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## Article

# Diversity of Trematodes in *Myotis* Bats (Chiroptera, Vespertilionidae) from the Samarskaya Luka (European Russia)

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**Simple Summary:** Different species of bats often inhabit the same territories but have differences in diet. Bats become infected with parasitic worms such as trematodes when they feed on insects. Fluke diversity will be determined by bat dietary breadth. The purpose of our research was to study the trematode diversity in five *Myotis* species inhabiting the Samarskaya Luka National Park. In 2005–2007 we studied parasitic worms in bats and found 11 trematode species. One parasite species, *Prosthodendrium cryptolecithum* was recorded for the first time in Russia. Only three fluke species, were revealed in all studied bat species. Our study shown that trematode species diversity is higher in *Myotis daubentonii* and *M. dasycneme*. The trematode fauna of *M. brandtii*, *M. nattereri* and *M. mystacinus* is less diverse. Our results confirm the similarity of habitats and diets among the five *Myotis* species, and show weak competition among bats for food items.

**Abstract:** Various bat species often occupy the same habitats. Cohabitation should induce different preferences in spatial or trophic components of the bat ecological niche, to reduce their competition. This determines the differences in the trematode fauna of *Myotis* spp. The purpose of our research was to study the biodiversity of trematodes in syntopic populations of five *Myotis* species in the Samarskaya Luka National Park. In 2005–2007 we studied 867 bat specimens by the methods of complete helminthological dissection. In total, 11 trematode species were found in *Myotis* bats. Only three trematode species, *Plagiorchis koreanus*, *Prosthodendrium chilostomum* and *Parabascus duboisi* were revealed in all studied *Myotis* spp. *Prosthodendrium cryptolecithum* was recorded for the first time in Russia. Digenean species diversity is higher in *Myotis daubentonii* and *M. dasycneme*. The trematode fauna of *M. brandtii*, *M. nattereri* and *M. mystacinus* is less diverse. The determining factor in the infection of bats by trematodes is feeding on semi-aquatic insects, possible second intermediate hosts of these parasites. Our results confirm the data that the ecological niches of the five *Myotis* species partially overlap. An analysis of the trematode fauna in *Myotis* spp. showed that in the Samarskaya Luka there may be weak competition for food items among bats.

**Keywords:** bats; digenea; *Myotis* spp.; Middle Volga region; Samarskaya Luka National Park

## 1. Introduction

Recently, more and more attention be paid to the study of groups of co-inhabiting and even taxonomically close bat species. In such communities, quite complex and contrasting relationships develop between their members [1–5]. Various bat species often occupy the same habitats. In this case, the competitive exclusion principle can be applied, according to which species with the same ecology cannot live in the same space. Species living together should show different preferences in some component of the ecological niche, spatial or trophic, to reduce their competition [6].

The territory of the European Russia is inhabited by 27 bat species. The bat fauna of the Middle Volga region includes 16 species [7–10]. Five bat species of the genus *Myotis* (Chiroptera, Vespertilionidae) are among them: the whiskered bat *Myotis mystacinus* (Kuhl, 1819), the Daubenton's bat *Myotis daubentonii* (Kuhl, 1817), the pond bat *Myotis dasycneme* (Boie, 1825), the Brandt's bat *Myotis brandtii* (Eversmann, 1845) and the Natterer's bat *Myotis nattereri* (Kuhl, 1817). All *Myotis* bats are sedentary species, wintering in underground shelters, caves and abandoned adits [2,9]. Among

sedentary bat species inhabiting the Samarskaya Luka the populations of *M. brandtii* and *M. daubentonii* reach the highest abundance [2].

Despite the relative similarity in the habitat preferences, various *Myotis* spp. have significant trophic and chorological differences [7,11,12]. The life style differences, in particular, feeding habits and spatial distribution, cause the features of the trematode fauna in *Myotis* spp. In this regard, it is of considerable interest to study the helminth fauna of syntopic *Myotis* bats, and especially their trematodes.

Previously, studies of helminths in various bat species of the genus *Myotis* were carried out in many European countries [13]. The helminth fauna in *M. mystacinus* is the most studied and was studied in Poland, Austria, Italy, Switzerland, the Czech Republic, Slovakia, Moldova, Ukraine, Belarus, Georgia and Azerbaijan. In this bat species, 25 species of parasitic worms were registered in Europe and the countries of the former USSR, including 15 trematodes [13–28].

The helminth fauna in *M. dasycneme* was studied in Poland and Hungary [15,16, 18,29]. Data on the helminths in *M. daubentonii* were obtained in Belarus, Hungary, Germany, Italy, Norway, Poland, Ukraine and Turkey [15,16,29–35]. Parasitic worms in *M. nattereri* were studied in Belarus, Hungary, Poland, Spain and Austria [15,16,29,34,36,37].

Data on *M. brandtii* helminths in West and Central Europe are known only from studies by Shimalov et al. [34,38]. Probably, the scarce data on the parasitic worms of *M. brandtii* in Europe are due to the fact that *M. mystacinus* and *M. brandtii*, as independent species, began to be distinguished only in the last 40 years [7,8]. Thus, the data of helminthological studies of *M. mystacinus* apparently refer to both sympatric bat species.

There is little information about trematodes, as well as about helminths in general, of *Myotis aurascens* Kuzyakin, 1935 and *Myotis alcathoe* Helversen et Heller, 2001, studied only in Turkey [35,39]; *Myotis oxygnathus* (Monticelli, 1885) – in Serbia [40]; *Myotis myotis* Borkhausen, 1797 and *Myotis emarginatus* (E. Geoffroy, 1806) – in Austria [36]. Recently Frank with coauthors [13] presented a summary of parasites (including seven trematode species) in *Myotis* bats inhabiting European countries. Unfortunately, this review did not include some works on parasites of bats from Eastern Europe.

