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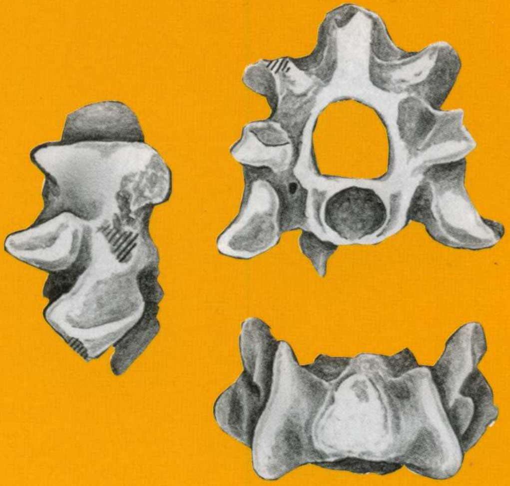

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Intestinal helminths parasitizing *Mauremys leprosa* (Chelonia: Bataguridae) from Extremadura (western Spain)

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Abstract: A survey of the intestinal helminth community of the Spanish terrapin, *Mauremys leprosa* (Schweiger, 1812), from Extremadura, Spain, was conducted in order to determine the presence, prevalence, intensity, and diversity of parasites in this freshwater turtle. The digenean *Patagium pellucidum* and the nematode *Spyroxis* sp. are recorded for the first time in freshwater turtles of Spain. The nematodes *Falcaustra* sp. and *Serpinema microcephalus* are core species in the helminth component community of *M. leprosa*. Helminth infracommunities of these turtles are depauperate and isolationist.
Key words: freshwater turtles, helminth communities, Spain.

Resumen: Helmintos intestinales parásitos de *Mauremys leprosa* (Chelonia: Bataguridae) de Extremadura (oeste de España). – Se ha llevado a cabo un análisis de la comunidad de parásitos intestinales de ejemplares del galápago leproso, *Mauremys leprosa* (Schweiger, 1812) procedentes de Extremadura, España, para determinar la presencia, prevalencia, intensidad y diversidad de parásitos. El digénido *Patagium pellucidum* y el nematodo *Spyroxis* sp. se citan por primera vez en galápagos españoles. Los nematodos *Falcaustra* sp. y *Serpinema microcephalus* se constituyen como especies del núcleo en la comunidad componente de *M. leprosa*. Las infracomunidades helmintianas de estos galápagos resultaron ser depauperadas y aislacionistas.
Palabras clave: comunidades helmintianas, España, galápagos.

INTRODUCTION

Studies of the helminth parasites of the two species of Spanish freshwater turtles, *Mauremys leprosa* (Schweiger, 1812) and *Emys orbicularis* (Linnaeus, 1758) have been partial and scarce. LÓPEZ-NEYRA (1947) and LÓPEZ-ROMÁN & GUEVARA-POZO (1977) recorded one species of Nematoda, LÓPEZ-ROMÁN (1976) recorded three species of Monogenea, and LÓPEZ-ROMÁN (1974) recorded one species of Digenea (see also LLUCH *et al.*, 1987). But these, primarily faunistic or taxonomic studies, did not include data on the composition and structure of the helminth communities. More recently, preliminary intestinal and fecal analyses have been conducted on both turtles (CRESPO-GONZÁLEZ *et al.*, 2002; HIDALGO-VILA *et al.*, 2004), and also on a wild population of *Trachemys scripta elegans* (Wied-Neuwied, 1839), a North American exotic turtle (HIDALGO-VILA *et al.*, 2004). However, none of these studies provided data on the helminth communities of freshwater turtles. In the rest of Eurasia and in North Africa (which encompasses the known distribution areas of *M. leprosa* and *E. orbicularis*, see DA SILVA, 2002; KELLER & ANDREU, 2002), parasitological knowledge is likewise scarce and does not include any data on communities of parasites (see DOLLFUS, 1963; GRABDA-

KAZUBSKA, 1972; KNOEPFFLER & COMBES, 1977; COMBES & THIERY, 1983). Regarding the knowledge of parasites of *T. scripta* (and other freshwater turtles from North America), there is more abundant data on helminths (BAKER, 1979, 1986; NICKOL & ERNST, 1987) referring to both biogeography and host-parasites relationships (ERNST & ERNST, 1980). Data on unicellular parasites have also been reported for these turtles (MCALLISTER & UPTON, 1988, 1989).

