

# Records of cysticercoids of *Fimbriarioides tadornae* Maksimova, 1976 and *Branchiopodataenia gvozdevi* (Maksimova, 1988) (Cyclophyllidea, Hymenolepididae) from brine shrimps at the Mediterranean coasts of Spain and France, with a key to cestodes from *Artemia* spp. from the Western Mediterranean

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## Abstract

Cysticercoids of two cestode species isolated from brine shrimps from the Mediterranean coasts of Spain and France are described. *Fimbriarioides tadornae* Maksimova, 1976 (adults known as parasites of *Tadorna tadorna*) was recorded from *Artemia parthenogenetica* and *A. franciscana* from Spain (Bras del Port and River Ebro Delta, respectively), and from *A. franciscana* from Aigues-Mortes, France (new geographical record). *Branchiopodataenia gvozdevi* (Maksimova, 1988) (adults known as parasites of *Larus genei*) was recorded from *A. parthenogenetica* (Bras del Port, Spain), *A. salina* (San Pedro del Pinatar, Spain) and *A. franciscana* (River Ebro Delta, Spain); this is the first record of the species in Europe. An illustrated key to cysticercoids of 12 cestode species from *Artemia* spp. from the Western Mediterranean is proposed.

## Keywords

Hymenolepididae, cysticercoids, *Artemia*, Mediterranean, Iberian Peninsula

## Introduction

Brine shrimps of the genus *Artemia* Leach, 1819 (Crustacea, Branchiopoda) have been recorded as intermediate hosts of cestodes parasitising aquatic birds from the Western Mediterranean and the southern Atlantic coast of Spain and Portugal (Gabrion and MacDonald 1980; Thiéry *et al.* 1990; Robert and Gabrion 1991; Amat *et al.* 1991a, b; Mura 1994; Varó *et al.* 2000; Georgiev *et al.* 2005, 2007). Nine cestode species were identified from *Artemia parthenogenetica* Bowen et Sterling, *A. franciscana* Kellogg and *A. salina* (L.) from various localities of the Iberian Peninsula (Amat *et al.* 1991a, b; Varó *et al.* 2000; Georgiev *et al.* 2005, 2007; Sánchez *et al.* 2006a, b). Descriptions of cysticercoids of 8 species were presented in a previous paper (Georgiev *et al.* 2005). Recently, we examined

*Artemia* spp. from four localities on the Mediterranean coasts of Spain and France and found a further hymenolepidid species in the samples from Spain, i.e. *Branchiopodataenia gvozdevi* (Maksimova, 1988) (adults known as parasites of slender-billed gull *Larus genei* Brème). Cysticercoids of the hymenolepidid species *Fimbriarioides tadornae* Maksimova, 1976 (adults known as parasites of the shelducks *Tadorna tadorna*) were also found in samples from Spain and France. The aim of this paper is to present the morphological results of the examination of cysticercoids of *F. tadornae* and *B. gvozdevi* from brine shrimps from the Western Mediterranean (first geographical record for *B. gvozdevi*). In addition, a key to cysticercoids of 12 cestode species from *Artemia* spp. in the Western Mediterranean and the southern Atlantic coast of the Iberian Peninsula is provided.

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## Materials and methods

Samples of *Artemia parthenogenetica* from Bras del Port (Spain) and Aigues-Mortes (France), *A. franciscana* from the River Ebro Delta (Spain) and Aigues-Mortes, and *A. salina* from San Pedro del Pinatar (Spain), were collected in 2007–2008. The sampled brine shrimps were killed by heating to 80°C and preserved in 70% ethanol. They were mounted in temporary glycerol mounts and examined under a stereomicroscope or compound microscope after gentle pressure on the coverslip; after that, if needed, the cysticercoids were isolated and mounted in Berlese's medium in order to facilitate observations on the morphology of rostellar hooks. Some brine shrimps were studied unfixed under microscope for the presence of cestodes and some measurements were taken at this stage (indicated in the text). More detailed data on the brine shrimps studied are given in the text for each cestode species (see below).

Voucher specimens of cysticercoids found are deposited in the Parasitic Worms Collection of The Natural History Museum, London (BMNH).

