Zoodiversity, 59(5): –, 2025

DOI 10.15407/zoo2025.05.
UDC 597.8:576.89(477.74)

A SURVEY OF HELMINTHS OF THE MARSH FROG, *PELOPHYLAX RIDIBUNDUS* (AMPHIBIA, RANIDAE) AND THE FIRST RECORD OF *OPHIOTAENIA SAPHENA* (CESTODA, PROTEOCEPHALIDAE) IN THE UKRAINIAN PART OF THE DANUBE DELTA

O. Greben [1], Ya. Syrota [1,2], I. Dmytrieva [1], A. Nechai [1], V. Dupak [1], O. Marushchak [1], Yu. Kuzmin [1,3] & R. Svitin [1,3,4]

[1] I. I. Schmalhausen Institute of Zoology, NAS of Ukraine; vul. B. Khmelnytskogo, 15, Kyiv, 01054 Ukraine
[2] Institute of Parasitology Slovak Academy of Sciences; Hlinkova 3, 04001, Košice, Slovakia
[3] African Amphibian Conservation Research Group, Unit for Environmental Sciences and Management, North-West University, Potchefstroom Campus; Private Bag X6001, Potchefstroom 20520, South Africa
[4] Taras Shevchenko Kyiv National Universityof Kyiv; vul.Volodymyrska, 60, Kyiv, 01033 Ukraine

* Corresponding author

E-mail: romasvit@izan.kiev.ua

O. Greben (https://orcid.org/0000-0001-9942-2542)
Ya. Syrota (https://orcid.org/0000-0002-8070-9823)
I. Dmytrieva (https://orcid.org/0000-0002-7907-4111)
A. Nechai (https://orcid.org/0009-0008-1014-336X)
V. Dupak (https://orcid.org/0000-0002-0957-3791)
O. Marushchak (https://orcid.org/0000-0001-9380-5593)
Yu. Kuzmin (https://orcid.org/0000-0002-1723-1265)
R. Svitin (https://orcid.org/0000-0002-5450-7240)

urn:lsid:zoobank.org:pub:CC2EF292-B07E-4546-A04C-591A429D6301

A Survey of Helminths of the Marsh Frog, *Pelophylax ridibundus* (Amphibia, Ranidae) and the First Record of Ophiotaenia saphena (Cestoda, Proteocephalidae) in the Ukrainian Part of the Danube Delta. Greben, O., Syrota, Ya., Dmytrieva, I., Nechai, A., Dupak, V., Marushchak O., Kuzmin, Yu. & Svitin, R. — In 23 examined marsh frogs, *Pelophylax ridibundus*, from the town of Vylkove (Odesa Region, Ukraine), 25 helminth species were found. Helminth infracommunities in the studied frogs comprised 3–14 species (mean 9.4; median

10.0) and intensity of infection ranged from 20–351 (mean 151; median 155). Six helminth species were classified as predominating in the helminth component community based on their prevalence and abundance: the nematode *Oswaldocruzia duboisi*, the cestode *Ophiotaenia saphena*, the trematodes *Diplodiscus subclavatus*, *Prosotocus confusus*, *Pleurogenoides medians*, and *Tylodelphys excavata* (metacercariae). The nematodes *Icosiella neglecta* and *Oswaldocruzia duboisi* and the cestode *Ophiotaenia saphena* are reported for the first time in marsh frogs from Vylkove. The latter species is known as a parasite of true frogs (Ranidae) in North America and was recently discovered in *Pelophylax* spp. in Greece and Romania. It is first reported in Ukraine and is regarded as an invasive alien parasite of *P. ridibundus* in the Ukrainian part of the Danube Delta. The morphological description and molecular characterisation of *O. saphena* from the new locality are provided.

Key words: helminths, frogs, *Ophiotaenia*, helminth community, Danube, Ukraine.

Introduction

Parasites and parasite communities are an essential part of any natural ecosystem, influencing the overall community structure (Hudson et al., 2006). Healthy ecosystems are expected to be rich in parasites; therefore, parasite diversity may be used as an indicator of ecosystem health (Marcogliese, 2005; Hudson et al., 2006), while the diversity within a parasite community may be indicative of the state of a particular host population. Amphibians often harbour a variety of parasites. It is known that many parasites, including helminths, use amphibians as definitive or intermediate hosts due to their intermediate position in the food webs of both aquatic and terrestrial ecosystems (Koprivnikar et al., 2012). Therefore, investigation of helminth communities in separate populations of amphibians may provide useful information on both the state of the host populations and the entire ecosystem community.

Pelophylax ridibundus (Pallas, 1771) is the most widespread species of amphibians in Ukraine, inhabiting all natural zones, except for high mountain areas in the Carpathians and Crimea (Shcherbak & Shcherban, 1980; Pysanets, 2007). The species prefers almost any type of water body, especially those situated in open areas, and is often found along the shoreline of rivers, lakes, ponds, ditches, and canals. It avoids wetlands and small bodies of water with stagnant water, except when the latter are located in close proximity to larger bodies of water. It is rarely found on land far from water bodies, except during daily migrations and movements between water bodies (Kotenko, 1999; Nekrasova, 2002). Within the area of the Danube Delta, three population systems of water frogs are known as consisting of only *P. ridibundus* (R), consisting of *P. ridibundus* and *P. esculentus* (Linnaeus, 1758) (RE), and consisting of *P. ridibundus*, *P. esculentus* and *P. lessonae* (Camerano, 1882) (REL). The marsh frog *P. ridibundus* is the dominant species and its local range expanding accidentally in response to changes (seasonal, historical) in the river (Nekrasova & Morozov-Leonov, 2012). The marsh frog is not only the most numerous amphibian within the fresh water bodies of the Danube Delta (Kotenko, 1999; Nekrasova & Morozov-Leonov, 2012, Vasyliuk et al., 2022), but it also has the biggest biomass (Török, 2002), with population density ranging from 5 to 57 individuals per 100 m^2 depending on the habitat type and peculiarities of the vegetation cover of the inhabited water bodies (Török, 2014).

In Ukraine, helminths of *Pelophylax* spp. and other amphibians have been widely studied, predominantly in the northern part of the country (Mazurmovich, 1951; Maguza, 1972; Kuzmin et al., 2020). The only report on the helminth parasites of anurans from south-western Ukraine and the Danube Delta in particular, is the paper by Volgar-Pastukhova (1959), which is based on material collected between 1946 and 1948. The author identified 34 helminth species collected from 206 adult *P. ridibundus* from two localities, including the town of Vylkove (Odesa Region). Seventeen species (50% of the total number) were found in their larval stages; this fact evidenced the important role of marsh frogs in helminth transmission in the Danube Delta ecosystem.

In July 2023, we collected 23 individuals of *P. ridibundus* in Vylkove and investigated the helminth parasites in the collected sample. In addition to the helminth species known as rather common parasites of this host, we identified the cestode *Ophiotaenia saphena* Osler, 1931, known as a parasite of *Rana clamitans* Latreille, 1801 and *R. catesbeiana* (Shaw, 1802) in North America (Scholz et al., 2023) and recently reported from *Pelophylax* spp. in two localities in Greece and one locality in Romania (de Chambrier et al., 2025). Identification of the species was confirmed by morphological studies and the analysis of its COX1 gene sequence.

The present study aimed to characterise the current composition and structure of the helminth community of *P. ridibundus* from the Ukrainian part of the Danube Delta (the town of Vylkove, Odesa Region) and to describe the cestode *O. saphena* based on a sample collected from a new locality.

