



## Parasitological Investigation of *Artemia parthenogenetica*: A Cestode Case from Ayvalık Saltwork

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Received: 24.02.2023

Accepted: 11.03.2024

Published: 19.03.2024

How to cite: Dinçtürk, E. & Tanrikul, T.T. (2024). Parasitological investigation of *Artemia parthenogenetica*: A cestode case from Ayvalık Saltwork. *J. Anatolian Env. and Anim. Sciences*, 9(1), 94-99. <https://doi.org/10.35229/jaes.1442106>

Atf yapmak için: Dinçtürk, E. & Tanrikul, T.T. (2024). *Artemia parthenogenetica*'nın parazitolojik incelenmesi: Ayvalık Tuzlası'nda bir sestod olgusu. *Anadolu Çev. ve Hay. Dergisi*, 9(1), 94-99. <https://doi.org/10.35229/jaes.1442106>

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**Abstract:** One of the primary food supplies for the aquaculture sector and wild animal populations, such as flamingos (*Phoenicopterus roseus*), is *Artemia* (*Artemia parthenogenetica*). Along with the rise in cases and reports from different salterns in recent years, the prevalence of *Artemia* cestode parasitism has also grown. *Flamingolepis liguloides* eggs are consumed by *Artemia* and transform into cysticercoids which mature into adult worms in the final host, flamingos. We described the first investigation of cestode parasitism in the natural populations of *Artemia parthenogenetica* from the Ayvalık saltwork (Balıkesir, Türkiye) in this study. A total of 965 *A. parthenogenetica* were examined and 320 of them had *F. liguloides* infection. The overall prevalence was estimated to be 33.2%. Most of the parasites were found in the abdomen and intestinal tract of *A. parthenogenetica*. Parasitism intensity ranged from 1 to 4 per individual, with a maximum of 4 cysticercoids detected in a single *Artemia* sample. To understand the influence of parasitism on *Artemia* populations and the transmission of species, it is important to establish *Artemia* parasitism in the saltwork. This initial report from the Ayvalık saltwork provides information on the infestation of *F. liguloides* in *Artemia*; therefore, the interactions between the saltworks' species should be investigated in depth.

**Keywords:** Cestode, parasite, saltwork, artemia, transmission.

## *Artemia parthenogenetica*'nın Parazitolojik İncelenmesi: Ayvalık Tuzlası'nda Bir Sestod Olgusu

**Öz:** Su ürünleri yetiştiriciliği sektörü ve flamingolar (*Phoenicopterus roseus*) gibi yabani hayvan popülasyonları için temel gıda kaynaklarından biri *Artemia*'dir (*Artemia parthenogenetica*). Son yıllarda farklı tuzlalardan gelen vaka ve raporların artmasıyla birlikte *Artemia* sestod parazitizminin yaygınlığı arttığı görülmektedir. *Flamingolepis liguloides* yumurtaları *Artemia* tarafından tüketilir ve son konakçı flamingolarda yetişkin solucanlara dönüşen sistiserkoidlere dönüşür. Bu çalışma Ayvalık tuzlasındaki (Balıkesir, Türkiye) *A. parthenogenetica*'nın doğal popülasyonlarındaki sestod parazitizminin ilk araştırmasıdır. Toplam 965 *A. parthenogenetica* incelendi ve bunların 320'sinde *F. liguloides* enfeksiyonu görüldü. Genel prevalans %33,2 olarak hesaplandı. Parazitlerin çoğu *A. parthenogenetica*'nın toraks, karın ve bağırsak kanalında bulundu. Parazit yoğunluğu birey başına 1 ile 4 arasında değişmekle birlikte tek bir *Artemia* örneğinde maksimum 4 sistiserkoid tespit edildi. Ayvalık tuzlasından bildirilen bu ilk rapor, *Artemia*'da *F. liguloides* istilası hakkında bilgi vermektedir ve tuzlalardaki türler arasındaki etkileşimler ayrıntılı şekilde araştırılmalıdır.

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**Anahtar kelimeler:** Sestod, parazit, tuzla, artemia, bulaşma.

### INTRODUCTION

A group of obligatory helminth endoparasites known as cestodes have several host life cycles and transmit trophic levels through interactions between predators and prey and the final host are mammals, birds or fish (Redon et

al., 2024). In hypersaline ecosystems, the most sufficient and prevalent cestodes infect especially the Greater Flamingo (*Phoenicopterus ruber*), which is the most abundant final host (Sanchez et al., 2013a). The most widespread genus, *Flamingolepis* (Cyclophyllidea, Hymenolepididae) is

transmitted by *Artemia* species as an intermediate host (Amarouayache et al., 2009).

