ON CYSTICERCOIDS OF THE GENUS FLAMINGOLEPIS SPASSKIJ & SPASSKAJA, 1954 (CESTODA: HYMENOLEPIDIDAE) PARASITIZING ARTEMIA FRANCISCANA KELLOG, 1906 (ARTHROPODA: ARTEMIIDAE) IN DUBAI, UNITED ARAB EMIRATES

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An examination of 300 *Artemia franciscana* Kellog, 1906 revealed an unusual high cysticercoid prevalence of 99 %. Large and small cysticercoids of the genus *Flamingolepis* were the most frequent cestode larvae. Digestion of crustaceans in artificial gastric juice resulted in a large number of cysticercoids of which 120 large and 60 small cysts were studied in light microscopy. A portion of the material was used for histological examination. As a result, elliptical shaped large cysts of 345–570 x 204–387 µm with rostellar hooks of 161–188 and a blade of 91–115 µm were considered *F. dolgushini* and cysts of 157–209 x 112–164 µm and rostellar hooks of 49–59 µm and a blade of 25–33 µm were attributed to *F. flamingo*. The material contained another species with rostellar hooks measuring 82–84 µm that most probably belong to *F. megalorchis*.

Keywords: *Artemia franciscana*, Cestoda, *Flamingolepis*, cysticercoids, Dubai.

Brine shrimps are phylogenetically old crustaceans belonging to the order Anostraca, class Branchiopoda. They inhabit hypersaline inland waters and can be found on all continents except Antarctica. The genus *Artemia* consists of seven species with *A. salina* (Linnaeus, 1758) and *A. franciscana* Kellogg, 1906 being the best investigated ones and parthenogenetic populations called *A. parthenogenetica* (Asem et al., 2010). Brine shrimps play an important role in aquaculture as their juvenile stages is food source for fish and crayfish larvae. *Artemia* spp. are also known to act as intermediate hosts for cestodes. The first description of a cysticercoid in *A. salina* was done roughly 100 years ago (Heldt, 1926). A.P. Maksimova rendered great service to the investigation of the role of *A. salina* as intermediate host for avian cestodes. Working at the Tengiz lake of Kazakhstan, the author described larval stages of 10 cestode species (Maksimova, 1973, 1977, 1981, 1986, 1987, 1988, 1991; Gvozdev, Maksimova, 1979). Research on the role of brine shrimps as intermediate hosts for avian cestodes in the French Camargue started in the early 1980's (Gabrion, Mac Donald, 1980;

Gabrion et al., 1982) and was extended to salterns of Spain by Amat et al. (1991). Mura (1995) examined brine shrimps in Sardinia. Georgiev et al. (2005) gave a comprehensive description of 8 cysticercoids found in *A. parthenogenetica* in the Odiel Marshes in Spain. One cysticercoid that struck due to its large dimensions and eight large skrjabinoid hooks measuring around 180 μm was frequently found hypersaline aquatic habitats in Italy, France, Spain, Portugal and Tunesia was attributed to *Flamingolepis liguloides* (Gervais, 1847) [1]. However, rostellar hooks of the adult *F. liguloides* were distinctly smaller and measured only 130 μm. Another frequently detected but considerable smaller cysticercoid was allocated to *Flamingolepis flamingo* Skrjabin, 1914). The aim of this paper was to describe the morphology of *Flamingolepis* cysticercoids in *A. franciscana* from Godolphin lakes in Dubai.

MATERIAL AND METHODS

In connection with a study of *Eurycestus* spp. in *A. franciscana* collected in the Godolphin lakes in Dubai (Schuster, 2019) there was also the possibility to examine the morphology of cysticercoids belonging to the genus *Flamingolepis* that occurred in high prevalences and intensites. The Godolphin lakes are artificial hypersaline ponds situated in the city of Dubai. A detailed description of this habitat was given by Sivakumar et al. (2018).

The sample collected by closed meshed net consisted of several thousand *A. franciscana* of all development stages (cysts, nauplii, metanauplii and adults). Part of the material was killed in hot (70 ᵒC) 70% alcohol. A total of 300 adult specimens were randomly selected and put for five days into a drop of glycerin to clear the body of the crustaceans and make all cysticercoids visible. Screening for the presence of cysticercoids was carried out at low magnification (40–100x).