The following descriptions present metrical data based on specimens mounted in glycerol unless otherwise stated. Metrical data are given as the range, with the mean and the number of measurements taken in parentheses. The measurements are in micrometres except where otherwise stated.

## Results

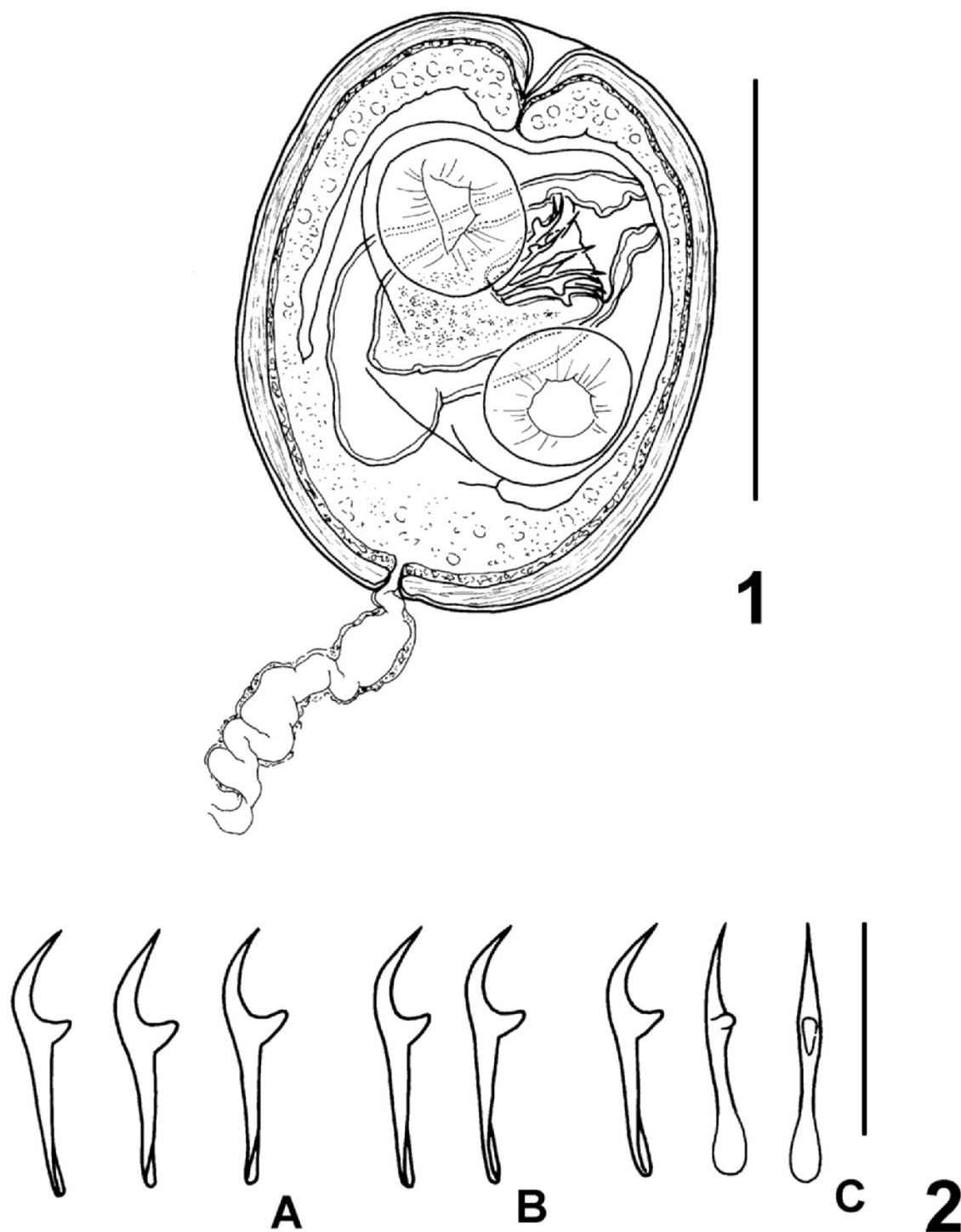
### *Fimbriarioides tadornae* Maksimova, 1976