Materials and Methods

Frogs were collected in a small creek near its confluence with the main stream of the Danube river (coordinates: 45° 24' 02.9" N 29° 34' 44.1" E). Collecting was performed at night using a small fishing net or manually. The gathered frogs were transported to the laboratory of the Department of Parasitology of the Institute of Zoology in Kyiv, Ukraine. For transportation, frogs were placed in two large plastic containers with a sufficient amount of water and some aquatic vegetation from the collection site. In the laboratory, the frogs were measured and then euthanised by lidocaine injection into the spinal cord, then autopsied and examined. The frog sample consisted of six adult females and 17 adult males. The snout-vent length of the frogs was 72 ± 5.9SD (63–86) mm.

Collected trematodes, cestodes and nematodes were washed in saline and fixed in hot 70% alcohol. Acanthocephalans were placed in distilled water and kept there for 2–24 hours until complete evagination of the proboscis and relaxation of the trunk. Thereafter, they were fixed in 70% alcohol. Encysted juvenile stages of helminths were extracted from the cysts with thin needles and fixed in hot 70% alcohol. Nematodes, acanthocephalans and trematodes were cleared in lactophenol (a mixture of equal volume parts of glycerine, phenol, water, and lactic acid) for 0.5–4 hours. Thereafter, they were examined and identified using a compound light microscope (AmScope T690B).

Adult cestodes and plerocercoids were stained with iron acetocarmine (Georgiev et al., 1986), dehydrated in an ascending alcohol series, cleared in clove oil and mount-

ed in Canada balsam. The morphology of *O. saphena* was investigated under a Zeiss AxioImager M1 microscope equipped with DIC and a digital imaging system.

A partial COX1 gene sequence was obtained from an adult tapeworm specimen preserved in 96% alcohol. Total genomic DNA was isolated using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. A ~700 bp region of the COX1 gene was amplified using the forward primer Dice1F (ATTAACCCTCACTAAATTWCNTTRGATCATAAG) and the reverse primer Dice14R (TAATACGACTCACTATACCHACMRTAAACATATGATG) (van Steenkiste et al., 2015). Thermal cycling included an initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 51 °C for 40 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplicons were purified using the ExpinTM Combo GP kit (GeneAll, Biotechnology, South Korea) and sent to Sanger sequencing at the Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia. Chromatograms were assembled and edited in Geneious Prime 2025.0.3 (Biomatters, Auckland, New Zealand; https://geneious.com). Subsequent analyses included the newly obtained partial COX1 sequence and nine additional sequences of *Ophiotaenia saphena* obtained from GenBank (Table 1). These nine sequences represent all publicly available COX1 gene sequences for this species at the time of the study. All sequences were aligned and trimmed using Geneious Prime. The alignment was then visualised as a haplotype network using the R programming language (R Core Team, 2024), specifically the "ape" (Paradis & Schliep, 2019) and "pegas" (Paradis, 2010) packages.

For each helminth species, prevalence (P), intensity of infection (I), and total (A) and mean (MA) abundance were calculated as defined by Bush et al. (1997). Additionally, the relative abundance (RA) of each species was calculated as the percentage of its specimens in the whole sample of helminths. The dispersion index (DI) was calculated as the variance to mean ratio following the recommendations of Rózsa et al. (2000). Confidence intervals of prevalence and mean abundance were calculated using the Quantitative Parasitology 3.0 software (Rózsa et al., 2000). The diversity indices and the estimated species richness (based on Chao1, jackknife, and bootstrap methods) in the helminth component community were calculated in PRIMER 6 software (Clarke & Gorley, 2006).

Table 1. **COX1 gene sequences of *Ophiotaenia saphena* from GenBank used for comparison and construction of the haplotype network**

Host species	Geographical origin	GenBank number	Reference
Pelophylax epeiroticus	Greece	PQ438704	Chambrier et al. (2025)
Pelophylax ridibundus	Romania	PQ438705	Chambrier et al. (2025)
Pelophylax ridibundus	Romania	PQ438706	Chambrier et al. (2025)
Pelophylax ridibundus	Romania	PQ438707	Chambrier et al. (2025)
Lithobates (=*Rana*) *clamitans*	USA	OQ200060	Scholz et al. (2023)
Lithobates (=*Rana*) *clamitans*	USA	OQ200061	Scholz et al. (2023)
Lithobates (=*Rana*) *clamitans*	USA	OQ200062	Scholz et al. (2023)
Lithobates (=*Rana*) *clamitans*	USA	OQ200063	Scholz et al. (2023)
Lithobates (=*Rana*) *clamitans*	USA	OQ200063	Scholz et al. (2023)

Results

Helminth species and communities

In the examined frog sample, we found 25 helminth species (Table 2), including 18 species of trematodes, one cestode species, five species of nematodes, and one acanthocephalan species. Eight species of trematodes were represented by metacercariae; the rest of the helminth species were in the adult stage. Adult trematodes were observed mostly in the intestinal lumen but also in the intestinal wall (*B. turgida* (Brandes, 1888)), stomach (*H. ovocaudatus* Vulpian, 1859), lungs (*H. variegatus* (Rudolphi, 1819) and *S. similis* (Looss, 1899)), urinary bladder (*G. varsoviensis* Sinitzin, 1905), and rectum (*D. subclavatus* (Pallas, 1760)). The majority of encysted trematode metacercariae were found attached to the internal organs and rarely in the muscles (*C. urniger* (Rudolphi, 1819)). Motile metacercariae of *T. excavata* (Rudolphi, 1819) and several encysted *C. urniger* were observed in the spinal canal. The cestode *O. saphena* Osler, 1931 (adult worms) dwelled in the intestine, and the posterior part of the strobila sometimes reached the rectum; procercoids and plerocercoids of the species were found embedded into the muscles of the intestinal wall closer to its inner surface. The nematodes *C. ornata* (Dujardin, 1845), *N. schneideri* (Travassos, 1931), and *O. duboisi* Ben Slimane, Durette-Desset et Chabaud, 1993 inhabited the intestine; *I. neglecta* (Diesing, 1851) was found in the muscles of the legs and throat; two specimens of *R. esculentarum* Cipriani, Mattiucci, Paoletti, Santoro et Nascetti, 2012 parasitised the lungs of one frog individual.

All the examined frogs were found to be infected with helminths. Helminth infracommunities included 3–14 species (mean 9.4; median 10.0) and intensity of infection ranged from 20–351 (mean 151; median 155). In the helminth component community, the estimated species richness reached 26 (Chao1), 30 (Jackknife1), or 27 (bootstrap) species. The Margalef's index of species richness was 2.9.

