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Microplastics in reptiles: Assessment of particle content in viviparous and sand lizard populations (*Zootoca vivipara*, *Lacerta agilis*, Lacertidae) and common adder (*Vipera berus*, Viperidae) from Western Siberia

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Abstract. The paper presents the results of quantitative assessment of the content and morphological characterization of microplastics (MPs) accumulated in the organs of adult individuals ($n = 43$) from three reptile species: the viviparous lizard *Zootoca vivipara* Lichtenstein, 1823, the sand lizard *Lacerta agilis* Linnaeus, 1758, and the common adder *Vipera berus* Linnaeus, 1758. The species inhabit overlapping ranges, are often syntopic, and exhibit zoophagous behavior. The studied individuals were collected in the spring-summer period of 2021-2023 from various localities in Tomsk Oblast (southeast of Western Siberia). MPs in the size range of 0.15-5 mm were detected in the gastrointestinal tract (GIT) of both lizard species (100%), as well as in the GIT and skin of *V. berus*. The maximum average MP content in the GIT was 6.80 ± 11.4, with a range of 0 to 32 particles in *Z. vivipara* collected in 2022. Interannual variations were noted in the contamination level of *Z. vivipara*: the average MP content in the GIT was 3.2-fold lower in 2023 versus 2022. The differences in MP content between *Z. vivipara* and *L. agilis* were not statistically significant ($p > 0.05$), as were the differences in MP content between the GIT and the skin of *V. berus*. In the GIT of adult individuals of the three species, MPs were represented by microspheres, microfilms, irregularly shaped fragments, with microfibers being predominant, comprising 64.5% in Z. *vivipara* and 82.0% in *L. agilis*, respectively. The study revealed the prevalence of MPs with sizes ranging from 0.3 to 1 mm, with the exception of Z. *vivipara*, where most particles did not exceed 300 μm (43.5%). The proportion of larger particles (> 3 mm) in the organs of *V. berus* was higher compared to *Z. vivipara* and *L. agilis*. MP detection in the GIT and skin of adult individuals indicates plastic pollution in the taiga zone of Western Siberia. The data obtained represent the first evidence of the presence of MPs in Palearctic reptiles in Russia.

The article contains 2 Figures, 1 Table, 40 References.

Keywords: microplastics, reptiles, bioindication, Western Siberia

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Микропластик в пресмыкающихся: Оценка содержания частиц в популяциях живородящей и прыткой ящериц (*Zootoca vivipara*, *Lacerta agilis*, Lacertidae) и обыкновенной гадюки (*Vipera berus*, Viperidae) Западной Сибири

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Аннотация. Представлены результаты исследований по количественной оценке содержания и характеристике морфологии микропластика (МП) в органах взрослых особей ($n = 43$) трех видов пресмыкающихся – прыткой ящерицы *Lacerta agilis* L., 1758, живородящей ящерицы *Zootoca vivipara* Lichtenstein, 1823 и обыкновенной гадюки *Vipera berus* L., 1758. Виды имеют перекрывающиеся ареалы, часто синтопичны, зоофаги. Исследованные особи отловлены в весенне-летний период 2021–2023 гг. в ряде локалитетов Томской области (юго-восток Западной Сибири). Частицы МП размерного диапазона 0,15–5 мм обнаружены в ЖКТ всех особей ящериц (100%), а также в ЖКТ и шкурах гадюки. Максимальное среднее содержание МП в ЖКТ составило 6,80 ± 11,4 с разбросом от 0 до 32 частиц у *Z. vivipara*, отловленных в 2022 г. Отмечены межгодовые отличия в уровне загрязнения *Z. vivipara*: в 2023 г. среднее содержание МП в ЖКТ в 3,2 раза ниже по сравнению с 2022 г. Различия между выборками живородящей ящерицы и прыткой ящерицы были незначимыми ($p > 0{,}05$), также как и различия в содержании МП между ЖКТ и кожей обыкновенной гадюки. В ЖКТ взрослых особей трех видов МП представлен микросферами, микропленками, фрагментами неправильной формы, но преобладают микроволокна: от 64,5% у *Z. vivipara* до 82,0% у *L. agiis*. В исследованных выборках преобладал МП размерами от 0,3 до 1 мм за исключением ЖКТ живородящей ящерицы, где большинство частиц меньше 300 мкм (43,5%). Доля более крупных частиц (> 3 мм) в органах змей выше по сравнению с ящерицами. Детекция микропластика в ЖКТ и коже взрослых особей ящериц и змей указывает на загрязнение таежной зоны Западной Сибири. Полученные данные являются первым доказательством наличия МП у пресмыкающихся Палеарктики на территории России.

