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Cestodes from *Artemia parthenogenetica* (Crustacea, Branchiopoda) in the Odiel Marshes, Spain: A systematic survey of cysticercoids

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Abstract

A total of 3,300 specimens of brine shrimps *Artemia parthenogenetica* from the Odiel Marshes, Huelva Province, SW Spain, were studied during several seasons of 2002 and 2003 for the presence of cestode infections. Cysticercoids were found in 26.8% of brine shrimps. Eight cestode species were recorded, i.e., Hymenolepididae: *Flamingolepis liguloides* (adults parasitic in flamingos) with prevalence (P) 18.5%, mean intensity (MI) 1.48 and mean abundance (MA) 0.28; *F. flamingo* (adults parasitic in flamingos), P 0.9%, MI 1.03, MA 0.01; *Confluaria podicipina* (adults parasitic in grebes), P 6.5%, MI 1.42, MA 0.09; *Wardium stellorae* (adults parasitic in gulls), P 0.2%, MI 1.00, MA 0.002; Dilepididae: *Eurycestus avoceti* (adults parasitic in waders and flamingos), P 2.7%, MI 1.08, MA 0.03; *Anomotaenia* sp., probably *A. microphallos* (adults parasitic in waders), P 0.8%, MI 1.04, MA 0.01; *A. tringae* (adults parasitic in waders), P 2.2%, MI 1.01, MA 0.02; Progynotaeniidae: *Gynandrotaenia stammeri* (adults parasitic in flamingos), P 0.6%, MI 1.00, MA 0.01. The cysticercoids are described and accompanying illustrations are presented. This study provides the first record of *Anomotaenia tringae* in an intermediate host and the first records of *C. podicipina*, *E. avoceti*, *A. tringae* and *G. stammeri* in Spain.

Key words

Cyclophyllidea, cysticercoids, life cycles, *Artemia*, intermediate host, Spain

Introduction

Previous studies revealed the role of brine shrimps of the genus *Artemia* Leach, 1819 (Crustacea, Branchiopoda) as intermediate hosts in the life cycles of cestodes parasitising aquatic birds. The most detailed studies were carried out at Tengiz Lake, Kazakhstan (Maksimova 1973, 1976, 1977, 1981, 1986–1989, 1991; Gvozdev and Maksimova 1979) and in the Camargue, France (Gabrion and MacDonald 1980, Thiéry *et al*. 1990, Robert and Gabrion 1991); in those places, cysticercoids of 11 and 6 avian cestode species, respectively, were found in brine shrimps. In Spain, only two cestode species, *Flamingolepis liguloides* (Gervais, 1874) and *Wardium stellorae* (Deblock, Biguet et Capron, 1960), were found in *Artemia* at the Mediterranean coast (Amat *et al*. 1991a, b; Varó *et al*. 2000).

The present study was carried out at one of the largest saltworks in Spain, which is also a site of major importance for waterbirds (Bernués 1998, Sánchez *et al*. in press). It is situated at the estuary of the Odiel and Tinto Rivers, Huelva Province, SW Spain, near the Atlantic coast of Andalusia. The aim of this article is to characterise the species composition of the avian cestodes utilising crustaceans of the genus *Artemia* as intermediate hosts in the Odiel Marshes. It also provides the taxonomic framework for ecological studies being carried out on brine shrimps and their cestode parasites (results to be reported elsewhere).

Materials and methods

The identification of the brine shrimps was based on the work by Abatzopoulos *et al.* (2002) and recent studies at the same site (Amat *et al.* 2005). In the Mediterranean Region, the native species of *Artemia* are the bisexual *A. salina* Leach, 1819 (synonym *A. tunisiana* Bowen et Sterling, 1978) togeth-

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er with a heterogeneous group of parthenogenetic populations known under the binomen *A. parthenogenetica* Bowen et Sterling, 1978 (see Abatzopoulos *et al*. 2002). *A. parthenogenetica* only has been recorded in the Odiel Saltworks (Amat *et al*. 2005).

Samples, each containing 500 specimens of brine shrimps, were collected from an evaporation pond (E-18) at the Odiel Saltworks in October and December of 2002 and in February, April, June and August of 2003. In addition, a sample of 200 specimens collected in October, 2002 and a sample of 100 specimens collected in November, 2003 were examined. In total, 3,300 specimens of brine shrimps were studied for the presence of cysticercoids. The collected 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. If the identification of the cysticercoids recorded was not possible at this stage, whole infected brine shrimps or isolated cysticercoids were prepared as permanent mounts in Berlese's medium in order to facilitate observations on the morphology of rostellar hooks. Data on the prevalence, intensity and abundance are based on these samples.

Another sample of 380 specimens collected from the same and neighbouring ponds (E-11, E-18 and E-17) were examined in July, 2004 in order to provide further information on the morphology of the cysticercoids, so as to complete that gathered on the basis of the fixed materials. The brine shrimps were examined alive under a stereomicroscope and a compound microscope. After observations of the cysticercoids *in situ*, each infected specimen was gently pressed under the coverslip and the observations continued on isolated cysticercoids. These were carried out initially in hypertonic conditions (in water from ponds) and later in hypotonic conditions (in tap water). The latter was found to provide longer survival of the cysticercoids (for about 1 hour) *ex situ* and, in some cases (*Eurycestus avoceti*, *Anomotaenia microphallos* and *Gynandrotaenia stammeri*), to provoke their excystation.

In July, 2004, samples of other invertebrates abundant in the same ponds were examined. These were adult coleopterans of the families Hydraenidae (*Ochthebius corrugatus* Rosenhauer, 1856 – 23 specimens and *O. notabilis* Rosenhauer, 1856 – 55 specimens) and Dytiscidae [*Nebrioporus ceresyi* (Aubé, 1836) – 70 specimens], chironomid larvae tentatively identified as *Chironomus salinarius* Kieffer in Thienemann, 1915 – 424 specimens, and harpacticoid copepods *Cletocamptus retrogressus* Shmankevich, 1875 – 160 specimens.

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

The metrical data in the following descriptions are based on specimens mounted in Berlese's medium only. They 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. The infection descriptors (prevalence, intensity and abundance) are after the definitions by Bush *et al*. (1997).

Results

Infection characteristics

Of 3,300 specimens of *Artemia* studied, 886 specimens (26.8%) were infected with cysticercoids. The intensity of the infection was 1–13 cysticercoids. The total values of the mean abundance and the mean intensity were 1.71 and 0.44, respectively. Cysticercoids of 8 cyclophyllidean species belonging to three families were recorded in the brine shrimps examined. Among them, *Flamingolepis liguloides* was the most prevalent and abundant species (Table I).

