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RESEARCH ARTICLE

Status of the alien pathogen *Paranosema locustae* (Microsporidia) in grasshoppers (Orthoptera: Acridoidea) of the Argentine Pampas

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After experimental introductions from North America in 1978–1982, the biocontrol agent *Paranosema locustae* became established in grasshopper communities of the western Pampas region of Argentina. The use and establishment of *P. locustae* in Argentina constitute both a case of neoclassical or new association biological control (use of an alien species against native pests) and a case of pathogen pollution (anthropogenic introduction–establishment of an infectious disease in populations of native species). Since *P. locustae* is a multihost pathogen among grasshoppers, its presence in the western Pampas represents an additional factor disrupting grasshopper communities according to the differential susceptibility of each species and possibly threatening some species. Microscopic examination of 504 grasshopper samples (the mean number of individuals per sample = 185) belonging to 43 species from 93 localities throughout the Pampas revealed an establishment area of approximate 90,000 km^2 from about 35° North to 38° South and from 61° East to 65° West. Field infections by *P. locustae* have now been detected in 21 grasshopper species in the western Pampas. Susceptible species with geographic distributions mostly restricted to the establishment area and with numerically small populations, like the melanopline *Scotussa daguerrei*, are predicted to be the ones facing higher risks of negative impacts.

Keywords: biocontrol agent; biological control; field host range; multihost pathogen; neoclassical biological control; pathogen pollution

Introduction

As in other temperate grasslands of the world, grasshoppers are among the most important herbivorous insects in the Argentine Pampas, a large biogeographic region (540,000 km^2) in the central-eastern part of the country (Morrone 2006). About one quarter of the approximately 200 species of grasshoppers known for Argentina were reported for the Pampas (Carbonell, Cigliano, and Lange 2006). Some of these species, up to six but notably *Dichroplus maculipennis* (Blanchard) and *Dichroplus elongatus* Giglio-Tos, constitute recurrent pests that cause serious losses to forage and various crops during outbreaks (Lange, Cigliano, and De Wysiecki 2005; Mariottini, De Wysiecki, and Lange 2011). However, many other species are ecologically beneficial. Because of their diversity and abundance in grasslands, grasshoppers are important in nutrient cycling and represent major links in a variety

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of food webs (Samways 1997). All grasshopper species of Argentina are native (Carbonell et al. 2006), and no reports exist on the endangerment status of any of the species.

The anthropogenic introduction and establishment of infectious diseases in populations of native species is recognised as one of the threats that fauna is increasingly facing. The process, termed 'pathogen pollution', is considered a worldwide problem that threatens biodiversity conservation (Cunningham, Daszak, and Rodriguez 2003). Although the concept was coined with an emphasis on vertebrate wildlife diseases, the principles and potential effects are valid for invertebrates as well. According to Daszak and Cunningham (2003) introduced, established pathogens can cause mass mortalities, population declines and extinctions of native species. Because the majority of available data supporting the contention that alien infectious diseases may cause extinction and endangerment is largely anecdotal (Smith, Sax, and Lafferty 2006), it is important to provide as much information as possible on the status of cases of introduction and establishment of disease-causing agents.

The microsporidium *Paranosema locustae* (Canning) is a spore-forming pathogen of the adipose tissue cells of orthopterans that was isolated from North American grasshoppers (state of Idaho) and developed in the USA as a long-term biocontrol agent of grasshoppers (Henry and Oma 1981; Johnson 1997). It was originally described by Canning (1953) as *Nosema locustae*, and transferred to genus *Paranosema* by Sokolova et al. (2003) and Sokolova, Issi, Morzhina, Tokarev, and Vossbrinck (2005). It develops slowly (normally 2–3 weeks before producing significant amounts of propagules, the spores) and causes a debilitative, chronic type of disease in susceptible hosts. Demonstrated effects of *P. locustae* on hosts include increase of mortality rates, reduction of fecundity and longevity, delay in development, decrease of activity, reduction of food consumption, changes in pigmentation, flying inability or difficulties and disruption of aggregation behaviour and morphological phase transformation (Henry and Oma 1981; Johnson 1997; Shi and Njaqi 2004; Fu, Hunter, and Shi 2010). In 1978–1982 and 1996, *P. locustae* was introduced from North America at several grasshopper-affected localities in Argentina. The pathogen became established in three different areas of the country: two in north-western Patagonia (Gualjaina and Loncopué in Chubut and Neuquén provinces, respectively) and the other in the Pampas (Buenos Aires and La Pampa provinces) (Lange and Cigliano 2005; Lange and Azzaro 2008).

