

# The helminth community of the wood mouse, *Apodemus sylvaticus*, in the Sierra Espuña, Murcia, Spain

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## Abstract

The helminth community of the wood mouse, *Apodemus sylvaticus*, in the Sierra Espuña was characterized after a complete analysis of its helminth community component and infracommunity structure relative to host age, sex and year of capture. The helminth community comprised 13 species: one trematode, four cestodes and eight nematodes. The cestode *Pseudocatenotaenia matovi* and the nematode *Syphacia frederici* were the most prevalent and abundant helminth species, respectively. Sixty four percent of mice analysed presented helminths with a direct cycle and 42% presented helminths with an indirect cycle. The helminth community presents a low diversity with infracommunities usually made up of only one or two helminth species. Host age and year of capture seem to play a major role in determining species richness and helminth diversity, but not in determining the abundance of helminths. Host sex does not seem to affect the infection rate nor the diversity. Further studies on more samples of wood mice and other small mammal species in this regional park are needed to explore any possible interactions between helminth communities in the host populations.

## Introduction

From early studies of González Castro (1944) and López-Neyra (1947) to the more recent studies of Fuentes *et al.* (2000) and Portolés *et al.* (2000), a great deal has been contributed to the knowledge of the helminth fauna of small mammals (insectivores and rodents) of the Iberian Peninsula. The majority of these contributions was compiled by Mas-Coma & Gállego (1980) and Cordero del Campillo *et al.* (1994). However, these studies produced a general overview of the helminth fauna of the various host species studied, primarily in the provinces, regions or in the Iberian Peninsula in general, e.g. Feliu *et al.* (1997). Thus, few studies have concentrated on ecologically-limited geographic areas, with the exception of Portolés *et al.* (1996, 2000) in the Natural Park of Albufera de València and Fuentes *et al.* (2000) in the Natural Park of the Serra Calderona. More studies

along these lines would make possible a helminthecological comparison between particular natural regions in the Peninsula. Moreover, the majority of these studies have mainly taken into account qualitative aspects, but very few quantitative data and/or a characterization of the helminth community have been provided, e.g. Fuentes *et al.* (2000).

Therefore the aim of the present paper is to analyse the helminth community of the wood mouse, *Apodemus sylvaticus* (Rodentia: Muridae), in the Regional Park of Sierra Espuña, a natural Mediterranean ecosystem located in the south-east of the Iberian Peninsula, with qualitative, quantitative and ecological aspects taken into account.

## Materials and methods

The Sierra Espuña, a regional park of approximately 18000 ha, is a Mediterranean ecosystem situated in the centre of the region of Murcia, in the south-east of the Iberian Peninsula. It belongs to the easternmost Cordillera Bética, located between the valleys of the

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Guadalentín and Pliego rivers (1°28'–1°35'W/37°46'–37°55'N). It has a very uneven topography with mountains orientated from east to west and ascending considerably from 200 to 1500 m. The vegetation is made up of a typical Mediterranean scrubland together with woodland dominated by Aleppo pine, although Austrian pine and maritime pine trees predominate in the highlands. Holm oaks, Portuguese oaks and maple trees can also be found in some areas.

After the completion of three expeditions to the Sierra Espuña in the spring/summer of 1999, 2000 and 2001, a total of 74 wood mice were examined for helminth parasites. The year of capture and host sex and age are summarized in table 1, with age being determined by weight, i.e. sub-adults between 15–25 g, and adults >25 g.

Helminths were collected and identified as previously described by Fuentes *et al.* (2000). Trematodes and cestodes were preserved in 70% ethanol, stained with Grenacher's boracic and alcoholic chlorhydric carmine, respectively, differentiated with acidified ethanol, dehydrated in an alcohol series, cleared with xylene and mounted in Canada balsam. Nematodes were preserved in 70% ethanol and cleared in Amann lactophenol.

The study of the helminth community composition and structure was carried out considering each particular biological cycle and the prevalence, mean abundance, range and total number of helminths calculated according to Bush *et al.* (1997). As Aznar *et al.* (1998) proposed, median intensity values are provided to maximize the amount of meaningful, non-redundant data about infection statistics, particularly as the distribution of some helminth species in the host was aggregated.