The majority of the infected brine shrimps contained cysticercoids of one cestode species; 171 specimens (19.3% of

Table I. Infection of brine shrimps with cysticercoids in the Odiel Marshes, Spain

Cestode species	Prevalence (%)	Intensity		Mean abundance ± SD
		range	mean ± SD	
Hymenolepididae				
Flamingolepis liguloides	18.5	1–8	1.48 ± 0.89	0.28 ± 0.01
Flamingolepis flamingo	0.9	1–2	1.03 ± 0.18	0.01 ± 0.10
Confluaria podicipina	6.5	1–7	1.42 ± 0.82	0.09 ± 0.40
Wardium stellorae	0.2	1–1	1.00 ± 0.00	0.002 ± 0.043
Dilepididae				
Eurycestus avoceti	2.7	1–3	1.08 ± 0.31	0.03 ± 0.18
Anomotaenia sp. (cf. *A. microphallos*)	0.8	1–2	1.04 ± 0.19	0.01 ± 0.10
Anomotaenia tringae	2.2	1–2	1.01 ± 0.12	0.02 ± 0.15
Progynotaeniidae				
Gynandrotaenia stammeri	0.6	1–1	1.00 ± 0.00	0.01 ± 0.08
Cysticercoids (total)	26.8	1–13	1.71 ± 1.28	0.44 ± 0.99

Table II. Multiple species infections of brine shrimps with cysticercoids in the Odiel Marshes, Spain

Cestode species	Number of cestode species participating in the multiple infection																														
	2																	3									4			5
Flamingolepis liguloides	+	+	+	+	+	+													+	+	+	+	+	+	+	+	+	+	+	+	+
Flamingolepis flamingo	+						+	+	+	+	+								+	+	+	+						+			
Confluaria podicipina		+					+					+	+	+	+	+			+				+	+	+	+		+	+	+	+
Wardium stellorae												+					+														+
Eurycestus avoceti			+					+					+					+		+			+				+		+	+	+
Anomotaenia sp. (cf. *A. microphallos*)				+					+					+							+			+					+		
Anomotaenia tringae					+					+					+		+	+							+					+	+
Gynandrotaenia stammeri						+					+					+						+				+	+	+			
No. of specimens of *Artemia* infected by this combination of species	10	41	29	3	7	8	1	1	1	1	1	2	3	3	19	2	1	2	5	2	1	1	7	8	3	4	1	1	1	1	1

the infected shrimps) harboured more than one. Two cestode species were recorded from 135 shrimps, three species from 32 specimens, four species from three specimens and one shrimp contained cysticercoids of 5 tapeworm species (Table II).

No cysticercoids were recorded in other invertebrate species studied.

Systematic survey of cysticercoids

Family Hymenolepididae
Flamingolepis liguloides (Gervais, 1847)

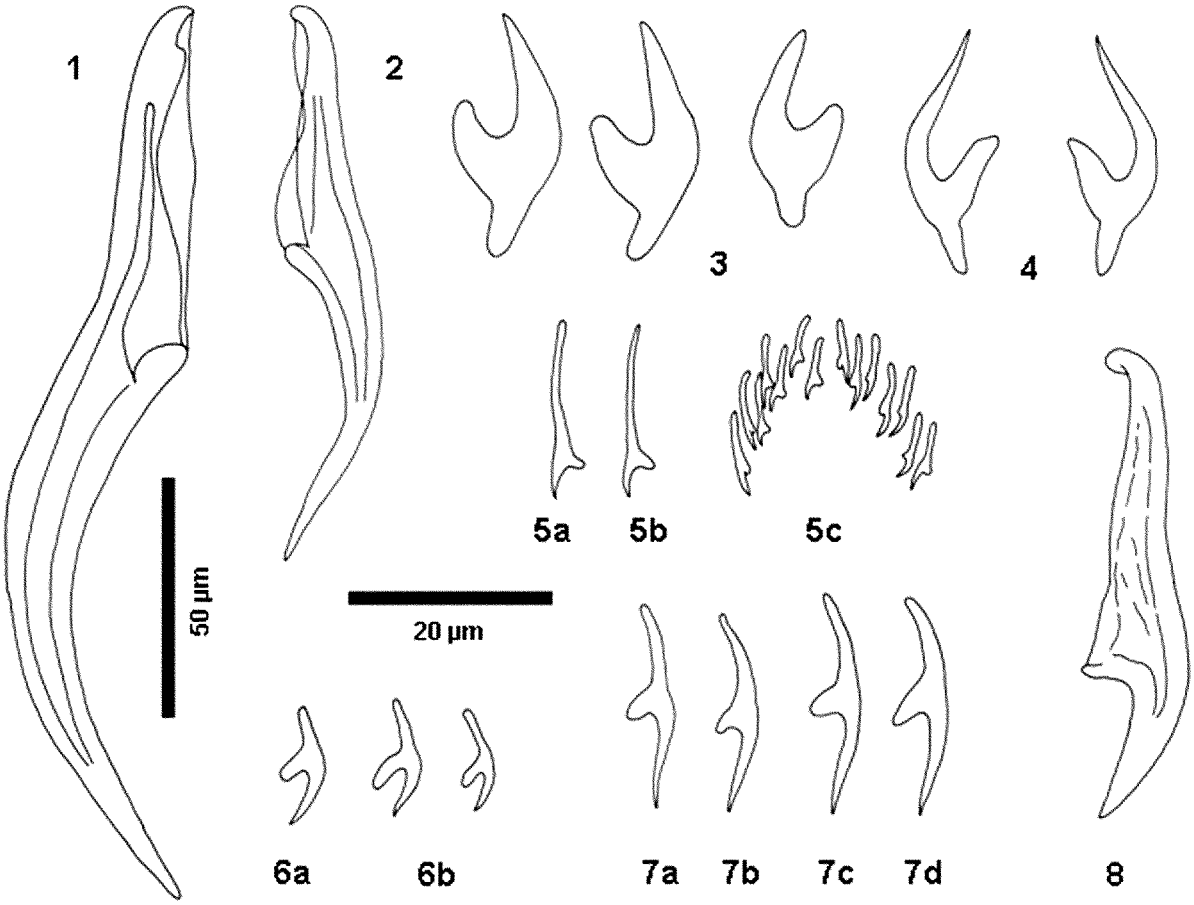
Description: Cyst oval (Figs 9 and 10), 560–810 × 372–597 (671 × 479, n = 14). Calcareous corpuscles numerous. Scolex 358–771 × 339–490 (614 × 423, n = 14). Suckers round or oval, with diameter 181–288 (233, n = 18). Rostellum elongate, 446–485 × 116–149 (465 × 125, n = 14), with strongly developed circular musculature. Rostellar hooks skrjabinoid, 8 (n = 15) in number, 186–201 (189, n = 15) long (Figs 1 and 11); length of blade 105–117 (110, n = 15). Ratio of length of blade/total hook length 0.56–0.60 (0.58, n = 15). When rostellum contracted, blades of hooks are posteriorly directed. Cercomer elongate (Fig. 10), with length comparable with that of cyst, 620–810 × 45–68 (710 × 55, n = 8).

Voucher specimens: BMNH 2005.2.23.1-5 (5 slides).

Remarks: The present material corresponds to the cysticercoids from *Artemia* sp. identified by Robert and Gabrion (1991) as *F. liguloides*, and from *Artemia* sp. (originally identified as *A. salina*) and *Branchinella spinosa* (M. Milne-Edwards) reported by Maksimova (1973, 1989) as *F. dolguschini* Gvozdev et Maksimova, 1968. The taxonomy of *Flamingolepis* Spasskii et Spasskaya, 1954 is poorly known. Amat *et al*. (1991b) believed that *F. dolguschini* is a synonym of *F. liguloides*. However, the adult cestodes described by Maksimova (1989) under these two names clearly differed from each other. Nevertheless, the affiliation of these cysticercoids from France, Spain and Kazakhstan, all with 8 skrjabinoid hooks 180–190 µm long, to a single species is justified by their morphology. Pending a revision of the hymenolepidids specific to flamingos, we follow Robert and Gabrion (1991) and accept *F. liguloides* as the most probable identification of these cysticercoids.

