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Helminths of the rock lizards *Darevskia dahli* and *D. armeniaca* in their invaded range in Ukraine

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Abstract

This study investigated the helminths of the mixed invasive population of *Darevskia armeniaca* and *D. dahli*, collected during two field trips in Denyshy, Zhytomyr region, Ukraine, in 2023. In total, 67 adult lizards (35 *D. armeniaca* and 32 *D. dahli*) were examined. Molecular and morphological approaches were used to identify the parasites. The analyses revealed six helminth species, including four nematodes (*Toxocara cati*, *Strongyloides darevskyi*, *Oswaldocruzia* sp., and Spirurida gen. sp.), one trematode (*Pleurogenes claviger*), and one cestode (*Mesocestoides litteratus*). *Toxocara cati* had the highest prevalence, found in cysts located primarily on the liver and in the body cavity of the hosts. The qualitative and quantitative comparative assessment of the helminth community suggests that, due to the introduction of these lizards, most helminth species from their native range have been lost. Additionally, most local helminth species have not yet adapted to parasitising these lizards as normal hosts of their life cycle.

Introduction

Human-caused biological invasions have become one of the most pressing challenges for ecosystems globally (Capinha *et al.* 2015; Ellis *et al.* 2012). Many invasive species, particularly plants and certain animals, can significantly alter ecosystems by transforming the habitat structure (Simberloff 2011). Numerous studies examining the negative impacts of invaders most frequently focus on their competition with native species and their effects on established food chains (Amundsen *et al.* 2013; Bellard *et al.* 2016; Meyer *et al.* 2015; Schoeman *et al.* 2019). Accidentally or deliberately introduced into the wild, often through illegal trade (Garcia-Diaz *et al.* 2016), alien species can become invasive and disrupt native ecosystems by interacting with local species. Invasive alien species may compete with native species for space, such as resting, nesting, and wintering areas, and for food, acting as additional consumers and significantly reducing the availability of local prey (Balzani *et al.* 2016; Manchester and Bullock 2000; Nekrasova *et al.* 2021; Pupina *et al.* 2018). They can also introduce new diseases and parasites into native ecosystems (Hidalgo-Vila *et al.* 2020). However, despite the risks associated with parasite co-introduction, the parasitological aspect of invasive animals often remains understudied (Blackburn and Ewen 2017). Additionally, some invasive species can hybridise with phylogenetically related indigenous species (Fong and Chen 2010), which can lead to genetic dilution of the latter and reduced biodiversity in native ecosystems. All of the abovementioned lead to substantial resources – time, effort, and money – being spent globally to control and minimise the effects of invasive alien species, though success is often limited (Nekrasova *et al.* 2024; Pimentel *et al.* 2005).

Among invasive animals, the cold-blooded terrestrial ones play a smaller role than other vertebrates, particularly fishes (Smit *et al.* 2017); however, their impact on ecosystems is substantial in particular cases (Meyer *et al.* 2015). Two of the well-studied introduced species in this group are the African clawed frog *Xenopus laevis* (Daudin, 1802) and the Red-eared slider *Trachemys scripta* (Thunberg in Schoepff, 1792), which were introduced to Europe from South Africa and the USA, respectively. Parasitological studies of both species within their invaded ranges have shown that these introduced animals can carry parasites from their native regions and transmit them to the native species. They can also serve as reservoirs for the native parasites, demonstrating spill-over and spill-back effects (Hidalgo-Vila *et al.* 2020; Meyer *et al.* 2015; Schoeman *et al.* 2019).

There are five invasive species of reptiles known in Ukraine. The Armenian lizards *Darevskia armeniaca* (Mehely, 1909) and Dahl's lizard *D. dahli* (Darevsky, 1957) were deliberately introduced by I. S. Darevsky in the 1970s to the Zhytomyr region (Darevsky, Shcherbak, 1968; Nekrasova, Kostiushyn, 2016). The Red-eared slider, *T. scripta*, is very abundant in urban and

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suburban water bodies due to uncontrolled releases by privates after unsuccessful attempts at keeping the species in captivity (Nekrasova *et al.* 2022). The Wall lizard *Podarcis muralis* (Laurenti, 1768) has a single recorded population in the Reni, Odesa region (Matveev *et al.* 2013; Oskyrko *et al.* 2019). The species may originate from Croatia (based on molecular data (Oskyrko *et al.* 2019) or neighbouring Romania, where the population of these lizards also exists (Cogalniceanu *et al.* 2013). Bogdanov's gecko *Tenuidactylus bogdanovi* Nazarov & Poyarkov, 2013 was probably brought to Odesa from the Middle East, and the breeding population was limited only to a few streets in the city (Duz *et al.* 2012).

