# Helminth diversity in brine shrimps (*Artemia*) from Ukraine

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Abstract

Brine shrimps (*Artemia* spp.) are aquatic crustaceans known as important intermediate hosts for a wide range of helminth species. From 2011 to 2021, 4,347 individuals of brine shrimp were collected for this study, investigating the diversity and infection rates of helminth species in *Artemia* spp. from hypersaline waters in southern and eastern Ukraine. Seven helminth species were found: six cestodes (*Anomotaenia tringae*, *Eurycestus avoceti*, *Branchiopodataenia gvozdevi*, *Confluaria podicipina*, *Fimbriarioides tadornae*, *Hymenolepis* s.l. *stellorae*) and one unidentified acuariid nematode (Acuariidae gen. sp.). All these helminths were recorded for the first time in intermediate hosts in Ukraine, although they had been known from other regions. Additionally, partial sequences of the 18S rDNA gene as well as the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*nad*1) genes were obtained for varying numbers of cestode and nematode isolates for the first time. The overall prevalence of helminth infection in *Artemia* spp. was 21.9%, and the intensity ranged from one to three specimens.

Introduction

Crustaceans of the genus *Artemia* (Crustacea: Artemiidae), commonly known as brine shrimps, are distributed worldwide and play an essential role as dominant grazers in hypersaline water ecosystems, contributing to nutrient cycling (Sánchez *et al.*, 2016; Triantaphyllidis *et al.*, 1998). One of their important ecological functions is serving as intermediate hosts for various species of waterfowl helminths, primarily cestodes. The first report of a metacestode in *Artemia salina* (Linnaeus, 1758) was from Tunisia in 1926 (Heldt, 1926). The author presented a brief description without identification but, based on the shape and length of the hooks (180 μm), it could be assumed that it was *Flamingolepis liguloides* (Gervais, 1847). Later on, a metacestode was found in *A. salina* from the United States (Young, 1952) and, after infecting a definitive host experimentally, it was identified as *Hymenolepis* s.l. *californicus* Young, 1950, a species parasitising gulls. Investigations of various brine shrimp species have been conducted in Kazakhstan (Maksimova, 1973, 1976, 1977, 1981, 1986, 1987, 1988, 1989, 1991, 1991; Gvozdev & Maksimova, 1979, 1985), Romania (Codreanu & Codreanu-Balcescu, 1978), France (Gabrion & MacDonald, 1980; Gabrion *et al.*, 1982; Thiéry *et al.*, 1990; Robert & Gabrion, 1991; Vasileva *et al.*, 2009; Sánchez *et al.*, 2012), Spain (Amat *et al.*, 1991a, b; Varó *et al.*, 2000; Georgiev *et al.*, 2005, 2007, 2014; Sánchez *et al.*, 2006, 2007, 2013; Vasileva *et al.*, 2009; Redón *et al.*, 2015c), Italy (Mura, 1995), United Arab Emirates (UAE) (Schuster, 2019; Sivakumar *et al.*, 2020), Algeria (Amarouayache *et al.*, 2009), United States (Redón *et al.*, 2015b), and Chile (Redón *et al.*, 2019). These studies established that *Artemia* spp. serve the intermediate host for 16 cestode species and one nematode species. The latter was identified only at the family level, the Acuariidae (Georgiev *et al.*, 2014; Redón *et al.*, 2015b). Some species, i.e. *Flamingolepis caroli* (Parona, 1887), *Flamingolepis tengizi* Gvozdev & Maksimova, 1968 and *Hymenolepis* s.l. *fusa* (Krabbe, 1869), were each reported in a single publication (Maksimova, 1973, 1987; Gabrion & MacDonald, 1980; Robert & Gabrion, 1991). *Flamingolepis megalorchis* (Lühe, 1898), found in *Artemia franciscana* Kellogg, 1906 in the UAE (Schuster, 2019), was previously recorded in chironomids (Gvozdev & Maksimova, 1978). These faunistic studies demonstrate a lack of strict metacestode specificity for Artemia species; however, Redón *et al.* (2015a) showed that parasite infection may vary depending on the *Artemia* species. In particular, they demonstrated that the prevalence and species richness of helminth infections in the same ecosystem are higher in the native *A. salina* compared to the invasive *A. franciscana*, which is non-native to the European region.

There are few partial sequences of small rRNA gene for metacestodes from *Artemia* spp. in GenBank: *Confluaria podicipina* (Szymanski, 1905), *F. liguloides* H. s.l. *californicus*, *Fimdriarioides* sp., and *Flamingolepis* sp. based on the material from Spain, United States, and Chile (Redón *et al.*, 2024).

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Despite being extensively studied worldwide, brine shrimp helminths have not been investigated in Ukraine. The present research aimed to study the species composition of *Artemia* spp. helminths in the South and East regions of Ukraine, where several hypersaline water bodies are located.

Materials and methods

Material collection

From 2011 to 2021, 4,347 individuals of brine shrimp (*Artemia* spp.) were collected for the study. The sample efforts were conducted over different years at three locations in Ukraine: small salt lakes near Sloviansk in Kramatorsk Raion, Donetsk Oblast; an industrial saltern in Skadovsk Raion, Kherson Oblast; and the Adzhibaychik and Sasyk lakes on the western coast of Crimea. The details regarding the brine shrimps collected are provided in Table 1. The brine shrimps were seized using an aquarium net, placed in plastic containers filled with salt water and transported to the laboratory for examination. Because of logistical capabilities, the host individuals obtained from Crimea were treated differently; they were preserved in 70% ethanol before being transported to the laboratory for further examination.

Brine shrimps were examined between the microscope slides under the dissecting microscope. The detected alive helminths were extracted from the brine shrimps using preparatory needles, and the unstained helminth specimens were subsequently studied in 0.9% NaCl under the microscope. Most of the helminth specimens were fixed in 70% ethanol. In addition, several specimens were fixed in 96% ethanol for molecular analysis.

