

Microsporidian parasites in malaria mosquitoes (Diptera: Culicidae) from water bodies of the Ob-Irtysh basin, West Siberia, Russia

Микроспоридии малярийных комаров (Diptera: Culicidae) из водоёмов Обь-Иртышского бассейна Западной Сибири в России

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Key words: Microsporidia, malaria mosquitoes, *Parathelohania*, *Anopheles*, water bodies.

Ключевые слова: микроспоридии, малярийные комары, *Parathelohania*, *Anopheles*, водоёмы.

Abstract. This study investigated larval infection of malaria mosquitoes with microsporidia of the genus *Parathelohania* Codreanu, 1966 in water bodies of the Ob-Irtysh basin during the summer seasons of 2020–2022 yy. A total of 4576 larval specimens from 54 water bodies were analyzed. Microsporidian infection was found in three of the four species of malaria mosquitoes recorded in West Siberia, namely: *Anopheles messeae* Falleroni, 1926, *An. daciae* Linton, Nicolescu, Harbach, 2004, and *An. beklemishevi* Stegnii, Kabanova, 1976. Overall infection prevalence (IP) ranged from 0.14–15.78 % and amounted to $2.97 \pm 0.02\%$, with varying IP values for *An. messeae* (IP = $0.78 \pm 0.01\%$), *An. daciae* (IP = $0.81 \pm 0.1\%$), and *Anopheles beklemishevi* (IP = $0.13 \pm 0.06\%$). The highest prevalence was observed in water bodies of the middle (IP = $2.36 \pm 0.02\%$) and upper Ob (IP = $2.28 \pm 0.02\%$) basins, while the lowest prevalence was recorded in the lower Ob basin (IP = $1.63 \pm 0.07\%$), which shows a statistically significant difference between these groups. The prevalence across years remained consistently low with insignificant fluctuations, and epizootic events were extremely rare. This study presents the first report of microsporidian infection in *An. daciae* mosquitoes, with infection prevalence comparable to that observed in *An. messeae*. Ultrastructural analysis identified four microsporidian species of the genus *Parathelohania*, namely: *P. divulgata* Simakova, Pankova, 2004, *P. formosa* Simakova, Pankova, 2004, *P. teguldeti* Simakova, Pankova, 2004, and *P. tomski* Simakova, Pankova, 2004. Our data confirm the previously reported low prevalence of microsporidia of the genus *Parathelohania* in malaria mosquito larvae in West Siberia.

Резюме. Исследована заражённость личинок малярийных комаров микроспоридиями рода *Parathelohania* Codreanu, 1966 в водоёмах Обь-Иртышского бассейна в летний период 2020–2022 гг. Всего обследовано 4576 экземпляров личинок из 54 водоёмов. Выявлено заражение микроспоридиями трёх из четырёх видов малярийных комаров, зарегистрированных в Западной Сибири: *Anopheles messeae* Falleroni, 1926, *An. daciae* Linton, Nicolescu, Harbach, 2004 и *An. beklemishevi* Stegnii, Kabanova, 1976. В целом эктенсивность инвазии личинок комаров колеблется в пределах 0,14–15,78 % и составляет $2,97 \pm 0,02\%$. *An. messeae* (ЭИ = $0,78 \pm 0,01\%$), *An. daciae* (ЭИ = $0,81 \pm 0,1\%$) и

Anopheles beklemishevi (ЭИ = $0,13 \pm 0,06\%$). Наибольшие показатели ЭИ личинок комаров наблюдались в водоёмах бассейна средней (ЭИ = $2,36 \pm 0,02\%$) и верхней Оби (ЭИ = $2,28 \pm 0,02\%$), наименьшие — в водоёмах нижней Оби (ЭИ = $1,63 \pm 0,07\%$), что значимо различается. Показатели заражённости личинок в разные годы стабильно низкие и колеблются в незначительных пределах, эпизоотии наблюдаются крайне редко. По ультарструктуре нами выявлено 4 вида микроспоридий, относящихся к роду *Parathelohania*: *P. divulgata* Simakova, Pankova, 2004, *P. formosa* Simakova, Pankova, 2004, *P. teguldeti* Simakova, Pankova, 2004, и *P. tomski* Simakova, Pankova, 2004. Впервые изучена заражённость микроспоридиями комаров *An. daciae*, показатели ЭИ сопоставимы с *An. messeae*. Наши данные подтверждают предыдущие исследования о низкой заражённости личинок малярийных комаров микроспоридиями рода *Parathelohania* в Западной Сибири.

