Title: The exotic weevil *Stenopelmus rufinasus* Gyllenhal, 1835 (Coleoptera: Curculionidae) across a "host-free" pond network

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Short title: Exotic weevils in "host-free" ponds

ABSTRACT

The exotic weevil *Stenopelmus rufinasus* Gyllenhal, 1835 (Coleoptera: Curculionidae) across a “host-free” pond network

The presence of the exotic weevil *Stenopelmus rufinasus* (Coleoptera: Curculionidae) is closely related to the occurrence of the exotic red water fern, *Azolla filiculoides*. Here we present the first records of *S. rufinasus* in the Doñana National Park (SW Spain), based on sampling of macroinvertebrates in 91 temporary ponds, including monthly samples of 22, during two successive years (2005-2007). The exotic weevil was present in 21 % of sampled ponds, where the host plant, *A. filiculiodes*, was not detectable. Because *A. filiculoides* can reach high densities in an adjacent area of marsh, we suggest that the occurrence of the exotic weevil in these ponds is a consequence of dispersal from nearby marshes. Our study demonstrates that *S. rufinasus* adults can occur at relatively high densities in ponds where the host plant is not present, suggesting that such apparently “host free” sites may act as stepping stones for the spread of this species.

Key words: *Azolla*, *Stenopelmus*, exotic species, Doñana, freshwaters, marshes, temporary ponds.

RESUMEN

Presencia del gorgojo exótico* Stenopelmus rufinasus *Gyllenhal, 1835 (Coleoptera: Curculionidae) en un sistema de lagunas libre de hospedadores

La presencia de la especie de gorgojo exótico Stenopelmus rufinasus (Coleoptera: Curculionidae) *está íntimamente relacionada con la planta acuática exótica* Azolla filiculoides*. En este estudio se registró por primera vez la presencia de S. rufinasus en el Parque Nacional de Doñana (SO España) tras realizar un muestreo de macroinvertebrados en 91 lagunas que incluye muestreos mensuales de 22 de las mismas durante dos años consecutivos (2005-2007). El gorgojo exótico estuvo presente en el 21 % de las lagunas muestreadas a pesar de que su supuesto hospedador*, A. filiculiodes*, no fue detectado. Dado que* A. filiculiodes *puede alcanzar grandes densidades en la marisma adyacente, sugerimos que la presencia del gorgojo exótico en las lagunas temporales se debe a su dispersión desde la marisma. Este estudio demuestra que individuos adultos de* S. rufinasus *pueden aparecer con densidades relativamente altas en lagunas donde su hospedador potencial no está presente, lo que sugiere que estos sitios libres de hospedador podrían actuar como zonas de paso para la dispersión de la especie*

Palabras clave: Azolla, Stenopelmus, *especies exóticas, Doñana, humedales, marisma, lagunas temporales.*

INTRODUCTION

The aquatic weevil *Stenopelmus rufinasus* Gyllenhal 1835 (Coleoptera: Curculionidae) is native to North America. It was first recorded in Europe (France) in 1898 (Bedel, 1901), expanding in only a few years to the United Kingdom and the Netherlands (http://www.gbif.org). Today, this exotic weevil is quite widespread in Europe also being recorded in Ireland, Germany, Belgium, Italy, Spain and the Ukraine (Pan-European Species directories Infrastructure, PESI). The introduction of *S. rufinasus* to Europe is probably related with the arrival of the exotic red water fern, *Azolla filiculoides* (Lamark 1783), as an ornamental plant in the mid-19th century (Sculthorpe, 1967). This water fern is native to the southern and western USA, today being distributed across most countries in Europe (Delivering Alien Invasive Species Inventories for Europe, http://www.europe-aliens.org) as a harmful invasive alien species causing high impacts on biodiversity in freshwater ecosystems (European Alien Species Information Network, http://easin.jrc.ec.europa.eu). The life cycle of *S. rufinasus* is strongly linked to *Azolla* ferns, the typical host plant in which this weevil oviposits. After emergence, the larvae feed on *Azolla* leaves for 4-7 days until pupation, giving rise an amphibious imago (Richerson & Grigarick, 1967).