Recently, the introduction in Spain of the red-eared slider, *T. s. elegans* and the settlement of wild populations in areas occupied by populations of autochthonous freshwater turtles has given rise to an increased interest among Spanish herpetologists for all aspects of the biology of these turtles (A. Andreu, personal communication; J. Lluch, personal communication). Regarding their parasites, interest is focused on the need to know the parasites of both groups of freshwater turtles (autochthonous and exotic), and to assess the possibility of parasite interchange between them.

In this paper, we report on the helminth community of a population of the Spanish terrapin, *M. leprosa*, from Extremadura. Our approach was to analyse the prevalence, intensity, and diversity of helminths from this host, specifically addressing the following points: (i) characterization of helminth community richness and diversity; (ii) characterization of helminths as either specialists or generalists; and (iii) characterization of the helminths as core, secondary or satellite species.

MATERIALS AND METHODS

In all, 72 digestive tracts of *M. leprosa* from Extremadura (Western Spain) were examined for helminths: eight were of males, 18 of females, and 46 of individuals of unknown sex. Unfortunately, we have no data on the localities where animals were caught or their measurements.

Digestive tracts fixed in 70% alcohol were sent to the laboratory for analysis (we do not know the previous procedures on the animals). They were opened and placed in distilled water for examination. Helminths were removed, washed, fixed and mounted according to standard techniques (for details see ROCA, 1985). Parasites were identified, when possible, to species, and the number and location of individuals of each species were recorded. After helminth examination, digestive tracts were deposited in the herpetological collection of the Department of Zoology, University of Valencia (Spain). Voucher specimens of helminths were deposited in the helminthological collection of the Department of Zoology, University of Valencia (Spain), with the following accession numbers: *Patagium pellucidum* ML07PP, *Telorchis solivagus* ML03TS, *Serpinema microcephalus* ML12SM, *Falcaustra* sp. ML01FSP, and *Spyroxis* sp. ML16SSP.

The descriptive ecological terms are basically prevalence (the number of infected hosts divided by the number of examined hosts, expressed as a percentage), mean intensity (the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite), and mean abundance (the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined, including both infected and uninfected hosts). Brillouin's index was used for calculating diversity according to MAGURRAN (2004). A prevalence of 10% was adopted as the lowest limit in identifying satellite species (KENNEDY & BAKKE, 1989). Species with prevalences 10%

< p < 30% were assigned as secondary species (HANSKI, 1982; ROCA, 1993). Species with prevalences of 30% or higher were considered as core species (ROCA, 1993).

RESULTS

In all, five helminth species were found in the gastrointestinal tract of *M. leprosa* (two Trematoda and three Nematoda). The total number of parasite species, the site in which they were found, and the infection parameters are in Table 1. The overall prevalence of infection of the sample of *M. leprosa* from Extremadura was 83.33%.

The intestinal helminth infracommunities of the turtles examined comprised trematodes and nematodes. The digenean *Telorchis solivagus* was only found in two hosts. The nematode *Spyroxis* sp. was found in four hosts. The mean values of species richness and abundance were 1.24 (SD = 0.80, range = 0-3), and 58.03 (SD = 95.71, range = 0-532) respectively. The mean value of Brillouin's index of diversity was 0.11 (SD = 0.17, range = 0-0.66).

Patagium pellucidum (Coil et Kuntz, 1958) (Digenea: Auridistomidae) was characterized by a spatulated body, an oral sucker with two pairs of conical lateral projections, the position of the gonads (testes diagonal, ovary posterolateral to acetabulum) and the position of the genital pore (median, a little anterior to acetabulum). Eggs (n = 35) measured 75.1 ± 5.6 µm length, 45.8 ± 4.5 µm wide.

Telorchis solivagus Odhner, 1902 (Digenea: Telorchiidae) was characterized by the position of the testicles and ovary and the length of the cirrus pouch. Eggs (n = 35) measured 26.5 ± 2.6 x 16.1 ± 2.3 µm.