The microcrustacean known as *Artemia* or brine shrimp (subphylum Crustacea; class Brachiopoda; order Anostraca), which is a non-selective filter feeder that tolerance to a broad range of salinity (5 to 250), is also referred to as euryhaline, and temperature (6 to 35 °C) is the primary trait of this group (Nunes et al., 2006; Oliveira & Vaz, 2018; Serrao & Marques-Santos, 2023). When marine fish and crustaceans are in the larviculture stage, its newly produced Nauplius larvae are vital sources of live food (Van Stappen et al., 2020). Depending on the environmental conditions, a female *Artemia* individual has the ability to switch reproductive modes, including parthenogenetic cyst production (Gajardo & Beardmore, 2012; Dong et al., 2023). Divided into six widely recognized bisexual species and numerous parthenogenetic populations, *Artemia* shares common characteristics like a short life cycle, high adaptability to unfavorable environmental conditions, high fecundity, a bisexual/parthenogenetic reproduction strategy (with the production of nauplii or cysts), a small body size, and adaptability to a variety of nutrient resources due to its non-selective filter feeding behavior (Nunes et al., 2006).

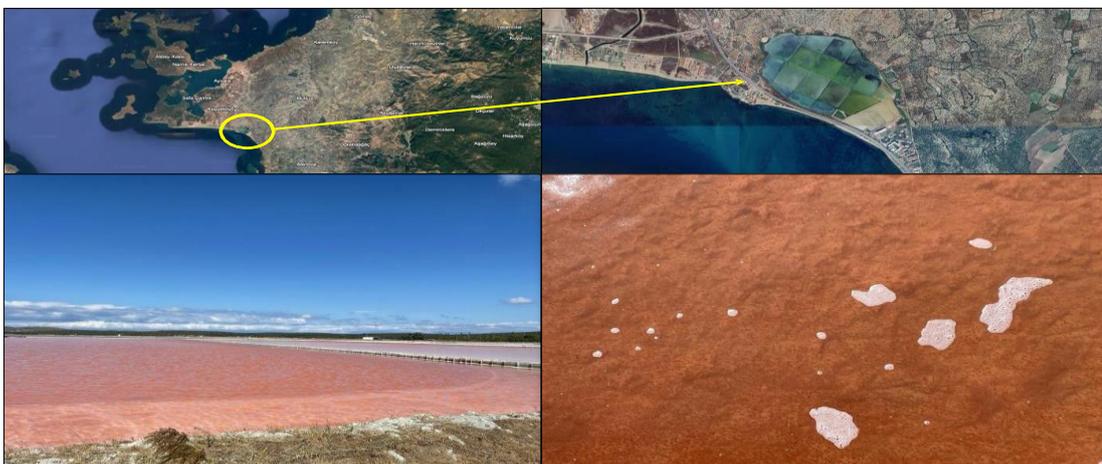
Besides the common use in the aquaculture industry, *Artemia* has a key role in hypersaline ecosystems as controlling the primary production by filter feeding behaviour and becoming a food source for several aquatic bird species (Sanchez, Green & Castellanos, 2006; Varo et al., 2011; Sanchez et al., 2016; Redon et al., 2019). *Artemia* has several adaptable characteristics to the environment such as being a non selective filter feeder, short generation time, long lifespan, and high fecundity, which make brine shrimps ideal for aquaculture use (Sivaji, 2016). Brine shrimp are also the main components of the flamingo diet and have several waders in saline aquatic habitats and during evolution, the relationships between these birds and their cestodes and *Artemia* as intermediate hosts were established (Schuster, 2018). It is also crucial to the food web in

ecosystems because it establishes intricate biotic interactions at several trophic levels, such as host-parasite and predator-prey relations (Redon et al., 2020). Therefore, it is important to detect the potential of *Artemia* to carry pathogens between themselves and other crustacean species (Wang et al., 2020; Dong et al., 2023). Up to now, 22 different species and undiscovered parasitic forms use *Artemia* as an intermediary host (Kornychuk et al., 2023). The most prevalent parasite of *Artemia* species is *Flamingolepis liguloides* (Cestoda, Hymenolepididae) cysticercoids, because this host is an important part of the food of definitive hosts, such as flamingos and several other water birds (Sanchez et al., 2006; Sanchez et al 2016a; Sanchez et al., 2016b; Redon et al., 2021; Kornychuk et al., 2023).