This exotic weevil was first detected in the Iberian Peninsula in 2002 (Fernández Carrillo *et al.*, 2005), being found in the surroundings of the Doñana National Park in 2003 (Dana & Viva, 2006). In 2001 the presence of *A. filiculoides* was first reported in the Doñana National Park (García-Murillo *et al.*, 2007), where the fern can reach high densities in the marshes (Fernández-Zamudio, 2011). The Doñana National Park has a high conservation status, being included in the RAMSAR convention since 1982 and designated as a World Heritage Site in 1995 by UNESCO. In this study, we first report the presence of this exotic weevil in the Doñana National Park and note its distribution in a natural pond network in which *A. filiculoides* appears only occasionally.

METHODS

Study area

We sampled 91 ponds distributed across the Doñana National Park (Fig.1, see Appendix 1 for detailed geographical coordinates at www.limnetica.com/internet) to analyse the macroinvertebrate composition of the pond network. This area is located between the mouth of the Guadalquivir River and the Atlantic Ocean in southwest Spain. In this area numerous temporary ponds are located on stable dunes, adjacent to an extensive marsh. Ponds vary greatly in size and permanence; temporary ponds being flooded after heavy rains, usually filling in autumn or winter, and persisting until late spring or early summer.

Sampling procedure

We compiled data from two kinds of samples: i) macroinvertebrate sampling performed from mid-March to mid-June 2007 in a total of 91 ponds encompassing a wide range of hydroperiods (Fig. 1; see Florencio *et al.*, 2011 for details); ii) monthly macroinvertebrate sampling of 22 ponds located in a Biological Reserve in the centre of the Park (Fig. 1) across two complete annual cycles of inundation to desiccation (October 2005 - August 2007; see Florencio *et al.*, 2009 for details). The use of a standardised sampling process for macroinvertebrates allowed us to compare exotic and native weevils between ponds differing in habitat heterogeneity and environmental variables, e.g. pond depth and surface area. The specimens recorded were preserved in

70 % ethanol and identified by one of the authors (DTB). Records of exotic weevils in 2011 were also considered to confirm its occurrence in the marsh. Aquatic plants were also visually recorded in each sampling unit; special attention was paid to the presence of *A. filiculoides*.

Biomass of the exotic red water fern

The biomass of *A. filiculoides* was obtained from monthly sampling of 10 different localities across the marsh area during the study period. Three different replicates of a 0.03 m^2 area were sampled at each locality. Plants were dried at 75 ºC until a constant dry weight was obtained (see Fernandez-Zamudio, 2011 for details).

RESULTS AND DISCUSSION

Distribution of the exotic weevil

In total we detected 48 adult *Stenopelmus rufinasus* across 17 temporary ponds. In these 17 ponds *Azolla filiculoides* was not detectable only occurring in two of the 91 sampled ponds. The exotic weevil was never detected in the South of the park (Fig. 1), where water bodies are few and isolated (see Díaz-Paniagua *et al.*, 2014). In the marsh, two individuals of the exotic weevil were also detected in May 2011, on leaves of *A. filiculoides* collected from the border of the marsh (Fig. 1). In contrast, during our study period, *S. rufinasus* was always recorded in ponds where the specific host plant, *Azolla filiculoides*, was not detectable. All specimens of *S. rufinasus* were collected in May-June, coinciding with the season when *A. filiculoides* was especially productive in the marsh (Fig. 2). Almost all specimens were recorded during 2006-2007, whereas its presence in 2005-2006 was limited to a single pond in May (Fig. 1 & 2). The exotic weevil exhibits excellent dispersal abilities, as it has even been recorded up to 300 km from sites where it was released (Hill, 2003). Therefore we suggest that the occurrence of *S. rufinasus* in the ponds of the park may reflect the seasonal high production of *A. filiculoides* in the marshes, from where adult weevils could have dispersed to the pond network. This could explain the higher abundance of the exotic weevil in 2007, which may be related to the higher production of *A. filiculoides* that year, probably associated with higher rainfall (2005-2006= 468 mm *vs.* 2006-2007= 717 mm). A reduced number of ponds were formed in the park in 2005-2006 as a consequence of the low precipitations (Florencio *et al.*, 2009), which could also have limited the occurrence of the exotic weevil in 2006, when it was only detected in a single pond.