Serpinema microcephalus (Dujardin, 1845) (Nematoda: Camallanidae) has a peculiar cephalic structure with buccal valves provided of cuticular ridges. Larvated eggs. Eggs (n = 35) measured 20.6 ± 2.4 x 13.4 ± 1.8 µm.

Falcaustra sp. (Nematoda: Kathlaniidae) is characterized by the presence of three big lips surrounding the mouth, and males possessing well-developed preanal muscles. Eggs (n = 35) measured 111.5 ± 6.3 x 82.5 ± 3.5 µm.

Only 13 specimens of *Spyroxis* sp. were found (females and larvae). As is typical of species belonging to the order Spirurida, in our sample individuals have a cylindrical oesophagus divided into an anterior muscular and a larger posterior glandular portion. Also,

TABLE 1. Infection parameters of helminth species parasitizing *Mauremys leprosa* from Extremadura. Int: intestine, Sto: stomach,*: larvae were found at other sites, such as the gall bladder or liver.

TABLA 1. Parámetros de infección de las especies parásitas de *Mauremys leprosa* de Extremadura. Int: intestino, sto: estómago,*: las larvas fueron encontradas en otras localizaciones, como la vejiga urinaria o el hígado.

Helminth species	Adult site	Prevalence (%)	Mean intensity	Mean abundance
Digenea				
Patagium pellucidum	int.	8.3	5.8 ± 4.0 (1-10)	0.5 ± 1.9 (0-10)
Telorchis solivagus	int..	2.8	3.0 ± 1.4 (2-4)	0.1 ± 0.5 (0-4)
Nematoda				
Serpinema microcephalus	int.	31.9	10.6 ± 14.9 (1-58)	3.4 ± 9.7 (0-58)
Falcaustra sp	int.*	75	71.9 ± 104.2 (1-532)	53.9 ± 95.3 (0-532)
Spiroxys sp	sto. & int.	5.6	3.3 ± 3.9 (1-9)	0.2 ± 1.1 (0-9)

two large lateral lips are present in our specimens. Eggs (n = 5) measured 37.8 ± 2.1 x 30.0 ± 2.8 µm.

DISCUSSION

Systematics and chorology of helminths

Based on morphological characters, several specimens of Digenean found in *M. leprosa* from Extremadura (Spain) were identified as *Patagium pellucidum* (YAMAGUTI, 1971). This species was previously recovered in Turkey from the same host (YAMAGUTI, 1971), but this is the first time that *P. pellucidum* is found in any turtle from Spain (CORDERO DEL CAMPILLO *et al*., 1994).

Telorchis solivagus is a common species parasitizing *M. leprosa* and *E. orbicularis* from many areas of Europe, Africa and Asia (HUGHES *et al*., 1942; JOYEUX & GAUD, 1945; DOLLFUS, 1963; MISHRA & GONZÁLEZ, 1978). In Spain, it was first found in *M. leprosa* from Granada (LÓPEZ-ROMÁN, 1974). Our results provide the second record for this species in Spanish freshwater turtles (CORDERO DEL CAMPILLO *et al*., 1994).

The nematode *Serpinema microcephalus* (and the congeneric *S. trispinosus*) may be the best species to use as an indicator of parasite interchange between autochthonous (*M. leprosa*, *E. orbicularis*) and American (mainly *T. scripta*) freshwater turtles. *Serpinema trispinosus* is known to be as parasite only in turtles of the Nearctic: Southeastern Canada and the Eastern United States south to Cuba and Texas (MACCALLUM, 1918; MAGATH, 1919; BARUS & MORAVEC, 1967), whereas *S. microcephalus* only occurs in turtles of the Western Palearctic (BAKER, 1979). The later is common in turtles of Western Europe, and has also been recorded in western Russia (SHARPILO, 1976) and in North Africa (IVASHKIN *et al*., 1971). Both species are very similar, but they differ in the morphology of the buccal valves. In *S. trispinosus* the valves are relatively wider and shorter than in *S. microcephalus*. The ridges in each buccal valve in *S. trispinosus* vary in number from approximately 15 to 19. In *S. microcephalus*, the ridges are less numerous (8-11) but markedly thicker (BAKER, 1979). *Serpinema microcephalus* was previously recorded in Spanish freshwater turtles by LÓPEZ-NEYRA (1947) and LÓPEZ-ROMÁN & GUEVARA-POZO (1977) in the province of Granada. HIDALGO-VILA *et al*. (2004) noted the presence of this species in wild populations of *Trachemys scripta elegans* from Andalucía, and suggested the possibility of transmission of this nematode from autochthonous to exotic turtles.

