Prevalence rates of microsporidia in locusts and grasshoppers in South-Western Russia

Aleksei Gerus*, *Anastasia* Ignatieva, and *Yuri* Tokarev

All-Russian Institute of Plant Protection, Podbelskogo 3, 196608, St. Petersburg, Pushkin, Russia

Abstract. Locusts and grasshoppers are dangerous polyphagous pests of agricultural crops. In the present paper, results of screening of Acridoidea populations in the South-Western Russia for microsporidia infections including locusts *Locusta migratoria*, *Dociostaurus maroccanus*, and *Calliptamus italicus* and grasshoppers *Chorthippus loratus*, *Oedipoda caerulescens*, and *Acrida bicolor,* are presented. Microsporidia prevalence rates were estimated using light microscopy of fresh smears. Out of 179 specimens of *L. migratoria* sampled between 2002 and 2019 in Krasnodar Territory, Astrakhan and Rostov Regions, none was infected with microsporidia. Similarly, 95 specimens of *D. marrocanus* from Krasnodar Territory (2017) and Dagestan Republic (2009) were also negative for microsporidia. Meanwhile, one positive case was detected for *C. italicus* corresponding to 0.5 % for the total amount of 192 exemplars collected from 2002 to 2019 in Krasnodar Territory, Astrakhan and Rostov Regions. As for grasshoppers, all *Ch. loratus* samplings in Krasnodar Territory in 2017-2019 were infected at the prevalence rates of 2.2-15 %, though no infection was found in 40 specimens in Crimea in 2019. In 56 individuals of *O. caerulescens* collected from Rostov Region and Krasnodar Territory, the microsporidia prevalence rate was 1.8 %. Among 96 specimens of *A. bicolor,* none was infected. In total, the microsporidia prevalence rates were higher in grasshoppers as compared to locusts, the difference being statistically significant at $p<0.01$.

1 Introduction

Modern plant protection implies the management of populations of organisms harmful to plants in agricultural ecosystems. Phytosanitary monitoring is among the most important prerequisites for effective pest management. Phytosanitary forecasts must meet the requirements of reliability, quality and accuracy, taking into consideration the effects of regulatory mechanisms as the key prognostic criterion, rather than merely a correction factor [1].

Locusts and grasshoppers (Acridoidea) are the most dangerous polyphagous agricultural pests. Huge territories of floodplain zones for centuries serve as a reservation of migratory locust, Italian locust and grasshoppers dwelling in the natural environment on perennial

*Corresponding author: gerus_13@mail.ru

grasses, roadsides and uncultivated lands. Over the past 20 years, outbreaks of locusts have been reported in the South of Russian Federation and the Republic of Kazakhstan, leading to enormous crop losses of a wide range of cultivated plants [2].

Biological formulations are being developed worldwide to substitute chemical pesticides against Orthoptera. To date, microorganisms of various groups are known as agents of Orthoptera diseases. Microsporidia are parasitic in representatives of all major taxa of Metazoa, as well as in some protists. The vast majority of microsporidia species develop in arthropods, mainly insects. Currently, 18 species of microsporidia are known as parasites of Orthoptera. In recent studies of microsporidia of Orthoptera, five new species have been described, namely *Liebermannia covasacrae* [3] from *Covasacris pallidinota* (Acridoidea, Acrididae), *Encephalitozoon romalae* [4] from *Romalea microptera* (Acridoidea, Romaleidae), *Endoreticulatus poecilimonae* [5] from *Poecilimon thoracicus* (Tettigonoidea, Tettigoniidae), *Liebermannia* sp. [6] from *Chorthippus loratus*, *Microsporidium* sp. [7] from *Gryllus bimaculatus*. One species, *Perezia dichroplusae* from *Dichroplus elongatus* (Acridoidea, Acrididae), has been redefined as *Liebermannia dichroplusae* [8].

However, the representative of the genus *Paranosema* should be recognized as the most promising for the development of microbial formulations to control a number of pests. Discovered in a culture of migratory locusts *Locusta migratoria,* the microsporidium *Paranosema locustae* (Antonospora*, Nosema*) [9] was found able to infect over 120 host species among Orthoptera [10, 11] and to retain infectivity for years [12]. In 1980, formulations were designed and registered in US (Nolo Bait™, Semaspore™) against Orthoptera, remaining the only mean for pest control based on microsporidia [13].

