***Nudaciraxine imperium* sp. n. (Monogenea: Axinidae) from the gills of Mediterranean needlefish *Tylosurus acus imperialis* (Teleostei: Belonidae)**

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Abstract: *Nudaciraxine imperium* sp. n. (Monogenea: Axinidae) is described from the gills of the needlefish *Tylosurus acus imperialis* (Rafinesque) caught in the Gulf of Gabès, Tunisia. This new species mainly differs from both previously described species, *Nudaciraxine gracilis* (Linton, 1940) Price, 1962 from *Strongylura marina* (Walbaum) and *Nudaciraxine cabosanlucensis* Payne, 1990 from *Ablennes* sp., by having more testes (42 vs. 20–22 and 32, respectively). *Nudaciraxine imperium* also differs from *N. gracilis* by having a median vaginal pore (submedian in *N. gracilis*), narrower clamps (52–70 µm vs. 75–100 µm) and differently shaped haptoral lateral hamuli. From *N. cabosanlucensis* it can be distinguished by a J-shaped rather than U-shaped ovary, a longer cirrus pouch (125–190 µm vs. 64–70 µm), an oesophageal bifurcation slightly anterior to genital complex (immediately after pharynx in *N. cabosanlucensis*) and a wider guard on the lateral hamuli. In agreement with Payne (1990), the diagnosis of the genus *Nudaciraxine* Price, 1962 is amended as follows: vaginal pore dorsal, median to submedian, armed or not with horn-like spines. This is the first report of the genus *Nudaciraxine* in the Mediterranean Sea. The potential use of axinid monogeneans as biological tags for subspecies of belonid fishes is discussed.

Keywords: Monogenea, Axinidae, *Nudaciraxine imperium*, gill parasite, *Tylosurus acus imperialis*, Belonidae, Tunisia, Mediterranean Sea

The needlefish *Tylosurus acus* (Lacépède) is an epipelagic teleost marine fish, widely distributed in the tropical and subtropical zones of the world. Four subspecies are recognised according to their geographical distribution (Collette 2003): *Tylosurus acus acus* (Lacépède) in the Western Atlantic, *Tylosurus acus rafale* Collette et Parin in the Gulf of Guinea, *Tylosurus acus imperialis* (Rafinesque) in the Mediterranean Sea, and *Tylosurus acus melanotus* (Bleeker) throughout the Indo-Western Pacific, extending to the Eastern Pacific.

Tylosurus acus has been reported as a host of monogeneans belonging to the Axinidae Unnithan, 1957 in different localities. *Axine belones* Abildgaard, 1794 has been reported in the Mediterranean Sea (Palombi 1949), *Nudaciraxine gracilis* (Linton, 1940) Price, 1962 in the Gulf of Mexico (Bravo-Hollis 1984), *Axine resplendens* Cabelero, Bravo-Hollis et Grocott, 1954 and *Axinoides raphidoma* Hargis, 1956 in the Eastern Pacific (Pérez-Ponce de León et al. 1999), *Neoaxine constricta* (Yamaguti, 1938) Price, 1946 in the South China Sea (Zhang et al. 2003) and *Chlamydaxine* sp. and *Nudaciraxine* sp. in the Western Atlantic (Tavares et al. 2004).

During a recent parasitological study on fishes in the Gulf of Gabès (Tunisia), three species of Monogenea

were collected from *T. a. imperialis*: *Aspinatrium gallieni* Euzet et Ktari, 1971 (Microcotylidae) on the inner side of the operculum and also two additional previously undescribed axinid species on the gills. In this paper we describe one of these, the most abundant axinid monogenean, as a new species of *Nudaciraxine*. The potential use of axinid monogenean as biological tags for subspecies of belonid fishes *Tylosurus* is discussed.

MATERIALS AND METHODS

Between May and July of 2004, 2006 and 2007, 74 specimens of *T. a. imperialis* caught in the Gulf of Gabès (Tunisia) by local fishermen using gill nets were examined. This fish inhabits offshore waters, but can be found in coastal waters during spring and summer. Specimens were identified using Bauchot (1987).

Fish were dissected and examined a few hours after capture. The operculum and gill arches were separated and placed in Petri dishes with filtered seawater using filter paper. Monogeneans were detected using a stereomicroscope, detached from the gills and operculum and then transferred to a dish containing filtered seawater. They were studied either alive or fixed between slide and coverslip in 70% alcohol. 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 CorelDRAW software. All measurements are given in micrometres (unless stated otherwise) as the mean $\pm$ standard deviation, with the range and the number of measurements (n) in parentheses.

RESULTS

Axinidae Unnithan, 1957

Nudaciraxine imperium sp. n.

