Annals of Parasitology 2026, 72, 27–37
doi: 10.17420/ap72.552

Original paper

Helminths of the common crane (*Grus grus*) from the South of Ukraine with new morphological and molecular data for *Gruitaenia latissima* (Cestoda, Dilepididae)

Oksana B. GREBEN[1], Yaroslav SYROTA[1,2], Olga I. LISITSYNA[1], Tetiana A. KUZMINA[1]

[1]I.I. Schmalhausen Institute of Zoology NAS of Ukraine, vul. B. Khmelnytskogo, 15, Kyiv, 01054 Ukraine
[2]Institute of Parasitology of Slovak Academy of Sciences, Hlinkova 3, Košice, 040 01, Slovakia

Corresponding Author: Yaroslav Syrota; e-mail: syrota@saske.sk

ABSTRACT. This study provides the first investigation of the helminth fauna of the common crane (*Grus grus*) in Ukraine. Fifty cranes were collected in southern Ukraine in February and March 2021 following a mass poisoning event caused by rodenticide brodifacoum. Eleven helminth taxa were identified, including six nematode taxa (Acuariidae gen. sp., *Eucoleus obtusiuscula*, *Physocephalus sexalatus*, *Porrocaecum ardeae*, *Strongyloides* sp., *Tetrameres paradisea*), four trematode taxa (*Bilharziella polonica*, *Strigea gruis*, *Strigea sphaerula*, *Strigea* sp.), and one cestode (*Gruitaenia latissima*). Four species, namely *E. obtusiuscula*, *T. paradisea*, *S. gruis*, and *G. latissima*, were reported for the first time in Ukraine. Among the recorded helminths, *E. obtusiuscula*, *T. paradisea*, *S. gruis*, and *P. ardeae* were the dominant taxa, with prevalence values of 50–88%, mean intensities of 6–25, and mean abundances of 4–20. Additionally, this study provides the first detailed description of the scolex morphology of the cestode *G. latissima* and the first molecular data for this species, represented by a partial sequence of the mitochondrial *nad1* gene. Our findings enrich knowledge of the helminth fauna of the common crane in Eastern Europe and establish reference data for further taxonomical investigations of the genus *Gruitaenia*.

Keywords: bird parasites, avian helminths, fauna, helminth community, *Gruitaenia latissima*, Ukraine, Europe

Introduction

The common crane, *Grus grus*, is one of the most widely distributed crane species in the Palaearctic with an estimated wild population size of more than 700,000. Its breeding range spans from Western and Northern Europe across the entirety of Russia to Eastern Siberia, with its southern limits reaching Mongolia and northern China [1]. During migration, these populations use multiple flyways to reach wintering grounds distributed across the Mediterranean basin, the Near East, north-east Africa, Iran, the Indian subcontinent, and China, with marginal numbers reaching as far as Myanmar and North Vietnam [1]. In Ukraine, the main breeding areas are located in the northern part of the country. In contrast, southern Ukraine, particularly the territory of the F. E. Falz-Fein Biosphere Reserve "Askania-Nova" and its vicinity in Kherson Region, serves mainly as an important migration stopover, with occasional wintering of small flocks recorded there [2].

Because crane species, including the common crane, are protected across large parts of their range [1], helminthological studies of these birds are usually limited to opportunistically collected material from individuals whose death was unrelated to research activities. According to published reports, 44 helminth species have been recorded in cranes, including 20 nematode species, 21 trematode species, two cestode species, and one acanthocephalan [3–10]. Of these, only ten species are considered host-specific to cranes. For *G. grus* specifically, 22 helminth species, comprising nine trematodes, one cestode, and 12 nematodes, have been documented [10–13]. The feeding ecology of

the common crane largely influences the composition of its helminth fauna. As an omnivorous bird, it consumes plant material and animal prey, including small vertebrates and a wide range of invertebrates [14–16]. Among the latter, arthropods, molluscs, and oligochaetes are particularly important for helminth transmission, as they may serve as intermediate hosts in helminth life cycles.

To date, the only published report on the helminths of the common crane from Ukraine concerns the nematode *Dispharynx nasuta* (Rudolphi, 1819), found in a captive individual at Kyiv Zoo [17]. In that case report, adult worms were recovered during necropsy, and transmission within the zoo was further supported by the occurrence of *D. nasuta* larvae in terrestrial isopods (*Oniscidea* spp.), which were abundant in the aviary where the crane had been kept. These isopods may have become infected after ingesting parasite eggs shed with the faeces of wild birds visiting the zoo. Thus, prior to the present study, published data on helminths of free-ranging common cranes in Ukraine were lacking. In this context, the present study aimed to characterise the helminth fauna of common cranes from southern Ukraine and provide a basis for future comparisons of *G. grus* helminths across its range.