Traditionally, the use of entomopathogens, including those for locust control, is carried out similarly to the application of chemical insecticides, i.e. by treating pests in the active phases of development, without taking into account further circulation of the pathogen in the host insect population [14]. For a long-term decline in numbers, it seems relevant to create long-term infectious foci in the biotopes inhabited by harmful insects, primarily in their reservation sites. This approach can be effective for obligate parasites, well adapted to the permanent presence in host populations. For example, long-term (over 10 years) circulation after introduction in local populations of grasshoppers has been shown for *P. locustae* in Argentina [15].

In one species of insects, there may be several different species of microsporidia. For example, different species of microsporidia can infect larvae of *Loxostege sticticalis,* в том числе *Nosema loxostegi* [16], *Tubulinosema* sp., *Nosema* sp., *Vairimorpha ceranae* [17], *Tubulinosema loxostegi* [18] and *Vairimorpha thomsoni* [19, 20].

Screening of locust and grasshoppers populations for parasitic microorganisms is important for understanding factors and patterns of density dynamics, to reveal novel isolates which may serve as promising producers of microbial formulations and to track previously released pathogens. The goal of the present study is access and compare prevalence rates of microsporidia in locusts and grasshoppers in their natural habitats in South-Western Russia.

2 Materials and Methods

To reveal microsporidia infections causing diseases of Acrididae, screening of populations of locusts and grasshoppers was performed in the South-Western Russia. Insects were caught between 2002 and 2019 by net or by hand in ten sampling sites (Fig. 1).

Locusts of the species *Locusta migratoria*, *Dociostaurus maroccanus,* and *Calliptamus italicus* and grasshoppers of the species *Chorthippus loratus*, *Oedipoda caerulescens*, and *Acrida bicolor* were sampled and maintained under laboratory conditions in cages, fed with foliage of monocotyledonous crops and weeds, including *Zea mais, Triticum durum, Poa pratensis* etc. Insects perished during transportation and maintenance were stored as dried cadavers at ambient temperature.

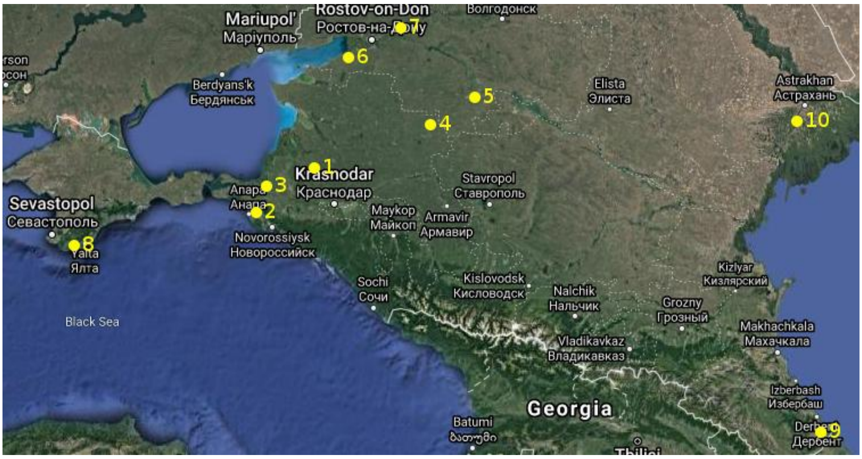

Figure 1. Sampling sites of locusts and grasshoppers: (1) Krasnodar Territory, Slavyansk District; (2) Krasnodar Territory, Anapa District; (3) Krasnodar Territory, Temryuk District; (4) Krasnodar Territory, Beloglinsky District (5) Rostov Region, Salsk District; (6) Rostov Region, Azov District; (7) Rostov Region, Bagaevsky District; (8) Crimea, Yalta District; (9) Dagestan Republic, Derbent District; (10) Astrakhan Region, Kamyzyak District

Live insects were dissected and smears were prepared from midgut, adipose and ovarian tissues. Cadavers were homogenized using mortar and pestle in a drop of water. Microsporidia spores were detected by smear examination under light microscope. Values of microsporidia prevalence rates were compared using Pearson's Chi-square [21].

