Lecithostaphylus tylosuri sp. nov. (Digenea, Zoogonidae) from the digestive tract of the needlefish Tylosurus acus imperialis (Teleostei, Belonidae)

Châari Manel, Derbel Hela and Neifar Lassâd*

Laboratoire de Biodiversité et Ecosystèmes Aquatiques, Département des Sciences de la vie, Faculté des Sciences de Sfax, Université de Sfax, BP 1171, 3000 Sfax, Tunisie

Abstract
Lecithostaphylus tylosuri sp. nov. (Digenea, Zoogonidae) specimen were collected from the digestive tract of Tylosurus acus imperialis (Teleostei, Belonidae) caught off the eastern coast of Tunisia. L. tylosuri is very similar to its closest relatives, L. retroflexus and L. nitens. It can be easily distinguished from L. retroflexus (Molin, 1859) in having a more extensive vitellarium, with follicles reaching from the posterior margin of the acetabulum and extending beyond the posterior margin of the testes and a coiled seminal vesicle. L. tylosuri differs from L. nitens as illustrated by Linton 1898, in having a longer cirrus pouch (0.7 mm vs 0.36 mm, respectively) overlapping the anterior edge of the ventral sucker and a submarginal genital pore (submedian in L. nitens). It’s also different from L. nitens as described by Manter 1947 in the vitelline disposition and in having the greater sucker ratio (1 : 1.3–2.1 vs 1 : 1.3–1.6, respectively). L. tylosuri differs from L. nitens as reported by Machida and Kuramochi 2000 by the absence of variations in the vitellarian disposition in all specimens. L. tylosuri is more similar to L. nitens from group A (considered synonym of L. ahaaha Yamaguti, 1970 = L. nitens by Bray 1987) by having vitelline follicles extending beyond the testes. L. tylosuri can be distinguished from L. ahaaha by its pedunculate rather than prominent acetabulum and its larger body size (4.10–7.85 mm long and 0.75–1.2 mm large vs 2.1–6 mm long and 0.45–1.1 mm large, respectively). The prevalence of L. tylosuri sp. nov. was negatively correlated with host length (decreasing with host size increasing). Host sex does not seem to affect infection parameters.

Keywords
Lecithostaphylus tylosuri sp. nov., Digenea, Zoogonidae, Tylosurus acus imperialis, Belonidae, Tunisia

Introduction
Members of the digenean family Zoogonidae (Odhner 1902) are common gastro-intestinal parasites mainly of marine fishes. This family includes at least 77 nominal species (Brooks 1990) and 32 valid genera (Bray 2008, Jezewski et al. 2009, Châari et al. 2011). The taxonomic status of this family has been discussed by Bray 1987 and was subdivided into two subfamilies, the Zoogoninae Odhner, 1902 and the Lepidophyllinae Stossich, 1903. Subsequently, Brooks 1990 re-analysed Bray’s work and reported a phylogenetic tree of zoogonids with an additional two subfamilies, the Deretrematinae Odhner, 1910 and the Lecithostaphylinae Odhner, 1911. Brooks and McLennan 1993 supported these results and republished the same tree. Bray 2008 did not recognized these additional subfamilies, but agreed with Brooks’ view that a satisfactory revision of this family awaited a larger database. The genus Lecithostaphylus (Digenea, Zoogonidae) belongs to the Lepidophyllinae according to Bray 2008 and the Lecithostaphylinae according to Brooks 1990. It was erected by Odhner 1911 for the type species L. retroflexus (Molin, 1859) and was defined as a subgenus of Steganoderma Stafford, 1904 (= S. L. retroflexum) by Bray 1987 and then elevated back to the generic rank by Brooks 1990.

Members of the genus Lecithostaphylus are cosmopolitan, frequently reported from beloniform fishes (Exocoetidae and Belonidae) (Brooks 1990), and recently mentioned in damselfishes (Pomacentridae) (Cribb et al. 1992, Toman 1992). In this work, we describe a new species of Lecithostaphylus from the digestive tract of the needlefish T. a. imperialis. This belonid is a marine epipelagic subspecies of Tylosurus acus (Lacépède, 1803) essentially restricted to the Mediterranean Sea. This new digenean species is described, characterised below, and its host specificity is discussed. In addition, the im-
pact of the host’s sex and size on infection parameters, such as prevalence and abundance, were also examined.

