Original Article

Registration of Fish Sanguinicolosis in the Republic of Armenia

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How to Cite This Article Hakobyan, A., Sukiasyan, K., Karapetyan, A., Grigoryan, A., Abgaryan, T., & Nikoghosyan, E. (2026). Registration of Fish Sanguinicolosis in the Republic of Armenia. *Iranian Journal of Veterinary Medicine, 20*(1), 153-166. http://dx.doi.org/10.32598/ijvm.20.1.1005747

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ABSTRACT

Background: The Republic of Armenia is poor in water resources; therefore, fish farming is based on growing freshwater fish in artificial reservoirs. A special feature of the republic's aquaculture is its focus on the breeding and cultivation of carp, as well as some species of predatory fish. Since sanguinicolosis especially affects cyprinid fish, the study of this disease is of great importance in the aquaculture of Armenia.

Objectives: The objective of our study was to assist fish farmers in implementing effective measures for the prevention and treatment of sanguinicolosis, thereby preventing its further spread within the country.

Methods: To identify the causative agent, parasitological and histological examinations were conducted on 200 fish specimens.

Results: The results indicated that 42.5% of the examined fish were infected with sanguinicolosis. The cercariae exhibited a clear localization within the fish tissues, with lengths ranging from 0.16 to 1.6 mm. Morphological changes of a destructive nature were observed in the gills of the studied silver crucian carp specimens. Disruptions in the histological structure of the intestinal wall were also documented. Similar changes in the swim bladder were attributed to the direct negative impact of the parasite.

Conclusion: The decline in fish populations in the pond farm specializing in the cultivation of crucian carp in the Armavir region of the Republic of Armenia is caused by infection with *Sanguinicola* sp. To determine the prevalence of the disease within the republic and identify the specific type of causative agent, it is deemed necessary to expand the scope and nature of the research.

Keywords: Cercariae, Gills, Intestinal vessels, *Sanguinicola* sp., Swim bladder

Article info:
Received: 26 Apr 2025
Accepted: 09 Jul 2025
Publish: 01 Jan 2026

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Introduction

Despite their widespread occurrence globally, blood flukes of freshwater fish remain insufficiently studied. Data on the taxonomy and ecology of sanguinicolids are particularly scarce. The classification of digenean trematodes is challenging due to a lack of genetic data and incomplete descriptions of presumed species (Outa & Avenant-Oldage, 2024). The limited knowledge about these parasites may be attributed to their small size (1–2 mm), transparency, and the ease with which they are lost due to damage to blood vessels during parasitological examination of fish.

Moreover, there is limited information regarding the larval stages of blood flukes in fish, and their intermediate hosts remain largely unknown (Zhokhov et al., 2021). According to Warren and Bullard, the scarcity of data on the parasite's larval stages is attributed to the challenges in identifying larvae, which are often rare in intermediate hosts. In addition to methodological difficulties hindering the study of blood-feeding mollusks, very few parasitologists focus on the study of blood flukes in fish.

Until recently, all blood flukes of fish were classified under the family Aporocotylidae (Odhner, 1912). However, phylogenetic analysis of the 28S rDNA sequences available for blood flukes of marine and freshwater fish has revealed five distinct lineages (Cutmore & Cribb, 2021; Cutmore et al., 2023).

Based on morphological characteristics and genetic data, Warren and Bullard classified blood flukes of fish into five families: Aporocotylidae Odhner, 1912; Sanguinicolidae Poche, 1926; *Chimaerohemecidae* Yamaguti, 1971; *Acipensericolidae*; and *Elopicolidae*. The species within the *Sanguinicolidae* and *Acipensericolidae* families primarily infect freshwater fish (Warren et al., 2020).

Genetic data for adult sanguinicolids are available for only seven species. These include *Sanguinicola volgensis* Rašín, 1929 and *Sanguinicola plehnae* (Truong & Bullard, 2013; Warren & Bullard, 2023), along with two unidentified Sanguinicolidae species from Vietnam: *Pseudosanguinicola occidentalis* (Warren & Bullard, 2023) and an unidentified species from the USA (Warren & Bullard, 2023). Additionally, DNA data are available for cercariae of 12 sanguinicolid species from freshwater gastropods in Australia, East Africa, Poland, and the USA (Zemmer et al., 2020; Preston et al., 2021; Cutmore et al., 2023). However, the identities of some cercariae whose sequences are available remain uncertain.

Sanguinicolosis most commonly affects carp, silver crucian carp, their hybrids, and black amur (Warren et al., 2023).

The development of the causative agent involves one intermediate host: 24 species of gastropod mollusks from 7 families of pond snails. Among these are Limneidae (such as *Lymnea stagnalis*, *Lymnea auricularia*, *Radix ovata*, and *Galba palustris*), in which the parasites undergo the stages of sporocyst, redia, and cercaria. The latter leave the mollusk and attack fish (Zhokhov et al., 2021). The lophocercous-apharyngeate cercariae of *Sanguinicola inermis* are a dangerous parasite infecting freshwater cyprinid fish. It causes serious pathological changes in the final host and significantly reduces the volume of fish production (Richards et al., 1996; Kirk, 2012). Considering the importance of wetlands in the Iranian province of Guilan for wildlife and migratory birds, as well as the number of fish and poultry farms in the area, efforts to combat *Lymnaea gedrosiana* snails are essential for the protection of wildlife and human health (Eslahi et al., 2023). Preserving infected fish makes little sense, and where possible, it is recommended that the affected stocks be completely destroyed and the farm disinfected (Iqbal & Summerville, 2008).

Objective of the study

The present study aimed at identifying the cause of fish mortality in one of the fish farming facilities in the Armavir region of Armenia.

Materials and Methods

The objective of this research was to detect mature *Sanguinicola* species, their eggs, and miracidia in both the blood and internal organs, including the gills of fish, through parasitological and histological methods.

The research was carried out in a fish farming facility in the Armavir region from June to November 2023. The study was prompted by the mass decline in juvenile silver crucian carp populations observed in the facility in June 2023.

Sample collection

Samples were collected from different age groups: Small fish, juveniles, one-year-old, two-year-old, and adult fish. The age of the fish was determined by count-

ing the number of paired opaque and translucent rings on their scales, as well as estimating the development stage of their gonads. Each group included an equal number of fish, and the total number of fish examined was 200 specimens. The fish were transplanted into clean, ventilated containers filled with filtered pond water (Mishanin, 2021).

The specimens underwent a complete parasitological survey (Bykhovskaya-Pavlovskaya, 1985). All organs and tissues of the fish were examined.