Introduction

Microsporidia represent a large and diverse group of obligate parasites found in natural populations of mosquitoes of the family Culicidae. Mosquitoes of this family host more than 150 microsporidian species belonging to 25 genera [Simakova, 2013].

To date, four species of malaria mosquitoes have been recorded in West Siberia, namely: *Anopheles messeae* Falleroni, 1926, *An. daciae* Linton, Nicolescu, Harbach, 2004, *An. beklemishevi* Stegnii, Kabanova, 1976 [Vaulin et al., 2018], and *An. claviger* Meigen, 1804 [Redkina, 2008].

Anopheles messeae sensu lato is the most widespread species of malaria mosquitoes in Russia. According to morphological analysis, this species has been recorded across a vast territory in Asian Russia, extending southward up to the southern border of Russia [Malkova et al., 2013].

Anopheles messeae, initially described as a subspecies of *An. maculipennis*, was later regarded as a separate species within the *maculipennis* complex. The

differentiation of *An. messeae* in West Siberia was first considered in 1973 [Stegniy et al., 1973]. Later, based on cytogenetic and population genetic [Novikov, Kabanova, 1979], and molecular genetic [Novikov, Shevchenko, 2001; Nicolescu et al., 2004] differences, a new species, *An. daciae*, was distinguished from *An. messeae*. The molecular marker for identifying *An. daciae* is the ITS2 nucleotide sequence [Nicolescu et al., 2004]. According to available studies, *An. daciae* is the most widespread species of the maculipennis group in central West Siberia, Tomskaya Oblast. However, further north and south of Tomskaya Oblast, its dominance decreases, with *An. messeae* becoming prevalent. This pattern is attributed to the lower tolerance of *An. daciae* to extreme temperature conditions [Vaulin, 2018].

Prior to 1976, *An. beklemishevi* was assigned to Siberian *An. maculipennis* [Stegniy, Kabanova, 1976]. Cytogenetic studies showed no records of *An. maculipennis* in Asian Russia; therefore, all earlier records of this species in West Siberia subsequently have been referred to *An. beklemishevi* [Gornostaeva, Danilov, 2002].

Anopheles claviger was previously found in the northern part of Tomskaya Oblast [Redkina, 2008], and is now regularly recorded in Tomskaya Oblast [Poltoratskaya et al., 2021].

Microsporidia infecting blood-sucking mosquitoes exhibit complex life cycles, including mono-, di-, tri-, or tetramorphic stages with obligatory host rotation. The parasite life cycle can span up to three years [Hazard et al., 1979]. To date, eight microsporidian genera have been found to infect malaria mosquitoes: *Amblyospora* Hazard, Oldacre, 1975, *Anncalitia* Issy, Krylova, Nikolaeva, 1993, *Crepidulospora* Simakova, Pankova, Issy, 2003, Simakova, Pankova, Issy, 2004, *Culicospora* Weiser, 1977, *Duboscqia* Perez, 1908, Hazardia Weiser, 1977, *Parathelohania* Codreanu, 1966, and *Senoma* Tokarev, Issi, 2005 [Simakova, Pankova, 2008]. *Parathelohania* is the most common and species-rich genus, comprising currently 23 species. Parasites of this genus destroy only male larvae of the genus *Anopheles*. They are characterized by one of the most intricate life cycles among microsporidia [Avery, Undeen, 1990].

Research findings suggest that microsporidia can have a substantial impact on malaria mosquito populations, particularly *An. messeae* sensu lato and *An. beklemishevi*. Infection by these parasites affects larval viability, which may lead to a potential decline in mosquito abundance. Microsporidia may function as sterilizes in mosquito populations, causing mortality of all males and infecting subsequent generations. This paves the way for developing promising eco-friendly approaches to biological control of malaria using natural pathogens [Simakova, 2013; Simakova, Pankova, 2008].

In addition, the evidence supports antagonistic relationships between microsporidia and malaria parasites of various species, and the development of the latter is inhibited in microsporidia-infected female mosquitoes [Anthony et al., 1972; Ward, Savage, 1972].