Since all grasshopper species inhabiting Argentina are native, the introduction and establishment of *P. locustae* in grasshopper communities of the Pampas and Patagonia represent a case of pathogen pollution. From a pest control point of view, the establishment appears successful. Although quantitative data linking *P. locustae* presence to diminished grasshopper abundance are not available, serious grasshopper outbreaks have not been reported in areas of establishment, but are still a recurrent problem outside of them, not only in Argentina but apparently also in some of the other areas of the world where it was introduced (Cuddeford 2007; Shi, Wang, Lv, Guo, and Cheng 2009). However, the situation might be different from the perspective of biodiversity conservation. Although *P. locustae* affects only species of Orthoptera, and in Argentina it was mainly targeted against four grasshopper species (*D. elongatus, D. maculipennis, Dichroplus pratensis* Bruner, *Scotussa lemniscata* Stål) (Hajek, McManus, and Delalibera 2005), it exhibits a wide host

range among that group. By 2010, 122 species of Orthoptera worldwide were known to be susceptible to *P. locustae*, either naturally or experimentally, most of which grasshoppers in the Superfamily Acridoidea (Lange 2005, 2010). Within the Acridoidea then, *P. locustae* qualifies as a generalist pathogen. As such, it can alter, through differences in host susceptibilities, the structure of grasshopper communities where it was not previously present (Bonsall 2004). Similarly pathogens that possess efficient transmission mechanisms like *P. locustae* (Lange and Cigliano 2005) may amplify their epizootics by having multiple hosts (Dobson 2004). By 2010, infections by *P. locustae* had been found in 20 grasshopper species in the Pampas and northwestern Patagonia (Lange 2010).

Here we report and discuss on the updated geographic distribution and host range of *P. locustae* in grasshopper communities of the Pampas, the area that has received comparatively more attention of the three establishment areas.

Materials and methods

Following the standard procedure of broadcasting wheat bran bait with spores (Henry and Oma 1981), *P. locustae* was experimentally applied between 1978 and 1982 to grasshopper communities in grasslands and improved pastures in the vicinity of seven localities in the Pampas region (Lange and De Wysiecki 1996). Five localities were in Buenos Aires province (Casbas, Coronel Pringles, Coronel Suárez, General Lamadrid, Gorchs) and two in La Pampa province (Macachín, Santa Rosa) (Figure 1). Except for Casbas, in all other localities *P. locustae* was applied in combinations with the carbamate insecticide Carbaryl, an approach employed at the time in order to obtain some short-term grasshopper control (Onsager, Rees, Henry, and Foster 1981), an effect not normally achieved when *P. locustae* is used alone (Onsager 1988), at least at the standard application rate of 2.5×10^9 spores/ha (Henry 1990). Previous publications have dealt with the establishment, prevalence, infection intensity and host range of the pathogen in Argentina (Lange 2003a, b, 2010; Lange and Cigliano 2005, 2010; Lange and Azzaro 2008). However, given that grasshopper sampling is conducted every summer (January and February) since 1994 throughout the Pampas, new information is obtained at a seasonal additive base.

Each sample processed for this study consisted of all grasshoppers captured with 300 sweeps with entomological nets (diameter: 40 cm, depth: 75 cm, sweep: 180°) along transects in each collecting site, a method acknowledged to provide acceptable representative estimates in terms of species diversity and relative abundance of the grasshopper communities sampled (Larson, O'Neill, and Kemp 1999). Samples included in the study spanned from 1994 to 2011. The total number of samples collected and examined was 504, and the mean number ($\pm$ SE) of grasshoppers per sample was 184.5 ± 32.7 (range: 18–1242; approximate number of grasshoppers examined = 93,000). Of the 93 collecting localities, 72 were visited several times over the years while 21 were visited only once. Grasshoppers examined belonged to 43 species of Acridoidea, 17 of them Acrididae in the Subfamily Melanoplinae, nine Gomphocerinae, one Oedipodinae, two Copiocerinae, five Acridinae, two Leptysminae, six Romaleinae of Family Romaleidae, and one Ommexechinae of Family Ommexechidae. The acridological zones proposed by Liebermann (1972) (Figure 2) based on presence and abundance of characteristic species were the template against
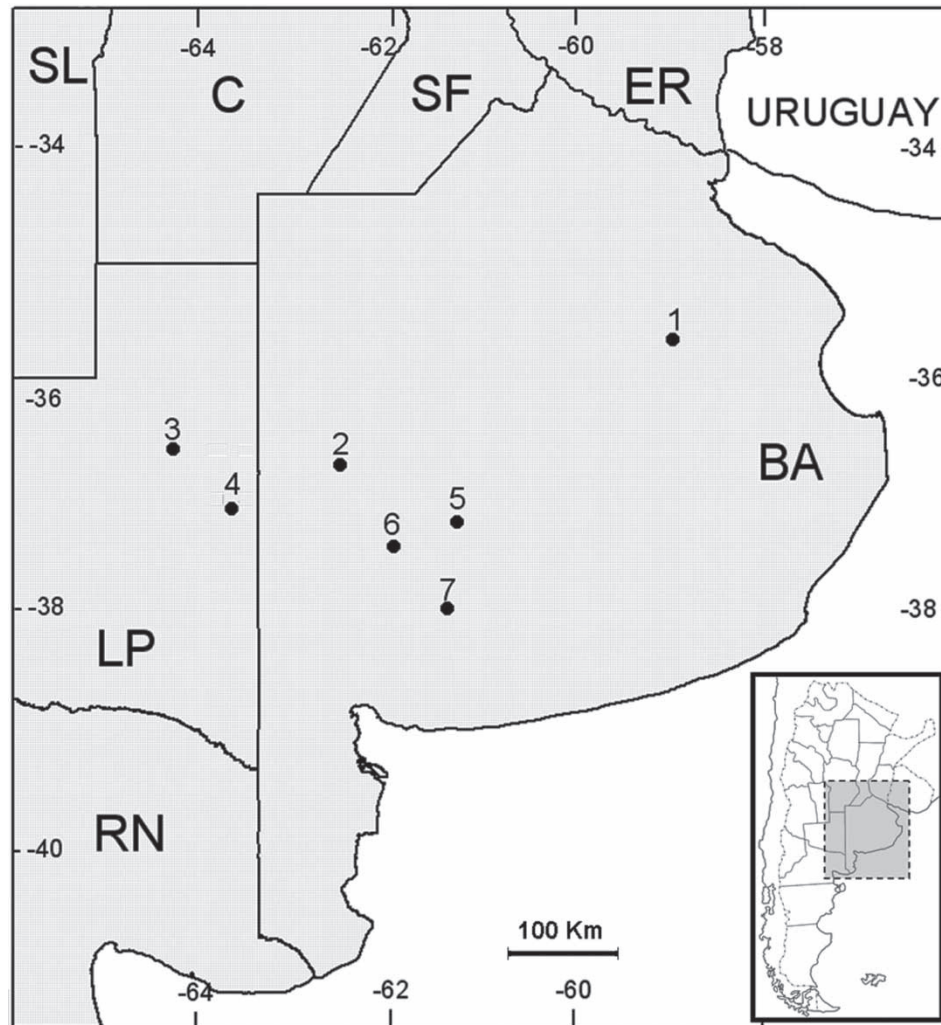
Figure 1. The seven localities of introduction of *Paranosema locustae* in grasshopper communities of the Pampas region. **1**, Gorchs; **2**, Casbas; **3**, Santa Rosa; **4**, Macachín; **5**, General Lamadrid; **6**, Coronel Suárez; **7**, Coronel Pringles. **BA**, Buenos Aires; **C**, Córdoba; **ER**, Entre Ríos; **LP**, La Pampa; **SF**, Santa Fe; **SL**, San Luis; **RN**, Río Negro.