Recent herpetological studies on all five species suggest that their populations are likely growing, though some uncertainty about the conclusion remains (Krasylenko and Kukushkin, 2017; Nekrasova and Kostiushyn 2016; Nekrasova *et al.* 2022; Oskyrko *et al.* 2019). This trend points to an increasing potential impact on populations of native species. However, parasitological studies on these invasive species within the region have yet to be published, leaving a gap in understanding the level of their integration into the ecosystems they were introduced to.

The only known mixed population of *D. armeniaca* and *D. dahli* in Ukraine exists in a single location – the Teteriv River canyon near the village of Denyshy in Zhytomyr region (Nekrasova and Kostiushyn 2016). This population originated in 1963 when 129 hermaphroditic *D. armeniaca* from the Semenov Mountain pass in Northern Armenia were intentionally released on the outskirts of the settlement. Five years later, in 1968, 11 male *D. mixta* from near Abastumani in Western Georgia were also released in the same area (Darevsky 2006; Nekrasova and Kostiushyn 2016). Monitoring efforts have since confirmed the presence of *D. armeniaca* and *D. dahli* in the area, but *D. mixta* has not been detected (Nekrasova and Kostiushyn 2016). The findings of D. *dahli*, which was not initially introduced, might be due to its accidental inclusion among the first *D. armeniaca* sample, as both species are common in the Armenian mountains. Alternatively, it could result from hybridisation between *D. armeniaca* and *D. mixta* already after introduction (Nekrasova and Kostiushyn 2016).

The information on parasites of the two lizard species in their native ranges is scarce; to our knowledge, the single helminth species, *S. darevskyi*, is found in *D. armeniaca* (Sharpilo, 1976). At the same time, there is a complete absence of data on helminths of the lizards from their invasive ranges. Therefore, we conducted the parasitological examination of *D. armeniaca* and *D. dahli* to fill this gap by analysing a representative sample of the lizards from their invaded range in Ukraine.

Materials and methods

Lizards were manually collected during the two short field trips to Denyshy (Zhytomyrska Oblast, Ukraine) taken on May 11, 2023, and July 1, 2023. The sampling area was rather limited and consisted of a rocky slope on the river shore with forestation on the top and close to the river. Adult lizards were captured at different sites along the slope on the ground and on rocks, and some on trees. During the first trip, 12 specimens of *D. armeniaca* and 16 of *D. dahli* were collected, and in the second, 23 and 16, respectively. The host individuals were identified based on their morphological features, mostly on peculiarities of prominent precloacal scutes (Tarkhnishvili *et al.* 2017). All lizards were transported to the parasitological laboratory of the I. I. Schmalhausen Institute of Zoology NAS of Ukraine and processed within a few days. The lizards were humanely euthanised by injecting 10% lidocaine in the brain stem and dissected. All organs were removed, placed in a separate Petri dish with 0.9% saline, and examined for parasites under the dissecting microscope. Found parasites were transferred into small Petri dishes and fixed accordingly to the taxonomic group. Nematodes and a trematode were fixed with hot 70% ethanol, while cestodes were priorly killed with hot water and then fixed with 70% ethanol. Larval stages of nematodes found in cysts were excysted and then fixed in either hot or room-temperature ethanol for morphological and molecular studies, respectively.

Morphological identifications of helminths and photomicrographs were performed using a ZEISS Axio Imager M1 light microscope. Specimens of *Pleurogenes claviger*, *Oswaldocruzia* sp., and Spirurida gen. sp. were found in only one or two host individuals each and thus observed and photographed on a temporary slide in distilled water to preserve DNA for molecular studies. Other nematodes were cleared in lactophenol for about 15–20 minutes and studied on temporary mounts in lactophenol. Specimens of *Mesocestoides litteratus* were stained with iron acetocarmine according to Georgiev *et al.* (1986), dehydrated in an ascending alcohol series, cleared in clove oil, and mounted in Canada balsam.