The genus *Falcaustra* Lane, 1915 (= *Spironoura* Leidy, 1856) contains many species with a worldwide distribution (YAMAGUTI, 1961; MANNA & MAHAPATRA, 1989). The taxonomy of the genus is unclear because of the large number of species (about 50 nominal species, BAKER, 1986) and the fact that many descriptions are incomplete. Thus, the specimens found in *M. leprosa* have been preliminarily identified as *Falcaustra* sp. until further studies clarify their taxonomic status. The species of this genus are found in many hosts, such as freshwater turtles, amphibians or fish (YORKE & MAPLESTONE, 1969; ERNST & ERNST, 1980; MUZZALL, 1991; GOLDBERG *et al*., 1996). In Spain, *Falcaustra* sp. has been recently recorded in *M. leprosa*, *E. orbicularis* and *T. scripta elegans* (HIDALGO-VILA *et al*., 2004), from Andalucía.

Thirteen of the nematodes found in *M. leprosa* had characters (see results) which allowed us to include them as species of the genus *Spyroxis* (YORKE & MAPLESTONE, 1969; CHABAUD, 1974; NAVARRO, 1987). Most likely, our specimens belong to the species

S. contortus (Rudolphi, 1819) since this is a common species parasitizing *M. leprosa* and *E. orbicularis* (SCHAD *et al.*, 1960; YAMAGUTI, 1961; YORKE & MAPLESTONE, 1969). *Spyroxis contortus* is a widespread species parasitizing mainly freshwater turtles and amphibians (YAMAGUTI, 1961; COY-OTERO & BARUS, 1979; COY-OTERO & LORENZO-HERNÁNDEZ, 1982; SHARPILO, 1983). In Spain, larvae of *Spyroxis* sp. (probably *S. contortus*) were found in the external wall of the stomach and intestine of *Rana perezi* (NAVARRO, 1987). Larvae of this species (and also of *Falcaustra* sp. and *S. trispinosus*) have also been found in the aquatic snail *Lymnaea stagnalis* in Ontario (BARTLETT & ANDERSON, 1985). Thus, this is the first record for *Spyroxis* sp. in Spanish freshwater turtles, and also for adults of this species in any Spanish host (CORDERO DEL CAMPILLO *et al.*, 1994).

Helminth community of *Mauremys leprosa*

Of the five helminth species found in this study, only one, *Falcaustra* sp. was previously known from *M. leprosa* (and also in *E. orbicularis*) from Andalucía, Spain (HIDALGO-VILA *et al.*, 2004). *Aplectana* sp. was also found in autochthonous turtles in Andalucía (HIDALGO-VILA *et al.*, 2004), but not in Extremadura. *S. microcephalus* and *Physaloptera* sp. (probably *Spyroxis*) were found in free populations of *T. scripta elegans* in Andalucía (HIDALGO-VILA *et al.*, 2004), and *M. leprosa* from Extremadura. On the other hand, eggs of nematodes and trematodes were recorded from faeces of *E. orbicularis* in areas of Galicia (North Western Spain) (CRESPO-GONZÁLEZ *et al.*, 2002). Three of the helminth species found in *M. leprosa* from Extremadura, *P. pellucidum*, *T. solivagus* and *S. microcephalus* can be considered, in adult stage, as freshwater turtles specialists (*sensu* EDWARDS & BUSH, 1989) since they parasitize only these reptiles. The nematodes *Falcaustra* sp. and *Spyroxis* sp. appear to be generalist species that parasitize freshwater turtles and also other amphibian, reptile and fish hosts (see previous references). Thus, it seems that interchanges of helminth species among autochthonous and exotic freshwater turtles may be possible depending on favourable environmental conditions permitting contact between the final host and the infective stages of the parasite. This requires hosts to live in similar habitats (in this case, aquatic habitats) and to feed on similar foods (ERNST & ERNST, 1980), as is probably the case with autochthonous and exotic freshwater turtles in Spain.