which the geographic distribution of each grasshopper species according to Carbonell et al. (2006) was depicted. The distribution and relative abundance of the grasshopper species in the samples were estimated using the percentage categories employed by Mariottini (2009). The distribution categories considered were narrow (presence of a species in less than 25% of the samples), limited (26–50%), wide (51–75%) and very wide (more than 75%). Abundance categories were termed as infrequent (presence of a species in less than 10% of the samples in no more than 10% individuals per sample), frequent (10–30% of samples having 11–30% individuals/sample), abundant (31–50% of samples with 31–50% individuals/sample)
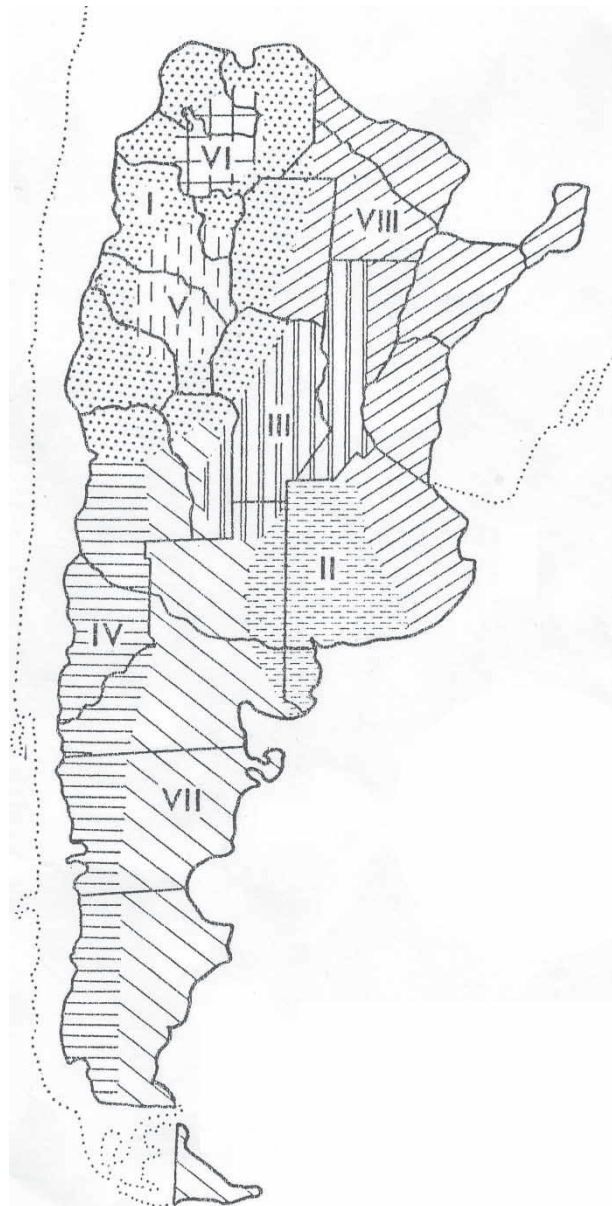
Figure 2. The eight acridological zones of Argentina as proposed by Liebermann (1972).

and very abundant (more than 50% of samples having more than 50% individuals/ sample).