Flamingolepis flamingo (Skrjabin, 1914)

Description: Cyst oval, thick-walled (Figs 12 and 13), 168–270 × 126–207 (231 × 177, n = 14). Calcareous corpuscles numerous, concentrated mostly in anterior part of cyst. Scolex 141–225 × 108–183 (182 × 145, n = 12). Suckers oval, with diameter 45–75 (66, n = 16). Rostellum highly elongate, with strong circular musculature, 108–180 × 33–48 (119 × 43, n = 5) when contracted or 177–225 × 33–39 (206 × 36, n = 3) when extended. Rostellar hooks skrjabinoid, 8 (n = 16) in number, 55–61 (57, n = 15) long (Figs 2 and 14); length of blade 28–30 (29, n = 15). Ratio of length of blade/total hook



Figs 1–8. Rostellar hooks and sucker hooklets of the cysticercoids found. **1.** *Flamingolepis liguloides*. **2.** *F. flamingo*. **3.** *Confluaria podicipina*. **4.** *Wardium stellorae*. **5.** *Eurycestus avoceti*: a – anterior hook, b – posterior hook, c – sucker hooklets. **6.** *Anomotaenia* sp., cf. *A. microphallos*: a – anterior hook, b – posterior hooks. **7.** *A. tringae*: a, b – rostellar hooks of a cysticercoid from a brine shrimp; c, d – rostellar hooks of a syntype specimen from *Tringa glareola* from Sri Lanka. **8.** *Gynandrotaenia stammeri*

length 0.47–0.53 (0.50, n = 15). When rostellum contracted, blades of hooks are posteriorly directed. Cercomer highly elongate (Figs 12 and 13), 6.5–7.4 mm (n = 3) long, 8–12 (10, n = 14) wide.

Voucher specimens: BMNH 2005.2.23.6-10 (5 slides).

Remarks: *F. flamingo* is a specific parasite of flamingos in Eurasia (Maksimova 1989). Robert and Gabrion (1991) found its cysticercoid in *Artemia* sp. in France. The present material is in agreement with their description.

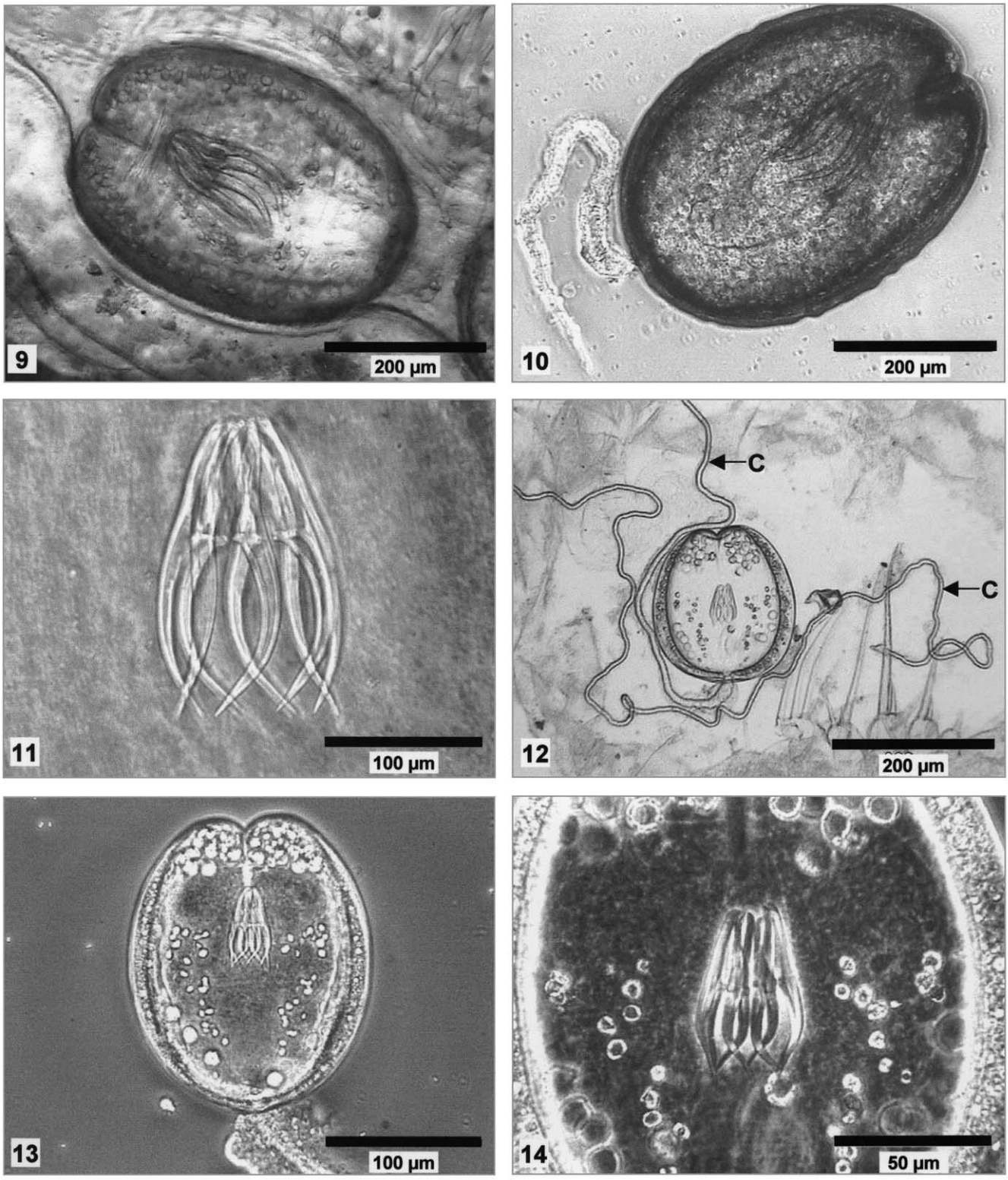
Confluaria podicipina (Szymanski, 1905)

Description: Cyst oval, thin-walled (Fig. 19), 93–147 × 47–87 (121 × 73, n = 12), often with distinct transverse striations. Calcareous corpuscles numerous, concentrated in anterior and in posterior part of cyst (Figs 18 and 19). Scolex 72–104 × 38–72 (92 × 59, n = 12). Suckers slightly oval, with diameter 26–32 (28, n = 10). Rostellum 42–47 (46, n = 7). Rostellar hooks 10 in number, aploparaksoid (Fig. 3), 21–24 (22, n = 12) long; when rostellum contracted, blades of hooks anteriorly directed. Cercomer very large. Anterior part of cercomer forms additional envelope surrounding cyst, thus forming external capsule (Figs 17 and 18) measuring 195–255 × 135–204 (215 × 152, n = 9). Posterior part of cercomer highly coiled, densely packed in thin membranous envelope (Fig. 16), which surrounds entire cysticercoid.