Materials and Methods

Collection of helminths

Fifty carcasses of common cranes were collected on a farmland near the F. Falz-Fein Biosphere Reserve "Askania Nova" (46°27′ N; 33°52′ E) in the Kherson Region, the South of Ukraine, in February and March 2021. The carcasses were collected following a mass mortality event in agricultural fields that was caused by accidental poisoning of the birds with the rodenticide brodifacoum [18]. The gastrointestinal tracts were removed, frozen at –18 °C and transported to the laboratory of the I.I. Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine in Kyiv, for parasitological examination, which was performed following standard parasitological procedures [19]. Helminth specimens were collected manually under a stereo microscope, fixed, and stored in 70% ethanol.

Morphological analysis

For morphological examination, the cestode and trematode specimens were stained with iron acetocarmine as described by Georgiev et al. [20]. Then, the helminths were dehydrated through a graded ethanol series, cleared in clove oil, and mounted in Canada balsam. For detailed morphological examination of the hooks and copulative organs, scolices and fragments of the cestodes were prepared separately by staining with iron acetocarmine diluted with 70% ethanol (1:1) and mounting in Berlese's medium. Nematodes were washed in distilled water and then cleared in lactophenol (an equal-parts mixture of distilled water, glycerol, phenol, and lactic acid). The morphology of helminths was examined under a Zeiss Axio Imager M1. Identification of helminths was based on published descriptions [11, 21–27]. The drawings of cestodes were made using a drawing tube RA-6. All measurements are in micrometres (µm) and are given as a range, followed by the mean and the number of measurements (n) in parentheses.

Molecular analysis

Due to the observation of novel morphological characteristics, the cestode *G. latissima* was selected for molecular analysis to establish a reference sequence. Total genomic DNA was extracted from a strobilar fragment of the voucher specimen using the FavorPrep^{TM} Tissue Genomic DNA Extraction Kit (Favorgen Biotech Corporation, Pingtung, Taiwan) according to the manufacturer's instructions. A partial sequence of the mitochondrial *nad1* gene, encoding NADH dehydrogenase subunit 1, was amplified using the following primers targeting an approximately 850 bp region [28]: *Cyclo_nad1F* 5′-GGNTATTSTCARTNTCGTAAG GG-3′ and *Cyclo_trnNR* 5′-TTCYTGAAGTTAAC AGCATCA-3′. The polymerase chain reaction (PCR) was performed in a final volume of 25 µl, containing 12.5 µl of MyTaq^{TM} HS Red Mix (Bioline Reagents, London, UK), 1 µl of each primer (10 µM), 2 µl of template DNA, and 8.5 µl of nuclease-free water. Thermal cycling conditions included an initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min. Subsequently, the PCR product was visualised on a 1.5% agarose gel alongside a 100 bp DNA ladder. Amplicons were purified using the GeneAll® ExpinTM Combo GP kit (GeneAll Biotechnology Co., Ltd., Seoul, South Korea) and sent for Sanger

Table 1. Infection parameters for helminth species collected from the common crane *Grus grus*. For infection prevalence (P) and mean abundance (MA), 95% confidence intervals are shown in brackets; intensity (I) is presented as mean followed by range in parentheses and median; A – abundance, RA – relative abundance (in %)

Species	P, in %	MA	I	A	RA, in %
PLATYHELMINTHES: TREMATODA					
Bilharziella polonica	10.0 [4.0–21.8]	0.10 [0.02–0.18]	1 (1–1), 1	5	0.2
Strigea gruis	50.0 [35.9–64.1]	12.36 [6.90–21.26]	24.7 (1–99), 12	618	26.8
Strigea sphaerula	4.0 [0.7–13.7]	0.20 [0.0–0.80]	5.0 (2–8), 5	10	0.4
Strigea sp.	2.0 [0.1–10.6]	0.28 [0.0–0.84]	14*	14	0.6
PLATYHELMINTHES: CESTODA					
Gruitaenia latissima	14.0 [6.7–26.8]	0.32 [0.12–0.72]	2.3 (1–5), 1	16	0.7
NEMATODA: CHROMADOREA					
Acuariidae gen. sp.	2.0 [0.1–10.6]	0.02 [0.0–0.06]	1*	1	0.04
Eucoleus obtusiuscula	62.0 [48.0–74.6]	6.96 [4.66–10.68]	11.2 (1–53), 6	348	15.1
Physocephalus sexalatus	18.0 [9.5–30.9]	0.34 [0.16–0.76]	1.9 (1–6), 1	17	0.7
Porrocaecum ardea	70.0 [56.0–81.2]	4.24 [3.06–6.46]	6.1 (1–31), 4	212	9.2
Strongyloides sp.	26.0 [15.5–39.9]	0.92 [0.44–1.94]	3.5 (1–13), 2	46	2.0
Tetrameres paradisea	88.0 [76.2–94.6]	20.4 [13.36–32.12]	23.2 (1–142), 10	1,020	44.2

* – only one host infected

sequencing using the amplification primers and an internal sequencing primer (*Cyclo_nad1Fb* 5′-AGG TTTGARGCKTGTTTTATG-3′) at SEQme s.r.o. (Dobříš, Czech Republic). Resulting chromatograms were assembled and edited in Geneious Prime 2026.0.2 (https://geneious.com). Sequence similarity searches were performed using the NCBI BLAST web interface. The nucleotide sequence was compared against the core nucleotide database using BLASTn.