Figs. 1–3

Description (based on 30 adult specimens): Body flattened dorsoventrally; 3.8 ± 0.3 mm (2.5–5.1 mm; n = 25) long by 500 ± 37 (300–700; n = 28) wide at level of ovary. Haptor asymmetrical, triangular, 1.3 ± 0.1 mm (0.7–1.9 mm; n = 28) in width, elongated with straight posterior base bordered by single row of 52 ± 2 (42–58; n = 25) clamps (Fig. 1). Seventy percent of specimens with haptor directed to right, line of clamps located on same side as dextral genito-intestinal canal; 30% of specimens with haptor directed to left, line of clamps facing the opposite genito-intestinal canal. Clamps 55 ± 1 (50–60; n = 15) long by 61 ± 3 (52–70; n = 15) wide. Clamp of “*Axine*-type” with marginal sclerites of anterior and posterior jaw, in two parts (Fig. 2C, D). Two pairs of hamuli and one pair of uncinuli situated 570 ± 60 (370–740; n = 13) from posterior end of haptor. Number of clamps at right side of hamuli and uncinuli 20 ± 1 (17–22; n = 17); at left side 34 ± 2 (22–41; n = 19). Median hamuli falciform 47 ± 1 (45–50; n = 10) long; blade, Y: 10 ± 1 (10–12; n = 11) articulated with long straight handle, X: 38 ± 1 (32–40; n = 11). Lateral hamuli 34 ± 1 (28–38; n = 21) long with wide guard, bent shaft and short pointed blade, Y: 21 ± 1 (18–23; n = 14), handle, X: 16 ± 1 (12–19; n = 21). Postero-lateral uncinuli 10 ± 1 (8–10; n = 10) long, between median and lateral hamuli (Fig. 2B). Anterior end bilobed, two muscular buccal suckers subcircular, aseptate, (Fig. 2A); 53 ± 3 (43–70; n = 21) long by 38 ± 3 (25–50; n = 21) wide. Mouth subterminal ventral, pharynx oval, 35 ± 3 (28–50; n = 14) long by 30 ± 4 (22–45; n = 12) wide. Oesophagus long, 400 ± 100 (300–500; n = 4) from apical margin of body, caeca dendritic diverticulate laterally and medially, not extending into haptor.

Testes intercaecal, arranged in two longitudinal rows in median field with smallest at posterior extremity, 42 ± 2 (37–48; n = 15) in number, 87 ± 5 (60–120; n = 26) long by 95 ± 6 (55–120; n = 26) wide. Genital atrium unarmed, slightly posterior to intestinal bifurcation. Cirrus unarmed with evaginable anterior globular part. Cirrus pouch with folded muscular wall 170 ± 9 (125–190; n = 20) long by 64 ± 5 (50–83; n = 15) in maximum width, 43 ± 3

Fig. 1. *Nudaciraxine imperium* sp. n., composite drawing of whole worm (dorsal view). Abbreviation: H – haptor width. Scale bar = 1 mm.

Fig. 2. *Nudaciraxine imperium* sp. n. **A** – anterior end; **B** – hamuli and uncinuli; **C** – closed clamp; **D** – open clamp. Abbreviations: Lh – lateral hamuli; Mh – median hamuli; Ms – marginal sclerite; Un – postero-lateral uncinuli; X – length of handle; Y – length of blade. Scale bars = 50 µm.

(30–60; $n = 21$) wide at proximal globular extremity followed with anterior part of vas deferens, tubular, muscular wall; medio-dorsal, narrowed and sinuous in posterior part (Fig. 3A).

Ovary pretesticular, J-shaped, intercaecal, pre-equatorial, 420 ± 61 (300–625; $n = 15$) long by 100 ± 15 (80–115; $n = 4$) maximum wide. Proximal extremity divided into three lobes. Oviduct directed anteriorly, emerging from distal extremity of ovary. Dextral genito-intestinal canal branching off near distal end of oviduct. Ovovitelline duct loops posteriorly, ascending limb widens to form ootype. Mehlis' glands not observed. Vitelline follicles lateral, surrounding dendritic intestinal caeca, extending posterior to level of haptor region. Transverse vitelloducts near halfway between vagina and ovary, joining at midline to form dorsomedian vitelloduct opening posteriorly at distal end of oviduct (Fig. 3B). Vagina 40 ± 3 (30–55; $n = 14$) in maximum width; entering median vitelloduct near median plane. Vaginal pore dorso-median, located at $20 \pm 2\%$ (14–31%; $n = 22$) of length from apical margin of the body; a tiny funnel-shaped sclerotised duct after vaginal pore. Vaginal chamber slightly folded, armed with paired sclerotised lamellae in anterior half. Uterus ventral, in midline, opening at level of genital atrium in common

genital pore. Egg fusiform, 590 (570–620; $n = 2$) long, 60 (60; $n = 2$) wide with two polar filaments of unequal length and curved at tips. Abopercular filament 230 (230; $n = 2$) long, opercular filament 185 (180–190; $n = 2$) long (Fig. 3C).