*Specimens studied:* From *A. parthenogenetica* (diploid), Bras del Port, Spain, 31 October 2007: adult brine shrimps, two cysticercoids; juvenile brine shrimps, two cysticercoids; Aigues-Mortes, France, 30 July 2008: adult brine shrimps, 10 cysticercoids. From *Artemia franciscana*: River Ebro Delta, Spain: October 2007, adult brine shrimp, one cysticercoid; May 2008, adult brine shrimp, one cysticercoid; July 2008, adult brine shrimp, one cysticercoid; Aigues-Mortes, France, 30 July 2008: adult brine shrimps, 10 cysticercoids.

*Redescription* (based on cysticercoids from *A. parthenogenetica*, Bras del Port; for metrical data see Table I): Cyst oval to lemon-shaped, with thick wall consisting of three layers (Fig. 1); when mounted in Berlese's medium, cysts becoming almost round, with measurements 154–175 × 139–154 (164 × 147, n = 3). Scolex almost round, with maximum width at level of suckers. Suckers oval, muscular. Rostellar sheath deep, thin-walled. Rostellum with apical enlargement, conically-tapering posterior part and invaginable anterior part. When rostellar withdrawn, blades of rostellar hooks directed anteriorly. Rostellar hooks 10 in number, diorchoid, with elongate handle ending with flatten enlarged portion; blade 9–10 (9, n = 12) long, sickle-shaped; distance between handle-tip and guard-tip 15–17 (16, n = 12), distance between blade-tip and guard-tip 9–10 (9, n = 12) (Fig. 2). Entire cercomer not isolated; length of cercomer exceeds 720 (judged from fragments); width variable 8–13.

Table I. Metrical data of cysticercoids of *Fimbriarioides tadornae* Maksimova, 1976 from *Artemia* spp.

Hosts	<i>A. salina</i>		<i>A. parthenogenetica</i>		<i>A. franciscana</i>		<i>A. parthenogenetica</i>		<i>A. franciscana</i>				
	Lake Tengiz (Kazakhstan)	Maksimova (1976)	Bras del Port (Spain)	River Ebro Delta (Spain)	Aigues Mortes (France)	Aigues Mortes (France)	Aigues Mortes (France)	Aigues Mortes (France)	Aigues Mortes (France)	Aigues Mortes (France)			
Source	range	range	present study	present study	present study	present study	present study	present study	present study	present study			
Cyst	length	164	126–146	138	4	118–159	–	114–158	135	10	114–158	135	10
	width	144	108–121	115	4	95–145	–	88–138	114	10	90–126	111	10
Scolex	length	108	85–98	91	4	59–105	–	79–114	98	10	84–110	96	10
	width	85	85–93	89	4	72–84	–	70–114	91	10	67–96	79	10
Suckers	diameter	50	33–39	36	16	33–37	35	32–40	35	28	33–39	36	25
	length	96	90–112	99	4	88	–	70–96	82	10	75–90	83	10
Rostellar sheath	length	30	41–46	44	4	47	–	35–47	42	10	35–56	42	10
	width	80	44–54	48	4	44–46	–	39–53	45	10	44–53	47	8
Rostellum	length	24	31–34	32	4	28–37	–	30–40	33	10	26–35	30	8
	width	28	24–26	25	12	25–27	26	25–27	26	19	25–27	26	10