After collection, samples were frozen ($-32°$C) until examination. Upon thawing, each grasshopper was examined either by the homogenisation or dissection methods (Henry, Tiahrt, and Oma 1973; Lange and Henry 1996; Undeen and Vávra 1997), observing wet mounts of homogenate aliquots or small pieces of tissues and organs in one-quarter-strength Ringer's solution (Poinar and Thomas 1984) under phase

contrast microscopy (400 ×, 1000 ×). *P. locustae* is readily distinguished at the light microscopy level from the native microsporidia of genus *Liebermannia* known to occur in Pampas grasshoppers by its larger spore size (5.2–2.8 μm vs. 3.5–1.5 μm in *Liebermannia dichroplusae* and 2.6–1.4 μm in *L. covasacrae*) and spore shape (oval vs. ovocylindrical in *Liebermannia* spp.), tissue affected (primarily adipose tissue vs. Malpighian tubules in *L. dichroplusae* and salivary glands in *L. covasacrae*), absence of moniliform plasmodia that occur in *Liebermannia* spp. and disporoblastic condition (two sporoblasts resulting from a sporont) (Canning 1953; Henry and Oma 1981; Lange 1987; Sokolova and Lange 2002; Sokolova et al. 2003; Sokolova, Lange, and Fuxa 2007; Sokolova, Lange, Mariottini, and Fuxa 2009). As depicted in Lange (2003a, 2010), the disporoblastic condition and absence of moniliform plasmodia can be clearly checked in Ringer's wet mounts of *P. locustae*-infected adipose tissue.

Results

As of 2011, the establishment area of *P. locustae* in grasshoppers of the Pampas region covers approximately 90,000 km^2 in the western Pampas (western Buenos Aires and eastern La Pampa provinces), stretching from about 35° North to 38° South and from 61° East to 65° West (Figure 3). Within this area, *P. locustae* was detected in 51.6% of the samples ($n = 190$) coming from 37 localities. Outside of the establishment area, *P. locustae* has never been found in any of the 314 samples examined from 56 localities (Figure 4), including those that were obtained in areas where there were introductions like in northeast (applications at Gorchs) and south (applications at Coronel Pringles, Coronel Suárez, and General Lamadrid) Pampas (Figure 1).

The 43 species of grasshoppers collected and examined from the Pampas are shown in Table 1. During the 2011 season, in addition to the detection of individuals of several grasshopper species that were found parasitised by *P. locustae* in previous seasons like *Baeacris pseudopunctulatus* (Ronderos), *D. pratensis*, *Ronderosia forcipata* (Rehn), *S. lemniscata*, and *Staurorhectus longicornis* Giglio Tos, the pathogen was found infecting two grasshopper species that were never before found to be infected. One was the melanopline (Subfamily Melanoplinae) *Dichroplus conspersus* Bruner in a sample from Trenque Lauquen (one infected individual out of two collected), and the other one was the copiocerine (Subfamily Copiocerinae) *Aleuas lineatus* Stål from Garré (two infected individuals out of 29), both localities in western Buenos Aires province (Figure 3). These new records expand the registered field host range of *P. locustae* in the Pampas to 21 species of grasshoppers out of the 22 recorded for the three establishment areas of the country (Table 1). Species with recorded field infections included 12 Melanoplinae, four Gomphocerinae, one Oedipodinae, one Copiocerinae, one Acridinae and two Romaleinae. Curiously, infections have not been found in *D. maculipennis* of the Pampas but were previously recorded at the two establishment areas of north-western Patagonia (Lange and Cigliano 2005, 2010).

