

Long-term Patterns of Occurrence of *Nosema locustae* and *Perezia dichroplusae* (Microsporidia) in Grasshoppers (Orthoptera: Acrididae) of the Pampas, Argentina

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Summary. The long-term (through the years) occurrence of the two known microsporidia parasitizing grasshoppers of the subfamily Melanoplinae in the Pampas of Argentina (vertically transmitted, native *Perezia dichroplusae* and horizontally transmitted, introduced *Nosema locustae*) was monitored at 13 localities from 1995 to 2003. *Nosema locustae* occurred at nine localities, and *P. dichroplusae* at five. There was almost no overlap of occurrence, and mixed infections were not registered. The presence and prevalences (1% to 6.1%) of *P. dichroplusae* were relatively stable over the years in all localities while *N. locustae* alternated years of presence and absence, and prevalences fluctuated markedly from 1.8% up to 41%. On four instances, each at a different locality, unusually high prevalences (epizootics) of *N. locustae* were recorded. Natural epizootics of such magnitudes are not known for *N. locustae* in other regions of the world.

Key words: enzootic, epizootic, horizontal transmission, Melanoplinae, prevalence, vertical transmission.

INTRODUCTION

Two species of microsporidia, *Nosema locustae* Canning and *Perezia dichroplusae* Lange, are known to occur in grasshopper species of the Argentine Pampas. *Nosema locustae*, a pathogen of the adipose tissue

with an unusually broad host range (today known to include more than 100 species, both natural and experimental; Brooks 1988, Henry 1990, Lange 2002), was experimentally introduced as a grasshopper biological control agent from North America at several localities between 1978 and 1982, and became established in the western Pampas (Lange and de Wysiecki 1996; Lange 2001, 2002). Recent ultrastructural studies have shown that the morphology and development of *N. locustae* remain essentially the same regardless of the host species involved (Sokolova and Lange 2002). Up to the present, infections by *N. locustae* in the Pampas have been detected in grasshoppers belonging to 16 species,

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mostly melanoplinae (subfamily Melanoplineae) (Table 1), which include some of the most harmful species for crops and forage (COPR 1982, Lange *et al.* 2003). Although vertical transmission have been shown to occur in *N. locustae* (Henry and Oma 1981, Raina *et al.* 1995), the primary route of transmission is horizontal through ingestion of propagules (spores), a characteristic that, together with its broad host range, was central for its development as a microbial control agent (Henry and Oma 1981, Johnson 1997, Lockwood *et al.* 1999, Lange 2002).

Perezia dichroplusae, a pathogen of the Malpighian tubules, is a native microsporidium of the melanopline grasshopper *Dichroplus elongatus* Giglio - Tos (Lange 1987a, b), normally one of the most common and harmful grasshopper species in the Pampas (Cigliano and Lange 1999, Cigliano *et al.* 2003). All attempts at transmitting *P. dichroplusae* to its natural host and other closely related species have systematically failed (Lange 1989, and unpublished results). Experimental studies have shown that its main mode of transmission is vertical through the female line (matroclinal) and within the egg (transovarial) (Lange 1997).

The purpose of this investigation was to record the long-term presence/absence of the two microsporidia in grasshoppers of the Pampas, trying to establish patterns of occurrence, and relating these to own characteristics of the pathogens.

MATERIALS AND METHODS

The study extended from 1995 to 2003, and included 13 localities of grasshopper collection. All localities but one (Benito Juárez) were in the western Pampas, eight in Buenos Aires province (Benito Juárez, Carhué, Casbas, Coronel Suárez, General Lamadrid, Laprida, Rivadavia, Trenque Lauquen), and five in La Pampa province (Alta Italia, Eduardo Castex, Ojeda, Riglos, Santa Rosa) (Fig. 1). Five of these localities (Casbas, Coronel Suárez, General Lamadrid, Riglos, Santa Rosa) were in close vicinity of five of the original introduction sites of *N. locustae* (Casbas in 1979, Coronel Suárez in 1981, General Lamadrid in 1981, Macachín in 1982, and Santa Rosa in 1982). Sampling was done always by the same two surveyors with sweep nets (diameter: 40 cm; depth: 75 cm; angle of sweep: 180°) along transects in natural grasslands and improved pastures in early February of each year, a time when grasshopper populations of most species are at their peak as young adults or older nymphs (COPR 1982, Lange *et al.* 2003). Larson *et al.* (1999) have shown that sweep sampling provides a fairly accurate estimate of the relative abundance of most grasshopper species. Since *N. locustae* and *P. dichroplusae* cause, as most microsporidia of insects do (Becnel and Andreadis 1999), chronic (i.e., prolonged course of infection) rather than acute diseases (Henry and Oma 1981, Lange 1987b), and most grasshopper

