DOI: 10.2478/s11686-011-0060-8

Acta Parasitologica, 2011, 56(3), 301–304; ISSN 1230-2821

VERSITA

Hypocreadium caputvadum sp. nov. (Digenea, Lepocreadiidae), an intestinal parasite of the grey triggerfish, *Balistes capriscus* (Teleostei, Balistidae) from the Gulf of Gabès, Mediterranean Sea

Hichem Kacem, Hela Derbel and Lassâd Neifar*

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

Abstract

Hypocreadium caputvadum sp. nov. (Digenea, Lepocreadiidae) is described from the intestine of *Balistes capriscus* Gmelin collected from the Gulf of Gabès (Tunisia) in the southern Mediterranean Sea. This new species can be distinguished by a combination of characteristics shared by no other described species of *Hypocreadium* Ozaki, 1936. These characteristics include the presence of a distinct anterior notch, the follicular vitellarium confluent in the forebody, the presence of a muscular sphincter at the level of the anterior third of the metraterm and the size and the position of the cirrus-sac.

Keywords

Digenea, *Hypocreadium caputvadum* sp. nov., Lepocreadiidae, *Balistes capriscus*, Gulf of Gabès, Tunisia

Introduction

Balistes capriscus Gmelin, 1789 is an amphi-Atlantic fish widely found in the eastern Atlantic from the British Isles to Angola, including the Mediterranean Sea (Harmelin-Vivien and Quero 1990) and in the western Atlantic from the Gulf of Mexico to Argentina (Robins and Ray 1986). In this last decade it has extended its northern range, perhaps as a result of global warming (Francour *et al*. 1994, Astraldi *et al*. 1995, Garrabou *et al*. 2003).

Several works have been published on the parasites of *B. capriscus* from the Atlantic Ocean (Linton 1907, Hanson 1950, Hargis 1955, Saunders 1959, Sogandares-Bernal 1959, Overstreet 1969, Ho and Rokicki, 1987, Alves *et al*. 2005) but nothing has been reported on the parasites of *B. capriscus* collected from the Mediterranean Sea.

During our investigation on the metazoan parasites of this fish, we found a new species of Digenea, Lepocreadiidae Odhner, 1905, in the intestine of *B. capriscus* caught in the Gulf of Gabès (southern Mediterranean Sea). This new species belongs to the genus *Hypocreadium* Ozaki, 1936. To our knowledge, this is the first record of a member of this genus found in a host from the Mediterranean Sea. In our paper we present a description of this new species.

Materials and methods

From June 2005 to May 2006, 23 specimens of grey triggerfish were collected from off the Gulf of Gabès at Chebba (34°14′N, 11°06′E), Kerkennah (34°45′N, 11°17′E) and Zarzis (33°41′N, 11°48′E) using specific gill nets and hand lines. Fish were identified using Fisher *et al*. (1987) and Whitehead *et al*. (1984). Each species was dissected as soon as possible after capture and opened with a circum-ventral incision. Then, all intestines were removed dissected and examined for metazoan parasites. Living parasites were partially compressed between slide and cover slip and examined using an optical microscope. Some parasites were washed in physiological saline water, slightly flattened between slide and cover slip and fixed in 70% alcohol or 5% formalin. Some living specimens were washed in cold saline then fixed in hot saline and preserved in 5% formalin. Whole mounts for light microscopy were stained with Semichon's acetic carmine, dehydrated using a graded ethanol series, cleared in clove oil, and mounted in Canada balsam.

Specimens prepared for scanning electron microscopy (SEM) were fixed in cold (4°C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.2 for a minimum of 2 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2,

*Corresponding author: lassad.neifar@fss.rnu.tn

postfixed in cold (4°C) 1% osmium tetroxide in the same buffer for 1 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2 and dehydrated in a graded ethanol series. After sonication in absolute alcohol for 30 s at 40 kHz, specimens were kept for 30 min in absolute alcohol, and successively immersed for 20 min in 3:1, 1:1 and 1:3 mixtures of absolute alcohol and n-amylacetate, washed in pure n-amylacetate for 20 min and dried to critical point with carbon dioxide. Specimens are mounted in aluminium stubs using colloidal silver, and sputter coated with 10 nm of gold. SEM was performed on a Zeiss DSM 940A Gemini field emission scanning electron.