To date, microsporidia are known to infect *An. beklemishevi* and *An. messeae* sensu lato in West Siberia; however, data on infection prevalence in *An. claviger* and *An. daciae* are lacking. Data concerning the distribution of microsporidia and their impact on the survival of individual species of malaria mosquitoes are needed to improve the accuracy and precision of mosquito infection prediction models. This can enhance the efficacy of malaria-control measures by maintaining the balance within the ecosystem inhabited by mosquitoes.

The aim of this study was to assess the prevalence of microsporidian infection among blood-sucking *Anopheles* mosquitoes in water bodies of the Ob-Irtysh basin, West Siberia, Russia.

Materials and Methods

Mosquito larvae were collected from inland and floodplain, permanent and temporary water bodies within the Ob-Irtysh basin (West Siberia, Russia) between 2020 and 2022. A total of 54 water bodies were surveyed. The studies were conducted in the forest-steppe zone (Novosibirskaya Oblast, Kemerovskaya Oblast, and the Altai Republic), the taiga zone (Tomskaya Oblast and Khanty-Mansi Autonomous Okrug), and steppe zone (Altai Republic and Altaiskii Krai) (Fig. 1, Table 1). Water bodies were divided based on their location within the Ob basins: the upper Ob basin (from the confluence of the Biya and Katun rivers to the mouth of the Tom river near Tomsk), the middle Ob basin (from the mouth of the Tom river to the confluence of the Irtysh and Ob rivers), and the lower Ob basin (from the confluence with the Irtysh to the Gulf of Ob) [Semenova, 2001]. Collected material from the upper and middle Ob basins included samples taken from water bodies within the Irtysh basin.

The larvae were examined for external signs of microsporidian infection, and then mechanically dissected into head, thorax, and abdomen.

The thorax was fixed in Carnoy's solution for cytogenetic analysis. The abdomen was placed in a 2.5 % glutaraldehyde solution in cacodylate buffer for electron microscopy, and the head was immersed in 96 % ethanol for molecular genetic analysis.

After dissection, smears were fixed with methanol on glass slides, followed by Romanovsky-Giemsa azure-eosin staining [Voronin, Issi, 1974].

Conventional methods were employed for electron microscopy of larvae [Reynolds, 1963; Weekly, 1975; Undeen and Vavra, 1997]. Sections were prepared using a Leica EM UC7 ultramicrotome (Leica Microsystems GmbH, Germany). Examination was performed using a Talos L120C electron microscope (THERMO FISHER SCIENTIFIC, USA) operating at an accelerating voltage of 80 kV. The dimensions of the structural elements of spores, sporogony stages, and sporophore vesicles were determined from micrographs based on 30 measurements.

Molecular genetic analysis was employed to confirm the identification of microsporidia and mosquito species based on their morphological characteristics.