Discussion

Since *P. locustae*, as all microsporidia, is a strictly obligate intracellular parasite unable of an active life outside of a host (Agnew, Becnel, Ebert, and Michalakis 2003), and the spores are relatively short-lived in the general environment (Germida,
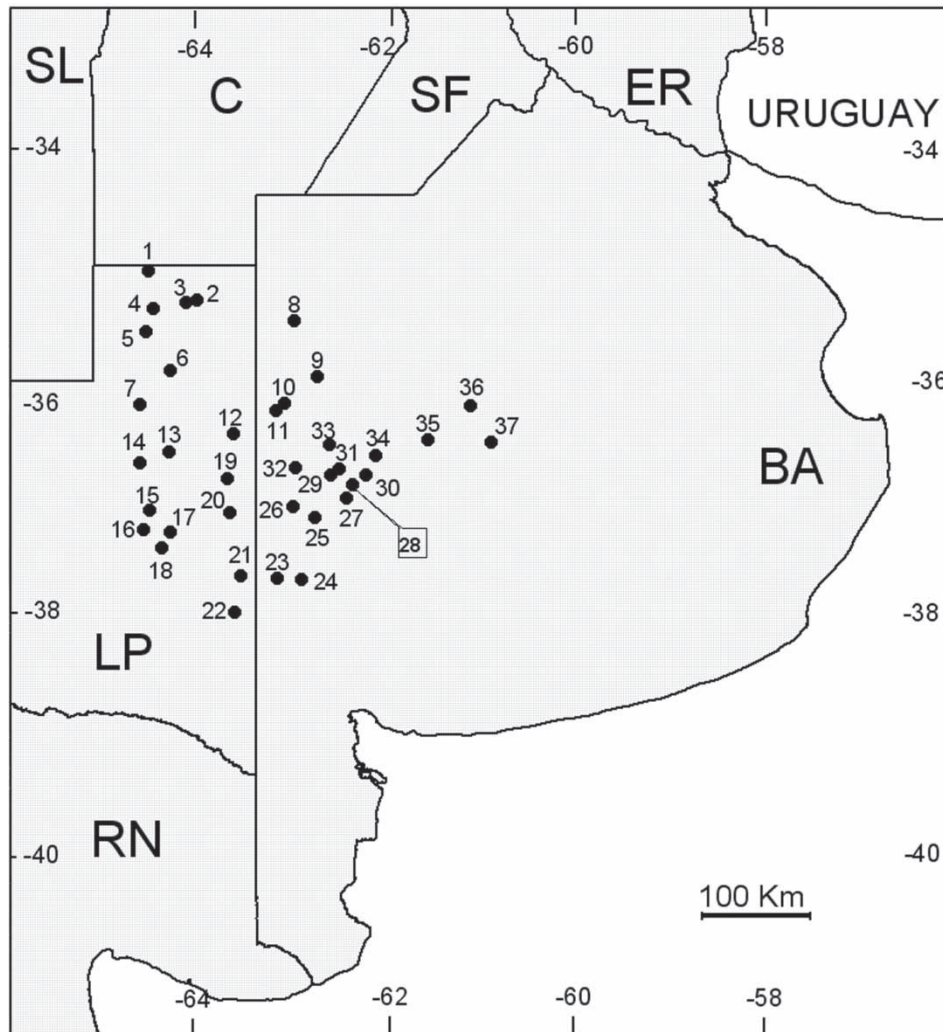
Figure 3.　The 37 localities of the Pampas region where grasshoppers infected with *Paranosema locustae* have been detected. **1**, Quetrequén; **2**, Ojeda; **3**, Alta Italia; **4**, Ingeniero Luiggi; **5**, Caleufú; **6**, Eduardo Castex; **7**, El Destino; **8**, Rivadavia; **9**, Trenque Lauquen; **10**, Bocayuva; **11**, Pellegrini; **12**, Lonquimay; **13**, Santa Rosa; **14**, Boliche El Araña; **15**, Quehué; **16**, Utracán; **17**, Padre Buodo; **18**, Gamay; **19**, Riglos; **20**, Macachín; **21**, Guatraché; **22**, San Martín; **23**, Darregueira; **24**, Azopardo; **25**, Carhué; **26**, Villa Sauri; **27**, Guaminí; **28**, Alamos; **29**, San Fermín; **30**, Estación Bonifacio; **31**, Casbas; **32**, Salliqueló; **33**, Garré; **34**, Luro; **35**, Pirovano; **36**, Bolívar; **37**, Blancagrande. **BA**, Buenos Aires; **C**, Córdoba; **ER**, Entre Ríos; **LP**, La Pampa; **SF**, Santa Fe; **SL**, San Luis; **RN**, Río Negro.