All the illustrations and measurements were made with the aid of a light microscope equipped with a drawing tube, and they were scanned and redrawn on a computer with Corel Draw Software.

Measurements are given in micrometres as the ranges followed by the mean and the number of measurements in parentheses.

Results

Class: Trematoda Rudolphi, 1808
Subclass: Digenea Carus, 1863
Superfamily: Lepocreadioidea Odhner, 1905
Family: Lepocreadiidae Odhner, 1905
Subfamily: Lepocreadiinae Odhner, 1905
Genus: *Hypocreadium* Ozaki, 1936

***Hypocreadium caputvadum* sp. nov.** (Figs 1 and 2)

Description based on 17 flattened specimens and 4 live specimens. Body more or less rounded, concave ventrally, edges frequently curl towards ventral surface, with distinct flat bottomed notch at anterior margin, 900–1,250 × 850–1,175 (1,079 × 1,015; n = 17); width 82.6–100 (94.14)% of body length (Fig. 1A and Fig. 2A). Tegumental spines apparently absent. Oral sucker subglobular, subterminal, with ventral aperture, 90–130 × 110–140 (113 × 118; n = 17) in diameter. Ventral sucker rounded, at midbody, 160–190 (183; n = 15) in diameter. Sucker-width ratio 1:0.53–0.76. Forebody: 435–450 (440; n = 15) long; 37–41 (39.34)% of body-length. Prepharynx absent. Pharynx well developed, globular, 80–100 × 90–110 (90 × 100; n = 15). Oesophagus short, 45–65 (60; n = 14) long. Intestinal bifurcation in mid-forebody. Caeca narrow, follow arcuate course around gonads, terminate blindly posteriorly to ovary.

Testes two, spherical to subspherical, symmetrical, separated by ovary and uterus, immediately postacetabular, contiguous with caeca, 150–250 × 100–190 (205 × 159; n = 15). External seminal vesicle, an elongate, straight sac, lying along right side of acetabulum. Cirrus-sac large, 250–320 × 100–135 (300 × 110; n = 14), claviform, with conspicuous diagonal or spirally arranged muscles, extending diagonally between the intestinal bifurcation and ventral sucker; surrounded by gland-cells. Internal seminal vesicle proximal, saccular. Pars prostatica with anuclear cell-like bodies (Fig. 2). Ejaculatory duct