In this study, *Flamingolepis liguloides* cestodes were determined in *A.parthenogenetica* individuals from Ayvalık saltworks (Balıkesir, Türkiye) and this is the first detailed investigation of this cestode infestation with clinical and pathological findings from *Artemia* individuals. The intensity, abundance, and prevalence were calculated and the clinical effect on *Artemia* individuals was observed.

## MATERIAL AND METHOD

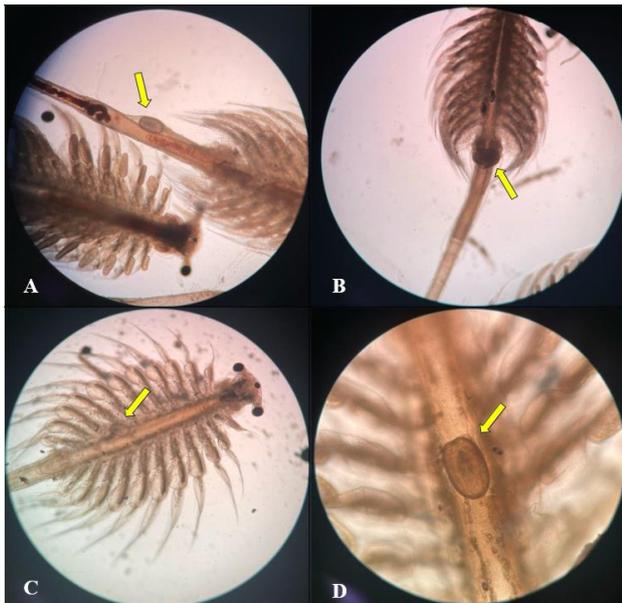
The studied population of *Artemia parthenogenetica* were collected from Ayvalık saltworks through an annual survey and the parasitic infestation was detected for the first time from this location (Figure 1). The study area has Mediterranean climate and waterbird community dominated by flamingos (*Phoenicopterus ruber*). *Artemia* samples were collected with mesh plankton net (125 µm), were washed with sterile physiological saline water and directly fixed in 3% formalin and preserved in 70% ethanol. The samples were examined with light microscope (Olympus CX22RFS1) and parasite individuals were counted. Each individual was studied under a microscope for the presence of cestodes. The identification of parasites was performed according to Georgiev et al. (2005).



**Figure 1.** Sampling area (Ayvalık saltworks / Balıkesir / Türkiye).

## RESULTS

This study is the first investigation of the cestode infestation of *A. parthenogenetica* from Ayvalık saltwork. The parasite was identified as *F. liguloides* from the samples collected from the saltwork. Only *F. liguloides* was determined in all *A. parthenogenetica* individuals, and the majority of the parasites were found in the brine shrimp's abdomen, particularly in the area close to the midgut and hindgut (Figure 2).



**Figure 2.** *F. liguloides* cestodes from different body parts of *A. parthenogenetica* individuals.

The temporal dynamics of cestode infection especially indicate higher prevalence in summer months and *F. liguloides* was detected at highest densities in July - August period. A total of 965 *A. parthenogenetica* were examined and 320 of them had *F. liguloides* infection. The overall prevalence was estimated to be 33.2%.

The fully developed oval shape cysts were determined from *Artemia* individuals. The identification of these cestodes were determined by the rostellar hooks in these oval cysts. In the anterior part, calcareous corpuscles were observed with species specific 8 rostellar hooks (Figure 3).



**Figure 3.** The rostellar hooks of *F. liguloides* cysticercoids.

The intensity of the parasitism was varied between one to four per individual. The maximum number of cysticercoids were detected as four for one individual (Figure 4).



**Figure 4.** Multiple cestode presence in *A. parthenogenetica*.

## DISCUSSION AND CONCLUSION

A comparative examination about cestode parasitism of *A. parthenogenetica* was studied and description of the infestation was reported in detail in this current research. It is difficult to understand the dynamics of a parasite's relationship with its host when the parasite has a complex life cycle (Sanchez et al., 2013b). Also, the diagnosis of parasitic infestations is important for both wild and cultured species that affect economic and ecologic losses (Schuster, 2018; Balta et al., 2019; Balta, 2020). In this case, flamingos (*P. ruber*) is the final host of *F. liguloides* cestodes in Ayvalık saltwork and the cestode parasitism spread through the feces, however *Artemia* plays the role of an intermediate host of the infestation.