Morphological analysis

For a detailed morphological examination, cestode specimens were stained with iron acetocarmine diluted with 70% ethanol at a 1:1 ratio and subsequently mounted in Berlese's medium. A cysticercoid of *Branchiopodataenia gvozdevi* (Maksimova, 1988) was stained with iron acetocarmine, dehydrated through a graded series of ethanol concentrations, cleared in clove oil, and mounted in Canada balsam. Before the examination, nematodes were rinsed in distilled water and cleared in lactophenol, a compound of equal parts water, glycerine, phenol, and lactic acid. The morphology of cestodes and nematodes was examined under a Zeiss Axio Imager M1 microscope equipped with DIC and an AmScope T690B microscope. The photos were made with digital cameras mounted on the microscopes. All measurements in the text are in micrometres and are given as a range followed by the mean and the number of measurements (n) in parentheses.

DNA extraction, polymerase chain reaction, and sequencing

DNA sequences of selected helminth species (10 isolates) were obtained. Total genomic DNA was isolated from cestode and nematode specimens preserved in 96% ethanol using the Gene-JET Genomic DNA Purification Kit (Thermo Fisher Scientific) or the Monarch Genomic DNA Purification Kit (New England Biolabs, Inc., Ipswich, MA, USA) according to the manufacturer's standard protocols. For each polymerase chain reaction (PCR) reaction, the mixture included 2.0 µL of gDNA, 12.5 µL of MyTaq HS Red Mix (Bioline), 1.0 µL of forward primer, 1.0 µL of reverse primer, and 5.5 µL of water.

The amplification of the 18S rDNA (~800 bp) fragment for nematode isolate was conducted using the forward primer 18SU467F (5′-ATC CAA GGA AGG CAG CAG GC-3′) and the reverse primer 18SL1170R (5′-GTG CCC TTC CGT CAA TTC CT-3′) (Indaryanto *et al.*, 2015). The cycling conditions included denaturation at 94 °C for 2 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 45 °C, and 1 min at 72 °C, with a final extension of 7 min at 72 °C.

The 1,450–1,500 bp long fragments of the 28S rDNA were amplified for cestode isolates using the forward primer ZX-1 (5′-ACC CGC TGA ATT TAA GCA TAT-3′) (Scholz *et al.*, 2013) and the reverse primer 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Tkach *et al.*, 2003). The cycling conditions included denaturation at 95 °C for 5 min, followed by 40 cycles of 30 s at 95 °C, 30 s at 55 °C, and 2 min at 72 °C, with a final extension of 7 min at 72 °C.

Fragments of approximately 500 bp and 800 bp of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene were amplified for cestode isolates using the forward primer Dig_cox1Fa (5′-ATG ATW TTY TTY TTY YTD ATG CC-3′) and the reverse primer Dig_cox1R (5′-TCN GGR TGH CCR AAR AAY CAA AA-3′) (Wee *et al.*, 2017) or the forward primer Dice1F (5′-ATT AAC CCT CAC TAA ATT WCN TTR GAT CAT AAG-3′) and the reverse primer Dice14R (5′-TAA TAC GAC TCA CTA TAC CHA CMR TAA ACA TAT GAT G-3′). The cycling conditions included denaturation at 94 °C for 4 min, followed by 40 cycles of 30 s at 94 °C, 30 s at 51 °C (Dig_cox primers) or 53 °C (Dice primers), and 30 s at 72 °C, with a final extension of 10 min at 72 °C.

For cestodes, partial *nad*1 + trnN region (~800 bp) was amplified using the forward primer Cyclo_nad1F (5′-GGN TAT TST CAR TNC GTA AGG G-3′) and the reverse primer Cyclo_trnNR (5′-TTC YTG AAG TTA ACA GCA TCA-3′) (Littlewood *et al.*, 2008).

Table 1. Summary data of sampling sites and sample size of *Artemia* spp. examined

Sampling site	Coordinates	Date	Sample size (specimens)
Sloviansk, Kramatorsk Raion, Donetsk Oblast	48°51′11″ N 37°36′21″ E	10 August 2013	363 (females)
		4–7 August 2021	747 (females)
Industrial saltern, Skadovsk Raion, Kherson Oblast	46°30′35″ N 31°54′04″ E	30 August 2011	89 (31 females, 58 males)
		3 August 2012	513 (124 females, 389 males)
		5–8 June 2021	1542 (516 females, 1,026 males)
Adzhibaychik Lake, Crimea	45°15′20″ N 33°05′48″E	29 July 2012	81 (females)
		24 July 2021	325 (females)
Lake Sasyk, Crimea	45°11′26″ N. 33°30′24″E	26 July 2021	687 (females)

The cycling conditions included denaturation at 94 °C for 3 min, followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1.5 min at 72 °C, with a final extension of 7 min at 72 °C.

Amplified DNA was purified using ExoSAP-IT PCR Cleanup enzymatic kit from Thermo Fisher Scientific, Inc. (Waltham, MA, USA) and sequenced from both strands using the PCR primers and additional internal sequencing primer 300F (5′-CAA GTA CCG TGA GGG AAA GTT G-3′) and ECD2 (5′-CTT GGT CCG TGT TTC AAG ACG GG-3′) (Littlewood *et al.*, 2000) for 28S rDNA and primer Cyclo_nad1Fb (5′-AGG TTT GAR GCK TGT TTT ATG-3′) for *nad*1 + *trn*N region. Sanger sequencing was conducted at the Faculty of Natural Sciences of Comenius University (Bratislava, Slovakia) or a commercial sequencing company, SEQme (Dobříš, Czech Republic). Chromatogram-based contigs were assembled and edited using Geneious Prime 2024.0.5 software (Biomatters, Auckland, New Zealand; https://geneious.com).

Results

Helminth infections were recorded in *Artemia* spp. at each of the studied locations. Of 4,347 examined individuals of *Artemia* spp., 953 (21.79%) were infected with helminths. The intensity of infection was low, with one to three specimens per individual. The total values of the mean abundance and the mean intensity were 0.22 and 1.03, respectively. Cysticercoids of six cyclophyllidean species of two families and one nematode species were recorded. The data on helminth prevalence and intensity for each location are provided in Table 2.

The information and the description of found helminths are given next.

CESTODA

Family Dilepididae Railliet et Henry, 1909

Anomotaenia tringae (Burt, 1940) (Fig. 1B)

Prevalence and intensity of infection: 0.3% and 1 specimen (salt lakes near Sloviansk, Donetsk Oblast).

