FIRST RECORD OF *ACANTHOCEPHALUS LUCII* (MÜLLER, 1776) LÜHE, 1911 (PALEACANTHOCEPHALA, ECHINORHYNCHIDAE) IN MINNOW (*PHOXINUS LUMAIREUL* LINNAEUS, 1758) FROM BELČIŠTA WETLAND (NORTH MACEDONIA)

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ABSTRACT

In our study, a total of 33 specimens of three cyprinid fish *(Squalius squalus, Phoxinus lumaireul*, and *Pelasgus minutus)* from Belčišta wetland (south-west Macedonia) were subjected to a parasitological investigation, by season. One specimen of *Acanthocephalus lucii* was found in autumn, in intestines of minnow (*Phoxinus lumaireul* Linnaeus, 1758). The record of *Acanthocephalus lucii* in minnow is considered as the first in N. Macedonia. At the same time, minnow represent a new host for this parasite worldwide.

RÉSUMÉ: Première mention de l'espèce *Acanthocephalus lucii* (Muller, 1776) Lühe, 1911 (Paleacanthocephala, Echinorhynchidae) chez le vairon (*Phoxinus lumaireul* Linnaeus, 1758) dans le Marais de Belčišta (Macédoine de Nord).

Durant notre étude, nous avons effectué des analyses parasitologiques saisonnières sur 33 individus appartenant à trois espèces de cyprinidés *(Squalius squalus, Phoxinus lumaireul* et *Pelasgus minutus)* du Marais de Belčišta (au sud-ouest de la Macédoine). Un exemplaire de *Acanthocephalus lucii* a été identifié durant l'automne dans les intestins d'un vairon (*Phoxinus lumaireul* Linnaeus, 1758). Celle-ci est la première mention de l'espèce *Acanthocephalus lucii* chez le vairon en Macédoine de Nord. Aussi, le vairon est la première mention en tant que nouvel hôte pour ce parasite au niveau mondial.

REZUMAT: Prima menționare a speciei *Acanthocephalus lucii* (Muller, 1776) Lühe, 1911 (Paleacanthocephala, Echinorhynchidae) la boiștean (*Phoxinus lumaireul* Linnaeus, 1758) în zona umedă Belčišta (Macedonia de Nord).

În lucrarea de față au fost supuse analizei parazitologice sezoniere un număr de 33 de indivizi din trei specii de ciprinide (*Squalius squalus, Phoxinus lumaireul* și *Pelasgus minutus)* din zona umedă Belčišta (sud-vestul Macedoniei). Un specimen de *Acantocephalus lucii* a fost găsit toamna în intestinul unui boiștean (*Phoxinus lumaireul* Linnaeus, 1758). Menționarea speciei *Acantocephalus lucii* la boiștean este considerată o premieră pentru Macedonia de Nord. Totodată, boișteanul reprezintă o gazdă nouă pentru acest parazit la nivel mondial.

INTRODUCTION

Belčišta wetland (also called Sini Viroj) is located in the municipality of Debrca, below Ilinska Mountain, at an altitude of about 767 m. It is a remnant of the former Desaret Lake, which flooded the Debrca Valley in the Pliocene. The wetland is fed with 11 karstic springs, known as Sini Viroj, which originate from the springs north and northeast of the village of Novo Selo, in the direction of the village of Belčišta. The surface of the wetland covers around 400 hectares. There are also several lakes in the wetland, three of which are larger: Belčiški Sin Vir, Novoselski Sin Vir 1, Novoselski Sin Vir 2, and Sino Duvlo. With the retreat of the Desaret Lake along the Sateska River, numerous endemic species of plant and animal life continued to exist in the waters of the wetland. Belčišta wetland is the largest and one of the most important wetlands in N. Macedonia, with flooded forests and wet pastures, because it is well preserved. Due to different types of wetland habitats, especially flooded alder forests, there is a relatively high diversity of flora and fauna. In this water ecosystem, according to research, a total of 55 different plant species and seven different fish species have been recorded. There are also nine representatives of birds, nine mammals, nine amphibians, and reptiles, as well as 14 representatives of invertebrates. Belčišta wetland is part of the Emerald National Network of the N. Macedonia, and is proposed to be nominated as a Natura 2000 location (Zoroski, 2022).

