HELMINTHS OF APODEMUS SYLVATICUS (MURIDAE) DISTRIBUTED ON THE SOUTHERN EUROPEAN BORDER (ITALIAN PENINSULA)

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HELMINTHES
APODEMUS SYLVATICUS
ITALIE
MURIDAE

RÉSUMÉ. – Cinq aires du sud de l'Italie ont été examinées et ont révélé la présence d'*Apodemus sylvaticus*. Au total, 14 espèces d'Helminthes intestinaux ont été identifiées : Trematoda (*Corrigia vitta)*, Cestoda *(*larves de *Taenia taeniaeformis*, *Gallegoides arfaai, Hymenolepis* sp. et *Rodentolepis straminea),* et Nematoda (*Trichuris muris, Aonchotheca muris-sylvatici, A.* sp.*, Mastophorus muris, Rictularia proni, Heligmosomoides polygyrus, Syphacia stroma, S. frederici* et *Aspiculuris tetraptera*). L'intensité d'infection par les Helminthes intestinaux est plus importante chez les adultes et chez les mâles. Le nombre d'espèces rencontrées en Calabre s'est révélé moins important que celui de la communauté de Vers identifiés en Europe du Nord et dans les aires continentales. La richesse spécifique, en effet, semble être identique à celle observée pour les vertébrés: dans une péninsule, le nombre d'espèces diminue parce que l'environnement réduit progressivement sa capacité à être colonisé par des vertébrés.

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ABSTRACT. – Five areas populated by *Apodemus sylvaticus* in South of Italy have been analyzed. In total, fourteen species of intestinal helminths belonging to trematodes (*Corrigia vitta),* cestodes *(Taenia taeniaeformis* larvae, *Gallegoides arfaai, Hymenolepis* sp. and *Rodentolepis straminea),* and nematodes (*Trichuris muris, Aonchotheca muris-sylvatici, A.* sp.*, Mastophorus muris, Rictularia proni, Heligmosomoides polygyrus, Syphacia stroma, S. frederici* and *Aspiculuris tetraptera*) were found. Mean intensities of intestinal helminths were higher in adults and males. The number of parasite species found in Calabria was lower compared to helminth communities of the northern part of the European peninsulas and of other continental areas. The specific richness was similar to that observed in vertebrate taxa: in a peninsula, the number of species decreases due to habitat barriers that progressively reduce the possibility of colonization.

INTRODUCTION

When describing the biodiversity of an area, parasites and infectious diseases are to be considered as "the forgotten half of biodiversity" (Dobson 1999). According to Dobson, there are ten species of parasites associated with any population of living mammals and each individual host contains a whole community of parasites. The helminth faunas of European *Apodemus* sp. have been well documented from extensive research mainly carried out in continental and insular areas. More than thirty helminth species have been reported parasitizing *A. sylvaticus, A. flavicollis, A. agrarius, A. microps* and *A. mystacinus* (Feliu *et al.* 1987, 1997, Montgomery & Montgomery 1990, Asakawa & Tenora 1996, Behnke *et al.* 1999, Abu-Madi *et al.* 2000). Part of these studies provide ecological data on host sex and/or host age compositions, seasonal compositions or habitat influences. From these studies, it is hypothetised that species diversity should be lower on islands than on the mainland (Goüy de Bellocq *et al.* 2003). Very few reports of *Apodemus* sp. helminths in the European peninsulas are available. Thus, the study of parasite communities in the southern parts of Europe (Iberian and Italian peninsulas) should be of great interest since these were subject to variations due to the fact that these areas established a shelter for faunas during the Quaternary period (Hewitt 1996, 1999). Data are scarce on parasitic helminths from rodents in Italy (Macchioni 1967, Virga *et al*. 1995, Goüy de Bellocq *et al*. 2003, Milazzo *et al*. 2003a, b). In

contrast, parasitic helminths of murine rodents, mostly of the woodmice, are well documented in different countries in Europe (Goüy de Bellocq *et al*. 2002). Helminths of Italian *Apodemus* sp. are almost unknown. Therefore, the aim of this research is to provide the first description of helminth communities of *A. sylvaticus* in the southern Italian Peninsula (Calabria). The species richness in *A. sylvaticus* from Calabria will be discussed, taking into account the biogeography of rodents in Italy.