An analysis of helminth community components was undertaken by calculating the frequency of occurrence of the number of helminth species, abundance index and the frequency distribution of helminths. The abundance index (I) was calculated according to Bush (1973) and Pence & Eason (1980), respectively, the helminth community of a host species being characterized by the presence of the following categories of species: dominant species ( $I > 1$ ); co-dominant species ( $0.1 \leq I \leq 1$ ); successful immigrant species ( $0 < I < 0.1$ ); unsuccessful immigrant species ( $I = 0$ ).

The frequency distribution of parasitic species of each infrapopulation was calculated using the Lefkovich index (L), where

$$L = (1/45)\text{tang}^{-1}(\text{variance/mean}) - 1$$

ranging from -1 (positive binomial or uniform distribution), 0 (Poisson or random distribution) and +1 (negative binomial or aggregated distribution).

Table 1. The number of *Apodemus sylvaticus* examined in Sierra Espuña, from 1999 to 2001.

Year	Sub-adult		Adult		Total
	Male	Female	Male	Female	
1999	17	17	7	2	43
2000	8	9	5	4	26
2001	1	–	3	1	5

The diversity/uniformity analysis of the helminth community was carried out using the Shannon index (Pielou, 1975; Magurran, 1988), Simpson index (D) (Simpson, 1949) expressed as 1-D (Magurran, 1988), Berger-Parker index (Berger & Parker, 1970; May, 1975; Magurran, 1988) and Shannon evenness index (Pielou, 1969; Magurran, 1988).

The helminth infracommunity structure was analysed using mean helminth abundance, mean species richness, the Brillouin index (Pielou, 1975; Magurran, 1988), the Brillouin index for infected hosts only and percentage of hosts infected relative to host age, sex and year of capture (only 1999 and 2000).

Where possible, standard non-parametric tests (chi-squared, Mann-Whitney and t tests) were applied (Sokal & Rohlf, 1981), and statistical significance was established at  $P < 0.05$ .

The role played by intrinsic (host age and sex) and extrinsic (year of capture – 1999, 2000) factors in determining species richness, helminth diversity and helminth abundance was analysed using a three-way analysis of variance (ANOVA).

## Results

A total of 55 mice (74.3%) were found to be infected with a total of 13 species: one trematode (7.7%), four cestodes (30.8%) and eight nematodes (61.5%) (table 2). *Pseudocatenotaenia matovi* showed the highest prevalence of infection (21.6%) and the most abundant was *Syphacia frederici* (15.7).

From the biological point of view, 63.5% of mice presented helminths with a direct cycle and 41.9% presented helminths with an indirect cycle, and the difference was statistically significant ( $\chi^2 = 6.10$ ,  $P = 0.0135$ ).

Forty percent of hosts presented between one and two helminth species, with infracommunities of up to five species and a high percentage of non-parasitized mice (table 3). Moreover, the frequency in the number of different helminth species present in each host showed a Poisson distribution.

The values of the Shannon (1.4), Simpson (0.7), Berger-Parker (0.5) and Shannon evenness (0.5) indices reflect the diversity/uniformity of the helminth community.

The abundance index values (table 4) make it possible to establish the following helminth community structure: *S. frederici*, *S. stroma* and *Aspicularis tetraptera* as dominant species; *Heligmosomoides polygyrus*, *P. matovi*, *Corrigia vitta*, *Eucoleus bacillatus*, *Aonchotheca annulosa* and *Trichuris muris* as co-dominant species; *Skrjabinotaenia lobata* and *Mastophorus muris* as successful immigrant species; and *Taenia parva* larvae and *Mesocestoides* sp. larvae as unsuccessful immigrant species.

The frequency distributions of the various helminth populations (table 4) show that all dominant species and the majority of co-dominant species present a negative binomial distribution.

The helminth infracommunity structure (table 5), except for the adult population, is essentially uniform, with between 23 and 37 helminths per host belonging to one or two species being the norm.

Table 2. The composition of helminth communities of *Apodemus sylvaticus* in the Sierra Espuña.