Materials and methods

*Tylosurus acus imperialis* is present in coastal Tunisian fisheries mainly during May to July off the eastern coast of Tunisia. 119 samples were obtained from local fishermen between 2004 and 2009. Fishes were caught by local fisherman using gillnets. Samples were identified using Collette and Parin 1970 and Bauchot 1987.

Specimens were dissected and digestive tracts were examined. Digeneans species were collected using a stereomicroscope under incident light. Individuals were fixed between a slide and coverslip with slight pressure in 70% alcohol or in Bouin’s fluid. Fixed specimens were stained in Semichon’s acetic carmine. After dehydration using a graded ethanol series they were cleared with clove oil and mounted in Canada balsam.

Illustrations and measurements were made on stained specimens with the aid of a Leitz microscope equipped with drawing tube then scanned and redrawn on a computer with Corel Draw Software. All measurements were given in micrometers (unless stated otherwise) as the mean ± standard deviation with the range and the number of measurements (n) in parentheses.

Prevalence (P), mean intensity (IM), and abundance (A) were determined following Margolis *et al.* 1982 and Bush *et al.* 1997. Prevalence and abundance of infestation in relation to body size and sex of the host were calculated. Hosts were grouped into 4 body size classes: 60–70, 71–80, 81–90, >90 cm. Spearman correlations coefficient was used to determine possible correlations between host length, parasite abundance, intensity and prevalence. The significance of sex differences on prevalence was assessed by the χ² test.

Results

Class: Trematoda Rudolphi, 1808
Subclass: Digenea Carus, 1863
Superfamily: Microphalloidea Ward, 1901
Family: Zoogonidae Odhner, 1902
Subfamily: Lepidophyllinae Stossich, 1903
Genus: *Lecithostaphylus* Odhner, 1911

*Lecithostaphylus tylosuri* sp. nov. (Figs 1–3)

Description based on 34 whole-mounts specimens. Body elongate, subcylindrical, 5.45 mm ± 300 (4.10–7.85 mm; n = 33) long by 993 ± 33 (750–1200; n = 34) wide at level of ventral sucker and 848 ± 44 (650–1200; n = 34) wide at level of testis. Tegument with small spines, denser anteriorly (Fig. 1). Forebody 1.40 mm ± 60 (1.10–1.90 mm; n = 33) long, 19–32 (26)% of body length. Hindbody 3.50 mm ± 204 (2.30–4.72 mm; n = 33) long, 50–80 (64)% of body length. Forebody-hindbody length ratio 0.40 ± 0.02 (0.27–0.63; n = 33). Oral sucker globular, subterminal, 361 ± 44 (280–475; n = 34) long by 392 ± 17 (300–520; n = 34) wide. Ventral sucker circular, pedunculate, with transverse slit like aperture, surrounded with circular folds ventrally, 622 ± 20 (520–750; n = 33) in diameter. Lips of ventral sucker armed with papillae-like denticles, which probably aid attachment. Aperture surrounded with glandular cells (Fig. 2). Sucker ratio 1:1.72 ± 0.06 (1.22–2.13; n = 33) long by 1:1.60 ± 0.06 (1.30–2.10; n = 33) wide. Prepharynx short, 55 ± 10 (25–105; n = 22) long. Pharynx oval, 250 ± 10 (185–300; n = 34) long by 271 ± 9 (220–350; n = 34) wide. Oral glands in prepharynx region, and lateral and basal region of pharynx. Oesophagus short, 145 ± 30 (60–240; n = 12). Intestinal bifurcation 771 ± 40 (600–950; n = 21) from anterior ex-
tremity. Caeca parallel, reaching beyond posterior extremity of testes, post-caecal region 672 ± 99 (475–920; n = 7) long. Testes two, longitudinally elongate, subsymmetric, oval, in mid-hindbody, post-testicular region 1840 ± 150 (1150–2750; n = 30) long, 28–35 (34)% of body length. Left testis 600 ± 48 (375–850; n = 25) long and 271 ± 21 (150–375; n = 25) wide. Right testis 583 ± 44 (400–850; n = 26) long and 271 ± 20 (200–375; n = 25) wide. Genital pore sinistral, submarginal, 783 ± 50 (500–1025; n = 34) from anterior extremity. Cirrus sac preacetabular, 700 ± 46 (435–1095; n = 33) long by 207 ± 13 (140–310; n = 33) wide, claviform, curved, thick walled, diagonally oriented, proximal end slightly overlaps anterior extremity of ventral sucker. Seminal vesicle, tubular, coiled, formed of two loops. Pars prostatica vesicular, surrounded by well-developed prostatic cells (Fig. 3A). Ovary subglobular, intercaecal, 287 ± 15 (200–360; n = 25) long by 309 ± 17 (200–390; n = 25) wide, closer to ventral sucker than to testes. Seminal receptacle oval, postovarian. Laurer’s canal opens submarginally, at level of seminal receptacle. Mehlis’ gland distinct, posterior to seminal receptacle. Distal part of uterus surrounded by glandular cells, forms distinct metraterm with sphincter distally. Uterus filling most of hindbody, including much of post-testicular space, passes anteriorly over dorsal surface of ventral sucker. Vitellarium follicular in two longitudinal lateral fields, extracaecal; follicles spherical, arranged with 9 on right side and 10 to 12 on left side; 150 ± 9 (120–190; n = 25) long by 130 ± 8 (100–180; n = 25) wide; anterior extremity of vitelline fields at level of posterior margin of ventral sucker; posteriorly fields extend well beyond posterior margin of testes particularly on poral side. Vitelline ducts from each follicle gather to form ducts running parallel to follicular fields, thence discharging into common, wider, transversally oriented, vitelline ducts just posteriorly to ovary. Vitelline reservoir between ovary and seminal receptacle (Fig. 3B). Eggs numerous, sometimes tanned, operculate, 36 ± 1 (30–45; n = 27) long by 19 ± 1 (15–25; n = 27) wide.

