Morphological variability in Acanthocephaloides irregularis Amin, Oğuz, Heckmann, Tepe & Kvach, 2011 (Acanthocephala: Arhythmacanthidae) from the European sea bass, Dicentrarchus labrax (L.) (Moronidae) in Bizerte Lagoon, Tunisia

Halima Jmii Chine, Omar M. Amin, Ana Elena Ahuir Baraja & Lamia Gargouri
Morphological variability in *Acanthocephaloides irregularis* Amin, Oğuz, Heckmann, Tepe & Kvach, 2011 (Acanthocephala: Arythmacanthidae) from the European sea bass, *Dicentrarchus labrax* (L.) (Moronidae) in Bizerte Lagoon, Tunisia

Halima Jmii Chine, Omar M. Amin, Ana Elena Ahuir Baraja, Lamia Gargouri

Received: 21 February 2018 / Accepted: 12 May 2018
© Springer Science+Business Media B.V., part of Springer Nature 2018

**Abstract** Specimens of an arhythmacanthid acanthocephalan were recovered from the European sea bass *Dicentrarchus labrax* (L.) (Perciformes: Moronidae) in Bizerte Lagoon, northern Tunisia. The specimens collected showed high morphological similarities to *Acanthocephaloides irregularis* Amin, Oğuz, Heckmann, Tepe & Kvach, 2011, recovered from four species of marine fishes in the Gulf of Odessa and Sukhyi Lyman off the Ukrainian Black Sea coast. Light and scanning electron microscopy revealed some morphological differences from the Black Sea specimens in trunk spine distribution, number of proboscis hooks in males (4 instead of 5), and lack of demonstrable trunk collar. These variations suggest that our specimens may only be a morphotype of *A. irregularis* with new host and locality records. A comparison between our specimens and those of the original description clarifies the possible reasons of this intraspecific morphological variability.

**Introduction**

The genus *Acanthocephaloides* Meyer, 1932 (Acanthocephala: Arythmacanthidae) currently includes 13 species (Amin, 2013). This genus is widespread in different localities in the world including the Mediterranean and Black Sea (Golvan, 1969; De Buron et al., 1986; Golvan & De Buron, 1988; Kvach, 2006; Amin et al., 2011a). It has been reported from more than 30 teleost fish species (Golvan & De Buron, 1988). So far, only one species of this genus, *Acanthocephaloides propinquus* Dujardin, 1845, has been reported in the sand steenbras *Lithognathus mormyrus* L. (Sparidae) in Tunisian waters (Gargouri et al., 2015). In the present study in Bizerte Lagoon (northern Tunisia), 11 specimens of an arhythmacanthid acanthocephalan were collected from the mid-intestine of the European sea bass *Dicentrarchus labrax* (L.) (Moronidae). This fish is a seasonally migratory species, moving further inshore and north in summer and feeding on small fish,
polychaetes, cephalopods and crustaceans (Freyhof & Kottelat, 2008; Froese & Pauly, 2017). Its habitats include estuaries, lagoons, coastal waters, and rivers. The European sea bass is found in the waters of Europe, including the Mediterranean Sea, and the Black Sea from where $A. \text{irregularis}$ Amin, Oğuz, Heckmann, Tepe & Kvach, 2011 was originally described. The newly collected worms appeared very similar to $A. \text{irregularis}$. However, the specimens identified in the present study have demonstrated some variations in their morphology compared to those in the original description from the Black Sea. Our worms are described in detail and a comparison between the two morphotypes of $A. \text{irregularis}$ is also presented.

**Materials and methods**

A total of 205 specimens of the European sea bass *Dicentrarchus labrax* (L.), ranging between 19 to 35 cm in length, were collected from Bizerte lagoon off north-eastern Tunisia (37°11′48.12″N, 9°51′22.68″E) between March 2016 and April 2017 by net fishing. Fish were examined for helminths under a stereomicroscope shortly after capture. The specimens of acanthocephalans collected were first placed in distilled water at 4°C for a few hours until the proboscis was everted.