In Russia, there are very few works containing data on helminths in *Myotis* bats. The first report on the parasitic worms *M. mystacinus* in Russia was given by Sten'ko et al. [41], where three species of trematodes were found in the Crimea. Podvyaznaya [42,43] studied one species, *Allasogonoporus amphoraeformis* (Mödlinger, 1930), from *M. brandtii* in the Voronezh Nature Reserve. Demidova and Vekhnik [44] studied the trematodes in *M. mystacinus* and *M. brandtii* from the Samarskaya Luka, in which two and nine species of trematodes were revealed, respectively. Five species of trematodes were found in *M. brandtii* from Karelia [45]. The study by Gulyaev et al. [46] provides data on one studied specimen of *M. brandtii* from the Magadan Oblast, in which was only *Plagiorchis* sp. was found. We studied the helminths in *M. dasycneme* and *M. daubentonii* from Mordovia, where three and six trematode species were identified in bats, respectively [47,48]. Our works on parasites in vertebrates from the Middle Volga region include the information on trematodes in all five species of bats [49–53].

Thus, the aim of our work was to study the biodiversity of trematodes in syntopic populations of five *Myotis* species in the Samarskaya Luka (European Russia).

## 2. Materials and Methods

The material for this research was collected from our own field studies on bat ecology and helminths in the territory of the Samarskaya Luka National Park (Samara Oblast, European Russia), which were conducted in the period from 2005 to 2007. The study area was the coastal zone of the Volga river in the northern part of the Samarskaya Luka (Samara Oblast). *Myotis* bats were studied in three trapping stations in the National Park near Solnechnaya Polyana, Bogatyr and Shiryaevo villages (Figure 1).



**Figure 1.** A map of bat trapping places in the Samarskaya Luka National Park. Red crosses in the map show the bat trapping sites.

In total, we studied 247 *Myotis brandtii*, 262 *Myotis daubentonii*, 135 *Myotis dasycneme*, 125 *Myotis mystacinus*, and 98 *Myotis nattereri*. Bats were caught by mist nets at nights. We used the common method of placing the net between two vertical sticks, which were telescopic fishing rods 6 m long [54]. Also, we studied bats that died from natural causes in the wintering places.

Chiropterans were examined by the methods of complete helminthological dissection [55,56]. Trematodes were collected from bats and fixed in 70% ethanol for further investigations. Digeneans were stained with aceto-carmine, dehydrated in a graded ethanol series (70–96%) and cleared in clove oil. Then parasitic worms were mounted in Canada balsam.

The indexes generally accepted in parasitology were used to characterize the helminth infection of *Myotis* bats: the prevalence ( $P$ , %), the mean abundance ( $MA$ ), and the intensity range ( $I$ , specimens) [57]. The Shannon ( $H'$ ) and Shannon evenness ( $E$ ) indexes were calculated to determine the species diversity of bat trematodes. The significance of the differences between the Shannon index values was measured using Student's  $t$ -test. The degree of similarity of the trematode faunas in *Myotis* spp. was revealed using the Jaccard ( $C_j$ ) (qualitative data) and Sørensen ( $C_N$ ) (quantitative data) similarity indexes [58]. The degree of similarity was assessed as low (0–0.33), medium (0.34–0.66), and high (0.67–1).

The dominance of species in the parasite community was determined using the the Paliakovnaty index of dominance ( $D$ ) [59]. Trematode dominance groups were as follows: 100–10, dominants; 10–1, subdominants; and 1–0.001, adominants. The Mann–Whitney ( $U$ ) tests was used to compare the total infestation of *Myotis* spp., and to assess the significance of differences in the infection of bats by individual trematode species. The similarity dendrogram of the helminth faunas in *Myotis* bats was made using the unweighted pair group method with arithmetic average (UPGMA) and the Morisita index as a distance measure in PAST 2.17 [60]. To standardize the bat sample size, we used the species richness index (bootstrap estimator), which makes it possible to predict the number of helminth species that were not included in the collections [61,62]. The taxonomy of digeneans is given according the Fauna Europaea (<http://www.fauna-eu.org>). Trematode voucher

specimens are stored in the parasitological collection of the Institute of Ecology of the Volga River Basin of the Russian Academy of Sciences (Togliatti, Russia).

3. Results

In total, 11 species of trematodes were revealed in five species of *Myotis* bats in the Samarskaya Luka National Park (Table 1).

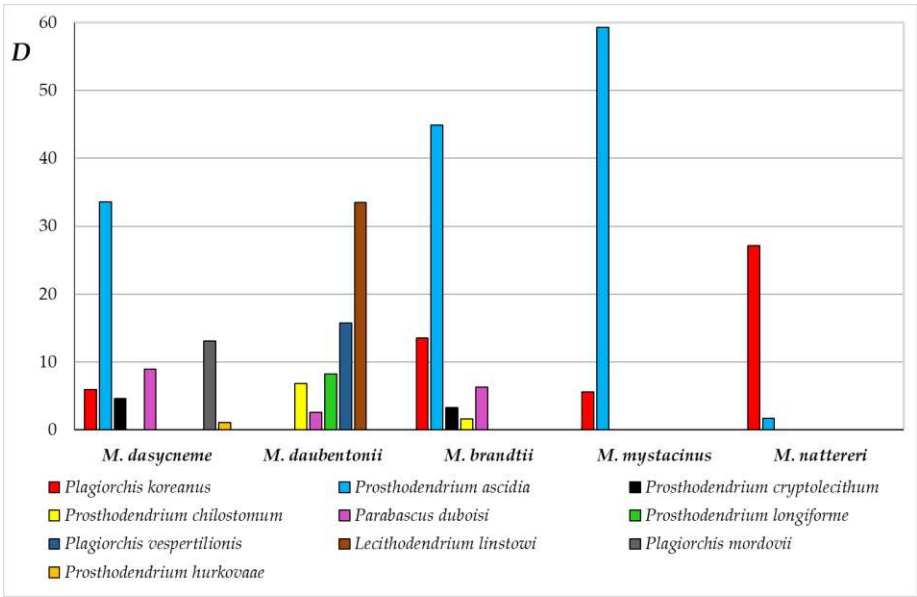
**Table 1.** Trematodes in *Myotis* bats from the Samarskaya Luka National Park.