Ключевые слова: микропластик, пресмыкающиеся, биоиндикация, Западная Сибирь

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Introduction

Plastic pollution is one of the most pressing environmental challenges of our time. Plastics exhibit high durability, exceptional wear resistance, and limited natural degradation; however, their widespread use leads to a concerning accumulation of plastic waste and its release into the natural environment. In recent years, there has been mounting concern about MPs, small plastic pieces ranging in size from 1 μm to 5 mm [1]. MPs found in the environment are classified as either primary or secondary. Primary MPs are mainly produced in the form of tiny granules and are used in production as a raw material for manufacturing plastic products [2]. Microgranules are components of cosmetics, household chemicals, and industrial abrasives. Secondary MPs, such as films and irregularly shaped fragments, are formed during degradation of larger plastic objects into smaller pieces under the influence of various environmental factors [2]. Plastic microfibers, originating from textiles (clothing, geotextiles) and fishing gear, are also widespread in the environment) [3].

The atmosphere, waterways, ocean currents, and animals can transport MPs over great distances. MPs have been found in various parts of the globe, including marine and freshwater habitats, as well as in soil, and they have a long residence time in the environment [4]. MPs can react with other pollutants to form more complex secondary pollutants with higher toxicity, facilitating their transport and transfer along the food chains [5-7].

MPs can directly or indirectly affect organisms and pose health risks to both animals and humans. Reptiles can easily become entangled in plastic nets and ingest bottles, caps, bags or straws, which can lead to physical injuries including asphyxiation, organ damage, and even death [8, 9]. The health risks posed by MPs to reptiles remain poorly understood. Studies are limited and primarily focus on aquatic reptiles, such as marine and freshwater turtles [10-15] and crocodiles [16]. The loggerhead sea turtle *Caretta caretta* is recognized as an indicator species to monitor MP pollution globally. It was found that MPs (polypropylene, polyethylene, and cotton fibers) accumulate not only in the GIT of *C. caretta*, but also in its reproductive organs and heart [15]. The impact of MPs on lizards and snakes in terrestrial ecosystems remains poorly studied, and the available data are extremely fragmentary [17-19].

The aim of this study was to identify the presence of MPs and assess their content in specific organs of three widespread Palearctic reptile species: the viviparous lizard, the sand lizard, and the common adder.

Material and methods

The subjects of the study were adult individuals of two lizard species *Zootoca vivipara* Lichtenstein, 1823 and *Lacerta agilis* Linnaeus, 1758, as well as the common adder *Vipera berus* Linnaeus, 1758. All the species are widespread in Eurasia, including Western Siberia.

Lacerta agilis, an oviparous species, inhabits a vast range of the territory that includes the European part of Russia and extends southward in Siberia to Lake Baikal. The sand lizard thrives in the steppe zone, where it inhabits open sunny areas. It is commonly found in pine forests, power line clearings, gardens and vegetable gardens, roadsides and embankments of roads and railways, as well as drained raised bogs, copses, hillsides, and ravines. In forested areas, it often lives near human settlements. In soft soil, the sand lizard digs shallow burrows up to 70 cm long and uses rodent burrows, voids in piles of stones, old stumps, and brushwood piles as shelters [20].