For the morphological description, 1 male and 1 female of the collected specimens were placed in a temporary mount of glycerol solution (50 ml: ½ glycerol and ½ 70% ethanol) to observe details of the proboscis and the internal structures. Nine specimens were fixed and preserved in 70% ethanol; some of these were stained with iron acetocarmine, destained in 1% hydrochloric acid in 70% ethanol, dehydrated in ascending concentrations of ethanol (70 to 100%), cleared in 98% dimethyl phthalate, and mounted in Canada balsam.

Line drawings were made with the aid of a drawing tube attached to a light microscope. Measurements were taken using Leica Application Suite (LAS) EZV2.0.0 software. Trunk length does not include neck, proboscis, or bursa. Length is given before width which refers to the maximum width. Measurements are in micrometres unless otherwise stated and measurements of egg length and width were made through the body wall of females.

For scanning electron microscopy (SEM) studies, one male and one female were dehydrated in an ascending ethanol series, placed in drying baskets, critical point dried in liquid CO$_2$, mounted on specimen’s stubs with conductive carbon paint, sputter-coated with gold-palladium to a thickness of 25–30 nm in a Bio Rad-Sc 500 coating unit, and examined in a HITACHI S-4100 scanning electron microscope at 5 kV at the Central Service for Experimental Research (SCSIE) of the University of Valencia, Spain.

Voucher specimens were deposited in the collections of the Muséum National d’Histoire Naturelle (MNHN HEL), Paris, France.

**Family Arhythmacanthidae, Yamaguti, 1935**

**Genus *Acanthocephaloideus* Meyer, 1932**

*Acanthocephaloideus irregularis* Amin, Oğuz, Heckmann, Tepe & Kvach, 2011

*Host: Dicentrarchus labrax* (Linnaeus) (Perciformes: Moronidae), European sea bass.

*Locality:* Bizerte Lagoon (37°11′48.12″N, 9°51′22.68″E), northern Tunisia.

*Site in host:* Mid-intestine.

*Voucher material:* MNHNHEL736 (2 males, 1 female on 1 slide); MNHNHEL737 (1 female).

*Prevalence and intensity:* One of 205 fish was infected with 11 worms.

**Description** (Figs. 1, 2)

**General.** Arhythmacanthidae, with characters of the genus *Acanthocephaloideus*. Worms small, fusiform (Figs. 1A, 2A–F). Females slightly larger than males. Anterior trunk cone undetectable. Trunk with micropores and many small cuticular spines in near transverse rows anteriorly (Fig. 2B), scarce in middle, and irregularly distributed posteriorly. Anterior trunk spines directed posteriorly, posterior spines pointed anteriorly, bearing micropores (Fig. 2D). No spines on copulatory bursa. Proboscis small, cylindrical with median swelling. Proboscis hooks in 12 rows each with 4 hooks in males and 5 hooks in females. First 3 proboscis hooks increase gradually in size posteriorly; posterior spiniform hooks almost equally long (Fig. 2B, C); all hooks with roots; hook surface smooth (Fig. 2C). Neck prominent with two sensory pores (Fig. 2B). Cephalic ganglion triangular and...
rounded posteriorly, at base of receptacle. Few nuclear pouches present at external posterior end of double-walled receptacle (Fig. 1B, C). Lemnisci digitiform almost equal in size and longer than receptacle. Gonopore terminal in males and near terminal in females (Figs. 1A–E, 2E–G).
Male [Based on 4 mature males, measurements in Table 1.] Proboscis short and cylindrical with 12 rows of 4 hooks each; each row with 2–3 rooted hooks and 1–2 spiniform hooks (Figs. 1B, 2B); largest hooks at middle of proboscis. Testes 2, spherical to elongate, contiguous, equatorial, almost equal in size. Cement glands pear-shaped, 6 in 3 pairs, with 2 cement gland ducts. Saefftigen’s pouch posterior to cement glands.
Copulatory bursa ornamented with circular ring of prominent sensory discs (Fig. 2E).