Helminth species	Cycle	Microhabitat	Prevalence			Mean abundance		Median intensity		Total number of parasites
			n	%	95% C.I.	SE	Range			
Trematoda										
<i>Corrigia vitta</i>	ILC	Pancreatic ducts	5	7	2–15	0.5	0.3	1	1–24	34
Cestoda										
<i>Taenia parva</i> larvae	ILC	Abdominal cavity	3	4	1–11	0.1	0.1	2	1–5	8
<i>Mesocestoides</i> sp. larvae	ILC	Abdominal cavity	7	10	4–19	3.3	2.1	8	1–147	244
<i>Pseudocatenotaenia matovi</i>	ILC	Small intestine	16	22	13–33	0.5	0.2	2	1–11	41
<i>Skrjabinotaenia lobata</i>	ILC	Small intestine	1	1	0–7	0.03	0.2	2	2	2
Catenotaeniidae gen sp.	ILC	Small intestine	4	5	1–13	0.07	0.04	1	1–2	5
Nematoda										
<i>Trichuris muris</i>	DLC	Caecum	8	9	4–18	0.1	0.05	1	1–2	9
<i>Eucoleus bacillatus</i>	DLC?	Stomach wall	5	7	2–15	0.3	0.1	3	1–8	20
<i>Aonchotheca annulosa</i>	ILC?	Small intestine	5	7	2–15	0.1	0.1	1	1–4	10
<i>Heligmosomoides polygyrus</i>	DLC	Small intestine	13	18	10–29	0.6	0.3	2	1–19	47
<i>Syphacia stroma</i>	DLC	Small intestine	13	18	10–29	8.8	4.5	8	1–272	652
<i>Syphacia frederici</i>	DLC	Intestine and caecum	14	19	11–30	15.7	10.1	5	2–698	1162
<i>Aspiculuris tetraptera</i>	DLC	Intestine	15	20	12–31	1.2	0.4	3	1–24	87
<i>Mastophorus muris</i>	ILC	Stomach	1	1	0–7	0.01	0.01	1	1	1

ILC, indirect life cycle; DLC, direct life cycle; n, number of infected hosts; %, prevalence; CI, confidence interval; SE, standard error.

The infracommunities determined by host age and year of capture present differences which are not statistically significant when referring to mean helminth abundance. These values are always below the adult and year 2000 sub-populations.

Table 3. The frequency (%) of helminths in *Apodemus sylvaticus* from Sierra Espuña.

No. of helminth species	n	%
0	19	25.7
1	23	31.1
2	17	23.0
3	10	13.5
4	3	4.1
5	2	2.7

n, number of infected hosts.

Table 4. Abundance index (AI) and Lefkovich index (L) of the helminth communities of *Apodemus sylvaticus* from Sierra Espuña.

Helminth species	AI	L
<i>Corrigia vitta</i>	0.46	0.93
<i>Taenia parva</i> larvae	0	0.66
<i>Mesocestoides</i> sp. larvae	0	0.99
<i>Pseudocatenotaenia matovi</i>	0.55	0.72
<i>Skrjabinotaenia lobata</i>	0.03	0.41
<i>Trichuris muris</i>	0.12	0.18
<i>Eucoleus bacillatus</i>	0.27	0.79
<i>Aonchotheca annulosa</i>	0.14	0.55
<i>Heligmosomoides polygyrus</i>	0.64	0.86
<i>Syphacia stroma</i>	8.81	0.99
<i>Syphacia frederici</i>	15.70	1.00
<i>Aspiculuris tetraptera</i>	1.18	0.90
<i>Mastophorus muris</i>	0.01	0

Species richness presents differences statistically significant only between the infracommunities determined by age,  $U = 405$ ,  $P = 0.0415$ .

The diversity of the infracommunities, determined by the Brillouin value index (table 5), takes into account the total of each infracommunity as well as that of infected hosts only, revealing a greater diversity in the adult compared with the sub-adult sub-population. However, the infracommunities determined by host sex show no significant differences of diversity.

High prevalence values are found in almost all infracommunities studied except for a slightly lower value in the sub-adult sub-population, but these differences were not statistically significant.

The ANOVA analysis shows that the most important source of variation for the mean species richness and the mean helminth diversity (expressed by Brillouin index) arose from the year of capture, host age and the interaction between two or three factors analysed, year of capture and host sex and age (table 6). However, the mean helminth abundance does not seem to be affected by any factor.