The species identity of infected larvae of *An. daciae*,

Table 1. Malaria mosquito larvae sampling sites

Таблица 1. Места сбора личинок малярийных комаров

No.	Sampling site locality	Coordinates	Sampling date
1	Berezovo urban-type settl., KhMAD, IO	63°55'46.8" N, 65°03'49" E	5.VIII.2020, 15.VI.2022
2	Priobye settl., KhMAD, IO	62°32'17.1" N, 65°35'15.4" E	4.VIII.2020
3	Priobye settl., KhMAD, IO	62°28'13.2" N, 65°34'20.1" E	18.VI.2021
4	Yaguryakh vill., KhMAD, IO	61°15'17.5" N, 67°40'01.5" E	4.VIII.2020
5	Khanty-Mansiysk city, KhMAD, IO	61°00'31.4" N, 68°59'34.1" E	3.VIII.2020
6	Strezhevoy city, Tomskaya Oblast, mO	60°42'59.5" N, 77°32'27.6" E	24.VII.2020
7	Aleksandrovskoye vill., Tomskaya Oblast, mO	60°25'55.1" N, 77°50'43.8" E	24.VII.2020
8	Novoyugino vill., Tomskaya Oblast, mO	58°59'11.3" N, 80°33'57.1" E	18.VII.2020
9	Maloye Nesterovo vill., Tomskaya Oblast, mO	58°40'13.4" N, 81°34'57.6" E	18.VII.2020
10	Togur vill., Tomskaya Oblast, mO	58°22'10.8" N, 82°51'06.0" E	3.VII.2020, 6.VII.2022
11	Priirtyshsky settl., Tomskaya Oblast, mO	58°09'50.4" N, 68°19'44.9" E	2.VIII.2020
12	Chazhemto vill., Tomskaya Oblast, mO	58°04'18.7" N, 82°50'38.4" E	18.VII.2020
13	Bolshaya Sarovka settl., Tomskaya Oblast, mO	58°06'09.7" N, 83°13'55.4" E	6.VII.2022
14	Maikovo vill., Tomskaya Oblast, mO	57°38'31.9" N, 83°38'13.6" E	18.VII.2020
15	Krivosheino vill., (water body A), Tomskaya Oblast, mO	57°21'11.5" N, 83°56'14.1" E	18.VII.2020
16	Krivosheino vill., (water body B), Tomskaya Oblast, mO	57°20'30.7" N, 83°56'26.9" E	18.VII.2020
17	Bakchar vill., Tomskaya Oblast, mO	57°01'59.6" N, 82°04'19.1" E	19.VI.2020
18	Starosaynikovo vill., Tomskaya Oblast, mO	57°05'48.2" N, 83°52'22.9" E	19.VI.2020
19	Zharkovka vill., Tomskaya Oblast, mO	56°51'50.6" N, 83°55'50.4" E	18.VII.2020
20	Yekaterininskoye vill., Omskaya Oblast, mO	56°52'46.4" N, 74°33'40.3" E	24.VII.2022
21	Verkh-Tarka vill., Novosibirskaya Oblast, uO	56°37'06.8" N, 77°31'10.9" E	19.VII.2022
22	Kyshtovka vill., Novosibirskaya Oblast, uO	56°33'03.0" N, 76°38'02.6" E	19.VII.2022
23	Severnoye vill., Novosibirskaya Oblast, uO	56°21'01.0" N, 78°23'02.0" E	19.VII.2022
24	Vengerovo vill., Novosibirskaya Oblast, uO	55°40'00.5" N, 76°43'47.9" E	19.VII.2022
25	Sargatskoye settl., Omskaya Oblast, uO	55°36'42.4" N, 73°29'30.6" E	24.VII.2022
26	Starogrebenchikovo vill., Novosibirskaya Oblast, uO	55°26'45.1" N, 78°05'07.5" E	20.VII.2022
27	Chany lake Novosibirskaya Oblast, uO	55°18'48.5" N, 76°45'51.2" E	23.VII.2022
28	Ubinskoye vill., Novosibirskaya Oblast, uO	55°17'31.4" N, 79°42'25.0" E	18.VII.2022
29	Chulym city, Novosibirskaya Oblast, uO	55°05'34.2" N, 80°59'10.4" E	18.VII.2022
30	Itkul lake, Novosibirskaya Oblast, uO	55°04'55.4" N, 81°01'37.1" E	18.07.2022
31	Bogdanovka vill., Omsk region, uO	55°01'54.2" N, 74°09'01.4" E	26.VII.2022
32	Yablonevka settl., Novosibirskaya Oblast, uO	54°42'58.1" N, 76°31'27.1" E	21.VII.2022
33	Chistoozerny settl., Novosibirskaya Oblast, uO	54°42'50.4" N, 76°35'50.5" E	21.VII.2022
34	Lyanino vill., Novosibirskaya Oblast, uO	54°22'06.7" N, 78°31'19.0" E	21.VII.2022
35	Bolshoy Atmas vill., Omskaya Oblast, uO	54°03'52.8" N, 74°55'27.9" E	23.VII.2022
36	Zakovryashino vill., Altaiskii Krai, uO	53°53'30.1" N, 81°11'44.2" E	22.VI.2022
37	Bezrukovo vill., Kemerovskaya Oblast, uO	53°46'54.0" N, 87°32'41.3" E	16.VI.2022
38	Road from Fantasia recreation centre to Chulzhan, Kemerovskaya Oblast, uO	53°38'10.5" N, 88°11'40.0" E	16.VI.2022
39	Ilyinka vill., Altaiskii Krai, uO	53°27'36.2" N, 82°20'05.4" E	22.VI.2022
40	Novoaltaisk city, Chesnokovka river, Altaiskii Krai, uO	53°24'20.5" N, 83°56'48.4" E	17.VIII.2020, 22.VI.2022
41	Martynovo vill., Altaiskii Krai, uO	53°14'59.9" N, 85°55'24.6" E	17.VI.2022
42	Verkh-Marushka vill., Altaiskii Krai, uO	52°55'25.9" N, 85°37'47.7" E	22.VI.2022
43	Neninka vill., Altaiskii Krai, uO	52°40'56.0" N, 86°13'41.0" E	10.VII.2022
44	Novikovo vill., Altaiskii Krai, uO	52°37'18.3" N, 85°58'34.8" E	6.VII.2022
45	Rodino vill., Altaiskii Krai, uO	52°29'22.7" N, 80°12'08.1" E	20.VI.2022
46	Aleysk city, Altaiskii Krai, uO	52°28'49.8" N, 82°44'37.9" E	19.VI.2022
47	Chuisky settl., Altaiskii Krai, uO	52°2644'26.4" N, 85°2949'50.4" E	23.VIII.2020
48	Pospelikha vill., Altaiskii Krai, uO	51°58'06.7" N, 81°51'16.9" E	19.VI.2022
49	Novosovetsky settl. (Kormikha river), Altaiskii Krai, uO	51°49'53.9" N, 80°38'42.8" E	20.VI.2022
50	Manzherokskoye lake, Altai Republic, uO	51°49'02.5" N, 85°48'04.5" E	19.VIII.2020
51	Novoegoryevskoye vill., Altaiskii Krai, uO	51°45'23.7" N, 80°52'46.7" E	20.VI.2022
52	Tolgoek vill., Altai Republic, uO	51°33'98.43" N, 86°05'86.49E	29.VI. 2021
53	Shebalino vill., Altai Republic, uO	51°17'17.2" N, 85°40'01.8" E	19.VIII.2020
54	Yustik settl., Altai Republic, uO	50°20'22.2" N, 85°15'49.2" E	22.VIII.2020