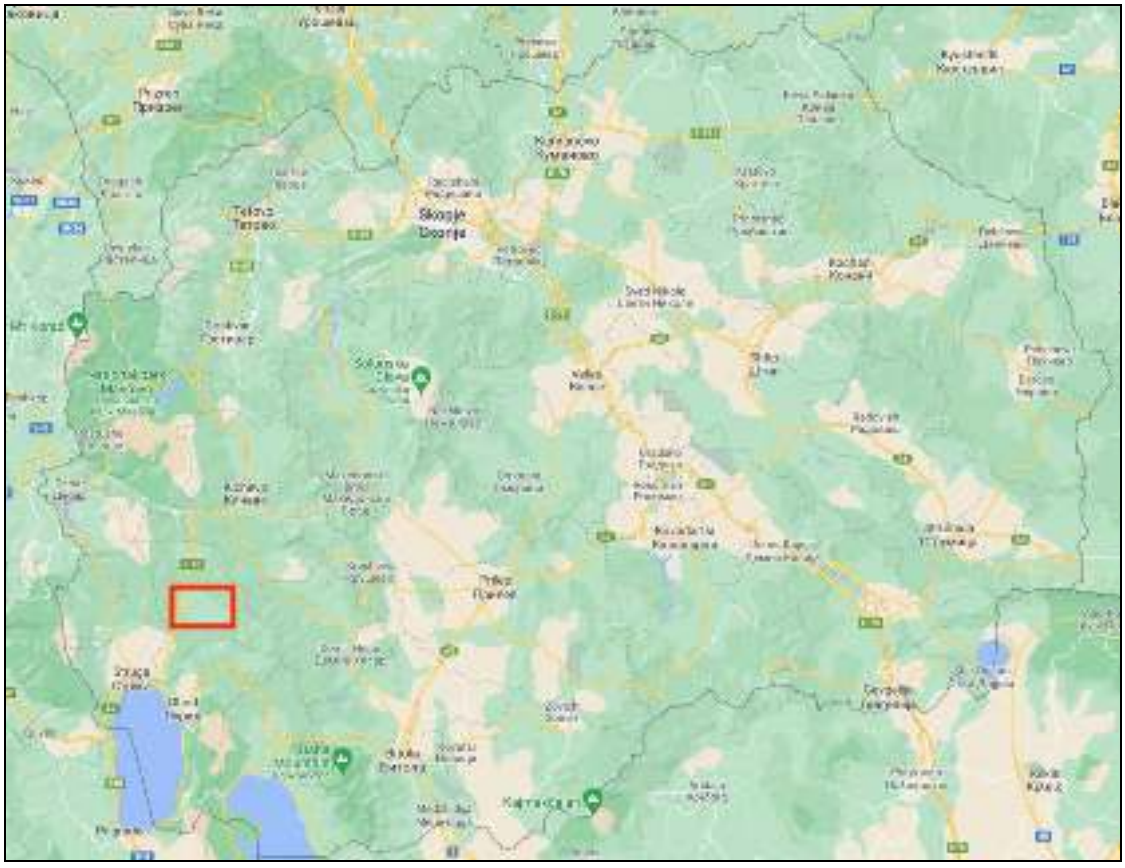

Figure 1: Map of N. Macedonia
– the red square indicates the area of Belčišta wetland.

Figure 2: Belčišta wetland – landscape.

Acanthocephala (spiny-headed worms or thorn-headed worms) are necrotrophic worms that live as adults exclusively within the small intestines. They have a retractable proboscis armed with spines that is inserted into the mucosa as a holdfast. They have separate sexes and a lack of circulatory, respiratory, and digestive systems.

They have an indirect life cycle, which utilizes an arthropod intermediate host. Various decapods and other crustaceans serve as intermediate hosts for those with aquatic life cycles. The most common intermediate host for fish Acanthocephala are amphipod crustaceans of the genus *Gammarus* or isopods of the genus *Asselus.* The intermediate host becomes infected by ingesting ova in fecally contaminated soil, food, water, or by feeding directly on the feces. Within the arthropod, an acanthor hatches from the egg and penetrates the gut wall and enter the haemocoele. There it develops into an acanthella, in which the body wall and rudiments of the internal organs begin to take form. The final larval stage is the cystacanth, which possesses the proboscis of the adult form.