Trematode species	<i>Myotis brandtii</i>	<i>Myotis daubentonii</i>	<i>Myotis dasycneme</i>	<i>Myotis mystacinus</i>	<i>Myotis nattereri</i>
<i>Plagiorchis koreanus</i> (Ogata, 1938)	81.4(1–111)/8.4 <sup>1</sup>	28.2(1–26)/1.5	63.7(1–52)/6.2	43.2(1–10)/1.7	40.8(1–13)/1.7
<i>Plagiorchis mordovii</i> Schaldybin, 1958	25.1(1–15)/1.0	3.8(1–5)/0.1	76.3(1–50)/11.6	–	–
<i>Plagiorchis muelleri</i> Tkach et Sharpilo, 1990	24.4(1–9)/0.7	–	–	13.6(1–6)/0.3	10.2(1–5)/0.2
<i>Plagiorchis vespertilionis</i> (Müller, 1780)	18.2(1–8)/0.5	79.4(1–99)/12.7	11.1(1–21)/0.8	–	–
<i>Prosthodendrium ascidia</i> (Beneden, 1873)	87.5(1–178)/25.9	–	74.8(3–225)/30.4	72.0(1–51)/10.5	12.2(1–5)/0.4
<i>Prosthodendrium chilostomum</i> (Mehlis, 1831)	32.8(1–62)/2.4	56.1(1–73)/7.7	16.3(1–13)/0.8	0.8(27)/0.2	6.1(1–3)/0.1
<i>Prosthodendrium cryptolecithum</i> Zdzitowiecki, 1969	39.7(1–70)/4.2	–	53.3(1–94)/5.8	–	–
<i>Prosthodendrium hurkovaee</i> Dubois, 1960	–	22.9(1–60)/2.0	29.6(1–21)/2.4	–	5.1(1–3)/0.1
<i>Prosthodendrium longiforme</i> (Bhalerao, 1926)	27.5(1–9)/0.8	66.8(1–110)/7.9	–	–	–
<i>Parabascus duboisi</i> (Hurkova, 1961)	54.7(1–59)/5.8	36.6(1–61)/4.6	63.9(1–58)/9.6	2.4(2–3)/0.1	3.1(1–5)/0.1
<i>Lecithodendrium linstowi</i> Dollfus, 1931	9.7(1–31)/0.9	76.7(1–125)/27.7	–	1.6(1)/0.02	–
<b>Total</b>	10	8	8(8.0) <sup>2</sup>	6(6.6)	6(6.1)

Note: 1 – prevalence of infection (*P*), in brackets – intensity range (*I*), beyond the line – mean abundance (*MA*); 2 – in brackets – predicted number of species not included in collections due to insufficient host sampling.

The greatest richness of the trematode fauna was found in *M. brandtii*, including 10 digenean species (Table 1). The total infestation of *M. brandtii* with trematodes reached 100%, *MA* = 50.4. According to the Palia-Kovnatsky dominance index, two species, *Pr. ascidia* (*D* = 44.9) and *P. koreanus* (13.5) dominated in the trematode fauna of this bat species. The group of subdominants included *P. duboisi* (6.3), *Pr. cryptolecithum* (3.3) and *Pr. chilostomum* (1.6) (Figure 2).





**Figure 2.** Dominant and subdominant species of trematodes in *Myotis* bats from the Samarskaya Luka (the Palia-Kovnatsky dominance index).

Eight species of trematodes were revealed in both *M. daubentonii* and *M. dasycneme*, the total infestation of which in both species was 100%, the abundance index was 64.2 and 67.6, respectively.

The trematode fauna of *M. daubentonii* is dominated by *L. linstowi* (33.5) and *P. vespertilionis* (15.7), while *Pr. longiforme* (8.2), *Pr. chilostomum* (6.8) and *P. duboisi* (2.6) are subdominants. In the trematode fauna of *M. dasycneme* dominants are *Pr. ascidia* (33.6) and *P. mordovii* (13.1); subdominants – *P. duboisi* (8.9), *P. koreanus* (5.9), *Pr. cryptolecithum* (4.6) and *Pr. hurkovaee* (1.1).

*Myotis mystacinus* has 6 species of trematodes, as well as *M. nattereri*. The total infection of *M. mystacinus* with trematodes was 60%, MA = 12.8. Among trematodes of *M. mystacinus* dominant is *Pr. ascidia* (59.3); while *P. koreanus* (5.6) is subdominant. The total infestation of *M. nattereri* compared to other *Myotis* bats is low and amounted to only 32%, and MA is 2.6. In the trematode fauna of *M. nattereri* dominant is *P. koreanus* (27.1), and subdominant is *Pr. ascidia* (1.7). The other four species in the trematode fauna of *M. nattereri* are adominants.

In the trematode fauna of *Myotis* bats, the structure and number of dominant and subdominant species are different (Figure 2). No common dominant or subdominant trematode species were revealed for all five *Myotis* species. Only two species of trematodes, *P. koreanus* and *Pr. ascidia* are dominant or subdominant species for four bat species, and *P. duboisi* for three *Myotis* species (Figure 2).

Only three of 11 trematode species were revealed in all five bat species: *P. koreanus*, *Pr. chilostomum* and *P. duboisi* (Table 1). *Prosthodendrium ascidia* was found in four species of bats. *Plagiorchis vespertilionis*, *P. mordovii*, *P. muelleri*, *Pr. hurkovaee* and *L. linstowi* have each been recorded in three host species. Two host bat species were noted for *Pr. cryptolecithum* and *Pr. longiforme* (Table 1).

Values of trematode species diversity in *Myotis* bats from the Samarskaya Luka National Park are shown in the Table 2.