Another portion of brine shrimps of the same sample was exposed for 30 min to artificial gastric juice on a magnet stirrer at 40 ᵒC. This portion was then washed 3 times in phosphate buffer solution (PBS) and the sediment with hundreds of cysticerci was kept for another 3 hours in an incubator at 40 ᵒC before it was fixed in hot 10% neutral formalin. For the morphological study, cysticercoids were put for 24 h into 30% lactic acid to dissolve calcareous bodies that otherwise reduce the transparency and visibility of structures of the cysticercoid. A total of 120 large and 60 small cysticercoids were measured and the following measurements were taken: length and width of the cysticercoid, the scolex, the suckers, the rostellum, width of cyst wall, length of the rostellar hooks and their blades. The cercomer of the cysticercoids was not considered since it was dissolved during artificial digestion.

The majority of the material was used to prepare histological sections. For this, cysticercoids were dehydrated in rising concentrations of alcohol and embedded in paraffin. Histological sections were hematoxylin-eosin (HE) and periodic-acid-Schiff (PAS) stained. Examination of both cysticercoids cleared in lactic acid and histological sections was performed an OLYMPUS BX51 microscope connected to an OLYMPUS DP27 camera with the software OLYMPUS cellSens Dimension.

Statistical analysis

For calculating prevalence and intensity of infection at their 95% lower and upper confidence intervals the software package Quantitative Parasitology 3.0 (QP WEB) (Rozsa et al., 2000) was used.

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[1] In a remark on *F. liguloides,* Georgiev et al. (2005) stated mildly that their material corresponds to that of Robert and Gabrion (1991) but also that of Maksimova (1973) but believed Amat et al. (1991) that *F. dolgushini* is a junior synonym of *F. liguloides*. In all following publications the large *Flamingolepis* cysticercoids were diagnosed as *F. liguloides.*

Of 300 examined *A. franciscana*, 134 were male of which 132 (= 98.5 %) harbored large *Flamingolepis* cysticercoids (Fig. 1) in numbers between one and twelve. Of the 166 female shrimps, 165 (= 99.4 % were infected with one to twenty-four of these large cestode larval stages (Tab. 1). Strikingly smaller *Flamingolepis* cysticercoids with a long coiled cercomer (Fig. 2) were present in 52 male (=38.8 %) and 72 (=43.4 %) female hosts. Cysticercoids of the genus *Eurycestus* were diagnosed in 83 male and 104 female shrimps. A single cysticercoid of *Gynandrotaenia stammeri* Fuhrmann, 1936 was detected in a female shrimp.

Peptic artificial digestion of fresh brine shrimps resulted in a large number of oval shaped large (345–570 x 204–387 μm) and small (157–209 x 112–164 μm) *Flamingolepis* cysticercoids (Figs 3, 4) with actively moving scolex structures. The average cyst walls of large and small cysticercoids measured 15 μm (range 9–34 μm and 14 μm (range: 10–21 μm), respectively. Measurements of cysts and subsequent scolex structures are given in Tab. 2 and 3 and compared to literature data. Histological examination showed that the wall of both cysticercoids consists of three layers (Figs 5, 6). Outer and inner layers are thin and compact while the intermediate stratum is wider and seem to be more fluffily. PAS staining revealed that the highest concentration of calcareous bodies is in tissues of the neck region, surrounding the scolex (Fig. 7).

Five large and one small cysticercoid hatched in PBS (Figs 8, 9). Amongst the examined material there were two cysts measuring 214–239 x 172–177 μm that were slightly bigger than any other of the small cysticercoids. The most striking difference were rostellar hooks of 82 and 84 μm in lengths with blades of 43 and 48 μm (Fig. 10).

Despite the relatively high number of *Eurycestus* cysticercoids seen in glycerin cleared shrimps no cysticercoids of this genus were detected after treatment with artificial gastric juice.