Helminth community description

For each helminth species, prevalence, intensity, total abundance, and mean abundance were calculated according to the definitions of Bush et al. [29]. Relative abundance was calculated as the percentage contribution of each species to the total number of helminths recovered. The dispersion index, expressed as the variance-to-mean ratio, was calculated for the whole sample to assess the overall distribution pattern of helminths, following the recommendations of Rózsa et al. [30]. The 95% confidence intervals for prevalence and mean abundance were calculated using Quantitative Parasitology 3.0 software [30]. Diversity indices for the helminth component community were calculated using PRIMER 6 software [31].

Results

Helminth species

Eleven helminth taxa were identified, which included six nematodes, four trematodes, and one cestode (Table 1). Eight taxa were identified at the species level. Two nematode and one trematode taxa could not be identified to the species level, but they were treated as separate taxonomic entities based on their morphological traits. The list of found taxa with remarks is presented below.

Phylum Nematoda

Class Chromadorea

Family Tetrameridae Travassos, 1914

Tetrameres (*Tetrameres*) *paradisea* Ortlepp, 1932 (Fig. 1D)

Syn: *Tetrameres* (*Tetrameres*) *grusi* (Shumakovich, 1946)

Site of infection: proventriculus

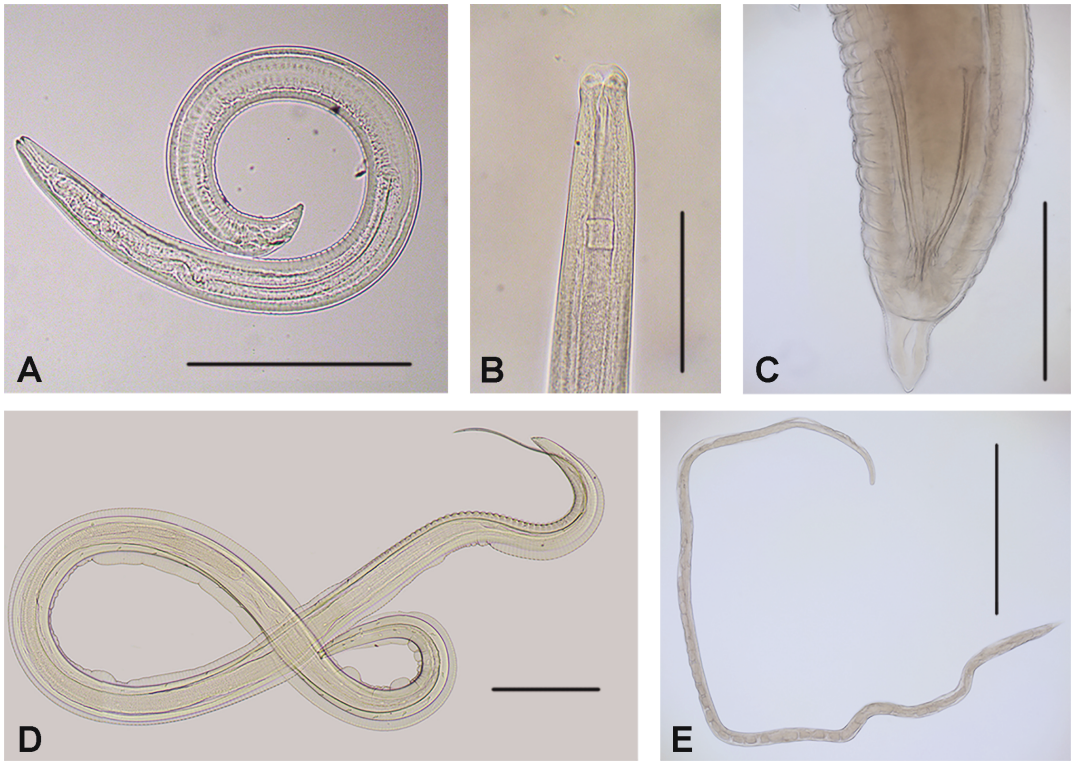

Figure 1. Nematodes found in *Grus grus* from the South of Ukraine: A – *Physocephalus sexalatus*, general view, scale bar 300 μm; B – *Eucoleus obtusiuscula,* posterior end, scale bar 100 μm; C – *Porrocaecum* (*Porrocaecum*) *ardeae*, posterior end, scale bar 300 μm; D – *Tetrameres* (*Tetrameres*) *paradisea*, general view, scale bar 300 μm; E – *Strongyloides* sp.*,* general view, scale bar 300 μm

Remarks. This species is reported exclusively from cranes. It was originally described from a host identified as "*Tetrapteryx* (*paradisea*)" in South Africa [32]; since then, it has been recorded in various crane species in Germany, Russia, and the USA [3,9,33–38], as well as in common cranes in Tajikistan [39] and Russia [40]. Small crustaceans were reported as intermediate hosts in the life cycle of this nematode [9]. The present study constitutes the first record of *T. paradisea* in Ukraine.