Description (based on one specimen from Sloviansk): Outer capsule oval, 220×230. Cyst rounded, 105×125. Cyst-wall consists of three layers: external 1–2 in thickness, median 9–14 in thickness, and internal 3–5 in thickness. Scolex rounded, 85×75. Suckers oval muscular, $38–45 \times 20–28$ (41.5×24, n=4). Rostellum 60 with maximum diameter at hooks level 28. Rostellar sheath 70×33. Rostellum armed with 18 hooks. Anterior surface of rostellum usually not invaginated and hook blades oriented posteriorly. Total length of hooks 19–20 (19.5, n=4), handle 9 (9, n=4), blade 9–10 (9, n=4). Invagination pore as narrow slit, 11 in depth, at anterior part of cyst. Excretory canal short, 8 in depth, opening at posterior part of cyst. Embryonic hooks 10–13 (12, n=6).

Remarks

Metacestodes of this species were first recorded from *Artemia parthenogenetica* Bowen et Sterling, 1978 in Spain (Georgiev *et al.*, 2005). In subsequent studies, metacestodes of *A. tringae* were found in *A. franciscana* in Portugal (Georgiev *et al.*, 2007) and in the UAE (Sivakumar *et al.*, 2020), as well as in *A. salina* in Spain (Sánchez *et al.*, 2013). The morphology of our specimens corresponds to that of specimens from Spain described by Georgiev *et al.* (2005) by the number, shape and size of rostellar hooks, and

Table 2. Summary data of species recorded in *Artemia* spp. and their infection rates

Helminth species	Sampling site and year	Prevalence (%)	Intensity (specimen)
A. tringae	Salt lakes near Sloviansk, 2021	0.3	1
E. avoceti	Salt lakes near Sloviansk, 2013	72.5	1–2
E. avoceti	Salt lakes near Sloviansk, 2021	42.8	1–2
E. avoceti	Industrial saltern, 2021	0.1	1
E. avoceti	Lake Adzhibaychik, 2012	11.1	1
E. avoceti	Lake Adzhibaychik 2021	3.4	1–2
E. avoceti	Sasyk Lake, 2021	1.0	1
B. gvozdevi	Industrial saltern, 2011	2.2	1
C. podicipina	Lake Adzhibaychik, 2012	4.9	1–2
C. podicipina	Lake Adzhibaychik, 2021	0.6	1
C. podicipina	Lake Sasyk, 2021	3.9	1–2
F. tadornae	Industrial saltern, 2021	0.1	1
F. tadornae	Lake Adzhibaychik, 2012	3.7	1
F. tadornae	Lake Adzhibaychik Lake, 2021	1.9	1
H. s.l. stellorae	Salt lakes near Sloviansk, 2013	9.9	1–2
H. s.l. stellorae	Salt lakes near Sloviansk, 2021	8.4	1–2
H. s.l. stellorae	Industrial saltern, 2011	23.6	1–2
H. s.l. stellorae	Industrial saltern, 2012	7.4	1–3
H. s.l. stellorae	Industrial saltern, 2021	0.4	1
H. s.l. stellorae	Lake Adzhibaychik, 2012	1.2	1
H. s.l. stellorae	Lake Adzhibaychik, 2021	0.6	1
H. s.l. stellorae	Lake Sasyk, 2021	0.4	1
Acuariidae gen. sp.	Salt lakes near Sloviansk, 2021	0.1	1
Acuariidae gen. sp.	Industrial saltern, 2021	5.7	1–3
Acuariidae gen. sp.	Lake Adzhibaychik, 2021	0.9	1
Acuariidae gen. sp.	Lake Sasyk, 2021	5.1	1–2

the shape of cysticercoid. Different charadriiform birds are definitive hosts of this species (Spasskaya & Spassky, 1978). In Ukraine, *A. tringae* was reported from *Tringa glareola* Linnaeus, 1758, *Tringa nebularia* (Gunnerus, 1767), *Tringa stagnatilis* (Bechstein, 1803), *Tringa totanus* (Linnaeus, 1758), *Gallinago gallinago* (Linnaeus, 1758) and *Limosa limosa* (Linnaeus, 1758) (Smogorzhevskaya, 1976; Greben & Kornyushin, 2001).
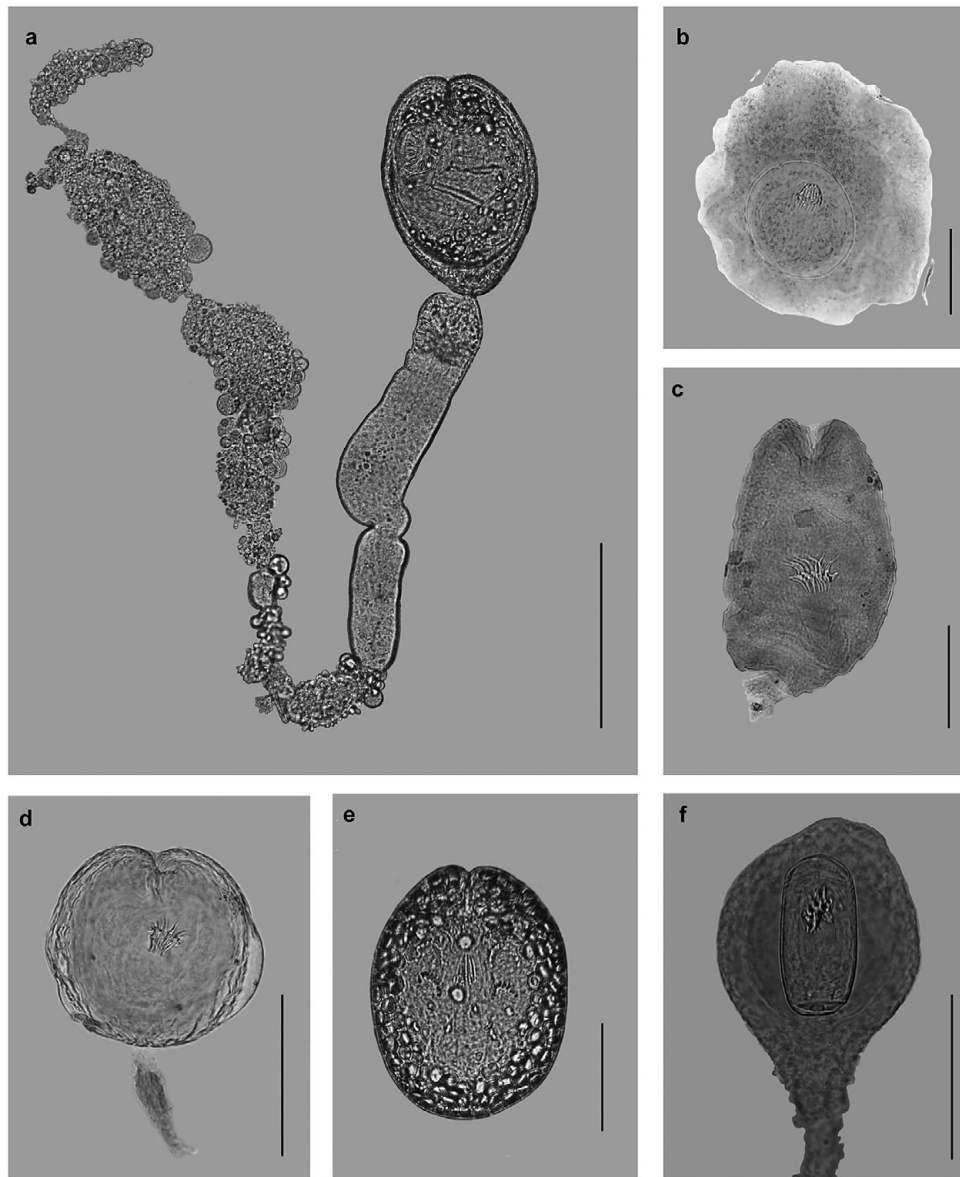
Figure 1. Metacestodes from *Artemia* spp. (A) *Hymenolepis* s. l. *stellorae* Deblock, Biguet et Capron, 1960; (B) *Anomotaenia tringae* (Burt, 1940); (C) *Branchiopodataenia gvozdevi* (Maksimova, 1988); (D) *Fimbriarioides tadornae* (Burt, 1940); (E) *Eurycestus avoceti* Clark, 1954; (F) *Confluaria podicipina* (Szymanski, 1905). Scale bars: (A), 200 μm; (B), (E), 50 μm; (C), (D), (F), 100 μm.

