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Diversity of monogenean parasites in belonid fishes off the Mediterranean Sea with redescription of *Aspinatrium gallieni* Euzet and Ktari, 1971 and *Axine belones* Abildgaard, 1794

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ABSTRACT

Objective: To provide informations on the diversity of Monogenea parasitizing belonid fishes from the Mediterranean Sea. Monogenean species *Aspinatrium gallieni* Euzet and Ktari, 1971 (*A. gallieni*) and *Axine belones* Abildgaard, 1794 (*A. belones*) from belonids *Tylosurus acus imperialis* Rafinesque, 1810 (*T. a. imperialis*) and *Belone belone* Lowe, 1839 (*B. belone*) off Tunisian coast were redescribed.

Methods: During the sampling period between 2004 and 2009, a total of 624 belonid fish belonging to three species, 453 *B. belone*, 45 *Belone svetovidovi* Collette and Parin, 1970 and 126 *T. a. imperialis* were investigated for monogenean parasites.

Results: Five Polyopisthocotylean monogenean parasites species were founded infected belonid fishes from Tunisian coast. These are, *Nudaciraxine imperium* Châari, Derbel and Neifar, 2010, *A. belones*, *Axine* sp., *Axinoides* sp. (Axinidae) and *A. gallieni* (Micrcotylidae). Among them, the most prevalent species *A. gallieni* from the inner gill cover and gill filaments of *T. a. imperialis* and *A. belones* from the gills of *B. belone* were redescribed. *A. gallieni* represented new host record for *T. a. imperialis*. A complete list of monogenean parasites found in belonid fishes from our study and those reported from the Mediterranean Sea was presented.

Conclusions: This paper enhances the current knowledge of Monogenea infecting belonids off the Mediterranean Sea. It's the first study on monogenean parasites of the overall belonid fishes off Tunisia.

1. Introduction

Due to their great diversity in terms of species number, but also their number of life history strategies, there is an increasing interest in using parasites as biological or ecological indicators of their fish host life conditions. Monogeneans are flatworms from a group of parasites commonly founded on the gills, skin or fins of fishes and lower aquatic invertebrates. There are more than 100 families of monogeneans found on fishes of the world, in fresh and salt water. Most species are host- and site-specific, requiring only one host to complete an entire life cycle[1].

In Tunisia, several studies on the monogenean parasites of fishes have been made[2-19]. Concerning those of belonid fishes, only two records have previously shown presence of *Axine belones* Abildgaard, 1794 (*A. belones*) on *Belone belone* Lowe, 1839 (*B. belone*)[20] and *Aspinatrium gallieni* Euzet and Ktari, 1971 (*A. gallieni*) on *Strongylura acus* Lacépède, 1803 (*S. acus*)[3]. During parasitological survey on various species of belonids along Tunisian coast, several Polyopisthocotylean monogeneans were recovered. We recently described monogenean species *Nudaciraxine imperium* Châari, Derbel and Neifar, 2010 (*N. imperium*) from the gills of *Tylosurus acus imperialis* Rafinesque, 1810 (*T. a. imperialis*)[18]. This paper enhances the current knowledge of monogeneans from belonid hosts off Tunisian waters. In this paper, we redescribed two others monogeneans *A. gallieni* from the inner gill cover and gill filaments of *T. a. imperialis* as a new host record and *A. belones* from the gills of *B. belone* to modern standards. Our study also aimed to list and give parameters and sites of infection of monogeneans species founded in belonid fishes off Tunisian coast. A complete list of the monogenean parasites reported in belonid fishes from the Mediterranean Sea is also presented.

2. Materials and methods

Between 2004 and 2009, a total of 624 specimens belonging to

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three species of belonid fishes: 453 *B. belone*, 45 *Belone svetovidovi* Collette and Parin, 1970 (*B. svetovidovi*) and 126 *T. a. imperialis* were collected. Fish were caught by local fishermen using gillnets from the eastern Tunisian coast, at the central area [Mahdia (35°30' N, 11°3' E), Sousse (35°50' N, 10°38' E), Monastir (35°46' N, 10°49' E) and Chebba (35°14' N, 11°8' E)] and at the southern area [Sfax (34°47' N, 10°49' E), Kerkennah (34°37' N, 11°6' E), Gabes (33°56' N, 10°4' E), Jerba (33°46' N, 10°44' E) and Zarzis (33°30' N, 11°7' E)]. Specimens were identified using the methods of Collette and Parin[21] and Bauchot[22].