species involved in the study are univoltine (only the species of *Baeacris* can be bivoltine) with winter egg diapause (COPR 1982), the time of year when sampling was conducted should provide an acceptable estimate of the yearly occurrence of both pathogens. One site (Carhué) was visited every year, three sites (Riglos, Santa Rosa, Trenque Lauquen) were visited eight years, one site (Benito Juárez) was visited six years, one site (Coronel Suárez) was visited five years, one site (Rivadavia) was visited four years, three sites (Alta Italia, Eduardo Castex, Ojeda) were visited three years, and three sites (Casbas, General Lamadrid, Laprida) were visited twice. For estimating prevalences of *P. dichroplusae*, each sample consisted of all the individuals of *D. elongatus*, the natural host of *P. dichroplusae*, that were captured with three hundred sweeps. Since previous studies (Lange and de Wysiecki 1999, Lange 2003) have shown that argentine melanopline species of grasshoppers have similar susceptibilities to *N. locustae*, for estimating prevalences of this pathogen each sample consisted of all the melanoplinae captured in the three hundred sweeps, namely *Baeacris punctulatus* Thunberg, *Baeacris pseudopunctulatus* Ronderos, *Dichroplus conspersus* Bruner, *Dichroplus elongatus*, *Dichroplus maculipennis* Blanchard, *Dichroplus pratensis* Bruner, *Dichroplus vitattus* Bruner, *Leiotettix pulcher* Rehn, *Neopedies brunneri* (Giglio-Tos), *Ronderosia forcipatus* (Rehn), *Scotussa lemniscata* (Stal), and *Scotussa daguerrei* Liebermann. Grasshoppers collected were placed in cages, and immediately taken to the laboratory where they were frozen at -32°C for later examination.

For disease diagnosis, frozen grasshoppers were thawed and examined by the homogenization method as described by Henry *et al.* (1973) or, less frequently, by ventral, longitudinal dissection and examination of tissues and organs as fresh preparations under phase contrast microscopy (x400; x1000) (Undeen and Vávra 1997). When using the homogenization method, trace infections (i.e. observation of only one spore per microscopic field; Henry *et al.* 1973) were not considered, avoiding, in doing so, biases that may occur due to contamination of homogenizers. *Nosema locustae* and *P. dichroplusae* are readily distinguished by several characters in addition to the organs affected. *Nosema locustae* has much larger spores (mean: 5.2 by 2.8 µm) with little variation in size except for some macrospores, developmental stages (meronts, sporonts) are mostly rounded and always with intimately paired nuclei (diplokaryotic), and sporogony is disporoblastic (Figs 2-4) (Canning 1953, Henry and Oma 1981, Sokolova and Lange 2002). Spores of *P. dichroplusae* are much smaller (mean: 3.5 by 1.5 µm) and vary greatly in size, moniliform developmental stages are present (sporogonial plasmodia), and sporogony is polysporoblastic (Figs 5-7) (Lange 1987a).

RESULTS

Nosema locustae occurred in nine out of the 13 sites (Alta Italia, Carhué, Casbas, Eduardo Castex, Riglos, Rivadavia, Ojeda, Santa Rosa, and Trenque Lauquen) while *P. dichroplusae* was present in five sites (Benito Juárez, Carhué, Coronel Suárez, General Lamadrid, and Laprida) (Table 2). The occurrence of both pathogens in a given site was registered only twice (1996, 1997) at one site (Carhué). Mixed infections (i.e. a single grass-