Ovoviviparous species, the viviparous lizard and the common adder, inhabit almost the entire forest zone of the Palearctic. In terms of biotopic distribution, both species prefer mesic habitats. The viviparous lizard prefers humid lowlands with natural shelters. It abundantly inhabits deciduous and coniferous forests, overgrown clearings, glades, forest edges, and swamps. In the south of Tomsk Ob region and the environs of Tomsk, it often shares the habitat with the sand lizard in the transitional zones between dry and humid biotopes. The viviparous lizard is often found near fallen logs, old stumps, and at the bases of large tree trunks. The species does not dig its own burrows, but uses voids between roots, moss hummocks, and abandoned burrows of small rodents as shelters. Of the 54 described winter burrows of *Z. vivipara*, 75.9% were found in agrogenic soil (vegetable gardens, arable land, and orchards). In soil at a depth of 15-25 cm, the number of winter burrows is 2.8-fold higher, and the number of hibernating lizards in these shelters is 1.5-fold higher than at a depth of 0-15 cm ($p < 0.01$) [21].

The common adder is unevenly distributed in the forest and forest-steppe zone, forming high-density clusters in certain areas known as hotbeds. It inhabits forest edges, glades, overgrown burnt areas, peat bogs, banks of reservoirs, and abandoned villages. The species hibernates at depths ranging from 40 cm to 2 m, below the freezing zone. It uses rodent burrows, soil cracks, voids among tree roots and peat bogs as winter burrows [20].

Lizards and snakes were collected in the first half of summer (June 2021-2023) from three localities of Tomsk district, Tomsk Oblast: localities 1 and 2 are situated on the right bank of the Tom River: the villages of Anikino (56°24'N, 84°59'E) and Zonalny (56°25'N; 85°01'E); locality 3 is located on the left bank of the Tom River: the village of Timiryazevsky (56°29'N, 84°52'E). The study areas are part of the Tomsk suburban area and are subject to anthropic load (arable land, garden plots, highways and railways, holiday homes and children's camps, and construction of microdistricts). Lizards were captured by hand or with a net; a stick with a hook was used to catch snakes. Fabric bags and specialized containers were employed to transport the specimens to the laboratory for further processing. Within 24-48 hours after capture, 43 individuals of lizards and snakes were anesthetized with tricaine; their body weight (g) was determined and body length (L - *Longitudo corporis*) was measured. For lizards, measurements were taken using a digital caliper (CHIZ SHTCTC-1-150-0.01) with an accuracy of 0.1 mm, and a tape measure was used for measuring snakes. Fixation of individuals and organs (skin, lungs, liver, intestines) was performed in 70% ethanol or a 10% formalin solution to determine the accumulation of MPs and sub-micron plastics (SMPs) using specialized methods. The procedures adhered to international and national requirements for appropriate and humane treatment of animals.

Sample preparations from reptile organs and MP extraction were performed using a method previously developed and tested for fish organs [22] with some modifications. The procedure included the following stages: (a) alkaline hydrolysis of organs in a 10% KOH solution at 55ºC for 48 h with periodic stirring; (b) separation of particles by density in a saturated NaCl solution for 24 h; (c) treatment of the upper phase with 96% ethyl alcohol (10% v/v) at 50°C to remove saponified lipids; (d) vacuum filtration to collect particles on a glass fiber filter with a pore size of 1 μm (Membrane Solutions, China) for subsequent quantitative and qualitative analyses. Blank samples containing reagent solutions (KOH, NaCl, ethanol) and undergoing the above processing stages were analyzed in parallel to assess external contamination during sample preparation. For each series of 10 samples of reptile organs, 5 control samples were prepared and examined. The content of fibers within the target size range of 0.15-5 mm in the control samples varied from 0 to 2 items/filter. When fibers were detected in the control samples, the results of quantitative assessment of MPs in the entire series of natural samples were corrected accordingly.