DNA was extracted using the ZYMO Research Quick-DNA Miniprep Kit following the manufacturer's protocol for further molecular identification. Two genetic regions, 18S rDNA and *cox1*, were chosen for PCR and subsequent sequencing because they are the most widely used in molecular genetic studies of helminths and represent the greatest diversity of these organisms in the GenBank. The 18S rDNA amplicons were obtained using the primer pair 18SU467F 5′ - ATC CAA GGA AGG CAG CAG GC - 3′ and 18SL1310R 5′ - CTC CAC CAA CTA AGA ACG GC - 3′ (Suzuki *et al.* 2008) with the following thermocycling protocol: 2 min denaturation at 94°C, 35 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 2 min for amplification, 72°C for 7 min for final extension. Additionally, 18S rDNA was amplified using the primers 635F 5′ - GAG GGC AAG TCT GGT GCC AGC AG - 3′) and 1754R (5′ - TAG CGA CGG GCG GTG GTA CA - 3′ with a thermocycling protocol of 94°C for 1 min for initial denaturation, 10 cycles of 94°C for 20 s, 55°C for 30 s, and 72°C for 1 min, followed by 25 cycles of 94°C for 10 s, 52°C for 15 s, and 72°C for 1.2 min, concluding with a final extension at 72°C for 7 min (Zaleśny *et al.* 2010). The *cox1* amplicons were obtained using the primers pair DICE1F 5′ - ATT AAC CCT CAC TAA ATT WCN TTR GAT CAT AAG - 3′ and DICE14R 5′ - TAA TAC GAC TCA CTA TAC CHA CMR TAA ACA TAT GAT G - 3′ and the thermocycling profile as follows: 3 min denaturation at 94°C, 35 cycles of 94°C for 40 s, 51°C for 40 s, 72°C for 1 min, 72°C for 10 min for final extension. After PCR, the presence of DNA of target length was confirmed by gel-electrophoresis in agarose gel, purified with ExoSAP-IT™ Express PCR Product Cleanup Reagent (Applied Biosystems, Lithuania) following the manufacturer instructions and sent for Sanger sequencing to the commercial company (Nanodiagnostika, LTD, Vilnius, Lithuania) or the Faculty of Natural Sciences of Comenius University (Bratislava, Slovakia). The same primers used for PCR were used for sequencing. Obtained sequences were assembled in Geneious Prime 2024.0.5 software (https://www.geneious.com). These sequences were then compared to sequences from the GenBank database using the BLAST tool and submitted to GenBank.

In addition to the collected material, archive data from the helminth collection catalogue of the Department of Parasitology of the I. I. Schmalhausen Institute of Zoology NAS of Ukraine was used for comparison. The data included results of identifications of parasites from 21 *D. armeniaca* collected by V. Sharpilo in 1974 from Armenia and Azerbaijan and five *D. dahli* collected in 1975 from Georgia.

Results

Six species of helminths, including four species of nematodes, one species of trematode, and one species of cestode, were found in *D. dahli* and *D. armeniaca*. Only a single adult specimen of *S. darevskyi* was observed, while the other species were found at various larval stages. All species, except *T. cati*, exhibited very low prevalence and intensity.

Mesocestoides litteratus (Batsch, 1786), larvae (Figure 1a)

Host: *Darevskia armeniaca* – observed in 1 out of 35 individuals, with an intensity of 3 specimens.

Infection site: body cavity.

Obtained sequences: 18S rDNA [PV069325], *cox1* [PV066090].