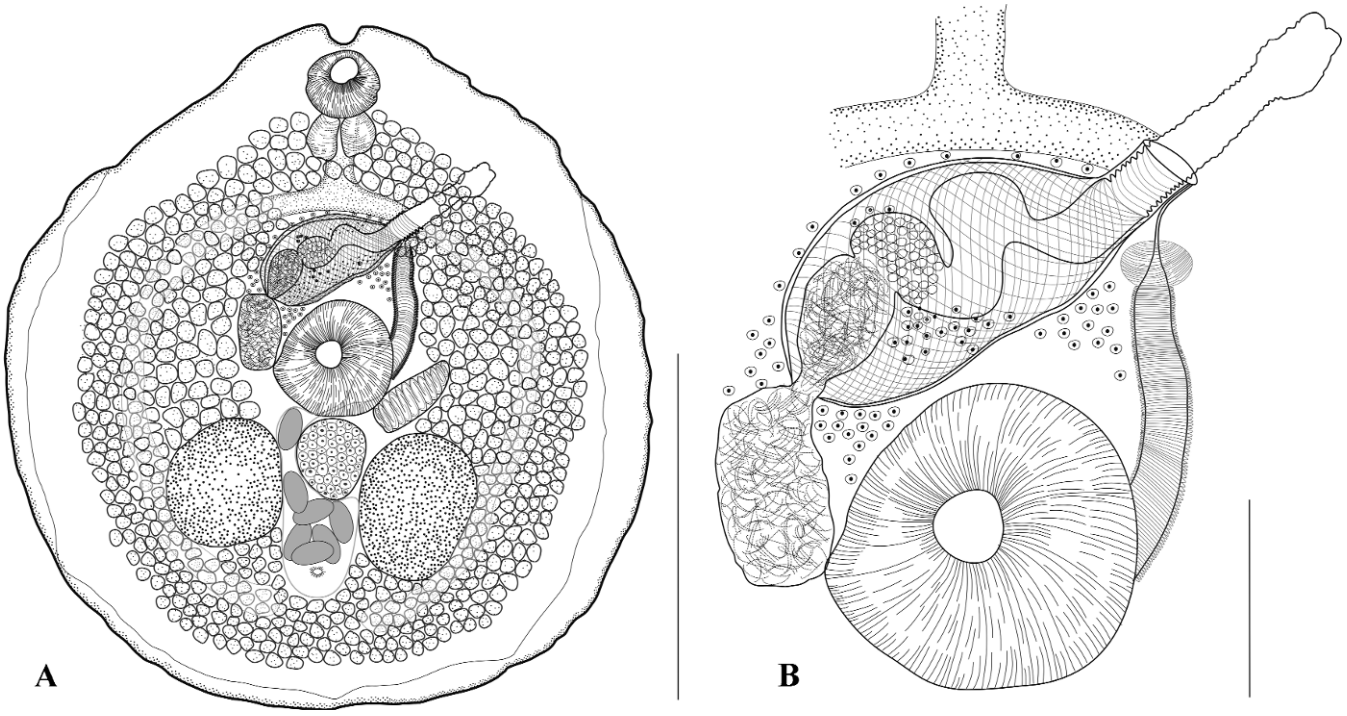

Fig. 1. *Hypocreadium caputvadum* sp. nov. from *Balistes capriscus*. **A.** Ventral view of flattened specimen. **B.** Ventral view of terminal genitalia. Scale bars = 500 µm