Microscopic examination

To detect parasites, the compressor method of diagnosis was used. Organs and tissues were placed on the lower slide of the compressor, covered with another slide, compressed, and examined under a microscope with 10x and 20x objectives and 10x eyepieces. The heart and its associated vascular system, blood, gills, liver, and kidneys were examined for the presence of adult *Sanguinicola* species and their eggs. To detect cercariae, the skin, fins, and gills were examined (Grishchenko et al., 1999). During the examination of the heart, blood, kidneys, liver, and gills, the high permeability of mature *Sanguinicola* species, their eggs, and miracidia made it difficult to obtain clear images (Figures 1B, 1C and 2A).

The contents of the intestine were examined through sequential flushing. In this method, fresh portions of feces are collected, placed in a container, and mixed with water in a 1:5 and 1:10 ratio. The mixture is thoroughly stirred and left to settle for 5 minutes. Then, the upper layer is decanted until the sediment is visible, after which water is added again, and these steps are repeated until the liquid above the sediment becomes clear. Finally, the supernatant liquid is discarded one last time, and the sediment is placed in a Petri dish for examination under a microscope (Golovina, 2010).

Histological examination

The gills, intestines, and swim bladder were fixed in 10% neutral buffered formalin. Properly fixed tissues were processed in the usual manner to obtain paraffin blocks, which were sectioned to a thickness of 4–5 microns (Culling, 1974).

Staining methods

The paraffin tissue sections of the affected fish were stained with hematoxylin and using the Van Gieson method (Sarkisov et al., 1996).

Microscopy image analysis

Images were captured using a phase contrast microscope connected to a computer, with magnifications of 10x, 20x, and 40x. Measurements (in micrometers) were obtained using an ocular micrometer.

Results

The extent of invasion is the ratio of infected animals to the total number of animals examined, expressed as a percentage. The degree of infection in different age groups ranged from 12.5%-77.5%.

Figures 1 and 2 shows the results of the compressor method.

Figure 3 shows the results of sequential flushing of the intestinal contents.

Figure 4 Shows the results of the histological examination of the intestine.

Figure 5 shows the results of the histological examination of the intestine.

Figure 6 shows the results of the histological examination of the swim bladder.

Figure 7 shows the results of the histological examination of the swim bladder.

Figure 8 shows the results of the histological examination of the gills.

Figure 9 shows the results of the histological examination of the gills.

Figure 10 shows the results of the histological examination of the gills.

Figure 11 shows the results of the histological examination of the gills.

Figure 12 shows the results of the histological examination of the gills.

Figure 13 shows the results of the histological examination of the gills."

Table 1. Fish infection rate in different age groups (n=200)

Age groups	Examined Population	Number of Healthy Fish	Number of Infected Fish	Extent of Infection (%)
Fry	40	9	31	77.5
Fingerlings	40	17	23	57.5
One-year-old	40	25	15	37.5
Two-year-old	40	29	11	27.5
Older age	40	35	5	12.5
Total	200	115	85	42.5

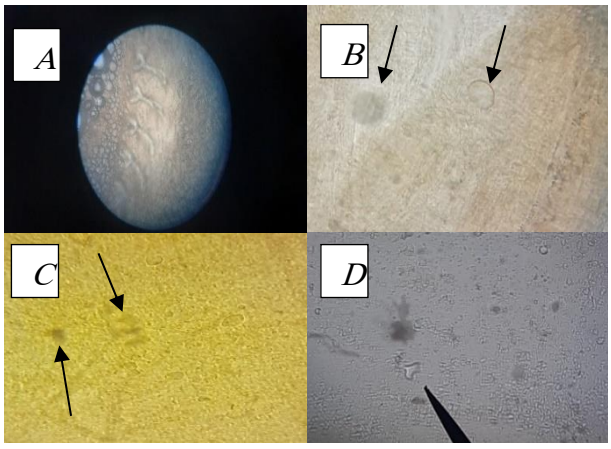

Figure 1. Compressor research method

A) Cercariae in the gill arch; B) Sanguinicola eggs in the blood; C) Eggs and mature Sanguinicola in the blood; D) Eggs in the kidneys Magnification: ×10 and ×10.

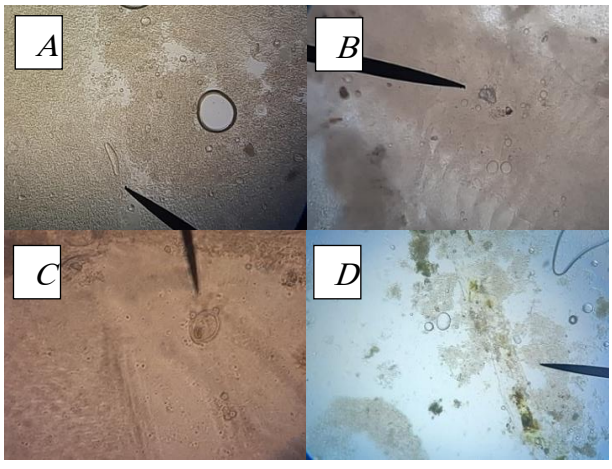

Figure 2. Compressor research method

A) Mature Sanguinicola in the liver; B) Eggs in the gills; C) Miracidia in the heart vessels; D) Miracidia in the blood vessels of the intestine Magnification: A, B, and D: ×10 and ×10; C: ×10 and ×20.

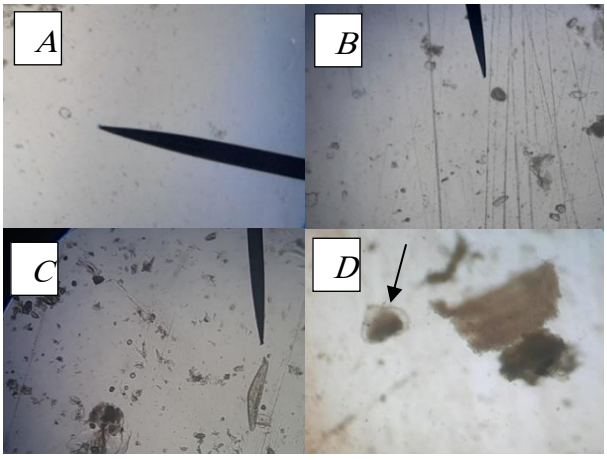

Figure 3. Examination of intestinal contents

A & B) Miracidia in the intestinal contents; C) Mature *Sanguinicola* in the feces; D) Eggs in the feces

Magnification: A, B, and C: ×10 and ×10; D: ×10 and ×20.