**Table 2.** Values of trematode biodiversity indexes in *Myotis* bats.

Index	<i>M. brandtii</i>	<i>M. daubentonii</i>	<i>M. dasycneme</i>	<i>M. mystacinus</i>	<i>M. nattereri</i>
Margalef index, $D_{Mg}$	0.955	0.719	0.768	0.678	0.906
Shannon index, $H'$	1.555	1.584	1.592	0.616	1.127
Shannon evenness index, $E$	0.675	0.762	0.766	0.344	0.629

Simpson index, <i>d</i>	3.175	3.817	3.718	1.439	2.110
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The diversity indices of the trematode fauna are significantly higher in *M. daubentonii* and *M. dasyncneme*, with the exception of the values of the Margalef index. The values of the Margalef index in these bat species are slightly lower than those in *M. brandtii* and *M. nattereri* (Table 2). The trematode fauna of *M. brandtii* and *M. nattereri* is less diverse. The least diversity of digenean fauna was observed in *M. mystacinus* (Table 2). This bat species has significantly lower Shannon diversity, Shannon evenness, Margalef and Simpson evenness indices than other species (Table 2). The differences in the Shannon species diversity index of the trematode fauna of the five species of moths are significant ( $P < 0.001$ ), with the exception of *M. daubentonii* – *M. dasyncneme* pair ( $P > 0.05$ ).

Comparison of the trematode infection of five *Myotis* species using the Kruskal-Wallis test revealed significant differences ( $H = 425.6$ ,  $P < 0.0001$ ). Pairwise comparison of infestation of different *Myotis* bats by digeneans using the Mann-Whitney test showed significant differences in most cases ( $P < 0.0001$ ), with the exception of the pair *M. daubentonii* – *M. dasyncneme* (Table 3).

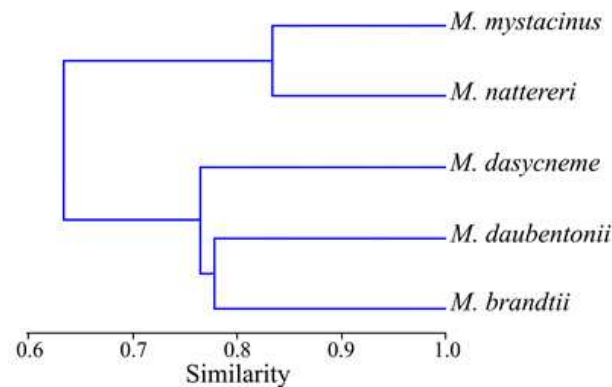
**Table 3.** Validity of differences in infection of *Myotis* spp. by common trematode species.

Bat species	<i>Plagiorchis koreanus</i>		<i>Parabascus duboisi</i>		<i>Prosthodendrium chilostomum</i>		Total infestation	
	<i>U</i>	<i>p</i>	<i>U</i>	<i>p</i>	<i>U</i>	<i>p</i>	<i>U</i>	<i>p</i>
<i>M. mystacinus</i> / <i>M. brandtii</i>	7549.0	0.0001	7222.0	0.0001	10540.0	0.0001	3895.0	0.0001
<i>M. mystacinus</i> / <i>M. daubentonii</i>	14310.0	0.016	10650.0	0.0001	7375.0	0.0001	2328.0	0.0001
<i>M. mystacinus</i> / <i>M. dasyncneme</i>	5496.0	0.0001	3214.0	0.0001	7141.0	0.0001	1156.0	0.0001
<i>M. mystacinus</i> / <i>M. nattereri</i>	<b>6048.0</b>	<b>0.858</b>	<b>6084.0</b>	<b>0.760</b>	5740.0	0.013	2433.0	0.0001
<i>M. dasyncneme</i> / <i>M. brandtii</i>	<b>14840.0</b>	<b>0.073</b>	13760.0	0.01	13860.0	0.001	1234.0	0.0001
<i>M. dasyncneme</i> / <i>M. daubentonii</i>	10210.0	0.0001	12500.0	0.0001	9814.0	0.0001	<b>17330.0</b>	<b>0.7455</b>
<i>M. dasyncneme</i> / <i>M. nattereri</i>	4296.0	0.0001	2551.0	0.0001	5970.0	0.027	69.5	0.0001
<i>M. brandtii</i> / <i>M. daubentonii</i>	13430.0	0.0001	27540.0	0.002	22380.0	0.0001	24650.0	0.0001
<i>M. brandtii</i> / <i>M. nattereri</i>	5873.0	0.0001	5738.0	0.0001	8847.0	0.0001	292.0	0.0001
<i>M. daubentonii</i> / <i>M. nattereri</i>	<b>11390.0</b>	<b>0.057</b>	8419.0	0.0001	6124.0	0.0001	166.5	0.0001

Note: insignificant differences are shown in bold ( $p > 0.05$ ).

Analysis of the infestation of bats by common species using the Mann-Whitney test showed significant differences in the infection of bats with *Pr. chilostomum* (in all cases), *P. koreanus* and *P. duboisi* (in most cases) (Table 3). Differences in the *P. koreanus* infestation in pairs *M. brandtii* – *M. dasyncneme*, *M. mystacinus* – *M. nattereri*, *M. nattereri* – *M. daubentonii*, as well as in the infection of *M. mystacinus* and *M. nattereri* with *P. duboisi* are not significant. There are no significant differences in the *P. koreanus* infestation in the pairs *M. brandtii* – *M. dasyncneme*, *M. mystacinus* – *M. nattereri*, *M. nattereri* – *M. daubentonii*, as well as in the infection of *M. mystacinus* and *M. nattereri* by *P. duboisi* (Table 3).

We conducted a comparative analysis of the helminth faunas in *Myotis* species inhabiting the Samarskaya Luka. The similarity dendrogram of the parasite faunas in five bat species is shown in Figure 3.



**Figure 3.** Similarity dendrogram of trematode fauna in *Myotis* bats obtained using Morisita index (UPGMA) from the Samarskaya Luka National Park. Cophen. corr.:  $r = 0.802$ .

The cophenetic correlation coefficient is 0.802, which confirms the validity of the cluster. As a result of the clustering analysis, the considered *Myotis* species were divided into two clusters, with the most similar helminth fauna (Figure 3).

The first cluster is formed by the trematodes of *M. mystacinus* and *M. nattereri*, in which the greatest similarity of digenean communities is observed (0.83). They differ maximally in the structure of trematodes from the other three *Myotis* species, which form the second cluster (Figure 3).