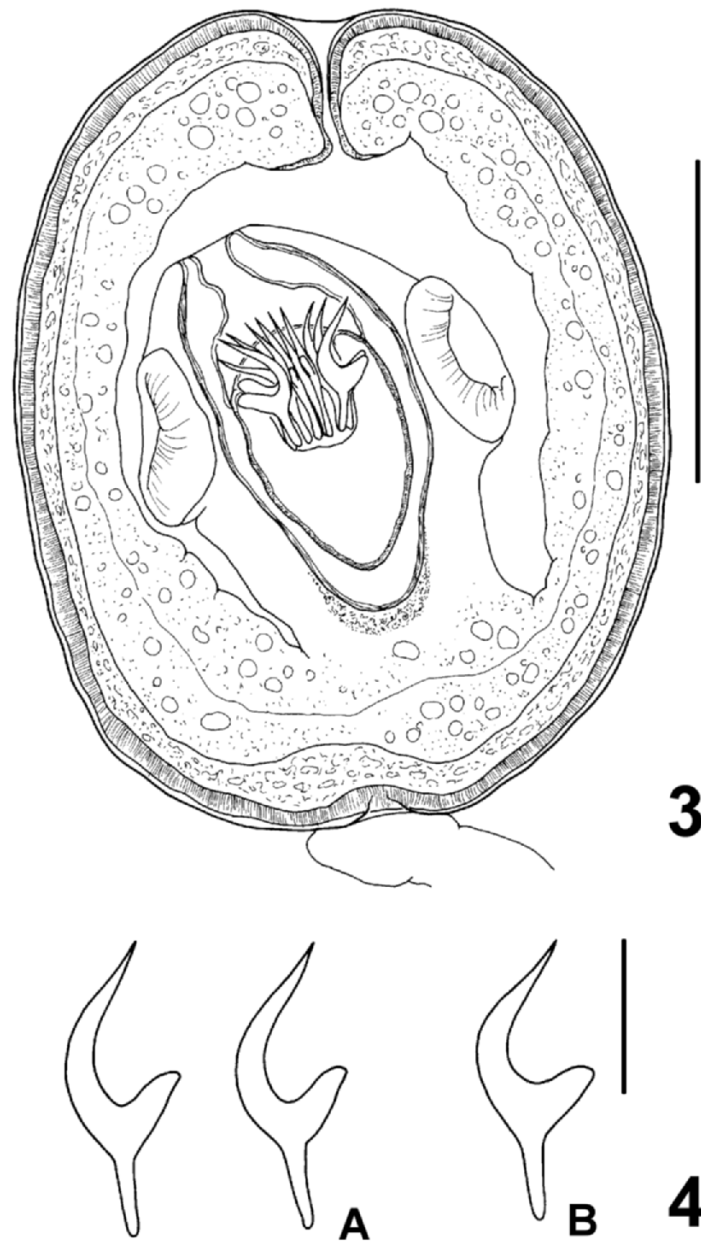


**Figs 1 and 2.** *Fimbriarioides tadornae* Maksimova, 1976 from *Artemia parthenogenetica*, Bras del Port, Spain (**1, 2A**); *A. franciscana*, River Ebro Delta, Spain (**2B**) and *A. franciscana*, Aigues-Mortes, France (**2C**). **1.** Cysticercoid. **2.** Rostellar hooks of cysticercoids. Scale-bars = 100  $\mu\text{m}$  (1), 20  $\mu\text{m}$  (2)

Additional measurements of cysticercoids from *A. franciscana* from the River Ebro Delta, and *A. franciscana* (Fig. 5) and *A. parthenogenetica* from Aigues-Mortes are presented in the Table I.

*Remarks:* The original description of this species was based on both adult cestodes from *Tadorna tadorna* (L.) and cys-

ticercoids that developed experimentally in '*Artemia salina*' from Tengiz Lake, Kazakhstan (Maksimova 1976). The present redescription corresponds with the metrical and morphological data of Maksimova (1976), with a few exceptions (see Table I). The specimens from *A. salina* from Kazakhstan have slightly longer hooks 27–28 (vs 24–26 or 25–27 in specimens



**Figs 3 and 4.** *Branchiopoddataenia gvozdevi* (Maksimova, 1988) Bondarenko et Kontrimavichus, 2004 from *Artemia parthenogenetica*, Bras del Port, Spain (**3, 4A**) and *A. franciscana*, River Ebro Delta, Spain (**4B**). **3.** Cysticercoid. **4.** Rostellar hooks of cysticercoids. Scale-bars = 100  $\mu\text{m}$  (3), 20  $\mu\text{m}$  (4)

from Spain and France) and a longer rostellum. In our material, there are withdrawn rostellum only and this might be the reason for the difference in the rostellar measurements. The metrical data of the specimens from the two species of brine shrimps and the various localities are within the limits of intraspecific variation.