Eurycestus avoceti Clark, 1954 (Fig. 1E)

Prevalence and intensity of infection: 52.5% and one to two specimens (salt lakes near Sloviansk, Donetsk Oblast); 0.1% and one specimen (industrial saltern, Kherson Oblast); 2.5% and one to two specimens (Adzhibaychik and Sasyk lakes, Crimea).

Representative sequence: PQ084087 (28S rDNA), PQ397346 (*cox*1), PQ156477 (*nad*1).

Description (based on 10 specimens from Sloviansk): Total length of cysticercoid 115–143 (126, n=10), 75–115 (101, n=10) in diameter. Cysticercoid surrounded by thick external capsule, with thickness of walls 40–70. Wall of cysticercoid delicate, 3–5 thick. Internal covering 10–28 thick. Calcareous corpuscles numerous. Scolex oval or rounded, 60–80 × 55–70 (75 × 62, n=10). Suckers oval, 23–30 × 16–23 (25 × 19, n=34), armed with small spines in anterior part. Rostellum thin and elongate, 33–60 (50, n=10) long; maximum diameter at hooks level, 18–20 (19, n=10). Rostellar sheath 45–71 × 25–30 (62 × 29, n=10). Rostellum armed with 14–16 hooks. Rostellum usually not invaginated; hook blades oriented posteriorly. Total length of hooks 14–16 (16, n=32), handle 10–13 (11, n=32) long, blade 3–4 (4, n=32) long. Invagination pore as narrow slit, 20–35 (26, n=9) in depth, at anterior part of cyst. Excretory canal very short, 1–5 (2, n=9) in depth, at posterior end of cyst. Embryonic hooks not distinct.

Remarks

Initially, metacestodes of *E. avoceti* were recorded from *Artemia* sp. in France (Gabrion & MacDonald, 1980). A detailed description of these metacestodes was presented based on the specimens from *A. salina* in Kazakhstan (Maksimova, 1991) and *A. parthenogenetica* in Spain (Georgiev *et al.*, 2005). This species was registered in *A. salina* in Spain (Georgiev *et al.*, 2007; Sánchez *et al.*, 2013), in *A. parthenogenetica* in Spain (Georgiev *et al.*, 2007; Sánchez *et al.*, 2006, 2007, 2013), and in France (Sánchez *et al.*, 2012), and in

A. franciscana in Spain and Portugal (Georgiev *et al.*, 2007), in France (Sánchez *et al.*, 2012), and in the UAE (Schuster, 2019; Sivakumar *et al.*, 2020). Our specimens are similar to the specimens described by Gabrion and MacDonald (1980) from France, by Maksimova (1991) from Kazakhstan, and by Georgiev *et al.* (2005) from Spain. They differ only from material from Spain by the smaller size of cysticercoid (126 × 101 *vs* 182 × 137) and smaller scolex (75 × 62 *vs* 135 × 98). However, the armament of the rostellum and suckers of specimens from Ukraine correspond to the same material described in the previous publications.

Various charadriiform birds are definitive hosts of *E. avoceti* (Spasskaya & Spassky, 1978). In Ukraine, this species was found in *Charadrius alexandrinus* (Linnaeus, 1758), *Himantopus himantopus* (Linnaeus, 1758) and *Recurvirostra avosetta* Linnaeus, 1758 (Smogorzhevskaya, 1976).

Family Hymenolepididae Ariola, 1899

Branchiopodataenia gvozdevi (Maksimova, 1988) (Fig. 1C)

Prevalence and intensity of infection: 0.1% and one specimen (industrial saltern, Kherson Oblast).

Description (based on two specimens from industrial saltern, Kherson Oblast, after Kornyushin and Greben, 2022, with additional data): Cysticercoid oval, massive, 250–330 in length and 150–200 in maximum width. Cyst-wall 20–30 in thickness. Scolex 150–180 in length and 110–163 in maximum diameter at sucker level. Suckers rounded 55–60 × 50–55. Rostellar sheath 140 × 70. Rostellum 90 in length, with maximum diameter at hooks level 60. Rostellum armed with 10 aploparaksoid hooks. Anterior surface of rostellum usually invaginated and hook blades oriented anteriorly. Total length of hooks 38–40 (39, n=5), handle 13 (13, n=5), blade 20–23 (22, n=5), guard 10–13 (11, n=5), base with guard 20–21 (21, n=5). Invagination pore 60–80 in depth, at anterior part of cyst. Excretory canal 20–30 in depth, at posterior part of cyst. Cercomer short, 630 long, 45–50 in diameter. Embryonic hooks 10–11 (10, n=5), usually localised in cercomer.