METHODS

A total of 697 *A. sylvaticus* were studied for intestinal helminths. The wood mice were trapped in five different areas of Calabria (South of Italy): Crati River Valley (N 16: 33, E 39: 21) (years 1990-91; n= 168; monthly captured from February 1990 to February 1991), Sila Grande (N 16: 35, E 39: 23) (years 1994-95; n= 88; monthly from June 1991 to July 1992), Sila Greca (N 16: 42, E 39: 29) (years 1994-95; n= 163; monthly from April 1994 to March 1995), Northern Jonian rivers ("fiumare") (Avena river: N 16: 33, E 39: 55, Satanasso river: N 16: 31, E 30: 52) (years 1993-94; n= 204; monthly July 1992 to July 1993) and Catena Costiera (N 16: 06, E 39: 22) (years 1988-02; n= 74; may 1989 to January 1990).

Crati River Valley: The valley, shaped by the main river of Calabria, is about 91 km long. The landscape shows a mosaic of biotopes consisting of small patches of residual *Quercus virgiliana* woods, olive groves, cereal farming, abandoned fields in which rudimentary vegetation prevails.

Sila Grande: This is an extensive undulating plateau ranging from a minimum of 800 m to a maximum of 1930 m in altitude in the northern part of the Calabrian Apennines. Between 600 and 1100 m of woodland is covered by chestnut *(Castanea sativa).* Some areas consist of deciduous woodland of different types of oak (*Quercus* spp.) and maple (*Acer* spp.). Above 1100 m, the forest is covered by the Calabrian black pine (*Pinus laricio),* beech *(Fagus sylvatica),* white fir *(Abies alba),* hazel (*Corylus avellana)* and poplar *(Populus tremula).*

Sila Greca: The massif is located in the north eastern part of Calabria, ranging from 40 to 1480 m. In the lower part prevails the sclerophyll vegetation with Mediterranean maquis and *Quercus ilex* woods. Instead, in the upper part, the deciduous woods prevail *(Quercus pubescens, Q. cerris, Q. frainetto, Q. virgiliana).*

Catena Costiera: This is a mountain range of the north western part of Calabria, with few peaks higher than 1400 m. This area is covered by *Quercus virgiliana* and *Castanea sativa* woods in the lower part and *Fagus sylvatica* woods in the upper part.

Northern Jonian rivers ("fiumare"): Rodents were collected in the two "fiumare" Avena and Satanasso, located in the north eastern coast of Calabria. The "fiumare" are temporary rivers characterized by a short and rapid route whose final part is wide and stony. The area's climate is that of a typical dry Mediterranean climate. Vegetation is scarce dowstream river and it is mainly dominated by *Chenopodium bothryos, Helicrisum italicum* and *Artemisia variabilis.* On the bedside of the river we can find wood of *Quercus ilex, Pistacia lentiscus* or *Pinus halepensis.*

Animals were trapped using pitfall traps, according to a standardized procedure that allows to compare the relative density of animals at different stations (Cagnin *et al.* 1998) and these were stored in a 70% ethanol solution for a later examination. We examined 382 males and 314 females (1 specimen remained undetermined for sex). These animals were classified into two different categories of age as a result of the four age classes established by Adamcewska-Andrejewska (1976): juveniles (Class I and II = younger than two months; 263 specimens), adults (Class III and IV = older than two months; 428 specimens) (6 specimens remain undetermined for age). The stomach, the intestine, the liver and the pancreas were split and examined for any presence of helminths. Parasitic worms were treated for permanent mounting in Canada Balsam (cestodes and trematodes previously stained with Semichon acetocarmine) or temporarily examined with Amann lactophenol (nematodes). For specific identification of helminths the works of Verster (1969), Tenora (1963), Tenora & Meszaros (1975), Tenora & Mas Coma (1977), Meszaros & Murai (1979), Moravec (1982), Moravec *et al.* (1987), Casanova *et al.* (2001), Feliu *et al.* (2000) were consulted.