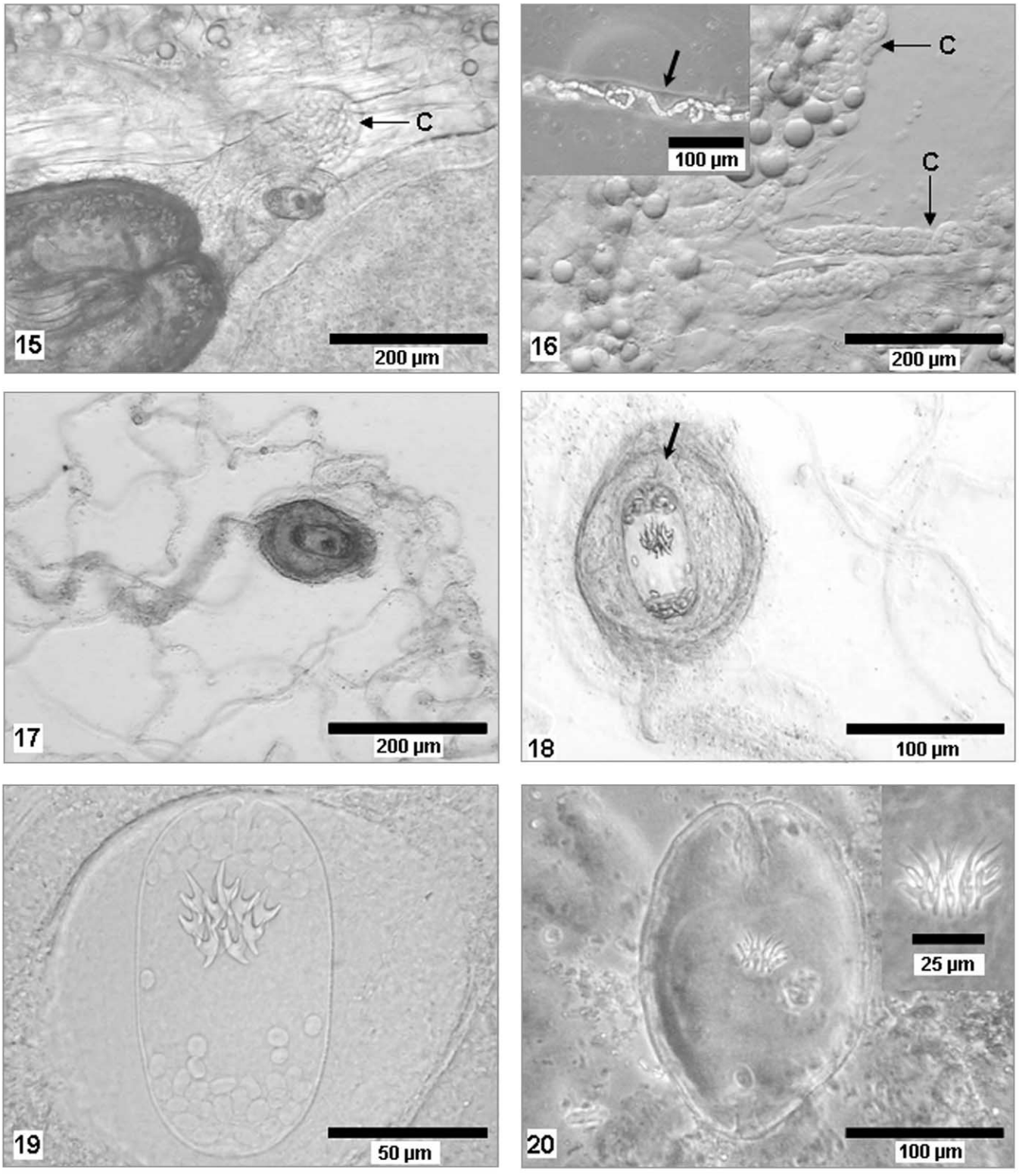
Voucher specimens: BMNH 2005.2.23.11-16 (6 slides).

Remarks: *C. podicipina* is a parasite of grebes (Podicipedidae) in the Holarctic Region (see Vasileva *et al*. 2000 for a survey). Its cysticercoid was described from *Artemia* in Kazakhstan (Maksimova 1981, 1989). We were not able to measure the length of the cercomer exactly because of its coiled configuration; however, it seems to be close to the length reported by Maksimova (1981), i.e. 14.5–16.5 cm. We also confirm that the cysticercoid rostellar hook of this species corresponds to the hook part referred to as the 'refractive particle' (Vasileva *et al*. 2000) or 'tip hooklet' (Maksimova 1981, 1989) of adults, and therefore that hooks do grow in the final host.

Our observations on living specimens lead us to an interpretation of the structure of the cysticercoid differing from that by Maksimova (1981). She believed that the external wall of the cyst consisted of 6 layers. In contrast, we consider that



Figs 9–14. *Flamingolepis* spp. **9–11.** *F. liguloides*: **9.** Cysticercoid *in situ*. **10.** General view of isolated cysticercoid, phase contrast. **11.** Rostellar hooks. **12–14.** *F. flamingo*: **12.** General view of isolated cysticercoid (C – cercomer). **13.** Cyst, phase contrast. **14.** Rostellar hooks, phase contrast



Figs 15–20. *Confluaria podicipina* and *Wardium stellorae*. **15–19.** *C. podicipina*: **15.** Cysticercoid *in situ*; compare the size with that of the cysticercoid of *F. liguloides* (C – cercomer). **16.** Highly coiled and densely packed cercomer (C) in the body cavity of the host. Inset: Portion of the cercomer, phase contrast. Note the thin membranous envelope (arrow). **17.** Isolated cysticercoid. **18.** Isolated cysticercoid under hypotonic conditions. Note the anterior orifice of the external capsule (arrow). **19.** Cysticercoid, mounted in Berlese's medium. **20.** *W. stellorae*, general view of the cysticercoid, mounted in Berlese's medium, phase contrast. Inset: Rostellar hooks, phase contrast

only the 'internal cyst' is homologous to the cysts of other hymenolepidid cysticercoids, while the external 3 layers described by Maksimova (1981) are a modification of the anterior part of the cercomer, forming an additional protective envelope surrounding the cyst. When placed in hypotonic conditions, the real cyst is readily detached from the bottom of the cavity formed by the anterior part of the cercomer (Figs 18 and 19); simultaneously, a distinct orifice can be seen at the anterior end of the 'external cyst' (Fig. 18).

Wardium stellorae (Deblock, Biguet et Capron, 1960)

Syns: *Hymenolepis stellorae* Deblock, Biguet et Capron, 1960; *Aploparaksis parafilum* Gąsowska, 1932 *sensu* Maksimova (1973).

Description: Cyst oval to lemon-shaped (Fig. 20), 189–213 × 153–189 (199 × 166, n = 5). Scolex 123–132 × 105–123 (126 × 111, n = 5). Rostellum oval, 39–45 × 25–30 (41 × 27, n = 5). When rostellum is contracted blades of rostellar hooks are directed anteriorly. Suckers slightly oval, with diameter 45–57 (50, n = 7). Rostellar hooks 10 in number, 23 (n = 4) long, aploparaksoid, with distinct handle; blade considerably longer than guard (Figs 4 and 20). Entire cercomer was not isolated; length of cercomer exceeds 400 (judging from fragmented cercomer), width 25–39.

