Protistology 13 (1), 14–18 (2019)

Protistology

Rare case of Microsporidia co-infection in the grasshopper *Dichroplus elongatus* (Orthoptera: Acrididae: Melanoplinae)

Carlos E. Lange[1,2] and María Marta Cigliano[1,3]

[1] *Centro de Estudios Parasitológicos y de Vectores (CEPAVE), CCT La Plata CONICET - Universidad Nacional de La Plata (UNLP), Argentina*
[2] *Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Argentina*
[3] *Museo de La Plata, División Entomología, Facultad de Ciencias Naturales, Universidad Nacional de La Plata (UNLP), Argentina*

| Submitted October 24, 2018 | Accepted November 23, 2018 |

Summary

Two Microsporidia species are known to parasitize *Dichroplus elongatus* in the Pampas region of Argentina, allochthonous *Paranosema locustae*, a generalist pathogen of the adipose tissue of grasshoppers with efficient horizontal and vertical transmission routes, and autochthonous *Liebermannia dichropluse*, a *D. elongatus*-specific pathogen of the Malpighian tubules with effective transovarial transmission but unknown horizontal transfer. Long-term monitoring of grasshopper communities for detection of microsporidioses revealed that it is not often that these two pathogens co-exist in populations of *D. elongatus*, even in different individuals. We report the unusual detection for the first time of a co-infection by *P. locustae* and *L. dichroplusae* in *D. elongatus* at a site in the southern Pampas. Given the own traits that characterize both pathogens we presume that the most likely scenario for the occurrence of the observed mixed infection is that the affected *D. elongatus* individual was already infected by *L. dichroplusae* when it contracted infection by *P. locustae*. Unfortunately, our attempts to induce infections with *L. dichroplusae* in the laboratory through experimental inoculations have not produced positive results, preventing efforts towards testing our presumption through laboratory bioassays. However, Microsporidia natural mixed infections appear to be so rare that we believe this case is worth reporting.

Key words: *Liebermannia dichroplusae, Paranosema locustae*, mixed infection, transmission

Introduction

The univoltine and polyphagous melanopline grasshopper *Dichroplus elongatus* is widely distributed in southern South America. It inhabits all of Argentina except the island of Tierra del Fuego at the southernmost tip of the country, Uruguay, much of Chile, and southern Brazil (Cigliano et al., 2014).

doi:10.21685/1680-0826-2019-13-1-2
© 2019 The Author(s)
Protistology © 2019 Protozoological Society Affiliated with RAS

It is particularly common in natural and disrupted grasslands, pastures, and crops in the Pampas region (provinces of Buenos Aires, eastern La Pampa, and southern Córdoba and Santa Fe) where it readily becomes a pest when outbreaks develop (Cigliano et al., 2014; Carbonell et al., 2017). Two species of Microsporidia are known to parasitize *D. elongatus* in the Pampas, allochthonous *Paranosema locustae* and autochthonous *Liebermannia dichropluse* (Sokolova et al., 2007; Bardi et al., 2012). *Paranosema locustae* became naturalized in grasshopper communities after introductions for biocontrol purposes were done between years 1978-1982 (Lange and Cigliano, 2005). *Liebermannia dichroplusae* appears to be endemic to the Pampas since it has never been detected anywhere else outside that region in spite of years of surveys in Argentina. While *P. locustae* infects primarily the adipocytes of the host's fat body tissue, exhibits an extremely broad host range among grasshoppers (at least 124 species are known to be susceptible worldwide; Lange, 2005, 2010, and unpublished observations; Phithalsoun and Zhang, 2018), has both horizontal and vertical transmission (Solter et al., 2012), and causes epizootics (Lange and Cigliano, 2005), *L. dichroplusae* develops in the Malpighian tubules of *D. elongatus*, appears to be host-specific, horizontal transmission has never been achieved, and occurs enzootically (Lange, 1987, 1997, 2003). After nearly thirty years of monitoring only a few sampling sites in the Pampas revealed the coexistence of *P. locustae* and *L. dichroplusae* albeit in different individuals of *D. elongatus* and not at the same time. Hereafter the first detection of a mixed infection (i.e. co-infection) by these two microsporidia is reported.