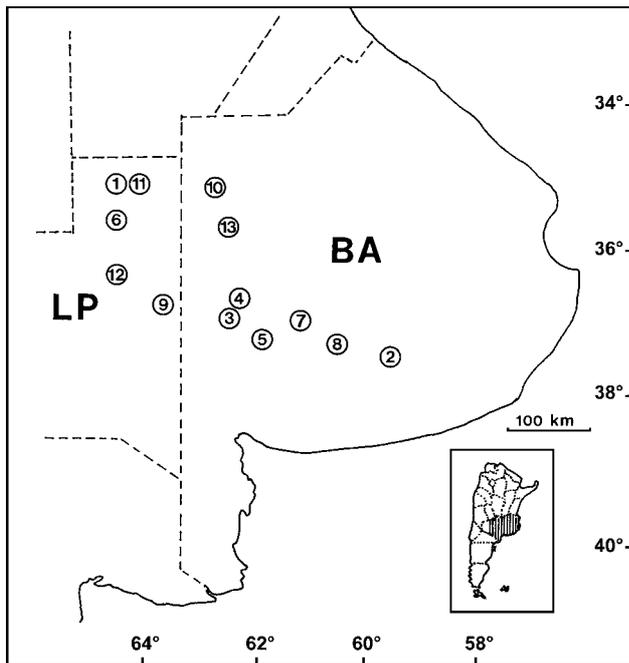


Fig. 1. The 13 sites of grasshopper collection in the Pampas. 1 - Alta Italia, 2 - Benito Juárez, 3 - Carhué, 4 - Casbas, 5 - Coronel Suárez, 6 - Eduardo Castex, 7 - General Lamadrid, 8 - Laprida, 9 - Riglos, 10 - Rivadavia, 11 - Ojeda, 12 - Santa Rosa, 13 - Trenque Lauquen, BA - Buenos Aires province, LP - Pa Pampa province.

Table 1. Species of grasshoppers in which infections of *Nosema locustae* have been found in the western Pampas of Argentina.

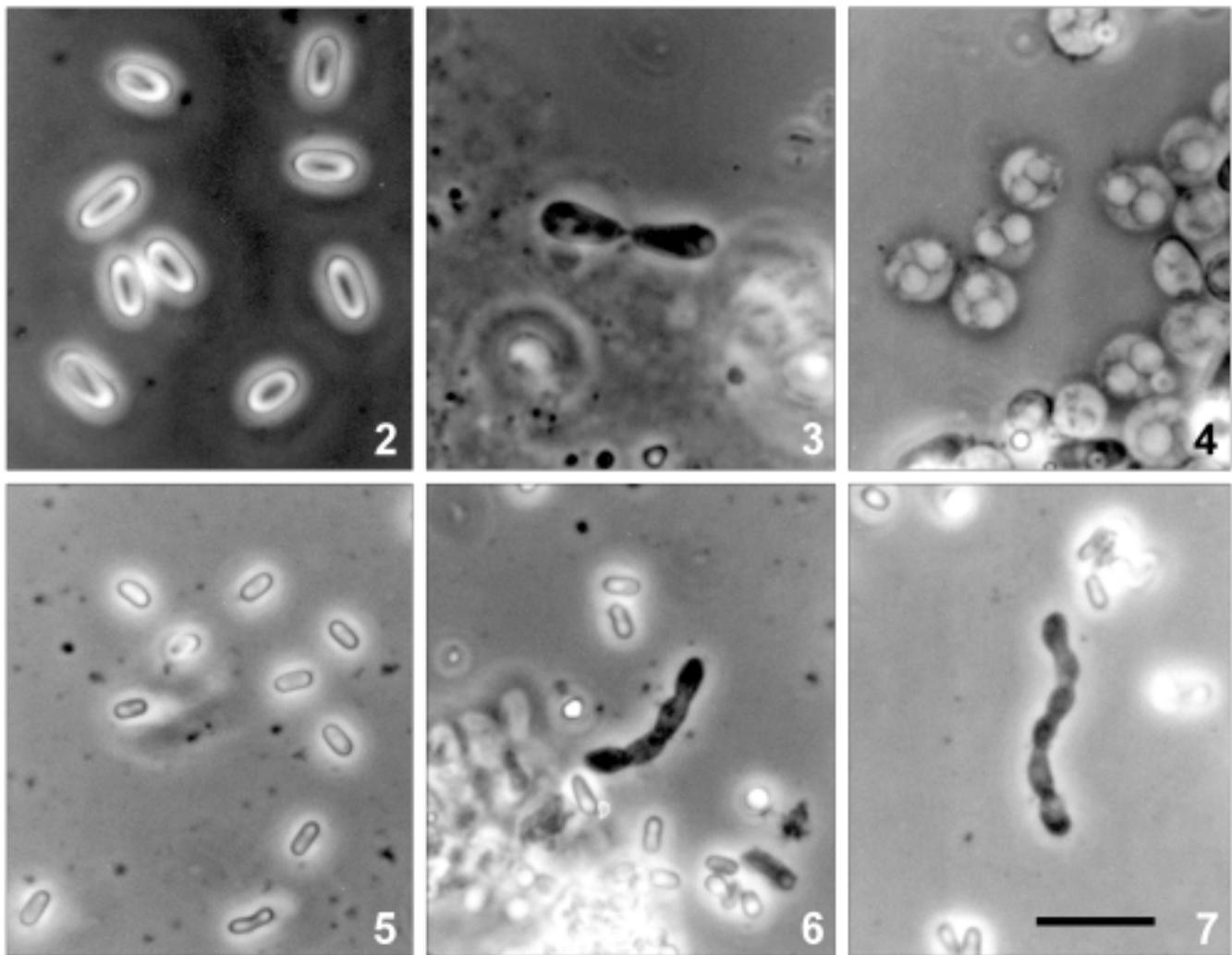
Acrididae
Acridinae
<i>Allotruxalis strigata</i> (Bruner)
<i>Euplectrotettix shulzii</i> Bruner
Gomphocerinae
<i>Rhammatocerus pictus</i> (Bruner)
<i>Staurorhectus longicornis</i> Giglio - Tos
Melanoplinae
<i>Baeacris punctulatus</i> (Thunberg)
<i>Baeacris pseudopunctulatus</i> Ronderos
<i>Dichroplus elongatus</i> Giglio - Tos
<i>Dichroplus pratensis</i> Bruner
<i>Dichroplus vittatus</i> Bruner
<i>Leiotettix pulcher</i> Rehn
<i>Neopedies brunneri</i> (Giglio - Tos)
<i>Ronderosia forcipatus</i> Rehn
<i>Scotussa lemniscata</i> (Stal)
<i>Scotussa daguerrei</i> Liebermann
Romaleidae
Romaleinae
<i>Diponthus argentinus</i> Pictet et Saussure
<i>Zoniopoda tarsata</i> (Serville)

