zone de pêche 37. Volume II.*

Vertébrés. [*FAO Files for the Identification of species for fisheries purposes. (Revision 1). Mediterranean and Black Sea. Fishing area 37. Volume II. Vertebrates*.]. *Publication prepared by FAO, the result of an agreement between FAO and the Commission of the European Communities (Project GCP / INT / 422 / EEC) financed jointly by these two organizations. Rome, FAO, Vo1.2: 761 – 1530* (In French)

Froese, R., Pauly, D., ed. (2016): *FishBase. World Wide Web electronic publication. www.fishbase.org*

Goto, S. (1894): *Studies on the ectoparasitic Trematodes of Japan.* Edition, Tokyo

Jovelin, R., Justine, J.-L. (2001): Phylogenetic relationships within the polyopisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. *Int. J. Parasitol.*, 31: 393 – 401

Justine, J.-L. (2011): *Protocotyle euzetmaillardi* n. sp. (Monogenea, Hexabothriidae) from the bigeye sixgill shark *Hexanchus nakamurai* Teng (Elasmobranchii, Hexanchidae) off New Caledonia. *Syst. Parasitol.*, 78: 41 – 55. DOI: 10.1007/s11230-010-9275-6

Justine, J.-L., Rahmouni, C., Gey, D., Schoelinck, C.Hoberg, E.P. (2013): The monogenean which lost its clamps. *PLoS ONE* 8(11): e79155. DOI: 10.1371/journal.pone.0079155

Kitamura, A., Ogawa, K., Taniuchi, T., Hirose, H. (2006): Two new species of hexabothriid monogeneans from the ginzame *Chimaera phantasma* and shortspine spurdog *Squalus mitsukurii*. *Syst. Parasitol.*, 65(2): 151 – 159. DOI: 10.1007/s11230-006-9046-6

Littlewood, D.T.J., Rohde, K., Clough, K.A. (1997): Parasite speciation within or between host species? – Phylogenetic evidence from site-specific polystome monogeneans. *Int. J. Parasitol.*, 27: 1289 – 1297. DOI: 10.1016/S0020-7519(97)00086-6

MacCallum, G.A. (1931): Four new species of Trematode worms of the subfamily Onchocotylinae. *Proc. U. S. Nat. Mus.*, 79: 1 – 8. DOI: 10.5479/si.00963801.79-2892.1

Maillard, C. (1966): *Études sur les Hexabothriidae (Monogenea) parasites des Sélaciens de la région de Sète*. [*Studies on Hexabithriidae (Monogenea) parasites in Selacians in the region of Sète*]. PhD Thesis, Thesis, Université de Montpellier, France (In French)

Maillard, C. (1970): Trois nouvelles espèces d'Hexabothriidae (Monogenea) parasites de Sélaciens du Golfe du Lion. [Three new species of Hexabothriidae (Monogenea) parasites of Selacians in the Gulf of Lion]. *Ann. Parasitol. Hum. Comp.*, 45(5): 563 – 576 (In French)

Maillard, C., Oliver, G. (1966): Monogenea, Hexabothriidae. *Vie Milieu A: Biol. Ma.*, 17(3A): 1201 – 1216

Martorelli, S.R., Marcotegui, P.S.Suriano, D.M. (2008): *Squalonchocotyle squali* MacCallum (Monogenoidea, Hexabothriidae) on *Squalus acanthias* Linneus (Elasmobranchii, Squalidae) from the Atlantic coast of Argentina. *Pan-Am. J. Aquat. Sci.*, 3(1): 14 – 17

Mollaret, I., Jamieson, B.G.M., Justine, J.-L. (2000): Phylogeny of the Monopisthocotylea and Polyopisthocotylea (Platyhelminthes) inferred from 28S rDNA sequences. *Int. J. Parasitol.*, 30: 171 – 185

Olson, P.D., Littlewood, D.T.J. (2002): Phylogenetics of the Monogenea – evidence from a medley of molecules. *Int. J. Parasitol.I,* 32: 233 – 244

Olsson, P. (1876): Bidrag till skandinaviens helminthfauna. I. [Study of scandinavian helminthofauna. I.] *K. Sven. vetensk. akad. handl.*, 14(1): 1 – 41 (In Swedish)

Patella, R., Bullard, S.A. (2013): Hexabothriids of devil rays (Mobulidae): new genus and species from gill of *Mobula hypostoma* in the Northern Gulf of Mexico and redescription of a congener from *Mobula rochebrunei* in the eastern Atlantic Ocean. *J. Parasitol.*, 99(5): 856 – 867. DOI: 10.1645/12-153.1

Price, E.W. (1942): North American monogenetic trematodes. V. The family Hexabothriidae n. n. (Polystomatoidea). *Proc. Helminthol. Soc. Wash.*, 9(2): 39 – 56

Tendeiro, J., Valdez, V. (1955): Helmintologica ictiológica. Helmintes de alguns peixes da Costa Oeste-Africana. [Ichthyological helminthology. Helminths of some fish of the West-African coast]. *Bol. Cult. Guiné. Port.*, 37: 129 – 166 (In French)

Van Beneden, P.-J. (1853): Espèce nouvelle du genre *Onchocotyle*, vivant sur les branchies du *Scimnus glacialis*. [New species of genus Onchocotyle, living on the gills of Scimnus glacialis]. *Bull. Acad. R. Belg.*, 20(9): 59 – 68 (In French)

Vaughan, D., Christison, K. (2012): Towards addressing the current state of confusion within the Hexabothriidae Price, 1942 (1908): *Callorhynchocotyle* Suriano & Incorvaia, 1982 (Monogenea: Hexabothriidae) re-visited, with the preliminary evaluation of novel parameters for measuring haptoral armature of hexabothriids. *Zootaxa*, 3229: 1 – 34. DOI: 10.15468/6tmpcn

Whittington, I.D., Chisholm, L.A. (2003): Diversity of Monogenea from Chondrichthyes: do monogeneans fear sharks? In: *Taxonomie, écologie et évolution des Métazoaires parasites.* [*Taxonomy, Ecology and Evolution of Metazoan Parasites*]. *Livre-hommage à Louis Euzet*, Vol II. Combes, C. & Jourdane, J. (Eds). Perpignan: Presses Universitaire de Perpignan, pp. 339 – 363 (In French)

Yamaguti, S. (1958): Studies on the helminth fauna of Japan. Part 53. Trematodes of fishes, XII. *Publ. Seto Mar. Biol. Lab.*, 7: 53 – 88