Note: water bodies of the upper Ob (uO), middle Ob (mO), and lower Ob (lO) basins.

Примечание: водные объекты верхнего бассейна Оби (uO), среднего бассейна Оби (mO) и нижнего бассейна Оби (lO).

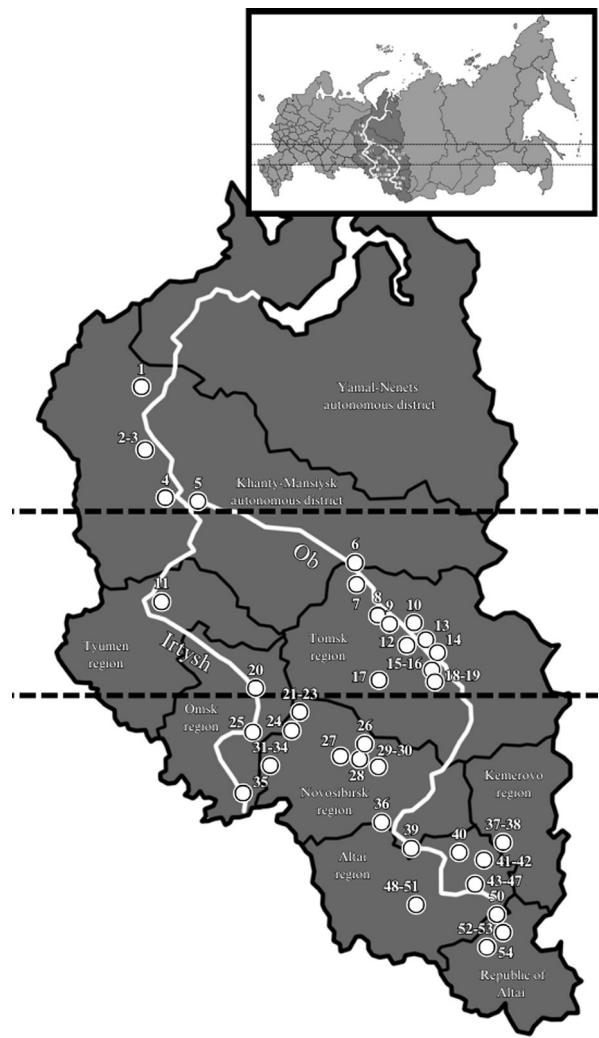


Fig. 1. Locality map showing the sampling sites for *Anopheles* mosquito larvae in the Ob-Irtysh basin. The upper, middle, and lower Ob basins are separated by horizontal dotted lines.