Remarks. Three larvae found in the body cavity of a lizard were identified at the species level primarily based on molecular-genetic data. The obtained partial sequences of the 18S rDNA gene from the larvae were 100% identical to those of *M. litteratus* from red foxes (*Vulpes vulpes* L.), GenBank accession numbers DQ643002-DQ642999. The

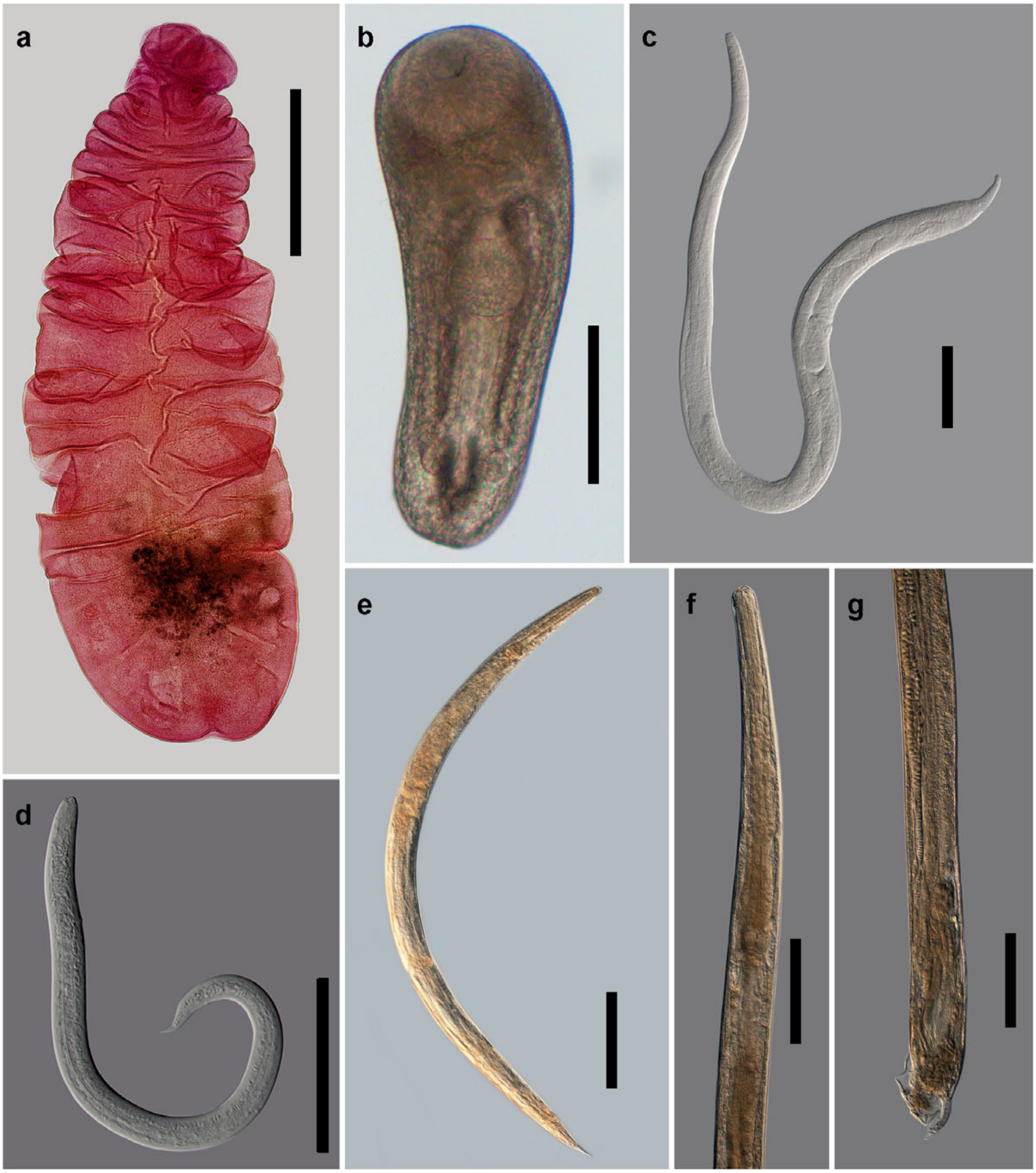

Figure 1. Photomicrographs of helminth species recovered from *Darevskia dahli* and *D. armeniaca* in Ukraine. a – *Mesocestoides literatus* from *D. armeniaca*; b – *Pleurogenes claviger* from *D. dahli*; c – *Strongyloides darevskyi* from *D. armeniaca*; d – *Toxocara cati* from *D.* armeniaca; e – Spirurida gen sp. From *D. armeniaca*; f, g – Anterior and posterior ends of *Oswaldocruzia* sp. From *D. dahli*; – . Scale bars: a – 1000 µm; b, d, f, g – 100 µm; c – 200 µm; e – 200 µm.