In the studied sample, seven helminth species had a prevalence (P) of about 70% or higher and thus predominated by their occurrence in the host: the nematode *O. duboisi* (P = 91.3%); the trematodes *D. subclavatus* (P = 91.3%), *Pleurogenoides medians* (Olsson, 1876) (87.0%), *Prosotocus confusus* (Looss, 1894) (P = 78.3%), and *T. excavata* (P = 69.6%); the cestode *O. saphena* (P = 78.3%); and the acanthocephalan *Acanthocephalus ranae* (Schrank, 1788) (P = 82.6%) (Table 2, Fig. 1). Four other species were common in the sample, each infecting more than a third of examined frogs: the nematode *C. ornata* (P = 52.2%), and the trematodes *Strigea sphaerula* Rudolphi, 1803) (P = 43.5%), *H. ovocaudatus* (P = 39.1%), and *Astiotrema monticelli* Stossich, 1904 (P =39.1%). The prevalence of five species ranged from 20% to 30%: the nematode *I. neglecta* (P = 26.1%), and the trematodes *Pleurogenes claviger* (Rudolphi, 1819) (P = 26.1%), *Paralepoderma cloacicola* (Luhe, 1909) (P = 26.1%), *H. variegatus* (P = 26.1%), and *Opisthioglyphe ranae* (Froelich, 1791) (P = 21.7%). The remaining nine species each infected a single host (*B. turgida*, *Encyclometra colubrimurorum* (Rudolphi, 1819), *N. schneideri*, *R. esculentarum*, *S. similis*), two hosts (Echinostomatidae gen. sp., *Gorgodera varsoviensis* Sinitzin, 1905), or three hosts (*C. urniger*, *N. attenuatum*) (Fig. 1). They all are classified as rare (statistically uncertain) species, for the lower value of the confidence intervals of their prevalence was less than 4.3% (the prevalence that reflects one infected host individual), and the lower value of the confidence intervals of their mean abundance was 0 (except for *N. attenuatum*) (Table 2).

Table 2. **Helminth parasites of *Pelophylax ridibundus* (n=23) from Vylkove**

#	Species	P, %	MA	I	A (RA)	DI
			Platyhelminthes: Trematoda			
1	*Brandesia turgida* (Brandes, 1888)	4.3 (0.2–21.2)	0.17 (0–0.52)	4	4 (0.12%)	4.0
2	*Diplodiscus subclavatus* (Pallas, 1760)	91.3 (72.2–98.4)	10.83 (7.26–16.13)	11.9 (10) 1–46	249 (7.17%)	11.1
3	*Gorgodera varsoviensis* Sinitzin, 1905	8.7 (1.6–27.8)	0.09 (0–0.17)	1 (1) 1–1	2 (0.06%)	0.9
4	*Haematoloechus variegatus* (Rudolphi, 1819)	26.1 (12.0–47.8)	0.96 (0.17–3.57)	3.7 (1) 1–16	22 (0.63%)	11.5
5	*Halipegus ovocaudatus* Vulpian, 1859	39.1 (21.3–61.1)	3.04 (1.48–5.78)	7.8 (6) 1–18	70 (2.02%)	8.3
6	*Opisthioglyphe ranae* (Froelich, 1791)	21.7 (9.0–43.3)	0.52 (0.13–1.22)	2.4 (2) 1–5	12 (0.35%)	2.9
7	*Pleurogenes claviger* (Rudolphi, 1819)	26.1 (12.0–47.8)	0.43 (0.17–0.83)	1.7 (1.5) 1–3	10 (0.29%)	1.6
8	*Pleurogenoides medians* (Olsson, 1876)	87.0 (67.6–96.3)	37.74 (25.09–53.78)	43.4 (35.5) 1–122	868 (24.99%)	33.0
9	*Prosotocus confusus* (Looss, 1894)	78.3 (56.7–91.0)	14.78 (8.91–23.65)	18.9 (10.5) 1–63	340 (9.79%)	21.5
10	*Skrjabinoeces similis* (Looss, 1899)	4.3 (0.2–21.2)	0.04 (0–0.13)	1	1 (0.03%)	1.0
11	*Astiotrema monticelli* Stossich, 1904 (mtc)	39.1 (21.3–61.1)	1.3 (0.65–2.7)	3.3 (3) 1–10	30 (0.86%)	4.1
12	*Codonocephalus urniger* (Rudolphi, 1819) (mtc)	13.0 (3.7–32.3)	1.74 (0.13–4.96)	13.3 (16) 3–21	40 (1.15%)	16.6
13	Echinostomatidae gen. sp. (mtc)	8.7 (1.6–27.8)	2.83 (0–8.57)	32.5 (32.5) 21–44	65 (1.87%)	35.3
14	*Encyclometra colubrimurorum* (Rudolphi, 1819) (mtc)	4.3 (0.2–21.2)	0.04 (0–0.13)	1	1 (0.03%)	1.0
15	*Neodiplostomum attenuatum* (Linstow, 1906) (mtc)	13.0 (3.7–32.3)	7.87 (0.52–23.04)	60.3 (84) 12–85	181 (5.21%)	75.1
16	*Paralepoderma cloacicola* (Luhe, 1909) (mtc)	26.1 (12.0–47.8)	2.7 (0.39–10.57)	10.3 (3) 1–47	62 (1.79%)	35.7
17	*Strigea sphaerula* Rudolphi, 1803) (mtc)	43.5 (24.7–64.0)	11.04 (4.04–29.74)	25.4 (5.5) 2–116	254 (7.31%)	63.4
18	*Tylodelphys excavata* (Rudolphi, 1803) (mtc)	69.6 (47.8–85.5)	28.26 (12.57–69.35)	40.6 (19.5) 1–276	650 (18.72%)	129.2
			Platyhelminthes: Cestoda			
19	*Ophiotaenia saphena* Osler, 1931	78.3 (56.7–91.0)	8.78 (4.83–16.87)	11.2 (5) 1–62	202 (5.82%)	22.2
			Nematoda: Chromadorea			
20	*Cosmocerca ornata* (Dujardin, 1845)	52.2 (32.4–72.2)	2.96 (1.57–5.83)	5.7 (4) 1–21	68 (1.96%)	8.4
21	*Icosiella neglecta* (Diesing, 1851)	26.1 (12.0–47.8)	0.35 (0.13–0.65)	1.3 (1) 1–2	8 (0.23%)	1.2
22	*Neyraplectana schneideri* (Travassos, 1931)	4.3 (0.2–21.2)	0.13 (0–0.39)	3	3 (0.09%)	3.0
23	*Oswaldocruzia duboisi* Ben Slimane, Durette-Desset et Chabaud, 1993	91.3 (72.2–98.4)	11.57 (7.39–19.26)	12.7 (7) 1–53	266 (7.66%)	16.0
24	*Rhabdias esculentarum* Cipriani, Mattiucci, Paoletti, Santoro et Nascetti, 2012	4.3 (0.2–21.2)	0.09 (0–0.26)	2	2 (0.06%)	2.0
			Acanthocephala: Palaeacanthocephala			
25	*Acanthocephalus ranae* (Schrank, 1788)	82.6 (61.1–93.8)	2.74 (1.78–4.74)	3.3 (2) 1–15	63 (1.81%)	3.8

For each species, prevalence (P) and mean abundance (MA) are shown with 95% confidence intervals in parentheses; intensity of infection (I) is shown as mean, median in parentheses, and range; DI — dispersion index (variance to mean ratio); A — total abundance, RA — relative abundance; mtc – metacercaria.