Рис. 1. Карта мест сбора личинок комаров рода *Anopheles* в водоёмах Обь-Иртышского бассейна. Горизонтальными пунктирными линиями разделены бассейны верхней, средней и нижней Оби.

An. messeae, and *An. beklemishevi* was determined using PCR-RFLP analysis of the mosquito ITS2 region. The ITS2 fragment was amplified using primers flanking 5.8S and 28S rDNA, followed by the RsaI restriction endonuclease in accordance with the established protocols [Vaulin et al., 2018; Artemov et al., 2021].

To differentiate between *An. daciae* and *An. messeae*, we employed PCR-RFLP analysis. A 100 ng aliquot of the PCR product containing 1×SE-buffer B (SibEnzyme, Novosibirsk, Russia) and 1 unit of RsaI (SibEnzyme, Novosibirsk, Russia) was added to the mixture. These species differ in the number of restriction sites for the RsaI endonuclease: *An. daciae* has three sites, while *An. messeae* has four sites. Restriction fragment lengths were 10, 47, 71, and 307 bp for *An. daciae*, and 10, 47, 71, 72, and 235 bp for *An. messeae*. The species were differentiated based on the longest ITS2 restriction fragments, which were visualized on a 1.5–2 % agarose gel

[Artemov et al., 2021]. DNA was extracted using the phenol-chloroform method, followed by a standard PCR protocol: 95 °C 6 min 20 cycles (94 °C 30 sec, 51 °C 1 min, 72 °C 1 min), 72 °C 5 min (T100 Thermal Cycler, Bio-Rad, USA).

The presence of microsporidia was confirmed using a standard PCR protocol: 94 °C 3 min 35 cycles (94 °C 45 sec, 45 °C 30 sec, and 72 °C 90 sec) [Andreadis, 2012]. The following primers were used for the reaction: 1492r (5'-GGTTACCTTGTACGACTT-3') and 18f (5'-CACAGGTTGATTCTGCC-3') [Andreadis, 2012].

The present work is registered in ZooBank (www.zoobank.org) under urn:lsid:zoobank.org:pub:4C3A6D50-3A5D-428D-B8E4-AE-3931A4491C.

Results

A total of 4576 larval specimens were collected, of which 89 specimens were found to be microsporidia-infected. Microsporidian spores were isolated from 40 mosquito larvae, and electron micrographs (40 images) were made to analyze spore morphology and classify them to genus and species based on ultrastructural analysis.

All identified species of malaria mosquitoes recorded in the study area (*An. messeae*, *An. daciae*, and *An. beklemishevi*) were found to be infected with microsporidia. The ultrastructural analysis showed that all detected microsporidia belong to the genus *Parathelohania*.

The overall prevalence of microsporidia of the genus *Parathelohania* in malaria mosquito larvae was low and amounted to $2.97 \pm 0.02\%$. The species with the highest prevalence were *Anopheles daciae* and *An. messeae* (IP = $0.81 \pm 0.01\%$ and $0.78 \pm 0.01\%$, respectively), while the prevalence in *An. beklemishevi* differed significantly ($0.13 \pm 0.06\%$) (Fig. 2).

Comparative analysis of infection prevalence across different areas revealed the highest number of microsporidia-infected mosquito larvae in water bodies of the middle (IP = $2.36 \pm 0.02\%$) and upper Ob (IP = $2.28 \pm 0.02\%$) basins, while the lowest number was recorded in the lower Ob basin (IP = $1.63 \pm 0.07\%$). This shows its statistically significant difference from infection prevalence in water bodies of the middle and upper Ob (non-overlapping confidence intervals) (Fig. 3).

The highest number of microsporidia-infected larvae was recorded in water bodies of the middle Ob basin, which corresponded to the region with the highest number of larvae analyzed. The lowest number was recorded in water bodies of the lower Ob basin, where only *An. messeae* and *An. daciae* were found to be infected, with no microsporidia-infected larvae of *An. beklemishevi*.

No statistically significant differences in infection prevalence were observed across different water bodies (IP = $2.29 \pm 0.34\%$ in permanent floodplain water bodies, IP = $2.28 \pm 0.32\%$ in permanent inland water bodies, IP = $2.21 \pm 0.77\%$ in temporary water bodies, and IP = $2.22 \pm 0.86\%$ in river backwaters).