Material and methods

In early March 2017 a sample of adults of *D. elongatus* (n = 87) was collected with entomological nets in a disrupted grassland close to the intersection of highways 76 and 85 (37°46'33.63"S, 61°42'53.52"W, San Eloy locality, approximately halfway between the towns of Coronel Suárez and Coronel Pringles) in southern Buenos Aires province. Grasshoppers were immediately taken to the laboratory and frozen at -32 °C until processing. Upon thawing, small samples of tissues and organs were examined as wet mounts in one-quarter-strength Ringer's solution (Poinar and Thomas, 1984) under phase contrast microscopy (400×, 1000×) after careful ventral longitudinal dissection of each individual (Lange and Henry, 1996). After examination by dissection each grasshopper was homogenized whole in 5 ml of double distilled water, filtered through cheesecloth, and aliquots of resulting homogenates were further scrutinized under the compound microscope (Plischuk et al., 2013). Intensity of infections was estimated by spore counts *per* individual using an improved Neubauer haemocytometer (Undeen and Vávra, 1997). *P. locustae* and *L. dichroplusae* are readily distinguished by size and shape of spores, type of development stages, tissue-organ tropism, and internal pathological changes (Lange, 1987, 2003; Henry and Oma, 1981).

Although transovarial transmission is a well-established trait of *L. dichroplusae* (Lange, 1997), we still believe that some contribution through horizontal transfer should occur because there is no assistance from the male parent and infections do not seem to impart a selective advantage to the host but are indeed detrimental (Lange, 1987), conditions which are considered as requirements for the maintenance of the pathogen through vertical transmission alone (Fine, 1984). Accordingly, in order to induce horizontally transmitted infections we performed over the years additional oral experimental inoculations to those already reported (Lange, 1989) on all postembryonic developmental stages of *D. elongatus* (nymphal instars I to V and young, one-week old adults) following protocols successfully employed previously with other pathogens (Habtewold et al., 1995; Lange and Wittenstein, 1998). The source of *L. dichroplusae* spores used was infections at the type locality (Brandsen) and other localities in Buenos Aires province (Lange, 2003). Briefly, a total of 291 individuals of *D. elongatus* reared at the Center for Parasitological Studies and Vectors (CEPAVE) as described by Mariottini et al. (2011) were individually placed in 20 or 40 ml foam-plugged glass vials and starved for 24 hours. A 5 mm diameter lettuce disk with either 10^4, 10^5 or 10^6 spores of *L. dichroplusae* each in aqueous suspension was then administer to each grasshopper. Only those that consumed the entire disk were considered inoculated, and were kept for 30 days, checking regularly for infection development through dissection and homogenization. Controls were identical but without adding spores to the lettuce disks. Table 1 shows the bioassays performed.

Table 1. *Per os* experimental inoculations (bioassays) with spores of *Liebermannia dichroplusae* on its natural host *Dichroplus elongatus*.

Host nymphal instar or stage	Number of inoculated individuals	Dose *per* individual	Infection development
I	24	10^4	No
I	23	10^5	No
I, II	20	10^6	No
II	30	10^5	No
II	30	10^6	No
II, III	24	10^5	No
III	20	10^5	No
III	32	10^6	No
IV	15	10^5	No
IV, V	25	10^6	No
V	28	10^6	No
Adult	20	10^6	No