Fish were dissected and examined carefully a few hours after capture for Monogenea. The gill covers and gill arches were separated and placed in Petri dishes with filtered seawater using filter paper. Monogenea were detected using a stereomicroscope, detached from the gills and gill covers and then were transferred to a dish containing filtered seawater. They were studied either alive or fixed between slide and coverslip in 70% alcohol. Some fixed specimens were stained with Semichon's acetic carmine. Other specimens were double-stained with light green and Semichon's acetic carmine to study the morphology of the clamps. After dehydration through a graded ethanol series, specimens were cleared with clove oil and mounted in Canada balsam. Some fixed specimens were mounted in Berlese's fluid in order to study the haptoral sclerites and the genital armature.

Illustrations and measurements were made of stained specimens with the aid of a Leitz light microscope equipped with a drawing tube. Illustrations were scanned and redrawn on a computer using Corel Draw software. All measurements were given in micrometres (unless stated otherwise) as the mean, with the range and the number of measurements (*n*) in parentheses.

Prevalence (P), mean intensity (MI) and mean abundance (MA) were determined following Bush *et al.*[23].

3. Results

Five Polyopisthocotylea monogenean parasites species from the axinid and the micrcotylid family were founded infecting belonid fishes from Tunisian coasts. These are: *N. imperium*, *A. belones*, *Axine* sp., *Axinoides* sp. (Axinidae) and *A. gallieni* (Micrcotylidae). The report of *A. gallieni* from the belonid *T. a. imperialis* represented a new host record. Data on parameters and infection site of monogeneans parasites are given in Table 1. *A. belones* and *A. gallieni* were the most prevalent monogenean species from belonids *B. belone* and *T. a. imperialis* respectively (Table 1). These monogenean parasites species are above redescribed. The monogenean parasites of belonid fishes from this study and from others previously reports are listed in Table 2.

Table 1
The prevalence (P%), mean intensity (MI), mean abundance (MA) and infection site of monogeneans in belonid fish from Tunisia.

Host species	Monogenean species	P (%)	MI	MA	Site of infection
B. belone	*A. belones*	59	4.36	2.57	Gills
B. svetovidovi	*Axine* sp.	36	4.87	1.73	Gills
T. a. imperialis	*A. gallieni*	55	3.07	1.70	Inner gill cover, gills
	N. imperium	43	2.63	1.14	Gills
	Axinoides sp.	3	1.75	0.06	Gills

Table 2
Checklist of monogenean parasites of belonids from Mediterranean Sea.

Species	Host	Area	Reference
Microcotylidae Tashenberg, 1879			
A. gallieni	*S. acus*	GG, GT	[3]
	T. a. imperialis	T	Present study
Axinidae Monticelli, 1903			
A. belones	*B. belone*	GL, GG	[20]
		M	[31]
		T	Present study
Axine sp.	*B. svetovidovi*	T	Present study
N. imperium	*T. a. imperialis*	T	[18]
Axinoides sp.	*T. a. imperialis*	T	Present study

GG: Gulf of Gabes; GT: Gulf of Tunis; T: Tunisian coast; GL: Gulf of Lion; M: Mediterranean Sea.

Family Microcotylidae Tashenberg, 1879

A. gallieni

Host: *T. a. imperialis*

Voucher specimens deposited: Muséum National d'Histoire Naturelle, Paris, No. MNHN HEL553.

Redescription is shown in Figure 1 (based on 15 adult whole mounted worms). Body flattened dorso-ventrally, cordiform, tapering in anterior region, 7.71 mm (5.20–9.90 mm, *n* = 15) long by 3.30 mm (2.30–4.00 mm, *n* = 15) wide at level of ovary. Haptor, roseate shaped, slightly wider than body, occupies the posterior third of body length, provided with several rows of 120 (81–157, *n* = 11) pedunculate clamps (Figure 1A). Largest clamps in the middle part measuring 196 (160–230, *n* = 13) in length and 128 (100–170) in width. Clamps with a well developed, muscular, cupuliform shaped body and sclerites of "*Microcotyle* type" with median sclerite "a" of the anterior jaw ends in T at its distal end extended proximally but not curved and not pass in the posterior jaw. Marginal sclerites, semicircular, end dorsally on "a". In posterior jaw, sclerite "c" arcuate, articulated proximally on "b", and distal ends quite discarded from the median plane which marks an important sclerification welded to the inner side at the end of "a" (Figure 1B, C).