## Discussion

The helminth community of *A. sylvaticus* is composed of 13 species only. Helminths with an aquatic cycle have not been detected due to the absence of permanent water during the greatest part of the year; the same is true for other enclaves, such as the Spanish Mediterranean enclave of Serra Calderona (Fuentes *et al.*, 2000).

The high prevalence values, contrasted with the infracommunity composition, consist mainly of one or two helminth species which correlates with the Shannon, Berger-Parker and Shannon evenness indices showing a low richness or diversity. However, the Simpson index presents higher values but this is due to the absence of a

Table 5. Diversity characteristics of the helminth infracommunities in *Apodemus sylvaticus* (A.s.) from Sierra Espuña.

Characteristics	Host age		Host sex		Year of capture	
	Adult	Sub-adult	Male	Female	1999	2000
Mean helminth abundance	22.7	36.7	31.3	31.5	44.9	11.8
SE	10.0	16.7	18.1	10.3	18.3	5.9
Mean species richness	1.9	1.2	1.5	1.5	1.6	1.3
SE	0.3	0.3	0.2	0.2	0.2	0.2
Brillouin index	0.29	0.18	0.21	0.21	0.23	0.18
SE	0.07	0.04	0.04	0.05	0.04	0.06
Max.	0.98	0.79	0.91	0.98	0.98	0.91
B.I. infected A.s. only	0.33	0.27	0.29	0.28	0.32	0.23
SE	0.07	0.05	0.05	0.06	0.05	0.07
% of A.s. infected	85.7	67.4	73.7	75.8	72.1	76.9

clearly dominant species. Only 22% of hosts were infected with *P. matovi*, the helminth with the highest prevalence, and the three dominant species present all have prevalences in the region of 20%.

Surprisingly, the helminth community structure has a low number of dominant species, namely three, and amongst these no prevalent species is found. Moreover, only five of 13 species of the helminth community are component species (prevalence >10%).

The helminth community has a low destabilizing capacity on the wood mouse population as the five component species, besides *C. vitta* and *E. bacillatus*, present a clearly negative binomial distribution.

The results of the ANOVA analysis show the factors which affect the variation of the mean species richness and the mean helminth diversity. The year of capture might have a clear influence as climatic differences occur between years. In the present study, 2000 was much drier than 1999 with respect to the region studied (Spanish Meteorological Institute data). Thus, some helminth species might have had difficulty in completing their life cycles, especially heteroxenous ones and geohelminths. Differences detected in the infracommunities determined by host age may be related to a more vegetarian diet in the sub-adult sub-population and a higher intake of invertebrates amongst the adult sub-population. In both

cases, these differences can be attributed to species with indirect cycles. They are more prevalent in the 1999 and in the adult sub-populations. The absence of differences between the infracommunities determined by host sex show that males and females of the wood mouse are equally exposed to infection with the parasite species.

In conclusion, intrinsic (host age) and extrinsic (year of capture) factors play a major role in determining species richness and helminth diversity but not in determining the abundance of helminths. The sex of the host does not seem to affect the infection rate nor the diversity.

These results correlate with the conclusions of Behnke *et al.* (1999) and Abu-Madi *et al.* (2000) for *A. sylvaticus* in southern England and south-east England, respectively, although these studies considered each helminth species separately.

Future studies using larger samples of *A. sylvaticus* from the Regional Park of Sierra Espuña and an analysis of a complete data set of seasonal climatic data for the years of capture are required. Moreover, a helminthological study of other small mammal species present in this regional park will permit us to assess their helminth fauna and the possible interactions with helminth communities in the wood mouse.

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Table 6. Species richness and mean helminth diversity in *Apodemus sylvaticus* from Sierra Espuña by year of capture, host age and host sex, through a three-way ANOVA, expressed by F-statistic values with associated probabilities (*P*).

Source of variation	Species richness		Mean helminth diversity	
	F	<i>P</i>	F	<i>P</i>
Year of capture	8.3780	0.0053	6.2680	0.0150
Host age	9.7812	0.0027	8.0257	0.0062
Year of capture/ host age	6.2865	0.0015	4.3141	0.0420
Year of capture/ host sex	5.6429	0.0207	6.6059	0.0147
Year of capture/ host age/sex	9.1584	0.0036	10.0954	0.0023

Only statistically significant values are reported.