The second cluster is formed by the clade of trematode fauna of *M. brandtii* and *M. daubentonii* (0.78), which is adjacent to the digenean fauna *M. dasyncneme*, more similar to *M. brandtii* (0.78); and less with *M. daubentonii* (0.75) (Figure 3). The other pairs showed an average degree of similarity from 0.57 to 0.63.

#### 4. Discussion

The analysis of the trematode fauna of *Myotis* bats in the Samarskaya Luka National Park showed that the species composition is the richest in *M. brandtii* (10 species). Slightly fewer species of digeneans were found in *M. daubentonii* and *M. dasyncneme* (eight species each), while the smallest number of trematode species was found in *M. mystacinus* and *M. nattereri* – six species each.

All species of trematodes were revealed in *Myotis* bats at the mature stage and are host-specific parasites of bats. Infection of bats with trematodes occurs entirely when feeding on semi-aquatic insects which serve as the second intermediate hosts of these digeneans. Thus, findings of *P. koreanus* and *P. vespertilionis* indicate the consumption by bats of adult forms of Diptera, Ephemeroptera, Megaloptera, Trichoptera and Odonata [25]. The presence of *Prosthodendrium chilostomum* in the helminth fauna of *Myotis* bats indicates that the animals consume imagoes of Trichoptera and, possibly, Odonata [25]. Second intermediate hosts of the trematode *Pr. ascidia* are chironomid larvae, such as *Chironomis plumosus* (Linnaeus, 1758) [25,63].

The life cycles of *P. mordovii*, *P. muelleri*, *L. linstowi*, *Pr. longiforme*, *Pr. cryptolecithum*, *Pr. hurkovaee*, *P. duboisi* and *P. lepidotus* are currently not studied. Probably, the second intermediate hosts of these digeneans, like other bat trematodes, are insects that develop in the aquatic environment [25].

The present differences in the trematode fauna of various *Myotis* species are associated mainly with preferences for certain food items. The widest feeding range was found in *M. daubentonii*, whose diet in the Samarskaya Luka includes 10 food items from nine insect orders. The diet of *M. dasyncneme*, *M. brandtii* and *M. mystacinus* is slightly smaller, numbering nine food objects from eight insect orders [12]. Also nine food items, but from seven insect orders are registered for *M. nattereri* [12].

Despite the similar diet composition of bats, the share of various food items in the diet of the five bat species is not the same [12]. Thus, the diet basis for *M. daubentonii* and *M. dasyncneme* consists of Trichoptera, Lepidoptera and Diptera. Coleoptera are somewhat less represented in the diet of these two species, and in *M. daubentonii*, members of the orders Hemiptera and Homoptera are also present. Insects of other orders are rare in the diet of *M. daubentonii* and *M. dasyncneme* [12].



The diet of *M. brandtii* is dominated by Lepidoptera and Diptera; Coleoptera are rather less represented; insects of the orders Trichoptera, Neuroptera, Homoptera and Hymenoptera are rarely found [12]. In *M. mystacinus*, the main diet components are Lepidoptera; Trichoptera are less represented, and Coleoptera, Homoptera and Diptera are rare. The diet of *M. nattereri* is dominated by Lepidoptera. And members of Trichoptera, Coleoptera, Diptera, Hymenoptera and Homoptera are present in the diet in equal proportions [12].

The differences identified in the infestation of chiropterans with common trematode species can be explained by different proportions of food items (second intermediate hosts of trematodes) in the diet of *Myotis* spp.

The diversity of the trematode fauna in bats, in addition to diet, also depends on the number of hunting grounds that chiropterans visit. Trematodes are parasites with a complicated life cycle, involving the change of hosts. Accordingly, the trematode fauna in *Myotis* bats is formed depending on the presence/absence of the different second intermediate trematode hosts (insects) in each site. Consequently, visiting a larger number of hunting sites by chiropterans helps to expand the diet of bats, which ultimately increases the likelihood of their infection with various species of trematodes.

*Myotis* spp. show unequal preferences for different types of hunting grounds. Among *Myotis* bats, the largest number of hunting sites was found for *M. brandtii*, *M. daubentonii* and *M. dasycneme* [64]. *Myotis nattereri* prefers to hunt in only one type of hunting sites, while *M. mystacinus* has two types of hunting grounds [64]. Our finding of a greater number of trematode species in *M. brandtii*, *M. daubentonii* and *M. dasycneme*, compared to *M. nattereri* and *M. mystacinus*, confirms these data (Table 1).

In addition, when hunting sites overlap, *Myotis* bats occupy different spatial niches [64]. So, *M. daubentonii* and *M. dasycneme* hunt above the water surface, *M. brandtii* feeds high in the tree crowns and *M. mystacinus* hunts immediately below (the middle and lower parts of the tree crowns). *Myotis nattereri* prefers to hunt in open, confined spaces above the ground, such as forest edges, wide forest glades and paths [64]. The confinement of various *Myotis* species to certain hunting grounds also affects the species structure and abundance of insects in the bat diet. Ultimately, this determines the species structure and abundance of trematodes in *Myotis* bats.

Analysis of the species diversity of trematodes in *Myotis* spp. showed that the trematode fauna is more diverse in *M. daubentonii* and *M. dasycneme*, despite the fact that a greater number of trematode species were found in *M. brandtii* (Table 2). The value of Shannon diversity index in *M. brandtii* is lower, which is associated with the high abundance and dominance of one species in its trematode fauna, *Pr. ascidia*. At the same time, the Margalef index, which takes into account species richness and the total number of parasite specimens, is significantly higher in *M. brandtii*, from which the largest number of trematodes was collected (Table 2). Abundance and dominance of *Pr. ascidia* in *M. mystacinus* also leads to a significant decrease in the Shannon index. As a result, the lowest species diversity is observed in this bat species.

A comparative analysis of the trematode fauna in five *Myotis* species inhabiting the Samarskaya Luka National Park showed a significant similarity in the structure of digeneans in different bat species. This is due to a similar lifestyle and feeding on semi-aquatic insects, which serve as the second intermediate hosts of trematodes. Thus, the finding of three common trematode species in bats (*P. koreanus*, *Pr. chilostomum* and *P. duboisi*) indicates the feeding on the same food objects (insects) – second intermediate hosts of these digenean species. Our data confirm the partial overlap of spatial and trophic niches of various *Myotis* spp. and their dividing thanks to food preferences and hunting grounds.