Comparison between exotic and native weevils

In contrast to *S. rufinasus*, only a total of 16 individuals of native weevils (*Bagous vivesi* González, 1967, *Bagous subcarinatus* Gyllenhal, 1836 and *Bagous revelierei* Tournier, 1884) were collected, across 9 ponds and in different months (Fig. 1 & 2). Although it has been shown that *S. rufinasus* can often occur at low density (Pemberton & Bodle, 2009), we found that it was more frequent than any native aquatic weevil in our study ponds. Exotic and native weevils were detected in sites exhibiting high vegetation cover (*ca.* 80 % vegetated) of similar species of aquatic plants: *Agrostis stolonifera, Panicum repens, Paspalum paspalodes, Juncus heterophyllus, Isolepis pseudosetaceus, Eleocharis palustris* and *Ranunculus peltatus*. Whilst native weevils occurred across different months in the study ponds, the exotic weevil only occurred during May-June (Fig. 2) suggesting that *S. rufinasus* may complete its life cycle in the marshes, where *A. filiculoides* is abundant, only appearing in ponds after adult dispersal. In this sense, the presence of *A. filiculoides* could be essential for the exotic weevil's reproduction but not necessary for adult survival. Although *A. filiculoides* is demonstrated to be the most suitable host plant for feeding, oviposition and larval development for *S. rufinasus* (Hill, 1998), *S. rufinasus* has also been detected on other plant species (Carrapiço *et al.*, 2011) suggesting that feeding on other plants cannot be completely discarded. The presence of both exotic and native weevils in similar aquatic plant assemblages showing dense cover of vegetation strengthens this possibility. The establishment of the exotic weevil on native aquatic plants could constitute a potential source for further dispersal when *A. filiculoides* reappears following annual inundation (McConnachie *et al.*, 2004). Our discovery of *S. rufinasus* at relatively high densities in apparently host free ponds suggests that the species may utilise alternative hosts in southern Europe, at least as an adult. Although we cannot discard the possibility that these occurrences in host-free ponds constitute sink populations, such populations may also represent an incipient case of niche shift following the introduction of an exotic species into a new area (Broennimann *et al.*, 2007). On the other hand, such adult populations may themselves act as sources of colonists; host free sites thus acting as stepping stones for the spread of this invasive species.

Sampling specifically designed to collect abundance data of *S. rufinasus* should be performed in the marshes in order to shed some light on its invasive potential. Further studies on these particular populations (e.g. demography, species distribution modelling, physiological competence experiments, propagule pressure, etc) should be performed as this is an interesting system for understanding invasion processes, which may lead to the rethinking of exotic species introductions as biological control agents. *S. rufinasus* has been already used as a successful biological control agent against *A. filiculoides* in South Africa (Hill, 2003; Hill & Julien, 2004), but its use in the United Kingdom has not had the same impact on the target plant (Gassmann *et al.*, 2006), and it has not been specifically employed in other European regions to date. In the light of our findings, future uses of *S. rufinasus* as a control agent should be preceded by host specificity tests, including how well the species can persist on other possible host/intermediate plants (Pratt *et al.*, 2013).

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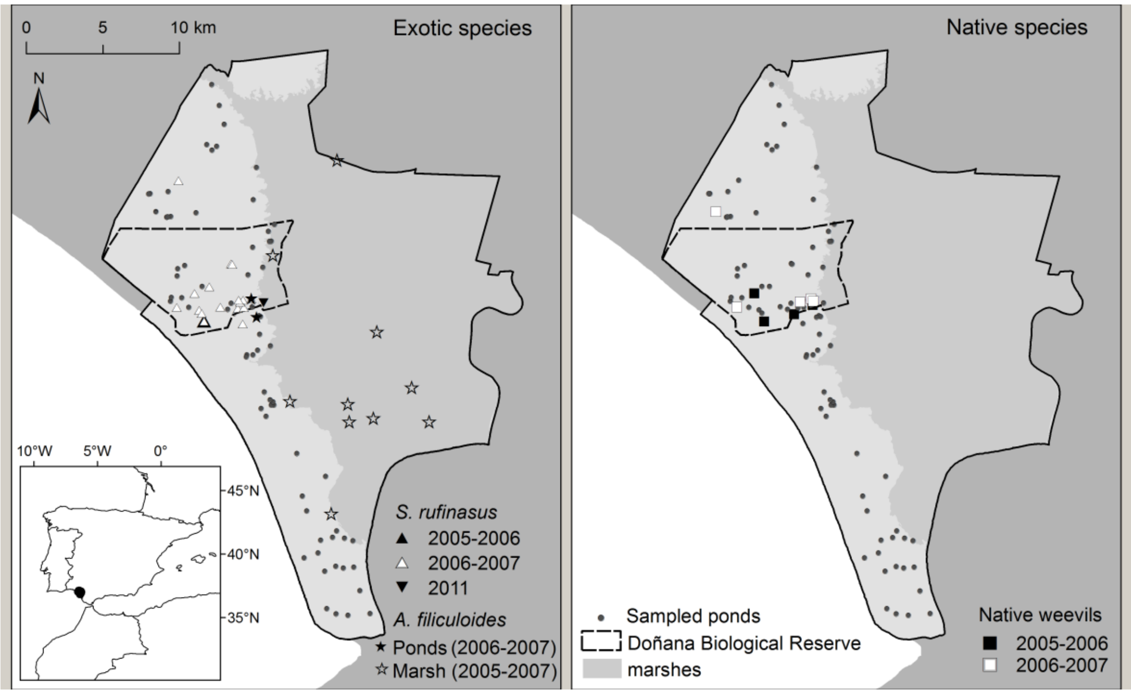
Figure legends