Type-host: Tylosurus acus imperialis (Rafinesque, 1810) (Teleostei, Belonidae).

Site of infection: Third anterior part of digestive tract.

Type-locality: Sfax (34°46´N; 10°48´E).

Other localities: Kerkennah (34°45´N, 11°17´E); Skhira (34°18´N, 10°10´E); Mahdia (35°30´N, 11°4´E); Chebba (35°13´N, 11°8´E); Zarzis (33°21´N, 11°09´E); Tunisia.


Material studied: Whole-mounts of 34 flattened adult specimens.

Etymology: The name tylosuri refers to the host generic name.

Differential diagnosis


Lecithostaphylus tylosuri is very similar to two of these species, L. retroflexus and L. nitens, in having its ovary separated from the testes by uterine coils and a significant proportion of the uterus pretesticular. L. tylosuri can be easily distinguished from L. retroflexus, as described by Bray and Gibson 1986 and Bartoli et al. 2003; by its vitellarium, which extends anteriorly as far as the posterior margin of the acetabulum and reaches beyond the posterior extremity of the testes instead of the vitellarium extending from just anteriorly to the ovary to just overlapping the anterior margin of testes at its posterior-

Fig. 2. Lecithostaphylus tylosuri sp. nov. Whole worm in lateral view. Composite drawing. Scale bar = 500 µm.
Lecithostaphylus tylosuri sp. nov. also differs in the shape of the seminal vesicle which is coiled instead of bipartite with a larger proximal part in L. retroflexus. L. tylosuri has testes approximately in the mid-hindbody whereas in L. retroflexus the testes are in the posterior half of the hindbody. It can be also distinguished by the size of the body which is longer than L. retroflexus [5.45 mm (4.10–7.85 mm) vs 2.67 mm (2.52–2.86 mm)].