Until now, *F. tadornae* has been recorded in Kazakhstan (Maksimova 1976), Ukraine (Kornyushin 1969) as '*Fimbriarioides intermedia* (Fuhrmann, 1913)' (re-identification proposed by Maksimova 1976) and Spain from *A. parthenogenetica* in the Odiel Saltpans (Georgiev *et al.* 2007). The present result is the first record of *F. tadornae* from France.

***Branchiopoddataenia gvozdevi* (Maksimova, 1988) Bondarenko et Kontrimavichus, 2004**

*Specimens studied:* From *A. parthenogenetica* (diploid), Bras del Port, Spain, 24 July 2007, five cysticercoids; 31 October 2007, two cysticercoids. From *A. salina*, San Pedro del Pinatar, Spain, 7 February 2008, 30 cysticercoids. From *A. franciscana*, River Ebro Delta, Spain, 30 April 2008, one cysticercoid; 23 May 2008, one cysticercoid.

*Redescription* (based on cysticercoids from *A. parthenogenetica*, Bras del Port; for metrical data see Table II): Cyst oval to lemon-shaped, with very thick wall (Figs 3, 6); mea-



**Table II.** Metrical data of cysticercoids of *Branchiopodataenia gvozdevi* (Maksimova, 1988) Bondarenko et Kontrimavichus, 2004 from *Artemia* spp.

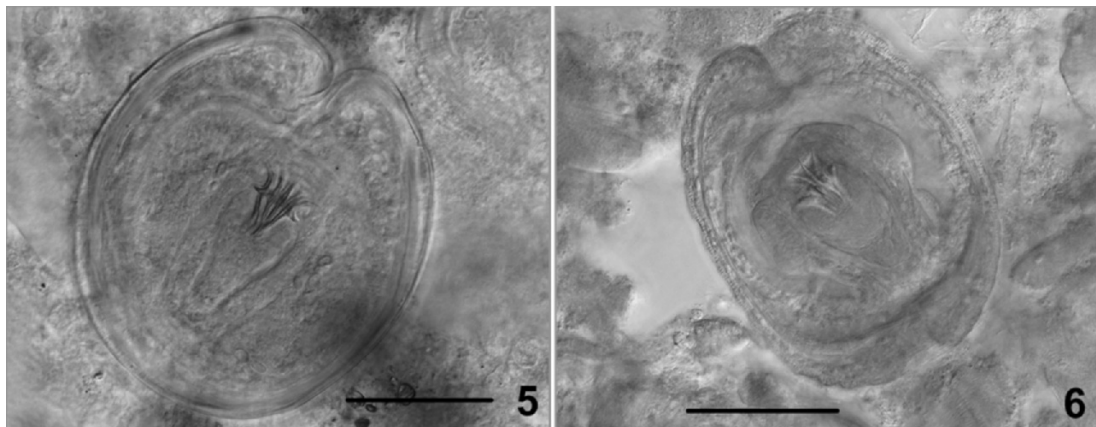
Hosts		<i>A. salina</i>	<i>A. parthenogenetica</i>			<i>A. franciscana</i>		
Locality		Lake Tengiz (Kazakhstan)	Bras del Port (Spain)			River Ebro Delta (Spain)		
Source		Maksimova (1988)	present study		present study			
		range	range	mean	n	range	mean	n
<b>Cyst</b>	length	270–320	218–318	260	6	284–295	–	2
	width	150–200	170–254	201	6	175	–	2
<b>Scolex</b>	length	151–160	123–141	132	5	147–149	–	2
	width	84–120	116–134	122	5	114–131	–	2
<b>Suckers</b>	diameter	60–65	46–57	51	22	40–53	46	4
<b>Rostellar sheath</b>	length	134–150	93–116	105	5	105–126	–	2
	width	58–60	46–67	59	5	63–79	–	2
<b>Rostellum</b>	length	84–140	54–62	57	5	70	–	2
	width	38–50	44–51	49	5	44	–	2
<b>Rostellar hooks</b>	length	42	38–40	39	9	39–40	39	10