Acanthocephala stick deep into the intestinal wall, causing severe destruction of the intestinal epithelium, with consequent proliferation of connective tissue. Perforation of the intestinal wall can also occur, when the parasites can be found attached to the liver parenchyma. Species from the genus *Acanthocephalus* are even more dangerous, because they cause similar changes and also change the place of fixation. In species with a small proboscis,

only the intestinal epithelium and lamina propria mucosae are damaged, with changes limited to the site of fixation. Signs of the disease include anemia, weight loss, and retardation in the growth and development of the young. Changes caused by acanthocephala can be followed by secondary bacterial infections that cause more severe inflammatory processes, intoxications, and mass deaths. In case of simultaneous infections, the disease also takes a more severe course, with frequent deaths (Stojanovski, 1997).

Acanthocephalus lucii (Müller, 1776) Lühe, 1911 exhibits a typical acanthocephalan life cycle (Schmidt, 1985); Arthropod, in this case an isopod (*Asselus aquaticus*), serves as intermediate host. Adult worms mate in the intestine of fish, and females release eggs into the environment with the feces. Isopods become infected by ingesting eggs. The parasite develops in the isopod to the infective cystacanth stage, and the life cycle is completed when an infected isopod is eaten by an appropriate definitive host (Benesh and Valtonen, 2007). Those authors conducted experimental infection of *A. lucii* in its isopod intermediate host (*Asellus aquaticus*) to investigate host survival and growth throughout the course of parasite development. Isopods exposed to *A. lucii* had reduced survival, but only early in the infection. Mean infection intensity was high relative to natural levels, but host mortality was not intensity dependent.

Dezfuli et al. (2018) investigated pike intestinal tissue response to *A. lucii.* Numerous mast cells (MCs) were seen throughout the mucosa and submucosal layers. In infected and uninfected intestines of pike, MCs were the dominant immune cell type encountered; they are the most common granulocyte type involved in several fish-helminth systems. Immunopositivity of MCs to nine out of 11 antibodies is of great interest and these cells could play an important key role in the host response to an enteric helminth.

Acanthocephalus lucii can be a sensitive bioindicator for lead pollution (Jankovska et al., 2011). They found that lead accumulates in higher concentration in *A. lucii* than in different tissues (liver, gonads, and muscle) of perch. The bioconcentration factors for lead indicated that parasites accumulate metals to a higher degree than fish tissues – lead concentrations in acanthocephalans were 9.32, 19.27, and 55.05 higher than in liver, gonads, and muscles of host, respectively.

MATERIAL AND METHODS

Fish material from a total of 33 specimens of three cyprinid fish from Belčišta wetland (southwest Macedonia) were subjected to a parasitological investigation, by seasons through this year. Only fresh fish were subjected to routine identification, dissection, and observation methods. Cleaned parasites were separated and put in certain fixatives, prepared for determination with determined techniques of staining and clearing (Gussev, 1983; Vasiljkov, 1983). For the collection of acanthocephalan species, intestines of fish were examined using the stereomicroscope “Zeiss Stemi 305” and microscope “Zeiss Primovert” and parasites were removed. For morphological examination, permanent slide of whole individual parasite was prepared by staining with acetocarmine, dehydrating with ascending grades of alcohol and mounting in Canada balsam. Identification was made throughout the morphology of proboscis with hooks and the reproductive system, using referent key for determination (Bauer, 1985).

RESULTS AND DISCUSSION

From Belčišta wetland three cyprinid fish species were investigated parasitologically: chub – *Squalius squalus* (Bonaparte, 1837) – 15 specimens, minnow – *Phoxinus lumaireul* (Schinz, 1840) – 10 specimens and Ohrid' grunče – *Pelasgus minutus* (Karaman, 1924) – eight specimens. We found one specimen of *Acanthocephalus lucii* – female in one minnow in autumn (Tab. 1).

Table 1: Infestation of cyprinid fish from Belčišta wetland with *Acanthocephalus lucii*.

Fish species	Parasite species	Season							
		Winter		Spring		Summer		Autumn	
		Examined	Infested	Examined	Infested	Examined	Infested	Examined	Infested
Squalius squalus	*Acanthocephalus lucii*	1	0	5	0	3	0	6	0
Phoxinus lumaireul		0	0	0	0	0	0	10	1
Pelasgus minutus		1	0	0	0	0	0	7	0
In total		2	0	5	0	3	0	23	1

Phyllum Acanthocephala

Class Paleacanthocephala

Ordo Echinorhynchida

Family Echinorhynchidae

Ordo Acanthocephalus

Species *Acanthocephalus lucii* (Müller, 1776) Lühe, 1911

The parasite species is determined morphologically through observation on the shape and measurements of proboscis with hooks, and the reproductive system.