Discussion

Out of the 200 fish examined, 85 were infected with sanguinicolosis. Among them, 23 were yearlings, 15 were one-year-olds, and 11 were two-year-olds. The highest mortality rate was recorded among fry (31 individuals), while the lowest mortality rate was observed in adult fish (5 individuals). The results of our study are in line with the data obtained by several other researchers. Infection with *Sanguinicola* sp. can cause significant damage to the fish gills and lead to respiratory distress, reduced growth, and increased mortality, especially in fish fry (Kirk, 2012; Ogawa, 1996; Padrós et al., 2001; Richards et al., 1996; Sommerville, 1991).

The target organ for cercarial penetration was the gills (Figure 1A). There were so many cercariae in the microscope's field of view that it was impossible to calculate their exact number or determine the intensity of the infection. Cercariae were not found in the skin, fins, or other organs of the fish. According to Somerville and Iqbal (Somerville & Iqbal, 1991), cercariae enter their hosts mainly through the gills and skin of the body or fins, especially through the caudal fin. Preference seems to be given to parts with smaller scales. Kua et al. (Kua et al., 2002) concluded that actively floating furcocercous cercariae of *Sanguinicola armata*, with alternating periods of passive buoyancy, infected the final host, white amur fry, by penetrating through the abdominal cavity.

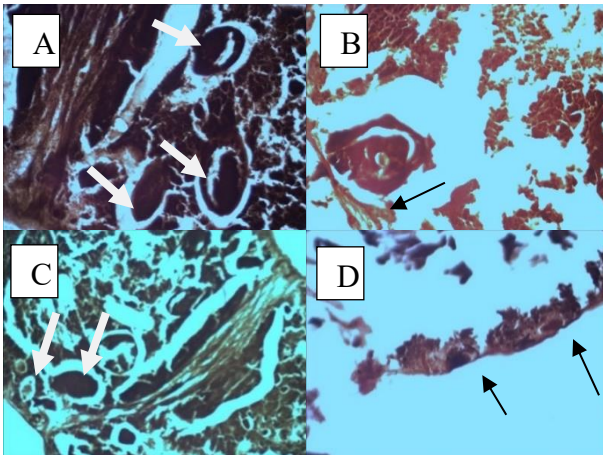

Figure 4. Histological examination of the intestine

A) Miracidia and mature *Sanguinicola* in the lumen of the intestine, B) Blood filling the blood vessels of the intestine; integrity of the serous membrane is compromised; C) Miracidia are visible in the blood vessels of the intestine; damage to the intestinal vessel wall, D) Mature *Sanguinicola* and their eggs are visible in the blood vessels of the intestine; destruction of intestinal villi and crypts in the mucous membrane is evident

Staining: Hematoxylin-eosin (H&E) and the Van Gieson methods. Magnification: ×10 and ×20.

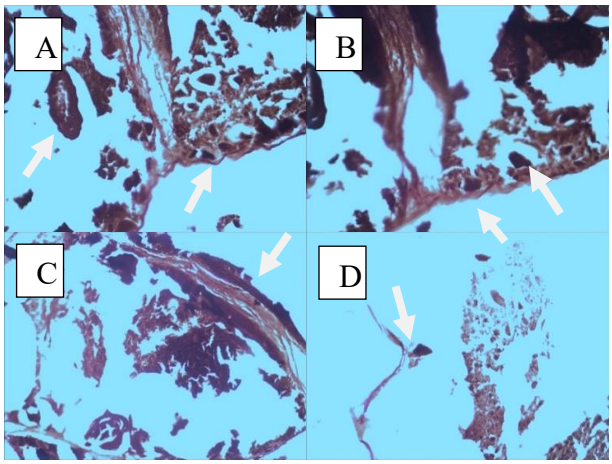

Figure 5. Histological examination of the intestine

A & B) Miracidia and eggs in the blood vessels of the intestine, and mature *Sanguinicola* and eggs in the lumen of the intestine, indicating damage to the intestinal vessel wall and compromise of mucosal integrity; C) Miracidia in the blood vessels of the intestine, showing breakdown of muscle cells in the muscle layer; D) Eggs in the lumen of the intestine, with disruption of the histological structure of the intestinal wall.

Staining: H&E. Magnification: ×10 and ×20.

When examining the skin and fins, only a small number of eggs and miracidia were found. In adult fish, the eggs of *Sanguinicola* were primarily found in the liver and kidneys (Figures 2A and 1D). In fry, yearlings, and two-year-old fish, the eggs were found in the highest numbers in the gills and heart (Figure 2B). Most of the eggs appeared to be newly laid. Miracidia were found in adult fish.

During a compression study, a large accumulation of miracidia was found in the heart vessels and in the lumen of the vessels supplying the intestines. In a parasitological examination of the intestinal contents of eight cru-

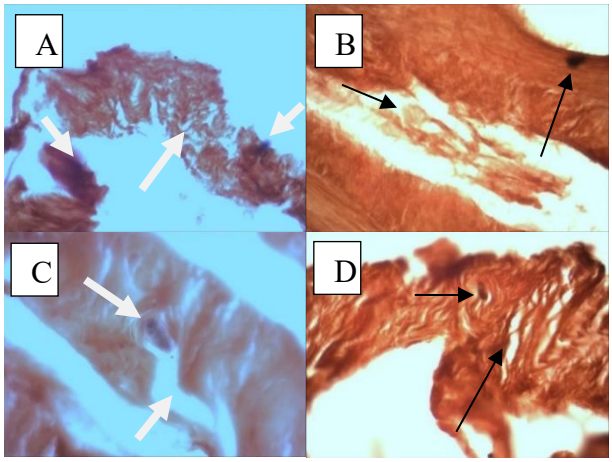

Figure 6. Histological examination of the swim bladder

A) The presence of mature *Sanguinicola*, along with hemorrhages and edema in the wall of the swim bladder; B) Eggs visible in the epithelial layer of the bladder and miracidia in the lumen of the vessel, with dilated and hemorrhaged blood vessels of the swim bladder observed; C) The presence of mature *Sanguinicola* in the blood vessel of the swim bladder, along with disruption of the blood vessel wall integrity, tissue edema in the bladder wall, and separation of the tissue; D) Coexistence of Sanguinicola in the epithelial layer of the swim bladder, with the accumulation of exudate in all layers of the bladder, tissue separation, and necrosis.

Stating: H&E and Van Gieson methods. Magnification: ×10 and ×20.