measurements of cysts mounted in Berlese's medium 299–384 × 221–241 (325 × 232, n = 4). Scolex with short, conically tapering rostrum; measurements of a scolex with everted rostellum 190 × 93. Suckers large, oval, muscular, unarmed. Rostellar sheath deep, thick-walled, crossing level of posterior margins of suckers. Rostellum with apical enlargement, conically-tapering posterior part and invaginable anterior part; measurements of partly everted rostellum 69 × 41 (n = 1). When rostellum withdrawn, blades of rostellar hooks directed anteriorly. Rostellar hooks 10 in number, almost aploparaksoid in shape, blade straight, 20–23 (21, n = 9) long, shorter handle and distinct guard (Fig. 4); distance between handle-tip and guard-tip 21–23 (22, n = 9), distance between blade-tip and guard-tip 17–20 (18, n = 9). Entire cercomer not isolated; length of cercomer exceeds 400 (judged from fragments), width 25–39.

Additional measurements: Unfixed cysticercoids from *A. salina*, San Pedro del Pinatar – cyst length 274–395 × 181–

248 (325 × 210, n = 30), length of cercomer 1.08–3.60 mm (2.30, n = 30), diameter of cercomer 40–80 (53, n = 15). Additional measurements of two cysticercoids from *A. franciscana* from the River Ebro Delta are presented in Table II.

*Remarks:* The species was described by Maksimova (1988) on the basis of adult cestodes from *Larus genei* Brème and cysticercoids isolated from 'A. salina' (both naturally and experimentally infected) from Tengiz Lake, Kazakhstan. The present results correspond with the morphology of the cysts and the metrical data of Maksimova (1988) (see Table II), with a few exceptions, which are within the limits of intraspecific variability. The rostellum of the cysts from Kazakhstan is longer (84–140 µm) compared to 54–62 µm (withdrawn rostellum) and 69 µm (a single protruded rostellum) in the material from Spain. In addition, Maksimova (1988) considered the cercomer as rather short (1.21–1.43 mm); our measurements of cercomers of unfixed cysts show that it is highly elongate and, when it is not coiled, it can extend up to 3.60 mm.



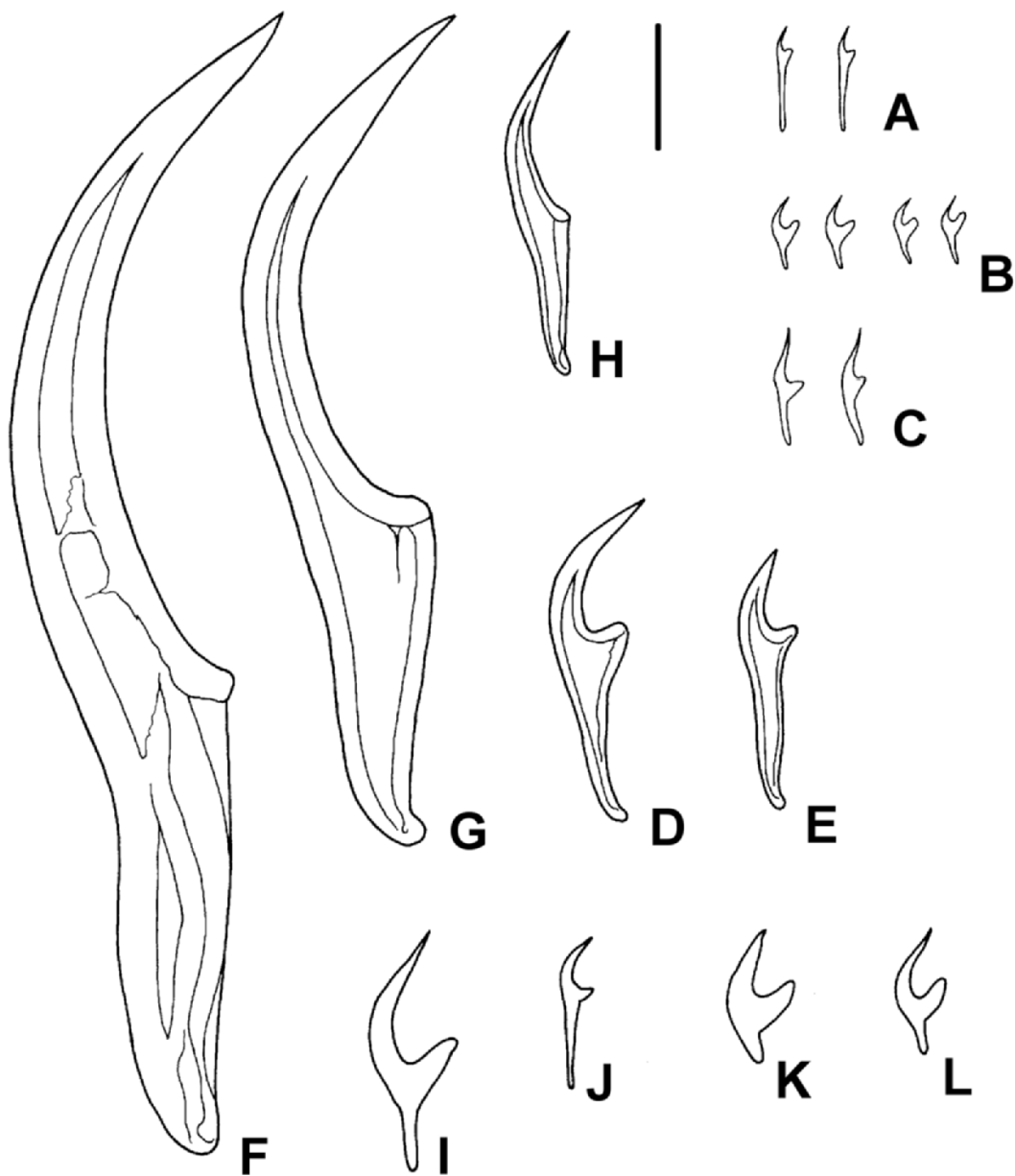
**Figs 5 and 6.** *Fimbriarioides tadornae* Maksimova, 1976 from *Artemia franciscana*, Aigues-Mortes, France (5). *Branchiopodataenia gvozdevi* (Maksimova, 1988) Bondarenko et Kontrimavichus, 2004 from *Artemia parthenogenetica*, Bras del Port, Spain (6). Scale-bars = 50 µm (5), 100 µm (6)

