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On two morphologically different cysticercoids of the genus *Eurycestus* (Cestoda: Dilepididae) in *Artemia franciscana* (Arthropoda: Artemiidae) in a hypersaline pond in Dubai, United Arab Emirates*

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Article info	Summary
Received January 10, 2018 Accepted March 28, 2018	During a survey on tapeworm larval stages in <i>Artemia franciscana</i> from an artificial pond in Dubai, United Arab Emirates, a high prevalence of <i>Eurycestus avoceti</i> -like cysticercoids was established. Adult male and female crustaceans showed a prevalence of 61.9 and 62.7 %, respectively. The intensity ranged from one to four and one to three cyst, respectively. Out of 215 examined cysticer- coids, 207 specimens had morphological features matching with <i>E. avoceti</i> . The flaky structure of the surrounding capsule, the elongated shape of the cysticercoid and the larger number of hooklets on the suckers suggest that the eight further larval cestodes belonged to another species of the genus <i>Eurycestus</i> . Keywords: Brine shrimps; <i>Artemia franciscana</i> ; cysticercoids; <i>Eurycestus</i> ; United Arab Emirates

Introduction

Brine shrimps of the genus *Artemia* are aquatic crustaceans that live in hypersaline environments. The genus *Artemia* consists of *A. salina* (Linnaeus, 1758), *A. monica* Verrill, 1869, *A. urmiana* Günther, 1899, *A. franciscana* Kellogg, 1906, *A. persimilis* Piccinelli & Prosdocimi, 1968, *A. sinica* Cai, 1989, *A. tibetiana* Abatzopoulos, Zhang & Sorgeloos, 1998 and parthenogenetic populations called *A. parthenogenetica* (Asem et al. 2010).

Serving as food for flamingos, waders, gulls and ducks, brine shrimps act as intermediate host for a number of avian cestodes of the Hymenolepididae family (*Aploparaksis parafilum* Gasowska, 1932, *Brachiopodataenia gvozdevi* Maksimova, 1988, *Confluaria podicipina*, (Szymanski, 1905), *Fimbriaruioides tadornae* Maksimova, 1976, *Flamingolepis caroli* (Parona, 1887), *F. flamingo* Skrjabin 1914, *F. liguloides* Gervais, 1847, *F. tengizi* Gvozdev & Maksimova, 1968, *Hymenolepis californicus* Young, 1950, *Wardium fusca* (Krabbe, 1869) and *W. stellorae* Deblock, Biguet et Capron, 1960), the Dilepididae family (*Eurycestus avoceti* Clark, 1954, *Ano*- *molepis averini* Spassky & Yurpalova, 1967, *Anomotaenia tringae* (Burt, 1940) and *A. microphallus* (Krabbe, 1869) and the Progynotaeniidae family *Gynandotaenia stammeri* Fuhrmann, 1936 and *Gynandrotaenia* sp. Redón et al., 2015b – a so far undetermined further species of this genus) (Schuster 2018).

One of the cysticercoids that was found in numerous studies was *E. avoceti* (Table 1). The description of its larval stage is based only on small numbers of cysticercoids. In a recent study on the location of cestode larvae in the body of *A. franciscana*, we examined a larger number of *Eurycestus* cysticercoids showing the existence of two morphologically different types.

Materials and Methods

Collection site of A. franciscana

The Godolphin lakes in the Al Quoz district of Dubai are small ponds of 2.5 and 2.9 ha, respectively and were created as satellite wetland to attract wader birds. A detailed description of this habitat was given by Sivakumar et al. (2018).

^{* –} This paper is dedicated to the 90th birthday of my teacher, Prof. Dr. Dr. h.c. mult. Theodor Hiepe, former director of the Parasitological Institute of the Humboldt-University of Berlin.