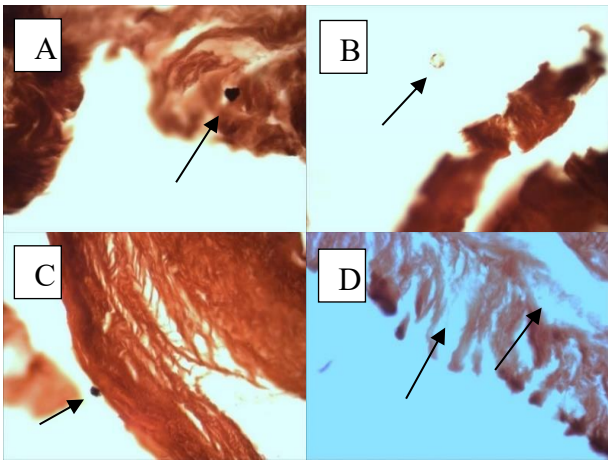

Figure 7. Histological examination of the swim bladder

A) Connective tissue capsule formed around the eggs, B) Miracidia in the cavity of the swim bladder, C) Dilated and hemorrhaged blood vessels of the swim bladder, with miracidia on the epithelial surface of the outer wall of the swim bladder, D) Disruption of the integrity of the blood vessel wall, edema in the swim bladder wall, necrotic changes, and tissue separation Staining: H&E and the Van Gieson method. Magnification: ×10 and ×20.

cian carp using the sequential washing method, mature forms of Sanguinicola, their eggs, and miracidia were detected, with the miracidia displaying clearly visible cilia and pigmented eyespots (Figures 2C, 2D, 3A, 3b, 3C and 3D).

In a study of 85 carp affected by sanguinicolosis, macroscopic changes in the swim bladder were observed in only five individuals. The damage was manifested by thickening of its walls, as well as the presence of dotted and stripe-shaped hemorrhages on the surface. The above changes formed the basis of a histological examination of the gills, intestines, and swim bladder, which revealed the progression from cercarial invasion to helminth migration, maturation, egg-laying, and miracidial hatching.

In the affected intestines of the fish, light microscopy at both medium and high magnification revealed that the structural integrity of the organ wall was disrupted (Figures 4C and 4D). Goblet-shaped cells, which are usually responsible for mucus production, were absent in the intestinal epithelium (Figure 5C). Additionally, the villi

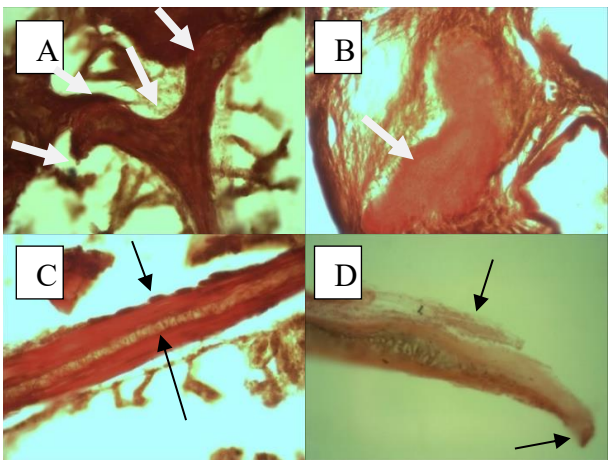

Figure 8. Histological examination of the gills

A) Body of the cercaria attached to the gill arch, showing the circular niche, dorsal fins, and oral sucker; B) Body of the cercaria from the upper position, with the oral sucker visible; C) Tail stalk of the cercaria, with the intestine visible and mature *Sanguinicola* on the upper outer surface; D) Caudal end of the cercaria with furcae visible, a sexually mature *Sanguinicola* is visible along the length of the upper outer surface.

Staining: Van Gieson method. Magnification: ×10 and ×40.

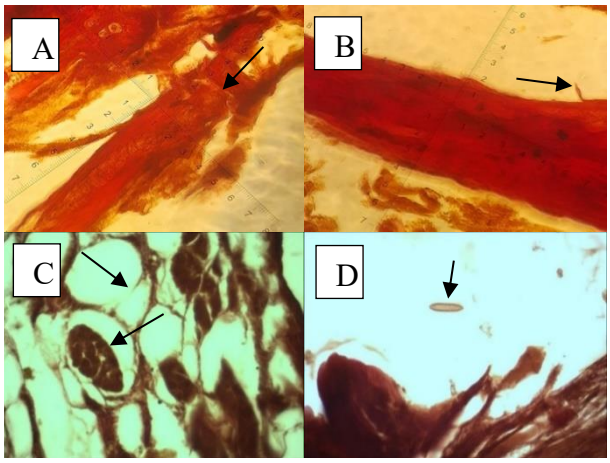

Figure 9. Histological examination of the gills

A) The junction between the body and tail stalk of the cercaria; B) Spines on the tail stalk of the cercaria, C) Sanguinicola in the gill arch, with necrosis of the gill arch and formation of vacuoles; D) Sanguinicola in the gills

Staining: Van Gieson method. Magnification: ×10 and ×40.

and crypts in the mucosal layer were found to be damaged; some were even completely erased (Figures 5A, 5B, and 5D). There were signs of degeneration in the muscle layer. The integrity of the serosal membrane surrounding the intestine was also compromised, contributing to the overall tissue damage.

The intestinal vessels are characterized by disrupted endothelial layers, blood-filled vessels, and the presence of miracidia and trematode eggs (Figures 4B, 4C and D). Mature forms of Sanguinicolus, their eggs and miracidia are also visible in the intestinal lumen (Figures 4A, 5A, 5B, and 5D). Miracidia, mature sanguinicolids, and their eggs can be released into the water through feces. When the miracidia use their sharp stiletto to drill into the walls of the intestinal blood vessels, bleeding occurs. Under pressure, along with the blood flow, sanguinicolids and their eggs enter the intestinal lumen. This process indicates a direct mechanism for the transmission of the parasites from the fish's internal systems into the

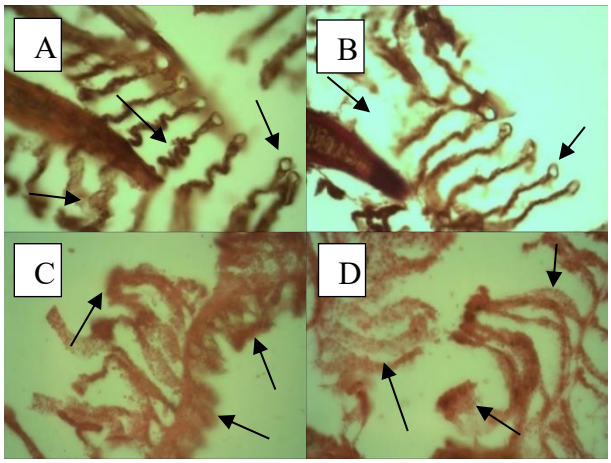

Figure 10. Histological examination of the gills

A) Twisting of respiratory lamellae, with edema of the epithelial petals; B) Growth of respiratory epithelium in the form of "drumsticks," accompanied by necrosis of the lamellae; C) Destruction of gill petals, with edema of the epithelial petals, curvature, and fusion of lamellae; D) Dysplasia and detachment of the epithelial lamellae, with necrosis of lamellae and the presence of mucous cells

Staining: Van Gieson method. Magnification: ×10 and ×40.