Remarks

Metacestodes of *B. gvozdevi* were first recorded in *A. salina* in Kazakhstan, where the species was described, and its development in the intermediate host was studied (Maksimova, 1988). Cysticercoids of this species were also found in *A. franciscana*, *A. salina* and *A. parthenogenetica* in Spain (Vasileva *et al.*, 2009; Redón *et al.*, 2015c).

The specimens in this study were generally similar to the metacestodes described by Maksimova (1988) and Vasileva *et al.* (2009). They differed from the specimens from Kazakhstan by having a wider scolex (110–163 *vs* 84–120), a larger rostellum (60 *vs* 38–50), and a shorter, slightly wider cercomer (630 × 45–50 *vs* 1.21–1.43 × 40–42). Our specimens differed from the specimens reported from Spain by having a longer scolex (150–180 *vs* 123–149), a more extended rostellar sheath (90 *vs* 54–70), a larger rostellum (90 × 60 *vs* 54–70 × 44–51), a large cercomer (630 × 45–50 *vs* 400 × 25–39) and a larger maximum scolex width (110–163 *vs* 84–120).

The gull *Chroicocephalus genei* (Brème, 1839) is the only known definitive host for *B. gvozdevi* in Ukraine (Kornyushin & Greben, 2013) and elsewhere (Maksimova, 1988).

Confluaria podicipina (Szymanski, 1905) (Fig. 1F)

Prevalence and intensity of infection: 3.0% and one to two specimens (Adzhibaychik and Sasyk lakes, Crimea).

Representative sequence: PQ084089 (28S rDNA), PQ096505 (*nad*1).

Description (based on seven specimens from Crimea): Cysticercoid oval, 160–240 × 130–190 (171 × 141, n=7), with very long

think cercomer 8–23 (11, n=14) in diameter. Length of cercomer tens of times greater than length of cysticercoid. Cyst oval, 66–125 × 40–80 (99 × 58, n=7). Cyst wall consists of three layers. External layer thin, 2.5–3 in thickness. Medial layer consists of three parts: first part 4–6 thick, friable part 8–15 thick, and threaded part 2–3 thick. Internal layer consists of three parts: basal part 9–30 thick, fibrous part 2–3 thick, and parenchymal part 2–4 thick. Calcareous corpuscles numerous. Scolex oval, 55–75 × 35–55 (66 × 48, n=7). Suckers slightly oval, 25–32 × 13–25 (30 × 22, n=14). Rostellum 35–65 (47, n=7), with maximum diameter at hooks level 20–40 (29, n=7). Rostellar sheath 55–65 × 35–45 (59 × 40, n=4) with thin wall. Rostellum armed with 10 aploparaksoid hooks. Anterior surface of rostellum usually invaginated and hook blades oriented anteriorly. Total length of hook 18–23 (21, n=18), handle 4–6 (5, n=18), blade 10–13 (11, n=18), guard 5–8 (7, n=18), base with guard 13–15 (13, n=18).

Invagination pore as slit, 13–20 (16, n=6) deep, at anterior part of cyst. Posterior canal short, 5–10 (7, n=6) in depth.

Remarks

Metacestodes of this species were described from *A. salina* in Kazakhstan (Maksimova, 1981). *Confluaria podicipina* was found in *A. parthenogenetica* and *A. salina* from Spain (Georgiev *et al.*, 2005, 2007; Sánchez *et al.*, 2006, 2007, 2013), in *A. franciscana* from the USA (Redón *et al.*, 2015b), the UAE (Sivakumar *et al.*, 2020), and Chili (Redón *et al.*, 2019). Cysticercoids identified *C. podicipina* from *Acanthocyclops viridis* in the Czech Republic (Tolkacheva, 1987) likely belong to a different species because this is the only record of this species in freshwater invertebrates; it is unlikely that eggs of the same cestode species can survive in both fresh and saltwater. There are several cestode species with aploparaksoid hooks of the same length as *C. podicipina* and with an unknown life cycle (Bondarenko & Kontrimavichus, 2006).

The morphology of our specimens corresponds to that of metacestodes from Kazakhstan (Maksimova, 1981) and Spain (Georgiev *et al.*, 2005). They differ from the specimens described by Maksimova (1981) in having a wider cysticercoid (130–190 *vs* 105) and from the specimens described by Georgiev *et al.* (2005) in having a wider range of the scolex length (55–75 *vs* 72–104).

Grebes are the definitive hosts of *C. podicipina* (Spasskaya, 1966; Vasileva *et al.*, 2000). This species was found in Ukraine in various species of grebes (Smogorzhevskaya, 1976).

Fimbriarioides tadornae (Burt, 1940) (Fig. 1D)

Prevalence and intensity of infection: 0.1% and one specimen (industrial saltern, Kherson Oblast); 0.8% and one specimen (Lake Adzhibaychik, Crimea).

Description (based on two specimens from industrial saltern, Kherson Oblast and one specimen from Crimea): Length of rounded cysticercoid 150–215 (185, n=3), diameter 130–150 (142, n=3). One specimen with delicate capsule 10–30 in thickness. Cyst 105–155 × 100–150 (130 × 132, n=3). Cyst-wall thick, consisting of three layers: external 5–6 thick, median 6–10 thick, and internal 10–15 thick. Scolex oval, 73–95 × 80–110 (90 × 86, n=3). Suckers oval, 33–45 × 27–45 (36 × 32, n=7). Rostellum 45–50 (48, n=3) long, with maximum diameter at hooks level 23–30 (28 × 33, n=3). Rostellar sheath 60–73 × 42–45 (68 × 44, n=3). Rostellum armed with 10 hooks. Anterior surface of rostellum usually invaginated and hook blades oriented anteriorly. Total length of hook 25–26 (26, n=7), handle 13–15 (14, n=7), blade 10–11 (10, n=7), guard 4–5 (4, n=7). Invagination pore as narrow slit, 33–43 (37, n=3) deep, at anterior part of cyst. Posterior pore

short, 9–18 (13, n=3) deep. Cercomer with maximum length 1.120 mm, 10–20 in diameter. In one specimen cercomer has small tear-shaped widening, 35 × 25, on the end. Embryonic hooks 9–11 (10, n=6), localised in cercomer.