Family Acuariidae Railliet, Henry et Sisoff, 1933
Acuariidae gen. sp.
Site of infection: gizzard

Remarks. One third-stage larva was found in a single crane. The presence of cuticular cords on the anterior end allowed the nematode to be assigned to the family Acuariidae.

Family Ascarididae Baird, 1853
Porrocaecum (*Porrocaecum*) *ardeae* (Frölich, 1802) (Fig. 1C)
Site of infection: gizzard, intestine

Remarks. This species was originally described from a heron in Europe [41]. Since then, it has been documented in ciconiiform, charadriiform, and phoenicopteriform birds, as well as in various crane species across Europe, Asia, Africa, and North America [23]. In the common crane, the species was recorded in Germany, Hungary, Bulgaria, Russia, and Azerbaijan [9,13,40,42,43]. The life cycle of *P. ardeae* was experimentally studied in Germany [9]; earthworms were identified as intermediate hosts of this nematode. In Ukraine, the species has been found in herons in the Danube Delta [44] and in the demoiselle crane (*Grus virgo*) at the "Askania Nova" Biosphere Reserve [45].

Family Spirocercidae Chitwood et Wehr, 1932
Physocephalus sexalatus (Molin, 1860) (Fig. 1A)
Site of infection: intestine

Remarks. In this study, larvae in capsules were recovered in the intestine of common cranes. This nematode parasitises the gastric mucosa of various ungulates: camels, donkeys, cattle, and pigs, which are the definitive hosts [27]; however, it is often registered in other groups of vertebrates, including birds, which serve as its paratenic hosts. Scarabaeidae beetles are the intermediate hosts for this species [27]. The nematode has a cosmopolitan distribution. Domestic geese, ducks, and reptiles

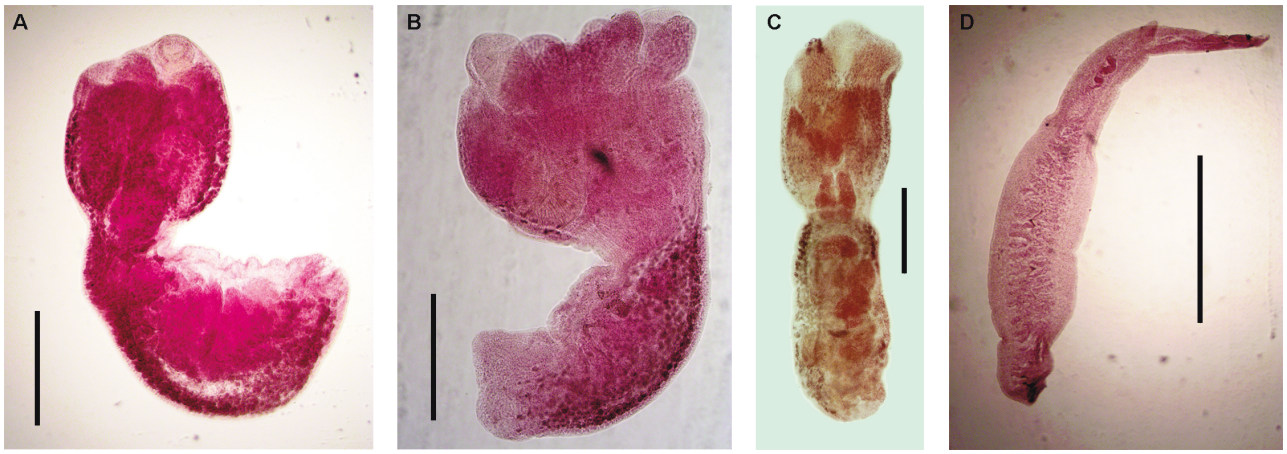

Figure 2. Trematodes found in *Grus grus* from the South of Ukraine: A – *Strigea gruis*, general view, scale bar 500 μm; B – *Strigea sphaerula,* general view, scale bar 300 μm; C – *Strigea* sp., general view, scale bar 500 μm; D – *Bilharziella polonica,* general view, scale bar 1000 μm

were recorded as paratenic hosts of *P. sexalatus* across Ukraine [44]. This is the first record of this parasite from the common crane in Ukraine.