Voucher specimens: BMNH 2005.2.23.17-18 (2 slides).

Remarks: *W. stellorae* was described as an intestinal parasite of *Larus ridibundus* L. on the French Atlantic coast (Deblock *et al*. 1960). Further studies recorded it from *L. genei* Breme in the Ukraine and Kazakhstan and from *L. californicus* Lawrence in North America (data summarised by Maksimova 1986, 1989). Its cysticercoid was reported from brine shrimps from Kazakhstan (Maksimova 1986), France (Robert and Gabrion 1991) and the Mediterranean coast of Spain (Varó *et al*. 2000). The rostellar hooks of the present material correspond with the previous descriptions of adults (Deblock *et al*. 1960; Maksimova 1986, 1989) and cysticercoids (Maksimova 1986, Robert and Gabrion 1991). Maksimova (1986) mentioned that the cysticercoids from *Artemia* identified by her (Maksimova 1973) as *Aploparaksis parafilum* Gąsowska, 1932 should also be considered as belonging to *W. stellorae*.

Recently, Bondarenko and Kontrimavichus (2004a) erected the genus *Branchiopodataenia* for a group of species from gulls and previously referred to *Wardium* Mayhew, 1925. They used the structure of the female copulatory ducts as a main distinguishing character and showed that the cysticercoids of these species develop in branchiopods. None of the cysticercoids described by Bondarenko and Kontrimavichus (2004b) has rostellar hooks identical to those of the present material. *B. anaticapicirra* Bondarenko et Kontrimavichus, 2004, a parasite of Arctic gulls, has rostellar hooks 18–22 long but with the blade only slightly larger than the guard. The cysticercoid of *B. arctowskii* (Jarecka et Ostas, 1984), described from Antarctic gulls and recorded at various places in the Holarctic Region, has hooks 15–16 long. *B. haldemani* (Shiller, 1951) and *B. pacifica* (Spassky et Jurpalova, 1969) from Arctic gulls are characterised by rostellar hooks 12–13 and 16–18 long, respectively (Bondarenko and Kontrimavichus 2004b). Thus, *W. stellorae* is a distinct form, differing from *Branchiopodataenia* spp. Although its cysticercoid develops in branchiopods, the morphology of the adult worms (Deblock *et al*. 1960) does not correspond to the diagnosis of *Branchiopodataenia*. Therefore, we prefer to consider it as belonging to *Wardium*.

Family Dilepididae

Eurycestus avoceti Clark, 1954

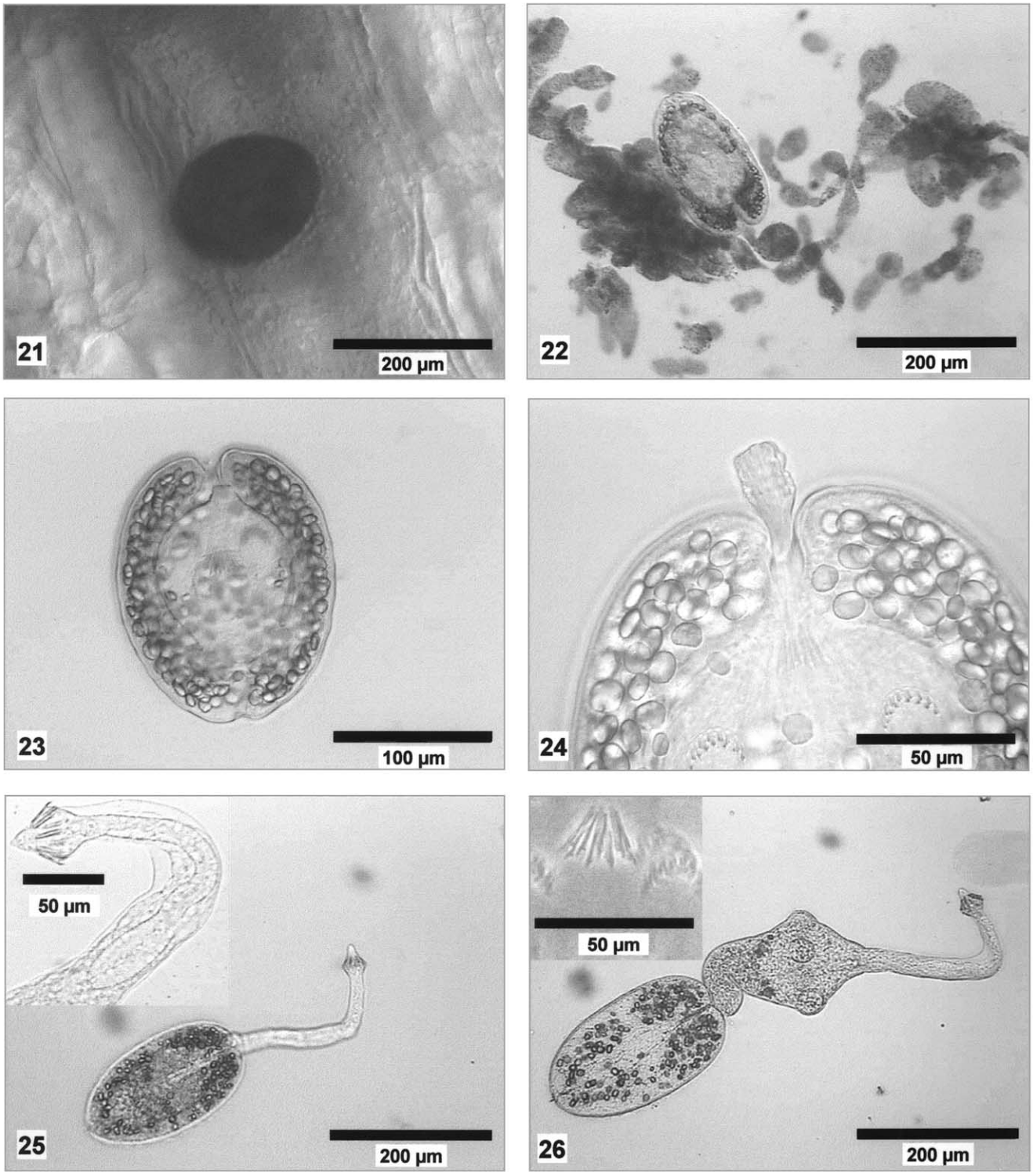
Description: Outer capsule oval or almost spherical (Fig. 21), brownish, with granular contents, 240–330 × 230–279 (295 × 255, n = 9). When compressed, outer capsule breaks down into cercomer fragments (Fig. 22). Cyst oval (Fig. 23), 141–228 × 99–163 (182 × 137, n = 12). Calcareous corpuscles

Table III. Morphological characteristics of the scolex armament of adult cestodes and cysticercoids of *Eurycestus avoceti* and species with similar scoleces

Species	Locality	Source	Rostellar hooks			Sucker hooklets	
			number	length		number	length
				anterior	posterior		
Eurycestus avoceti							
adult cestodes	France	Baer (1968)	14–16	14–16*	14–16*	10–14	5–6
	Kazakhstan	Maksimova (1991)	16	16–18	10–12	30–32	5–6
cysticercoids	France	Gabrion and MacDonald (1980)	16	18	12	15–16	7
	Kazakhstan	Maksimova (1991)	16	16–18	10–12	30–32	4–5
	Spain	present study	14–16	16–18	15–16	9–17	6–8
Paraliga oophorae							
adult cestodes	Russia (White Sea)	Belopol'skaya and Kulachkova (1973)	16	18	14	34–37	4
Paraliga celermatus							
adult cestodes	France	Deblock and Rosé (1962)	16	24–26*	24–26*	25–30	4–5

