First finding of spirurid larva (Chromadorea, Spirurida) in the common European viper Vipera berus (Linnaeus, 1758) of the Russian fauna

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Abstract. The National park “Smolny” (Republic of Mordovia, Russia) is located in the Basin of Alatyr River, a second-order tributary of the Volga River. Favorable conditions are created for the development of helminths and infecting reptiles with them in floodplain biotopes. In 2018–2020 in this Protected Area we examined 25 individuals of the common viper Vipera berus (Linnaeus, 1758) by the method of complete helminthological dissection. We studied only road-killed vipers and reptiles killed by local residents. In total, eight species of helminths were registered in vipers: Leptophallus nigrovenosus, Telorchis assula, Alaria alata, larvae, Rhabdias fuscovenosa, Oswaldocruzia filiformis, Physocephalus sexalatus, larvae, Physaloptera clausa, larvae and Agamospirura minuta, larvae. Larva of spirurid nematodes Ph. sexalatus, Ph. clausa and A. minuta were recorded in the common viper for the first time in Russia. We found these nematode juveniles in walls and lining of stomach and intestine of snakes. We gave original drawings and morphological descriptions of these nematodes. Vipera berus was noted as a new host for Physaloptera clausa. Currently the helminth fauna of Vipera berus of the Russian fauna includes 15 species. Helminth infection of Vipera berus strongly depends on habitat and, especially, on the diet features. Snakes that live in dry habitats and feed on mouse-like rodents, as a rule, are weakly infested by helminths. Conversely, helminth infection is higher in vipers living in near-water habitats and feeding tailless amphibians. Participation of paratenic hosts in the helminth life cycles plays an important role in the distribution and preservation of parasites in the wild, and increases the invasion probability of the final hosts.

1. Introduction
The National Park “Smolny” (Republic of Mordovia, Russia) is located in the Alatyr River Basin, a second-order tributary of the Volga River. The Alatyr River traverses through Nizhny Novgorod Oblast, the Republic of Mordovia, the Republic of Chuvashia and flows into the Sura River [1]. There are many small streams, rivers, ponds and lakes in the Alatyr basin. The complex structure of floodplain biotopes contributes to the development of species richness and abundance of invertebrates – intermediate hosts of helminths with a complex life cycle. On the other hand, favorable conditions are created in moist near-water habitats for the development of helminths with direct life cycle.

Despite the broad distribution of reptiles, there is very little information about their helminths. The parasites of reptiles of the European Russia and, in particular, of Vipera berus (Linnaeus, 1758), have been not fully studied. Data about the helminths of the European vipers is presented in only a few
In Russia, the helminth fauna of *Vipera berus* was studied in the Republic of Karelia, the Republic of Mordovia, Leningrad, Volgograd, Samara and Ulyanovsk Oblasts [11-16]. Currently, 27 species of parasitic worms were registered in this reptile species in Russia and adjacent countries [10, 11, 13, 15].

The purpose of this study is to describe the larva of spirurid nematodes, found in *Vipera berus* of the Russian fauna for the first time.

2. Materials and Methods

We examined 25 specimens of *Vipera berus* by the method of complete helminthological dissection (Skryabin, 1928). In this work, we studied snakes that died on the roads passing through the National park “Smolny” and reptiles killed by local residents in 2018–2020. The helminth fauna of *Vipera berus* we studied in six sites of the National park (figure 1).

![Figure 1. Schematic map showing research sites in the National park “Smolny”; red circles – research sites of *Vipera berus* helminths, 1 – Obrezki village, 2 – Tashkinskiy pond, 3 – Kuzoley Streem, 4 – Alatyr River floodplain, 5 – Rezovatovskiy cordon, 6 – Mitryashki Lake.](image)

Research site near Obrezki village in the Tesovka River floodplain (54.835554 N, 45.380793 E) (figure 1, site 1) represents forest edges and abandoned vegetable gardens with dense herbaceous vegetation where predominance of *Urtica dioica* L. There are clumps of *Salix* spp. and *Alnus glutinosa* (L.) in some places along riverbank. Sedge-cereal forb grows at the edge of the mixed forest.

Tashkinskiy pond (54.747151 N, 45.262525 E) is a small water reservoir located on the Chernushka River (figure 1, site 2). Clumps of willow and alder grow along the banks of the pond. Around the pond, on the north and south sides, there are glades overgrown with grasses and sedges with gazebos and recreation areas for tourists. Open spaces near pond surrounded by an old pine forest. A dirt road runs along the pond to the Malye Ichalki village.
Research site near automobile bridge over the Kuzoley Stream (54.756020 N, 45.390809 E) (figure 1, site 3). The Kuzoley Stream with a sandy-silty bottom is a fourth order tributary of the Volga River. It dries out by midsummer. The stream banks overgrown with alders and, singly, Tilia cordata Mill. and Betula pendula Roth. Meadow with sedge-cereal grasses adjoins the floodplain forest. Dirt road runs along the eastern edge of the meadow.

