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3 **HELMINTHS COMMUNITIES OF AN INTRODUCED HARE (*LEPUS***
4 ***GRANATENSIS*) AND A NATIVE HARE (*LEPUS EUROPAEUS*) IN SOUTHERN**
5 **FRANCE**

6

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14 ABSTRACT We investigated the parasite communities of introduced Iberian hares (*Lepus*
15 *granatensis*) and native European hares (*Lepus europaeus*) in southern France, where Iberian
16 hares were introduced locally 20 yr ago as a game animal. Parasite communities of sympatric
17 populations of the two hare species and of allopatric populations of European hares were
18 compared. Iberian hares in France harbored a depauperate community of parasites, relative to
19 population in its native habitat in Spain. European hares in areas of sympatry also were
20 infected by *Nematodiroides zembrae*, which normally infects Iberian hares on their native
21 range.

22

23 *Keys words* : Introduced populations, European hare, Iberian hare, *Lepus europaeus*, *Lepus*
24 *granatensis*, macroparasites, *Nematodiroides zembrae*,

25

26 Iberian hares (*Lepus granatensis*) are endemic to the Iberian Peninsula and the island
27 of Mallorca and live in a strict parapatry with the European hare (*Lepus europaeus*). *Lepus*
28 *granatensis* is present throughout the Peninsula except in the center and eastern areas of
29 northern Spain. This northern limit of the natural range of the Iberian hare in the Iberian
30 Peninsula is distant (200-300 km) from southern France, where only *L. europaeus* is naturally
31 present (Palacios 1983; Palacios 1998; Palomo and Gisbert 2002). Iberian hares have been
32 introduced as game animals during the last 20 yr in the department of Pyrénées Orientales
33 (southern France), and they are now found in sympatry with European hares in some
34 localities. Iberian hare populations have reached high densities, and in some areas of
35 sympatry, outnumber European hares (unpublished data, Departemental Federation of
36 Hunters). In this study, we recorded and compared the helminth communities of *L.*
37 *granatensis* and *L. europaeus* in sympatric and allopatric areas.

38 The study was conducted in the department of Pyrénées Orientales in southern France.
39 The study area was subdivided into two sectors including several localities. The first sector
40 (longitude 2°30' -3°03' E, latitude 42°32' - 42°54' N), the Roussillon lowland, consists of
41 vineyards where both species are present (sympatric area). The second sector (longitude
42 1°55' -2°20' E, latitude 42°24' - 42°40' N), including the Conflent and Cerdagne, is a middle
43 mountain sector in which only European hares were present at the time of the study (allopatric
44 area). Hares were obtained during the hunting season, between September and December
45 2003 and species identification was based on morphological differences: length, weight,
46 color, and fur pattern according to Palacios (1989). The demographic structure (age and
47 gender distribution) was recorded. Differences between species were tested using Fisher's
48 exact tests.

49 To detect internal macroparasites, the abdominal cavity was first examined for cysts.
50 The alimentary tract and the liver then were removed and frozen for future examination at the
51 laboratory. Eighty hares were examined, 58 in the sympatric area (47 *L. granatensis* and 11 *L.*
52 *europaeus*) and 22 in the allopatric area (all *L. europaeus*). The alimentary tract was divided
53 into three regions (stomach, small intestine and large intestine) before inspection. Viscera
54 were examined under stereoscopic microscope, and helminths were counted and preserved in
55 70% alcohol. Identification of parasites was made according to previous descriptions
56 (Erhardova 1957; Bernard 1965; Tenora and Murai 1978; Durette–Desset 1979; Hugot 1983;
57 Genov et al. 1990). The terminology for describing parasite infestation (prevalence, mean
58 intensities and abundances) followed Bush et al. (1997). Differences in abundance were
59 tested using the Mann-Whitney U Test.

60 Significant differences in age (adults and juveniles) and gender distribution between
61 samples from sympatric European and Iberian hare populations or between sympatric and
62 allopatric European hare populations were not detected ($P>0.05$). Only two species of

63 nematodes were recorded in the Iberian hare samples in the sympatric area: *N. zembrae* in the
64 small intestine, and *Graphidium strigosum*, which was found in the stomach of a single
65 animal (Table 1). *Nematodiroides zembrae* is known to be endemic to the Iberian Peninsula
66 and Zembra Island (near the coast of Tunisia), where it is a common parasite of both
67 European rabbits (*Oryctolagus cuniculus*; Blasco 1996) and Iberian hares (Moreno Montanez
68 et al. 1979; Martinez Gomez et al. 1987; Molina i Figueras 1998).

69 The parasite species community of European hares in the sympatric area was very
70 poor with only one parasite species (*N. zembrae*) detected (Table 1). The prevalence and
71 intensity of *N. zembrae* in the sympatric area, however, were high in both hare species (Table
72 1), but differences between species were not detected (Mann-Whitney U Test; $P > 0.9$).

73 The helminth fauna of the European hares in the allopatric area was richer despite a
74 smaller sample size from the mountain localities. Three nematodes (*Passalurus ambiguus*,
75 *Graphidium strigosum* and *Trichostrongylus retortaeformis*) and two cestodes (*Mosgovoyia*
76 *ctenoides* and *Leporidotaenia. Cf wimerosa*) were recorded (Table 1). *Nematodiroides*
77 *zembrae* was not observed in this allopatric area. The species composition of the parasite
78 community of European hares in the allopatric area is similar to that of parasite communities
79 investigated in other countries, but differs by the presence of the cestode *Leporidotaenia. cf*
80 *wimerosa*. The cestode *L. cf wimerosa* has only recorded in mountain hares (*Lepus timidus*)
81 and rabbits (Genov et al. 1990) and this is the first report for the European hare.