The characteristics of helminth faunas were analyzed in terms of prevalence (P) and the Average Intensity (AI) according to Bush *et al.* (1997). The diversity index of Gini-Simpson were calculated according to Gini (1912).

RESULTS

Out of a total of 697 specimens of *A. sylvaticus* examined (Table I), 311 were infested (prevalence: P = 44.6) by 15790 parasites (average intensity AI = 51). The parasites belonged to 14 species (Table I) of intestinal helminths: one trematode *(Corrigia vitta* Dujardin, 1845*),* four cestodes *(Taenia taeniaeformis* (Batsch, 1786) larvae, *Gallegoides arfaai* (Mobedi et Ghadirian, 1977), *Hymenolepis* sp. and *Rodentolepis straminea* (Goeze, 1782) and nine nematodes (*Trichuris muris* (Schrank, 1788*), Aonchotheca muris-sylvatici* (Dujardin, 1843*), Aonchotheca* sp.*, Mastophorus muris* (Gmelin, 1790*), Rictularia proni* Seurat, 1915*, Heligmosomoides polygyrus* (Dujardin, 1845*), Syphacia stroma* (Von Linstow, 1884*), S. frederici* Roman, 1945 and *Aspiculuris tetraptera* (Nitsh, 1821). Only one species of trematode was collected in the pancreatic ducts of ten animals. Three species of cestodes are intestinal worms whereas *H. taeniaeformis* was found in the liver of the larval stage from one host. All species of nematodes are intestinal worms except *M. muris* and *R. proni* that are stomachal parasites. General helminth infections in *A. sylvaticus* are low not exceeding half of the studied animals (Table I). Only two species

Table I. – Value of prevalence (P%) and mean intensity (MI) for each recorded parasite. N = number of hosts; 95% l.c.l.–u.c.l. = lower and upper confidential limits at 95% of significativity; s^2 = variance; Max-Min = maximum and minimum number of parasites; N_p= number of parasites.

	N	**P%** (95% l.c.l.-u.c.l.)	**MI**	**σ^2**	**Max-Min**	N_p
TREMATODA						
C.vitta	10	1.4 (0.7-2.7)	5.1	17.8	14-1	51
CESTODA						
T. taeniaeformis (l)	1	0.1 (0.0-0.9)	1.0	0.0	1-1	1
G. arfaai	9	1.3 (0.6-2.5)	2.4	3.5	6-1	22
Hymenolepis sp.	2	0.3 (0.0-1.2)	1.0	0.0	1-1	2
R. straminea	1	0.1 (0.0-0.9)	1.0	0.0	1-1	1
NEMATODA						
T. muris	10	1.4 (0.7-2.7)	4.3	18.6	14-1	43
A. muris-sylvatici	13	1.9 (1.0-3.3)	6.5	59.6	24-1	84
Aonchotheca sp.	3	0.4 (0.1-1.4)	3.3	10.3	7-1	10
M. muris	1	0.1 (0.0-0.9)	1.0	0.0	1-1	1
R. proni	30	4.3 (3.0-6.2)	5.8	114.4	49-1	173
H. polygyrus	193	27.7 (24.4-31.2)	34.4	11,103.8	1350-1	6,643
S. stroma	50	7.2 (5.4-9.4)	39.6	3,426.5	310-1	1,981
S. frederici	142	20.4 (17.5-23.6)	43.7	7,356.3	782-1	6,212
A. tetraptera	26	3.7 (2.5-5.5)	21.8	2,909.1	255-1	566
TOTAL	697	44.6 (40.9-48.4)	51	12,444.9	1374-1	15,790