Collection site	Country	Examined host species	Number examined	Prevalence (%)	Average intensity	Reference
Camargue	F	Artemia sp.	64.640	0.09	1 – 2	Robert & Gabrion (1991)
Odiel Marsh	Es	A. parthenogenetica	3,300	2.5	1 – 3	Georgiev et al. (2005)
Odiel Marsh	Es	A. parthenogenetica	200	7.0	-	Sanchez et al. (2006)
Odiel Marsh	Es	A. parthenogenetica	100	17.0	-	Sanchez et al. (2007)
Salinas Castro Marim	Р	A. franciscana	240	4.0	1	Georgiev et al. (2007)
Odiel Marsh	Es	A. parthenogenetica	200	3.0	1	Georgiev et al. (2007)
Salinas de Nuestra	Es	A. parthenogenetica	200	7.0	1.14	Georgiev et al. (2007)
Salinas Portuguesas	Es	A. parthenogenetica	200	30.0	1.15	Georgiev et al. (2007)
Salinas de Animas	Es	A. parthenogenetica	70	4.3	1	Georgiev et al. (2007)
Salinas de St. Barbara	Es	A. franciscana	200	2.0	1.4	Georgiev et al. (2007)
Salinas de Cerillos	Es	A. salina	200	8.0	1	Georgiev et al. (2007)
La Mata Lagoon	Es	A. parthenogenetica	195	2.6	1	Redon et al. (2011)
Odiel Marsh	Es	A. parthenogenetica	3,000	2.13	1.08	Sanchez et al. (2013)
La Tapa salters	Es	A. franciscana	949	2.3	1.09	Georgiev et al. (2014)
Ebro delta	Es	A. franciscana	9,293	7.6	1.2	Redon <i>et al</i> . (2015a)
Ebro delta	Es	A. franciscana	487	8.6	1.02	Redon et al. (2015b)
Ebro delta	Es	A. salina	381	3.9	1	Redon et al. (2015b)
Godolphin lakes, Dubai	AE	A. franciscana	1,840	4.5	1.1	Sivakumar et al. (2018)

Table 1. Frequency of *Eurycestus avoceti* in brine shrimps in different studies. (F: France, Es: Spain, P: Portugal, AE: United Arab Emirates)

Examination of shrimps

Brine shrimps of the species A. franciscana were caught by net in the Godolphin lakes in Dubai, in May 2018 for a study of the location of cysticercoids in the body of the shrimps. Shrimps were killed in hot 70 % alcohol and 300 adult specimens were randomly selected. Prior to examination, they were placed for 5 days on a slide in a drop of glycerin and were covered with a cover slip. Glycerin cleared the body of the shrimps and made the parasites visible. Examination for the presence of cysticercoids was carried out at low magnification (40 - 100x). Special attention was paid to cysts surrounded by a brown capsule as well as to those with a transparent capsule and suckers exhibiting hooklets. Measurements of the outer capsule were taken at a magnification of 400x. Shrimps were then dissected and cestode larvae were individually placed into a drop of glycerin. Prior to putting a cover slip, the capsule surrounding the cysticercoid was destroyed by fine preparation needles. At a magnification of 600x, the following parameters of each cysticercoid matching the description of the genus Eurycestus were determined: length and width of the cysticercoid, width of the scolex at the level of suckers, length and width of suckers, distribution, number and length of hooklets on suckers, length and width of the rostellum, number and length of rostellar hooks. In order to determine the exact length of sucker hooklets and rostellar hooks as well as the number of rostellar hooks most of the cysticercoids had to be squashed by pressing on the cover slip. Measurements were taken using an OLYMPUS BX51 microscope connected to an OLYMPUS DP27 camera with the software OLYMPUS cellSens Dimension.

Ethical Approval and/or Informed Consent

All applicable national and institutional guidelines for the care and use of animals were followed.



Fig. 1. *Eurycestus avoceti* cysticercoid surrounded by a homogenous brown capsule and found in the thorax of *Artemia franciscana*.