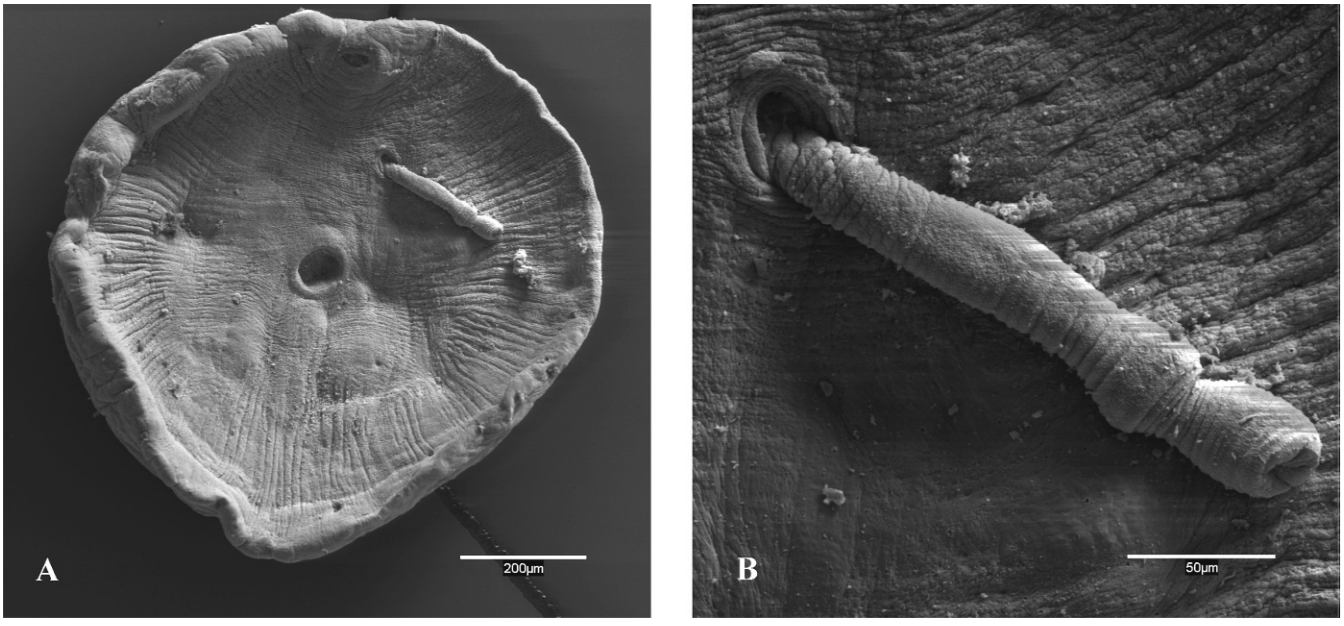

Fig. 2. Scanning electron micrographs of *Hypocreadium caputvadum* sp. nov. from *Balistes capriscus*. **A.** Ventral surface of body. **B.** Ventral view of everted cirrus.

muscular, long, folded, becoming larger distally in everted cirrus. Genital atrium distinct. Sinistral genital pore near level of intestinal bifurcation.

Ovary more or less oval, intertesticular, 120–140 × 90–130 (135 × 125; n = 14) usually contiguous with sinistral testis, immediately posterior to acetabulum. Seminal receptacle large, saccular, between sinistral testis and ventral sucker. Uterus passes between ovary and dextral testis into post-ovarian region. Metraterm muscular, extending from left side of acetabulum to genital pore with muscular sphincter at level of anterior third. Eggs relatively few, elongate oval, 90–110 × 60–70 (100 × 66; n = 13). Vitellarium follicular; follicles numerous, dorsal and ventral fields vary anteriorly between posterior edge of oral sucker and middle of pharynx, extending posteriorly to mid-post-testicular region, well separated from body-margins, surround gonads, fields confluent in forebody in unflattened worms, appear separated in some flattened worms, confluent posteriorly, fields lie lateral, dorsal and median to caeca. Excretory pore dorsal, anterior to ends of caeca, muscular, with 8 or 9 radially arranged spine-like projections. Excretory vesicle tubular, extending to mid-testicular level where two cornua branch off, one on each side of body, giving Y-shaped appearance; not visible anteriorly to seminal receptacle.

Type- host: *Balistes capriscus* Gmelin, 1789 (Balistidae).

Type- locality: Off Chebba (Tunisia) (35°14′N, 11° 06′E).

Other localities: Kerkennah (Tunisia) (34°45′N, 11°17′E), Zarzis (Tunisia) (33°41′N, 11°48′E).

Type-material: Holotype British Museum (Natural History), London, No. NHMUK 2011.4.11.1; paratypes British Museum (Natural History), London, No. NHMUK 2011.4.11.2-8; Muséum National d'Histoire Naturelle, Paris, Nos. HEL 205 (Tf 224) and HEL 206 (Tf 225).

Site: Posterior intestine.

Material studied: Whole-mounts of 17 flattened adult specimens.

Infection index: Prevalence of 23.72%, intensity 1–13.

Etymology: The name "*caputvadum*" refers to the ancient Roman name of the type locality Chebba "Caput vada".

Discussion

The new species belongs to *Hypocreadium* Ozaki, 1936 as recognised by Bray and Cribb (1996), Bray (2005) and Bray *et al*. (2009). This species presents a combination of characteristics that have not been described for other *Hypocreadium* spp. These characteristics include the presence of a distinct anterior notch, the vitelline follicles confluent in the forebody, the presence of a muscular sphincter at the level of the anterior third of the metraterm and the size and the position of the cirrus-sac.