Ewen, and Onofriechuk 1987), its geographic distribution is intimately interwoven with its host range. Only where susceptible hosts are present is *P. locustae* expected to be able to persist. Regardless of the fact that demonstrating absolute freedom from a multihost disease agent in communities of a large area is virtually unattainable, the trend or pattern of distribution observed in the occurrence of *P. locustae* in the
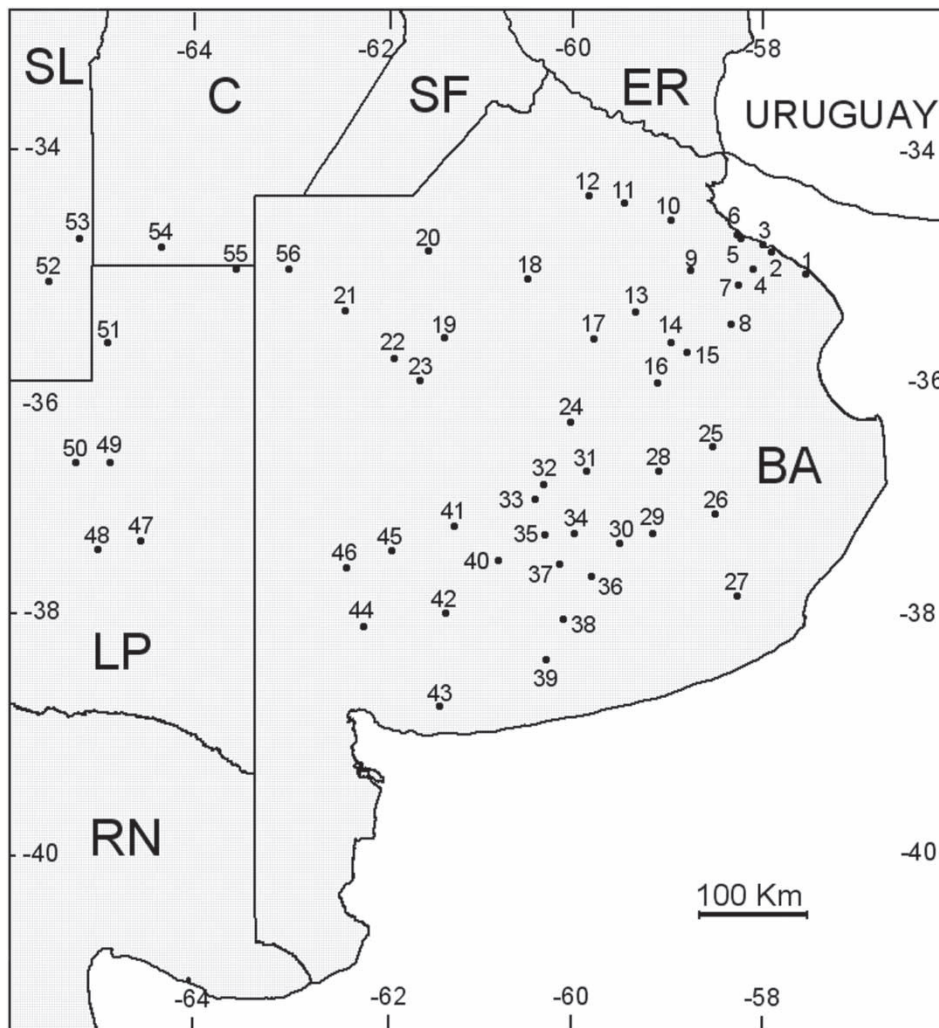
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Figure 4. The 56 localities of the Pampas region where *Paranosema locustae* has never been detected. **1**, Magdalena; **2**, Berisso; **3**, Punta Lara; **4**, Etcheverry; **5**, Berazategui; **6**, Quilmes; **7**, Brandsen; **8**, Ranchos; **9**, Cañuelas; **10**, General Rodríguez; **11**, San Andrés de Giles; **12**, Carmen de Areco; **13**, Roque Pérez; **14**, Gorchs; **15**, La Chumbiada; **16**, Las Flores; **17**, Saladillo; **18**, Bragado; **19**, Carlos Casares; **20**, Lincoln; **21**, Carlos Tejedor; **22**, Pehuajó; **23**, Girondo; **24**, Tapalqué; **25**, Udaquiola; **26**, Ayacucho; **27**, Balcarce; **28**, Rauch; **29**, Tandil; **30**, María Ignacia; **31**, Azul; **32**, Olavarría; **33**, Empalme Querandíes; **34**, Chillar; **35**, El Luchador; **36**, Benito Juárez; **37**, Coronel Bunge; **38**, González Chávez; **39**, Tres Arroyos; **40**, Laprida; **41**, General Lamadrid; **42**, Coronel Pringles; **43**, Calvo; **44**, Tornquist; **45**, Coronel Suárez; **46**, Pigué; **47**, General Acha; **48**, El Carancho; **49**, El Tropezón; **50**, El Durazno; **51**, La Maruja; **52**, Bagual; **53**, Buena Esperanza; **54**, Huinca Renancó; **55**, Larroude; **56**, General Villegas. **BA**, Buenos Aires; **C**, Córdoba; **ER**, Entre Ríos; **LP**, La Pampa; **SF**, Santa Fe; **SL**, San Luis; **RN**, Río Negro

Table 1. The 43 species of grasshoppers collected and examined from the Pampas region between 1994 and 2011, their geographic range according to the eight acridological zones of Liebermann (1972), and their presence in samples according to categories of Mariottini (2009).

Family, subfamily, species	Liebermann's acridological zone								Presence in samples	
	I	II	III	IV	V	VI	VII	VIII	Distribution	Abundance
Acrididae: Melanoplinae										
Atrachelacris unicolor	+	+	+	N	+	+	N	+	Nw	I
*Baeacris pseudopunctulatus**	+	+						+	W	F
*Baeacris punctulatus**	+	+	+		+	+		+	W	F
*Dichroplus conspersus**	+	+	+		+	+	N	+	Nw	I
*Dichroplus elongatus**	+	+	+	+	+	+	+	+	Vw	Va
Dichroplus exilis	+	+	+		+	+			Nw	I
Dichroplus maculipennis	S	+	+	+			+		L	A
*Dichroplus pratensis**	+	+	+	+	+	+	+	+	Vw	A
Dichroplus schulzi	+	+	+		+	+		+	Nw	I
*Dichroplus vittatus**	+	+	+	N	+	+	N		L	F
*Leiotettix pulcher**		+	+			+		+	L	F
*Neopedies brunneri**	+	+	+				N		L	F
*Ronderosia bergi**	+	+	+		+	+		+	L	F
*Ronderosia forcipata**		+	+					+	Nw	I
Scotussa cliens		+	+					+	Nw	I
*Scotussa daguerrei**		+							Nw	I
*Scotussa lemniscata**		+	+	N				+	W	A
Acrididae: Gomphocerinae										
Amblytropidia australis	+	+	+		+	+		+	L	F
Borellia bruneri	+	+	N	+				+	L	F
Borellia pallida		+	+	N				+	Nw	I
*Euplectrotettix schulzi**	+	+	+						Nw	F
Euplectrotettix ferrugineus		+	+				N	+	Nw	I
Laplatacris dispar		+	+		+		N	+	Nw	I
*Rhammatocerus pictus**	+	+	+		+	+		+	L	F
*Sinipta dalmani**	S	+	+					+	L	I
*Staurorhectus longicornis**	+	+	+	N	+		N	+	W	A
Acrididae: Oedipodinae										
*Trimerotropis pallidipennis**	+	+	+	+	+	+	+	+	Nw	I
Acrididae: Copiocerinae										
*Aleuas lineatus**	S	+	+			N	+	+	W	A
Aleuas vitticolis		+	+				+		Nw	I
Acrididae: Acridinae										
*Allotruxalis gracilis**	+	+	+		+	+	N	+	L	F
Covasacris pallidinota	S	+					+	+	L	F
Metaleptea adspersa	+	+	+					+	Nw	I
Orphulella punctata	+	+	+	+	+	+	+	+	Nw	I
Parorphula graminea		+	+	+			+		Nw	F