Figure 1. Distribution of the exotic weevil (*Stenopelmus rufinasus*) and the three species of native weevils in 91 ponds sampled across the Doñana National Park. The boundaries of the Doñana Biological Reserve, the sampling period considered (2005-2006, 2006-2007 and 2011), those sampling sites in the marsh where the biomass of *Azolla filiculoides* was sampled and those ponds in which *A. filiculoides* was detected (only detected in 2006-2007) are also indicated (see Appendix 1 for detailed geographical coordinates). *Distribución del gorgojo exótico (*Stenopelmus rufinasus*) y de las tres especies de gorgojos nativos en las 91 lagunas muestreadas en el Parque Nacional de Doñana. Se indican los límites de la Reserva Biológica de Doñana, el periodo de muestreo considerado (2005-2006, 2006-2007 y 2011), los puntos de la marisma donde se realizaron los muestreos de biomasa de* Azolla filiculoides *y aquellas lagunas donde* A. filiculoides *fue detectada (sólo detectada en 2006-2007) (ver el Apéndice 1 para las coordenadas geográficas detalladas).*

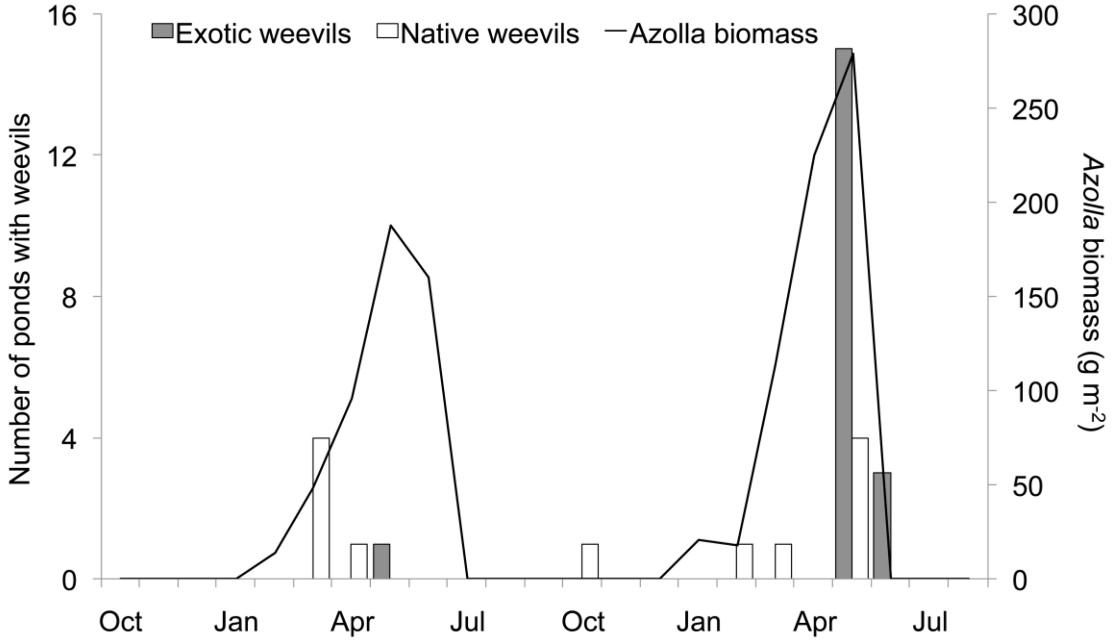
Figure 2. Number of ponds in the sandy area of Doñana National Park where native and the exotic weevils were detected (from October, 2005 to August, 2007) and monthly *Azolla filiculoides* biomass (g m^{-2}) collected in the marshes. *Número de lagunas de las arenas estabilizadas del Parque Nacional de Doñana donde se detectaron las especies de gorgojo exótico y nativo (desde Octubre de 2005 hasta Agosto de 2007) y biomasa mensual de* Azolla filiculoides *(g m^{-2}) recolectada en la marisma.*