Table 2. Prevalence and burden of Eurycestus cysticercoids in A. f	franciscana from Godolphin lakes of	Dubai in May 2018
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Sex	Num	ber of	Prevalence	Inten	sity
	examined	parasitised	(%)	average	range
males	134	83	61.94	1.14	1 – 4
females	166	104	62.65	1.15	1 – 3
total	300	187	62.33	1.15	1 – 4

Results

Of 300 examined *A. franciscana*, 134 were males and 166 were females. A total of 187 (= 62.33 %) of crustacean hosts harbored between one and four *Eurycestus* cysticercoids. There was no sex related difference in prevalence and burden (Table 2). Out of 95 *Eurycestus* cysticercoids in male shrimps, 93 were located in the thorax and each one in the abdomen and phyllopods. Female shrimps contained a total of 120 *Eurycestus* cysticercoids. Of these, 114 were detected in the thorax, four and two were found in head and abdomen, respectively.

The majority (n=207) of the examined 215 cysticercoids was surrounded by a more or less homogenous, transparent to dark



Fig. 2. *Eurycestus avoceti* removed from its capsule.

brown irregularly rounded capsule (Fig 1). The decapsulated cestode larval stages were heart shaped to oval round (Fig 2.) The anterior margin of the oval suckers was furnished with two layers of hooklets in total numbers between 8 and 15. In most of the cases 12 hooklets were counted (Table 3). Their length was $5-7 \mu m$. There were 14 to 16 arcuatoid rostellar hooks arranged in two circles. Their length varied between 12 to 16 and 14 to 18 μm , respectively.

Eight other *Eurycestus* cysticercoids were surrounded by an irregularly elongated, flaky and very fragile capsule (Fig. 3). The decapsulated cysticercoids were elongated (Fig. 4) and differed from the above mentioned by a larger number of hooklets on the suckers ranging between 24 and 32 (Table 3). Sixteen arcuatoid rostellar hooks arranged in two circles measured 11 – 14 and 17 – 19 $\mu m,$ respectively.

Discussion

While previous publications reported prevalence data of *Eurycestus* cysticercoids in *Artemia* spp. between 0.09 and 30.0 % (Table 1), the current study revealed an unusually high prevalence of 62.33 %. The reasons for this might be the small size of the habitat, a large number of birds visiting the ponds and a specific moment in time for the collection when *Artemia* population was in full bloom in the month of May.

The species inventory of the genus *Eurycestus* consists so far of three species. In addition to the description of *E. avoceti* from its final host, the American avocet (*Recurvirostra americana* Gmelin, 1789) by Clark (1954), Burt (1979) described two further species of the genus *Eurycestus* found in the same final host. *E. falciformis* and *E. latissimus* differed from *E. avoceti* in the shape of strobila, size of cirrus sac, number of testes and armature of the cirrus. All three descriptions were based on strobilae without scolex.



Fig. 3: *Eurycestus* sp. cysticercoid surrounded by a flaky capsule fragile capsule and found in the thorax of *Artemia franciscana*.

In addition to American and pied avocets from Nebraska and France, respectively, *E. avoceti* was found also in black necked stilts (*Himantopus mexicanus* (Müller, 1776) in Texas (Hinojus & Canaris 1988). Maksimova (1991) mentioned greater flamingos (*Phoenicopterus roseus* Pallas, 1811), black-winged stilts (*Himan*-

Type		Outer (capsule	Cystice	rcoid	Skolex	SUI	ckers			Rost	ellum	Å	ostellar hoo	ks
									hooklets					small	large
		length	width	length	width	width	length	width	۲	length	length	width		length	length
A	average	173.7	152.2	124.1	100.1	63.8	18.6	15.5			58.1	20.1		13.8	16.2
N=207	range	130 – 246	102 – 215	100 – 153	70 – 146	50 – 94	14 – 25	10 – 20	8 – 15	5 – 7	44 – 88	14 – 26	14 – 16	12 – 16	14 – 18
в	average	211.5	123	157.9	90.5	78	22.4	20.3		7	65.8	18.8	16	12	17.8
N=8	range	182 – 245	93 – 160	145 – 186	76 – 113	63 – 91	21 – 24	18 – 22	24 – 30		58 – 78	13 – 21		11 – 14	17 – 19