Study of the long-term dynamics of malar-

ia mosquito larval infection revealed that infection prevalence across years fluctuated insignificantly. In the middle Ob basin, water bodies exhibited minor fluctuations, ranging from 2.2 % to 3.5 % ($IP_{2022} = 2.39 \pm 0.93 \%$, $IP_{2022} = 3.21 \pm 0.24 \%$). In the upper Ob basin, infection prevalence ranged from 2.2 % to 2.6 % ($IP_{2022} = 2.37 \pm 0.3 \%$, $IP_{2022} = 2.5 \pm 6.09 \%$, $IP_{2022} = 2.31 \pm 0.35 \%$). The lowest prevalence was recorded in the lower Ob basin, ranging between 0.14 % and 0.57 % ($IP_{2022} = 0.54 \pm 0.99 \%$, $IP_{2022} = 3.6 \pm 0.84 \%$). The highest prevalence ($IP = 15.78 \pm 0.98 \%$) was solely observed in specimens collected in 2021 from the lower Ob basin (water body No.3), while the lowest prevalence ($IP = 0.14 \%$) was recorded in specimens collected in 2020 from the lower Ob basin (water body No.1).

The ultrastructural analysis revealed four microsporidian species, namely: *Parathelohania divulgata* Simakova, Pankova, 2004, *P. formosa* Simakova, Pankova, 2004, *P. teguldeti* Simakova, Pankova, 2004, and *P. tomski* Simakova, Pankova, 2004.

Thus, infection prevalence in malaria mosquito larvae within the Ob-Irtysh basin was low, and epizootic events were extremely rare. All infected larvae harbored parasites of the genus *Parathelohania*.

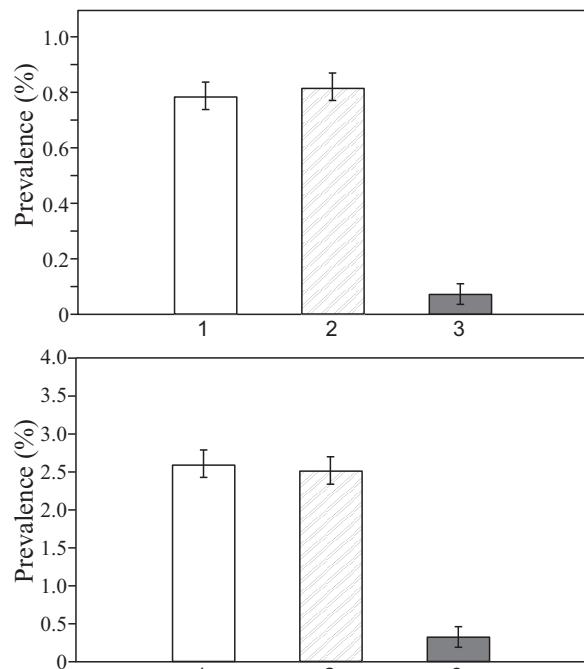
Discussion

To date, no large-scale study on the prevalence of microsporidian infection in malaria mosquitoes has been conducted in West Siberia. Previous studies were limited to Tomskaya and Kemerovskaya Oblasts (water bodies of the middle and upper Ob) [Simakova, Pankova, 2004, 2005; Simakova, 2013]. We investigated the prevalence of microsporidian infection in *Anopheles* mosquito larvae across different water bodies of the Ob-Irtysh basin.

The study of *Parathelohania* microsporidian infection in malaria mosquitoes in water bodies began in 1978. At that time, infection prevalence reached a peak value of 62 %. Subsequently, in 1979, the prevalence decreased sharply to 17.1 % and did not exceed 3–4 % until 1988 [Simakova, Pankova, 2008]. From 1992 to 2013, infection prevalence remained low, ranging from 0.1 % to 2.6 %, and no epizootic events were recorded [Simakova, Pankova, 2008; Simakova, 2013].

The study conducted between 2020 and 2022 also recorded low infection prevalence in malaria mosquito larvae (an average of $2.97 \pm 0.02 \%$). These data are consistent with findings reported in previous studies. The lowest prevalence was observed in previously unstudied water bodies of the lower Ob basin. The reduced prevalence in the lower Ob basin may be associated with lower water temperatures, which are unfavorable for microsporidia development.