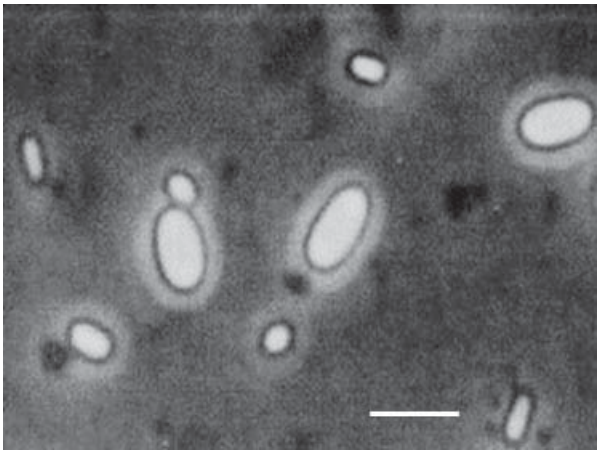

Fig. 1. Mature spores of *Paranosema locustae* (larger ones) and *Liebermannia dichroplusae* (smaller ones) from the co-infection in *Dichroplus elongatus*. Scale bar: 5 μm.

Results

Only six individuals of *D. elongatus* had infections. Three of them were infected with *P. locustae* alone, two were infected with *L. dichroplusae* alone, and one individual had a co-infection with both microsporidia (Fig. 1). Therefore both pathogens occurred at low prevalence, *P. locustae* at 4.6 % and *L. dichroplusae* at 3.4 %. Spore loads *per* individual were 1.7×10^9, 8.6×10^8 and trace as defined by Henry (1972) for *P. locustae*, 4.1×10^8 and 3.4×10^7 for *L. dichroplusae*, and 1.0×10^6 and 2.9×10^5 for *P. locustae* and *L. dichroplusae*, respectively in the mixed infection. Heaviest infection by each pathogen alone (1.7×10^9 for *P. locustae*, 4.1×10^8 for *L. dichroplusae*) showed typical internal signs of infection following ventral dissection. Creamy color with some pink tinge and heavily hypertrophied fat body in the case of *P. locustae* (Fig. 2), and whitish, also greatly hypertrophied Malpighian tubules in the case of *L. dichroplusae* (Fig. 3). No obvious internal signs of infection by either of the pathogens were observed in the mixed infection. All bioassays gave negative results (i.e. no infection development).

Discussion

This is the first detection of the microsporidia *P. locustae* and *L. dichroplusae* sharing the same individual host (i.e. co-infection). Since both pathogens are found with relative frequency in *D. elongatus* of the Pampas and considering that it took approximately three decades of monitoring of thousands of *D. elongatus* from nearly 100 localities (Lange, 2003; Bardi et al., 2012) prior to finding the co-infection here reported it might be assumed that such an event is highly unusual. Indeed, natural mixed infections by entomopathogenic microsporidia species appear to be a rare phenomenon possibly due to competition issues (Solter, 2014). A possible antagonistic interaction was previously hypothesized between *P. locustae* and *L. dichroplusae* (Lange and Cigliano, 2005), as it was also suggested for *P. locustae* and an undescribed microsporidium in North America (Streett and Henry 1984), because by 2003 at only one locality (Carhué, western Buenos Aires province) and for just two seasons both pathogens were found to coexist in *D. elongatus*, albeit in different individuals (Lange, 2003). Since then, further monitoring revealed the coexistence of *P. locustae* and *L. dichroplusae* in populations of *D. elongatus* in the surroundings of five additional localities in the Pampas, also in western Buenos Aires province (Alamos, Blancagrande, Casbas, Espartillar, Trenque Lauquen) but without the occurrence of mixed infections. The co-infection herewith reported shows that there is no absolute antagonism between *P. locustae* and *L. dichroplusae* but possibly a more subtle interaction that under a very narrow set of opportunistic-based type of conditions may allow simultaneous occurrence in a single host. Unfortunately, at the moment we cannot test in the laboratory the interactions between both microsporidia simul-taneously within the host due to the inability of inducing

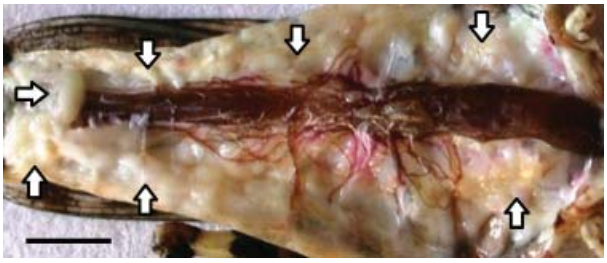

Fig. 2. Ventrally dissected adult of *Dichroplus elongatus* depicting heavily infected fat body tissue with *Paranosema locustae*. Scale bar: 20 mm.