## References

- Abu-Madi, M.A., Behnke, J.M., Lewis, J.W. & Gilbert, E.S.** (2000) Seasonal and site specific variation in the component community structure of intestinal helminths in *Apodemus sylvaticus* from three contrasting habitats in south-east England. *Journal of Helminthology* **74**, 7–15.
- Aznar, F.J., Badillo, F.J. & Raga, J.A.** (1998) Gastrointestinal helminths of loggerhead turtles (*Caretta caretta*) from the western Mediterranean: constraints on community structure. *Journal of Parasitology* **84**, 474–479.
- Behnke, J.M., Lewis, J.W., Mohd Zain, S.N. & Gilbert, E.S.** (1999) Helminth infections in *Apodemus sylvaticus* in southern England: interactive effects of host age, sex and year on the prevalence and abundance of infections. *Journal of Helminthology* **73**, 31–44.
- Berger, W.H. & Parker, F.L.** (1970) Diversity of planktonic Foraminifera in deep sea sediments. *Science* **168**, 1345–1347.
- Bush, A.O.** (1973) *An ecological analysis of the helminth parasites of the white ibis in Florida*. 115 pp. M.S. thesis, University of Florida.
- Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W.** (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575–583.
- Cordero del Campillo, M., Castañón Ordóñez, L. & Reguera Feo, A.** (1994) *Índice-catálogo de zooparásitos ibéricos*. 650 pp. León, Universidad de León, Secretariado de Publicaciones.
- Feliu, C., Renaud, F., Catzefflis, F., Hugot, J.P., Durand, P. & Morand, S.** (1997) A comparative analysis of parasite species richness of Iberian rodents. *Parasitology* **115**, 453–466.
- Fuentes, M.V., Cerezuela, A.M. & Galán-Puchades, M.T.** (2000) A helminthological survey of small mammals (insectivores and rodents) in the Serra Calderona mountains (Valencian Community, Spain). *Research and Reviews in Parasitology* **60**, 25–35.
- González Castro, J.** (1944) Contribución al estudio del parasitismo por helmintos o sus fases larvarias de diversos múridos capturados en Granada. *Revista Ibérica de Parasitología* **4**, 38–60.
- López-Neyra, C.R.** (1947) *Helmintos de los vertebrados ibéricos*. Granada, Imprenta Urania.
- Magurran, A.E.** (1988) *Ecological diversity and its measurement*. 179 pp. London, Croom Helm Ltd.
- Mas-Coma, S. & Gállego, J.** (1980) Conocimientos actuales sobre la helmintofauna de micromamíferos (Insectivora; Rodentia) en España. pp. 533–573 in Cordero del Campillo, M. *et al.* (Eds) *Índice-catálogo de zooparásitos ibéricos*. Madrid, Ministerio de Sanidad y Seguridad Social.
- May, R.M.** (1975) Patterns of species abundance and diversity. pp. 81–120 in Cody, M.L. & Diamond, J.M. (Eds) *Ecology and evolution of communities*. Cambridge, Harvard University Press.
- Pence, D.B. & Eason, S.** (1980) Comparison of the helminth faunas of two sympatric top carnivores from the rolling plains of Texas. *Journal of Parasitology* **66**, 115–120.
- Pielou, E.C.** (1969) *An introduction to mathematical ecology*. 250 pp. New York, John Wiley & Sons.
- Pielou, E.C.** (1975) *Ecological diversity*. 165 pp. New York, John Wiley & Sons.
- Portolés, E., Granel, P. & Esteban, J.G.** (1996) Helminth-faunistic analysis of *Crocidura russula* (Hermann, 1780) (Insectivora: Soricidae) from the Albufera Natural Park (Valencia, Spain). *Research and Reviews in Parasitology* **56**, 203–211.
- Portolés, E., Granel, P. & Esteban, J.G.** (2000) Helminth-faunistic analysis of *Apodemus sylvaticus* (Linnaeus, 1758) (Rodentia: Muridae) from the Albufera Natural Park (Valencia, Spain). *Research and Reviews in Parasitology* **60**, 17–23.
- Simpson, E.H.** (1949) Measurement of diversity. *Nature* **163**, 688.
- Sokal, R.R. & Rohlf, F.J.** (1981) *Biometry. The principles and practice of statistics in biological research*. 2nd edn. 859 pp. New York, W.H. Freeman and Co.

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