## 5. Conclusions

Thus, 11 trematode species were found in five species of *Myotis* bats in the Samarskaya Luka National Park. The trematode *Prosthodendrium cryptolecithum* was recorded for the first time among bats of the Russian fauna. A comparative analysis of the trematode fauna of *Myotis* bats showed that the species diversity of digeneans is higher in *M. daubentonii* and *M. dasycneme*. The trematode fauna

in *M. brandtii* and *M. nattereri* is less diverse. The least number of trematodes was found in *M. mystacinus*.

The determining factor in the infection of bats by trematodes is feeding on semi-aquatic insects. The diversity of the trematode fauna in *Myotis* spp. is caused by the number of hunting sites that bats visit. Various *Myotis* species visiting the same hunting areas and consuming the same food objects (but in different proportions), have an average degree of similarity in trematode fauna. The revealed differences in the trematode fauna in *Myotis* spp. are associated both with the food preferences of various bat species and with the variety of hunting grounds used by the bats.

Our parasitological results confirm the data that the ecological niches of the five *Myotis* species partially overlap in the spatial and trophic components. An analysis of the trematode fauna in *Myotis* spp. showed that in the Samarskaya Luka there may be weak competition for food items among bats because of their specialization and divergence of home ranges.

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## References

1. Carter, T.C.; Menzel, M.A.; Chapman, B.R.; Miller K.V. Partitioning of Food Resources by Syntopic Eastern Red (*Lasiurus borealis*), Seminole (*L. seminolus*) and Evening (*Nycticeius humeralis*) Bats. *Amer. Midl. Nat.* **2004**, *151*, 186–191.
2. Smirnov, D.G.; Vekhnik, V.P. Abundance and structure of bat communities (Chiroptera: Vespertilionidae), wintering in the artificial caves in the Samarskaya Luka. *Rus. J. Ecol.* **2011**, *1*, 64–72.
3. Mancina, C.A.; García-Rivera, L.; Miller, B.W. Wing morphology, echolocation, and resource partitioning in syntopic Cuban mormoopid bats. *J. Mammal.* **2012**, *93*(5), 1308–1317. <https://doi.org/10.1644/11-MAMM-A-331.1>
4. Andreas M., Reiter A., Cepakova E., Uhrin M. Body size as an important factor determining trophic niche partitioning in three syntopic rhinolophid bat species. *Biologia* **2013**, *68*(1), 170–175. <https://doi.org/10.2478/s11756-012-0139-1>
5. Salinas-Ramos V.B., Ancillotto L., Bosso L., Sanchez-Cordero V., Russo D. Interspecific competition in bats: state of knowledge and research challenges. *Mammal Review* **2020**, *50*(1), 68–81. <https://doi.org/10.1111/mam.12180>
6. Gause, G.F. *The struggle for existence*. Williams&Wilkins: Baltimore, USA, 1934, 1–163.
7. Strelkov, P.P.; Ilyin, V.Yu. Bats of the South of the Middle and Lower Volga regions. *Proceedings of Zoological Institute of the Academy of Sciences of the USSR*. 1990. 225: 42–167.