The body of *Acanthocephalus lucii* is cylindrical, slightly spread on the front part, with a length of 15 mm. Proboscis is cylindrical and medially swollen, with a bold, flat end. Hooks are well-developed, uniformly slender throughout the length of theproboscis, but smallest posteriorly. The root of the hooks is spread, but without lateral extensions. The hooks are in 16 longitudinal rows, eight in each row. The length of the proboscis is about 0.13 mm, and the length of front hooks are about 0.05 mm. The female gonopore is terminal, slit-shaped laterally in an elevated orifice. Eggs are elongate-fusiform, smooth, with no corrugations or special topography. Eggs are 0.10 x 0.02 mm (Fig. 3).

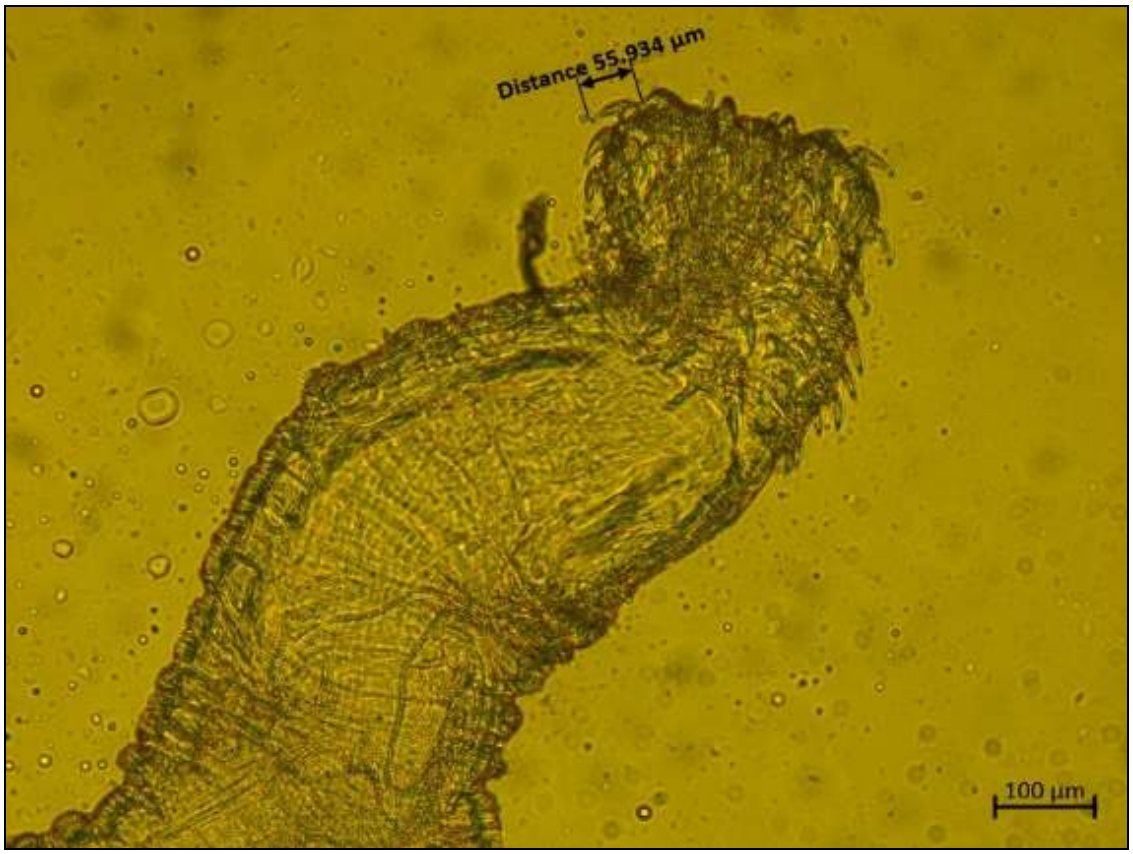

Figure 3: *Acanthocephalus lucii* from minnow (*Phoxinus lumaireul)* from Belčišta wetland – proboscis of a female worm with hooks.