hopper individual infected with both pathogens) were never found. In all five sites where *P. dichroplusae* was

present, it occurred in all years but one (Coronel Suárez in 2002, albeit the sample size was too small) at fairly stable prevalences that ranged from a minimum of 1% at Benito Juárez in 1995 to a maximum of 6.1% at Carhué in 2001. On the contrary, in all nine sites where *N. locustae* occurred, it alternated years of presence with years of absence (only at Riglos presence was almost permanent). Prevalences of *N. locustae* ranged from a minimum of 1.8% at Trenque Lauquen in 2001 to a maximum of 41% at Riglos in 1996. Aside from Riglos in 1996, there were three other instances where unusually high prevalences of *N. locustae* were detected: 37.5% at Trenque Lauquen in 1996, 21.8% at Rivadavia in 1997, and 20% at Casbas in 2003. The high prevalence registered at Rivadavia in 1997 was followed by three years (1998, 1999, 2002) of absence of *N. locustae* although hosts were still abundant. The high prevalence at Casbas in 2003 was preceded by a year (2002) of absence of *N. locustae* although hosts were relatively abundant.

DISCUSSION

Once a space-time context has been established (in this study, five localities for *P. dichroplusae* and nine for *N. locustae* along two to nine years), the state at which a pathogen occurs in the host population normally fluctuates along a range or continuum with two opposite ends: the enzootic and epizootic states (Fuxa and Tanada 1987). An enzootic disease is usually of low prevalence and is constantly present in the host population while an epizootic disease, on the contrary, is sporadic and limited in duration, and characterized by abrupt changes in prevalence. There are pathogens that tend to occur enzootically, and others that are more prone to vary sharply between states. The long-term occurrence registered for *N. locustae* and *P. dichroplusae* in grasshoppers of the Pampas seems to fit well in this framework. In those localities where *P. dichroplusae* was present, it occurred constantly season after season at relatively low, stable prevalences in what can be considered a typical enzootic state. On the other hand, *N. locustae* exhibited at least four instances in which prevalences that can be considered epizootics (i.e., unusually large number of cases of the disease; Tanada and Kaya 1993) were observed (41% at Riglos in 1996; 37.5% at Trenque Lauquen in 1996; 21.8% at Rivadavia in 1997; 20% at Casbas in 2003), and several instances of sudden “appearances” or “disappearances” of the



