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Ecological aspects of microsporidia parasitizing in natural populations of the *Aedes* (Diptera: Culicidae) blood-suking mosquitoes in Western Siberia

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We examined microsporidia (1976-2014) in natural populations of blood-sucking Aedes mosquitoes of Western Siberia with the focus on their biodiversity and ecology. In total, we recorded 31 species of Culicidae family mosquitoes in Western Siberia; 22 species of them belong to the univoltine genus Aedes, producing one generation per year (spring). As it has been shown, microsporidians infect 13 mosquito species. In this study, we isolated 26 microsporidian species representing five genera (Amblyospora, Andreanna, Dimeiospora, Novothelohania, and Trichoctosporea) from mosquitoes; of them, the Amblyospora species were the most abundant. The majority of species display a high level of host specificity. Microsporidians are found in all types of aquatic habitats; however, the highest diversity of parasites was observed in mainland temporary ponds. During the period of study, the prevalence rates of microsporidians infecting Aedes mosquitoes varied from 0.05 to100%, with the maximal parasite prevalence during mass mosquito pupation and imago emergence (mid-May - beginning of June). Low parasite loads (0.05-6.5%) are characteristic of the last 20 years.

Key words: *Aedes; Amblyospora;* microsporidia; blood-sucking mosquitoes; ecology; epizootiology; host specificity.

Introduction

The intracellular parasites of a Microsporidia type are abundant in the blood-sucking mosquitoes of the family Culicidae. These parasites are the most diverse in the mosquitoes belonging to the genus *Aedes* Meigen. Currently, 14 genera and over 100 species of microsporidians in these mosquitoes have been described, being the largest number as compared with other mosquito genera [1-6]. Representatives of some genera of these parasites have complex mono-, di-, tri, and tetramorphic life cycles with changing hosts.

Microsporidians are observed in the populations of blood-sucking mosquitoes at rather low abundance but are able to cause the disease outbreaks affecting a large number of individuals in the population. The infection rate of natural populations varies depending on the specific features of the parasite-host system itself, particular biotope, season and year.

The insight into epizootics in natural populations of blood-sucking mosquitoes is of great practical importance, making it possible to assess the role of the disease agent in the control of mosquito populations and giving the background for elaboration of the corresponding control methods. Many mosquito species of the genus *Aedes* are vectors of dangerous diseases. Correspondingly, the study of antagonistic interactions between microsporidians and various pathogens of medicinal and veterinarian importance that stop or slow down their development in the female mosquitoes invaded by microsporidians is also of great practical value.

This work summarizes the long-term studies (1976-2014) of the species composition of Microsporidia, their host specificity, as well as seasonal and long-term rates of microsporidian infection of the *Aedes* mosquitoes in Western Siberia.

Materials and methods

Microsporidians from the univoltine *Aedes* mosquitoes were studied in Western Siberia (Russia) during 1976-2014. Western Siberia has an area of about 2.5 million km, spanning from north to south for almost 3000 km.

The studies were performed in the taiga landscape zone (middle and southern subzones, Tomsk oblast), forest-steppe zone (aspen-birch forests and forest-steppe of Novosibirsk and Kemerovo oblasts), and the Salair mountain area near Kopna Mountain (altitude, 509 m, Kemerovo oblast).

The work over this period comprised stationary observations and surveys at over 200 water bodies of various types (mainland, floodplain, constant, temporary, open, closed, natural, artificial, formed by snow melting, meadow and forest bogs, as well as sphagnum, raised and reed bogs). The long-term annual microsporidian infection prevalence in mosquitoes was monitored in several model water bodies.

The mosquito larvae were harvested according to Pavlovskii (1935) [7]. The samples in water bodies with a circumference up to 200 m were taken every 10 m with a dip net. In water bodies with a shore length of 200-1000 m, sites differing in their vegetation and illumination were examined. The surveys were performed every 5 days over the entire season. Concurrently, copepods were collected in water habitats.

The larvae of blood-sucking mosquitoes were identified according to their morphological characters [8] and with the help of molecular genetic methods [5].

The collected larvae and copepods were examined in the laboratory against a dark background using an “MBS-10” (Lytkarino Optical Glass Factory, Russia) or an “MSP-1” (Leningrad Optical Mechanical Association, Russia) microscope (20×) to detect the individuals infected with microsporidians.