Type host: *Tylosurus acus imperialis* (Rafinesque, 1810) (Beloniformes: Belonidae).

Type locality: Sidi Mansour, Tunisia (34°46'N, 10°48'E).

Other localities: Kerkennah (34°45'N, 11°17'E) and Skhira (34°18'N, 10°10'E), both Tunisia.

Site of infection: Gill filaments, between secondary lamellae.

Material deposited: Holotype, Muséum National d'Histoire Naturelle, Paris, No. HEL 54 (Th 146), paratype No. HEL 55 (Th 146 bis); paratype, Natural History Museum, London, No 2008.6.3.1; voucher specimens, Natural History Museum, London, No. 2008.6.3.2; Institute of Parasitology, Biology Centre of the Academy of Sciences, České Budějovice, Czech Republic, No. M-503.

Prevalence: 39% (29 fish infected / 74 examined).

Mean intensity: 3.1 (90 specimens / 29 infected fish).

Mean abundance: 1.2 (90 specimens / 74 examined fish).

Etymology: The name “*imperium*” refers to the subspecies name of the host.

Fig. 3. *Nudaciraxine imperium* sp. n. **A** – dorsal view of anterior part of reproductive system; **B** – dorsal view of posterior part of reproductive system; vas deferens not drawn; **C** – egg. **Abbreviations:** C – cirrus; Cp – cirrus pouch; Def – vas deferens; Ga – genital atrium; Gic – genito-intestinal canal; M vit – median vitelloduct; Oot – ootype; Ov – ovary; Ovd – oviduct; T vit – transverse vitelloduct; Ut – uterus; Va – vaginal chamber; Va p – vaginal pore. Scale bars = 50 µm.

Remarks. The asymmetrical haptor with “*Axine*-type” clamps on only one side and the persistence of larval hamuli in adult specimens place this species within the Axinidae. The presence of numerous clamps and an unarmed genital atrium places this species within the Axinoidinae Price, 1962. The presence of an unarmed cirrus and unarmed genital atrium is an exclusive feature of the genus *Nudaciraxine* Price, 1962. Within this subfamily, two species have previously been described from belonid hosts: the type species *N. gracilis* (Linton, 1940) Price, 1962 found in *Strongylura marina* (Walbaum) from the Atlantic Ocean and *N. cabosanlucensis* Payne, 1990 collected from *Ablennes* sp. in the Eastern Pacific Ocean (California). The new species resembles *N. gracilis* in the shape and size of the body, but it differs in the higher number of testes and their arrangement (not tandemly arranged in

N. imperium). The number and width of clamps are also different. *Nudaciraxine imperium* has fewer and narrower clamps than *N. gracilis* (42–58 vs. 42–82 and 52–70 µm vs. 75–100 µm). It differs also in the position of the vaginal aperture, which is medially located in *N. imperium* but between the median line and the body margin in *N. gracilis*. The position of the vaginal aperture and the number and size of the clamps make *N. imperium* similar also to *N. cabosanlucensis*, but *N. imperium* has a J-shaped rather than U-shaped ovary. These two species differ also in the level of the oesophageal bifurcation (anterior to the genital complex in *N. imperium* vs. immediately posterior to the pharynx in *N. cabosanlucensis*) and *N. imperium* has more testes (42 vs. 20–22). Comparative measurements are listed in Table 1. These characteristics are consistent and allow us to regard *Nudaciraxine imperium* as a new species.

DISCUSSION

Nudaciraxine Price, 1962 was established with the type species *Nudaciraxine gracilis* (Linton, 1940) Price, 1962 collected from *Strongylura marina* on the Atlantic coast of the United States (Price 1962). This species was first described by Linton (1940) as *Axine gracilis* and later re-assigned to *Axinoides* Yamaguti, 1938 by Sproston (1946) from the same host, *S. marina*, from Woods Hole (North America). Since Price (1962), this species has been reported in *Tylosurus acus* from Veracruz (Gulf of Mexico) (Bravo-Hollis 1984).

As mentioned by Payne (1990) and after the description of *N. imperium*, the original diagnosis of the genus *Nudaciraxine* should be amended as follows: vaginal aperture dorsal, median to submedian, armed or not with horn-like spines. This genus now includes three species, *N. gracilis*, *N. cabosanlucensis*, and *N. imperium*.