Alatyr River floodplain near the sanatorium “Alatyr” (54.737837 N, 45.393652 E) (figure 1, site 4). There are floodplain sedge-grass meadows with low grass to the South of the sanatorium. Dense floodplain forest of Salix spp., Ulmus laevis Pall., Prunus padus L., Quercus robur L. and Euonymus verrucosus Scop. grows along the Alatyr River.

The research site at the Rezovatovsky cordon (54.739156 N, 45.473950 E) (figure 1, site 5) is a forest glade along the road leading to the floodplain meadows of the Alatyr River, rarely overgrown with Pinus sylvestris L. and Betula pendula. Herbaceous vegetation is represented by cereal herbs. The glade from the North, East and South is surrounded by oak forest, from the west – by pine forest.

Research site near the Mitryashki Lake (54.744966 N, 45.502406 E) (figure 1, site 6) is a dirt road in forest glade located on the first terrace above floodplain, on the edge of the Alatyr River floodplain with sedge-cereal herbs. The glade is bounded from the North by pine forest with Quercus robur, Betula pendula and Tilia cordata; from the South in the floodplain – clumps of alders and Salix sp. singly Prunus padus.

Snake dissection, fixation and handling of helminths were conducted according to standard techniques [17-19]. Collected nematodes were killed by heating in water, clarified in lactic acid and mounted in Glycerin-Jelly. Nematode identification was made according to Sharpilo [11]. Parasite taxonomy is provided according to the Fauna Europaea (http://www.fauna-eu.org/). Morphological descriptions and original drawings of nematode juveniles were given. Drawings of viper parasites were made using MBI-9 light microscope with drawing tube RA-7. Nematode and viper specimens were deposited in the helminth and reptile collections of Institute of Ecology of Volga River Basin of RAS (Togliatti).

3. Results and Discussion
A total of eight species of helminths were collected from 25 Vipera berus: trematodes Leptophallus nigrovenosus (Bellingham, 1844), Telorchis assula (Dujardin, 1845), Alaria alata (Goze, 1782), msc., nematodes Rhabdias fuscovenosa (Railliet, 1899), Oswaldocruzia filiformis (Goze, 1782), Physocyclus sexualus (Molin, 1860), juv., Physaloptera clausa Rudolphi, 1819, juv. and Agamospirura minuta Sharpilo, 1963, juv. Larva of nematodes of the order Spirurida Physocyclus sexualus, Physaloptera clausa and Agamospirura minuta were recorded in Vipera berus of the Russian fauna for the first time.

A total of 45 larvae of Physocyclus sexualus, Physaloptera clausa and Agamospirura minuta were collected from six Vipera berus out of 25 studied. Six juveniles of Physocyclus sexualus were recorded in reptile from the Rezovatovskiy cordon (figure 1, site 5) and 22 specimens in snake from vicinity of the Tashkinskiy pond (figure 1, site 2) in 2019. Four juveniles of Physaloptera clausa were noted in viper from the vicinity of the Mitryashki Lake in 2019. Seven juveniles of Agamospirura minuta were collected in Vipera berus from Alatyr River floodplain in 2018; one specimen in snake from bank of Kuzoley Stream, three juveniles in reptile from Rezovatovskiy cordon and two specimens in viper from vicinity of the Obrezi village in 2019. Below we give morphometric description of the found nematode larvae.

3.1. Morphological description of Physocyclus sexualus (Molin, 1860), juv. (10 specimens) (figure 2).
Body 1.30–1.54 mm long, maximum width 0.057–0.071 mm. Cuticle with slight transversal striations. At anterior end of body there are two lips, tops of which are in the form of “auriculate” protrusions. Each lip has a pair of submedial papillae. The funnel-shaped oral cavity passes into a narrow pharynx 0.074–0.087 mm long with dense sclerotized walls. Oesophagus is 0.579–0.669 mm long and is
divided into two parts: the muscular one, 0.083–0.102 mm long, and the glandular one, 0.496–0.567 mm. Nerve ring surrounds oesophagus approximately in the middle of the muscular part (slightly displaced to pharynx) at a distance of 0.114–0.126 mm from the anterior end of the body. Excretory pore is located at a distance of 0.126–0.154 mm from the anterior body end. Tail is 0.047–0.059 mm long, tapering, with a slight thickening at the end, seated with small spines. Larvae enclosed in connective tissue capsules 0.339–0.377 mm in diameter. Cysts localized in the walls of stomach and intestine. One cyst can contain two nematode larvae.

![Figure 2. Physocephalus sexalatus (Molin, 1860), juveniles from Vipera berus; (a) – general view, (b) – anterior end of body, (c) – tail end of body.](image)

The final hosts of *Physocephalus sexalatus* are mammals of family Suidae. The nematode parasitizes in the stomach lining of wild boars and domestic pigs. The parasite also was noted in camels, horses, donkeys, cattle and lagomorphs. Intermediate hosts are coprophagous beetles of the family Scarabaeidae. Final hosts become infected when eaten coprophagous beetles [20]. The parasite has a wide range of paratenic hosts, which include many species of vertebrates of different classes, including reptiles. *Physocephalus sexalatus* parasitizes in the stomach and intestine walls in reptilian hosts.