Family Strongyloididae Chitwood & McIntosh, 1934
Strongyloides sp. (Fig. 1E)
Site of infection: intestine

Remarks. Due to the absence of diagnostic morphological characters, identification to the species level was not possible.

Class Enoplea
Family Capillariidae Railliet, 1915
Eucoleus obtusiuscula (Rudolphi, 1819) (Fig. 1B)
Site of infection: gizzard, intestine

Remarks. This species was described from a crane in Europe; however, it was often recorded in sandpipers in Europe, Asia, Africa and North America [12,46,47]. A later record from the common crane is known from Russia [40]. The life-cycle of *E. obtusiuscula* is undescribed. The finding represents the first record of *E. obtusiuscula* in Ukraine.

Phylum Platyhelminthes
Class Trematoda
Family Strigeidae Railliet, 1919
Strigea gruis Dubois et Rausch, 1964 (Fig. 2A)
Site of infection: intestine

Remarks. This species was described in North America [21]; it has been reported only from cranes and is considered a typical helminth of these birds. The life cycle of *S. gruis* is undescribed. This finding represents the first record of the species in Ukraine and Europe.

Strigea sphaerula (Rudolphi, 1803) (Fig. 2B)
Site of infection: intestine

Remarks. This species is the common parasite of corvids and other birds of the order Passeriformes in Europe, Asia and North America [22]; the common crane is an accidental host for this species. In Ukraine, *S. sphaerula* was recorded in rook and crow [47,48]. Cercariae develop in Planorbidae molluscs; the second intermediate hosts are amphibians and reptiles [22].

Strigea sp. (Fig. 2C)
Site of infection: intestine

Remarks. Fourteen unidentified *Strigea* specimens were recovered from a single host individual. Based on morphological characteristics, the specimens could not be reliably identified to the species level.

Family Schistosomatidae Stiles et Hassall, 1898
Bilharziella polonica (Kowalewski, 1895) (Fig. 2D)
Site of infection: veins of the abdominal cavity
Site of recovery: washing from the proventriculus, intestine, and caeca

Remarks. This species is a non-specific, widespread parasite of birds, predominantly waterfowl. In the common crane, *B. polonica* has been documented in France [8]. Although the species is widely distributed across Ukraine [44], this study represents the first record of it parasitising the common crane in the country. Planorbidae and Lymneidae molluscs are the intermediate hosts of this trematode species [49–52].

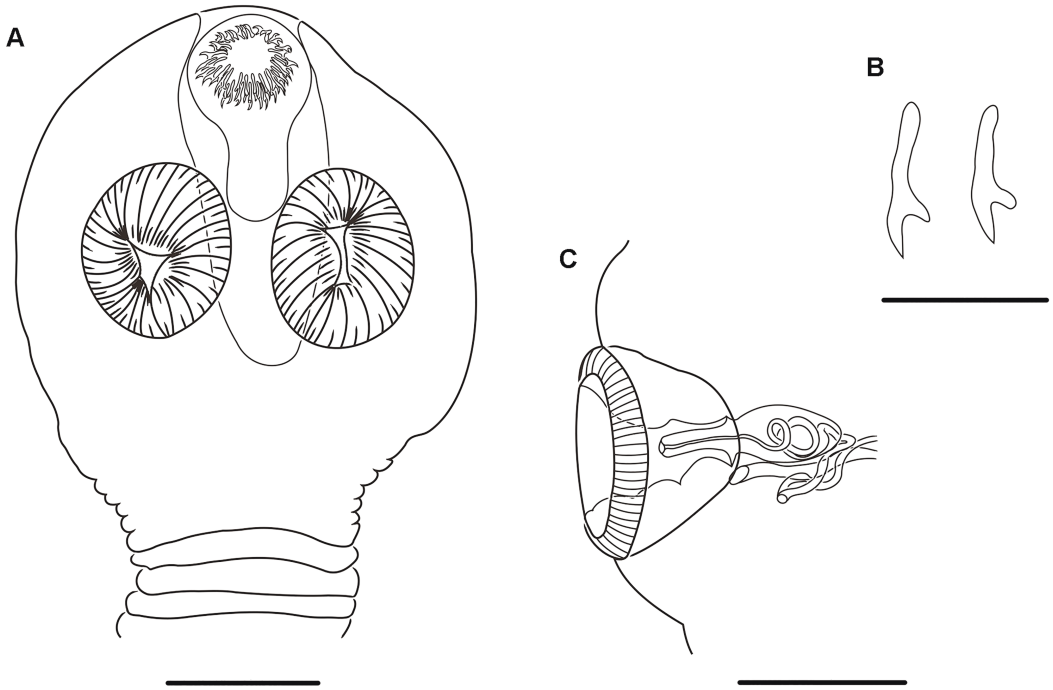

Figure 3. Morphology of *Gruitaenia latissima* from *Grus grus*: A – scolex, scale bar 100 μm; B – rostellar hooks, scale bar 200 μm; C – genital atrium, scale bar 300 μm