The species composition of Microsporidia was studied using standard procedures of light and electron microscopies as well as molecular genetic tools [4, 9].

Results

1.1. Species compositions of parasites and insect hosts. In total, 45 species of *Aedes* mosquitoes have been recorded in Siberia [10]. A systematic and intensive study of the blood-sucking mosquito fauna and ecology in the Tomsk Ob River area commenced in the 1970s-1980s [11-14]. The list of blood-sucking mosquitoes of that time comprised 38 species and eight subspecies belonging to five genera (*Anopheles* Meigen*, Aedes, Culex* L.*, Culiseta* Felt*,* and *Mansonia* Dyar), the majority of species (25 species) being the members of the genus *Aedes* [14].

The data of the last decade demonstrate that the taiga zone (Tomsk oblast) houses 31 mosquito species of the family Culicidae; of them, 22 species belong to the univoltine genus *Aedes,* which produced one generation a year and develop during the spring season [15]. The forest-steppe zone of Western Siberia (Novosibirsk oblast) houses 20 species of the *Aedes* mosquitoes [16], most of which are ubiquitous in Western Siberia.

Microsporidians were detected in 13 *Aedes* species (Table 1).

Table 1

Microsporidian species from univoltine *Aedes* mosquitoes in Western Siberia

Microsporidian species, author	Mosquito species, author
1. *Amblyospora baritia* Andreadis et al., 2012	*Aedes cataphylla* Dyar, 1916
2. *Amblyospora bogashovia* Andreadis et al., 2012	*Aedes excrucians* Walker, 1856
3. *Amblyospora caspius* Pankova, Issi et Simakova, 2000	*Aedes caspius* Pallas, 1771; *Aedes communis* De Geer, 1776
4. *Amblyospora chulymia* Andreadis et al., 2012	*Aedes caspius*
5. *Amblyospora flavescens* Simakova et Pankova, 2005	*Aedes flavescens* Muller, 1764; *Aedes diantaeus* Howard, Dyar et Knab, 1912
6. *Amblyospora hristinia* Andreadis et al., 2012	*Aedes communis*
7. *Amblyospora jurginia* Andreadis et al., 2012	*Aedes excrucians*
8. *Amblyospora kolarovi* Simakova et Pankova, 2005	*Aedes communis*, *Aedes punctor* Kirby, 1837
9. *Amblyospora mavlukevia* Andreadis et al., 2012	*Aedes cinereus* Meigen, 1818
10. *Amblyospora orbiculata* Simakova et Pankova, 2005	*Aedes diantaeus*
11. *Amblyospora rugosa* Simakova et Pankova, 2005	*Aedes euedes* Howard, Dyar et Knab, 1912*;* *Aedes cataphylla;* *Acanthocyclops venustus* Norman, Scott, 1906
12. *Amblyospora salairia* Andreadis et al., 2012	*Aedes cinereus*
13. *Amblyospora severinia* Andreadis et al., 2012	*Aedes excrucians*
14. *Amblyospora shegaria* Andreadis et al., 2012	*Aedes cinereus*
15. *Amblyospora timirasia* Andreadis et al., 2012	*Aedes cinereus*
16. *Amblyospora urski* Simakova et Pankova, 2005	*Aedes communis*
17. *Amblyospora* sp. 1	*Aedes cantans* Meigen, 1818
18. *Amblyospora* sp. 2	*Aedes cyprius* Ludlow, 1919
19. *Amblyospora* sp. 3	*Aedes hexodontus* Dyar, 1916

Table 1 (end)

Microsporidian species, author	Mosquito species, author
20. *Amblyospora* sp. 4	*Aedes intrudens* Dyar, 1906
21. *Amblyospora* sp. 5	*Aedes leucomelas* Meigen, 1804
22. *Andreanna caspii* Simakova, Vossbrinck et Andreadis, 2008	*Aedes caspius*
23. *Dimeiospora palustris* Simakova, Pankova et Issi 2003	*Aedes punctor*
24. *Novothelohania ovalae* Andreadis et al., 2012	*Aedes caspius*
25. *Trichoctosporea colorata* Simakova et Pankova, 2004	*Aedes euedes*, *Aedes punctor*, *Aedes cinereus*
26. *Trichoctosporea pygopellita* Larsson, 1994	*Aedes flavescens; Aedes excrucians; Aedes cyprius* Ludlow, 1919; *Acanthocyclops reduculus* Chappuis, 1925

Note: The mosquito species infected with several microsporidian species are underlined.