In the Middle Volga region, larva of *Physocephalus sexalatus* was noted in 17 species of vertebrates of different classes [21-31]. Parasite broadly distributed among reptiles of Eastern Europe and Central Asia [10, 11]. Previously *Physocephalus sexalatus*, juv. recorded in *Vipera berus* in Ukraine and Belarus [10, 11].

3.2. Morphological description of *Physaloptera clausa* Rudolphi, 1819, juv. (4 specimens) (figure 3). Body length 2.34–2.69 mm, width at mid-length 0.140–0.152 mm. Cuticle with slight transversal striations forms a “collar” at the anterior part of the body. Mouth with two lips. Each lip has well-defined medial tooth. The triple tooth is poorly expressed. Each lip has pair of large submedial rounded papillae and amphid. Oesophagus is 0.896–0.979 mm long. Oesophagus is clearly divided into two parts: the anterior muscular with a length of 0.157–0.170 mm, the posterior glandular – 0.735–0.822 mm. Nerve ring surrounding muscular part of oesophagus near its base, at distance of 0.122–0.136 mm from the anterior end of the body. Excretory pore is located at a distance of 0.195–
0.215 mm from the anterior end of the body. The tail is conical, 0.104–0.119 mm long, with a rounded end. Larva localized in the stomach lining without cysts.

**Figure 3.** Physaloptera clausa Rudolphi, 1819, juveniles from common European viper; (a) – general view, (b) – anterior end of body, (c) – tail end of body.

*Physaloptera clausa* parasitizes in the stomach of hedgehogs. At the larval stage, it is a common and widespread parasite of reptiles, more often found in lizards. Reptiles are paratenic hosts. In the Middle Volga region, we found parasite in the final host – the Northern white-breasted hedgehog *Erinaceus roumanicus* Barrett-Hamilton, 1900, in paratenic hosts – insectivores (*Sorex araneus* Linnaeus, 1758) and other species of reptiles [12, 15, 16, 23, 25]. The nematode juveniles were recorded in many species of reptiles in the former USSR [10, 11]. On the territory of Russia, it was found in *Lacerta agilis* from the Crimea [32]. *Vipera berus* represents a new host record for *Physaloptera clausa*, juv.

### 3.3. Morphological description of Agamospirura minuta Sharpilo, 1963, juv. (13 specimens) (figure 4).

Body 0.630–0.841 mm long with maximum width 0.037–0.047 mm. The body is covered with a slightly striated cuticle. Two cuticular crests protrude above the body surface and running along the larva body laterally. The lips are indistinguishable. Oesophagus length 0.280–0.356 mm, consists of two poorly differentiated parts. The walls of the oesophagus anterior part are sclerotized. The oesophagus is greatly widened at the base; it exceeds the intestine in width. The nerve ring surrounding the muscular part of the oesophagus approximately in its middle part, at a distance of 0.035–0.043 mm from the anterior end of the body. The excretory pore is located at a distance of 0.091–0.106 mm from the anterior end of the body. The genital primordium is oval in shape, located approximately at the mid-length of the intestine. The tail is conical, 0.037–0.049 mm long, sharpened at the end. The nematode juveniles enclosed in connective tissue capsules 0.235–0.322 mm in diameter. Cysts localized in the intestinal wall.

*Agamospirura minuta*, juv. is a specific parasite of reptiles, usually parasitizing the slowworm *Anguis fragilis* Linnaeus, 1758 and less common in other lizards and snakes [11]. Final host are unknown. Lewin [33] found similar nematode larva in reptiles of Poland and identified them as
Protostrongylidae sp. In his opinion, the definition by Sharpilo [11] of Agamospirura larva as Spirurida is erroneous.

Previously Agamospirura minuta was found in reptiles in Ukraine (including Vipera berus), Belarus, Georgia and Azerbaijan [10, 11, 28].

Figure 4. Agamospirura minuta Sharpilo, 1963, juveniles from common European viper; (a) – general view, (b) – anterior end of body, (c) – tail end of body.

4. Conclusion
The helminth fauna of Vipera berus of the Russian fauna before our research included 12 species [11]. As a result of our research, the lists of viper helminths currently consist of 15 species. And the helminth fauna of Vipera berus in Russia and adjacent countries has increased to 28 species. Helminth infection of Vipera berus strongly depends on habitat and, especially, on the diet features. Snakes that live in dry habitats and feed on mouse-like rodents, as a rule, are weakly infested by helminths. Conversely, helminth infection is higher in vipers living in near-water habitats and feeding tailless amphibians [13, 15].

Vipera berus, as a paratenic host, take part in the life cycles of biohelminths and transmitting infective larva along the food chains to the final hosts of parasites – birds of prey and mammals. Thus, the reptile is included in the diet of hedgehogs and wild boar Sus scrofa Linnaeus, 1758 [34]. Participation of paratenic hosts in the helminth life cycles plays an important role in the distribution and preservation of parasites in the wild and increases the invasion probability of the final hosts.

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