Acanthocephalus lucii (Müller, 1776) Lühe, 1911, also described as *Echinorhynchus angustus* Rudolphi, 1802 is found in various European freshwater fishes where it is widely distributed. Bauer (1985) cited that more than 40 species of freshwater fish are host for this parasite. Petrochenko (1956) stated that *A. lucii* is found within the fishes of families: Cyprinidae, Percidae, Esocidae, Anguillidae, Cottidae, and others.

According to Bauer (1985) and Benesh and Valtonen (2007), intermediate host for *A. lucii* is *Asselus aquaticus* that is already found in Belčišta wetland (Zoroski, 2022).

According to parasitological reference from the Balkans and widely (Ergens, 1960, 1970; Čhanković et al., 1968; Kazic, 1970; Brglez, 1973; Hristovski, 1983; Kakacheva-Avramova, 1983; Kyškaroly and Tafro, 1988; Nedeva-Lebenova, 1991; Cakic, 1992; Stojanovski, 1997, 2003, etc.), this parasite is found in: 1. Bulgaria, in *Barbus meridionalis petenyi, Leuciscus cephalus, Silurus glanis*, *Perca fluviatilis* (Kakacheva-Avramova, 1983); 2. Serbia, in *Cottus gobio* (Cakic, 1992); 3. Bosnia and Hercegovina, in *Salmo trutta, Salvelimus fontinalis, Esox lucius, Tinca tinca, Cyprinus carpio, Silurus glanis, Perca fluviatilis, Lucioperca lucioperca*, and *Acerina cernua* (Čhanković, 1968), as well as *Umbra krameri, Aspius aspius, Pelecus cultratus, Noemachilus barbatulus, Perca fluviatilis*, and *Acerina cernua* (Kiškaroly and Tafro, 1988); 4. Slovenia, in *Esox lucius, Salmo trutta, Oncorhynchus mykiss*, and *Lota lota* (Brglez, 1973).

A. anguillae is the parasite species of the genus *Acanthocephalus* previously found in N. Macedonia. Hristovski (1983) found this parasite in *Salmo letnica, S. ohridanus, S. macedonicus, S. peristericus, Oncorhynchus mykyss, Tinca tinca, Anguilla anguilla*, *Silurus glanis, Squalius squalus, Gambusia affinis, Cyprinus carpio*, *Perca fluviatilis*, and *Barbus macedonicus* in different lakes and rivers from N. Macedonia. Stojanovski (1997) found this parasite at *Barbus rebeli, Scardinius knezevici*, and *Anguilla anguilla* from Lake Ohrid.

The record of *Acanthocephalus lucii* in minnow in this study is considered as the first in N. Macedonia. At the same time, minnow represents a new host for this parasite worldwide.

Fish parasites have been used as biological indicators to determine fish dynamics and status (MacKenzie, 2002; Öktener et al., 2023). This parasite species lead to ruining of fish health, by causing severe destruction of the intestinal wall, and they can be followed by secondary bacterial infections. This emphasizes the danger of the spread of parasites.

The problem with introducing fish species from other regions of the world arises because almost completely unknownspecies of parasites are introduced with them. Data on non-native fish parasites are important for evaluating the health status and their general impact on native fish populations. The introduction of non-native species can have notable effects on the populations of native species, the ecosystem, but can also result in socio-economic consequences, caused by major damages to fisheries and aquaculture.

During the investigations that should follow, we expect to find more species of parasites in the fish in the Belčišta wetland, some of which will certainly be new to the parasite fauna of fish from N. Macedonia, and this will contribute to supplementing knowledge and obtaining a more complete picture of the pathology of fish in N. Macedonia and Balkans, but also the measures that would be taken to prevent the diseases.

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REFERENCES

1. Bauer O. N., 1985 – Key for determination of freshwater fish parasites in the fauna of USSR, II, First part, Akademia Nauk S. S. S. R., Izdateljstvo Nauka, L., 335-337. (in Russian)
2. Benesh D. P. and Valtonen E. T., 2007 – Effects of Acanthocephalus lucii (Acanthocephala) on intermediate host survival and growth: implications for exploitation strategies, *Journal of* Parasitology, 93, 4, 735-741.
3. Brglez J., 1973 – Parasites and parasitic diseases of freshwater fish in Slovenia, *Zbornik Biotehni, Fakultete Univerze v Ljubljani – Veterinarstvo*, Suplement 1, Ljubljana, 153. (in Slovenian)