8. Il'in, V.Yu.; Vekhnik, V.P.; Smirnov, D.G.; Kurmaeva, N.M.; Zolina, N.F.; Matrosova, O.M. Dynamics of abundance of bats (Chiroptera: Vespertilionidae) during hibernation in caves of the Samarian Luka over a 20-year period. *Rus. J. Ecol.* **1999**, *30*(6), 428–431.
9. Smirnov, D.G.; Vekhnik, V.P.; Kurmaeva, N.M.; Shepelev, A.A.; Il'in, V.Yu. Species structure and dynamics of bat communities (Chiroptera: Vespertilionidae) hibernating in artificial caves of Samara Luka. *Biol. Bull.* **2007**, *34*(5), 507–516.
10. Mammals of Russia. Available online: <http://rusmam.ru/mammal/index?sort=sort> (accessed on 25 September 2023).
11. Smirnov, D.G.; Vekhnik, V.P. Biotopic structure of bat communities inhabiting flood plain ecosystems of the Samarskaya Luka. *Proc. Samara Sci. Cent. RAS* **2012**, *14*(1), 177–179.
12. Smirnov, D.G.; Vekhnik, V.P. Ecology of nutrition and differentiation of the trophic niches of bats (Chiroptera: Vespertilionidae) in Floodplain Ecosystems of the Samara Bend. *Biol. Bull.* **2014**, *1*, 53–64.
13. Frank, R.; Kuhn, T.; Werblow, A.; Liston, A.; Kochmann, J.; Klimpel, S. Parasite diversity of European *Myotis* species with special emphasis on *Myotis myotis* (Microchiroptera, Vespertilionidae) from a typical nursery roost. *Parasite & Vectors* **2015**, *8*, 101–114. <https://doi.org/10.1186/s13071-015-0707-7>
14. Matsaberidze, G.V.; Khotenovsky, I.A. To the trematode fauna of bats in Georgia. In *Helminth fauna of animals and plants in Georgia*; Kurashvili B.E., Ed.; Metsniereba: Tbilisi, Georgia, 1967; pp. 83–94.
15. Zdzietowiecki, K. Helminths of bats in Poland. II. Trematodes of the subfamily Lecithodendriinae. *Acta Parasitol. Pol.* **1969**, *16*(24), 208–226.
16. Zdzietowiecki, K. Helminths of bats in Poland. III. Trematodes of the subfamily Lecithodendriidae, except for Lecithodendriinae. *Acta Parasitol. Pol.* **1969**, *16*(24), 227–237.
17. Skvortsov, V.G. Trematodes of the genus *Prosthodendrium* (family Lecithodendriidae) from bats in Moldavia. In *Parasites of vertebrate animals*; Spassky A.A., Ed.; Stiintsa: Kishinev, Moldova, 1969; pp. 87–97.
18. Zdzietowiecki, K. Helminths of bats in Poland. I. Cestodes and trematodes of the family Plagiorchiidae. *Acta Parasitol. Pol.* **1970**, *17*(20), 175–188.
19. Khotenovsky, I.A. Family Pleurogenidae Looss, 1899. In *Trematodes of animals and man. Essentials of trematodology*; Skryabin K.I., Ed.; Nauka: Moscow, Russia, 1970; Volume 23, pp. 135–306.
20. Skvortsov, V.G. Trematodes of the family Lecithodendriidae from bats in Moldavia. In *Parasites of animals and plants in Moldavia*, Spassky A.A., Ed.; Stiintsa: Kishinev, Moldova, 1970, Volume 5; pp. 17–36.
21. Skvortsov, V.G. Nematodes of bats from Moldavia (Report 2). In *Parasites of animals and plants in Moldavia*, Spassky A.A., Ed.; Stiintsa: Kishinev, Moldova, 1971, Volume 7; pp. 75–93.
22. Shakhtakhtinskaya, Z.M.; Mustafaev, Yu.Sh.; Sailov, D.I. About helminths of some bats of Azerbaijan. *Proc. Azerb. St. Univ. Biol. Ser.* **1971**, *2*, 25–30.
23. Skvortsov, V.G. Ecological and faunistic analysis of helminth fauna of bats in Moldavia. In *Parasites of animals and plants*, Spassky A.A., Ed.; Stiintsa: Kishinev, Moldova, 1973, Volume 9; pp. 92–155.
24. Melnichenko, E.D.; Panasencko, N.A. To the helminth fauna of bats in the Middle Dnieper region. *Vest. zool.* **1979**, *3*, 76–78.
25. Sharpilo, V.P.; Iskova, N.I. *Trematodes. Plagiorchiata. Fauna of Ukraine*. Vol. 34(30). Naukova Dumka: Kiev, Ukraine; 1989. pp. 1–280.
26. Tkach V.V. First finding of males of *Pterygodermatites bovieri* (Nematoda, Rictulariidae) parasitizing bats. *Zool. Zhurn.* **1991**, *70*(9), 125–127.
27. Tkach V.V. Helminths of bats from the fauna of Ukraine. In: *Bats: materials of 6th meeting on bats of the CIS countries*. Khujand State University: Khujand, Tajikistan, 1995; pp. 90–95.
28. Bychkova, E.I.; Akimova, L.N.; Degtyarik, S.M.; Yakovich, M.M. *Helminths of vertebrates and man in Belarus*; Belarusskaya Nauka Publish.: Minsk, Belarus, 2017; pp. 1–316.
29. Matskási, I. The Systematic–faunistic survey of the Trematode fauna of Hungarian bats. I. *Annales Hist.-Nat. Mus. Nation. Hung. Pars Zool.* **1967**, (59), 217–238.
30. Odening, K. Exkretionssystem und systematische stellung einiger fledermaustrematoden aus Berlin und umgebung nebst bemerkungen zum Lecithodendrioiden komplex. *Zeitsch. Parasitenk.* **1964**, *24*(5), 453–483.
31. Bakke, T.A.; Mehl, R. Two species of fluke recorded in bats in Norway. *Fauna, Oslo, Norway* **1977**, *30*(4), 224–226.
32. Ricci, M. Report on trematode parasites of bats in Italy. *Parasitologia* **1995**, *37*(2-3), 199–214.
33. Tkach, V.V.; Pawlowski, J.; Sharpilo, V.P. Molecular and morphological differentiation between species of the Plagiorchis vespertilionis group (Digenea, Plagiorchiidae) occurring in European bats, with a re-description of *P. vespertilionis* (Muller, 1780). *Syst. Parasitol.* **2000**, *47*, 9–22. <https://doi.org/10.1023/A:1006358524045>
34. Shimalov, V.; Demyanchik, M.; Demyanchik, V. A study on the helminth fauna of the bats (Mammalia, Chiroptera: Vespertilionidae) in Belarus. *Parasitol. Res.* **2002**, *88*(11), 1011.
35. Sumer, N.; Yildirimhan, H.S. Intestinal Helminth Parasites of Two Bat Species of the Genus *Myotis* Kaup, 1829 (Chiroptera: Vespertilionidae) from Turkey, with DNA Sequencing of Helminth Nuclear LSR rDNA. *Acta Zool. Bulg.* **2019**, *71*(2), 273–278.