L. tylosuri also differs in having the vitellarium extending posteriorly beyond the testes. The main difference seems to be the size of the cirrus pouch. Linton 1940 gave measurements of nearly ‘to 0.036 by 0.018 mm’ which, when compared with the sizes of others organs, appears be in error. The actual size of the cirrus pouch seems to be 0.36 by 0.18 mm. L. tylosuri has a longer cirrus pouch (0.7 mm) overlapping the anterior edge of the ventral sucker. L. nitens differs also in the position of the genital pore which is to the left of the median line rather than submarginal in L. tylosuri. L. nitens (= Stegandermo elongatum) as described by Manter 1947 differs from L. tylosuri in the vitelline disposition which extends from just

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Fig. 3. Lecithostaphylus tylosuri sp. nov. A – terminal genitalia. Abbreviations: Ej, ejaculatory duct; Ga, genital atrium; Gp, genital pore; Mt, metraterm; Pp, pars prostatica; Sv, seminal vesicle; B – female genital system. Abbreviations: Cvd, common vitelline duct; Dc, digestive caecum; Lc, Laurer canal; Ov, ovary; Rc, seminal receptacle; Vit, vitellarium; Vr, vitelline reservoir. Scale bars = 100 µm.
anterior to the ovary to the anterior edge of the testes or slightly beyond, never extending posteriorly to the testes rather than extending from the posterior edge of the acetabulum and reaches beyond the posterior extremity of the testes. It differs also in having a smaller sucker ratio (1 : 1.3–1.6 in \textit{L. nitens} vs 1 : 1.3–2.10 in \textit{L. tylosuri}).

\textit{Lecithostaphylus tylosuri} has its vitellarium extending posteriorly beyond the testes in all specimens whereas variation in the posterior vitellarium disposition of \textit{L. nitens} has been also reported by Machida and Kuramochi 2000 who divided their specimens into two groups A and B mainly by the vitelline distribution. In group A, the vitellarium extends beyond the testes and in group B the vitellarium does not reach posteriorly beyond the testes. By having vitelline follicles extending beyond the testes, \textit{L. tylosuri} resembles \textit{L. nitens} from group A. Whereas, Machida and Kuramochi 2000 considered their group A specimens similar to those of Manter 1947 except that in Manter’s specimens the vitellarium does not extend into the posttesticular region. \textit{L. nitens} from group A also corresponds to \textit{L. ahaaha} Yamaguti, 1970 (synonymised with \textit{L. nitens} by Bray 1987). \textit{L. tylosuri} can be also distinguished from \textit{L. ahaaha} by its pedunculate rather than prominent acetabulum. \textit{L. tylosuri} reaches a larger size than \textit{L. ahaaha} (4.10–7.85 mm long and 0.75–1.2 mm large vs 2.1–6.0 mm long and 0.45–1.1 mm large, respectively). It differs also by the higher upper limit of the sucker-ratio range 1 : 1.3–2.10 vs 1 : 1.3–1.75, respectively.

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\textit{Lecithostaphylus tylosuri} sp. nov. were found in the digestive tract of \textit{T. a. imperialis} from eastern coast of Tunisia with prevalence, mean intensity and abundance of, 33.61%, 3.33 and 1.12, respectively. The highest values of prevalence were encountered in needlefishes in the 60–70 cm length class, but the highest values of mean intensity and abundance were encountered in fishes in the 71–80 cm length class (Table I). Lower values of infection parameters were found in specimens larger than 90 cm of total length.

The prevalence showed a negative correlation with total host length (\(r = –0.1, P<0.05\)) whereas no significant differences of mean intensity and abundance in relation to fish length were found (\(r = –0.4, P>0.05\)) and (\(r = –0.8, P>0.05\)), respectively. Comparisons of parasitism in relation to host sex showed no significant differences for prevalence (\(\chi^2 = 0.0067, P>0.05\)), mean intensity (\(\chi^2 = 0.0004, P>0.05\)) and abundance (\(\chi^2 = 0.007, P>0.05\)) (Table II).

\textbf{Discussion}

Using the host specificity classification introduced by Euzet and Combes 1980, \textit{L. tylosuri} seems to exhibit oioxenic specificity. So far it has been collected only from the digestive tract of the Mediterranean subspecies of \textit{T. acus}. The closest related species to \textit{L. tylosuri} are known as having wider specificity towards mainly belonid hosts. Indeed, \textit{L. nitens} were placed by Bray 1987 among the strictly stenoxenic zoogonids restricted to belonids. This species has been reported from the North Western Atlantic, the Pacific and the Indian Oceans; from the belonid fishes, \textit{Ablennes hians} (Valenciennes, 1846), \textit{Belone platyura} Bennett, 1882, \textit{Platybelone argalus} (Lesueur, 1821), \textit{S. marina} (Walbaum, 1792), \textit{Tylosurus acus} (Lacépède, 1803), \textit{T. crocodilus} (Péron et Lesueur, 1821) and \textit{Xenentodon can-
Lecithostaphylus tylosuri sp. nov.

cila (Hamilton, 1822) (Bray 1987). Bray and Justine 2008 reported it also from T. crocodilus in New Caledonia.