Microscopic, morphological and photomicrographic analyses of particles were conducted using an MSP-1 stereomicroscope (LOMO, Russian Federation), a ToupView USB 2.0 CMO S digital camera (ToupTek Photonics, China), and ToupView 3.7.6273 software. The hot needle test was employed to identify MPs among particles extracted from the reptiles. This method enables the classification of synthetic polymer particles based on the plastic/non-plastic principle [23, 24]. A heated dissecting needle was brought into contact with the suspected particle under microscopic control; plastic particles melted, while organic particles darkened or burned. The MP content was expressed in terms of MP items per individual. MPs exhibited a variety of shapes, including spheres, films, fibers, and irregularly shaped fragments. Depending on their size, MPs were divided into small particles of 150-300, 301-1000 μm and further up to the maximum size of 5000 μm with a step of 1000 μm, as was previously described [25].

Primary data processing was performed using Microsoft Excel 2010. The statistical analysis was conducted with Statistica 8.0 (Statsoft). The following indicators were calculated: sample size (n), arithmetic mean ($\bar{x}$), standard deviation (SD), standard error of mean ($m_{\bar{x}}$), range of values (lim). For pairwise comparison, Student's t-tests ($p < 0.05$) were employed for variables with a normal distribution, and the Mann-Whitney test ($p \leq 0.05$) was used for comparison when data were not normally distributed.

Results and discussion

MPs with different sizes (0.15-5 mm) and morphologies were found in the GIT and skin of *V. berus*, as well as in the GIT of both lizards, *Z. vivipara* and *L. agilis* (Fig. 1).

The results of quantitative assessment of MPs accumulated in the organs of reptiles are summarized in Table 1. The MP content in the GIT of *Z. vivipara* collected in 2022 attained 6.80 ± 11.4. Interannual variations were recorded in the contamination level of *Z. vivipara*: the average MP content in the GIT was

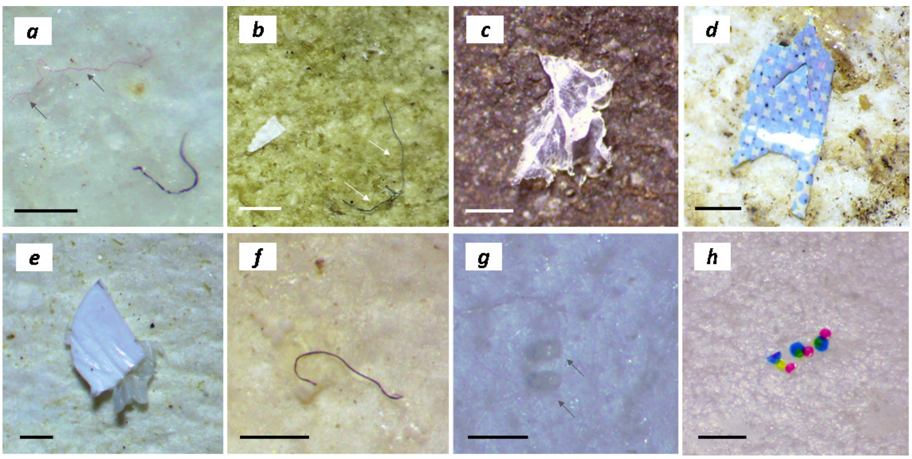

Fig. 1. Light micrographs of MPs: fibers and fragment from the GIT of *V. berus* (*a*, *b*); a film (*c*) and a fragment from the skin of *V. berus* (*d*), a fragment (*e*) and a fiber (*f*) from the GIT of *Z. vivipara*; spheres (*g*) and a fragment (*h*) from the GIT of *L. agilis*. Scale bar: 500 µm

Table 1

Quantitative content of MPs in the organs of reptiles (Tomsk district, Tomsk Oblast, 2021-2023)

Indicator	GIT				*V. berus* (skin)
	L. agilis	*Z. vivipara* (2022)	*Z. vivipara* (2023)	*V. berus*	
n	20	7	4	12	12
$\bar{x}$	3.42	6.80	2.10	3.86	5.64
SD	4.40	11.4	0.96	2.43	6.37
$m_{\bar{x}}$	0.98	4.32	0.48	0.70	1.84
lim	0-19	0-32	1-3	1-8	0-20

Note. *n* - sample size, $\bar{x}$ - arithmetic mean, *SD* - standard deviation, $m_{\bar{x}}$ - standard error of mean, lim - range of values.