**Female** [Based on 5 females with eggs and ovarian balls, measurements in Table 1] Proboscis with 12 longitudinal rows of 5 hooks each; 3 rooted hooks and 2 spiniform hooks (Fig. 1C). Uterus relatively long, uterine bell with nucleated cells (Fig. 1E). Eggs elongated, fusiform and with polar prolongation of fertilization membrane (Fig. 1F).

**Remarks**

The material found in the present study is regarded as a morphotype of *A. irregularis* described by Amin et al. (2011a) based on the morphometric data presented in Table 1.
Table 1. The obvious overlap in measurements and counts of morphometric characters (Table 1) suggests that both morphotypes belong to the same species. In spite of these similarities, specimens found in *D. labrax* exhibit several morphological variations. Compared with specimens described by Amin et al. (2011a), the Tunisian worms are somewhat smaller and have fewer hooks (4) in the male proboscis. The female gonopore is near terminal in our specimens but terminal in the Black Sea specimens. We have previously observed the same displacement of the female gonopore in other species of acanthocephalans including *Pomphorhynchus kashmirensis* Kaw, 1941 (*Pomphorhynchidae*) from *Schizothorax plagiostomus* Heckel (Cyprinidae) in Jammu–Kashmir (Amin et al., 2012) and *Echinorhynchus salmonis* Müller, 1784 (Echinorhynchidae) from *Coregonus lavaretus* (L.) (Salmonidae) in Lake Baikal (Amin et al., 2015). Furthermore, the collar at the anterior trunk, the micropores on hook surface, and the cuticular spines on the copulatory bursa were not demonstrable in our specimens. All these characters appear to represent intraspecific variation characteristic of the Tunisian material. The presence of a circular ring of prominent sensory discs on the bursa in both populations and the nuclear pouch at the external base of the receptacle further confirm the specific identity.

The description of *A. irregularis* from the Black Sea was based on several morphological features especially on the irregular distribution of spines on the trunk. In our specimens, the anteriormost trunk spines were distributed in rather circular transverse circles (Fig. 2B) but were irregular in the middle and posterior trunk. Therefore, the noted disparities are attributed to an intraspecific variability.

We have also noticed that there are some similarities between our specimens and *A. propinquus* which has been reported from a variety of fish species in the Mediterranean Sea (Golvan, 1969) and from gobid fishes from the Black Sea (Kvach, 2006; Oğuz & Kvach, 2006). However, they are distinguished by the following features: the disposition of the hooks on the proboscis, the trunk length, the shape of the cephalic ganglion and the absence of nucleated pouches at the posterior end of proboscis receptacle. For further details, see the key to species of *Acanthocephaloides* in Amin et al. (2011a).

**Discussion**

The prevalence of the parasites encountered during our study is quite low (only one of 205 examined fish was infected) which suggests that *D. labrax* may not be the main definitive host of *A. irregularis*. The presence of other definitive hosts in the same lagoon from which a spill over to the sea bass by piscivory (ichthyophagy) is possible. De Buron & Maillard (1987) indicated that “adult intestinal parasites (acanthocephalans and digeneans) can be experimentally transferred to predator fishes without any alteration”. Indeed, the sea bass is carnivorous, and it spends the first stages of its life in lagoons where it feeds mostly on a variety of crustaceans such as small amphipods “Gammarus” and isopods “Idotea” (Ktari et al., 1978) but also including a significant proportion of gobid fish that may serve as final host for *A. irregularis*. Summer migrations of sea bass from the Tunisian lagoon to the Black Sea (Smith, 1990) where a larger population of *A. irregularis* appears to be present may have served as a historical connection bringing in this acanthocephalan to the Tunisian coast where they may overwinter.