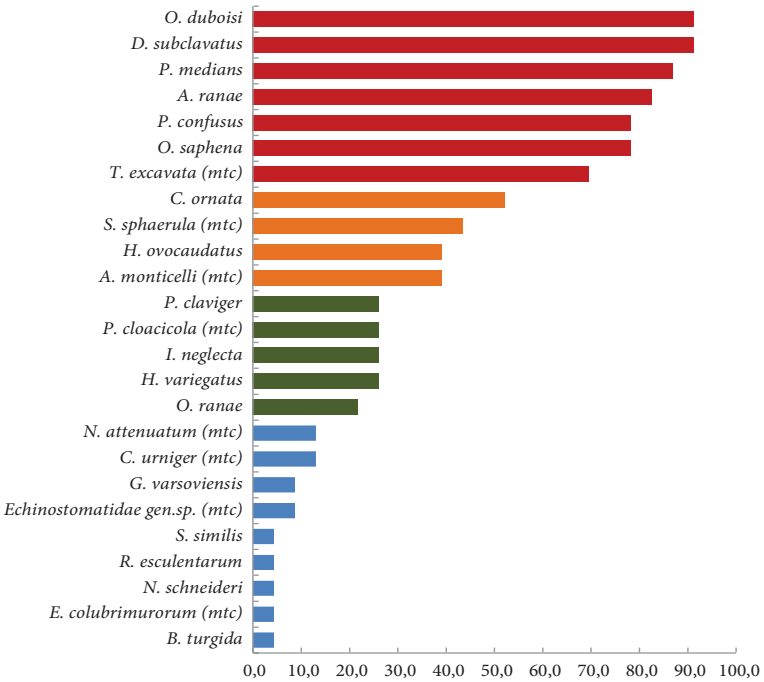

Fig. 1. Prevalence (%) of helminth species in the studied sample of *Pelophylax ridibundus*

Fourteen helminth species in the sample had a relative abundance (RA) of 1% or higher (Fig. 2); eight had a relative abundance higher than 5%: *P. medians* (RA=25.0%), *T. excavata* (RA = 18.7%), *P. confusus* (RA = 9.8%), *O. duboisi* (RA = 7.7%), *S. sphaerula* (RA = 7.3%), *D. subclavatus* (RA = 7.2%), *O. saphena* (RA = 5.8%), and *N. attenuatum* (RA = 5.2%). Three trematode species, namely *P. medians*, *T. excavata*, and *P. confusus*, constituted more than 50% of the total count of helminths in the sample. Considering both the prevalence and abundance of helminth species in the sample, six of them were classified as predominating in the helminth component community: the nematode *O. duboisi*, the cestode *O. saphena*, the trematodes *D. subclavatus*, *P. confusus*, *P. medians*, and *T. excavata*.

The diversity indices in the helminth community were the following: Shannon diversity index 2.36; Simpson's diversity index (1-λ) 0.87; Pielou evenness 0.73; Berger-Parker dominance 0.25.

Description of *Ophiotaenia saphena* (Figs 3–5)

Class: Cestoda
Order: Proteocephalidea
Family: Proteocephalidae

Ophiotaenia saphena Osler, 1931
Host: Marsh frog *Pelophylax ridibundus* (Pallas, 1771)
Site in host: Intestine lumen, intestinal wall
Locality: Danube River, Vylkove, Odeska Oblast, Ukraine; coordinates: 45°24' 02.9" N 29°34' 44.1" E

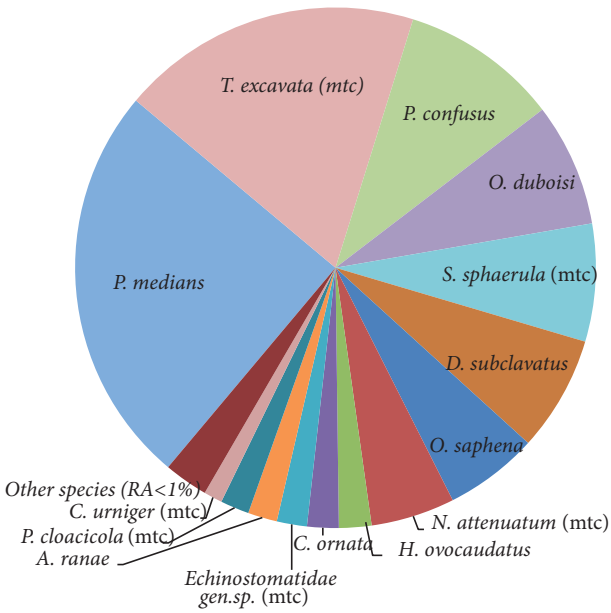

Fig. 2. Relative abundance of helminth species in the studied sample of *Pelophylax ridibundus*

Prevalence: 78.3%.

Intensity of infection: 1–62 (mean 11.2).

Material deposited in the collection of the Department of Parasitology of the Institute of Zoology NAS of Ukraine (IZSHK) under accession numbers: CA001.01; CA001.2; CA001.3; CA001.4; CA001.5; CA001.6; CA001.7; CA001.8; CA001.9; CA001.10; CA001.11; CA001.12; CA001.13; CA001.14.; CA001.5; CA001.16; CA001.17.

Description (based on nine specimens; all measurements are in micrometres unless otherwise indicated). Large cestodes. Strobila delicate. Total length of mature specimens up to 168–208 mm, with maximum width 1.24–1.44 mm at level of gravid proglottids. Immature proglottids wider than long, length/width ratio 0.45–0.60 (Fig. 3, *b*); mature and pregravid proglottids wider than long to longer than wide, length/width ratio 0.65–1.75 (Fig. 3, *c*); gravid proglottids longer than wide, length/width ratio 1.20–2.70 (Fig. 3, *d*).

Scolex oval, 245–415 × 320–475 (mean 343 × 418, n=9), without rostellum. Four cup-shaped suckers 170–240 × 170–240 (mean 207 × 183, n = 36), slightly muscular, unarmed. Apical organ not visible. Width of neck varying from 110 to 340 (mean 261, n = 10) (Fig. 3, *a*).

Genital pores irregularly alternating. Ratio of distance of genital pore from anterior margin of proglottid to proglottid length 14–27%. Two pairs of osmoregulatory canals, ventral canals wide, 25–45 in diameter; dorsal canals narrow, 5–7 in diameter, situated at same level as ventral canals. Testes in two lateral fields. Some testes overlapping vitelline follicles. Number of testes varying from 84 to 158 (mean 109, n = 30), testis size 40–65 × 35–55 (mean 55 × 45, n = 40). Vas deference forming loops from proximal end of cirrus-sac to median line of proglottid. Thick-walled cirrus-sac elongated, 115–290 × 75–130 (mean 197 × 95, n = 40), opening into shallow genital atrium (Fig. 3, *f*). Ratio of cirrus-sac length to proglottid width 15–29%. Invaginated cirrus forming loops in cirrus-sac. Evaginated cirrus cylindrical, unarmed, 200–380 × 35–60 (mean 268 ×47, n = 6) (Fig. 3, *e*).

Ovary large, bilobed and broader laterally, 405–830 (mean 617, n = 30) wide, located in posterior part of proglottid. Ratio of ovary width to proglottid width 71–84%. Mehlis' gland irregular in shape, slightly knobbly, 115–165 × 50–90 (mean 139×73, n = 30), located posterior to centre of ovary. Ratio of Mehlis' gland width to proglottid width 13–25%. Vitelline follicles in two longitudinal bands in lateral fields of proglottid, outside

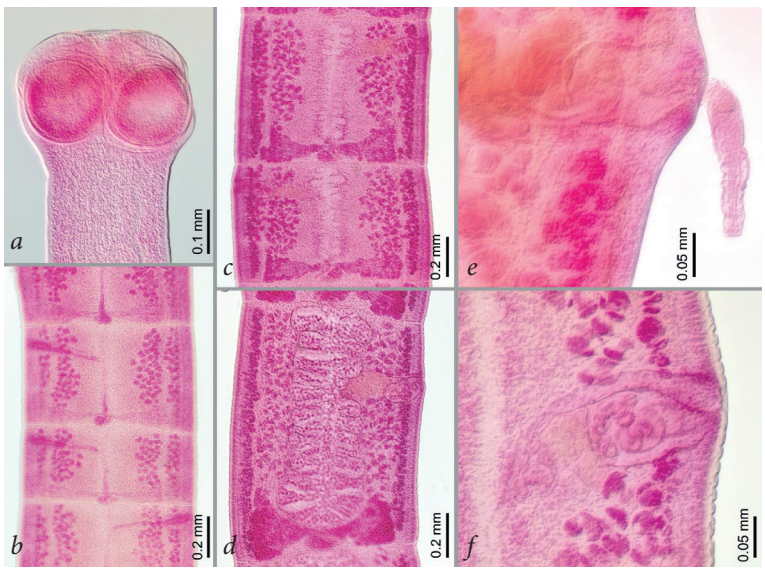

Fig. 3. *Ophiotaenia saphena* from *Pelophylax ridibundus*. *a* — scolex; *b* — immature proglottids; *c* — mature proglottids; *d* — gravid proglottid; *e* — evaginated cirrus; *f* — terminal genitalia