Remarks

Metacestodes of this species were first recorded from *A. salina* in Kazakhstan with the description of the species (Maksimova, 1976). Also, *F. tadornae* has been registered in *A. salina* in Spain (Sánchez *et al.*, 2013), in *A. parthenogenetica* in Spain (Georgiev *et al.*, 2007; Vasileva *et al.*, 2009), in France (Sánchez *et al.*, 2012), in *A. franciscana* in Spain (Vasileva *et al.*, 2009) and France (Sánchez *et al.*, 2012; Vasileva *et al.*, 2009).

Our specimens are similar to those reported from Kazakhstan (Maksimova, 1976), Spain and France (Vasileva *et al.*, 2009). They differ from the specimens described by Maksimova (1976) in a shorter scolex (73–95 *vs* 108), rostellum (45–50 *vs* 80), rostellar sheath (60–73 *vs* 96) and a smaller diameter of the suckers (33–45 *vs* 50). From specimens described by Vasileva *et al.* (2009), they differ by a longer cercomer (1.120 *vs* 720). However, the length and size of rostellar hooks of specimens from Ukraine correspond well to the specimens described in all the previous publications.

Fimbriarioides tadornae is a parasite of *Tadorna tadorna* (Linnaeus, 1758). In Ukraine, this species was reported as *Fimbriarioides intermedia* (Fuhrmann, 1918) in this bird (Kornyushin, 1969; Smogorzhevskaya, 1976).

Hymenolepis s.l. stellorae Deblock, Biguet et Capron, 1960 (Fig. 1A)

Prevalence and intensity of infection: 8.9% and one to two specimens (salt lakes near Sloviansk, Donetsk Oblast); 0.3% and one to three specimens (industrial saltern, Kherson Oblast); 0.5% and one specimen (Adzhibaychik and Sasyk lakes, Crimea).

Representative sequences: PQ084088 (28S rDNA), PQ397347 (*cox*1), PQ156478 (*nad*1).

Description (based on 10 specimens from Sloviansk): Total length of metacestode 1.32–3.48 (2.33, n=5). Cysticercoid oval, 200–280 × 100–175 (249 × 143, n=6). Cyst oval, 165–190 × 120–145 (181 × 127, n=10). Cyst-wall 9–15 in thickness. Calcareous corpuscles numerous, concentrated in anterior and posterior parts of cyst's cavity. Scolex oval, 85–120 × 75–105 (100 × 92, n=10). Suckers oval, muscular, 35–55 × 30–45 (44 × 35, n=29). Rostellum 52–60 (57, n=9) with maximum diameter at hooks level 35–45 (42, n=9). Rostellar sheath 70–90 × 40–60 (57 × 42, n=9). Rostellum armed with 10 aploparaksoid hooks. Anterior surface of rostellum usually invaginated and hook blades oriented anteriorly. Total length of hook 21–24 (22, n=19), handle 4–6 (5, n=19), blade 13–15 (13, n=19), guard 9–10 (10, n=19), base with guard 14–15 (14, n=19). Invagination pore as narrow slit 45–50 (48, n=10) in depth, at anterior part of cyst. Excretory canal short, 5–10 (7, n=10) in depth, at posterior part of cyst. Cercomer elongate 1.06–3.26 mm (1.94 mm, n=9) and 90–180 (128, n=10) in diameter. It has rare, not deep, protrusions. Embryonic hooks 13–15 (14, n=22), usually localised in cercomer.

Remarks

Cysticercoids of this species were described from *A. salina* as *Aploparaksis parafilum* Gasowska, 1932 (Maksimova, 1973) and later as *Wardium stellorae* (Maksimova, 1986) in Kazakhstan. This species was registered in *Artemia* spp. from France (Robert & Gabrion, 1991), *A. salina* from Spain (Georgiev *et al.*, 2007; Varó *et al.*, 2000), *A. parthenogenetica* from Spain (Georgiev *et al.*,

2005, 2007; Sánchez *et al.*, 2006, 2013; Varó *et al.*, 2000) and France (Sánchez *et al.*, 2012) and *A. franciscana* from UAE (Sivakumar *et al.*, 2020).

The description of metacestodes of *H. s. l. stellorae* by Maksimova (1973) and Robert and Gabrion (1991) were relatively poor and limited to the size of cysticercoid and hooks. A detailed description of these metacestodes was presented on the material from *A. salina* in Kazakhstan (Maksimova, 1986) and *A. parthenogenetica* in Spain (Georgiev *et al.*, 2005). The morphology of our specimens corresponds to that of cysticercoids described in Kazakhstan and Spain. They differ from metacestodes reported from Spain only by the larger size of the rostellum (52–60 × 35–45 *vs* 39–45 × 23–30) and from metacestodes from Kazakhstan by a shorter rostellum (52–60 *vs* 73).

Chroicocephalus genei is the only known definitive host of *H. s. l. stellorae*. In Ukraine, it was reported in birds on the Black Sea coast (Smogorzhevskaya, 1976).

NEMATODA

Acuariidae gen. sp. (Fig. 2 A–H)

Prevalence and intensity: 0.1% and one specimen (salt lakes near Sloviansk, Donetsk Oblast); 4.1% and one to three specimens (industrial saltern, Kherson Oblast.); 3.5% and one to two specimens (Adzhibaychik and Sasyk lakes, Crimea).

Representative sequences: PQ084085, PQ084086 (18S rDNA).

Description based on five specimens from Kherson Oblast and five from Crimea (Lake Sasyk).