common definitive hosts of this helminth species are carnivores, most often foxes (Literak *et al.* 2006). Also, previous studies have documented *M. litteratus* larvae from various vertebrates, including different species of lizards, which serve as paratenic hosts for this parasite (Sargsyan *et al.* 2014). Although we did not observe any carnivores or direct evidence of their presence in the study area, the habitat is suitable for foxes and other carnivores. Therefore, we believe that infections of rock lizards with *M. litteratus* in the area might be relatively common.

Pleurogenes claviger (Rudolphi, 1819) Looss, 1896, larva (Figure 1b)

Host: *Darevskia dahli* – observed in 1 out of 32 individuals, with an intensity of 1 specimen.

Infection site: intestine.

Obtained sequences: 18S rDNA [PV069323].

Remarks. This species is a common parasite of various amphibians, primarily inhabiting the intestines of aquatic frogs and newts and, less frequently, toads and other amphibians (Ryzhykov *et al.* 1980). The life cycle of this trematode involves a planorbid snail as the first intermediate host and an aquatic arthropod as the second (Ryzhykov *et al.* 1980). In the study area, lizards often prey along the riverbank, where frogs of the genus *Pelophylax* (a common host for *P. claviger*) were also observed. The lizards were likely infected incidentally by ingesting an arthropod with the metacercaria.

Strongyloides darevskyi Sharpilo, 1976 (Figure 1c)

Host: *Darevskia armeniaca* – observed in 1 out of 35 individuals, with an intensity of 1 specimen.

Infection site: intestine.

Remarks. The species was described as a parasite of rock lizards from Azerbaijan, Armenia and Georgia (Sharpilo, 1976). It was distinguished from its congeners based on several characteristics: body length of less than 2 mm, a female carrying no more than three eggs, and host specificity to rock lizards. Our study found only one specimen corresponding to the original description, having one developed egg and a body length of 1 mm. The available data on the prevalence of *S. darevskyi* in populations of *D. armeniaca* and *D. dahli* within their native range shows much higher prevalence and intensity (see below). Moreover, its prevalence in other rock lizards, such as *D. saxicola* (Eversmann, 1834) and *D. rudis* (Bedriaga, 1886) in their native populations exceeds 20% (Roca *et al.* 2015).

Toxocara cati (Schrank 1788), larvae (Figure 1d)

Hosts: *Darevskia armeniaca* – observed in 25 out of 35 individuals (71%), with an intensity ranging from 2 to 131 cysts per host; *Darevskia dahli* – observed in 3 out of 32 individuals (9%), with an intensity of two cysts per host.

Infection site: cysts on liver and other organs, body cavity.

Obtained sequences: 18S rDNA [PV069324].

Remarks. All excysted larvae were minute and in the early stages of development, making morphological identification impossible. However, comparing the obtained partial sequences of the 18S rDNA gene from some larvae with sequences from GenBank identified them as *T. cati.* As the obtained sequences demonstrated a 100% match to sequences of *T. cati* from a captive leopard cat (*Prionailurus bengalensis*) from China (GenBank: JN256973) (Li *et al.* 2012) and a domestic cat from the USA (GenBank: EF180059) (Nadler *et al.* 2007). The cysts were predominantly localised in the liver. Summarising the data for the entire mixed population, the number of cysts per lizard ranged from 1 to 131; all cysts contained a yellowish mass. While some cysts harboured motile, live larvae, others contained dead or entirely disintegrated larvae. Since some lizards were only affected by cysts with degenerated mass, the number of cysts with intact larvae per lizard, considering the mixed population, ranged from 2 to 93. Also, it was observed that smaller cysts tended to contain live nematodes, whereas larger cysts usually contained dead larvae or degraded material. Despite the high prevalence of *T. cati* in the studied lizards, the parasite does not appear to be fully adapted to these hosts, as many cysts contained non-viable larvae, which suggests that these lizards scarcely serve as a reliable paratenic host for the life cycle of this nematode.

