

Naturalized, newly-associated microsporidium continues causing epizootics and expanding its host range

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| Submitted January 4, 2020 | Accepted January 24, 2020 |

Summary

Examination of grasshoppers sampled at two sites, one in western Pampas and one in northwestern Patagonia, both in Argentina, revealed the occurrence of two new epizootic events of the allochthonous microsporidium *Paranosema locustae* which became naturalized in grasshopper communities of those areas after introductions from its native land in North America in the late seventies–early eighties and mid-nineties, respectively. In the Pampas site, *P. locustae* was found infecting six out of eight grasshopper species collected at an overall prevalence of 38.8 % and a maximum of 50 %. In the Patagonia site, *P. locustae* was detected in four out of six species at an overall prevalence of 10.5 % and a maximum of 34.3 %. Two of the species affected, one in the Pampas, the gomphocerine *Amblytropidia australis*, and one in Patagonia, the melanopline *Dichroplus vittigerum*, constitute new host records for *P. locustae*, expanding its field host range in Argentina to 24 species of grasshoppers. In addition, bioassays were performed on three other grasshopper species selected based on several criteria. The tristirid *Bufoacris claraziana* and the gomphocerine *Borellia bruneri* turned out to be not susceptible to *P. locustae* while the melanopline *Scotussa cliens* was highly susceptible. All in all, results highlight not only the uniqueness of *P. locustae* among the Microsporidia that allowed for its development as a biocontrol agent but also the suitability of grasshopper communities in the Pampas and Patagonia to fully express such uniqueness, possibly even more so as an allochthonous parasite against autochthonous novel hosts.

Key words: Argentina, biocontrol agent, grasshoppers, *Paranosema locustae*, prevalence

Introduction

An allochthonous species is considered to be naturalized if after a prolonged period of time and a number of generations it is still established or expanding in the new area, not requiring further inputs for survival and reproduction. If the species is parasitic and the hosts affected are autochthonous, then new parasite–host associations occur (Perlman and Jaenike, 2003). The more species of hosts that are susceptible to the novel parasite in a community the greater the chances are of naturalization (Bonsall, 2004). The fate of naturalized parasites on naïve, newly-associated hosts tends to be unpredictable but the lack of co-evolutionary experience of the new associations may result in enhanced impact or unexpected outcomes.

The microsporidium *Paranosema locustae* has been developed as a long-term grasshopper biocontrol agent (Henry, 2017; Lange and Sokolova, 2017) and it is known to occur in grasshopper communities of areas in northwestern Patagonia (Neuquén and Chubut provinces) and Pampas regions (Buenos Aires and La Pampa provinces) of Argentina since it was re-isolated in 1991 (Lange, 1992) after its introduction from North America more than forty years ago in the Pampas and Chubut (Lange and Cigliano, 2005; Bardi et al., 2012) and 24 years ago in Neuquén (Lange and Azzaro, 2008; Lange, 2010). *Paranosema locustae*, an intracellular microparasite, develops in the grasshoppers adipocytes of the fat body, depriving the host of vital energy for development and reproduction and causing not an acute disease but rather a chronic, debilitating illness characterized by numerous sub-lethal effects. These include reductions of fecundity and longevity, delays in development and molting abnormalities, decrease of activity and lethargy, reduction of food consumption, flying inability or difficulties, disruption of aggregation behavior, and alteration of phase transformation (Lange and Cigliano, 2005; Fu et al., 2010; Feng et al., 2015).

In the present contribution we report the detection of two new epizootic events of *P. locustae*, the finding of field infections in two grasshopper species not previously recorded to be susceptible, and the result of laboratory bioassays against three grasshopper species of unknown susceptibility