Figs 2-7. Developmental stages and spores of *Nosema locustae* and *Perezia dichroplusae* as fresh preparations under phase contrast microscopy. **2** - mature spores of *N. locustae*, **3** - disporoblastic sporogony of *N. locustae*, **4** - diplokaryotic stages (meronts) of *N. locustae*; **5** - mature spores of *P. dichroplusae*, **6, 7** - moniliform plasmodia and polysporoblastic sporogony of *P. dichroplusae*. Scale bar 10 μ m.

disease in a given locality, most notably at Casbas and Rivadavia, respectively.

Among the many factors that govern the occurrence of an infectious disease in a host population, the ability of the pathogen to be transmitted is of central relevance, and may greatly influence the way the pathogen occurs. In those host-pathogen relationships where horizontal transmission predominates, prevalence typically varies markedly, often culminating in epizootics, while in those relationships where vertical transmission prevails, prevalence is usually maintained in a steady, enzootic state (Andreadis 1987). The predominant transmission routes of *N. locustae* (horizontal) and *P. dichroplusae* (verti-

cal) appear to account for the long-term occurrence that was observed in the Argentine Pampas.

Due to the efficient horizontal transmission that characterizes *N. locustae* (Henry and Oma 1981, Henry 1985, Lange 2003), and the recognized high susceptibility to infection of the melanoplinae (Henry 1969, Henry *et al.* 1973, Bomar *et al.* 1993, Lange 2003), the occurrence of the observed epizootics in grasshoppers of the Pampas should probably be considered as an expected phenomenon. However expected, the occurrence of such epizootics are still intriguing. Natural epizootics of *N. locustae* (i.e. those not immediately following its application as a biocontrol agent) of the magnitude

Table 2. Yearly occurrence of *Nosema locustae* in melanopline grasshoppers (top row at each site and year) and *Perezia dichroplusae* in *Dichroplus elongatus* (second row) at 13 sites in the Pampas. The first number indicates prevalence (% of infection) and the one between parenthesis is the sample size (n).

	1995	1996	1997	1998	1999	2000	2001	2002	2003
A. Italia			2.9 (103) 0 (6)	0 (101) 0 (13)	0 (26) 0 (11)				
B. Juárez	0 (750) 1 (584)				0 (98) 2.8 (71)	0 (329) 1.2 (257)	0 (348) 1.7 (288)	0 (400) 1.3 (318)	0 (340) 2.2 (240)
Carhué	0 (67) 2.2 (90)	2.8 (36) 4 (25)	8 (25) 5.9 (17)	0 (62) 1.3 (74)	0 (44) 3.8 (26)	0 (156) 1.9 (105)	0 (42) 6.1 (33)	0 (47) 4.1 (121)	0 (213) 5.8 (138)
Casbas								0 (97) 0 (15)	20 (190) 0 (8)
C. Suárez		0 (302) 3.4 (156)			0 (257) 3.2 (157)	0 (310) 2.1 (94)		0 (180) 0 (6)	0 (188) 1.7 (59)
E. Castex	4.5 (312) 0 (257)	2.4 (84) 0 (25)	0 (150) 0 (256)						
G. Lamadrid		0 (205) 1.5 (129)				0 (175) 1.2 (87)			
Laprida		0 (371) 2.1 (136)				0 (168) 3.1 (127)			
Riglos	9 (379) 0 (79)	41 (166) 0 (26)	11.4 (44) 0 (25)	11.1 (36) 0 (8)	5.4 (55) 0 (0)	2 (195) 0 (4)		1 (95) 0 (39)	0 (61) 0 (60)
Rivadavia			21.8 (350) 0 (307)	0 (254) 0 (215)	0 (74) 0 (74)			0 (117) 0 (108)	
Ojeda			1.2 (159) 0 (44)	0 (89) 0 (35)	0 (92) 0 (60)				
S. Rosa	0 (274) 0 (104)	3 (269) 0 (96)	8.2 (97) 0 (90)	0 (215) 0 (147)	0 (171) 0 (160)	0 (34) 0 (7)	0 (53) 0 (0)	0 (20) 0 (12)	
T. Lauquen	3.8 (158) 0 (85)	37.5 (40) 0 (22)	15.8 (19) 0 (38)	0 (25) 0 (16)	2.5 (40) 0 (14)	0 (70) 0 (11)	1.8 (55) 0 (16)	0 (45) 0 (29)	