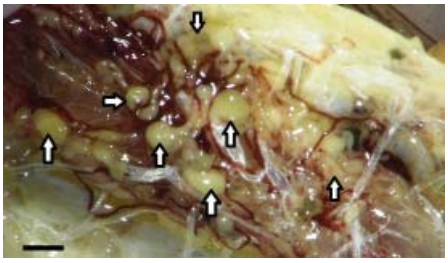

Fig. 3. Malpighian tubules of *Dichroplus elongatus* heavily infected with *Liebermannia dichroplusae*. Scale bar: 0.5 mm..

infections with *L. dichroplusae*. We speculate that given the characteristics inherent to both pathogens a conceivable scenario would be that the individual of *D. elongatus* was already infected with *L. dichroplusae* when it contracted infection by *P. locustae*. Horizontal transmission is highly efficient, spore loads are frequently heavy, and host range is unusually wide in *P. locustae,* whereas vertical transmission seems to predominate, spore loads are seldom too heavy, and host range is monospecific in *L. dichroplusae*. In any case, we feel that the rarity of the finding merits publication.

Acknowledgments

To Dr. S. Plischuk for discussing an earlier version of the manuscript.

References

Bardi C., Mariottini Y., Plischuk S. and Lange C. E. 2012. Status of the alien pathogen *Paranosema locustae* (Microsporidia) in grasshoppers (Orthoptera: Acridoidea) of the Argentine Pampas. Biocontrol Sci. Technol. 22, 497–512.

Carbonell C.S., Cigliano M.M. and Lange C.E. 2017. Acridomorph (Orthoptera) species of Argentina and Uruguay. https://biodar.io/acridomorph/.

Cigliano M.M., Pocco M.E. and Lange C.E. 2014. Acridoideos (Orthoptera) de importancia agroeconómica en la República Argentina. In: Biodiversidad de Artrópodos Argentinos. Vol. 3. (Eds Roig-Jucent S.A., Claps L.E. and Morrone J. J.). INSUE - Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina, pp. 1–36 (In Spanish with English summary).

Fine P.E.M. 1984. Vertical transmission of pathogens of invertebrates. In: Comparative Pathobiology. Vol. 7. (Ed. Cheng T.C.). Plenum Press, NY, pp. 205–241.

Habtewold T., Landin J., Wennergen U. and Bergman K.O. 1995. Life table for the tef grasshopper, *Aiolopus longicornis* under laboratory conditions and demographic effects of the pathogen *Nosema locustae*. Biol. Control. 5, 497–502.

Henry J.E. 1972. Epizootiology of infections by *Nosema locustae* Canning (Microsporida: Nosematidae) in grasshoppers. Acrida. 1, 111–120.

Henry J. E. and Oma E. A. 1981. Pest control by *Nosema locustae*, a pathogen of grasshoppers and crickets. In: Microbial control of pests and plant diseases 1970-1980. (Ed. Burges D.) Academic Press, NY, pp. 573–586.

Lange C.E. 1987. Histopathology in the Malpighian tubules of *Dichroplus elongatus* (Orthoptera: Acrididae) infected with *Perezia dichroplusae* (Microspora: Pereziidae). J. Invertebr. Pathol. 50, 146–150.

Lange C.E. 1989. Prevalence of *Perezia dichroplusae* (Microspora: Pereziidae) in Argentine grasshoppers (Orthoptera: Acrididae). J. Invertebr. Pathol. 54, 269–271.

Lange C.E. 1997. Vertical transmission of *Perezia dichroplusae* Lange (Protozoa: Microspora) in its natural host, *Dichroplus elongatus* Giglio-Tos (Orthoptera: Acrididae). Neotrópica. 43, 39–42 (In Spanish with English summary).