Two muscular suckers subcircular, aseptate, 171.54 (130–240, *n* = 13) long by 143.57 (100–190, *n* = 14) wide. Mouth subterminal, ventral. Pharynx, oval, 158.46 (90–250, *n* = 13) long by 86.54 (70–130, *n* = 13) wide. Oesophagus, short. Intestinal bifurcation immediately anterior to common genital pore. Two intestinal caeca, lateral, dendritic diverticulate laterally and medially, united posteriorly.

Testes numerous, 196 (70–290, *n* = 14), in posterior body field, between ovary and intestinal caeca. Vas deferens, dorsal, 70.71 (25–100, *n* = 7) in diameter, narrow and straight in anterior part, sinuous in posterior part plunges to ventrally where it opens in a small genital atrium, unarmed, median, slightly posterior to intestinal bifurcation.

Ovary median, tubular, pretesticular, folded, starts from the right side of the body, rises slightly to median line, pass to the left then loops anteriorly to the right. Oviduct first sinuous, becoming straight and crosses distal ascendant extremity of ovary. Genito-intestinal canal opening into right intestinal caecum. Ootype, smooth

walled. Mehlis' gland in form of crown at base of ootype. Uterus, extending along body midline to genital atrium as relatively straight tube ventral to ovary and vas deferens. Vitelline follicles, lateral, surrounding intestinal caeca, extending posterior to level of haptoral region. Transverse vitelloducts united near ovary to form madian vitelloduct directed to left side and joining distal end of oviduct. Vagina, unarmed, opening via single mediodorsal pore, located at 1 280 (650–2 200, n = 14) from anterior extremity, connected with globular vaginal chamber. Vaginal canal short, dividing posteriorly into two lateral vaginal canals connecting with vitelline ducts. Egg, fusiform, 90 (60–120, n = 4) in width, with two polar filaments of unequal length (Table 3).

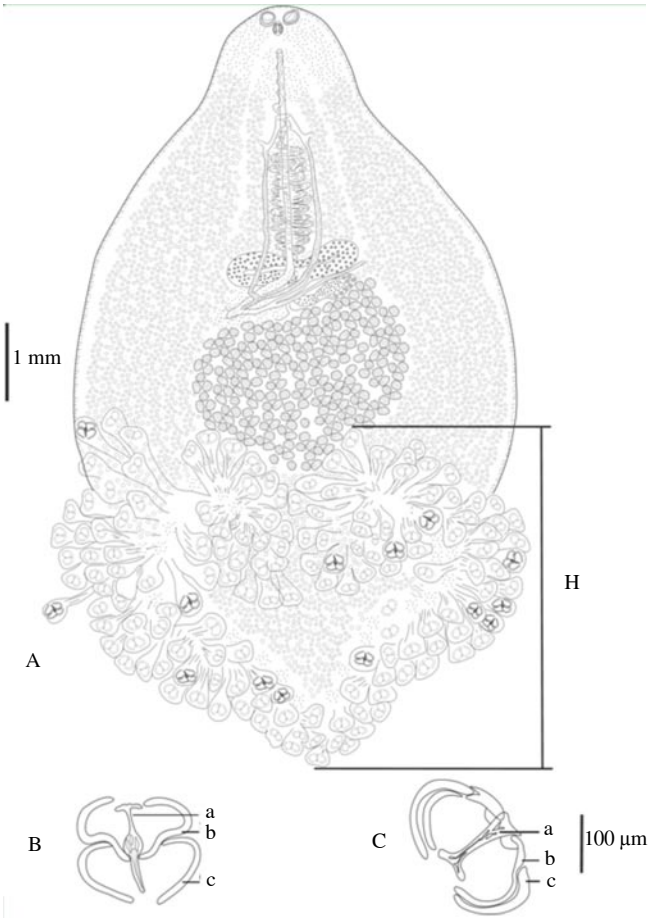

Figure 1. *A. gallieni* Euzet and Ktari, 1971.
A: Composite drawing of whole worm in ventral view, scale bar; B: Armature of open clamp; C: Armature of closed clamp. a: Median sclerite; b and c: Marginal sclerites; H: Haptor.

Table 3
Morphometrics of *A. gallieni* for comparisons with Euzet and Ktari (1971).

Morphometrics	Study of Euzet and Ktari (1971)	Present study
Body length (mm)	6.00–11.00	7.71 (5.20–9.90)
Body width (mm)	3.00–6.00	3.30 (2.30–4.00)
Buccal sucker diameter (µm)	150–200	171×143
Clamp number	100–150	120 (81–157)
Clamp size (µm)	250–150	196×128
Testes number	200–250	196 (70–290)
Testes diameter (µm)	75–150	-
Egg width (µm)	90	90 (60–120)

Family Axinidae Monticelli, 1903

A. belones

Host: *B. belone*

Voucher specimens deposited: Muséum National d'Histoire Naturelle, Paris, No. MNHN HEL554.