Table 1. Prevalence and burden and their 95% confidence intervals (C.I.) of cysticercoid infection in 300 Artemia fanciscana collected from Godolphin lakes in Dubai in May 2018

| Host sex	Cysticercoid type	Number of infected	Prevalence		Burden		
			(%)	95% C.I.	average	95% C.I.	range
males (n=134)	*Flamingolepis (large)*	132	98.5	94.7;99.8	5.52	5.08;5.99	1–12
	Flamingolepis (small)	52	38.8	30.5;47.6	1.81	1.54;2.12	1–5
	Eurycestus spp.	83	61.9	53.2;70.2	1.14	1.06;1.29	1–4
females (n=166)	*Flamingolepis (large)*	165	99.4	96.7;100	7.64	7.08;8.3	1–24
	Flamingolepis (small)	72	43.4	35.7;51.3	2	1.72;2.39	1–7
	Eurycestus spp.	104	62.7	54.8;70.0	1.15	1.08;1.23	1–3
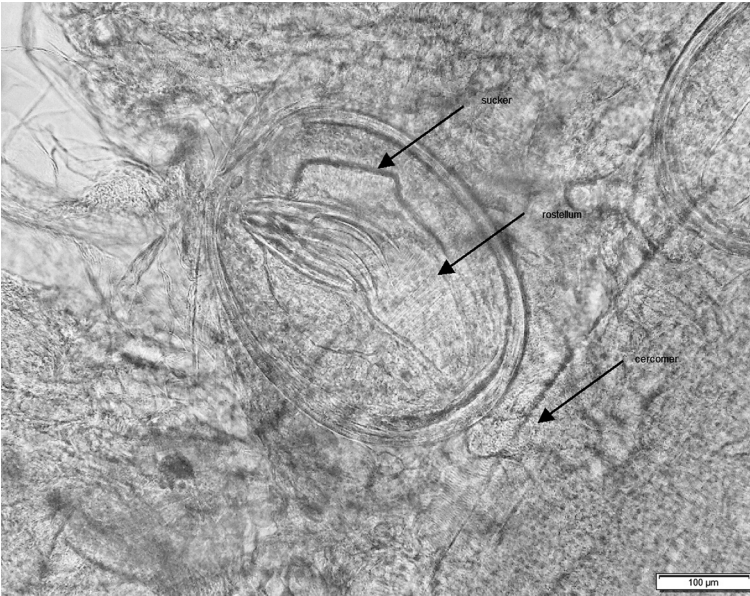
Figure 1. Large *Flamingolepis* cysticercoid in the distal thorax of *A. franciscana* with a relatively short cercomer, not longer than the double of the cyst length

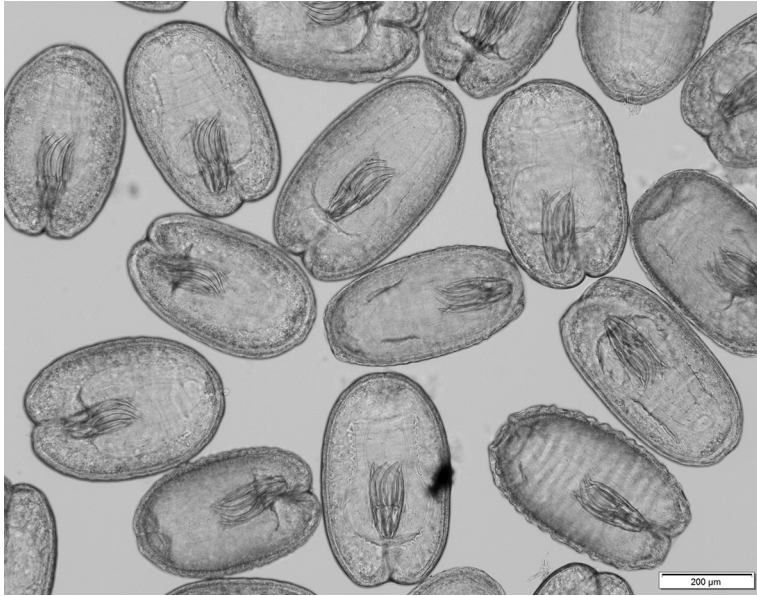**Figure 2.** Small *Flamingolepis* cysticercoid with a coiled long, thin cercomer in the abdomen of *A. franciscana*

Figure 3. Large *Flamingolepis* cysticercoid after treatment of infected brine shrimps with artificial gastric juice.