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Fig. 4: Eurycestus sp. cysticercoid removed from its capsule.

topus himantopus Linnaeus, 1758) and slender-billed gulls (*Chroi-cocephalus genei* Breme, 1839) as final hosts in Kazakhstan. This wide spectrum of phylogenetically distant hosts suggests that also other birds that feed on brine shrimps might play a role in the *Eurycestus* life cycle.

The assignment of *E. avoceti* to the Anoplocephalidae family by Yamaguti (1959) was proven incorrect after Baer (1968) found whole cestode including scolex in avocets^a in Carmargue, France and published a redescription of the species. The width of the extremely small scolex varied between only 45 and 53 µm and could reach 61 µm when the rostellum (52 µm long and 20 µm in diameter) is retracted. There were 14 to 16 rostellar hooks in a length of 14 to 16 µm, arranged in two circles. Suckers bore 10 to 14 hooklets in a length of 5 - 6 µm. Baer (1968) allocated E. avoceti to the Dilepididae family. Based on the morphology of the scolex, Georgiev et al. (2005) considered cysticercoids found in A. parthenogenetica in the Odiel Marshes in Spain, the larval stage of *E. avoceti*. The majority of cysticercoids found in the current study (type A in Table 3) seem to belong to the same species, E. avoceti. Eight other cysticercoids in the recent study (type B, in Table 3) however, showed striking differences in the structure of the capsule, surrounding the larval stage and in the shape of the cysticercoid. A further difference was the number and the distribution of the hooklets on the suckers. Already Gabrion & Mac Donald (1980) when examining Artemia sp. from Camarge, gave a description of an elongated cysticercoid (170 x 90 µm) suckers of which were furnished with 30 to 32 hooklets covering their anterior and lateral rims. Other parameters (number and size of rostellar hooks) did not differ significantly from above mentioned E. avoceti. Robert & Gabrion (1991) examined a total of 64,604 Artemia sp. in the same habitat and found among others, a total of 59 cysticercoids matching the description by Gabrion et Mac Donald (1980). In a survey on cestode larval stages in A. salina from Tengiz lake in Kazakhstan, Maksimova (1991) described elongated cysticercoids measuring 140 – 190 x 100 – 130 µm being surrounded by a dark brown but very fragile capsule of $180 - 320 \times 150 - 220 \mu m$ in size. The scolex measured $45 - 76 \times 42 - 71 \mu m$ and had four armed suckers with 30 to 32 hooklets arranged in two rows on their anterior and lateral margins. The rostellum had a length of $50 - 60 \mu m$ and a width of $17 - 22 \mu m$. It was armed with 16 hooks arranged in two rows. Larger hooks measured $16 - 18 \mu m$, smaller ones $10 - 12 \mu m$. The prevalence of these cysticercoids in *A. salina* in lake Tengiz was low and varied between 0.03 and 0.5 %. Referring to Gabrion et Mac Donald (1980) the author believed that this was *E. avoceti*.

Based on the appearance of the surrounding capsule and on the morphology of the cysticercoid, it can be concluded that *A. franciscana* in the Godolphin lakes of Dubai were infected with two different *Eurycestus* species one of which type A can be affiliated with *E. eurycestus*. The second species (type B) comparable to those found *Artemia* sp. in Carmargue and in *A. salina* in Tengiz lake remains unnamed until the adult cestode is described.

Conflict of Interest

There is no conflict of interests.

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a - The bird species was not mentioned in the paper but most probably it was the pied avocet (R. avosetta).

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