observed in the Pampas are not known in other regions of the world where *N. locustae* is native. In North America, where melanoplins are often numerically dominant in grasshopper communities, prevalences are normally less than 1 % with rare peaks of 2 to 5 % (Henry and Oma 1981, Ewen 1983). In India, prevalences were 1 to 2 %, sporadically reaching 5 % (Raina *et al.* 1987). Almost 10 % has been reported in the South Africa record but the sampled grasshoppers were kept in cages for some time before diagnosis (Whitlock and Brown 1991). Even repeated field applications of

N. locustae in North America did not result in significant increases of prevalence (Johnson and Dolinsky 1997). Reasons that would explain the occurrence of epizootics of *N. locustae* in the Pampas are not at hand but it should be kept in mind that its introduction resulted in a number of new host-pathogen associations (Hokkanen and Pimentel 1984, Hajek *et al.* 2000) with unpredictable outcomes.

Another intriguing aspect is how *N. locustae* “appears” and “disappears” at a given locality through the seasons. In this sense, persistence and dissemination of

a microsporidian pathogen in host populations is known to be governed by many factors, such as modes of transmission, behavior and movements of hosts and nonhost carriers, and physical agents (Andreadis 1987), and a number of possible scenarios would account for the sudden "appearances" of *N. locustae* in a given locality. In *N. locustae*, the main horizontal route of transmission is complemented by vertical transmission (Henry and Oma 1981, Raina *et al.* 1995), a way that may transfer infection from one season to the following. None of the grasshopper species known to be affected by *N. locustae* in the Pampas is an overwintering species (COPR 1982). Most of the grasshoppers affected in the Pampas have winged adults, and an infected grasshopper still capable of flying may disperse the infection. Among potential nonhost carriers, many insectivorous and opportunistic birds are known to prey on grasshoppers in the general area of establishment of *N. locustae* in the Pampas, notably the Swainson's hawk, *Buteo swainsoni*, the large flocks of which feed almost exclusively on grasshoppers (Goldstein *et al.* 1999). Physical agents such as wind and water would probably play a very minor role or no role at all in the spread of *N. locustae*. Spores of microsporidia are normally short lived in the environment (Becnel and Andreadis 1999), and Germida *et al.* (1987) found that *N. locustae* spores persisted very poorly in soils, and do not persist at all in vegetation of treated fields.

The absence of *N. locustae* in a locality where it was present in the previous season and where susceptible hosts were still abundant (notably Carhué, Rivadavia and Santa Rosa) remains unexplained. One would expect that as long as reasonable numbers of potential hosts are available, *N. locustae* should be able to persist season after season, especially considering that it has a vertical route of transmission.

Finally, another interesting outcome delivered by the study was that there was almost no geographical overlap between records of the two pathogens. *Perezia dichroplusae* was recorded at the southernmost sites (Benito Juárez, Coronel Suárez, General Lamadrid, Laprida) and *N. locustae* at the western sites (Alta Italia, Casbas, Eduardo Castex, Riglos, Rivadavia, Ojeda, Santa Rosa, Trenque Lauquen). Only at Carhué, and is just two seasons (1996, 1997), there were records of both pathogens in different individuals of *D. elongatus* (i.e., not mixed infections). Considering that some of the introductions of *N. locustae* were made where the southernmost sites are, the possibility that this pathogen did not established there because a native microsporidium

(*P. dichroplusae*) was already present can not be ruled out. Antagonistic interactions between *N. locustae* and an undescribed microsporidium have been suggested (Streett and Henry 1984), and Solter *et al.* (2002) have recently demonstrated through experimental work antagonistic interactions for other microsporidia of terrestrial insects.

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