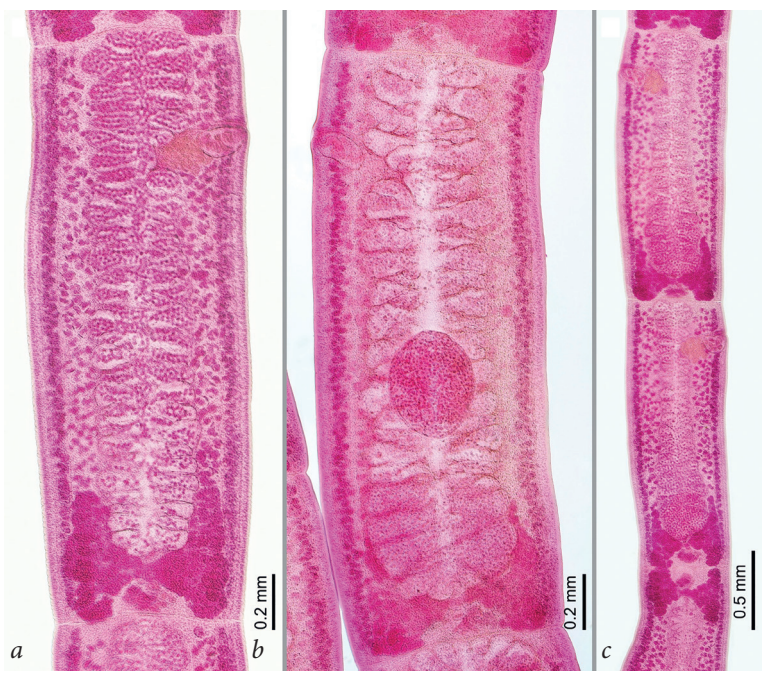

Fig. 4. *Ophiotaenia saphena* from *Pelophylax ridibundus*. *a* — gravid proglottid, normal morphology; *b* — gravid proglottid with spherical protrusion of the uterus; *c* — abnormal (mirrored) position of some proglottids

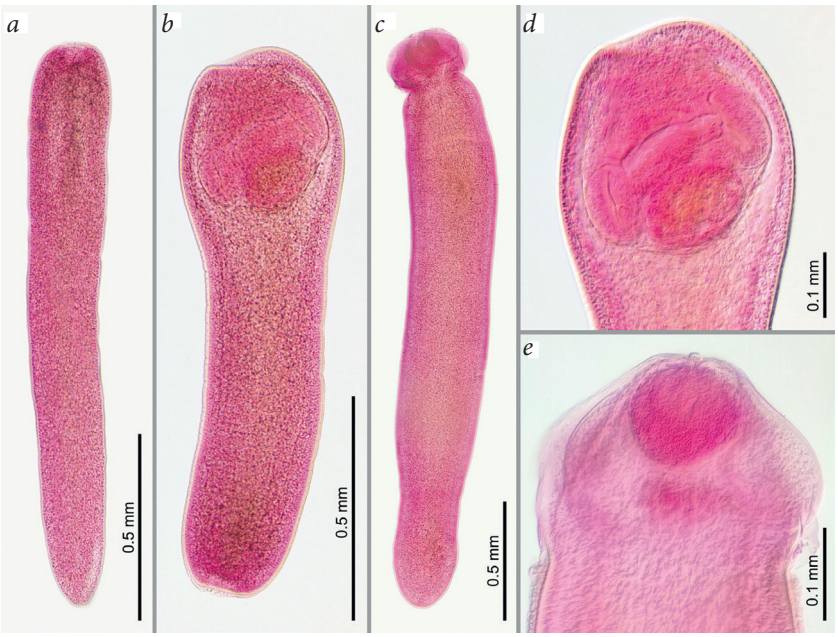

Fig. 5. *Ophiotaenia saphena* from *Pelophylax ridibundus*, juvenile specimens. *a* — procercoid; *b*, *d* — plerocercoid with invaginated scolex; *c, e* — plerocercoid with evaginated scolex. *a–c* — general view; *d, e* — anterior part

osmoregulatory canals. Ratio of length of poral band of vitelline follicles to proglottid length 76–98%. Ratio of length of aporal band of vitelline follicles to proglottid length 86–99%. Vagina opening into genital atrium anterior to cirrus-sac (Fig. 3, *f*). Diameter of copulative part of vagina 20–45 (mean 31, n = 23). Uterus appearing as long stem along median line of proglottid. In gravid proglottids, uterus forming transverse diverticula, their numbers varying from 10 to 23. Gravid eggs spherical, oncosphere 11–16 (mean 14, n = 30) in diameter.

In addition to adult worms, procercoids (Fig. 5, *a*) and plerocercoids at different stages of growth and development (Fig. 5, *b–e*) were found in the intestinal wall of examined hosts.

Description of plerocercoid (based on eight specimens). Length 1.24–2.59 mm (mean 1.92 mm, n = 7), width 310–434 (mean 388, n = 7). Evaginated scolex 220–260 × 315–420 (mean 243 × 363, n = 3); invaginated scolex 240–300 × 290–420 (mean 270 × 316, n = 5). Suckers oval, slightly muscular, 130–170 × 95–170 (mean 148 × 131, n = 17). Apical organ oval, 120–155 × 125–165 (mean 139 × 141, n = 7).

Remarks. The morphology of the studied specimens generally agrees with the description of *O. saphena* by Osler (1931) from *Rana clamitans* , the description by Scholz et al. (2023) from *Rana* spp. from the USA, and the description by de Chambrier et al. (2025) from *Pelophylax* spp. from Europe. The dimensions of the present specimens fall within the range of measurements reported in these publications. Specimens from *P. ridibundus* from Ukraine differed from those from *Rana* spp. in the USA in the wider range of the number of testes (84–158 *vs* 88–120 (Osler, 1931) and 84–140 (Scholz et al., 2023)), larger width of the cirrus-sac (75–130 *vs* 50–80 (Osler, 1931) and 50–90 (Scholz et al., 2023)), and wider range of the cirrus length (200–380 *vs* 250–330 (Osler, 1931)). Also, the studied specimens differed from those previously reported from Europe (de Chambrier et al.,

2025) in the wider range of the scolex length (245–415 *vs* 260–380), lower limit for the scolex width (320–475 *vs* 405–580), and smaller number of testes (84–158 *vs* 174–237 in specimens from *P. ridibundus* from Romania).