3 Results and Discussion

Out of 179 specimens of *L. migratoria*, sampled between 2002 and 2019 in Krasnodar Territory, Astrakhan and Rostov Regions, none was infected with microsporidia. Similarly, 95 individuals of *D. marrocanus* from Krasnodar Territory (2017) and Dagestan Republic (2009) were also negative for microsporidia. Meanwhile, one positive case was detected for *C. italicus* in Western part of Krasnodar Territory, corresponding to 5 % (number of examined insects in the local sampling N=20), or 0.5 % for the total amount of 192 exemplars of this species collected from 2002 to 2019 in Krasnodar Territory, Astrakhan and Rostov Regions (Table 1). To summarize, overall prevalence rate of microsporidia in the three locust species reached 0.2 % (N=466, Table 2).

As for grasshoppers, all *Ch. loratus* samplings in Krasnodar Territory in 2017-2019 were infected at the prevalence rates ranging from 2.2 to 15 %. Conversely, *Ch. loratus* from Crimea (2019, N=40) were free from microsporidia infection. In *O. caerulescens* collected from Rostov Region and Krasnodar Territory, the microsporidia prevalence rates were 5 % (N=20) and 0 % (N=36), or 1.8 % in total (N=56). Among 96 specimens of *A. bicolor*, none was infected. Mean prevalence rate of microsporidia of the overall grasshopper dataset was 4 % (N=371, Table 2).

Notably, prevalence rate differences between total locust and grasshopper samplings were statistically significant at $p<0.01$ (Table 2).

Table 1. Microsporidia prevalence rates of locusts and grasshoppers in South-Western Russia

Insect species (Taxonomy)	Sampling site		Sampling date (month, year)	Number of insect examined	Microsporidia prevalence rate, %
	Region	District			
Calliptamus italicus (Catantopinae: Calliptamini)	Astrakhan Region	Kamyzyak	Jun 2002	30	0
			Jun 2004	50	0
	Rostov Region	Azov	Jul 2009	20	0
		Salsk	Aug 2011	36	0
		Bagaevsky	Jun 2019	36	0
	Krasnodar Territory	Slavyansk	Jul 2019	20	5
Locusta migratoria (Acridinae: Locustini)	Astrakhan Region	Kamyzyak	Jun 2002	33	0
	Krasnodar Territory	Beloglinsky	Jun 2017	41	0
	Rostov Region	Bagaevsky	Jun 2019	105	0
Dociostaurus marrocanus (Acridinae: Dociostaurini)	Dagestan Republic	Derbent	2009	45	0
	Krasnodar Territory	Temryuk	2017	50	0
Chorthippus loratus (Acridinae: Gomphocerini)	Krasnodar Territory	Anapa	Sep 2017	61	15*
			Sep 2018	29	7
			Jul 2019	44	4.5
			Aug 2019	45	2.2
	Crimea	Yalta	Sep 2019	40	0
Oedipoda caerulescens (Acridinae: Oedipodini)	Krasnodar Territory	Anapa	July 2019	36	0
	Rostov Region	Bagaevsky	Jun 2019	20	5
Acrida bicolor (Acridinae: Acridini)	Krasnodar Territory	Slavyansk	Aug 2012	30	0
			Jul 2016	20	0
			Jun 2017	20	0
			Aug 2019	26	0
* See [22]					

Table 2. Summarized data of microsporidia prevalence rates of locusts and grasshoppers of South-Western Russia in 2002-2019

Acridoidea species	Insect number		Microsporidia prevalence rate %
	Examined	Infected	
Calliptamus italicus	192	1	0.5
Locusta migratoria	179	0	0
Dociostaurus marrocanus	95	0	0
Locusts, total	466	1	0.2*
Chorthippus loratus	219	14	6.4
Oedipoda caerulescens	56	1	1.8
Acrida bicolor	96	0	0
Grasshoppers, total	371	15	4*
* values are significantly different at p<0.01			

It can be concluded that populations of grasshoppers are infected with mcirosporidia at higher degree as compared to the locust populations. This could be influenced by various environmental factors associated with the properties of both parasites and insect hosts, as well as environmental conditions. In particular, it is known that fever can directly kill the infection in the body of insects and also stimulate the immune system and increase resistance to disease; different types of insects have a certain innate level of resistance to various pathogens; biological properties of the pathogen affect its adaptability to a particular insect species, including virulence, ability to horizontal and vertical transmission, etc. [23,24].