Class Cestoda
Family Dilepididae Railliet et Henry, 1909
Gruitaenia latissima Spasskii, Borgarenko et Spasskaya, 1971 (Figs. 3 and 4)
Site of infection: intestine

GenBank accession representative DNA sequence: PZ397833

Remarks. This species is considered a host-specific parasite of the common crane. It was originally described from a crane in a zoo in Tajikistan [11]; subsequently, it was recorded in common cranes in Tajikistan and Germany [9,53]. This report represents the first record of the species in Ukraine. Since the species' morphology was described only in the original description, which lacked details on the scolex morphology and the number, shape, and size of the rostellar hooks, we provide a detailed description of our specimens here. We also obtained a 789 bp partial *nad1* sequence from the morphologically identified *G. latissima* specimen. The closest match was a record attributed to *Eurycestus avoceti* (GenBank accession number PQ156477) [54]; however, the similarity was only 80.23%.

Description (based on four specimens, three of them have scolices).

Strobila thin and wide. Total length of specimens with scolex up to 649.7 mm. Maximum width at level of proglottids with gravid uterus 5 mm. Proglottids winder than longer with weakly developed sail. Only gravid proglottids longer than wide. Scolex very small and delicate, 230–290 × 275–295 (257 × 287, n = 3), with oval muscular suckers. Suckers 90–130 (120, n = 12) in length and 70–105 (97, n = 12) in width. Rostellar sheath sac-like, 100–220 × 80–110 (153 × 90, n = 3), slightly overlaps posterior borders of the suckers. Rostellum small, 90–130 × 60–70 (110 × 63, n = 3), armed with 38–42 hooks in two rows. Total hook length 18–25 (23, n = 15), handle 10–14 (13, n = 15), blade 5–8 (7, n = 15), guard 4–6 (7, n = 15). Neck width about 170–185.

External and internal segmentation of strobila present. Genital pores alternate irregularly. They located in anterior half of lateral margin of proglottid. Wide osmoregulatory canals, 2 pairs in lateral fields, with transverse anastomoses. Diameter of ventral osmoregulatory canals 90–320, of dorsal canals 40–50. Genital atrium well developed, muscular 140–200 (172, n = 20) in depth. Genital ducks located dorsal to osmoregulatory canals. Ontogeny of proglottids protandrous. Oval testes numerous, occupy almost entire proglottid. Most of them located in posterior and lateral parts of proglottid. In hermaphroditic proglottids, testes envelop female gonads. The total number of testes varies from 340 to

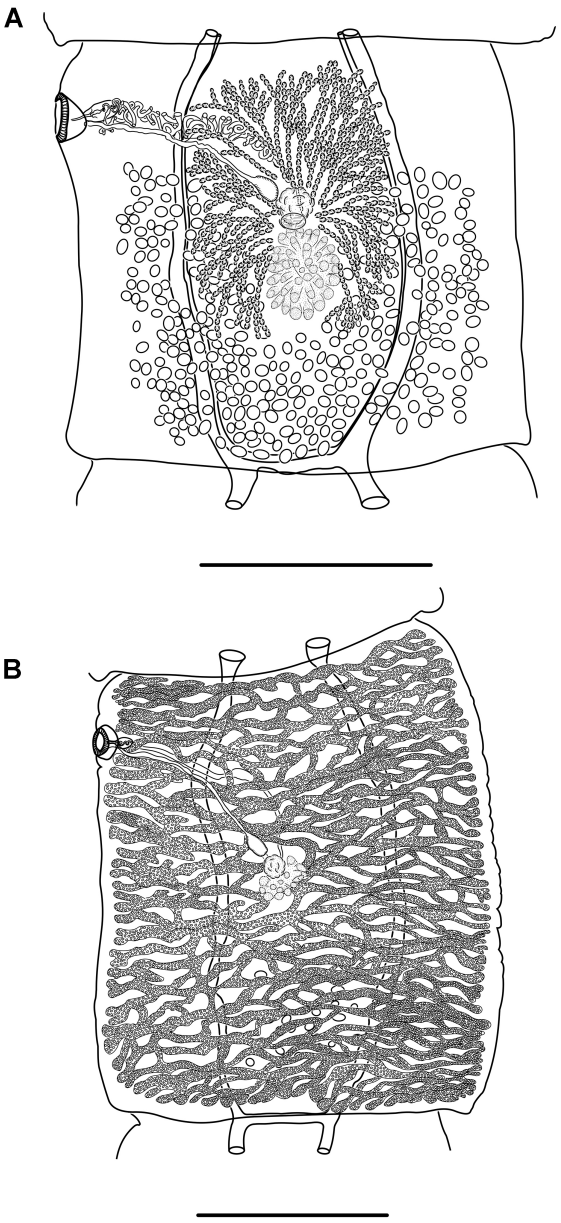

Figure 4. Proglottids of *Gruitaenia latissima* from *Grus grus*: A – hermaphroditic proglottid, scale bar 1500 μm; B – gravid proglottid, scale bar 2500 μm