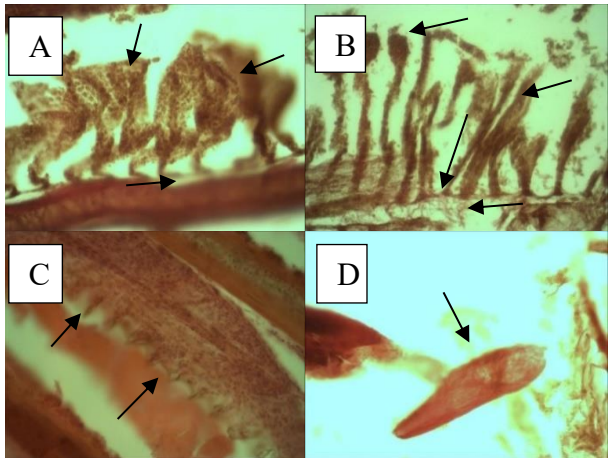

Figure 11. Histological examination of the gills

A) Hyperplasia and fusion of the gill lamellae; B) Club-shaped changes in the shape of the lamellae, with destruction of the cartilaginous elements and vascular layer of the lamellae; C) Constricted secondary filament; D) Presence of mature *Sanguinicola* in the gill filaments

Staining: Van Gieson method. Magnification: ×10 and ×40.

aquatic environment, contributing to the continuation of the parasitic life cycle and further spread of the infection. The ultrastructure of the apical gland, especially the stylet and rodlet complex, is related to the requirement for sanguinicolid miracidia to penetrate the double barrier of fish and snail hosts (McMichael-Phillips et al., 1992).

Histological examination of the swim bladder affected by sanguinicolids revealed microscopic changes in both the anterior and posterior chambers of the bladder. In all cases, the inflammatory processes began in the vascular layer of loose connective and muscle tissue, with simultaneous damage to the epithelium lining both the internal and external surfaces of the bladder (Figure 6A and 6B). The inflammatory process, gradually spreading, affected all layers of the bladder wall. The presence of mature forms of sanguinicolids, their eggs, and miracidia in the vessel lumen led to endothelial hyperplasia and vessel occlusion. The blood vessels supplying the swim bladder became dilated and filled with blood (Figure 6D). The static phenomena contributed to the accumulation of exudate in the loose connective tissue and muscle fibers

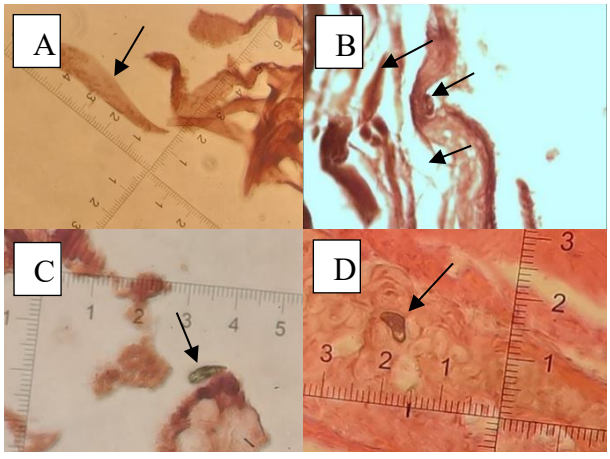

Figure 12. Histological examination of the gills

A) 120 µm long mature lanceolate Sanguinicolia in gills; B) Edema, necrosis, and sloughing of gill arch tissue; mature *Sanguinicola* of gill arch; C) Mature Sanguinicola of the gill arch; D) Mature ova in the gill arch, with miracidia visible

Staining: Van Gieson stain. Magnification ×10 and ×40.

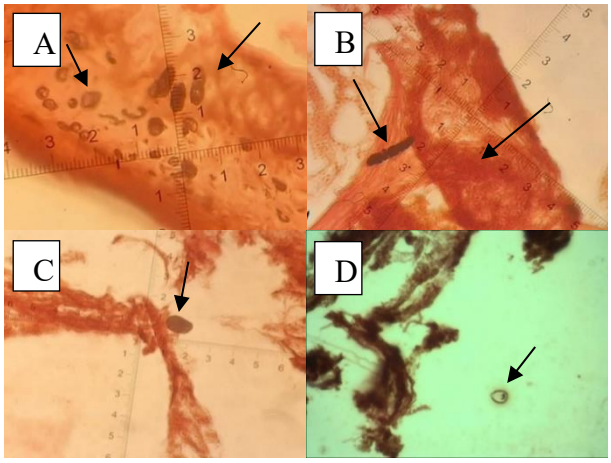

Figure 13. Histological examination of the gills

A) Mature Sanguinicola, eggs, and miracidia in a single field of view under the microscope in the gill arch; B) The body of a cercaria and a mature Sanguinicola on it; C) Miracidia in the gills, with the proboscis visible; D) Miracidia in the gills, with pigmented eyes visible

Staining: Van Gieson method. Magnification: ×10 and ×40.

of the swim bladder wall, causing edema of the tissues, thickening of the wall, and its separation (Figure 6C). By violating the integrity of the vascular wall, migrating miracidia led to ischemia, which in turn led to focal necrosis of both the outer and inner membranes of the swim bladder wall (Figures 6C and 7D). As a result of miracidia migration, they not only infiltrated the cavities of the anterior and posterior chambers of the swim bladder but also damaged collagen fibers and the epithelial tissue of the outer wall, penetrating its surface (Figures 7B and 7C). The silver crucian carp is classified as a fish with an open swim bladder. Therefore, the release of miracidia, found in the chambers of the swim bladder, into the aquatic environment is theoretically possible. However, in practice, unlike the miracidia found in the intestinal lumen, the probability of their release into the external environment is low. This is likely due to the structural and functional characteristics of the swim bladder, which may not facilitate the efficient expulsion of parasites into the water, compared to the digestive system, where parasites can exit more easily through feces.