Material and methods

Field grasshopper samples consisted of all individuals captured alive with three hundred sweeps of

entomological nets at each of two grassland sites, one in the Pampas region on February 11, 2017 (15 km North of Santa Rosa, La Pampa province; 36°28'15.85"S, 64°16'47.03"W) and the other in north-western Patagonia on February 3, 2016 (3 km SW of Loncopué, Neuquén province; 38°05'30.50"S, 70°39'28.82"W), areas where *P. locustae* is known to be established in grasshopper communities since long ago (Lange and Azzaro, 2008; Bardi et al., 2012). In the Pampas site, a total of 286 grasshoppers belonging to eight species (*Amblytropidia australis*, *Baeacris pseudopunctulata*, *Borellia bruneri*, *Dichroplus elongatus*, *D. pratensis*, *D. vittatus*, *Rhammatocerus pictus*, *Staurorhectus longicornis*) were collected. In the Patagonia site, 496 grasshoppers of six species (*Borellia bruneri*, *Dichroplus elongatus*, *D. maculipennis*, *D. vittigerum*, *Scotussa lemniscata*, *Scyllinula signatipennis*) were obtained. Soon after collection, the samples were frozen (−32 °C) until processing. Individual examination of each grasshopper was done either by organ/tissue scrutiny after ventral, longitudinal dissection and/or whole-body homogenization in double distilled water followed by phase contrast microscopy (400×, 1000×) as already described in Lange and Cigliano (2019). Changes in the gross appearance of the grasshopper's fat body infected with *P. locustae* are a pathognomonic sign (Henry and Oma, 1981; Lange and Cigliano, 2019) and both the size and shape of spores and type of developmental stages of *P. locustae* are easily distinguished under phase contrast microscopy from all known autochthonous grasshopper microsporidia, all of them belonging to genus *Liebermannia* (Lange, 2003, 2010; Sokolova et al., 2009; Lange and Cigliano, 2019).

Bioassays in the form of oral experimental inoculations as described by Mariottini and Lange (2014) and Lange and Cigliano (2019) were performed on juvenile individuals (third and fourth nymphal instars) of three species of grasshoppers not previously challenged with *P. locustae*. Source spores employed in the bioassays as aqueous suspensions at a dose of 10⁵ per each challenged insect were obtained from infections in the establishment area of the Pampas (Bardi et al., 2012). Grasshopper species challenged were *B. bruneri*, *Scotussa cliens*, and *Bufonacris claraziana*. Individuals inoculated, 40 *B. bruneri*, 43 *S. cliens*, and 58 *B. claraziana*, were F1 laboratory generation of grasshoppers collected as adults in the vicinity of Laprida county, southern Pampas (Buenos Aires province), La Plata and Lobos counties, northeastern Pampas (Buenos Aires province), and Cushamen, northwestern Patagonia

Table 1. Host range and prevalence of the microsporidium *Paranosema locustae* in grasshoppers collected in the Pampas site (15 km North of Santa Rosa, La Pampa province).

Subfamily of Acrididae	Species	Infected/Collected	Prevalence (%)
Melanoplinae	<i>Baeacris pseudo-punctulatus</i>	2/4	50.0
	<i>Dichroplus elongatus</i>	2/6	33.3
	<i>Dichroplus pratensis</i>	85/203	41.9
	<i>Dichroplus vittatus</i>	1/5	20.0
Gomphocerinae	<i>Amblytropidia australis</i>	1/10	10.0
	<i>Borellia bruneri</i>	0/4	00.0
	<i>Rhammatocerus pictus</i>	0/1	00.0
	<i>Staurorhectus longicornis</i>	10/53	18.9

(Chubut province), respectively. Criteria for selecting these three species for performing bioassays were as follows. *Borellia bruneri* belongs to a subfamily of Acrididae, the Gomphocerinae, whose specific members tend to be susceptible to *P. locustae* (Lange, 2005). However, in spite of *B. bruneri* being relatively common in some areas where *P. locustae* became naturalized, it has never been found infected. In outbreak years, *B. bruneri* is considered an actual agricultural pest in the Pampas of Uruguay and has the potential to become so in areas of the Argentine Pampas and Patagonia (Cigliano et al., 2014). *Scotussa cliens* also belongs to a subfamily, the Melanoplinae, whose members are possibly the most susceptible to *P. locustae*. Since *S. cliens* overwinters as nymphs (Liebermann, 1952) it might have the ability to carry the disease from one season to the next. The Tristiridae *B. claraziana*, a heavy-built, large (up to 36.7 mm long) apterous grasshopper endemic by Patagonia causes serious rangeland forage losses when outbreaks occur (Cigliano et al. 2014). None to the 25 known species of tristirids inhabiting South America (Cigliano et al., 2019) have ever been challenged with *P. locustae* which means that there is no clue about the intrinsic suitability of tristirids for infection development.