36. Kochseder, G. Untersuchungen über trematoden und cestoden aus fledermäusen in der Steiermark. In *Untersuchungen über Trematoden und Cestoden aus Fledermäusen in der Steiermark. Sitzungsberichten der Österr. Akademie der Wissenschaften*, Springer: Berlin, Heidelberg, Germany, 1969, Volume 8; pp. 205–232. [https://doi.org/10.1007/978-3-662-24784-6\\_1](https://doi.org/10.1007/978-3-662-24784-6_1)
37. Alvarez, F.; Rey, J.; Quinterios, P.; Iglesias, R.; Santos, M.; Sanmartin, M.L. Helminth parasites in some Spanish bats. *Wiad. Parazytol.* **1991**, *37*(3), 321–329.
38. Shimalov, V.V.; Demyanchik, M.G.; Demyanchik, V.T. The helminth fauna of bats (Microchiroptera) in the Republic of Belarus. *Proc. Nation. Acad. Sci. Belarus. Biol. Ser.* **2011**, *3*, 104–110.
39. Sumer, N.; Yildirimhan, H.S. Helminth parasites of the Whiskered brown bat, *Myotis aurescens* (Kuzyakin, 1935) (Chiroptera: Vespertilionidae) from Turkey. *Acta Zoo. Bulg.* **2018**, *70*(1), 113–116.
40. Horvat, Z.; Cabrilo, B., Paunovic, M., Karapandza, B., Jovanovic, J., Budinski, I., Bjelic Cabrilo, O. Gastrointestinal digeneans (Platyhelminthes: Trematoda) of horseshoe and vesper bats (Chiroptera: Rhinolophidae and Vespertilionidae) in Serbia. *Helminthologia* **2017**, *54*(1), 17–25.
41. Sten'ko, R.P.; Dulitskiy, A.I.; Karpenko, O.V.; Dushevskiy, V.P. Helminth fauna of Crimean chiropterans. *Zool. Zhurn.* **1986**, *65*(8), 1133–1139.
42. Podvyaznaya, I.M. The fine structure of the digestive tract of *Allassogonoporus amphoraeformis* (Trematoda: Allassogonoporidae). *Parazitologiya* **1994**, *28*(5), 403–412.
43. Podvyaznaya, I.M. The fine structure of the male reproductive system and genital atrium of bat parasite *Allassogonoporus amphoraeformis* (Trematoda: Allassogonoporidae). *Parazitologiya* **1996**, *30*(3), 229–235.
44. Demidova, T.N.; Vekhnik, V.P. Trematodes (Trematoda, Monorchidae) of *Myotis brandtii* and *M. mystacinus* (Chiroptera, Vespertilionidae) from the Samarskaya Luka (Russia). *Vestn. Zool.* **2004**, *38*(5), 71–74.
45. Lebedeva, D.I.; Belkin, V.V.; Stanyukovich, M.K.; Bespyatova, L.A.; Bugmyrin, S.V. First records of bat parasites in Karelia. *Transact. Karel. Res. Cent. RAS* **2020**, *8*, 120–125. <https://doi.org/10.17076/bg1142>
46. Gulyaev, V.D.; Orlovskaya, O.M.; Dokuchaev, N.E. Helminths of bats from Magadan region. *Plecotus et al.* **2002**, *5*, 86–92.
47. Kirillov, A.A.; Ruchin, A.B.; Artaev, O.N. Helminths of bats (Chiroptera) from Mordovia. *Bull. Volzhsk. Univ. Tatisch.* **2015**, *4*(19), 319–328.
48. Ruchin, A.B.; Kirillov, A.A.; Chikhlyayev, I.V.; Kirillova, N.Y. *Parasitic worms of land vertebrates of the Mordovia Nature Reserve*. Flora and fauna of reserves; Volume 124; Committee of RAS for the Conservation of Biological Diversity: Moscow, Russia, 2016, pp. 1–72.
49. Kirillov, A.A.; Kirillova, N.Yu.; Chikhlyayev, I.V. *Trematodes of terrestrial vertebrates of the Middle Volga region*; Cassandra: Togliatti, Russia, 2012, pp. 1–329.
50. Kirillov, A.A.; Kirillova, N.Yu.; Vekhnik, V.P. Trematodes (Trematoda) of bats (Chiroptera) from the Middle Volga region. *Parazitologiya* **2012**, *46*(5), 384–413.
51. Kirillova N.Yu., Kirillov A.A., Vekhnik V.P. Comparative analysis of the helminth fauna of *Myotis brandtii* and *Myotis mystacinus* (Chiroptera, Vespertilionidae) in the Samarskaya Luka National Park (Russia). *Nat. Conserv. Res.* **2022**, *7*(3), 1–14. <https://dx.doi.org/10.24189/ncr.2022.026>
52. Kirillova, N.Yu.; Shchenkov, S.V.; Kirillov, A.A.; Ruchin, A.B. Trematodes of genera *Gyrabascus* and *Parabascus* from bats in European Russia: morphology and molecular phylogeny. *Biology* **2022**, *11*, 878. <https://doi.org/10.3390/biology11060878>
53. Kirillova, N.Yu.; Kirillov, A.A.; Vekhnik V.A.; Ruchin A.B. Trematodes of small mammals (Soricomorpha, Erinaceomorpha, Chiroptera, Rodentia) in the Middle-Volga region (European Russia). 2023. Version 1.6. Institute of Ecology of the Volga river basin of Russian Academie of Sciences. Occurrence dataset. <https://doi.org/10.15468/gmt9ct> accessed via GBIF.org on 2023-09-26.
54. Jones, C.; McShea, W.J.; Conroy, M.J.; Kunz, J.H. Capturing mammals. In *Measuring and Monitoring Biological Diversity: Standard Methods for Mammals*; Wilson, D.E., Cole F.R., Nichils J.D., Rudran R., Foster M.S., Eds.; Smithsonian Institution Press: Washington DC, USA, 1996; pp. 115–155.
55. Ivashkin, V.M.; Kontrimavichus, V.L.; Nasarova, N.S. *Methods of the collection and studies of helminths of land mammals*; Nauka: Moscow, Russia, 1971; pp. 3–123.
56. Anikanova, V.S.; Bugmyrin, S.V.; Ieshko, E.P. *Methods of the collection and studies of helminths of small mammals*; Karelian Scientific Center of RAS: Petrozavodsk, Russia, 2007; pp. 3–145.
57. Bush, A.O.; Lafferty, K.D.; Lotz, J.M.; Shostak, A.W. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* **1997**, *83*, 575–583.
58. Magurran, A.E. *Ecological diversity and its measurement*. Mir: Moscow, Russia, 1992, pp. 3–182.
59. Bakanov, A.I. *Quantitated estimation of dominance in ecological communities*. Borok, Russia, 1987, 64 p. The manuscript was deposited in All-Union Institute of Scientific and Technical Information (VINITI) by 08.12.1987. No. 8593–B87.
60. Hammer, O.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. Version 2.16. *Palaeontol. Electron.* **2001**, *4*, 9.
61. Smith, E.P.; van Belle, G. Nonparametric estimation of species richness. *Biometrics* **1984**, *40*, 119–129.

62. Poulin, R. Comparison of three estimators of species richness in parasite component communities. *J. Parasitol.* **1998**, *84*(3), 485–490.
63. Lühe, M. *Parasitische Plattwürmer. I. Trematodes*. Die Süsswasserfauna Deutschlands. Verlag von Gustav Fischer: Jena, Germany, **1909**, *17*, 1–218.
64. Smirnov, D.G.; Vekhnik, V.P. Association of bats to different types of hunting grounds in floodplain ecosystems in the Samarskaya Luka. In *Recent problems of biological research in western Siberia and adjacent territories*; Starikov V.P., Ed.; Timer Publish.: Surgut, Russia, 2011, pp. 98–101.

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