Lange C.E. 2003. Long-term patterns of occurrence of *Nosema locustae* and *Perezia dichroplusae* (Microsporidia) in grasshoppers (Orthoptera: Acrididae) of the Pampas, Argentina. Acta Protozool. 42, 309–315.

Lange C.E. 2005. The host and geographical range of the grasshopper pathogen *Paranosema* (*Nosema*) *locustae* revisited. J. Orthoptera Res. 14, 137–141.

Lange C.E. 2010. *Paranosema locustae* (Microsporidia) in grasshoppers (Orthoptera: Acridoidea) of Argentina: field host range expanded. Biocontrol Sci. Technol. 20, 1047–1054.

Lange C.E. and Henry J.E. 1996. Métodos de estudio y producción de protistas entomopatógenos. In: Microorganismos Patógenos Empleados en el Control Microbiano de Insectos Plaga (Ed.: Lecuona R.). Mariano Mas, Buenos Aires, pp. 169–176 (In Spanish).

Lange C.E. and Wittenstein E. 1998. Susceptibility of the locust *Schistocerca cancellata* (Orthoptera: Acrididae) to different entomopathogens. Rev. Soc. Entomol. Arg. 57, 19–22 (In Spanish with English summary).

Lange C.E. and Cigliano M.M. 2005. Overview and perspectives on the introduction and establishment of the grasshopper biocontrol agent *Paranosema locustae* (Microsporidia) in the western Pampas of Argentina. Vedalia. 12, 61–84.

Mariottini Y., De Wisiecki M.L. and Lange C.E. 2011. Postembryonic development and consumption of the melanoplines *Dichroplus elongatus* Giglio-Tos and *Dichroplus maculipennis* (Blanchard) (Orthoptera: Acrididae: Melanoplinae) under laboratory conditions. Neotropical Entomol. 40, 190–196.

Phithalsoun G. and Zhang L. 2018. An effective biological control method of yellow-spined bamboo locust (*Ceracris kiangsu*) has been developed in Lao PDR and Vietnam. Metaleptea 38, 15–16.

Plischuk S., Bardi C.J. and Lange C.E. 2013. Spore loads of *Paranosema locustae* (Microsporidia) in heavily infected grasshoppers (Orthoptera: Acridoidea) of the Argentine Pampas and Patagonia. J. Invertebr. Pathol. 114, 89–91.

Poinar G.O. and Thomas G.M. 1984. Laboratory guide to insect pathogens and parasites. Plenum Press, NY, p 392.

Sokolova Y.Y., Lange C.E. and Fuxa J.R.. 2007. Establishment of the new combination *Liebermannia dichroplusae* on the basis of molecular characterization of *Perezia dichroplusae* Lange, 1987. J. Eukaryotic Microbiol. 54, 223–230.

Solter L. 2014. Epizootiology of microsporidiosis in invertebrate hosts. In: Microsporidia: Pathogens of opportunity (Eds: Weiss L.M. and Becnel J.J.). Wiley-Blackwell, Ames, Iowa, pp 165–194.

Solter L.F., Becnel J.J. and Oi D.H. 2012. Microsporidian entomopathogens. In: Insect Pathology, 2nd edition (Eds: Vega F.E. and Kaya H. K.). Elsevier, London, pp 221–263.

Streett D. A. and Henry J. E. 1984. Epizootiology of a microsporidium in field populations of *Aulocara elliotti* and *Psoloessa delicatula* (Insecta: Orthoptera). Can. Entomol. 116, 1439–1440.

Undeen A. and Vávra J. 1997. Research methods for entomopathogenic Protozoa. In: Manual of techniques in insect pathology (Ed.: Lacey L.). Academic Press, San Diego, pp. 117–151.

Address for correspondence: Carlos E. Lange. CEPAVE, Boulevard 120 s/n entre Av. 60 y Calle 64, La Plata (1900), Argentina; e-mail: *carlosl@cepave.edu.ar.*