H. polygyrus and *S. frederici* show prevalences higher than 10% and could be considered as members of the component community of the woodmouse in Calabria. Differences in the composition of the community were found between males and females and between juveniles and adults. General prevalences and number of species in males and females are similar (Table II). In general all species found in both sexes show low prevalences and only *H. polygyrus* and *S. frederici* could be considered in the component community of these animals (Table II). Young individuals are found parasitised by 11 worm species versus 13 found in adult animals (Table III). General prevalence and all prevalences of individual species in young host were lower than in adults (Table III). Also, both in adult and young animals only *H. polygyrus* and *S. frederici* show prevalences higher than 10%. The helminth communities structure was tested with the diversity index Gini-Simpson which shows to be fairly similar between adults and juveniles (0.38 versus 0.34) and quite different between males and females (0.31 versus 0.43).

Table II. – Value of prevalence and mean intensity for every recorded parasite inside sex variable. Abbreviations: see table I.

	P% (95% l.c.l.-u.c.l.)		MI		σ^2		Max-Min		N_p	
	F (N = 314)	M (N = 382)	F	M	F	M	F	M	F	M
TREMATODA										
C. vitta	1.6 (0.6-3.9)	1.3 (0.5-3.2)	4.4	5.8	12.3	26.7	10-1	14-1	22	29
CESTODA										
T. taeniaeformis	-	0.3 (0.0-1.7)	-	1.0	-	0.0	-	1-1	-	1
G. arfaai	0.6 (0.1-2.5)	1.8 (0.8-3.9)	1.0	2.9	0.0	3.8	1-1	6-1	2	20
Hymenolepis sp.	0.3 (0.0-0.2)	0.3 (0.0-1.7)	1.0	1.0	0.0	0.0	1-1	1-1	1	1
R. straminea	0.3 (0.0-2.0)	-	1.0	-	0.0	-	1-1	-	1	-
NEMATODA										
T. muris	1.0 (0.2-3.0)	1.8 (0.8-3.9)	1.3	5.6	0.3	21.6	2-1	14-1	4	39
A. muris-sylvatici	1.0 (0.2-3.0)	2.6 (1.3-4.9)	9.0	5.7	169.0	39.1	24-1	20-1	27	57
Aonchotheca sp.	0.3 (0.0-2.0)	0.5 (0.1-2.1)	7.0	1.5	0.0	0.5	7-7	2-1	7	3
M. muris	0.3 (0.0-2.0)	-	1.0	-	0.0	-	1-1	-	1	-
R. proni	3.8 (2.1-6.8)	4.7 (2.9-7.5)	5.7	5.8	107.1	125.9	37-1	49-1	68	105
H. polygyrus	25.8 (21.1-31.1)	29.3 (24.9-34.2)	36.7	32.8	22,598.2	2,913.0	1,350-1	279-1	2,974	3,669
S. stroma	5.4 (3.3-8.7)	8.6 (6.1-12.0)	30.9	44.1	2,092.2	4,139.2	183-1	310-1	525	1,456
S. frederici	15.0 (11.3-19.5)	24.9 (20.7-29.6)	28.4	51.3	3,294.6	9,246.8	299-1	782-1	1,336	4,876
A. tetraptera	2.5 (1.2-5.2)	4.7 (2.9-7.5)	6.0	28.8	51.4	4,087.9	18-1	255-1	48	518
TOTAL	40.4 (35.0-46.1)	48.2 (43.1-53.3)	39.4	58.5	16,674.3	9,451.7	1374-1	795-1	5016	10,774