In hypersaline habitats, *Artemia* spp. can be considered important hosts of cestodes; it is reported to serve as intermediate host in the life cycles of 22 different cyclophyllidean cestode species (Redon et al., 2020). *Artemia* are infected by ingestion of cestode eggs released into the water along with the final host's feces, the newly spawned oncosphere (the embryo of tapeworms) enters the hemocoel and develops into a cysticercoid (the larvae stage). When birds feed on infected *Artemia*, they get the disease agent through trophic transmission, and the parasite completes their life cycle in the intestines of birds. In the intestine of birds, adult tapeworms mature and multiply, finishing their life cycle (Sanchez et al., 2013b). And as being the main phytoplankton consumer in hypersaline waters, *Artemia* spp. are an intermediate host of most

parasite species (Jia et al., 2015; Kornyychuk et al., 2023). Besides, the frequency of avian cestodes in brine shrimps may be influenced by the seasonal presence of definitive hosts at specific hypersaline wetlands, according to earlier research conducted in the Western Mediterranean and North America (Georgiev et al., 2005; Georgiev et al., 2014; Sánchez et al., 2013; Redón et al., 2015a; Redon et al., 2019). Considering that the majority of their definitive hosts are migrating birds, these observations are compatible with their extensive geographic spread (Redon et al., 2020). Similarly, Koru (2022) concluded that the most abundant *F. liguloides* infection was detected in July which is the most suitable time for presence of flamingos.

The flamingo parasite *Flamingolepis liguloides* (Cyclophyllidea, Hymenolepididae) is a particularly common cestode found in brine shrimps (Sanchez et al., 2013b). Redon et al. (2019) reported cestode infection in *Artemia persimilis* from the Southern Chilean Patagonia during the spring and autumn seasons. The dominant species was recorded as *Flamingolepis sp.* (65% of the total species) and it is underlined that further research are necessary for better understanding the life cycle of parasites and interactions with hosts and other aquatic birds. Georgiev et al. (2005) stated the presence of cestode infections from *A. partenogenetica* and *F. liguloides* was detected with 18.5 % prevalence, 1.48 mean intensity and 0.28 mean abundance records. Similarly, *F. liguloides* was determined from *A. salina* individuals with 5 different cestode species in another study by Georgiev et al. (2007). Amarouayache et al (2009) defined *F. liguloides* parasitism in *A. salina* and the prevalence detected between 10 and 33 % in two different Artemia populations. The infection was mostly detected in the abdomen (95 %) and intensity was reported as 1 to 3 cysticercoids per individual. The prevalence of *F. liguloides* parasitism was calculated as 33.2 % in the total examined population from Ayvalık saltwork and the intensity was varied between one to four per individual. It is apparent that the prevalence and intensity remain in balance in the studied Artemia populations. The enhance in the intensity of parasites were linked to the death of intermediate hosts to be able to transmit the parasite to the final host (Parker et al., 2003; Amarouayache et al., 2009).

In this study, cestodes were mostly determined in the abdomen, especially in the midgut and hindgut of Artemia individuals and only female individuals were found to be infected. Likewise, Sivakumar et al. (2018) determined a distinct increase of parasitism in female individuals in March sampling and explained this situation as the higher occurrence of cysticercoid infection in females could be a greater need for nutrients associated with reproduction, which would lead to a higher intake of

cestode egg more frequently. The parasitism in Ayvalık saltwork was determined in the summer period (July-August), and the results support this proposition.

In conclusion, this study is the first detailed research on *F. liguloides* parasitism from Ayvalık/Balıkesir region with the prevalence, intensity, and clinical findings in *A. parthenogenetica*. The transmission of this cestode parasite should be investigated in detail through the migration route of flamingos and related hypersaline ecosystems to understand the host-parasite interactions comprehensively.

## ACKNOWLEDGEMENTS AND FUNDING

This research was presented at 2nd International Symposium on Fisheries and Aquatic Sciences “SOFAS 2023” as oral presentation. Authors have not received a specific grant, fund, or other support from any funding agency in the public, commercial, or non-profit sectors for this research.

## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this document.

## AUTHOR CONTRIBUTIONS

All authors contributed equally to the article as conceptualization, methodology, research, writing, and editing. All authors approved the final draft.

## ETHICS APPROVAL

No specific ethical approval was necessary for this study.

## DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author on reasonable request.

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