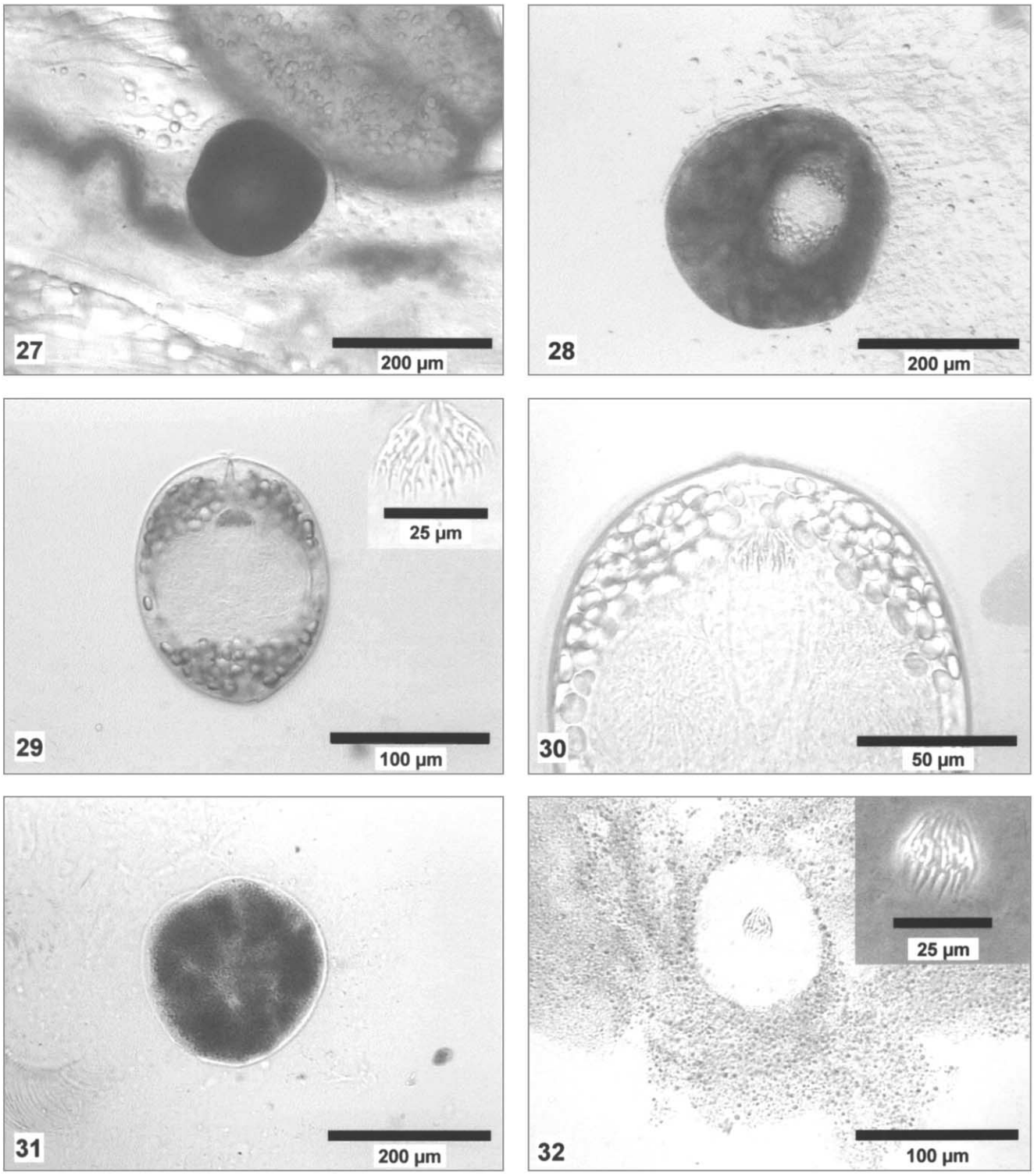
*Length of anterior rostellar hooks and length of posterior rostellar hooks not given separately.

numerous. Scolex rounded (Fig. 23), 111–150 × 81–114 (135 × 98, n = 8). Suckers round or slightly oval, with diameter 26–36 (30, n = 15); their anterior margin armed with hooklets, 6–8 long, arranged in 1–2 layers (Figs 5c and 24); number of hooklets on 1 sucker 9–17 (n = 18): 9 (n = 1), 10 (n = 2), 11 (n = 6), 12 (n = 1), 13 (n = 3), 14 (n = 1), 16 (n = 3) and 17 (n =



Figs 21–26. *Eurycestus avoceti*. **21.** Cysticercoid *in situ*. **22.** Isolated cysticercoid with outer capsule breaking down into cercomer fragments. **23.** Isolated cyst. **24.** Anterior end of isolated cyst. Note protruding rhynchus and sucker hooklets. **25.** Isolated cysticercoid with entirely protruded rhynchus. Inset: Rostellum. **26.** Cysticercoid excysted under hypotonic conditions. Inset: Rostellar hooks, phase contrast

1). Rhynchus very long (Figs 25 and 26). Rostellar hooks 14 (n = 1) or 16 (n = 12), in 2 rows (Figs 25 and 26). Anterior hooks 16–18 (17, n = 12) long, blade 3–4 long (Fig. 5a). Posterior hooks 15–16 (n = 10) long, blade 3 long (Fig. 5b).



Figs 27–32. *Anomotaenia* spp. **27–30.** *Anomotaenia* sp., cf. *A. microphallos*: **27.** Cysticercoid *in situ*; compare the size with that of the cysticercoid of *F. liguloides*. **28.** Isolated and slightly flattened cysticercoid. **29.** Isolated cyst. Inset: Rostellar hooks, phase contrast. **30.** Anterior part of isolated cyst. **31–32.** *A. tringae*: **31.** Isolated cysticercoid. **32.** Cysticercoid squashed in Berlese's medium; note the granular contents of the external capsule. Inset: Rostellar hooks, phase contrast

Voucher specimens: BMNH 2005.2.23.19-23 (5 slides).

Remarks: Adults of *E. avoceti* are parasites of charadriiform birds, mostly *Recurvirostra* spp., throughout the Holarctic Region (Baer 1968, Spasskaya and Spasskii 1978, Maksimova 1991). In Kazakhstan, this species was also recorded in 23.3% of flamingos studied (Maksimova 1991). The present identification is based on a correspondence between the cysticercoids found and previous descriptions of adults (Baer 1968, Maksimova 1991) and cysticercoids (Gabrion and MacDonald 1980, Maksimova 1991). However, there are some substantial differences in the lengths of the posterior rostellar hooks and the number of the sucker hooklets (Table III), which suggest that cysticercoids from France, Spain and Kazakhstan may represent more than one species. Until now, *E. avoceti* is the only species of the genus that has been recorded in the Palaearctic Region. Two further species occur in North America (Burt 1979) but their scoleces have not been described. Furthermore, the two Palaearctic species of *Paraliga* Belopol'skaya et Kulachkova, 1973, which includes dilepidids from charadriiform birds (Deblock and Rosé 1962, Belopol'skaya and Kulachkova 1973), are also characterised by a similar scolex armament (Table III). Therefore, although the identification of our material as *E. avoceti* is the most probable based on a comparison with Baer's (1968) description, it is somewhat provisional.