There are no data in the literature on the presence of sanguinicolids in the swim bladder. Once inside the host, the larvae migrate to the bloodstream, where they develop into adults that release eggs. The eggs trapped in the gills then mature and release miracidia. These miracidia then migrate through the tissues of the host organismHistological examination of the gillsinto the water (Kirk & Lewis, 1992). Blood trematodes are localized in the blood, body cavity, and rarely in other organs (Alama-Bermejo et al., 2011). Sanguinicola platyrhynchi n. sp is parasitic in the vascular system or visceral cavity of freshwater fish (Guidelli et al., 2002).

In fish affected by sanguinicolosis, most of the gill lamellae exhibited edema of the primary gill epithelium, with the intensity being more pronounced in the basal layer (Figure 10C). Hyperplasia of respiratory cells is visible, which often leads to fusion of the gill filaments (Figure 11A). In most fish, the secondary gill filaments were curved and club-shaped (Figure 11B). At the tips of the lamellae, proliferations of the respiratory epithelium were observed in the form of "drumsticks" (Figures 10A and 10B). Edema of the secondary gill epithelium and its detachment were noted, along with a reduction of secondary plates (Figures 10A, 10C, and 10D and 11C). Twisting and fusion of the respiratory filaments were noted, as well as degenerative changes in the gill cartilage itself (Figures 10A, 10 D and 11B). In the gills, destruction and damage to the vessels were observed, leading to extensive hemorrhages and necrosis, as well as the formation of vacuoles in the gill arches (Figure 9C). Foci of necrosis and tissue detachment were present in the gill's liplets and arches, especially at the attachment sites of the cercariae (Figure 12B). This observation matches that of Kirk and Lewis (Kirk & Lewis, 1998).

Histopathological studies of infected carp showed that young and adult Sanguinicola inermis caused mechanical damage to tissues during invasion and migration. Adults may have partially occluded blood vessels and

may have reduced blood circulation. In the initial phase of egg production, eggs and emigrating miracidia in gill tissue caused breakdown of vascular integrity, necrosis, hyperplasia, hemorrhage, and eosinophilic infiltration of epithelial tissue. A granulomatous inflammatory response encapsulates eggs lodged in the gills, visceral sites, and connective tissue, displacing normal tissue.

The eggs of S. armata cause hyperplasia of the primary gill plates. The filaments and secondary plates then fuse together, resulting in chronic hyperplasia (Shaharom-Harrison et al., 2011). Eggs of Sanguinicola inermis are the main cause of pathology in the definitive fish host (McMichael-Phillips et al., 1992).

The cercariae attached to the gill arches were in a dormant state. Their total length ranged from 160 to 1600±2.5 µm, which is approximately 0.16–1.6 mm. The elongated body of the cercarial was divided into two sections: The main body and the tail. A round depression was present at the front of the body. The dorsal surface displayed dorsal fins, while the anterior-lateral end featured an oral sucker (Figures 8A and 8B). The ventral sucker was absent. In the transverse section, the body had a cylindrical shape. The body was connected to the stem, narrowing sharply (Figure 9A). The stalk at its distal end was divided into two short, slightly curved, symmetrical branches (Figure 8D). There were irregularly arranged thorns on the stem (Figure 9B). In longitudinal sections, the intestine was clearly visible, extending along the stalk. It was a well-defined cluster of cells with large light nuclei (Figure 8C). We were unable to identify other structural features of the cercariae. Many researchers have characterized cercariae belonging to the Sanguinicolidae family of bloodsuckers, including features such as an oral sucker modified into a cephalic penetration organ, the absence of a pharynx (apharyngeate), the presence of a dorsal finfold on the body, brevifurcate furcae that are shorter than the tail stem and symmetrical, the presence of dorsoventral finfolds on the furcae, and the absence of a ventral sucker (Simon-Martin & Gomez, 1986; Sendersky & Dobrovolsky, 2004; Schell, 1974). Studies of the freshwater mollusk genus Burnupia (Burnupiidae) from South Africa have shown that 3.10% of specimens were infected with Sanguinicolidae larvae. The four species of Sanguinicolidae found differed in terms of body size, number of penetrating glands, pattern of scaly spines, and relative sizes of fin folds and grooves (Outa & Avenant-Oldewage, 2023).

The sizes of *Sanguinicola* sp. found in the histological preparations ranged from 12 to 120±2.5 µm. Additionally, the *Sanguinicola* species differed in shape depending on the developmental stage. Immature *Sanguinicola* species had an oval shape, while mature ones had a lancet-like shape, concave on the bottom and convex on the top (Figures 9D, 12A-C, 13A, 13B, and 11D). The tail was slightly bent upwards. Along the entire length of the body, lateral spines directed backwards were visible. The mouth sucker was absent. In mature forms, thin spines were visible around the well-developed mouth (Figure 12A). There are six rows of minute, tegmental spines on the most anterior tip of Aporocotyle simplex. Occasional cilium-like structures and different types of bulbs, with or without apical cilium-like structures, all presumed to be sensory receptors, are found on different parts of the body (Thulin, 1980). The body of S. volgensis has serrations on the sides, with bristles measuring 0.038 mm in length at the tops of these serrations (Warren et al., 2023). Scanning electron microscopy of the surface relief and transmission electron microscopy revealed shell details and confirmed the absence of spines in *S. inermis* (Iqbal & Sommerville, 2008). According to studies by Poddubnaya et al. (Poddubnaya et al., 2023), *S. volgensis* has a pharynx but lacks a mouth sucker.

At different stages of development, the sizes of the eggs and miracidia were also different. The eggs were triangular, measuring 3.7–37±2.5 µm. Miracidia were observed in mature eggs (Figure 12D). The size of the hatched miracidia varied between 3 and 30±2.5 µm, and they were covered with cilia on the outside. The pigmented eyes and the proboscis, located at the front of the body, were clearly visible (Figures 13C and D). Many digeneans possess miracidia that use an active strategy for infection. These larvae swim by ciliary action to specific mollusks and penetrate into them (Smirnov & Dobrovolskij, 2019).

In most histological preparations, a connective tissue capsule was visible, formed around the remnants of Sanguinicola, especially their eggs (Figures 6A, 6B and 7A). The encapsulation of eggs and migrating miracidia occurs when they enter the host tissue and are surrounded by inflammatory cells, and are then encapsulated by collagen produced by fibroblasts (Kirk & Lewis, 1998).

A definite pattern was established in the minimum and maximum sizes of cercariae, adult *Sanguinicola*, their eggs, and miracidia. The largest of them was ten times bigger than the smallest.