Parona in Palombi (1949) apparently observed a monogenean “very similar” to *Axine belones* on *Belone imperialis* (old, invalid synonym of *T. a. imperialis*), but they did not describe this species. Euzet and Lopez-Roman (1973) redescribed *A. belones* from the north and south occidental Mediterranean Sea and confirmed the host of this monogenean to be *Belone belone* (Linnaeus). The observation of Parona might be a misidentification of *N. imperium*.

Nudaciraxine appears to be cosmopolitan as its species have been collected from the Atlantic and the Pacific Oceans and the Mediterranean Sea. However, this genus seems to be specific to belonid fishes. A recent molecular phylogenetic analysis has shown that the belonid genera *Strongylura*, *Ablennes* and *Tylosurus*, recorded as hosts of *Nudaciraxine*, have close phylogenetic relationships (Banford et al. 2004).

Some authors have reported axinids from *Tylosurus acus* but did not identify the fish subspecies. However, with reference to the geographical distribution of *T. acus* given by Collette (2003) and that of the axinid monogeneans, we can predict the corresponding subspecies of the host. Bravo-Hollis (1984) collected *N. gracilis* from Veracruz (Gulf of Mexico) and this locality corresponds to the Western Atlantic subspecies *T. a. acus*. Alternatively, this report could be a misidentification of the host because this monogenean was first described from *Strongylura marina*, which was also found in this area (Price 1962).

Pérez-Ponce de León et al. (1999) mentioned *Axine resplendens* and *A. raphidoma* from *T. acus* in the Eastern Pacific; the corresponding host subspecies might be *T. a. pacificus* (Steindachner). *Tylosurus acus pacificus* has been elevated to the rank of species as *T. pacificus* by Collette and Banford (2001).

On the Tunisian coast, *Aspinatrium gallieni* (Monogenea: Microcotylidae) was described from *Strongylura*

Table 1. Comparison of the morphology of *Nudaciraxine imperium* sp. n. with that of *N. gracilis* and *N. cabosanlucensis*. Measurements are in μm , unless otherwise stated.

Species	<i>N. imperium</i> sp. n.	<i>N. gracilis</i>	<i>N. cabosanlucensis</i>
Authority	Present study	Price 1962	Payne 1990
Host	<i>T. a. imperialis</i>	<i>S. marina</i>	<i>Ablennes</i> sp.
Body length	3.8 (2.5–5.1) mm	1.9–5.1 mm	1.762–1.959 mm
Body width	500 (300–700)	200–500	329–439
Haptor width	1.3 (0.7–1.9) mm	700–1.6 mm	705
Buccal sucker length	53 (43–70)	38–47	–
Buccal sucker width	38 (25–50)	28–38	35–42
Clamps number	52 (42–58)	42–82	48–54
Clamps length	55 (50–60)	–	31–33
Clamps width	61 (52–70)	75–100	48–57
Lateral hamuli	34 (28–38)	32–38	30
Median hamuli	47 (45–50)	44–47	43
Testes number	42 (37–48)	20–22	32
Cirrus length	170 (125–190)	–	64–70
Cirrus maximum width	64 (50–83)	–	22–31
Egg length	590 (570–620)	–	–
Egg width	60 (60)	–	–

acus (Lacépède) on the inner side of the operculum (Euzet and Ktari 1971). In our study we found *A. gallieni* on *T. a. imperialis* in the same microhabitat (inner side of the operculum). Contrary to the majority of microcotylid species, which are attached by their clamps on the gill filaments, *A. gallieni* is fixed on the operculum epithelium. Open clamps work like a sucker.

Strongylura acus, the host erroneously reported by Euzet and Ktari (1971), has not been recorded in the Mediterranean Sea so far. Three species of belonid fishes are present in this area: *Belone belone gracilis* Lowe, *Belone svetovidovi* Collette et Parin, and *T. a. imperialis* (see Bauchot 1987, Collette 2003). According to Collette and Parin (2005), the genus *Tylosurus* has been widely confused with *Strongylura* for more than 100 years; *S. acus* is placed now as an invalid synonym (misidentification) of *T. a. imperialis* and the host of *A. gallieni* must be corrected as *T. a. imperialis*.

Four geographical subspecies of *T. acus* have been recorded (Collette 2003); for three of them the axinid monogeneans seem to have oixenic specificity. *Nudaciraxine imperium* is described from the Mediterranean subspecies *T. a. imperialis*. *Neoaxine constricta* was reported from the Indo-Western Pacific subspecies *T. a. melanotus* (Zhang et al. 2003) and *N. gracilis* was found on the Atlantic subspecies *T. a. acus* (Bravo-Hollis 1984). The forth subspecies, *T. a. rafale*, seems not have been examined for monogenea.

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