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Table 1 (*Continued*)

Family, subfamily, species	Liebermann's acridological zone								Presence in samples	
	I	II	III	IV	V	VI	VII	VIII	Distribution	Abundance
Acrididae: Leptysminae										
Leptysma argentina		+		+				+	Nw	I
Tucayaca gracilis	+	+	+		+	+		+	Nw	I
Romaleidae: Romaleinae										
Chromacris speciosa	+	+	+	N	+	+	N	+	Nw	I
*Diponthus argentinus**	N	+	+					+	Nw	I
Staleochlora viridicata		+	+					+	Nw	I
Xyleus laevipes	N	+	+	+				+	Nw	I
*Zoniopoda tarsata**	+	+	+					+	L	F
Zoniopoda omnicolor	+	+	+		+	+	+	+	Nw	I
Ommexechidae: Ommexechinae										
Graea horrida	+	+	+	N	+		N	+	Nw	I

Note: Species followed by an asterisk (*) indicate that field infections by *Paranosema locustae* have been detected in the Pampas. (+) denotes presence. A, abundant; F, frequent; I, infrequent; L, limited; N, North; Nw, narrow; S, South; Va, very abundant; Vw, very wide; W, wide.

Pampas region after more than 30 years after introductions appears clear (i.e. well-defined area sustained over an extended period of time).

In our view, considering the available knowledge on the pathogen [see reviews by Johnson (1997) and Lange and Cigliano (2005) for salient characteristics] and on the actual or potential hosts in Argentina, the observed distribution seems somewhat unexpected in the sense that it should probably be wider, covering most of the Pampas, not just the western section. For instance, it is not known which factors seem to have allowed *P. locustae* to establish and persist or expand within the recorded establishment area of the western Pampas but apparently not in the southern and northeastern Pampas, areas where there even were introduction localities and grasshopper species of known susceptibility occur (Carbonell et al. 2006; Lange 2010). The apparent lack of occurrence of *P. locustae* in the southern Pampas is particularly intriguing because this area is similar to the establishment area not only in terms of the species composition of grasshopper communities, which is probably the most important factor due to the obligatory parasitic nature of the pathogen, but also in climate, physiography and land use (Liebermann 1972; Cabrera and Willink 1973; Torrusio, Cigliano, and De Wysiecki 2002; Carbonell et al. 2006). We can hypothesise on just one conceivable reason for the observed pattern of distribution: the establishment of *P. locustae* in the western Pampas could be the result of the introduction in Casbas, where the pathogen was applied alone (i.e. not in combination with Carbaryl). The simultaneous use of a chemical insecticide in the other introduction localities may have had an antagonistic effect on *P. locustae* by killing most individuals of *P. locustae*-susceptible grasshopper species over a short period of time (hours to days, Onsager 1988; Foster et al. 1998), depriving the pathogen of opportunities to either initiate infections or complete development after infection initiation.

The recording of infections during the 2011 season in *A. lineatus* and *D. conspersus* expands the known field host range of *P. locustae* in Argentina to 22 species of grasshoppers (Table 1). Although both newly recorded infected species had been shown to be susceptible to *P. locustae* in laboratory bioassays (Luna, Henry, and Ronderos 1981), finding infected individuals in the field so many years after the introductions is of much more relevance because laboratory or physiological host range may be misleading for predicting actual field or ecological host range. The latter is usually more restricted than the former (Solter and Becnel 2007). Additionally, the detections suggest that most grasshopper species of the Pampas may be susceptible and confirm the patchiness pattern of occurrence that *P. locustae* tends to show in contrast to more epizootiologically stable pathogens like native *L. dichroplusae* (Lange 2003b; Sokolova, Lange, and Fuxa 2007). In this sense, it is remarkable that after years of examining hundreds of individuals from many localities of the widely distributed and abundant *A. lineatus,* infections were recorded for the first time only in 2011. Patchiness may also possibly account for the absence of detections of *P. locustae* in *D. maculipennis* in the Pampas while infections in this species were recorded in the two establishment areas of north-western Patagonia (Lange and Cigliano 2005, 2010; Lange 2010).