Anomotaenia sp. [cf. *A. microphallos* (Krabbe, 1869)]

Description: Outer capsule spherical to slightly oval (Figs 27 and 28), brownish, with granular contents, 228–316 × 192–283 (272 × 233, n = 11). Cyst oval (Figs 28 and 29), 186–209 × 121–150 (194 × 136, n = 12). Calcareous corpuscles numerous, concentrated mostly in anterior and posterior part of cyst. Scolex rounded (Figs 29 and 30), 135–165 × 105–120 (150 × 109, n = 8). Suckers slightly oval (Fig. 30), with diameter 48–60 (54, n = 14). Rostellum elongate (Fig. 30), 65–85 × 25–36 (76 × 31, n = 7). Rostellar hooks 26 (n = 1), 28 (n = 8) or 30 (n = 2), form irregular crown (Fig. 29). Anterior hooks situated one by one; 2 or 3 posterior hooks situated between each 2 anterior hooks; sometimes, posterior hooks at different level, thus creating impression of crown consisting of three rows. Anterior hooks with guard almost perpendicular to hook axis (Fig. 6a), 12–13 (n = 10) long; posterior hooks with guard almost parallel to blade (Fig. 6b), 11–12 (n = 10) long.

Voucher specimens: BMNH 2005.2.23.24-28 (5 slides).

Remarks: The number of rostellar hooks of the present material (26–30) suggests its affiliation with the family Dilepididae. The only dilepidid species from Palaearctic aquatic birds characterised by numerous (more than 20) rostellar hooks with a length of about and less than 15 µm is *Anomotaenia microphallos* (for surveys of Palaearctic dilepidids from charadriiform birds and from fish-eating birds, see Spasskaya and Spasskii 1978 and Ryzhikov *et al*. 1985, respectively). This species was originally described from *Vanellus vanellus* (L.) in Germany as having a crown of 24 hooks with a length of 14–16 µm (anterior) and 12–14 µm (posterior) (Krabbe 1869). Further studies added to the range of its final hosts waders of the genera *Charadrius* L., *Calidris* Merrem, *Gallinago* Brisson, *Philomachus* Merrem and *Tringa* L. (see Spasskaya and Spasskii 1978). However, none of the descriptions of adult cestodes of this species surveyed by Spasskaya and Spasskii (1978) reported more than 24 hooks. Therefore, we prefer to retain the identification at the generic level only.

Anomotaenia tringae (Burt, 1940)

Description: Outer capsule oval (Fig. 31), yellowish-brown, with granular contents, 249–316 × 218–297 (279 × 236, n = 12). Cyst oval (Fig. 32), 107–139 × 84–102 (131 × 93, n = 14). Calcareous corpuscles numerous, concentrated mostly in anterior part of cyst. Scolex rounded, 76–85 × 66–72 (82 × 70, n = 9). Suckers indistinct in available mounts in Berlese's medium, obviously with weak musculature. Rostellum 62–66 × 23–27 (n = 3). Rostellar hooks 18 (n = 6) or 20 (n = 4), in 2 rows forming compact crown (Fig. 32); blade slightly shorter than handle (Fig. 7a, b); anterior hooks 20–21 (n = 9) long; posterior hooks 19–20 (n = 8) long.

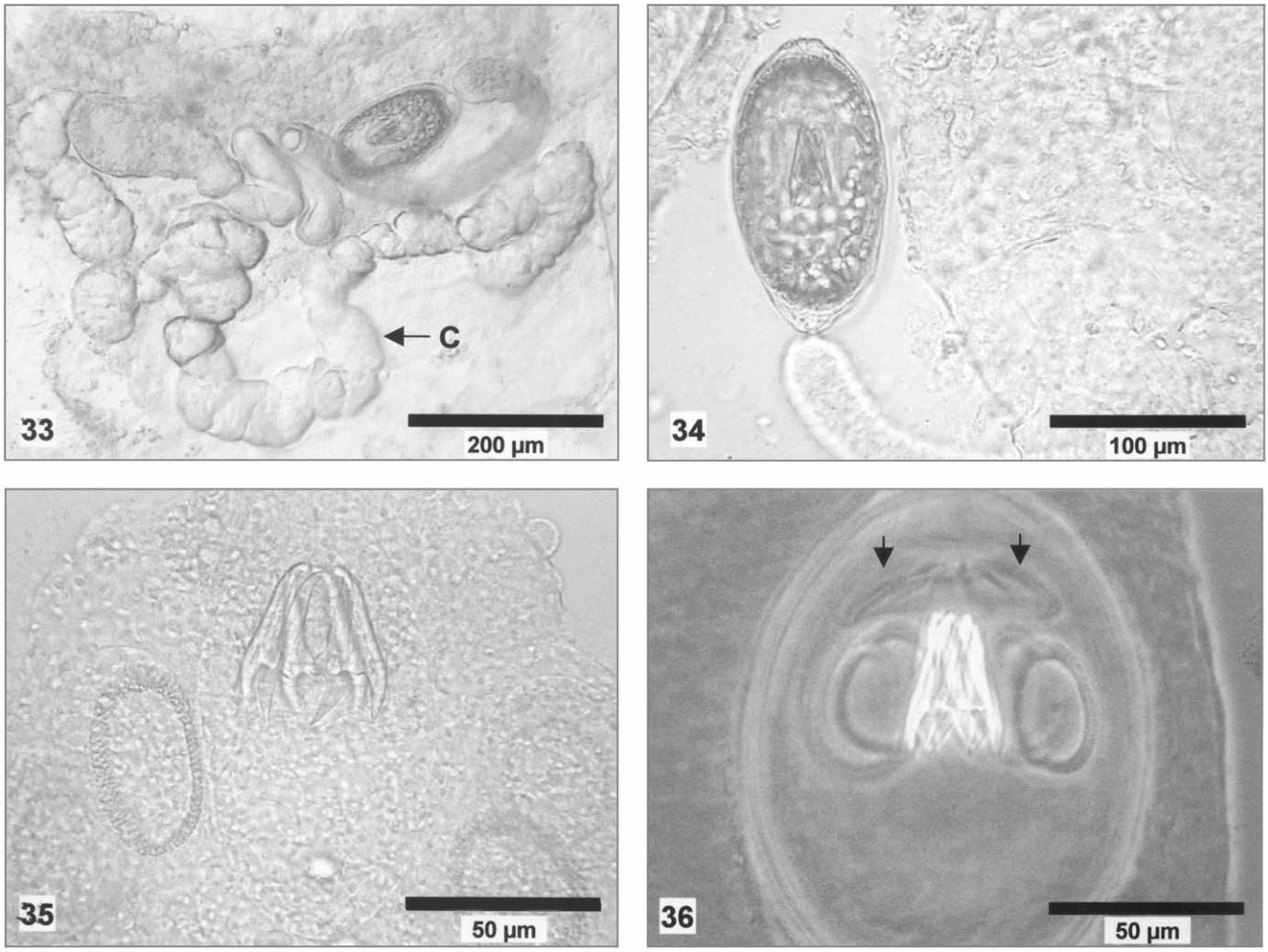
Voucher specimens: BMNH 2005.2.23.29-33 (5 slides).