In total, 26 parasite species belonging to five genera were isolated from mosquitoes, namely, the genera *Amblyospora* Hazard et Oldacre, 1975; *Andreanna* Simakova et al., 2008; *Dimeiospora* Simakova, Pankova et Issi, 2003; *Novothelohania* Andreadis et al, 2012 and *Trichoctosporea* Larsson, 1994 (Fig. 1). Note that 20 microsporidian species and three genera were regarded as new for science (Table 1), [4, 5, 17-20]; and five microsporidian specimens were not identified to the level of species. The largest number of species belongs to the genus *Amblyospora* (16 identified and five unidentified species).

1.2. Host specificity. Microsporidians were recorded in mass (*Aedes cinereus, Ae. communis, Ae. diantaeus,* and *Ae. punctor*) and abundant (*Ae. excrucians*) mosquito species as well as in common (*Ae. cantans, Ae. flavescens,* and *Ae. cyprius*) and low-abundance species (*Ae. caspius, Ae. cataphylla, Ae. euedes, Ae. leucomelas,* and *Ae. hexodontus*). Many mosquito species carry several microsporidian species as parasites, namely, seven species were recorded in *Ae. excrucians,* five in *Ae. cinereus,* three in *Ae. caspius* (three species)*,* and two in *Ae. diantaeus* (Table 1) [5, 17, 19, 20].

Our studies have shown that the 15 microsporidian species detected in the *Aedes* mosquitoes infect only one host mosquito species each and six microsporidian species have several insect host species, namely, *Amblyospora caspius*, *A. flavescens*, *A. kolarovi, A. rugosa, Trichoctosporea colorata,* and *T. pygopellita* (Table 1) [17, 19, 20].

1.3. The microsporidian infection rate in mosquitoes in different biotopes. According to our long-term studies, the mosquito larvae are most abundant in small mainland temporary ponds of natural (bogginess and depressions filled with spring waters) and artificial (pits and roadside ditches) origins. The abundance of larvae in these water bodies varies in the range of 2-57 individuals/m^2 and 4-50 individuals/m^2 in the floodplain water bodies versus a considerably lower abundance, 7-18 individuals/m^2, in the constant mainland water bodies.

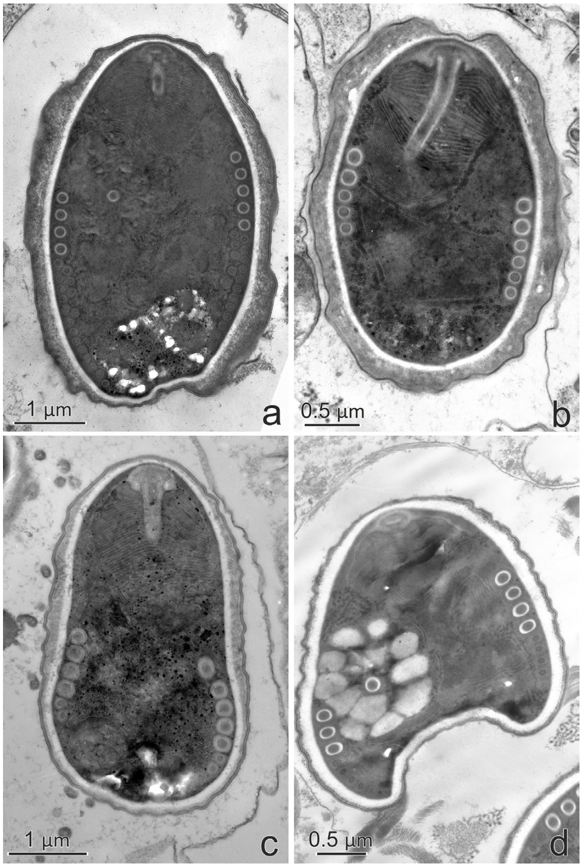

Fig. 1. Ultrastructural morphology of the meiospores of the microsporidian genera isolated from *Aedes* mosquitoes:
a - *Amblyospora*, b - *Andreanna,* c - *Novothelohania,* d - *Trichoctosporea*
(Photos are taken by AV Simakova, AA Miller authors)

Microsporidians are present in all types of water bodies in Western Siberia, being the most diverse (22 species) in the mainland temporary ponds (forest and meadow bogginess after snow melting, sphagnum bogs, and so on), since characteristic of these habitats is the mass emergence of *Aedes* mosquitoes, the major host for these parasites.