Spirurida gen. sp., larva (Figure 1e)

Host: *Darevskia armeniaca* – observed in 1 out of 35 individuals, with an intensity of 1 specimen.

Infection site: intestine.

Remarks. A single third-stage larva was recovered from the intestine of *D. armeniaca.* Despite numerous attempts, we could not extract any DNA of the nematode. Based on the oesophagus shape and the morphology of the anterior end, the larva was identified as belonging to the order Spirurida.

Oswaldocruzia sp., larvae (Figures 1f, 1g)

Host: *Darevskia dahli* – observed in 1 out of 32 individuals, with an intensity of 2 specimens.

Infection site: intestine.

Obtained sequences: 18S rDNA [PV069322].

Remarks. Two fourth-stage larvae of *Oswaldocruzia* sp. were collected from the intestine of a single *D. dahli* individual. The obtained 18S rDNA gene sequences confirmed the genus identification via a BLAST search in GenBank, but no sequences were available to confirm the species identification. The slowworm (*Anguis colchica*), the grass snake (*Natrix natrix*), and marsh frogs (*Pelophylax ridibundus*) were observed at the location, all of which could potentially host species of *Oswaldocruzia.* Additionally, various toad species, also potential hosts of *Oswaldocruzia*, may inhabit the area. Since several species of this genus occur in Ukraine (Marushchak *et al.* 2024), we cannot identify our specimens to the species level. Thus, it could not be reasonably hypothesised in this study which host interaction led to the lizard acquiring the nematode.

Archive data

The results of the helminthological surveys on various reptiles from the territory of the former USSR, including several rock lizards, were published by V. Sharpilo (1976). However, the publication did not provide data on the infection level of different parasites from each host. Luckily, this data is stored in the identification catalogue of the Department of Parasitology of the Institute of Zoology (collection numbers 253–290 for *D. armeniaca* and 46–50 for *D. dahli*). It allowed us to bring to light the details of the parasite infection for *D. armeniaca* and *D. dahli* in their native ranges. Thus, 15 of the 21 *D. armeniaca* were infected only with *S. darevskyi*, with the intensity of infection varying from 1 to 15. All five *D. dahli* were infected with at least one species of helminths. Three were infected

Table 1. Catalogue collection data on parasites from *Darevskia armeniaca* and *D. dahli* from their native regions (Armenia, Azerbaijan and Georgia) collected and identified by V. Sharpilo (1976). Data on infection parameters are presented as prevalence, followed by intensity of infection as ranges and mean values in parentheses

	Infection parameters: Prevalence, %; intensity limits and mean values					
	Strongyloides darevskii	*Spauligodon saxicolae*	*Acuariidae* gen. sp.	Unidentified Nematoda	*Oochoristica* sp.	Unidentifies Cestoda
Darevskia armeniaca (N = 21)	60; 1–15 (12.8)					
Darevskia dahli (N = 5)	60; 2–5 (3.3)	60; 1–4 (2.7)	20; 1	20; 2	20; 1	40; 2–4 (3)

with *S. darevskyi*, with an intensity ranging from 2 to 5; three with nematode *Spauligodon saxicolae* Sharpilo, 1961, with an intensity ranging from 1 to 4; one with one specimen of unidentified acuariid nematode; one with two specimens of unidentified nematode larvae; one with one specimen of the cestode *Oochoristica* sp.; and two lizards with 2 and 4 specimens of unidentified cestode larvae (Table 1).

Discussion

The helminth community of *D. dahli* and *D. armeniaca* from the mixed invasive population in Ukraine, with six helminth species documented, was found to be significantly less diverse compared to recent parasitological studies of other species of the genus *Darevskia* from their native range (Roca *et al.* 2015; Sargsyan *et al.* 2014). The studied lizard population was predominantly infected with a single species of helminth, *T. cati.* The prevalence and intensity of infection by five other identified helminth species were scarce. It is also worth noting that the condition of *T. cati* cysts and larvae indicates that these lizards are currently unsuitable hosts for this parasite. It is important to note that during field research, we did not observe domestic cats or wild definitive hosts; however, species such as the European lynx *Lynx lynx* L., European wild cat *Felis silvestris* Schreber, 1777, and domestic cats *Felis catus* L. could inhabit the area. Nevertheless, the prevalence of *T. cati* infection indicates their presence in the region. Infection with this parasite may occur through direct contact of lizards with felid faeces. However, the life cycle of this parasite may also involve invertebrate paratenic hosts, such as earthworms and various insects, which creates additional pathways for lizard infection (Sprent 1956).