490. More than half of them, 116–213 (144, n = 20) lie in posterior part of middle field of the proglottid, between osmoregulatory canals. 75–105 (90, n = 20) testes localised in left lateral field, 76–126 (97, n = 20) in right lateral field. A small part of testes overlaps osmoregulatory canals: on left – 8–28, (16, n = 20), on right – 10–30, (19, n=20). Testes 75–130 × 65–100 (105 × 81, n = 50) in size. Cirrus sac small and oval, 190–250 × 80–150 (212 × 110, n = 50), not reaches ventral osmoregulatory canals. Cirrus cylindrical, unarmed, located in genital atrium. Length of cirrus 80–150 (122, n = 34) with diameter 35–45 (40, n = 34). Vas deferens forms several bends inside the cirrus sac, emerges from its bottom and extends to Mehlis' gland, which located in anterior part of medial field of proglottid. Vas deferens forms numerous bends along its entire length.

Ovary fan-shaped, occupies almost entire anterior part of medial field of proglottid. It consists of elongated thin tubular lobules that capable of forming anastomoses and branching. Rounded Mehlis' gland, 150–310 × 140–280 (247 × 210, n = 50), localised behind anatomical centre of ovary. Oval ootype, 90–200 × 60–150 (129 × 98, n = 30), posterior to Mehlis' gland. Vitellarium, 500–920 × 460–700 (711 × 581, n = 50) occupies central position in proglottid. It consists of slender lobules diverged in different directions. Vagina opens into genital atrium posteriorly to cirrus sac. Copulative part of vagina as slightly tortuous tube with a diameter of 35–100 (64, n = 34), lies behind the cirrus sac and extends along the loops of vas deferens. It has length of about 529–980 (741, n = 30). Near poral osmoregulatory canals in median field of proglottid copulative part of vagina passes into narrower conducting part with diameter of 20–50. Conducting part of vagina flows into seminal receptacle. Size of seminal receptacle 180–480 × 90–240 (325 × 170, n = 50). Uterus first appears as narrow tubule at level of ovary and vitellarium, gradually maturing, forming numerous tubes with anastomoses. Gravid uterus becomes reticular, occupies entire median field of proglottids and enters lateral fields. Mature eggs absent in material.

Helminth communities

All 50 examined common cranes were infected with at least one specimen of gastrointestinal helminth. Helminth infracommunity species richness ranged from 1 to 7, with a mean of 3.5 (standard deviation [SD] = 1.2) and a median of 3.5. Total helminth abundance in the infracommunities ranged from 1 to 167 specimens per host, with a mean of 46.1 (SD = 43.1) and a median of 30 specimens. At the component community level, the dispersion index was 92.9, indicating a highly aggregated distribution of helminths among hosts, with most parasite specimens concentrated in a relatively small number of individuals. A total of 2,307 specimens of gastrointestinal helminths were collected. Nematodes were the most common, infecting 100% of the examined cranes, whereas trematodes and cestodes were found in 54% and 14% of the cranes, respectively. The Berger-Parker dominance index was 0.44, reflecting the highest

relative abundance of *T. paradisea*. Four helminth species, namely *P. ardea*, *E. obtusiuscula*, *S. gruis*, and *T. paradisea*, together accounted for 95% of the total helminth specimens collected. The Shannon diversity index was 1.44, the Simpson diversity index was 0.70, and Pielou's evenness was 0.60. Margalef index of species richness was 1.29.

Discussion

Although the common crane is a relatively well-studied bird species, knowledge of its helminth fauna remains fragmentary. In Ukraine, available information is also limited, consisting of a single case report [17]. Our present study helps to fill this gap by documenting eight helminth species: *T. paradisea*, *P. ardeae*, *P. sexalatus*, *E. obtusiuscula*, *S. gruis*, *S. sphaerula*, *B. polonica*, and *G. latissima*, and three indeterminate taxa, each representing at least one distinct species. Four of the documented species, namely *E. obtusiuscula*, *S. gruis*, *T. paradisea*, and *G. latissima*, represent first records for the helminth fauna of Ukraine. Our findings not only considerably expand the known helminth fauna of *G. grus* in the region but also reveal a clear community pattern: the helminth community was characterised by a core group of dominant species. In particular, four species, the nematodes *P. ardeae*, *E. obtusiuscula*, and *T. paradisea*, and the trematode *S. gruis*, dominated the community in terms of prevalence, intensity and abundance. An interesting feature of our material is that, despite the largely winter and early spring timing of our study, nearly all identified species were helminths with indirect life cycles, and total nematode prevalence reached 100%. This observation may reflect the long survival of adult helminths acquired earlier in the annual cycle, although additional infections acquired at wintering or stopover sites, where suitable intermediate hosts are available, cannot be excluded.