The specimens of *O. saphena* studied by Scholz et al. (2023) had some morphological anomalies: the presence of two or three cirrus-sacs in a proglottid, a duplicated ovary, and an extra ovary near the anterior margin of the proglottid. The tapeworms from Ukraine also demonstrated anomalies of morphology; however, they differed from those in the material from the USA (Fig. 4). Some proglottids had an abnormal (mirrored) position of the uterus (Fig. 4, *c*); other proglottids had large protrusions of the uterus on the surface of the strobila (Fig. 4, *b*). Interestingly, the eggs in these protrusions were in a more advanced stage than those in the uterus of the same proglottid. It is possible that these anomalies are associated with the development of the cestode in a new host.

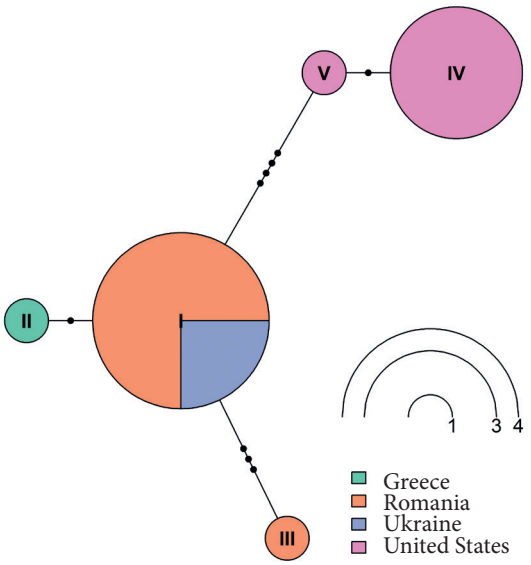

Fig. 6. Haplotype network of a 398-bp region of the COX1 gene. Circle sizes are proportional to the number of each haplotype, and colours indicate geographical origin. Roman numerals label distinct haplotypes. Each small black dot on a connecting line represents one point mutation

Molecular identification of *O. saphena* from Ukraine

A partial sequence of the COX1 gene was obtained from a single tapeworm specimen and deposited in GenBank under accession number PX35334. According to a recent study by de Chambrier et al. (2025), nine sequences (see Table 1), which we also used in our comparative analysis, belong to the species *O. saphena*. The similarity of our sequence with those nine sequences ranges from 98% to 100%. In particular, our sequence is identical to three of the Romanian samples (Fig. 6). Additionally, the haplotype network clearly illustrates a separation between North American and European haplotypes of *O. saphena*. Considering the high level of similarity, especially the identity with Romanian samples, we conclude that the examined specimen belongs to *O. saphena* (European form).

Discussion

The number of helminth species (25) recorded from *P. ridibundus* in Vylkove in the present study is somewhat smaller than the number (29) reported in the previous survey by Volgar-Pastukhova (1959). A possible reason for this is the smaller number of examined hosts, 23 in the present study, compared to more than 100 in Volgar-Pastukhova (1959). The association between sampling effort and species richness in parasite component communities has been demonstrated in a number of studies, e. g.

Poulin (1998). The species richness in the helminth component community estimated based on the examined frog sample in the present study (26–30 species) reached the number of species in the previous survey. In similar studies in other parts of the distribution area of *P. ridibundus*, a smaller number of helminth species was usually recorded, e. g. 2–5 species in separate regions of Poland (Popiolek et al., 2011); eight species in the Hortobágy National Park in Hungary (Herczeg et al., 2016); 15 species in the Kharkiv Region of Ukraine (Rezvantseva et al., 2010); 16 species in Antalya province, Turkey (Düşen & Öz, 2006); 18 species in several localities in Iraq (Saeed et al., 2007). On the other hand, Dubinina (1950) reported 29 helminth species in *P. ridibundus* from the Volga Delta, with 12 species (41%) being represented by larval stages. Similarly, Volgar-Pastukhova (1959) reported 11 out of 29 helminth species (38%) being represented by larval stages in *P. ridibundus* from Vylkove, while in the present study, we found eight species (32%) represented by larval stages only. Moreover, three of them, namely *T. excavata* (parasite of storks), *S. sphaerula* (parasite of corvids), and *A. monticelli* (parasite of snakes) reached a prevalence higher than 30% (see Table 2, Fig. 2). These data, along with the helminth component community diversity indices confirming high species richness and equitability, corroborate the high diversity of the helminth community of *P. ridibundus* as an important component of the rich biodiversity in the Danube Delta.

Some helminth species represented by larval stages in the study of Volgar-Pastukhova (1959) had a rather low prevalence, for example, 3.75% in *Centrorhynchus aluconis*, (Müller, 1780), 2% in *Psilochasmus* sp., 2.61% in *Spirometra erinaceieuropaei* (Rudolphi, 1819), Mueller, 1937 and 1.92% in *Alaria alata* (Goeze, 1792). They could be present in the helminth community but absent in the smaller host sample investigated in the present study. Other species reported by Volgar-Pastukhova (1959) were represented by adult stages with a higher prevalence (9–30%); however, they were not found in the present study. We did not record the lung trematode *Hematoloechus asper* Looss, 1899, the intestinal trematode *Candidotrema loossi* (Africa, 1930), and the trematode *Gorgodera cygnoides* (Zeder, 1800) from the bladder in the examined marsh frogs. Instead, the nematodes *O. duboisi* and *I. neglecta*, and the trematode *G. varsoviensis* were found in the present study but were not reported by Volgar-Pastukhova (1959). Interestingly, both nematode species had a rather high prevalence in the examined sample of marsh frogs (see Table 2). *Oswaldocruzia duboisi* was previously reported from France, Bulgaria, and several localities in Ukraine, from various amphibian hosts, including *Pelophylax* spp. (Ben Slimane et al., 1993; Durette-Dessetet al., 1993; Svitin and Kuzmin, 2012). The Danube Delta is a new locality record for this species. *Icosiella neglecta* is widely distributed in the western Palaearctic, including Ukraine, parasitizing mainly *Pelophylax* spp. (Mikulíček et al., 2021; Kuzmin et al., 2023). We conclude, therefore, that the presence of these two species in the studied material reflects changes in the composition of the helminth community of *P. ridibundus* from Vylkove that have occurred over the last 80 years.

The lung nematode *Rhabdias bufonis* (Schrank, 1788), reported by Volgar-Pastukhova (1959) in *P. ridibundus* from Vylkove (P = 5.2%), was not found in the present study. Instead, we found two specimens of *R. esculentarum* in one of the examined frogs (P = 4.3%). The latter species was described in 2012 from *Pelophylax* spp. in Italy (Cipriani et al., 2012); it was later recorded by Herczeg et al. (2016) in Hun-

gary. In the present study, we report this species for the first time in Ukraine and presume that *R. bufonis* from *Pelophylax* spp. recorded by Volgar-Pastukhova (1959) in the Danube Delta was, in fact, *R. esculentarum*.

Further evidence of changes in the helminth community structure in *P. ridibundus* from the Danube Delta may be seen in differences regarding the prevalence of some of the species. Almost all species assigned to the group of predominating species based on their prevalence in the present study were less prevalent in the study conducted by Volgar-Pastukhova (1959): *D. subclavatus* (91.3% *vs* 45.9%), *P. medians* (87.0% vs 39.4%), *P. confusus* (78.3% vs 30.8%), and *A. ranae* (82.6% *vs* 9.5%). All these differences were significant, considering the confidence intervals of their prevalence (see Table 2). On the other hand, the trematodes *O. ranae* and *P. claviger* and the nematode *C. ornata* had similar values of prevalence in the present study and in Volgar-Pastukhova (1959): 21.7% vs 35.2%, 26.1% vs 28.4%, and 52.2% vs 59.9%, correspondingly.