Until now the genus *Hypocreadium* includes 15 species. Only four species have a distinct anterior notch they are *H. toombo* Bray et Justine, *H. cavum* Bray et Cribb, *H. patellare* Yamaguti and, possibly, *H. lamelliforme* (Linton). As mentioned by Bray *et al*. (2009), *H. lamelliforme* described from a balistid and from two ostraciids remains poorly known and our comparison is based on Figure 77 in Linton (1907).

Hypocreadium caputvadum sp. nov. can be distinguished from species cited previously as follows:

Hypocreadium toombo differs in that the caecal terminations are directed distinctly anteriorly rather than transversely

and the external seminal vesicle is bi- or tripartite (Bray and Justine 2006) rather than undivided.

Hypocreadium cavum differs in that the vitelline fields are distinctly separated in the forebody and the uterus is always restricted to the pre-ovarian region rather than passing into the post-ovarian region (Bray and Cribb 1996).

Hypocreadium patellare differs from *H. caputvadum* sp. nov. by the absence of a muscular sphincter at the level of the anterior third of the metraterm, the ovary being inter to post-testicular rather than always intertesticular and the vitelline fields distinctly separated in the forebody rather than confluent (Bray *et al*. 2009).

Hypocreadium lamelliforme differs in that the vitelline fields are separated in the forebody and widely separated in the hindbody rather than confluent in the forebody and in the hindbody.

Three species of *Hypocreadium* are now reported from *Balistes capriscus* in different localities of the world: *H. lamelliforme* was recorded in the Bermudas (Linton 1907), *H. biminensis* in Bimini (Sogandares-Bernal 1959) and Rio de Janeiro (Alves *et al*. 2005) and *H. caputvadum* sp. nov. from the Gulf of Gabés (Tunisia). There are several differences between the *Hypocreadium* species found in these other places and *H. caputvadum* sp. nov. collected from the Gulf of Gabès. For example, the position of the cirrus sac in *H. biminensis* is different from that found in *H. caputvadum*. While in *H. biminensis* the cirrus sac is located sinistrally to the acetabulum, reaching to the posterior edge of the ventral sucker, in *H. caputvadum* the cirrus sac is located on the right side of the acetabulum, reaching to the mid-part of the ventral sucker. Also, in *H. biminensis* the external seminal vesicle lies transversally between the ovary and the ventral sucker rather than longitudinally and lying along the right side of the ventral sucker as in *H. caputvadum*. The species also differ in body shape and tegumental armament in that *H. caputvadum* is rounded and without spines whereas *H. biminensis* is elliptical and spined.

Acknowledgements. Authors wish to thank the "Serveis Científics i Tècnics" of the University of Barcelona (Spain) for their support in the preparation of samples of *Hypocreadium* for scanning electron microscopy (SEM). We are grateful to Dr. R.A. Bray for commenting on the manuscript.

References

Alves D.R., Paraguassú A.R., Luque J.L. 2005. Community ecology of the metazoan parasites of grey triggerfish, *Balistes capriscus* Gmelin, 1789 and queen triggerfish *B. vetula* Linnaeus,1758 (Osteichthyes: Balistidae) from the state of Rio de Janeiro Brasil. *Revista Brasileira Parasitologia Veterinária*, 14, 71–77.

Astraldi M., Bianchi C.N., Gasparini G.P., Morri C. 1995. Climatic fluctuations, current variability and marine species distribution: a case study in the Ligurian Sea (north-west Mediterranean). *Oceanologica Acta*, 18, 139–149.

Bray R.A. 2005. Family Lepocreadiidae Odhner, 1905. *In*: (Eds. A. Jones, R.A. Bray and D.I. Gibson) *Keys to the Trematoda. Volume 2*. CABI Publishing and the Natural History Museum, Wallingford, pp. 545–602.

Bray R.A., Cribb T.H. 1996. The Australian species of *Lobatocreadium* Madhavi, 1972, *Hypocreadium* Ozaki, 1936 and *Dermadena* Manter, 1945 (Digenea: Lepocreadiidae), parasites of marine tetraodontiform fishes. *Systematic Parasitology*, 35, 217–236. DOI: 10.1007/BF00009641.