Among the examined species of Acridoidea, the highest level of infection with microsporidia was observed in *Ch. loratus*. This is also noteworthy that insects of this species captured in the summer period were infected to a lesser extent as compared than insects captured in the autumn period. The microsporidia prevalence rate in autumn samples was 2.5 times higher than in summer samples.

Table 3. Summarized data of microsporidia prevalence rates of *Chorthippus loratus* sampled during summer and autumn seasons in 2017-2019

Sampling season	Insect number		Microsporidia prevalence rate %
	Examined	Infected	
Summer	90	3	3.4
Autumn	130	11	8.5

The authors are grateful for the assistance with collection of insects to Bondarev E.S., Khomitskaya L.N. (Russian Agricultural Center), Levchenko M.V., Lednev G.R., Kononchuk A.G., Pogrebnyak S.M., Gerus E.Y. (All-Russian Institute of Plant Protection). The work was financially supported by the Russian Foundation for Basic Research, Grant No. 20-016-00263.

References

1. A.N. Frolov, Plant Protection News, **3**, 4–33 (2019) https://doi.org/10.31993/2308-6459-2019-3(101)-4-33
2. M.G. Sergeev, A. V. Lachininski, *Entomologicheskie issledovaniya v Severnoj Azii.* Novosibirsk, 284-286 (2006)
3. Y.Y. Sokolova et al., J. Invertebr. Pathol., **1**, 34-42 (2009)
4. C.E. Lange et al., J. Parasitol., **4**, 976-986 (2009)
5. D.K. Pilarska et al., J. Invertebr. Pathol., **124**, 23-30 (2015)
6. A.N. Ignatieva et al., J. Euk. Microbiol., **4**, 680-683 (2019)
7. Y.S. Tokarev et al., J. Euk. Microbiol., **3**, 296-307 (2018)
8. Y.Y. Sokolova, C. E. Lange, J. R. Fuxa, J. Euk. Microbiol., **3**, 223-230 (2007)
9. E.U. Canning, Parasitology, **43**, 287-290 (1953)
10. C.E. Lange, J. Orthoptera Res., **2**, 137-141 (2005)
11. C.E. Lange, Biocontrol Sci Technol., **10**, 1047-1054 (2010)
12. J.V. Maddox, L. F. Solter, J. Euk. Microbiol., **3**, 221-225 (1996)
13. J.E. Henry, Protistology, **3** (2017)
14. G.I. Sukhoruchenko, L.A. Burkova, G.P. Ivanova, T.I. Vasilyeva, O.V. Dolzhenko, S.G. Ivanov, V.I. Dolzhenko, Plant Protection News, **1**, 05–24 (2020) https://doi.org/10.31993/2308-6459-2020-103-1-05-24
15. C.E. Lange, F. G. Azzaro, J. Invertebr. Pathol., **3,** 357-359 (2008)
16. I.V. Issi, P.A. Simchuk, D.F. Radischeva, Bulletin of the All-Soviet Institute of Plant Protection, **48**, 3-6 (1980)
17. J.M. Malysh et al., Parasitol. Res., **9**, 3039-3044 (2018)
18. J.M. Malysh et al., Acta Protozool., **4** (2013)
19. Y.M. Malysh et al., Evraziatskij entomologicheskij zhurnal, **6**, 543-548 (2013)

20. J.M. Malysh, A.G. Kononchuk, A.N. Frolov, Plant Protection News, **2**, 45–51 (2019) https://doi.org/10.31993/2308-6459-2019-2(100)-45-51
21. K.Pearson, Philosoph Magaz Ser, **50,** 157–175 (1900) doi:10.1080/14786440009463897
22. A.N. Ignatieva et al, J. Euk. Microbiol., **4,** 680-683 (2019)
23. L.A. Lacey, D. Grzywacz, D.I. Shapiro-Ilan, R. Frutos, M. Brownbridge, M.S. Goettel, J. Invertebr Pathol., **132**, 1–41 (2015). https://doi.org/10.1016/j.jip.2015.07.009
24. M.V. Levchenko, A.G. Kononchuk, A.V. Gerus, G.R. Plant Protection News, **103**