Results

In the Pampas site, *P. locustae* was found infecting six out of the eight species collected in an overall prevalence of 38.8 % (Table 1). The highest *per* species prevalence was recorded in *B. pseudo-*

Table 2. Host range and prevalence of the microsporidium *Paranosema locustae* in grasshoppers collected in the Patagonia site (3 km SW of Loncopué, Neuquén province).

Subfamily of Acrididae	Species	Infected/Collected	Prevalence (%)
Melanoplinae	<i>Dichroplus elongatus</i>	22/83	26.5
	<i>Dichroplus maculipennis</i>	23/67	34.3
	<i>Dichroplus vittigerum</i>	2/45	4.4
	<i>Scotussa lemniscata</i>	5/56	8.9
Gomphocerinae	<i>Borellia bruneri</i>	0/160	00.0
	<i>Scyllinula signatipennis</i>	0/85	00.0

punctulata (50 %) and the lowest in *A. australis* (10 %). However, prevalence of 41.9 % in *D. pratensis* and 18.9 % in *S. longicornis* are the ones sustained on considerable sample size, 203 and 53 individuals, respectively.

In the Patagonia site, *P. locustae* was found infecting four out of the six species collected in an overall prevalence of 10.5 % (Table 2). The highest *per* species prevalence was 34.3 % in *D. maculipennis* (n=67) and the lowest was 4.4 % in *D. vittigerum* (n=45).

Infections in both *A. australis* and *D. vittigerum* constituted the first records of *P. locustae* parasitizing such host species. None of the experimentally inoculated *B. bruneri* and *B. claraziana* developed infections while infection development occurred in 97.7 % of inoculated *S. cliens*.

Discussion

As pointed out by Lange and Sokolova (2017), *P. locustae* exhibits a combination of traits that makes it a remarkable, unique microsporidium: (i) it has an unusual wide host range among acridomorphs (up until this contribution 124 species were known to be susceptible worldwide), (ii) *per os* horizontal transmission is highly efficient and it is complemented with a vertical route through the female line, (iii) infections tend to be chronic rather than acute, typically allowing for the production of heavy spore loads *per* infected grasshopper host, and (iv) spores maintain viability at freezing temperatures for extended periods of time (years). These attributes confer *P. locustae* an exceptional ability for wide-spread occurrence and long-term persistence in grasshopper communities having susceptible spe-

Table 3. Host range of the microsporidium *Paranosema locustae* in grasshoppers of Argentina.

Family	Subfamily	Species	Field infection	Experimental infection	Challenged but no infection
Acrididae	Melanoplinae	<i>Baeacris pseudopunctulata</i>	X		
		<i>Baeacris punctulata</i>	X	X	
		<i>Dichroplus conspersus</i>	X		
		<i>Dichroplus elongatus</i>	X	X	
		<i>Dichroplus maculipennis</i>	X	X	
		<i>Dichroplus pratensis</i>	X	X	
		<i>Dichroplus schulzi</i>		X	
		<i>Dichroplus vittatus</i>	X		
		<i>Dichroplus vittigerum</i>	X		
		<i>Leiotettix pulcher</i>	X		
		<i>Neopedies brunneri</i>	X		
		<i>Ronderosia bergii</i>	X	X	
		<i>Ronderosia forcipata</i>	X		
		<i>Scotussa cliens</i>		X	
		<i>Scotussa daguerrei</i>	X		
	<i>Scotussa lemniscata</i>	X			
	Gomphocerinae	<i>Amblytropidia australis</i>	X		
		<i>Borellia bruneri</i>			X
		<i>Euplectrotettix schulzi</i>	X		
		<i>Laplatacris dispar</i>		X	
<i>Rhammatocerus pictus</i>		X			
<i>Sinipta dalmani</i>		X			
<i>Staurorhectus longicornis</i>		X			
Cyrtacanthacridinae	<i>Schistocerca cancellata</i>		X		
Oedipodinae	<i>Trimerotropis pallidipennis</i>	X	X		
Copiocerinae	<i>Aleuas lineatus</i>	X	X		
Acridinae	<i>Allotruxalis gracilis</i>	X			
Romaleidae	Romaleinae	<i>Diponthus argentinus</i>	X		
		<i>Staleochlora viridicata</i>			X
		<i>Tropidacris collaris</i>		X	
		<i>Zoniopoda tarsata</i>	X		
Tristiridae	Tristirinae	<i>Bufonacris claraziana</i>			X