The cestode *O. saphena* is a further helminth species recorded for the first time in the Danube Delta. This species was known from *R. clamitans* from Michigan, USA (Osler, 1931) and *R. catesbeiana* from Arkansas, Michigan, and Wisconsin in the USA and from New Brunswick in Canada (Scholtz et al., 2023). Recently, the species was registered in *Pelophylax epeiroticus* (Schneider, Sofianidou et Kyriakopoulou-Sklavounou, 1984) and *Pelophylax* sp. from Greece, and in *P. ridibundus* from Romania (de Chambrier et al., 2025). Our data, both morphological and molecular, proved that the cestodes found in *P. ridibundus* in Vylkove and *O. saphena* from North American frogs and from frogs in three other localities in Europe are conspecific.

Thomas (1931) experimentally identified *Megacyclops viridis* (Copepoda: Cyclopidae) as the intermediate host of *O. saphena* and successfully infected frog tadpoles with procercoids from copepods (Thomas, 1934). The author noted that the morphology of the procercoids from experimentally infected copepods resembled in all details the pleroceroids removed from the intestine of *R. clamitans* (Thomas, 1931). In our study, the plerocercoids at different stages of development were located in the intestinal wall, while developed strobilae were present in the intestinal lumen. The localisation of developing plerocercoids in the intestinal wall is probably associated with the helminth development in a new host. In an experimental study Thomas (1934), the plerocercoids were found only in the intestinal lumen of infected tadpoles of *R. clamitans*. Plerocercoids collected from frogs in the present study were similar in size to those described from experimentally infected tadpoles in Thomas (1934): up to 2.59 mm vs 3.15 mm, respectively.

The American bullfrog, *R. catesbeiana* is known as an alien species in several European countries: Belgium, France, Germany, Greece, Holland, Italy, Spain, and the United Kingdom; it has established populations detected in Belgium, France, Germany, Greece, and Italy (Johovic et al., 2020). We agree with the assumption of de Chambrier et al. (2025) about the introduction of *O. saphena* to Europe along with its natural host, *R. catesbeiana*. However, we found no official or unofficial information about the introduction of any North American frogs in or around the town of Vylkove. Interestingly, the prevalence of *O. saphena* was significantly higher in the studied sample of *P. ridibundus* from Vylkove (78%) than in other European localities, namely in Limanaki, Greece (20%), Loutros River, Greece (10%), and Chilia Veche, Romania (27%) (de Chambrier et al., 2025), even though the latter

locality is just 23.5 km apart from Vylkove. Now, it looks like the parasite has established its distribution area in the lower Danube, with separate *P. ridibundus* populations harbouring component populations of *O. saphena*.

We also consider the possible scenario in which *O. saphena* was carried to the lower Danube with its intermediate hosts, crustaceans of the Cyclopidae, after transmission had been established through an introduced bullfrog population elsewhere in the Danube or its tributaries. The infected crustaceans might have been transported to the lower Danube and its delta either by the water current or in ballast water of cargo ships.

The present study is the first record of an invasive parasite species in the amphibians of Ukraine. Further investigations of the life-cycle of the parasite in the locality are necessary to identify its intermediate hosts and the routes of *P. ridibundus* infection. In our opinion, the high levels of infection with *O. saphena* observed in the present study in the Ukrainian part of the Danube Delta corroborate the assumption that ecosystems with high biodiversity are more vulnerable to parasite spill-over due to a wide range of possible hosts, both intermediate and definitive.

Acknowledgements. The authors express their sincere thanks to Dr. Volodymyr Gorobchishin for his help in the organisation of the field trip to Vylkove and to Yana Koval for providing accommodation during the field work. The authors also wish to express their sincere thanks to the Armed Forces of Ukraine for enabling research to continue even during the full-scale Russian aggression. The study was supported by the National Research Foundation of Ukraine project No. 2023.03/0068.

REFERENCES

Ben Slimane, B., Durette-Desset, M. C. & Chabaud, A. G. 1993. *Oswaldocruzia* (Trichostrongyloidea) parasites d'Amphibiens des Collections du Museum de Paris. *Annales de Parasitologie Humaine et Comparée*, **68** (2), 88–100. https://doi.org/10.1051/parasite/199368288.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, **83** (4), 575–583. http://dx.doi.org/10.2307/3284227

De Chambrier, A., Kuchta, R., Kyslik, J., Benovics, M. & Scholz, T. 2025. The North American tapeworm *Ophiotaenia saphena* (Cestoda: Proteocephalidae) in frogs (Ranidae) of Europe: Introduction via the American bullfrog (*Lithobates catesbeianus*)? *Systematics and Biodiversity*, **23** (1), 2435831. https://doi.org/10.1080/14772000.2024.2435831

Cipriani, P., Mattiucci, S., Paoletti, M., Santoro, M., & Nascetti, G. 2012. *Rhabdias esculentarum* n. sp. (Nematoda: Rhabdiasidae) from green frogs of the *Rana esculenta* species complex in Italy: molecular evidence, morphological description and genetic differentiation from its congeners in frogs and toads. *Systematic Parasitology*, **82**, 131–146. https://doi.org/10.1007/s11230-012-9355-x

Clarke, K. R. & Gorley, R. N. 2006. *PRIMER v6: User Manual/Tutorial (Plymouth Routines in Multivariate Ecological Research)*. PRIMER-E, Plymouth.

Dubinina, M. N. 1950. Ecological study of the parasite fauna of the marsh frog (*Rana ridibunda* Pall.) of the Volga Delta. *Parazitologicheskiy Sbornik Zoologicheskogo Instituta AN SSSR*, **12**, 300–350 [In Russian].

Durette-Desset, M. C., Batcharov, G., Ben Slimane, B. & Chabaud, A. G. 1993. Some *Oswaldocruzia* (Nematoda: Trichostrongyloidea) parasites of Amphibia in Bulgaria. Redescription of *Oswaldocruzia bialata* (Molin, 1860). *Helmintologia*, **30**, 99–104.

Düşen, S. & Öz, M. 2006. Helminths of the Marsh Frog, *Rana ridibunda* Pallas, 1771 (Anura: Ranidae), from Antalya Province, Southwestern Turkey. *Comparative Parasitology*, 73(1), 121–129.

Georgiev, B., Biserkov, V. & Genov, T. 1986. In toto staining method for cestodes with iron acetocarmine. *Helminthologia*, **23** (4), 121–129.

Herczeg, D., Vörös, J., Végvári, Z., Kuzmin, Y. & Brooks, D. R. 2016. Helminth parasites of the *Pelophylax esculentus* complex (Anura: Ranidae) in Hortobágy National Park (Hungary). *Comparative Parasitology*, **83** (1), 36–48. https://doi.org/10.1654/1525-2647-83.1.36

Hudson, P. J., Dobson, A. P. & Lafferty, K. D. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution*, **21** (7), 381–385.

Johovic, I., Gama, M., Banha, F., Tricarico, E. & Anastácio, P. M. 2020. A potential threat to amphibians in the European Natura 2000 network: Forecasting the distribution of the American bullfrog *Lithobates catesbeianus*. *Biological Conservation*, **245**, 108551. https://doi.org/10.1016/j.biocon.2020.108551

Koprivnikar, J., Marcogliese, D. J., Rohr, J. R., Orlofske, S. A., Raffel, T. R. & Johnson, P. T. 2012. Macroparasite infections of amphibians: what can they tell us? *EcoHealth*, **9** (3), 342–360. https://doi.org/10.1007/s10393-012-0785-3

Kotenko, T. I. 1999. Amphibians and reptiles. *In*: Shelyag-Sosonko, Y. R., ed., *Biodiversity of the Danube Biosphere Reserve, conservation and management*. Naukova Dumka, InterEco-Center, Kyiv, 139–145 [In Ukrainian].