*Acanthocephaloides irregularis* has been reported from hosts in the Gobiidae (Amin et al., 2011a; Krasnovyd et al., 2012), Belinniidae, Syngnathidae (Amin et al., 2011a), Scorpaeinidae (Tepe & Oğuz, 2013) and Mullidae (Öztürk & Yesil, 2018). This is the first report of this acanthocephalan in a host of the Moronidae and in a coastal Mediterranean lagoon since all previous records are in the Black Sea. Intraspecific morphological variability reported above could be attributed to a different host species and/or to geographical and habitat divergence. The Black Sea is connected with the Mediterranean by the Bosphorus Strait, the Sea of Marmara and Dardanelles Strait. This communication between both seas has sparked interest of several oceanologists and marine geologists who proposed the inflow of the Mediterranean Sea waters into the Black Sea after the last glacial period (Yüce, 1990; Ryan et al., 1997; Yanchilina et al., 2017). The flooding caused an invasion of some marine organisms and to date there is still an exchange between the two seas. It has been reported that fish species, mainly gobids, with a Mediterranean origin are present in the Black Sea waters (Miller, 1965; Fricke et al., 2007; Kvach & Kutsokon, 2017). Despite this connection, Mediterranean waters are still highly more saline than
the brackish waters of the Black Sea (Yüce, 1990, 1996; Yanchilina et al., 2017) leading to the notion that the fish hosts of A. irregularis have different environmental requirements related to hydrochemical characteristics. These factors may contribute to other morphological variations found between the two morphotypes of A. irregularis. A recent common ancestral population could likely exist either in the Mediterranean or in the Black Sea; if not extinct.

Explaining the complexities of speciation, cryptic or not, is helped by several studies of inter- and intraspecific variations within acanthocephalans. Morphological variations in proboscis armature, trunk spination and reproductive structures were studied in several species (Amin & Huffman, 1984; Perrot-Minnot, 2004; García-Varela et al., 2012; Wayland, 2013; Amin et al., 2015; Amin et al., 2016). Amin et al. (2011b) distinguished an isolated population of Corynosoma strumosum (Rudolphi, 1802) Lu¨he, 1904 in the land-locked Caspian Sea from others reported from open waters elsewhere by the distribution of trunk spines, consistently smaller size of the trunk and testes, larger eggs, and fewer proboscis hooks; a situation comparable to ours. Amin et al. (2016) demonstrated the evolutionary origins and the geological history involved in the diversification of Echinorhynchus baeri Kostylew, 1928 in Turkey and Armenia. Seven percent of males of Acanthocephalus jacksoni Bullock, 1962 (synonym of A. dirus Van Cleave, 1931 in Amin, 1984) population were monorchid (Bullock, 1962). Differences in the shape of the neck bulb (an important taxonomic feature in the Pomphorhynchidae) related to speciation was observed in specimens of Pomphorhynchus zhoushanensis Li, Chen, Amin & Yang, 2017 (see Li et al., 2017). Relevant studies on species of the genus Acanthocephalus and intraspecific variability in Acanthocephalus dirus (see Amin, 1984; 1985) explored the adaptability of acanthocephalans to a wide range of intermediate and definitive hosts affecting population dispersal and diversification. These examples, among others, point to plasticity in the acanthocephalans and the influence of biotic factors on their diversification.

Our study bears out the view as to the existence of polymorphic forms of Acanthocephaloides, another demonstration of the variability within the phylum Acanthocephala. This case of closely related populations, yet distinguishable, of one species can serve as a model for determining host- and habitat-related specificity mechanisms.

Acknowledgements Thanks are due to Dr Francisco Esteban Montero (Cavanilles Institute of Biodiversity and Evolutionary Biology, Science Park, University of Valencia, Parc Scientific) for his technical assistance. We thank the SCSIE of the University of Valencia for their technical support for SEM micrographs.

Funding This study was funded by the Ministry of Higher Education and Scientific Research of Tunisia.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

References