To date, the most comprehensive study of helminths in the common crane was conducted in Germany [9, 44, 55], where 167 individuals were examined over a 10-year period (1998–2008). The total number of helminth taxa reported there was higher than in our study (14 *vs* 11). This difference may partly reflect the larger sample size and longer sampling period of the German study, but it was also associated with greater trematode diversity. Despite these differences, the dominant component of the helminth fauna was similar in both countries and included the same nematode species: *P. ardeae*, *E. obtusiuscula*, and *T. paradisea*. The predominance of the same helminth species in both Ukraine and Germany suggests a relatively stable core helminth fauna, at least in the European part of the host range.

A similar pattern has also been reported for sandhill cranes (*Grus canadensis*) in North America [3, 36, 38]. These studies were conducted in different regions and during different phases of the annual cycle, yet each described a relatively depauperate helminth fauna, dominated by only a few species. Although direct comparisons with our material are limited by differences in host species, geography and sampling design, these observations support the view that cranes may maintain a small but stable set of dominant helminths across different phases of the annual cycle.

In our study, *Gruitaenia latissima*, a host-specific parasite of the common crane, was found in 14.0% of the birds examined. This cestode was originally described from a common crane at the Tajikistan zoo based on specimens without scolices [11]; however, the unique morphology of the strobilae allowed the erection of a new genus, *Gruitaenia* Spasskii, Borgarenko et Spasskaya, 1971, within the family Dilepididae. The second species of the genus, *Gruitaenia gruis* Rausch et Rausch, 1985, was later described from *G. canadensis* in the USA [56]. The authors provided a detailed description, noting that while these two species are morphologically similar, they can be distinguished by the following criteria:

(i) The length of strobila. According to Spassky et al. [11], the strobila in *G. latissima* is shorter and wider (180−200 × 6.7) than in *G. gruis* (314 × 3.4).

(ii) The position of the testes in the proglottid. The testes in *G. latissima* do not overlap the ventral osmoregulatory canals. The testes in *G. gruis* overlap the ventral osmoregulatory canals in the posterior part of the proglottid.

(iii) The length of the cirrus-sac. The cirrus-sac in *G. latissima* is 225 in length and 112 in diameter, and does not reach the poral osmoregulatory canals. The cirrus-sac in *G. gruis* is 233−300 in length and 50−90 in diameter, and reaches the poral osmoregulatory canals.

(iv) The position of the lateral margins of the ovary's lobes. In *G. latissima,* lobules of the lateral margins of the ovary do not reach the ventral osmoregulatory canals. Numerous testes are present between the margins of the ovary and ventral osmoregulatory canals. In *G. gruis*, along the

lobules of the lateral margins of the ovary overlap the ventral osmoregulatory canals and in the middle field of the proglottids. Testes are not present lateral to the margins of the ovary.

(v) The morphology of the scolex. Since the scolex of *G. latissima* was unknown, and *G. gruis* was described with a scolex, the authors considered that the additional difference would be in the size, shape and number of rostellar hooks.

Before our study, the morphology of the *G. latissima* scolex had not been described. We determined that the scolex was armed with 38–42 hooks in two rows, measuring 18–25 μm in length. The shape, length, and number of hooks in both species were similar; *G. gruis* possesses 40 hooks, ranging from 14 to 19 μm in length.

The strobiles in our material were longer than those described by Spassky et al. [11] and Rausch and Rausch [56]. We suggest that strobila length is not a reliable diagnostic criterion for species differentiation, as it is highly variable across fixation methods. We also observed that the position of the testis in our specimens is variable: in some proglottids, they overlap the osmoregulatory canals, a feature consistent with the description of *G. gruis*.

The size of the cirrus sac in our *G. latissima* specimens (190–250×80–150) encompasses the ranges reported for both *G. latissima* (225×112; [11]) and *G. gruis* (233–300 × 50–90; [56]). Consequently, based on our data and literature description, *G. latissima* differs from *G. gruis* only by the position of the cirrus sac, which never reaches the poral osmoregulatory canals. While this criterion is quite subtle for the morphological differentiation between these two species, we refrain from proposing synonymy until the original type material of *G. gruis* can be examined.

In conclusion, our study considerably expands the documented diversity of crane helminths in Ukraine and Eastern Europe. By combining faunistic records with morphological and molecular data, it provides an important basis for future studies on the diversity, host associations, and biogeography of helminths parasitising cranes.

Acknowledgements

The authors are grateful to Natalya S. Zvegintsova from the F. Falz-Fein Biosphere Reserve “Askania Nova”, Kherson region, Ukraine, for her assistance in collecting the material.

This study was supported by the National Research Foundation of Ukraine (project number 2023.03/0068).

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Received 31May 2026
Accepted 08 August 2026