The study of distribution of the *Amblyospora* species over the territory of Western Siberia has shown that the species *A. kolarovi, A. rugosa,* and *A. severinia,* parasitizing a single or several host species and inhabiting various types of water bodies, have a wide distribution range. Characteristic of the remaining species is a local distribution.

1.4. Seasonal distribution of the *Amblyospora* **microsporidians in natural** ***Aedes*** **mosquito populations**. The *Aedes* larvae displaying evident signs of microsporidian infection were recorded from the first molts to the third-fourth instar (first 5-day period in May) and to the end of the larval season (late May-early June). An increase in the infection extensity was proportional to the increase in the number of larvae and pupae. By the end of May, when the healthy larvae massively pupate and imagoes massively emerge, the rate of infected larvae increases, while the share of the fourth instar larvae decreases. This is explainable by a delay in the development of infected individuals and their accumulation in water bodies.

The data of annual studies at the raised sphagnum bog near the village of Kolarovo (56º19'52.19" N, 84º58'18.74" E) in 1979-1984 demonstrate that the first larvae of the fourth instar appear during the first 5-day period of May concurrently with the first recorded infected larvae. The infection rate in mosquito population at that time is low (not exceeding 1%). Then the rate of healthy larvae decreases, whereas the number of infected larvae grows. In the fifth 5-day period of May, the number of larvae drastically decreases because of mass pupation of healthy larvae and emergence of imagoes, while the number of infected individuals in the population drastically increases (Fig. 2).

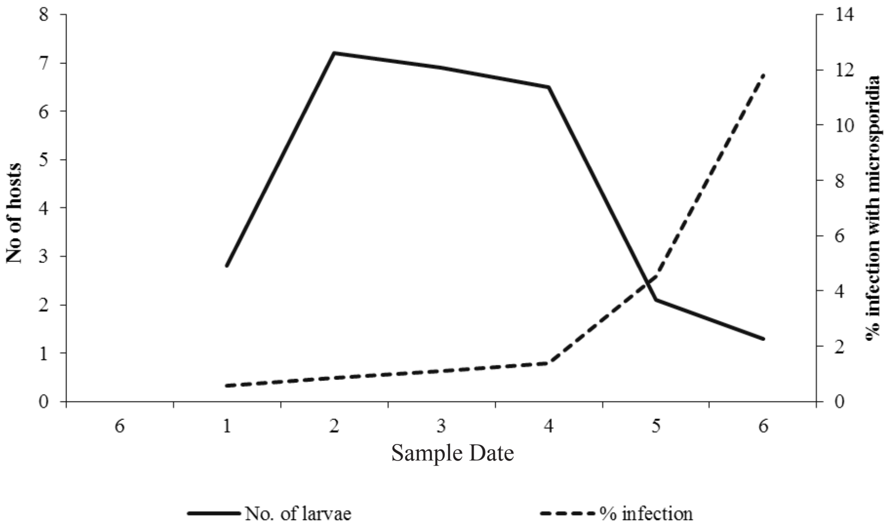

Fig. 2. Seasonal abundance of *Aedes* mosquitoes and prevalence of *Amblyospora* infection: 1 - Abundance of larvae, 2 - Microsporidian prevalence rates.

1.5. Long-term data on the rate of microsporidian infection in natural mosquito populations. The annual monitoring of the microsporidian infection prevalence in *Aedes* mosquitoes in 1976-2014 in the raised sphagnum bog near the village of Kolarovo demonstrated the following patterns. A large focus of microsporidian infection was observed in 1976: almost 100% of the larvae died as a result of this outbreak. In 1977-1978, a decrease in the infection rate was observed followed by an increase in 1979-1984 (Fig. 3).