Figure 1



Exotic species
Native species
0
5
10 km
N
10°W
5°W
0°
45°N
40°N
35°N
S. rufinasus
2005-2006
2006-2007
2011
A. filiculoides
Ponds (2006-2007)
Marsh (2005-2007)
Sampled ponds
Doñana Biological Reserve
marshes
Native weevils
2005-2006
2006-2007

Figure 2



Exotic weevils
Native weevils
Azolla biomass
Number of ponds with weevils
Azolla biomass (g m-2)
16
12
8
4
0
300
250
200
150
100
50
0
Oct
Jan
Apr
Jul
Oct
Jan
Apr
Jul

Appendix 1. UTM geographical coordinates (X and Y) of the 91 sampled ponds across the Doñana National Park, indicating those ponds where the exotic weevil (*Stenopelmus rufinasus*), the three species of native weevils and *A. filiculoides* (only detected in 2006-2007) were detected during the sampling period considered (2005-2006, 2006-2007 and 2011). The ten sampling sites in the marsh where the biomass of *Azolla filiculoides* was collected are also indicated. *Coordenadas geográficas en UTM (X e Y) de las 91 lagunas muestreadas en el Parque Nacional de Doñana, indicando aquellas lagunas en las que se detectó el gorgojo exótico (*Stenopelmus rufinasus*), las tres especies de gorgojos nativos y* A. filiculoides *(sólo detectada en 2006-2007) durante el periodo de muestreo considerado (2005-2006, 2006-2007 y 2011). Además se indican los diez puntos de muestreo de la marisma donde la biomasa de* Azolla filiculoides *fue colectada.*

			S. rufinasus		Native weevils		*A. filiculoides* in ponds	*A. filiculoides* in marsh
X	Y	2005-2006	2006-2007	2010- 2011	2005-2006	2006-2007	2006-2007	2005-2007
193299	4108588							
190047	4110088							
188194	4107742		1					
187502	4107003							
186326	4106871							
186234	4106855							
189342	4105603							
187677	4105360							
187406	4105327							
187467	4105343							
193057	4103349							
191644	4102277		1					
191714	4102234		1					

192752	4101524		
188408	4100024		
187660	4099807		
191632	4099662		
189545	4099232	1	
189725	4099016	1	
193466	4098846		
193572	4098791		
193314	4098759		1
193499	4097621		
194214	4096875		
193375	4096573		
193069	4096274		
192660	4096274		
192624	4096149		
197828	4088279		
196307	4086978		
199059	4084212		
197507	4084093		
199000	4082253		
200068	4080763		
188128	4101930		
194476	4104855		
194447	4092980		
190355	4114027		
193595	4092757		
198510	4079254		
200740	4079274		

198283	4084267			
199613	4084055			
190849	4112664			
191184	4111414			
193089	4106473			
194262	4092984			
195940	4089794			
193931	4092229			
196599	4086010			
198552	4084699			
198497	4082362			
197772	4082047			
196422	4082931			
197341	4083246			
199681	4082311			
197899	4079585			
192403	4098334		1	
192085	4099343		1	
191405	4099264			
187708	4100045			
189239	4100331		1	1
189728	4100771			
193718	4102021			
194273	4103734			
189877	4098482	1	1	1
194080	4104386			
190694	4109969			
190388	4109732			

194384	4093197						
194198	4093316						
193807	4093846						
199303	4079152						
186712	4105691				1		
186712	4105691						
190218	4100778	1					
192470	4099403	1					
194160	4103726						
192239	4099766	1			1		
192417	4099902	1					
191843	4098964			1			
188593	4102147						
190932	4099434	1					
192968	4099961	1			1	1	
193067	4099570			1			
188101	4101464						
192142	4099888	1					
188862	4099391						
193011	4099416						
193089	4099801				1		
188082	4099411	1		1	1		
193772	4099675		1				
194379	4102834						1
195516	4093245						1
198583	4109083						1
199304	4093065						1
200967	4092117						1

201197	4097797	1
199384	4091901	1
203486	4094140	1
204634	4091892	1
198204	4085830	1