3.2-fold lower in 2023 versus 2022. The differences in MP content between *Z. vivipara* and *L. agilis* were not statistically significant ($p > 0.05$), nor were the differences in MP content between the GIT and skin of the common adder.

The studied reptiles exhibit different ingestion patterns for MPs of different shapes and sizes (Fig. 2). The majority of ingested MPs were fibers (from 64.5% in *Z. vivipara* to 82% in *L. agilis*). The GIT of *Z. vivipara* was found to contain 2-fold more fragments compared to *V. berus* and almost 5-fold more fragments compared to *L. agilis* (Fig. 2*a*). Spheres were identified only in the GIT of both lizards. The revealed differences are associated with different dietary patterns, trophic links, and habitat conditions.

Zootoca vivipara from the studied sample consumed more MPs < 0.3 mm (43.5% of the total MPs) compared to *L. agilis* with only 16.2% of the fine particles and the majority of larger MPs of 0.3-1 mm in size (Fig. 2). In the GIT and skin of *V. berus*, 0.3-1 mm MPs were the most abundant. Compared to both lizards, the common adder consumed and accumulated in the skin were the most abundant. Compared to both lizards, the common adder consumed and accumulated in the skin more particles of 3-4 and 4-5 mm in size ($p < 0.05$). Moreover, the proportion of larger MPs (> 3 mm) accumulated in different organs of the common adder was approximately the same, amounting to 13.6% in the GT tract and 13% in skin (Fig. 2).

In the taiga zone of Western Siberia, reptiles, being poikilotherms, spend most of their annual cycle hibernating, with the active period lasting 4.4-5.5 months. Sand and viviparous lizards, as well as the common adder, are diurnal. The daily time budget divided into nighttime sleep and daytime activity is affected by different environmental factors. The soil characteristics are of great importance. Body temperature is regulated behaviorally, primarily via daily movements between shaded and sunny areas, or out and into the shelter. Active

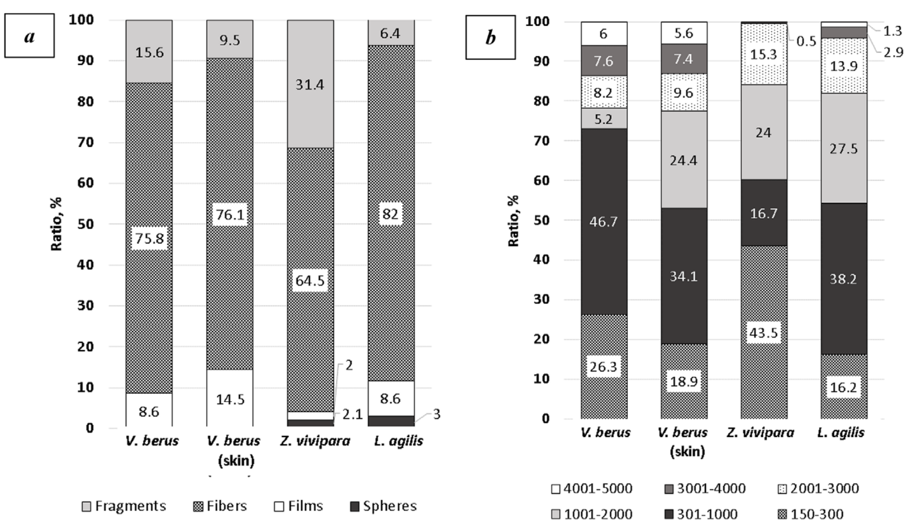

Fig. 2. Percentage of MPs from the GIT and skin of *V. berus* and the GIT of *Z. vivipara* and *L. agilis* by shape (*a*) and size (*b*)

behavioral forms include mating games, feeding, basking, and locomotor activity (foraging behavior) during molting. Inactive behavior includes nighttime sleep and daytime rest in the shelter. At the Timiryazevo site, we have estimated the time budget (24 hours) for lizards. Adult males and females of the sand lizard ($n = 7$) spend 78-89% of their time resting in the shelter, 2% basking, 2-4% moving, 3-5% hunting, and 2-13% slumbering during the daytime [26].