Redescription is shown in Figure 2 (based on 18 adult whole mounted worms). Body, flattened dorso-ventrally; 5.40 mm (3.90–7.25 mm, n = 17) long by 0.68 (0.40–1.05, n = 18) wide at level of ovary. Haptor asymmetrical, 1.52 mm (1.15–2.00 mm, n = 18) in width, directed to left, armed with single row of 60 (43–66, n = 15) clamps (Figure 2A, Table 4). Clamps, 87 (70–110, n = 10) long by 38 (20–60, n = 10) wide, *"Axine* type" with marginal sclerites of anterior and posterior jaw, in two parts (Figure 2E). Two pairs of hamuli and one pair of uncinuli situated 600 (520–690, n = 4) from posterior end of haptor (Figure 2D). Number of clamps at right side of hamuli and uncinuli 25 (23–27, n = 4), at left side 31 (20–39, n = 4).

Table 4
Morphometrics of *A. belones* for comparisons with Euzet and Lopez-Roman (1973).

Morphometrics	Euzet and Lopez-Roman (1973)	Present study
Body length (mm)	5.60–9.70	5.40 (3.90–7.25)
Body width (mm)	1.40–2.20	0.68 (0.40–1.05)
Haptor width (mm)	-	1.52 (1.15–2.00)
Buccal sucker length (µm)	47–49	36 (30–40)
Buccal suckers width (µm)	30–33	27 (20–35)
Clamp number	68 (59–77)	60 (43–66)
Clamp lengths (µm)	-	87 (70–110)
Clamp width (µm)	-	38 (20–60)
Testes number	40–50	36 (30–41)
Spines of genital atrium		
Posterior crown (µm)	17–24	16 (14–22)
Median (µm)	10–12	10 (8–12)
Two lateral groups (µm)	20–28	13 (10–19)

Anterior end bilobed. Two muscular buccal suckers subcircular, aseptate, 36 (30–40, n = 10) long by 27 (20–35, n = 10) wide. Mouth subterminal, pharynx oval, 30 (25–35, n = 4) long by 25 (20–30, n = 4) wide. Oesophagus long, from anterior extremity, caeca dendritic diverticulate laterally and medially, extending into haptoral area, with right branch longer than left.

Testes intercaecal, arranged in two longitudinal rows in median field, 36 (30–41, n = 7) in number. Genital atrium armed with three groups of spines, two lateral groups with 13 (10–19, n = 6) spines each, a median group of about 10 (8–12, n = 7) spines. Cirrus, muscular, with evaginable conical shaped, posterior part armed at base with crown of 16 (14–22, n = 6) spines followed with anterior part of vas deferens, tubular, narrow, muscular wall, medio-dorsal (Figure 2B).

Ovary, pre-testicular, J-shaped, intercaecal, in anterior third of body. Oviduct directed anteriorly from distal extremity of ovary. Dextral genito-intestinal canal immediately posterior to level of right vitelline duct. Ovovitelline duct loops posteriorly, ascending

limb widens to form ootype. Seminal receptacle present. Vitelline follicles lateral, surrounding dendritic intestinal caeca, extending from genital pore into haptoral region. Vagina, muscular, lined with heavy cuticula bearing prominent papilliform projections. Vaginal pore, dorso-lateral, situated on right side immediately posterior to intestinal bifurcation. Uterus ventral, in midline, opening at level of genital atrium in common genital pore (Figure 2C). Egg is not seen.