Most helminth species occurred at low prevalence. The nematodes *Falcaustra* sp. and *S. microcephalus* can be considered as core species, whereas the remaining helminths are satellite species; there are no secondary species. The low values of prevalences and mean intensities of infection (Table 1) indicate that many members of the helminth infracommunities occurr only irregularly and occasionally (MARTIN & ROCA, 2005). This occurs in *M. leprosa* in the cases of the trematodes *P. pellucidum* and *T. solivagus* and the nematode *Spyroxis* sp. Only the nematode *Falcaustra* sp. appears to be a common parasite of this turtle. This agrees with the typical pattern of helminth infection in many reptiles, i.e. few species occur frequently, few species occur with moderate prevalence (e.g. *S. microcephalus* in *M. leprosa*), and many species are rare (AHO, 1990; MARTIN & ROCA, 2005).

Patterns of diversity and abundance of helminths in *M. leprosa* from Extremadura are similar to those observed in other reptiles. Thus, the mean value of Brillouin's diversity index is the same as that for *Podarcis lilfordi* from Balearic Islands (ROCA & HORNERO, 1994), and close to the values observed for other insectivorous reptiles (ROCA, 1999). As in the later hosts, helminth communities of *M. leprosa* are depauperate and isolationist, a pattern probably widespread in other populations of freshwater turtles in Spain. Some characteristics of these reptile hosts, such as ectothermy, simplicity of the alimentary canal, generalist diet, helminths with indirect life cycles, and low vagility (KENNEDY *et al.* 1986; ROCA & HORNERO, 1994), may be responsible for this pattern.

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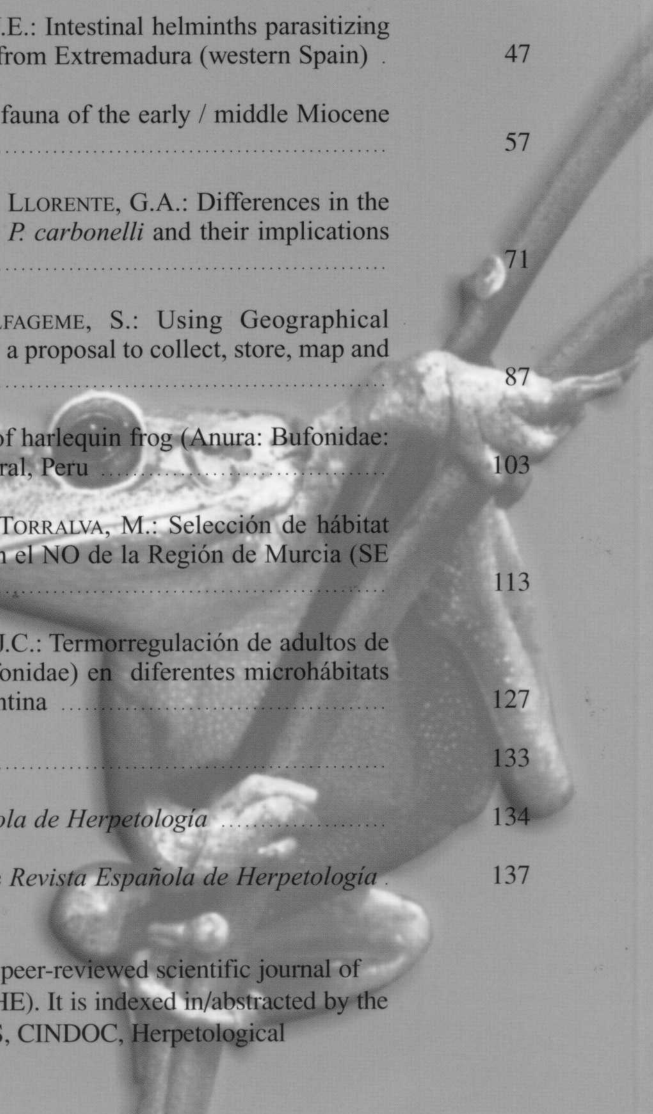

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