Third-stage larvae. Total length of body 2.912–3.968 mm (3.428 mm, n=10), maximum width 65–85 (76, n=10). Anterior end with two triangular pseudolabia, each bearing one pair of papillae. Cuticle with longitudinal ridges extending along body. Lateral alae absent. Lateral longitudinal ridges smaller than others. Cuticle forms transverse folds on tail at anus level. Cordons not observed. Deirids spine-like, 5–6 (5, n=16) long, situated at 68–80 (73, n=16) from anterior end, at level of posterior part of buccal cavity. Excretory pore at 175–245 (207, n=8) from anterior end. Tail 120–140 (131, n=8) long; width at anus 40–46 (45, n=8). End of tail with narrowing 13–15 (14, n=10) long, 7–10 (8, n=10) in diameter of proximal part, and 4–6 (5, n=10) in diameter of distal part. Buccal cavity 65–95 (86, n=10) long, 4–6 (4, n=10) wide. Anterior part triangular, posterior part circular. Muscular oesophagus 160–210 (184, n=10) long, 15–27 (21, n=10) wide. Glandular oesophagus 830–1.040 (912, n=10) long, 50–65 (58, n=10) wide. Nerve-ring 20–35 × 25–45 (26 × 33, n=10), at 113–135 (126, n=10) from anterior end. Relative length (ratio) of muscular and glandular oesophagus to body length 0.283–0.376 (0.320, n=10). Relative length of muscular oesophagus to glandular oesophagus (ratio) 0.154–0.205 (0.203, n=10).

Remarks

The presence of muscular and glandular portions of the oesophagus, lateral triangular pseudolabia, and elongated buccal cavity allows for the identification of the studied nematodes as Acuariidae gen. sp. (Chabaud, 1975; Anderson, 2000). We identified these nematodes at the family level only because the genera and species of the Acuariidae have been differentiated based on the morphology of the adult stage. Most of these nematodes are parasites of the stomach (under the gizzard lining), proventriculus or oesophagus of birds (Smogorzhevskaya, 1990). The absence of cordons is characteristic for nematodes of some genera of Acuariidae, in
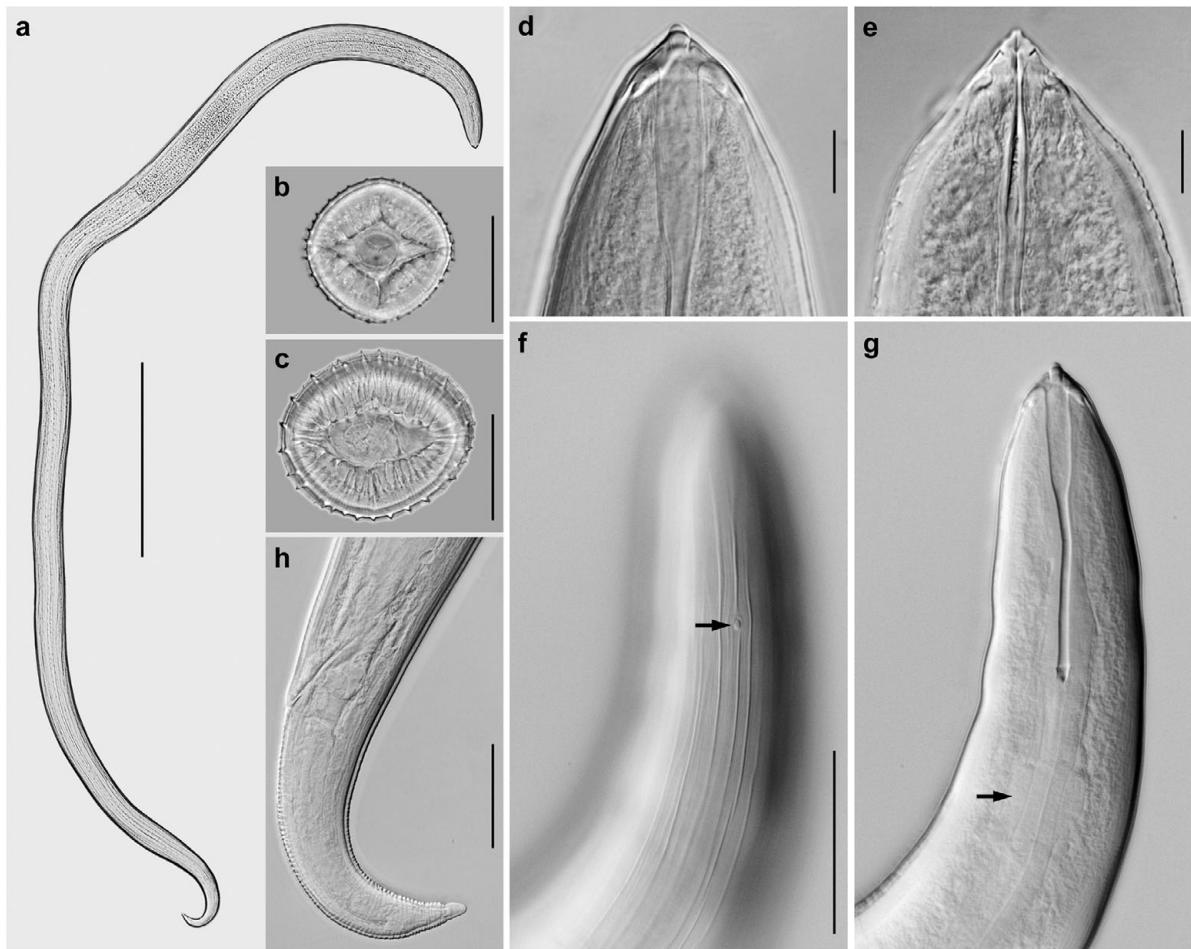
Figure 2. Acuariidae gen. sp. larva from *Artemia salina*. (A) general view; (B) transverse section of the body at level of muscular oesophagus; (C) transverse section of the body at mid-length; (D) anterior end, lateral view; (E) anterior end, dorso-ventral view; (F) surface of anterior part of the body showing cuticular ridges and deirid (arrow); (G) anterior part of the body showing the shape of the stoma and the nerve ring (arrow), lateral view; (H) posterior part of the body, lateral view. Scale bars: (A), (B), (C), (F), (G), (H), 50 μm; (D), (E), 10 μm.

particular for the genus *Paracuaria* Rao, 1951 (Mutafchiev et al., 2020). There is one species of the genus, *Paracuaria adunca* (Creplin, 1846), in Ukraine. It is a common parasite of gulls and terns and is found throughout the entire territory. However, freshwater crustaceans are intermediate hosts of this species of nematodes (Smogorzhevskaya, 1990). It is unlikely that invertebrates living in hypersaline waters can also participate in the circulation of these nematodes.