In all the studied species, MPs of different shapes and sizes were detected in the GIT and (in the common adder) in the skin. This indicates plastic contamination of the substrate surfaces, shelters and winter burrows, and most importantly, food items.

The atmosphere is an important transport medium for anthropogenic polymeric particles, including MPs [27]. Analysis of particles deposited to snow cover allows for tracking the amount and transport of atmospheric MPs. For example, anthropogenic polymeric particles, including various types of plastic and viscose fibers and microfragments, were identified in snow samples from Western Siberia [28]. The maximum estimated particle mass loading was 4444 ± 1530 mg/m^2 or 2817 ± 915 items/m^2. Fibers were the dominant shape of MPs, accounting for more than 90% of the total particles, due to their aerodynamic properties and large atmospheric transport potential. Fibers are the most abundant form of MPs found in the environment. Compared to secondary forms of particles (fragments, films) and spheres, microfibers are one of the most common microparticle pollutants in surface water bodies of Western Siberia and other regions of the Russian part of Eurasia [29].

MPs were found in samples of agrogenic gray and dark gray forest soils from the taiga forest zone, as well as in samples of southern and ordinary agrogenic chernozems from the steppe region of Western Siberia taken from a depth of 0-10 cm. The most common MPs ($> 80\%$) in samples of agrogenic soils from the two natural climatic zones were transparent fibers of different sizes [30, 31]. MPs are readily transported in soil layers by invertebrates, including termites, ants, and earthworms [32]. Invertebrates facilitate the transfer of organic and inorganic substances within and between soil systems. In plastic-contaminated environments, insects and worms moving through the soil horizons facilitate the dissemination of plastic particles and synthetic fibers via active transportation of soil and debris between habitats [33].

Both lizards and young snakes feed on various systematic and ecological groups of invertebrates that can be involved in MP circulation. Reptiles are not apex consumers in trophic chains, being prey items for numerous vertebrates and hosts for ecto- and endoparasites.

The sand lizard predominantly inhabits artificial pine plantations and coastal slopes. The viviparous lizard prefers humid lowlands with well-developed herbaceous vegetation and diverse and abundant invertebrates. The sand lizard and the viviparous lizard often live together, but their diet varies significantly. The study conducted in Tomsk Ob region has revealed that the sand lizard’s diet primarily consists of invertebrates: herpetobionts (38.7%) and chortobionts (47.1%) (hymenopterans, orthopterans, and coleopterans); less common are herpetobiont arthropods (10.3%) and hydrobionts (mollusks) (3.5%). The diet depends on the season, the type of biotope, and the sex and age of the lizard. Juvenile lizards generally prefer smaller and softer food items: spiders, caterpillars and cicada larvae. The average weight of a food bolus is 500 mg (max 1530 mg). Compared to *L. agilis*, about 40% of the viviparous lizard's diet consists of forest pests (leaf beetles, click beetles, weevils, leafhoppers, aphids, bugs, and caterpillars), mollusks, cicadas, as well as aphids, stoneflies, mosquitoes, and millipedes. An adult lizard consumes from 121 to 295 mg of invertebrates per day. Juvenile lizards prefer small spiders, leafhopper larvae, and caterpillars. Lizards primarily affect zoo- and phytophages, with the annual instantaneous consumption up to 20% of the available prey biomass [20].