The species has been described as a member of the genus *Wardium* Mayhew, 1925 and then transferred in *Branchiopodaena* Bondarenko et Kontrimavichus, 2004 on the basis of its aploparaksoid rostellar hooks, the life cycle including branchiopod crustaceans as intermediate hosts and the peculiar 'latch-like' structure of the copulatory part of the vagina (Bondarenko and Kontrimavichus 2004).

Until now, *B. gvozdevi* was known from its original hosts (both definitive and intermediate) and from the type locality only. The present report from Spain is the first European record of this species.

## Discussion

Until now, 13 cyclophyllidean cestode species have been reported to use brine shrimps of the genus *Artemia* as intermediate host in their life cycles (Georgiev *et al.* 2005). The observations on populations of *A. parthenogenetica*, *A. franciscana* and *A. salina* from various localities of the Western Mediterranean region and the southern Atlantic coast of Spain and Portugal revealed the presence of cysticercoids of twelve cestodes species (Robert and Gabrion 1991; Georgiev *et al.* 2005, 2007; present study). They are seven



**Fig. 7.** Rostellar hooks of twelve cestode species from *Artemia* spp. from the Western Mediterranean and the southern Atlantic coast of the Iberian Peninsula: **A** – *Eurycestus avoceti*; **B** – *Anomotaenia microphallos*; **C** – *Anomotaenia tringae*; **D** – *Gynandrotaenia* sp.; **E** – *Gynandrotaenia stammeri*; **F** – *Flamingolepis liguloides*; **G** – *Flamingolepis caroli*; **H** – *Flamingolepis flamingo*; **I** – *Branchiopodaena gvozdevi*; **J** – *Fimbriarioides tadornae*; **K** – *Confluaria podicipina*; **L** – *Wardium stellorae*. Scale-bar = 20  $\mu$ m