The first record of *Artemia* as the intermediate host for the nematodes was published by Georgiev *et al.* (2014). The authors found Acuariinae gen. sp. from *A. franciscana* in Spain, presented a photo of helminth without description, and noted that the larvae with similar morphology were also recorded in *A. parthenogenetica* and *A. salina*. An unidentified nematode of the Acuariidae with detailed description and illustrations was recorded in *A. franciscana* from the USA (Redón *et al.*, 2015b). The authors admitted similar morphology of their specimens and larvae from Spain and supposed that they belong to the same species.

The morphology of our material differs from the morphology of specimens from Spain and USA by the absence of lateral alae and cordons and the presence of longitudinal ridges extending along the body. Our specimens have a constriction on the distal end of the tail, which is not described in the nematode larvae from Spain and the United States (Georgiev *et al.*, 2014; Redón *et al.*, 2015b).

We found Acuariidae gen. sp. in brine shrimps in all examined localities, which likely suggests that *Artemia* is the intermediate host for these nematodes.

Discussion

The present study reported seven helminth species (six cestodes and one nematode) in *Artemia* spp. Two of these species, *H. stellorae* and *E. avoceti*, were genetically characterised (*cox*1 and *nad*1 mtDNA) for the first time. All identified species of cestodes and the nematode were registered for the first time in intermediate hosts in Ukraine, although they have been recorded elsewhere worldwide. The number of helminth species found in the present study is consistent with those found in other regions where the helminths of *Artemia* were studied. For instance, nine species were found in both Kazakhstan and Spain (Maksimova, 1989; Georgiev *et al.*, 2005, 2014; Sánchez *et al.*, 2013), seven species in France (Robert & Gabrion, 1991; Sánchez *et al.*, 2012) and the UAE (Sivakumar *et al.*, 2020), and five species in the United States (Redón *et al.*, 2015b).

Notably, our study did not detect any species of the genus *Flamingolepis* Spasskii et Spasskaya, 1952, which are considered typical helminths of brine shrimp and have been found in studies from Italy (Mura, 1995), Spain (Amat *et al.*, 1991b; Varó *et al.*,

2000; Georgiev *et al.*, 2005, 2007; Sánchez *et al.*, 2013; Redón *et al.*, 2015c), France (Thiéry *et al.*, 1990; Robert & Gabrion, 1991; Sánchez *et al.*, 2012), Algeria (Amarouayache *et al.*, 2009), Kazakhstan (Maksimova, 1973), and the UAE (Sivakumar *et al.*, 2020). This absence is obviously due to the lack of flamingos in our study localities.

The overall prevalence of helminths in *Artemia* from hypersaline waters in southern and eastern Ukraine was 21.9%. This prevalence was lower than those reported for *Artemia* in Spain (up to 51.95%) (Sánchez *et al.*, 2013), France (up to 70.9%) (Sánchez *et al.*, 2012), and the UAE (36.03%) (Sivakumar *et al.*, 2020). Studies in these countries were conducted in protected areas with a high diversity of definitive hosts (birds). In contrast, our research was conducted in water bodies frequently visited by humans – such as resorts in Sloviansk and Crimea – and industrial salt extraction areas in Kherson Oblast, which have lower bird diversity. Anthropogenic habitat alterations and human presence may explain the difference in bird prevalence and diversity between our study and those conducted in protected areas, as human activities can make areas less favourable for bird visits.

Currently, the most accurate method to test whether an adult helminth specimen and a larva belong to the same species is to compare their DNA sequences. This comparison can sometimes be a basis for identifying shared morphological features specific to larval and adult stages. However, this approach can be challenging due to the lack of reliable morphological characters in larval stages, the absence of reference material, or poor species descriptions. Unfortunately, our study did not include adult specimens of cestode species. Nonetheless, we were able to identify the metacestode species based on morphological features that are identical in both adult and larval stages, as is known from studies on the life cycles of specific species (Maksimova, 1989). These features include the size, shape and number of rostellar hooks (Spasskaya, 1966; Spasskaya & Spassky, 1978). A search in GenBank revealed a very limited number of nucleotide sequences for the cestode species we studied, reflecting a gap in research on cyclophyllidean cestodes (Waeschenbach & Littlewood 2017). This lack of genetic data highlights the need for further molecular studies of the taxa.

Another issue in the molecular identification of helminth species is that some sequences are submitted to GenBank under specific scientific names without accompanying morphological data for the specimens from which they were obtained in related publications. This is particularly problematic when the scientific name is being introduced into the database for the first time because the lack of morphological confirmation leads to inaccuracies, requiring additional efforts to correct. We encountered this problem when analysing sequences of the nematode larvae. The 18S rDNA gene region in the two nematode larvae we sequenced is identical. The GenBank BLAST search service found a nearly identical sequence (EF180064) differing from ours only in a single nucleotide position. The authors of that sequence (Nadler *et al.*, 2007) reported that it was obtained from a specimen of *Echinuria borealis* Mawson, 1956 parasitising *Somateria mollissima* L. Since the 18S rDNA gene is highly conserved, the minor difference indicates very close phylogenetic relationships between our samples and the sequence from GenBank. According to BLAST results, we should classify the nematodes we examined as *E. borealis* or at least within the genus *Echinuria*. However, we have some doubts preventing us from doing so. The reason for doubt is that third-stage larvae of the family Acuariidae typically have well-formed cephalic structures (Anderson, 2000; Smogorzhevskaya, 1990). We did not observe these structures in our specimens. Considering the

sequence similarity and our results of morphological examination, we have chosen to identify our specimens only at the family level for now. To achieve a more accurate identification based on sequencing data, further research is needed to compare the morphology of adult nematodes with the sequences we have obtained.

Overall, studies that provide detailed morphological and molecular information for distinguishing helminth species and contribute verified sequences to public genetic databases are vital for enhancing our understanding of helminth systematics. Such studies are also essential for ecological investigations of parasites as the advancements in next-generation sequencing technologies create new opportunities to study helminths (Thomas *et al.*, 2022), especially for monitoring purposes. Robust genetic databases with comprehensive reference sequences are essential for the broader implementation of these advanced methods.

Competing interest declaration. The authors declare that they have no conflicts of interest.

Ethical standard. Not applicable.

Statement on the use of AI-assisted technologies. During the preparation of this article, the authors used Grammarly (https://www.grammarly.com/) to correct grammatical errors and improve readability. After using this tool, the authors reviewed and edited the content as needed. The authors take full responsibility for the content of the published article.

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