82 The parasite community of European hares in the sympatric area in the department of
83 Pyrénées Orientales is poor and limited to a single nematode species, *N. zembra*. The paucity
84 of the parasite community could be related to the small sample size, the low densities of
85 European hares in this area, or to ecological differences between the sympatric area (lowland
86 agriculture area) and the allopatric area (subalpine). Concerning the third assumption, it is
87 know that parasite transmission rates can be affected by moisture availability (Wilson et al.

88 2001; Hulbert and Boag 2001). Previously, *N. zembrae* was only recorded in the Iberian
89 Peninsula and Zembra Island where it occurs only in rabbits and in Iberian hares (Blasco
90 1996; Moreno Montanez et al. 1979; Martinez Gomez et al. 1987; Molina i Figueras, 1998).
91 Many studies have been conducted on the helminth fauna of *L. europaeus* in other countries
92 outside of the Iberian Peninsula, and there have been no reports of *N. zembrae* from this
93 species (Sharpilo 1975; Kutzer and Frey 1976; Sugar et al. 1978; Forstner and Ilg 1982;
94 Soveri and Valtonen 1983; Boag 1987; Canestri-trotti et al. 1988; Allgoewer 1992; Poglayen
95 et al. 1994; Molina i Figueras 1998; Shimalov and Shimalov 2001). This nematode species
96 also was not recorded from European hares in northern Spain where Iberian hares are absent
97 (Molina i Figueras 1998). Our study is the first record of this nematode for *L. europaeus* and
98 France. These data and the absence of *N. zembrae* in the allopatric samples in our study,
99 strongly support the hypothesis that *N. zembrae* was introduced with Iberian hares in the
100 Roussillon and now parasitizes European hares on this area.

101 The parasite community of the introduced Iberian hares was species-poor and did not
102 include parasite species previous reported from Iberian hares sampled from its native range
103 (Iberian Peninsula). In comparison with previous published parasitological surveys on *L.*
104 *granatensis*, the parasite community in the introduced area is reduced to two nematodes, both
105 of which are characterised by a direct life cycle (Table 2). Parasite species with complex life
106 cycles (which involve invertebrate intermediate hosts) were absent in our study area; these
107 include two cestode species (*Mosgovoyia pectinata* and *Cysticercus pisiformi*) and one
108 trematode species (*Dicrocoelium dentriticum*) that occur in Iberian hares in Spain (Table 2).
109 The observed loss of parasite diversity can be explained by a founder effect. In addition, for
110 those parasites with complex life cycles, the potential lack of suitable intermediate hosts in
111 the introduction area also may have limited the potential for parasite introduction (Dobson

112 and May 1988; Goüy de Bellocq et al. 2002; Torchin et al. 2003). In Iberian hares, a founder
 113 effect seems likely because releases of this game animal are usually limited in number.

114 In contrast to the overall reduction in parasite diversity, a high prevalence (72% and
 115 74 %) and intensity of *N. zembrae* was observed in areas where Iberian hares have been
 116 introduced compared to native areas. Our restricted collection period may have affected both
 117 intensity and prevalence estimates as parasite burdens are seasonally variable (Boag et al.
 118 2001); however, the high densities of Iberian hares, and resulting high transmission rates, may
 119 explain the high prevalence of *N. zembrae*.

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207 **Table 1.** Prevalence (P) and mean intensity (MI, with range of infection) of parasites species
 208 identified from *Lepus europaeus* and *L.granatensis* in allopatric and sympatric areas.
 209

Parasite species	Sympatric area				Allopatric area	
	<i>Lepus granatensis</i> (n= 47)		<i>Lepus europaeus</i> (n= 11)		<i>Lepus europaeus</i> (n=22)	
	P ^a (%)	MI	P (%)	MI	P (%)	MI
<i>Passalurus ambiguus</i>	0	0	0	0	30	4 (1-14)
<i>Mosgovoyia ctenoides</i>	0	0	0	0	14	1,5 (1-2)
<i>Trichostrongylus retortaeformis</i>	0	0	0	0	9	2
<i>Graphidium strigosum</i>	0	0	2	5	5	150 (100-200)
<i>Leporidaenia. Cf wimerosa</i>	0	0	0	0	5	25
<i>Nematodiroides zembrae</i>	72	128 (27-286)	74	161 (2-1200)	0	0

210 ^aP=prevalence, MI=mean intensity (range)

211 **Table 2.** Surveys of parasite species richness of *Lepus granatensis* in Iberian Peninsula
 212 (native area) and Pyrenees Orientales (present study).
 213

References	Moreno Montanez et al. 1979	Martinez- Gomez et al. 1987	Molina i Figueras 1998	Present study 2003
Sites	Provincia de Cordoba ^a	Provincia de Cordoba ^a	Provincia de Navarra ^a	Pyrenees Orientales (France)
<i>n</i>	42	45	19	47
Species richness of helminths ^b	7	8	5	2
Cestodes ^c	2	2	1	0
Nematodes	5	6	3	2
Trematodes	0	0	1	0
Prevalence (%) of.	5	10	28	71
<i>Nematodiroides zembrae</i>				

214 ^a Iberian Peninsula

215 ^b Digestive tract, including peritoneal cavity

216 ^c Larval and adult forms