Figure 4. Small *Flamingolepis* cysticercoids after treatment of infected brine shrimps with artificial gastric juice.

Figure 5. Histological section of a large *Flamingolepis* cysticercoid.
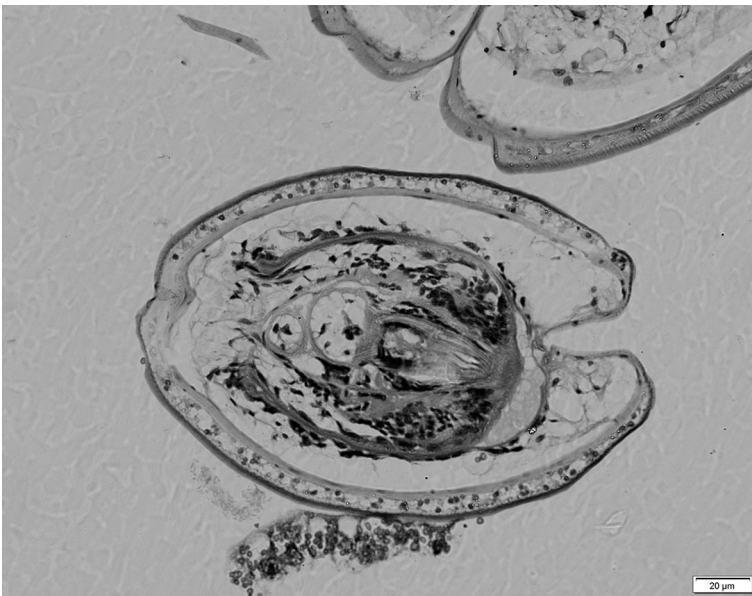The thin wall consists of three layers.

Figure 6. Histological section of a small *Flamingolepis* cysticercoid.
Compared to the total size of the cysticercoid, its wall is relatively wide

Table 2. Metrical data (in µm) of large Flamingolepis cyticercoids from own examination and literature data

Host	Cyst		Cercomer	Scolex		Suckers		Rostellum		Rost. hooks	Reference
	length	width	length	length	width	length	width	length	width	length	
A. salina	430	350	600	not given	not given	not given	not given	not given	not given	180	Heldt (1926)
A. salina	418	287	not given	254	164	121	62	254	62	184	Maksimova (1973)
Artemia sp.	470	320	800	340		120	120	450		170–180	Gabrion et al. (1982) Robert & Gabrion (1991)
Artemia sp.	434–488	281–314	800	317–330	244–256	152–163	97	280–305	122–134	175–185	Armat et al. 1991
A. parthenogenetica	671* (560–810)	479* (372–597)	710* (620–810)	614* (358–771)	423* (339–490)	233* (181–288)		453* (446–485)	125* (116–149)	189* (186–201)	Georgiev et al. 2005
A. franciscana	456* (345–570)	298* (204–387)	not measured	306* (182–346)	176* (155–200)	110* (98–125)	76* (62–96)	287* (223–326)	92* (65–116)	178* (161–188)	this paper

* Average data, figures in parentheses give the range.

Table 3. Metrical data (in µm) of small Flamingolepis cyticercoids from own examination and literature data

Host	Cysticercoid		Scolex		Suckers		Rostellum		Rost. hooks	Reference
	length	width	length	width	length	width	length	width	length	
A. salina	180	130	96	84	40	42	not given	not given	53–54	Maksimova, 1973
Artemia sp.	200	120	100	120	50		50	not given	55–62	Robert, Gabrion, 1991
A. parthenogenetica	231* (168–270)	177* (126–207)	182* (141–225)	145* (108–183)	66* (45–75)		119* (108–180)	43* (33–48)	57* (55–61)	Georgiev et al., 2005
A. franciscana	184* (157–209)	140* (112–164)	100* (71–124)	95* (74–109)	43* (31–51)	35* (28–45)	100* (80–130)	32* (23–46)	55* (49–59)	this paper

* Average data, figures in parentheses give the range.