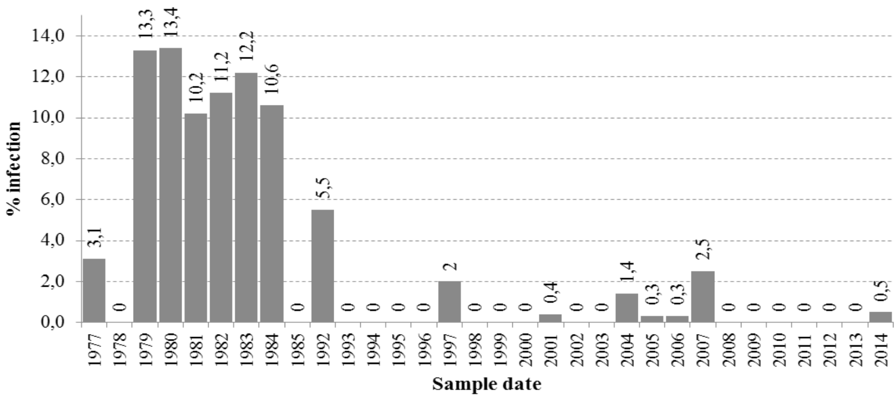

Fig. 3. Yearly prevalence of microsporidian infections in the *Aedes* larval populations in the raised sphagnum bog near the village of Kolarovo, Tomsk raion, Tomsk oblast, Russia, 1977-2014

In the subsequent years, no outbreaks of the disease were observed and the infection prevalence in mosquito larvae in this bog did not exceed tenths of a percent (Fig. 3). The *Ae. punctor* and *Ae. communis* were predominantly infected; *Ae. euedes, Ae. flavescens, Ae. diantaeus,* and *Ae. cinereus* were infected to a lesser degree; and the *Ae. cantans, Ae. cataphylla, Ae. excrucians,* and *Ae. intrudens* larvae, rarely. The main parasitic species were *Amblyospora flavescens*, *A. kazankia*, *A. kolarovi*, and *A. orbiculata*.

The observations of the *Aedes* mosquito larvae in temporary ponds formed by melting snow (Universitetskaya Roshcha of Tomsk State University, Tomsk; 56°28'12.78" N, 84°56'59.98" E) succeeded in detecting a large focus of microsporidian infection in 1994. By the end of May, the infection rate of the fourth instar larvae reached 40%. The *Ae. caspius* and *Ae. communis* mosquitoes were similarly infected with the *Amblyospora caspius.* The larval infection rate in this water body in 1995 amounted to 19%; in 1996, to only 1.9%; and no infected larvae were recorded during several subsequent years. A few infected larvae were found only in 2005 (infection extensity, 0.05%). In 2006-2014, any infected larvae were undetectable.

In 2002, a focus of the microsporidian infection was recorded in a constant mainland pond in the city of Tomsk (56°30'6.09" N, 85°2'0.23" E); the larval infection rate there during mass pupation reached 25%. The *Ae. excrucians* and *Ae. flavescens* larvae were mainly infected and the *Ae. euedes* and *Ae. punctor* larvae, to a lesser degree. The parasites were identified only to the level of genus and belonged to *Amblyospora.*

No mass epizootics of microsporidian infection in the larvae of univoltine *Aedes* mosquitoes were recorded in the remaining water bodies of the examined territory. The infection extensity in the natural mosquito populations was at a stably low level, amounting to 0.05-6.5% in different water bodies. The infection rate observed in the fourth instar larvae during the second week of May was 0.05-0.4% elevating to 1.2-6.5% by the end of May - beginning of June.

Thus, the long-term monitoring of the microsporidian infection prevalence in *Aedes* mosquito larvae in natural populations of Western Siberia demonstrates a significant variation in infection extensity from 0.05 to 100%.

Summing up the results, we may state that the microsporidians - parasites of the univoltine *Aedes* mosquitoes - mainly cause enzootics rather than epizootics (which are very rare) in the natural populations of blood-sucking mosquitoes in Western Siberia.

1.6. The specific features in the life cycles of *Amblyospora* and *Trichoctosporea* microsporidians. The microsporidians belonging to the genera *Amblyospora* and *Trichoctosporea* are polymorphic and heteroxenous parasites. The sequencing data for the small ribosome subunit rRNA of the microsporidians isolated from mosquitoes and copepods as well as numerous ecological observations suggest that the *Amblyospora* and *Trichoctosporea* microsporidians in Western Siberia have complex trimorphic life cycles with the univoltine *Aedes* mosquitoes as the definitive host and the *Acanthocyclops* (Norman et Scott) copepods as a potential intermediate host [21].