Conclusion

The decline in the number of silver crucian carp in the pond economy of the Armavir region is a serious issue that requires a comprehensive approach. The parasite *Sanguinicola* sp. not only threatens the aquatic ecosystem but also directly impacts the fish farming economy of the region. Given the importance of this issue, it is essential to take steps to conduct more detailed studies and monitor the situation. Expanding the scope of research will help determine not only the scale of the problem but also the patterns of pathogen spread among different fish populations. Protecting the health of fish populations should become a priority for both government authorities and scientific institutions. Collaboration between fish farmers, researchers, and natural resource management bodies will aid in developing a strategy for preserving biodiversity and ensuring the sustainable development of fish farming.

Ethical Considerations

Compliance with ethical guidelines

The animals used during the research belonged to a private farmer who was fully informed of the research objectives. Sampling procedures were carried out by qualified veterinarians with the explicit consent of the animal owner. All methods used in this research comply with the Armenian regulations on the handling and treatment of domestic animals, veterinary activities, and animal protection.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interests.

Acknowledgments

The authors express their sincere gratitude to the staff and management of the fish farm for their support during the research.

References

Alama-Bermejo, G., Montero, F. E., Raga, J. A., & Holzer, A. S. (2011). Skoulekia meningialis n. gen., n. sp. (Digenea: Aporocotylidae Odhner, 1912) a parasite surrounding the brain of the Mediterranean common two-banded seabream Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817) (Teleostei: Sparidae): description, molecular phylogeny, habitat and pathology. *Parasitology International, 60*(1), 34–44. [DOI:10.1016/j.parint.2010.10.001] [PMID]

Bykhovskaya-Pavlovskaya, I. Ye. (1985). [*Fish parasites: Study manual* (Russian)]. Leningrad: L.: Science. [Link]

Culling, C. F. A. (1974). *Handbook of Histopathological and Histochemical Techniques. (including museum techniques).* London: Butterworths. [Link]

Cutmore, S. C., & Cribb, T. H. (2021). A new order of fishes as hosts of blood flukes (Aporocotylidae); description of a new genus and three new species infecting squirrelfishes (Holocentriformes, Holocentridae) on the Great Barrier Reef. Un nouvel ordre de poissons comme hôtes de digènes du sang (Aporocotylidae) ; description d'un nouveau genre et de trois nouvelles espèces infectant les poissons-écureuils (Holocentriformes, Holocentridae) de la Grande Barrière de Corail. *Parasite (Paris, France), 28*, 76. [DOI:10.1051/parasite/2021072] [PMID]

Cutmore, S. C., Littlewood, D. T. J., Arellano-Martínez, M., Louvard, C., & Cribb, T. H. (2023). Evidence that a lineage of teleost-infecting blood flukes (Aporocotylidae) infects bivalves as intermediate hosts. *International Journal for Parasitology, 53*(1), 13–25. [DOI:10.1016/j.ijpara.2022.09.007] [PMID]

Eslahi, A. V., Aligolzadeh, A., Pirestani, M., Gharibi, Z., Abdoli, A., & Hatam-Nahavandi, K., et al. (2023). Trematode Cercariae from Lymnaea gedrosiana in the Caspian Sea Littoral in Iran: a one health concern. *Frontiers in Microbiology, 14*, 1222599. [DOI:10.3389/fmicb.2023.1222599] [PMID]

Golovina, N.A. (2010). [*Ichthyopathy* (Russian)]. Moscow: Kolos.

Grishchenko, L. I., Akbaeva, M. Sh., Vasilkova, G. V. (1999). [*Diseases of fish and fisheries* (Russian)]. Moscow: Kolos. [Link]

Guidelli, G. M., Isaac, A., & Pavanelli, G. C. (2002). Sanguinicola platyrhynchi n. sp. (Digenea: Sanguinicolidae) parasite of visceral cavity of Hemisorubim platyrhynchos (Valenciennes, 1840) (Pisces: Pimelodidae) from the floodplain of the upper Paraná River, Brazil. *Brazilian Journal of Biology = Revista brasleira de Biologia, 62*(4B), 801–806. [DOI:10.1590/s1519-69842002000500009] [PMID]

Iqbal, N. A. M., & Summerville, C. (2008) Effects of Sanguinicola inermis Plehn, 1905 (Digenea: Sanguinicolidae) infection on growth performance and mortality in carp, Cyprinus carpio L. *Aquaculture Research, 17*(2), 117 -122. [DOI:10.1111/j.1365-2109.1986.tb00092.x]

Kirk, R. S. (2012). Sanguinicola inermis and related species. Home Aquaculture. In P. T. K. Woo & K. Buchmann (Eds.), *Fish parasites: pathobiology and protection* (270-281). Wallingford UK: CABI. [DOI:10.1079/9781845938062.0270]

Kirk, R. S., & Lewis, J. W. (1992). The laboratory maintenance of Sanguinicola inermis Plehn, 1905 (Digenea: Sanguinicolidae). *Parasitology, 104* (Pt 1), 121–127. [DOI:10.1017/S0031182000060868] [PMID]

Kirk, R. S., & Lewis, J. W. (1998). Histopathology of Sanguinicola inermis infection in carp, Cyprinus carpio. *Journal of Helminthology, 72*(1), 33–38. [DOI:10.1017/s0022149x00000948] [PMID]

Kua B.C., Shaharom F., Jambali A. (2002) Life cycle of the blood fluke Sanguinicola armata Plehn, 1905 (Digenea: Sanguinicolidae), parasite of freshwater fishes in Malaysia. In C. R. Lavilla-Pitogo & E. R. Cruz-Lacierda (Eds.), *Diseases in Asian aquaculture IV: Proceedings of the Fourth Symposium on Diseases in Asian Aquaculture, 22-26 November 1999, Cebu City, Philippines* (269-277). Bethesda: Fish Health Section. [Link]

McMichael-Phillips, D. F., Lewis, J. W., & Thorndyke, M. C. (1992). Ultrastructural studies on the miracidium of Sanguinicola inermis (Digenea: Sanguinicolidae). *Parasitology, 105*(3), 435-443. [DOI:10.1017/S0031182000074618]

McMichael-Phillips, D. F., Lewis, J. W., & Thorndyke, M. C. (1992). Ultrastructure of the egg of Sanguinicola inermis Plehn, 1905 (Digenea: Sanguinicolidae). *Journal of Natural History, 26*(5), 895-904. [DOI:10.1080/00222939200770541]

Mishanin, Yu.F. (2021). [*Ichthyopathy and veterinary and sanitary examination of fish* (Russian)]. St.Peterburg: Lan.