Remarks: This species was originally described from *Tringa glareola* L. and *T. totanus* L. in Sri Lanka (Burt 1940). Subsequently, it was recorded from charadriiform birds of the genera *Tringa*, *Calidris*, *Charadrius*, *Gallinago*, *Philomachus* and *Vanellus* Brisson throughout the Old World, from Iceland to Zambia and from Kamchatka to Borneo (Spasskaya and Spasskii 1978). The present study is the first record of *A. tringae* in an intermediate host. Our identification is based on a comparison of the number, size and the shape of rostellar hooks of the cysticercoids with those of the adult worms [syntypes from *Tringa glareola*, British Museum (Natural History) Collection no. 1983.7.11.16]. The original description reported 18 rostellar hooks with a length of 17–18 µm "in what is practically a single row, but they show a slight alteration in arrangement into two levels but they are all similar in shape and size" (Burt 1940). Our observations show that the rostellar hooks of the syntypes studied are 18 in number, 21–22 µm long and have a similar shape (Fig. 7c, d) to those of the cysticercoids from Spain (Fig. 7a, b).

Family Progynotaeniidae

Gynandrotaenia stammeri Fuhrmann, 1936

Description: Cyst oval or lemon-shaped (Fig. 34), 142–192 × 95–170 (166 × 124, n = 17). Two layers distinguished in cyst wall: external layer hyaline, 9–11 thick; internal layer finely striated, 5–7 thick. Calcareous corpuscles 70–80. Scolex 113–147 × 89–121 (133 × 103, n = 14). Suckers oval, with diameter 36–45 (39, n = 62); margins armed with densely arranged spines (Figs 35 and 36). Invaginable anterior part of scolex (termed 'proscolex', see Fuhrmann 1936) distinct (Fig. 36), covered with small spines. Rostellum 65–74 × 25–34 (70 × 29, n = 11). Rostellar hooks 6 (n = 17), 40–46 (43, n = 15) long



Figs. 33–36. *Gynandrotaenia stammeri*: **33.** Cysticercoid, general view (C – cercomer). **34.** Cyst. **35.** Armament of rostellum and suckers, mounted in Berlese's medium. **36.** Cyst, mounted in Berlese's medium, phase contrast. Note invaginated proscolex (arrows)

(Figs 8, 35 and 36); length of blade 13–15 (13.5, n = 15). Ratio length of blade/total hook length 0.28–0.35 (0.31, n = 15). Cercomer highly elongate, convoluted, much larger than cyst (Fig. 33), 2.2–2.5 mm long and 47–78 wide (n = 3).

Voucher specimens: BMNH 2005.2.23.34-38 (5 slides).

Remarks: The morphology of these cysticercoids is in agreement with the previous descriptions (Gvozdev and Maksimova 1979, Robert and Gabrion 1991). Adults of *G. stammeri* have been recorded from *Phoenicopterus roseus* Pallas and *P. minor* Geoffroy Saint-Hilaire in Europe (Fuhrmann 1936a, b; Robert and Gabrion 1991), Kazakhstan (Gvozdev and Maksimova 1979) and Kenya (Jones and Khalil 1980). Cysticercoids were reported from *Artemia* from Kazakhstan (Gvozdev and Maksimova 1979) and France (Robert and Gabrion 1991).

Robert and Gabrion (1991) cited the paper by Gvozdev and Maksimova (1979) as a record of cysticercoids of *G. stammeri* in an ostracod species. However, the only intermediate host species mentioned in the latter article is *Artemia*.

Discussion

According to the terminology proposed by Chervy (2002), the cysticercoids of *Flamingolepis liguloides*, *F. flamingo*, *Wardium stellorae* and *Gynandrotaenia stammeri* belong to the group of the cercocysticercoids, while those of *Eurycestus avoceti*, *Anomotaenia tringae* and *Anomotaenia* sp. are considered monocysticercoids. The cysticercoid of *Confluaria podicipina* is close to the modification termed 'ramicysticercoid' but its cercomer is not branching. This suggests the necessity of further improvement of the terminology proposed by Chervy (2002).

The following 13 cyclophyllidean cestode species were previously known to use brine shrimps of the genus *Artemia* as intermediate host in their life cycles:

Hymenolepididae (10 species): *Confluaria podicipina* (see Maksimova 1981, 1989), *Fimbriarioides tadornae* Maksimova, 1976 (see Maksimova 1976, 1989), *Flamingolepis liguloides* (syn. *F. dolguschini*) (see Maksimova 1973, 1989;

Thiéry *et al*. 1990; Amat *et al*. 1991a, b; Robert and Gabrion 1991; Varó *et al*. 2000), *F. caroli* (Parona, 1887) (see Robert and Gabrion 1991), *F. flamingo* (see Robert and Gabrion 1991), *F. tengizi* (see Maksimova 1973, 1989), *Hymenolepis californicus* Young, 1950 (see Young 1952), *Wardium fusa* (Krabbe, 1869) (see Maksimova 1987, 1989), *W. gvozdevi* Maksimova, 1988 (see Maksimova 1988, 1989) and *W. stellorae* (Deblock, Biguet et Capron, 1960) (see Maksimova 1986, Robert and Gabrion 1991, Varó *et al*. 2000).

Dilepididae (2 species): *Eurycestus avoceti* (see Gabrion and MacDonald 1980, Maksimova 1991, Robert and Gabrion 1991) and *Anomolepis averini* (Spasskii et Yurpalova, 1967) (see Maksimova 1977).

Progynotaeniidae (1 species): *Gynandrotaenia stammeri* (see Gvozdev and Maksimova 1979, Robert and Gabrion 1991).

For 6 of these species, i.e. *C. podicipina*, *F. liguloides*, *F. flamingo*, *W. stellorae*, *E. avoceti* and *G. stammeri*, our study confirms the role of brine shrimps as an intermediate host in their life cycle. In addition, *Anomotaenia tringae* is recorded for the first time in its intermediate host. Therefore, brine shrimps participate in the transmission of the cestode parasites of flamingos, waders, grebes and gulls at the Odiel Marshes.

Robert and Gabrion (1991) examined more than 64,000 brine shrimps in the Camargue, France, and recorded a prevalence of cestode infection of between 1.56 and 5.12% in various seasons; the values of the mean abundance for the species in common with these in our study are: *F. liguloides* 0.0506, *F. flamingo* 0.000743, *W. stellorae* 0.000077, *G. stammeri* 0.000031 and *E. avoceti* 0.00091. The data obtained for the prevalence of cysticercoids in the Odiel Marshes (Table I) are 6 times higher, and those for the mean abundance are between 5 and 322 times higher. These differences suggest much higher rates of cestode transmission compared to those recorded in the Camargue. In contrast to the high infection rates in *Artemia*, no cysticercoids were found in any of the other 5 invertebrate species studied from the same water body.

The present study is the first record of *C. podicipina*, *E. avoceti*, *A. tringae* and *G. stammeri* in Spain.

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