DISCUSSION

The helminth fauna of *A. sylvaticus* in Calabria is composed of common species of *Apodemus* spp. in Europe but highest species richness has been detected in continental Europe (Tenora 1962, Asakawa & Tenora 1996, Goüy de Bellocq *et al.* 2002, 2003). Peninsular studies on helminths of *A. sylvaticus,* mostly carried out in the Iberian Peninsula, show that wood mice have a richer fauna than other rodents in the Peninsula, with a number of species similar to continental Europe (Feliu *et al.* 1997). Insular helminth populations parasitizing *A. sylvaticus* show a species richness that ranged from typical configurations in the British Isles (9 species from Montgomery & Montgomery 1990; eight species reported by Behnke *et al.* 1999) to those configurations reached in the Balearic Islands (see Mas Coma & Feliu 1984). In Calabria, the rodents' fauna is composed of a reduced number of species (31) compared to the rodents' fauna of northern regions, which is probably due to a "peninsular effect" (Simpson 1964, Cagnin *et al.* 1998, Baquero & Telleria 2001). To date known helminth faunas of rodents in Calabria seem also

Table III. – Value of prevalence and mean intensity for every recorded parasite within age variable. Abbreviations: see table I.

	P% (95% l.c.l.-u.c.l.)		MI		σ^2		Max-Min		N_p	
	J (N = 263)	A (N = 428)	J	A	J	A	J	A	J	A
TREMATODA										
C. vitta	0.4 (0.0-2.1)	2.1 (1.0-4.1)	1.0	5.5	0.0	17.7	1-1	14-1	1	50
CESTODA										
T. taeniaeformis	-	0.2 (0.0-1.5)	-	1.0	-	0.0	-	1-1	-	1
G. arfaai	0.8 (0.1-2.7)	1.6 (0.7-3.5)	1.0	2.9	0.0	3.8	1-1	6-1	2	20
Hymenolepis sp.	-	0.5 (0.1-1.9)	-	1.0	-	0.0	-	1-1	-	2
R. straminea	0.4 (0.0-2.1)	-	1.0	-	0.0	-	1-1	-	1	-
NEMATODA										
T. muris	0,8 (0.1-2.7)	1,9 (0.9-3.8)	5,5	4	24.5	20.0	9-2	14-1	11	32
A. muris-sylvatici	1.5 (0.4-3.8)	2.1 (1.0-4.1)	3.5	7.8	19.0	75.9	10-1	24-1	14	70
Aonchotheca sp.	0.4 (0.0-2.1)	0.5 (0.1-1.9)	7.0	1.5	0.0	0.5	7-7	2-1	7	3
M.muris	-	0.2 (0.0-1.5)	-	1.0	-	0.0	-	1-1	-	1
R. proni	1.9 (0.6-4.4)	5.8 (3.9-8.6)	2.0	6.5	3.0	134.2	5-1	49-1	10	163
H. polygyrus	17.5 (13.1-22.6)	34.1 (29.7-38.8)	13.5	41.2	626.9	14,315.8	129-1	1350-1	620	6,020
S. stroma	4.6 (2.4-7.8)	8.9 (6.4-12.1)	34.3	41.3	2,486.3	3,786.3	178-1	310-1	411	1,570
S. frederici	13.3 (9.4-18.0)	25.0 (21.0-29.4)	24.3	50.1	1,558.2	9,119.1	200-1	782-1	849	5,363
A. tetraptera	1.5 (0.4-3.8)	5.1 (3.3-7.8)	3.0	25.2	3.3	3,383.4	5-1	255-1	12	554
TOTAL	27.8 (22.4-33.6)	55.4 (50.5-60.1)	26.5	58.8	1,625.7	15,710.1	200-1	1374-1	1,938	13,849