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Figure 7. PAS stain of a histological section of a large *Flamingolepis* cysticercoid show a dense concentration of calcareous bodies in the tissues surrounding the scolex.

Figure 8. Hatched scolex of a large *Flamingolepis* cysticercoid with a retracted rostellum after peptic digestion of the host and neutralization in PBS. Contrary to invaginated cysticercoids the suckers are round. The basal part of the rostellum reach deep into the neck region.

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Figure 9. Hatching scolex of a small *Flamingolepis* cysticercoid with an everted rostellum after peptic digestion of the host and neutralization in PBS.

Figure 10. *Flamingolepis* cysticercoid with 84 µm long rostellar hooks.

Brine shrimps of the genus *Artemia* act as intermediate host for a number of cestode species and cysticercoids of at least 16 different species were described in the literature. In publications on cestode larval stages in *Artemia* spp. from hypersaline inland waters in Asia (Kazakhstan and United Arab Emirates) and salters of the Mediterranean costs of southern Europe and northern Africa *Flamingolepis* cysticercoids with eight skrjabinoid rostellar hooks were reported. The prevalence and burdens of these cysticercoids in the current sample were extraordinary high. In a previous publication on *A. franciscana* in the Godolphin lakes (Sivakumar et al., 2018) it was shown that there were considerable variations in the cysticercoid prevalence throughout the annual cycle. When water temperatures in June exceed 35 °C brine shrimps die, leaving resistant metabolic inactive cysts behind. In connection with the refilling of the ponds and falling temperatures in autumn, cysts hatch and the artemias gain life again attracting large amounts of waders and shore birds that contaminate the habitat with cestode eggs. This resulted in a relatively high prevalence of 16 % already in November. Falling temperatures in winter reduced the activity and food intake of the crustaceans and led to lower prevalence rates in the following months. Rising temperatures in spring resulted again in increasing activity of the brine shrimps and subsequently increased cysticercoid prevalence. The highest prevalence rate of 70 % in that research was found in May. Out of a total of 1.840 *A. franciscana* examined over an 8 month's period, large and small *Flamingolepis* cysticercoids were present in 25.3 and 10.7 % respectively.

Spasskij and Spasskaja (1954) erected the genus *Flamingolepis* and used *F. liguloides* as type species. *F. liguloides* was mentioned for the first time as *Halysis liguloides* by Gervais (1847). The original description supported by a drawing describes the cestode as small (60 mm) and slender (2 mm), with a globose scolex bearing four suckers and a small rhynchus[2]. Lühe (1898) who examined intestinal parasites of greater flamingos in Tunisia recognized the same cestode species by the specific appearance of its strobili resembling the body of a ligula and added other essential morphological details like the unilateral situation of genital pores and diameter of suckers. The most important completion however, was the presence of eight slim and elongated rostellar hooks measuring 130 μm in length with blade and handle being 70 and 60 μm long, respectively. The maximum width of the hooks was 30 μm at the guard. Lühe (1898) described a further cestode, *Flamingolepis megalorchis* (Lühe, 1898), that consisted only of 30 to 40 segments and stroke because of three large testes. Rostellar hooks had the same shape as *F. liguloides* but measured only 90 μm. He also put *Flamingolepis caroli* (Parona, 1886) as junior synonym for *F. liguloides*. Cohn (1901) reexamined *F. liguloides* collected by Lühe (1898) and enhanced the description by a histological study of hermaphrodite segments. With a maximum length of 40 mm Lühe's cestodes were 2 cm shorter than described by Gervais (1847) and histological sections did not reveal any traces of a uterus, indicating their prepatent status. Based on these histological

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[2] Gervais (1847) description did not mention rostellar hooks. They most probably got lost during preparation. The main feature of the cestode was the ligula like strobila.

sections, Fuhrmann (1906) described the configuration of the testes which are arranged in the shape of a triangle (type III). Linstow (1906) published a description of helminths of the Colombo museum. Amongst them was a 75 mm long cestode from flamingo with short but relatively broad segments. The scolex carried eight slim 140 µm long hooks. It is worth mentioning that Shachtatinskaja (1952) mentioned a *Hymenolepis* species found in the flamingo in Azerbaidjan. The description and the pictured scolex[3] matched with *F. liguloides*.