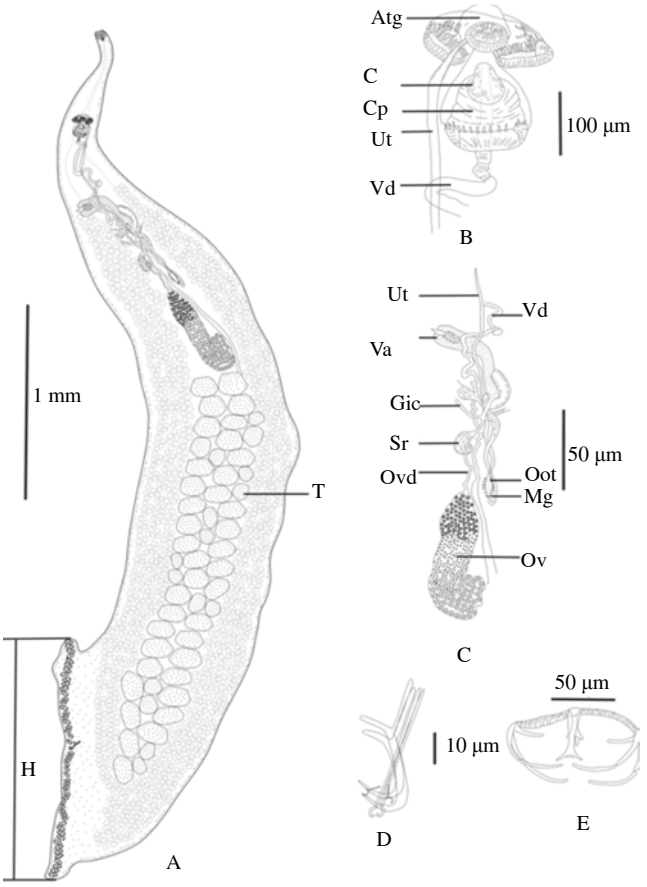

Figure 2. *A. belones* Abildgaard, 1794.
A: Composite drawing of whole worm in dorsal view; B: Armature of anterior genital complex; C: Posterior genital complex; D: Hamuli and uncinuli; E: Clamp. Atg: Atrium genital; C: Cirrus; Cp: Cirrus pouch; Gic: Genito-intestinal canal; H: Haptor; Mg: Mehlis gland; Oot: Ootype; Ovd: Oviduct; Ov: Ovary; Sr: Seminal receptacle; T: testis; Ut: Uterus; Va: Vagin; Vd: Vas deferens; Vt: Vitellogen gland.

4. Discussion

A. gallieni was first described by Euzet and Ktari from *S. acus* from Gulfs of Tunis and Gabes, Tunisia[3]. We collected this species for the first time from *T. a. imperialis* from Tunisia. The description and measurements of *A. gallieni* given in the present study are in close agreement with those of Euzet and Ktari for specimens from *S. acus*[3]. Whereas, specimens from the present study are slightly smaller in almost all measurements (Table 3). It is possible that there is confusion by Euzet and Ktari in determining the host because *S. acus* is not recorded yet in the Mediterranean Sea or in Tunisia. Belonid fishes were represented in Tunisian coast by *B. belone*, *B. svetovidovi* and *T. a. imperialis*[22,24].

We collected *A. gallieni* mainly from the inner side of the gill cover of *T. a. imperialis*. This helminth has a preferential specific site. Monogenea are known to be highly host-specific, and they also have a specific sites within hosts[25,26].

In this study, the prevalence of *A. gallieni* is more important in the left side (64%) than the right side (36%). This result has also been found by Euzet and Ktari[3]. It seems that the left inner side of the gill cover offers a favourable environment for the attachment and development of the oncomiracidium that may be in relation with a well vascular area.

Monogenean parasite species collected from the gills of *B. belone* belongs to the Axinidae based on the asymmetrical haptor with "*Axine*-type" clamps on only one side and the persistence of larval hamuli in adult specimens. The presence of numerous clamps and genital atrium armed with 3 groups of spines places these worms within the Axininae Monticelli, 1903. The presence of median spines of the genital atrium in single row in distal portion is an exclusive feature of the genus *Axine* Abildgaard, 1794. Specimens studied here agree well morphologically with *A. belones* described by Euzet and Lopez-Roman from *B. belone* off the Gulf of Lion and the Gulf of Tunis in the north and south occidental Mediterranean Sea[20]. Nevertheless, specimens from the present study are smaller in almost all measurements (Table 4).

A. belones appears to be cosmopolitan. It has been collected from *B. belone* in the Adriatic Sea by Radujkovic and Euzet[27], in the black sea by Dmitrieva[28] and Oktener[29], in the Baltic Sea by Braun[30], from the Mediterranean Sea by Euzet and Lopez-Roman[20] and Naidenova and Mordvinova[31].

Due to the scanty information on monogenean parasites of belonid fishes off the Mediterranaean Sea, only three studies have been so far reported by Euzet and Ktari[3] and Euzet and Lopez-Roman[20], Naidenova and Mordvinova[31]; we should here mention further results of our observations (Table 2). A comprehensive list of the monogenean parasites of belonid fishes is therefore presented in Table 2.

This work enhances the current knowledge of monogeneans parasites infecting belonid fishes from the Mediterranean Sea. This is the first study on the Monogenea of all belonid species from Tunisian coast.

Conflict of interest statement

We declare that we have no conflict of interest.

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