Kuzmin, Y., Dmytrieva, I., Marushchak, O., Morozov-Leonov, S., Oskyrko, O. & Nekrasova, O. 2020. Helminth species and infracommunities in frogs *Pelophylax ridibundus* and *P. esculentus* (Amphibia: Ranidae) in northern Ukraine. *Acta Parasitologica*, **65** (2), 341–353. https://doi.org/10.2478/s11686-019-00164-3

Kuzmin, Y., Dmytrieva, I. & Svitin R. 2023 *Icosiella neglecta* (Nematoda, Onchocercidae) in Ukraine: Occurrence, Hosts, Morphological and Molecular Characterisation. *Zoodiversity*, **57** (1), 75–92. https://doi.org/10.15407/zoo2023.01.075

Maguza, V. S. 1972. Helminths of amphibians of Polissia of Ukraine. Dissertation, Institute of Zoology, Kyiv [In Russian].

Marcogliese, D. J. 2005. Parasites of the superorganism: are they indicators of ecosystem health? *International Journal for Parasitology*, **35** (7), 705–716.

Mazurmovich, B. N. 1951. Parasitic worms of amphibians, their relationships with hosts and environment. Izdatelstvo Kievskogo Gosudarstvennogo Universiteta im. T. G. Shevchenko, Kyiv [In Russian].

Mikulíček, P., Mešková, M., Cyprich, M., Jablonski, D., Papežík, P., Hamidi, D., Pekşen, A. Ç., Vörös, J., Herczeg, D. & Benovics, M. 2021. Weak population-genetic structure of a widely distributed nematode parasite of frogs in the western Palearctic. *Journal of Zoological Systematics and Evolutionary Research*, **59** (8), 1689–1702. https://doi.org/10.1111/jzs.12575

Nekrasova, O. D. 2002. Population structure and hybridization of green frogs of the "*Rana esculenta*" complex from urban territories of Middle Dnieper basin. Dissertation, Institute of Zoology, Kyiv [In Ukrainian].

Nekrasova, O. D. & Morozov-Leonov, S. Y. 2012. Population systems of the green frogs *Pelophylax esculentus* complex of the Danube river and their composition. *In* Proceedings of the 5th Congress of the Alexander M. Nikolsky Herpetological Society. *Questions of Herpetology*. Minsk, Belarus, 206–209 [In Russian].

Osler, C. P. 1931. A new cestode from *Rana clamitans* Latr. *Journal of Parasitology*, **17** (4), 183–186. https://doi.org/10.2307/3271453

Paradis, E. 2010. pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, **26**, 419–420. https://doi.org/10.1093/bioinformatics/btp696

Paradis, E. & Schliep, K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, **35**, 526–528. https://doi.org/10.1093/bioinformatics/bty633

Popiołek, M., Rozenblut-Kościsty, B., Kot, M., Nosal, W. & Ogielska, M. 2011. Endoparasitic helminths of water frog complex in Poland: do differences exist between the parental species *Pelophylax ridibundus* and *Pelophylax lessonae*, and their natural hybrid *Pelophylax esculentus*? *Helminthologia*, **48** (2), 108–115. https://doi.org/10.2478/s11687-011-0020-8

Poulin, R. 1998. Comparison of three estimators of species richness in parasite component communities. *Journal of Parasitology*, **84** (3), 485–490.

Pysanets, Y. M. 2007. Amphibians of Ukraine (a guide to identifying amphibians of Ukraine and neighbouring countries). Vydavnytstvo Rayevskogo, Kyiv [In Ukrainian].

R Core Team. 2024. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org

Rezvantseva, M. V., Lada, G. A., Aksyonov, D. S., Shabanov, D. A., Korshunov, A. V., Chikhlyaev, I. V., Borkin, L. J., Litvinchuk, S. N. & Rozanov, J. M. 2010. Data on helminth fauna of green frogs (*Rana esculenta* complex) in the Kharkov Province. *In* Theoretical and Practical Problems of Parasitology, Materials of the International Conference (November 30–December 3, 2010, Moscow), Moscow, 308–312 [In Russian].

Rózsa, L., Reiczigel, J. & Majoros, G. 2000. Quantifying parasites in samples of hosts. *Journal of Parasitology*, **86** (2), 228–232. https://doi.org/10.1645/0022-3395(2000)086[0228:QPISOH]2.0.CO;2

Saeed, I., Al-Barwari, S. E. & Al-Harmni, K. I. 2007. A metazoan parasitological research of some Iraqi amphibians. *Türkiye Parazitoloji Dergisi*, **31** (4), 337–345.

Scholz, T., de Chambrier, A., Kudlai, O., Tkach, V. V. & McAllister, C. T. 2023. A global survey of tapeworms (Cestoda: Proteocephalidae) of 'true' frogs (Amphibia: Ranidae), including a tabulated list of all proteocephalids parasitizing amphibians. *Folia Parasitologica*, **70**, 009. https://doi.org/10.14411/fp.2023.009

van Steenkiste, N., Locke, S. A., Castelin, M., et al. 2015. New primers for DNA barcoding of digeneans and cestodes (Platyhelminthes). *Molecular Ecology Resources*, **15**, 945–952. https://doi.org/10.1111/1755-0998.12358

Svitin, R. S. & Kuzmin, Y. I. 2012. *Oswaldocruzia duboisi* (Nematoda, Molineidae): Morphology, hosts, and distribution in Ukraine. *Vestnik Zoologii*, **46** (3), 195–203.

Shcherbak, N. N. & Shcherban, M. I. 1980. Amphibians and reptiles of the Ukrainian Carpathians. Naukova Dumka, Kyiv [In Russian].

Thomas, L. J. 1931. Note on the life history of *Ophiotaenia saphena* from *Rana clamitans* Latr. *Journal of Parasitology*, **17** (4), 187–195. https://doi.org/10.2307/3271454

Thomas, L. J. 1934. Further studies on the life cycle of a frog tapeworm, *Ophiotaenia saphena* Osler. *Journal of Parasitology*, **20** (5), 291–294. https://doi.org/10.2307/3272191

Török, Z. 2002. Aspects related to the sustainable exploitation of frog stocks from the Danube Delta Biosphere Reserve (Romania). *Scientific Annals of the Danube Delta National Institute for Research and Development*, **4**, 185–190.

Török, Z. 2014. Assessing the stocks of green frogs (*Rana esculenta* / *Pelophylax esculentus* complex) from the fluviatile Danube Delta, in the early stage of their reproduction period. *In*: Book of Abstracts of International Conference "Environmental aspects and available scientific tools for Black Sea Basin protection — DELTAS AND WETLANDS", Tulcea, Romania, **2**, 40.

Vasyliuk, O., Prylutskyi, O., Marushchak, O., Kuzemko, A., Kutsokon, I., Nekrasova, O., Raes, N. & Rusin, M. 2022. An extended dataset of occurrences of species listed in Resolution 6 of the Bern Convention from Ukraine. *Biodiversity Data Journal*, **10**, e84002. https://doi.org/10.3897/bdj.10.e84002

Volgar-Pastukhova, L. G. 1959. Parasite fauna of tailless amphibians of the Danube Delta. *In*: Ecological Parasitology, ed. Yu. I. Polianskiy, Izdatelstvo Leningradskogo Universiteta, 58–95 [In Russian].

Received 19 December 2024
Accepted 21 October 2025