Thus, mosquito abundance in the studied water bodies fluctuates and occasionally reaches considerable levels, while infection prevalence exhibits minimal variation in nearly all cases. Epizootic events are extremely rare and are likely stochastic in nature.



Figs 2–3. Prevalence of microsporidian infection in malaria mosquitoes in water bodies of the Ob-Irtysh basin during 2020–2022. 2 — infection prevalence in different mosquito species, designations: 1 — *An. messeae*, 2 — *An. daciae*, 3 — *An. beklemishevi*; 3 — infection prevalence in different areas, designations: 1 — upper Ob, 2 — middle Ob, 3 — lower Ob.

Рис. 2–3. Экстенсивность инвазии малярийных комаров микроспоридиями в водоёмах Обь-Иртышского бассейна в 2020–2022 г. 2 — заражённость разных видов комаров, обозначения: 1 — *An. messeae*, 2 — *An. daciae*, 3 — *An. beklemishevi*; 3 — заражённость комаров в разных районах, обозначения: 1 — верхняя Обь, 2 — средняя Обь, 3 — нижняя Обь.

This study presents the first report of microsporidian infection in *An. daciae*, which shows that its prevalence does not differ significantly from that in *An. messeae*. A narrow host specificity of microsporidia infecting mosquitoes may indicate a close phylogenetic relationship between these two malaria mosquito species.

Previous studies showed that *Anopheles* mosquitoes in West Siberia are typically infected with four microsporidian genera: *Amblyospora*, *Crepidulospora*, *Parathelohania*, and *Senoma* [Simakova et al., 2003; Simakova, Pankova, 2004; Simakova et al., 2005; Simakova, Pankova, 2008]. Among these, microsporidia of the genus *Parathelohania* were reported as the most common in *Anopheles* mosquitoes. A total of 23 species of this genus have been described in literature. Six species were recorded in the middle Ob basin: *Parathelohania divulgata* Simakova, Pankova, 2004, *P. formosa* Simakova, Pankova, 2004, *P. illinoiensis* var. *messeae* [Pankova et al., 1991], *P. sibirika* Simakova, Pankova, 2004, *P. teguldeti* Simakova, Pankova, 2004, and *P. tomski* Simakova, Pankova, 2004 [Simakova, Pankova, 2008]. The ultrastructural analysis revealed four *Parathelohania* species in *Anopheles* mosquitoes, underscoring a broad distribution of this genus among malaria vectors in the study area. The detected species are endemic to the study area. All microsporidia exhib-

ited morphological criteria characteristic of this genus, which is evidenced by literature data. *Parathelohania* infecting malaria mosquitoes is reported to be the most prevalent and dominant microsporidian genus in permanent water bodies in this area [Pankova et al., 1996; Simakova, Pankova, 2008].

Thus, the obtained data confirm and extend previous data on microsporidian infection in malaria mosquitoes. The low prevalence and a complex life cycle of *Parathelohania* microsporidia hosting *Anopheles* mosquitoes suggest a long-term co-evolution of these organisms. This stable host-parasite association is evidenced by low infection prevalence observed in natural populations of malaria mosquito larvae.

Conclusions

The overall prevalence in mosquito larvae ranged from 0.14 % to 15.78 %, with an average of 2.97 ± 0.02 %. Three mosquito species were found to be infected with microsporidia: *An. messeae* (IP = 0.78 ± 0.01 %), *An. daciae* (IP = 0.81 ± 0.01 %), and *An. beklemishevi* (IP = 0.13 ± 0.06 %).

The highest prevalence was observed in water bodies of the middle ($IP = 2.36 \pm 0.02$ %) and upper Ob ($IP = 2.28 \pm 0.02$ %) basins, while the lowest prevalence was found in the lower Ob basin ($IP = 1.63 \pm 0.07$ %), which shows a statistically significant difference between these groups.

The ultrastructural analysis showed that all identified microsporidia infecting malaria mosquitoes belong to the genus *Parathelohania*.

The prevalence of microsporidian infection in mosquito larvae remained low across different years, fluctuating within a narrow range, and epizootic events were extremely rare.

Microsporidian infection in *An. daciae* has been investigated for the first time, indicating that its prevalence is comparable to that in *An. messeae*.

Our data confirm the previously reported low prevalence of *Parathelohania* microsporidia in malaria mosquito larvae in West Siberia.

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