species of Hymenolepididae, i.e. *Confluaria podicipina* (Szymanski, 1905), *Flamingolepis liguloides* (Gervais, 1847), *F. flamingo* (Skrjabin, 1914), *F. caroli* (Parona, 1887), *Wardium stellorae* (Deblock, Biguet et Capron, 1960), *Branchiopoddataenia gvozdevi* and *Fimbriarioides tadornae*, three species of Dilepididae, i.e. *Eurycestus avoceti* Clark, 1954, *Anomotaenia microphallos* (Krabbe, 1869) and *A. tringae* (Burt, 1940), and two species of Progynotaeniidae, *Gynandrotænia stammeri* Fuhrmann, 1936 and *Gynandrotænia* sp. Cysticercoids of the later species were found in *Artemia franciscana* sampled from the River Ebro Delta during the present study. The observations on the cysticercoid of *Gynandrotænia* sp. indicated that it differs significantly from that of *G. stammeri* in the shape and length of the rostellar hooks (see Fig. 7D, E) and probably belongs to another species. The elucidation of its status requires additional studies and comparisons with adult specimens and will be discussed elsewhere (Nikolov *et al.*, in preparation).

The detailed observations on the morphology of the cysticercoids from *Artemia* spp. exhibit several features, which are of great importance for distinguishing cestode larvae parasitising brine shrimps, i.e. the presence of outer larval envelope, the modifications of the cercomer, the armaments of the suckers and the number, shape and length of the rostellar hooks (Fig. 7). On this basis, we propose the following identification key to the cestode species parasitising *Artemia* spp. from the Western Mediterranean and the southern Atlantic coast of Spain and Portugal.

#### Key to the cestode species from *Artemia* spp. on the Iberian Peninsula and in the Western Palearctic

- 1a. Cyst surrounded by outer brownish envelope formed by the modified cercomer; no tail-shaped cercomer (i.e. monocysticercoids) ..... 2
- 1b. Cyst usually not surrounded by outer envelope, cercomer tail-shaped (i.e. cercocysticercoid); if outer envelope present, then its posterior end continues as a long tail-shaped appendage (i.e. caudate diplocyst) ..... 4
- 2a. Suckers armed with fine hooklets; rostellar hooks 14–16 in number ..... *Eurycestus avoceti*
- 2b. Suckers without armament; rostellar hooks more than 16 in number ..... 3
- 3a. Hooks 18–20 in number, in two rows, 19–21 µm long ..... *Anomotaenia tringae*
- 3b. Hooks more than 20 in number (26–30), in irregular crown, 11–13 µm long ..... *Anomotaenia microphallos*
- 4a. Hooks 6 in number; margins of suckers armed with fine spines ..... 5
- 4b. Hooks more than 6 in number; suckers unarmed ..... 6
- 5a. Hooks shorter than 45 µm (40–43 µm), blade straight ..... *Gynandrotænia stammeri*
- 5b. Hooks longer than 45 µm (50–52 µm), blade curved ..... *Gynandrotænia* sp.
- 6a. Hooks 8 in number, skrjabinoid, i.e. with long blade, well-developed handle (shorter than blade) and rudimentary guard ..... 7
- 6b. Hooks 10 in number, with other shape and more or less well-developed guard ..... 9
- 7a. Length of hooks more than 180 µm (186–201); length of cercomer compatible with cyst-length ..... *Flamingolepis liguloides*
- 7b. Length of hooks less than 150 µm; cercomer highly elongate ..... 8
- 8a. Length of hooks 110–130 µm ..... *Flamingolepis caroli*
- 8b. Length of hooks 55–61 µm ..... *Flamingolepis flamingo*
- 9a. Hooks more than or equal to 38 µm long ..... *Branchiopoddataenia gvozdevi*
- 9b. Hooks less 38 µm long ..... 10
- 10a. Hooks aploparaksoïd, with well-developed blade and guard, and handle shorter than blade ..... 11
- 10b. Hooks diorchoid, i.e. handle longer than blade, guard short but distinct ..... *Fimbriarioides tadornae*
- 11a. Cyst surrounded by additional envelope formed by anterior part of cercomer; posterior part of cercomer highly coiled, densely packed in thin membranous envelope ..... *Confluaria podicipina*
- 11b. Cyst thin-walled, without additional envelopes; cercomer elongate ..... *Wardium stellorae*

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