A comparison of studied lizards' helminth fauna with the composition of the helminth fauna of other species of genus *Darevskia* from their native range reveals that only one helminth species, *S. darevskyi*, was successfully transferred and continues to circulate in the new environment (Sargsyan *et al.* 2014). However, the observed prevalence and intensity of this species were low in the study. Given the high specificity of the parasite, which strongly supports the hypothesis that it was introduced alongside its host, and the direct life cycle of this genus of nematodes, we may hypothesise that its circulation is complicated at free-living stages due to unfavourable climatic conditions in the new range. Our assumption may also be supported by the fact that the infection prevalence and intensity of *S. darevskyi* in both lizards within their native range was quite high (Table 1), while the density of the Ukrainian population is exceptionally high, which should potentially have promoted easy transmission of the parasite across the population. The other five helminth species were acquired from local amphibians, reptiles, and mammals, illustrating the spill-back effect, where invasive hosts obtain new helminth species from native fauna.

A sample of *D. armeniaca* from Azerbaijan (three individuals) and Armenia (18 individuals) showed the presence of only one parasite species – *S. darevskyi.* In contrast, five *D. dahli* were infected with a total of six different parasite species, including *S. darevskyi*, another nematode *Spauligodon Saxicola*, and larval stages of poorly identifiable nematodes and cestodes. Considering both the qualitative and quantitative patterns of the studied helminth community, we conclude that, due to introduction, the lizards have lost most of their native helminth species, and even one parasite that accompanied lizards to the new region displayed a much lower level of infection. Such findings correspond to a similar study of invasive populations of the clawed frog *X. laevis* that lost most of their native parasites, and of the accompanying ones, at least one (monogenean *Protopolystoma xenopodis* Price, 1943) demonstrated significantly lower prevalence (Schoeman *et al.* 2018). It is worth noting that *P. xenopodis* also (like *S. darevskyi*) has a direct life cycle, and the environmental parameters are likely the reason preventing it from high abundance in dense invasive populations of clawed frogs collected from France. Furthermore, local helminth species have not yet adapted to using these lizards as normal hosts for sustaining their life cycles, although attempts at parasitism are ongoing. Although we do not have a directly comparable data (e.g., identified helminths collected from both species within the native range), the comparison to the archive data may support the Enemy Release Hypothesis (Heger *et al.* 2024) for invasive animals. Unfortunately, finding of only one specimen of *S. darevskii* from invaded territory does not allow us to conduct a comprehensive statistical analysis in order to confirm it.

Both lizard species, *D. armeniaca* and *D. dahli*, were observed across different parts of the biotope, particularly near riverbanks and higher up on forested slopes. However, despite inhabiting the same biotope and presumably sharing similar food sources, a notable difference was observed in their infection with *T. cati.* Specifically, 35 *D. armeniaca* individuals had 403 cysts, including 178 live larvae, whereas only 6 cysts with 2 larvae were found in 32 *D. dahli.* We believe that the higher infection rate in *D. armeniaca* may be due to the peculiarities of the host-parasite interactions, particularly related to the host's immune response to this nematode. The extremely low prevalence of other helminth species limits the ability to assess their host specificity.

In the studied location, the number of lizard individuals has increased dramatically over the years (Nekrasova and Kostiushyn 2016), and observations have shown a notable lower abundance of

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invertebrates (mainly insects and spiders) in biotopes where these lizards have spread compared to similar nearby areas without their presence (V. Gorobchyshyn – personal communication). There is no prior data on the parasite infection in *D. dahli* and *D. armeniaca* from their invasive range, making it difficult to speculate how these species may interact with the native ecosystems, although the results of the present study infer the high level of involvement of lizards in a local trophic network. All things considered, we assume these lizards may have a greater impact on the local ecosystem than previously supposed, a topic that future research should investigate in more detail.

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Competing interest. All authors declare that they have no conflicts of interest.

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