affected by the "peninsula effect" that influenced both prevalences and infection intensities of helminth parasites. The helminth fauna of *C. glareolus* was well sudied in Calabria (Milazzo *et al*. 2003 b). The results of this study show a helminth community with a low species richness with a range of prevalences between 0,5 to 5,1%. The only data previously available on rodent parasitic helminths in Calabria comes from the study of Di Bella *et al.* (1997) which shows that *A. sylvaticus* and *A. flavicollis* are parasitized by the same and lower number of species. In Goüy de Bellocq *et al*. (2003), 12 helminth species were cited in *A. sylvaticus* from Italy, all of these present in Calabria. No data are available on the prevalence or infection intensities of these species in the host population. In our study a general prevalence of 44,6% was found but only two species *H. polygyrus* and *S. frederici* show a prevalence higher than 10%. The rest of the species are not present in the component community of *A. sylvaticus* in Calabria. This fact is rare in the known helminth communities of this host in Europe. Normally specific species of *Apodemus* spp. are in the component communities of this host in Europe and, in several islands, also less specific species show

prevalences higher than 10%. In Sicily, despite the close proximity to Calabria, the number of species found in *A. sylvaticus* is higher than in Calabrian populations of this host. In another study of Goüy de Bellocq *et al*. (2003) all the species found are members of the component community of woodmouse. Probably, the non specific parasites of rodents such as *C. vitta*, *T. muris*, *A. dujardini* and *Mesocestoides* sp. are found in high prevalences in Sicilian woodmouse by the absence of other habitual host of these parasites as different species of Arvicolinae (i.e. *Clethrionomys glareolus*, habitual host of these worms in Europe mainland) (Feliu *et al*. 1997, Milazzo *et al*. 2003 b). Unfortunately, the rodent helminth faunas in Sicily are poorly known. To date only three rodent species in Sicily are studied helminthologically, *A. sylvaticus*, *Mus domesticus* and *Rattus rattus* and only three common species are found in these hosts, *H. diminuta*, *M. muris* and *T. muris* (Goüy de Bellocq *et al*. 2003, Milazzo *et al*. 2003a, Virga *et al*. 1995). In Calabria, the helminth faunas of *C. glareolus* and *M. domesticus* are known and are also characterised by a low helminth richness with several species (*C. vitta*, *M. muris*) parasitizing Murinae and Arvicolinae and *H. polygyrus* and *A. tetraptera* present in Murinae (*M. domesticus*, *A. sylvaticus*, *A. flavicollis*) (Di Bella *et al*. 1997, Milazzo *et al*. 2003, 2004). Prevalences of these common species in the different hosts are always lower than 10% except for *H. polygyrus* in *A. sylvaticus* found in our study (Di Bella *et al*. 1997, Milazzo *et al*. 2004). Concerning the continental populations of *A. sylvaticus*, the results found in Calabria are surprising. In the study of Behnke *et al*. (1999) in England nine helminth species were found, all of these except *A. muris sylvatici*, *H. taeniaeformis* and *Brachylaima recurva* with prevalences higher than 8,2%.

Different factors could be contributes to increase helminth richness in the different European populations of the woodmouse. In Iberia, *A. sylvaticus* is parasitized by endemic species which are mostly found in the Pyrenean mountains that separate the peninsula from the continental areas (Feliu *et al.* 1987). Most of these species are digenetic Trematodes with two intermediate hosts (in most cases also endemic species) with a distribution influenced by paleobiogeographic factors. The absence of several species in the helminth fauna of *A. sylvaticus* in Calabria could be explained by the historical biogeography of this host because the Italo-Balkan populations of woodmouse are characterised by a lower genetic diversity (Michaux *et al*. 2003). Sicily, on the contrary, as it is demonstrated in Michaux *et al*. (2003) represents a genetic hot spot of *A. sylvaticus* and probably this could explain the high helminth richness in this host (Goüy de Bellocq *et al*. 2003).

Our results are in accordance with the reports showing a lesser role of sex in the structure of the helminth communities in several small mammals (Kisielewska 1970, Abu-Madi *et al*. 2000, Milazzo *et al*. 2003 a, b). After Haukisalmi *et al*. (1988), Montgomery & Montgomery (1990), Gregory (1992), Behnke *et al*. (1999) host age play a significant role in the structure of the component communities. Our results about *A. sylvaticus* in Calabria suggest that the host age is not an important factor determining the structure of the helminth community since species richness are similar in young and adult animals.

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