cies. In spite of this, occurrence appears not as wide as might be expected in its native land in North America (Arizona, Colorado, Idaho, Minnesota, Montana, North Dakota, Oregon, and Wyoming in the USA as well as southwestern Canada; Lockwood and Larsen, 1988) and prevalence is known to be normally less than 1% with rare peaks of 5% (Henry and Oma, 1981; Ewen, 1983). Despite heavy survey efforts in North America only once a natural epizootic (i.e., not following a field application) was detected (Henry, 1972). It might be possible that such low rates of natural prevalence where *P. locustae* is autochthonous would be somehow related with the longtime association between *P. locustae* and North American grasshoppers, where

pathogen and hosts probably co-evolved towards a sort of “win-win” equilibrium for both parties. The high prevalence and wide host range here reported in samples at both the Patagonia and Pampas sites not only corroborate the occurrence of significant epizootics in grasshopper communities in areas of naturalization of *P. locustae* in Argentina but also suggest that such an outcome might be related with the novel pathogen-hosts associations generated. We think it is relevant to report about these two new epizootic events for several reasons: i) as mentioned above, the apparent extreme rarity of such events in North America, ii) the fact that similar events were observed long ago in other four different localities in the Pampas (Lange and de Wysiecki,

2005) but at a time when monitoring surveys were much more intense. The intensity of surveys was relatively high until 2011 (Bardi et al., 2012) but was severely relaxed since then which might indicate that epizootics could be a more common outcome in more recent years, ii) although a quantitative assessment of the effect of naturalized *P. locustae* on grasshopper communities is not possible due to a lack of pre-introduction grasshopper abundance data, epizootics might explain into some extent why the frequency and severity of pest grasshopper outbreaks appear to have diminished in areas of naturalization.

The detection of infections in *A. australis* and *D. vittigerum* expands the known field host range of *P. locustae* in Argentina to 24 grasshopper species (Table 3). In a sense, these findings should not be considered as unexpected because both *A. australis* and *D. vittigerum* belong to subfamilies of Acrididae, the Gomphocerinae and Melanoplinae, respectively, which are of known susceptibility to *P. locustae* (Lange, 2005). However, the detection of field infections are the actual or ultimate confirmation of susceptibility as opposed to infections induced in the laboratory where unknown ecological barriers or other factors may be bypassed by the forced inoculation procedure.

Results from the bioassays felt within our presumptions. As should be expected from a Melanoplinae, *Scotussa cliens* resulted to be highly susceptible which means that in a field situation scenario infected overwintering nymphs could be able to carry the pathogen from one season to the next and conceivably be the source of a new appearance of the disease in a given area and eventually an epizootic. *S. cliens* is widespread and common in the Pampas (Cigliano et al., 2019). The negative outcome of the bioassays (i.e. lack of infection development) in *B. bruneri* concurs with the fact that this species has never been found infected in the field in spite of belonging to a subfamily (Gomphocerinae) of recognized general susceptibility. Although close phylogenetic relatedness seems to be generally a predictor of susceptibility to *P. locustae* among the Acrididae exceptions clearly do occur and ultimate susceptibility seems to be at the host species level. Absence of infections in the bioassays on *B. claraziana* is not surprising considering the phylogenetic distance between Tristiridae and Acrididae (Song et al. 2015).

Our results highlight not only the uniqueness of *P. locustae* among the Microsporidia that allowed

its development as a biocontrol agent but also the suitability of grasshopper communities in the Pampas and Patagonia to fully express the combination of traits that account for such uniqueness, possibly even more so as an allochthonous parasite against novel hosts rather than as an autochthonous one in co-evolved hosts. Lange and Cigliano (2005) and Bardi et al. (2012) discussed at length the potential of *P. locustae* to alter the structure and dynamics of native grasshopper communities in Argentina through differences on the inherent susceptibility of the specific components, even singling out species that might be at risk due to *P. locustae* as a threat, and our new findings here reported reinforce that particular quality of this remarkable microsporidium.

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