Lecithostaphylus retroflexus was placed among the predominantly stenoxenic zoogonids which exhibit a strong predilection for fish of the genus Belone (Bray 1987). It has been reported mainly from the belonids, Belone belone (= acus) Risso, 1827 (13 records), B. b. euxeni Günther, 1866 (2 records) and recently from B. b. gracilis Lowe, 1839 (1 record) (Bartoli et al. 2003). Examination of digeneans parasites of belonid fishes from Tunisian waters revealed the presence of L. retroflexus from the digestive tract of B. b. gracilis and B. svetovidovi Collette et Parin, 1970 (personal observation). However, L. retroflexus has been occasionally recorded in species from other groups such as Exocoetidae, Labridae, Scorpaenidae, Sparidae (Bray 1987), but some doubts accompany these records (Bartoli et al. 2003). These can probably be explained by misidentification of the parasite or host species.

Morphologically, L. tylosuri most closely resembles L. nitens, which is known to parasitize species of the genus Tylosurus. Whereas, L. retroflexus seems to more closely associated with belonids of the genus Belone and has never been reported from species of the genus Tylosurus. The molecular phylogenetic hypothesis for beloniform fishes proposed by Lovejoy et al. 2004 shows that species of the genus Belone share a close ancestor and species of the genus Tylosurus were in a widely separated clade. It appears that specificity to the host genus may be related to host phylogeny reflecting host parasite co-evolution, although the co-evolution of hosts and their parasites is a multidimensional process that is not well understood.

The subspecies of T. acus have been recognized depending to their geographical distribution (Collette 2003). Tylosurus acus acus (Lacépède, 1803) is found in the Western Atlantic; Tylosurus acus rafale Collette et Parin, 1970 is mainly distributed in the Gulf of Guinea while Tylosurus acus melanotus (Bleeker, 1850) is found throughout the Indo-Western Pacific, extending to the Eastern Pacific (Collette 2003). The digenean fauna of only two subspecies of T. acus are known. T. a. melanotus has been reported as host for bucephalid, lecithasterid and zoogonid digeneans from the coast of Japan (Machida and Kuramochi 2000). In the South China Sea, Liu et al. 2010 also listed digeneans belonging to the Bucephaliinae and the Monorchidae from this needlefish. Tylosurus acus acus harbours didymozoid, bucephalid, haploplanchnid and hemiurid digenean species from Brazil. No Lecithostaphylus species were founded in the Western Atlantic subspecies T. a. acus (Tavares et al. 2004). It appears that specificity is related to the geographical distribution of the host. In addition, Châari et al. 2010 founded that the Monogenea of T. a. imperialis differ from those of others subspecies of T. acus.

The prevalence of infection of L. tylosuri showed a negative correlation with host total length. This can be probably related to its immune system and physiology of the host. T. a. imperialis congregate in coastal waters mainly between May and July for the purpose of spawning. Most specimens caught were in the size range between 70 and 80 cm of total length and have the most abundant infection with L. tylosuri. Fish in these size classes spawn (TL of first maturity about 80 cm), but a few specimens were >90 cm in total length indicating that they are at the end of their spawning migration. As suggested by Rohlenová et al. 2011, energy resources are in a trade-off between demands of immunity and other physiological demands like spawning.

In this study, the sex of the host has no effect on the infection parameter. Thus is probably related to the similarity in feeding habits of male and female. Our findings show that coefficients of emptiness (% of total empty stomachs/total stomachs) of male and female (43.24%, 60.87% respectively) does not differ significantly ($\chi^2 = 2.63$, P>0.05). Similar findings are presented by Al Zubaidy 2010, who found that the sex of the host Carangoides bajad seems not to influence the prevalence of infection with the digenean Lecithochirium sp. in the Red Sea, and by Oliva et al. 1996 who found that the sex of the host did not affect the mean abundance in most parasites of the flatfish Paralichthys adspersus off northern Chile.

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References


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