Bray R.A., Cribb T.H., Justine J.L. 2009. New observations on the genus *Hypocreadium* Ozaki, 1936 (Digenea: Lepocreadiidae) in the Indo-West Pacific region, including the description of one new species. *Zootaxa*, 2110, 22–40.

Bray R.A., Justine J.L. 2006. *Hypocreadium toombo* n. sp. (Digenea: Lepocreadiidae) in the yellow-spotted triggerfish *Pseudobalistes fuscus* (Perciformes: Balistidae) and additional lepocreadiids parasitizing fishes from the waters off New Caledonia. *Zootaxa*, 1326, 37–44.

Fischer W., Bauchot M.L., Schneider M. 1987. Ficher FAO d'identification des espèces pour les besoins de la pêche. Méditerranée et Mer Noire. Zone de pêche 37. Vertébrés. Vol. 2. FAO, Rome, 761–1530.

Francour P., Boudouresque C.F., Harmelin-Vivien M., Harmelin J.-G., Quignard J.-P. 1994. Are the Mediterranean waters becoming warmer? Information from biological indicators. *Marine Pollution Bulletin*, 28, 523–526.

Garrabou J., Pérez T., Chevaldonné P., Bensoussan N., Torrents O., Lejeusne C., Romano J.C., Vacelet J., Boury-Esnault N., Harmelin-Vivien M., Verlaque M., Boudouresque C.F., Zibrowius H., Harmelin J.G. 2003. Is global change a real threat for conservation of the NW Mediterranean marine biodiversity? EGS-AGU-EUG joint assembly. *Geophysical Research Abstracts*, 5, 10522.

Hanson M.L. 1950. Some digenetic trematodes of marine fishes of Bermuda. *Proceedings of the Helminthological Society of Washington*, 17, 74–89.

Hargis W.J., Jr. 1955. Monogenetic trematodes of Gulf of Mexico fishes. Part II. The superfamily Gyrodactyloidea. *Journal of Parasitology*, 41, 185–193.

Harmelin-Vivien M.L., Quéro J.C. 1990. Monacanthidae, pp. 1061–1066. *In*: (Eds. J.C. Quero, J.C. Hureau, C. Karrer, A. Post and L. Saldanha) *Check-list of the fishes of the eastern tropical Atlantic* (*CLOFETA*). JNICT, Lisbon; SEI, Paris; and UNESCO, Paris. Vol. 2.

Ho J.S., Rokicki J. 1987. Poecilostomatoid copepods parasitic on fishes off the west coast of Africa. *Journal of Natural History*, 21, 1025–1034.

Linton E. 1907. Notes on parasites of Bermuda fishes. *Proceedings of the United States National Museum*, 33, 85–126.

Overstreet R.M. 1969. Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida. *Tulane Studies in Zoology and Botany*, 15, 119–176.

Ozaki Y. 1936. Two new genera of the trematode family, Allocreadiidae. *Zoological Magazine*, 48, 513–519.

Robins C.R., Ray G.C. 1986. A field guide to Atlantic coast fishes of North America. Houghton Mifflin Company, Boston, USA, pp. 354.

Saunders D.C. 1959. *Trypanosoma balistes* n. sp. from *Balistes capriscus* Gmelin, the common triggerfish, from the Florida Keys. *Journal of Parasitology*, 45, 623–626.

Sogandares-Bernal F. 1959. Digenetic trematodes of marine fishes from the Gulf of Panama and Bimini, British West Indies. *Tulane Studies in Zoology and Botany*, 7, 69–117.

Whitehead P.J.P., Bauchot M.L., Hureau J.C., Nielson J., Tortonese E. 1984. Fishes of the north-eastern Atlantic and Mediterranean. Vol. 1. UNESCO, Paris, 510 pp.

(Accepted May 07, 2011)