Ogawa, K. (1996). Marine parasitology with special reference to Japanese fisheries and mariculture. *Veterinary Parasitology, 64*(1-2), 95–105. [DOI:10.1016/0304-4017(96)00962-4] [PMID]

Outa, J. O., & Avenant-Oldewage, A. (2024). Underreported and taxonomically problematic: Characterization of sanguinicolid larvae from freshwater limpets (Burnupiidae), with comments on the phylogeny and intermediate hosts of sanguinicolids. *Parasitology, 151*(1), 108–124. [DOI:10.1017/S003118202300121X] [PMID]

Padrós, F., Zarza, C., & Crespo, S. (2001). Histopathology of cultured sea bream Sparus aurata infected with sanguinicolid trematodes. *Diseases of Aquatic Organisms, 44*(1), 47–52. [DOI:10.3354/dao044047] [PMID]

Poddubnaya, L.G., Warren, M. B., & Bullard, S. A. (2023) Foregut ultrastructue of adult Sanguinicola Volgensis (Rasin, 1929) Mcintosh, 1934 (Digenea:Aporocotylidae). *Journal of Parasitology, 109*(1), 27-34. [DOI:10.1645/22-79]

Preston, D. L., Layden, T. J., Segui, L. M., Falke, L. P., Brant, S. V., & Novak, M. (2021). Trematode parasites exceed aquatic insect biomass in Oregon stream food webs. *The Journal of Animal Ecology, 90*(3), 766–775. [DOI:10.1111/1365-2656.13409] [PMID]

Richards, M., Côrte-Real, H., Forster, P., Macaulay, V., Wilkinson-Herbots, H., & Demaine, A., et al. (1996). Paleolithic and neolithic lineages in the European mitochondrial gene pool. *American Journal of Human Genetics, 59*(1), 185–203. [PMID]

Sarkisov, D. S., Perov, Yu. L. (1996). [*Microscopic technique. Guide for doctors and laboratory workers* (Russian)]. Moscow; Medicine.

Schell, S. C. (1974). The life history of Sanguinicola idahoensis sp. n. (Trematoda: Sanguinicolidae), a blood parasite of steelhead trout, Salmo gairdneri Richardson. *The Journal of Parasitology, 60*(4), 561–566. [DOI:10.2307/3278706] [PMID]

Sendersky, I.V., Dobrovolsky, A.A. (2004). [*Morphology and heterotaxy of cercaria Sanguinicola Armata (Trematoda: Sanguinicolidae)* (Russian)]. Parasitology, 38, 4. [PMID]

Shaharom-Harrison, F., Seman, N. A., & Suhairi, M. (2011). Laboratory experimental infection of the freshwater snail Gyraulus convexiusculus (Hutton, 1849) and the bighead carp Aristichthys nobilis (Richardson, 1845) with the blood fluke Sanguinicola armata Plehn, 1905. In: M. G. Bondad-Reantaso, J.B. Jones, F. Corsin & T. Aoki (Eds.), *Diseases in Asian Aquaculture VII*. Bangkok: Fish Health Section, Asian Fisheries Society. [Link]

Simon-Martin, F., & Gomez-Bautista, M. (1986). Preliminary SEM observations of the cercaria of a Sanguinicola sp. (Digenea: Sanguinicolidae). *Annales de Parasitologie Humaine et Comparée, 61*(5), 529-531. [DOI:10.1051/parasite/1986615529] [PMID]

Smirnov, P. A., & Dobrovolskij, A. A. (2019). What is hidden under an eggshell? Ultrastructural evidence on morphology of "passive" Prosorhynchus Squamatus Miracidium (Digenea: Bucephalidae). *Invertebrate Zoology, 16*(1), 361-376. [DOI:10.15298/invertzool.16.4.04]

Sommerville, C., & Iqbal, N. A. M. (1991). The process of infection, migration, growth and development of Sanguinicola inermis Plehn, 1905 (Digenea: Sanguinicolidae) in carp, Cyprinus carpio L. *Journal of Fish Diseases, 14*(2), 211-219. [DOI:10.1111/j.1365-2761.1991.tb00590.x]

Truong, T. N., & Bullard, S. A. (2013). Blood flukes (Digenea: Aporocotylidae) of walking catfishes (Siluriformes: Clariidae): New genus and species from the Mekong River (Vietnam) with comments on related catfish aporocotylids. *Folia parasitologica, 60*(3), 237–247. [DOI:10.14411/fp.2013.027] [PMID]

Thulin, J. (1980) Scanning electron microscope observations ofAporocotyle simplex Odhner, 1900 (Digenea: Sanguinicolidae). *Zeitschrift für Parasitenkunde, 63,* 27-32. [DOI:10.1007/BF00927723]

Warren, M. B., Bakenhaster, M. D., Scharer, R. M., Poulakis, G. R., & Bullard, S. A. (2020). A new genus and species of fish blood fluke, Achorovermis testisinuosus gen. et sp. n. (Digenea: Aporocotylidae), infecting critically endangered smalltooth sawfish, Pristis pectinata (Rhinopristiformes: Pristidae) in the Gulf of Mexico. *Folia Parasitologica, 67*, 2020.009. [DOI:10.14411/fp.2020.009] [PMID]

Warren, M. B., & Bullard, S. A. (2023). Systematic Revision of the Fish Blood Flukes with Diagnoses of Chimaerohemecidae Yamaguti, 1971, Acipensericolidae N. Fam., Sanguinicolidae Poche, 1926, Elopicolidae N. Fam., And Aporocotylidae Odhner, 1912. *Journal of Parasitology, 109*(4), 401-418. [DOI:10.1645/23-13]

Warren, M. B., Poddubnaya, L. G., Zhokhova, A. E., Reyda, F. B., Choudhury, A., & Bullard, S. A. (2023). Revision of Sanguinicola Plehn, 1905 with Redescription of Sanguinicola Volgensis (Rašín, 1929) McIntosh, 1934, description of a new species, proposal of a new genus, and phylogenetic analysis. *Journal of Parasitology, 109*(4), 296-321. [DOI:10.1645/23-14]

Zemmer, S. A., Detwiler, J. T., Sokol, E. R., Da Silva Neto, J. G., Wyderko, J., & Potts, K., et al. (2020). Spatial scale and structure of complex life cycle trematode parasite communities in streams. *Plos One, 15*(11), e0241973. [DOI:10.1371/journal.pone.0241973] [PMID]

Zhokhova, A. E., Pugacheva, M. N., & Poddubnaya, L. G. (2021). Freshwater Trematodes Sanguinicola (Digenea: Aporocotylidae) in Europe: Distribution, Host Range, and Characteristics of Fish and Snail Infestation (Review). *Inland Water Biology, 14*(3), 301-315. [DOI:10.1134/S1995082921020164]

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