Our study [21] of the life cycles of *Amblyospora* microsporidians completely confirmed the data by other researchers for several other species of the same genus [3, 22-31]. In Western Siberia, the copepods inhabiting temporary water bodies, which were infected by meiospores from mosquito larvae, emerge from diapause by the end of April. The parasites there start to develop and form spores. Copepods die at the beginning of May (the infection extensity in natural mosquito populations is 0.01-2.5%) [32]. At the same time (early May), the larvae of univoltine *Aedes* mosquitoes emerge, are orally infected with the copepod spores and develop to imagoes. In the female mosquitoes, the parasites undergo sexual process and give diplokaryotic spores for transovarial transmission. The females lay infected eggs over the summer. The mosquito eggs overwinter with the microsporidian meronts.

Next spring, microsporidians form monokaryotic meiospores in the infected male fourth instar larvae; such mosquito larvae die. The healthy larvae pupate and emerge as imagoes (end of May–beginning of June). The meiospores are invasive for copepods, which are still abundant at that time. Copepods are orally infected with meiospores and enter their diapause, carrying meronts of the parasite, to remain dormant until next spring, since the water bodies they inhabit dry up.

Thus, the life cycle of the *Amblyospora* and *Trichoctosporea* parasites is not only synchronized with the life cycles of both hosts (mosquitoes and copepods), but is also adapted to specific ecological features of the region. This allows microsporidians both to exist in populations for rather a long time and to avoid causing epizootics despite their high abundance.

Consequently, a complex life cycle of the *Amblyospora* microsporidians, which guarantee a long-term existence of this host-parasite system, can be regarded as an adaptation providing the preservation of both the parasite and its host. A large number of adaptations of these microsporidians to the development in mosquitoes as well as their uniquely complex life cycles suggest long-term interactions between the parasite and the host established during their coevolution.

Discussion

Intensive research into the microsporidians of blood-sucking mosquitoes, which started in the second half of the last century in all developed countries, was driven by the search for highly pathogenic microorganisms with the potential to control the insects of medical and veterinary importance.

The earlier studies and identification of the octospores microsporidians parasitizing various mosquito species suggested a wide specificity of many species indistinguishable according to their taxonomic characters at the level of light microscopy. However, involvement of electron microscopy data on the developmental stages and mature spores of microsporidians confirmed that in the majority of cases that the microsporidians of blood-sucking mosquitoes displayed narrow host specificity. It was concurrently found out that the microsporidians with a narrow specificity for their definitive host were able to utilize several crustacean species as an intermediate host [30].

We have shown that most microsporidian species from the univoltine *Aedes* mosquitoes display narrow host specificity; however, several microsporidian species can parasitize different mosquito individuals of the same host species [4, 5].

Numerous long-term epizootological studies in Russia, Karelia, Ukraine, Uzbekistan and Azerbaijan, as well as in various regions of North and South Americas [22, 33-46] have demonstrated that microsporidians cause enzootics in larval population of the univoltine and polyvoltine *Aedes* species with a stably low infection rate (several tenths of a percent to 10-20%) during all examined years, whereas epizootics are a very rare event. As has been observed, the infection extensity in natural copepod populations is somewhat higher as compared with the mosquito larval populations.

In Western Siberia, the *Aedes* mosquitoes infected by microsporidians are recorded from the very emergence of the fourth instar larvae and to the end of the larval season (early May to mid-June), i.e., to the emergence of adult mosquitoes. Outbreaks of microsporidian infections were recorded in the 1970s-1980s; at that time, the microsporidian infection prevalence in natural populations of univoltine *Aedes* larvae reached 100%. Most likely, this is explainable by the fact that the larval infection is assessed when the healthy larva undergo mass pupation. Low microsporidian prevalence, varying from 0.05 to 6.5% in different water bodies, is observed in the natural populations of blood-sucking mosquitoes during the last two decades. The major mechanism underlying stabilization of the parasitic system of *Amblyospora* microsporidians-blood-sucking *Aedes* mosquitoes is a complex life cycle of the parasite, which is synchronized with the life cycles of its hosts, blood-sucking mosquitoes and copepods.

Conclusion

These results evidently suggest that microsporidians have no potential as producers of microbiological preparations for controlling blood-sucking mosquitoes. However, it cannot be excluded that some species with less narrow host specificity, which have relatively recently commenced parasitizing mosquitoes, are able to influence the abundance of mosquito populations to a considerably greater degree.

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