In the collection of the Naturkundemuseum of Berlin there is a syntype (5226-E) of *Flamingolepis flamingo* (Skrjabin, 1914) from greater flamingo of unknown origin. According to Skrjabin (1914), the cestode was 18–25 mm long and up to 1 mm wide. The long rostellum (150 x 37 µm) was armed with eight hooks, 62 µm in length.

Scientists from Kazakhstan published the description of two further flamingo cestodes from Tengiz lake in Kazakhstan. *Flamingolepis dolgushini* Gvozdev and Maksimova, 1968 with a length of up to 10 mm and a maximum width of 0.96 mm had relatively large suckers of 170 µm in diameter and a rostellum armed with eight skrjabinoid hooks of 176–182 µm in length. Their blades measured 103 µm. The basal part of the rostellum reached deep into the neck region. The other species, *Flamingolepis tengizi* Gvozdev and Maksimova, 1968, measured only 5–6 mm with a maximum width of 1.2 mm. The rostellum was armed with eight skrjabinoid hooks in a length of 53 µm, length of the blade was 28 µm.

The most reliable features to allocate a cysticercoid to the adult cestode are number, shape and length of rostellar hooks. In this regard, it is difficult to understand why large cysticercoids were determined as *F. liguloides* by Gabrion et al. (1982).

The description of cysticercoids in this paper is based on a large number of examined cysticercoids. Georgiev et al. (2005) measured each 14 *Flamingolepis* cysticercoids of species they believed to belong to *F. liguloides* and *F. flamingo,* respectively. Other researchers gave only measurements not mentioning the number of examined specimens (Tabl. 2 and 3). Measurements of the large cysticercoids in the own material matched with data published by Heldt (1926), Maksimova (1973), Gabrion et al. (1982), Robert, Gabrion (1991) and Amat et al. (1991) while parameter established by Georgiev et al. (2005) exceeded those measurements. Measurements of the small cysticercoids coincide with those given by Maksimova (1973) for those of *F. tengizi* and by Robert and Gabrion (1991) and Georgiev et al. (2005) for those of *F. flamingo*.

Based on the size of rostellar hooks it can be excluded that large cysticercoids belong to *F. liguloides* and it is more likely that they are *F. dolgushini* while small cysticercoids are the larval stage of *F. flamingo* and *F. tengizi* has to be treated as its junior synonym. The two other *Flamingolepis* cysticercoids had rostellar hooks comparable to *F. megalorchis*.

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[3] The scolex is pictured in a book by Spasskaja (1966).

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О ЦИСТИЦЕРКОИДАХ РОДА *FLAMINGOLEPIS* SPASSKIJ & SPASSKAJA, 1954 (CESTODA: HYMENOLEPIDIDAE), ПАРАЗИТИРУЮЩИХ В *ARTEMIA FRANCISCANA* KELLOG, 1906 (ARTHROPODA: ARTEMIIDAE) В ДУБАЕ, ОБЪЕДИНЕННЫЕ АРАБСКИЕ ЭМИРАТЫ

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РЕЗЮМЕ

Исследование 300 особей *Artemia franciscana* Kellog, 1906 в Дубае выявило их необычайно высокую зараженность цистицеркоидами – 99 %. При этом, среди личинок цестод преобладали крупные и мелкие цистицеркоиды рода *Flamingolepis*. После переваривания ракообразных в искусственном желудочном соке было изолировано множество цистицеркоидов, из которых 120 крупных и 60 мелких цист были изучены в световом микроскопе. Часть материала была использована для гистологического исследования. В результате крупные цисты эллиптической формы размером 345–545 х 204–387 мкм с ростеллярными крючками 161–188 и лезвием 91–115 мкм были определены как *F. dolgushini*, а цисты размером 157–209 х 112–164 мкм с ростеллярными крючками 49–59 мкм и лезвием 25–33 мкм были приписаны *F. flamingo*. Материал содержал еще один вид, предположительно *F. megalorchis*, с ростеллярными крючками размером 82–84 мкм.