Data on the interaction between insects and MPs in terrestrial and aquatic ecosystems, as well as the associated ecotoxicological consequences, are fragmentary. Recent studies have shown various adverse effects of MPs on the diet, growth, reproduction, and behavior of insects [34]. It has been revealed that MP consumption is detrimental to growth and development of some insect species, while others exhibit tolerance to MP exposure. For example, mosquitoes provide a new pathway for MP accumulation from aquatic to terrestrial environments, preserving particles during metamorphosis and exacerbating the issue of plastic contamination [35]. Certain representatives of beetles and lepidopterans can digest plastic. *Zophobas morio* larvae can survive and even thrive on a diet consisting solely of plastic, which is assimilated with the help of their gut microbiota [36]. Significant amounts of MPs have been found in insect larvae from natural populations [37] and in bee products [38]. Since many invertebrates are part of the diet of amphibians, reptiles and birds, MPs can be easily transferred through the food chain.

The common viper primarily feeds on small mammals, amphibians (brown frogs, newts), less often shrews, lizards, small passerines and their chicks. Juvenile snakes prefer insects, worms, mollusks, and underyearling frogs. In certain years, the common viper's diet is dominated by mouse-like rodents (up to 62%), amphibians (sharp-nosed frogs, 43-72%), with reptiles (viviparous lizards), shrews, and chicks of small passerines being less frequently consumed. The common viper's diet composition increases due to the stomach content of prey species. In years when the primary food source (mouse-like rodents) is scarce, the diet mainly consists of amphibians. Insect and arachnid remains, as well as plant remains consisting of sphagnum, cranberry leaves, and wild rosemary were found in 35% of stomachs and guts. In addition, four red-backed vole cubs, five common whitethroat chicks, three adult moor frogs, and juvenile viviparous lizards were identified in the GIT of the common adder [20].

In 2022-2023, a quantitative and qualitative analysis was conducted on the composition of MPs of similar size found in the GIT of rodents (order Rodentia) ($n = 45$) from areas where individuals of the common adder were collected. These included the striped field mouse *Apodemus agrarius* Pallas, 1771, common tundra vole *Mycrotus oeconomus* Palas, 1776, gray red-backed vole *Clethionomys rufocanus* Sundevall, 1846, and northern red-backed vole *Cl. rutilus* (order Rodentia) [39, 40]. It was shown that in the striped field mouse with a high valence for food items, the average MP content in the GIT was 1.7-fold higher than that in the common tundra vole. The GIT of the field mouse contained an average of 0.6 film-shaped particles, 0.5 fibers, and 0.1 fragments, while the corresponding figures for the root vole were 0.1, 0.2, and 0.4 [39]. In the forest vole (genus *Clethionomys*), MPs were detected in 27% of cases ($n = 24$): 0.3-1 and 2-3 mm films were found in *Cl. rutilus* (plant polyphage), and 0.3-1 mm films and 1-2 mm fibers dominated in *Cl. rufocanus* (phytophagous) [40]. The common adder can consume both juvenile and adult amphibians that have ingested MPs [25].

Thus, the entry of MPs into the organism of reptiles living in the study area can occur both directly from the environment and indirectly through trophic chains.

Conclusion

The study was the first to describe accumulation of MPs ranging from 0.15 to 5 mm in size in the GIT and skin of widespread Palearctic reptile species, in particular, the viviparous lizard, the sand lizard, and the common adder. The presence and accumulation of MPs of various shapes and sizes in the GIT of two lizard species, as well as in the GIT and skin of the common adder, are most likely associated with the dietary patterns, trophic links, and habitat conditions. MP consumption by reptiles is affected by the level of plastic pollution of food items, substrate surfaces for insolation and hunting, shelters, and winter burrows in the soil. The MPs detected in the GIT and skin of reptiles restricted to the study area were mainly represented by irregularly shaped fragments, spheres, and films, with fibers being predominant (64.5-82.0%), which is consistent with data previously reported for soils, snow cover, and surface waters in Western Siberia. In general, MP accumulation in the GIT and skin of reptiles indirectly indicates the terrestrial ecosystem pollution within the taiga zone. Lizards and snakes are involved in the MP circulation in Western Siberia and can serve as biological indicators of environmental pollution.

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