Regardless of the beneficial effect that *P. locustae* may have or have had as a pest biocontrol agent in the western Pampas, its current status, both in terms of host species affected and area, appears to depict its capacity for invasiveness. Through its ability to develop in a variety of different hosts of differing susceptibilities and varying commonness and abundance, *P. locustae* might be disrupting the grasshopper communities in the establishment area. Unfortunately, data on grasshopper diversity and abundance prior to the introductions that would allow for a quantitative comparison between pre- and post-introduction scenarios are not available. In addition, few studies on introduced microsporidia are available. Recently, Solter et al. (2010) showed no effects of two introduced microsporidia on non-target Lepidoptera in Slovakia, albeit both species appear to have narrow host ranges as opposed to *P. locustae.*

The establishment of a previously absent multihost pathogen in sympatric populations of novel host species is a highly complex issue driven by numerous factors such as host susceptibility, availability, and interactivity, the pathogen's transmission modes and genetic variability (Woolhouse, Taylor, and Haydon 2001; Holt, Dobson, Begon, Bowers, and Schauber 2003). Of primary importance when attempting an assessment of the risks faced by each grasshopper species in the establishment area of *P. locustae* is actual or confirmed field susceptibility. In addition to recorded field susceptibility, other two factors of possible central relevance are the geographic range and the rarity of a species. Susceptible species with restricted geographic ranges and small populations should be under higher risk than those with wide ranges and large populations, although some formerly widespread and abundant species have become extinct elsewhere by anthropogenic impacts (Samways and Lockwood 1998). Even if individuals of a susceptible host species are numerically too low for allowing persistence of *P. locustae*, other grasshopper species may act as source of infection or reservoirs. Under this perspective, *Scotussa daguerrei* Liebermann emerges as the only species under possible risk of extinction. It was seldom present during sampling (categorised as narrowly distributed and infrequent), field infections have been found and its known geographic range is restricted to the western Pampas (Table 1). All other

species in which infections have been detected have wide geographic ranges, extending well beyond the establishment area (Table 1), and with the exception of *Diponthus argentinus* Pictet and Saussure and *D. conspersus* (both categorised as narrowly distributed and infrequent), were common in samples. Because of their apparent low numbers, these last two species may also be under risk within the establishment area (i.e. may face local extinction).

For those species in which field infections have not being recorded (Table 1), close relatedness to a field-infected species (same genus, same subfamily) should be an appropriate predictor. Species of Subfamilies Melanoplinae, Oedipodinae and Gomphocerinae are generally acknowledged to be more susceptible to *P. locustae* than species in other Subfamilies (Lange and Cigliano 2005), while the Acridinae are believed to be less susceptible, possibly because of their low content of adipose tissue (Henry and Oma 1981). Nothing is known on the susceptibility to *P. locustae* of the five species of Family Ommexechidae, a group of grasshoppers endemic to South America (Ronderos 1979), that have been reported in the establishment area. Although all five species (*Neuquenina fictor* (Rehn), *Clarazella patagona* Pictet and Saussure, *Spathalium audouini* Ronderos, *Calcitrena maculosa* Eades, *Graea horrida* Philippi) have geographic ranges that extend far from the establishment area, they were not collected except for a few individuals of *G. horrida*. The same situation (unknown susceptibility) applies to the seven species of the endemic Neotropical Subfamily Leptysminae of the Family Acrididae (Cigliano and Lange 1998). Although the ranges of *Cylindrotettix dorsalis* (Burmeister), *Leptysma argentina* Bruner, *Leptysma gracilis* Bruner, *Leptysma pallida* Giglio Tos, *Tucayaca gracilis* Giglio Tos, *Tucayaca parvula* Roberts and *Haroldgrantia lignose* Carbonell et al. extend well away from establishment area (Carbonell et al. 2006), they are not prevalent grasshoppers. Only a few specimens of *L. argentina* and *T. gracilis* were sampled.

In assessing the threat posed by *P. locustae* to grasshopper diversity in the Pampas, it should be kept in mind that a number of other anthropogenic factors must have been gradually but steadily affecting grasshopper communities in the region for many decades. The original grasslands where native grasshoppers evolved and diversified turned into a different environment. From radical changes in land use and species additions and deletions (whether domestic or wild, microbial or not) to massive chemical input (pesticides, fertilisers), the grasslands were changed into an intensely exploited new environment: an agroecosystem (Ghersa, Martínez Ghersa, and León 1998). Habitat destruction and introduction of alien species have been blamed as responsible for the extinction of *Melanoplus spretus* (Walsh), the most serious agricultural pest in western North America before 1900 (Lockwood and Debrey 1990). In this sense, the establishment of *P. locustae* should be viewed as just one more additional factor altering the grasshopper communities that may be perceived as beneficial or deleterious according to the perspective considered (farmers-ranchers or conservationists). We anticipate conducting further, more intense and wider monitoring in order to detect possible expansions in host and geographic ranges of *P. locustae* in grasshoppers of the Pampas.

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