4. Cakic P., 1992 – Fish parasites in the waters of Sjenica-Peshter Plateau and possibilities of their prevention, PhD Thesis, Faculty of Veterinary Medicine, University of Belgrade, Yugoslavia, 277. (in Serbian)
5. Čanković M., Delić S., Kiškarolj M. and Rukavina J., 1968 – Parasite fauna of freshwater fish in Bosnia and Hercegovina (Trematoda, Cestoda, Nematoda, Acantocephala), Academy of Sciences and Arts of Bosnia and Hercegovina, Department of Natural and Mathematical Sciences, Sarajevo, Yugoslavia, 158. (in Serbian)
6. Dezfuli B. S., Giari L., Lorenzoni M., Carosi A., Manera M. and Bosi G., 2018 – Pike intestinal reaction to Acanthocephalus lucii (Acanthocephala): immunohistochemical and ultrastructural surveys, *Parasites & Vectors*, 11, 424, https://doi.org/10.1186/s13071-018-3002-6.
7. Ergens R., 1960 – Helminth fauna of some fishes in Albania, *Ceskoslovenska Parasitologie*, 7, 49-90. (in Russian)
8. Ergens R., 1970 – Parasite fauna of fishes in Montenegro I., Polyonchoinea (Monogenoidea) of some fishes from the Lake Skadar and Great Black Lake, *Poljoprivreda i Šumarstvo*, XVI, 1-2, 149-192. (in Russian)
9. Jankovska I., Miholova D., Petrtyl M., Romocusky S., Kalous L., Vadlejch J., Chadkova S. and Langrova I., 2011 – Intestinal parasite Acanthocephalus lucii (Acanthocephala) from European perch (Perca fluviatilis) as a bioindicator for lead pollution in the stream Jevansky potok near prague, Czech Republic, *Bulletin of Environment Contamination Toxicology*, 86, 342-346, DOI: 10.1007/s00128-011-0210-6.
10. Hristovski N. D., 1983 – Fauna of fish endohelminths in the lakes from Macedonia, PhD Thesis, Faculty of Natural Sciences and Mathematics, Novi Sad, 242. (in Serbian)
11. Kakacheva-Avramova D., 1983 – Helminths of freshwater fishes in Bulgaria, Bulgarian Academy of Sciences, Sofia, 261. (in Bulgarian)
12. Kazic D., 1970 – Endohelminths of economically most important fish from Lake Skadar, PhD thesis, Veterinary Faculty, Sarajevo, 128. (in Serbian)
13. Kiškaroly M. and Tafro A., 1988 – Contribution to the knowledge of helminth fauna of some fish from a part of Danube, *Veterinaria*, 37, 2-3, 211-221. (in Serbian)
14. MacKenzie, K. 2002 – Parasites as biological tags in population studies of marine organisms: an update, *Parasitology*, 124, S153-S163.
15. Nedeva-Lebenova I., 1991 – Morphology, fauna and ecology of fish helminths from the reservoir Pcelina, PhD Thesis, Bulgarian Academy of Sciences, Institute of Parazitology, Sofia, 177. (in Bulgarian)
16. Öktener A. and Bănăduc D., 2023 – Ecological interdependence of pollution, fish parasites, and fish in freshwater ecosystems of Turkey, *Water*, 15, 1385, DOI: 10.3390/w15071385.
17. Petrochenko V. I., 1956 – Acanthocephala of domestic and wild animals, Academy of Science of Moscow, 435. (in Russian)
18. Schmidt G. D., 1985 – Development and life cycles, in Biology of the Acanthocephala, Nickol B. B. and Crompton D. W. T., (eds), Cambridge University Press, Cambridge, U.K., 273-306.
19. Stojanovski S., 1997 – Ecto and endoparasites of fishes from Lake Ohrid, MSc Thesis, Faculty of Veterinary Medicine. Belgrade, Serbia, 199. (in Serbian)
20. Stojanovski S., 2003 – Fauna of monogenean trematodes – parasites of fishes from natural lakes in Macedonia, PhD Thesis, Faculty of Veterinary Medicine, Skopje, Macedonia, 202. (in Macedonian)
21. Vasiljkov G. V., 1983 – Gelmintozi ryb. Izdateljstvo Kolos, 207. (in Russian)
22. Zoroski D. J., 2022 – Study on the valorization of Belčišta wetland – Park of nature, Final report, 105. (in Macedonian)