Gastrointestinal helminths found in the three freshwater turtles (*Erymnochelys madagascariensis*, *Pelomedusa subrufa* and *Pelusios castanoides*) from Ankarafantsika National Park, Madagascar

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Summary

We conducted a survey of the presence, prevalence and diversity of gastrointestinal helminths in faecal samples and stomach contents of three turtle species, - *Erymnochelys madagascariensis* (Chelonia: Podocnemididae), *Pelomedusa subrufa* and *Pelusios castanoides* (Chelonia: Pelomedusidae), - from several localities in Madagascar. Four nematode species were detected: *Atractis chabaudi*, *Camallanus chelonius*, *Falcaustra pelusios*, and *Spiroxys* sp. *E. madagascariensis*, with all four species, had the greatest helminth diversity. *A. chabaudi* was the community's core species, whereas *Spiroxys* sp. was a satellite species. Only two species (*A. chabaudi* and *Spiroxys* sp.) were found in *P. subrufa* and only one, *Spiroxys* sp., in *P. castanoides*. These are the first helminthological data on *E. madagascariensis*, one of the most threatened freshwater turtles in the world.

Key words: turtles; nematodes; Madagascar

Introduction

Madagascar, with its high levels of species endemism, is considered a global hotspot of high conservational importance (García, 2005). Four species of freshwater turtles are found there: *Pelomedusa subrufa*, *Pelusios castanoides*, *P. subniger* (Pelomedusidae) and *Erymnochelys madagascariensis* (Podocnemididae) (Glaw & Vences, 1994). *Erymnochelys* is a monotypic genus endemic to Madagascar (Bonm *et al.*, 2006). It is a relict form of the fauna of Gondwana, and the only extant Old World representative of the family Podocnemididae. With populations in severe decline (García, 2005), it is one of the 15 chelonian species with the highest conservation priority rating in the IUCN/SSC.

Previously there had been no studies on the helminth fauna of *E. madagascariensis*, and only a few partial studies of

P. subrufa and *P. castanoides* from Madagascar (Euzet & Combes, 1965). In this paper, we report the helminths found in the gastrointestinal tracts of these hosts. Our approach was to analyse the prevalence, intensity, and diversity of their helminths specifically addressing the following points: (i) characterisation of taxonomy and chorology of the helminths; (ii) characterisation of the helminths as either specialists or generalists; and (iii) characterisation of the helminths as core, secondary or satellite species in the helminth community of *E. madagascariensis*.

Material and Methods

Study sites

The area where the sample was carried out was The Ankarafantsika National Park, in NW Madagascar, between the Marovoay and Ambato-Boeni regions in Mahajanga province (16°00'-16°20'S, 49°15'-49°31'E) (Fig. 1). Nine sites were selected, including three lakes (Antsilomba, Ravelobe and Antsiloky), two rivers (Andranomiditra and Ambavan'Ampijoroa River) and an area modified by human activity, Ambarindahy.

Sampling of hosts

Three techniques for capturing turtles were used: trapping, netting, and fishing rods.

Baited traps have the advantage of not requiring much maintenance, apart from changing the bait. The major disadvantage is that they require regular checking since turtles may escape. The traps used were designed, described and built by Kuchling (2004).

In rivers, long nets were also used. No netting was possible in the lakes because it was culturally prohibited. Nets were used to trawl for turtles and were especially useful under aquatic vegetation along riverbanks.

Indirect capture through incidental collection by villagers

during routine fishing excursions occurred in some areas using a number of traditional fishing techniques (see García, 2005). These incidental captures provided a source of turtles additional to direct trapping.

Obtaining parasites

The method of stomach flushing, described by Legler (1997), enabled the collection of animal and vegetable matter as well as nematodes in the stomach.

Faecal sampling is a good method to obtain parasites when examination of the intestine by sacrificing animals is not possible (Roca *et al.*, 1999). We did not use destructive sampling because of the highly-threatened status of *E. madagascariensis* and because alternative techniques were available.

Six samples from *P. castanoides* (stomach contents), 12 from *P. subrufa* (stomach contents) and 95 from *E. madagascariensis* (8 stomach contents, 87 faecal samples) were obtained.

Samples were fixed in 70 % alcohol. Parasites were identified to species when possible and the number of individuals of each species was recorded.

The use of descriptive eco-parasitological terms follows Bush *et al.* (1997) and 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 a prevalence of 30 % or higher were considered as core species (Roca, 1993).

Results

Four nematode species were found. All were in the gastrointestinal tract of *E. madagascariensis*; two were in the stomach of *P. subrufa*, and only one in the stomach of *P. castanoides*. The total number of nematode species, the site in which they were found, and the infection prevalence are shown in Table 1. Specimens were deposited in the Helminthological Collection of the Department of Zoology, University of Valencia, Spain, accession numbers S3SA, S63SD, P75SB, P89SA, S91SA, P94SB, C24SB, C48SA, C52SA, C67SA, C112SA, C128SB, C141FA, C205SC, C209FA, C228SA, C239FA, C297FA, EF1 – EF95.

From a quantitative point of view, the most important nematode found was *Atractis chabaudi* Petter, 1966 (Atractidae) (Fig. 2). Monodelphic females with two or three lar-

ge eggs and many larvae in the uterus were identified. Males had two unequal spicules, cuticular ornamentation formed by one ventral range of small plectanes, and 10 pairs of caudal papillae: 3 precloacal, 1 adcloacal, and 6 postcloacal pairs.

Four specimens, one male, one female and two larvae, of *Camallanus chelonius* Baker, 1983 (Camallanidae) were found. They showed a peculiar cephalic structure with buccal valves made up of 8 – 10 cuticular ridges (Fig. 3A).

Seventy-one specimens of *Falcaustra pelusios* Baker, 1983 (Kathlaniidae) were found. They were characterised by the presence of a spherical oesophageal isthmus, the absence

Fig. 1. Location (arrow) of Ankarafantsika National Park in Madagascar

Table 1. Nematode species and their prevalence, parasitising turtles from Madagascar

Species	Site			Prevalence (%)		
	<i>E.m.</i>	<i>P.s.</i>	<i>P.c.</i>	<i>E.m.</i>	<i>P.s.</i>	<i>P.c.</i>
<i>Atractis chabaudi</i>	FC	SC		80	33.3	
<i>Camallanus chelonius</i>	SC			3.2		
<i>Falcaustra pelusios</i>	FC			16.9		
<i>Spiroxys</i> sp.	SC	SC	SC	2.1	83.3	100

E.m. – *Erymnochelys madagascariensis*; *P.s.* – *Pelomedusa subrufa*; *P.c.* – *Pelusios castanoides*; SC – stomach contents; FC – faecal sample

Fig. 2. *Atractis chabaudi*. A – ♀ caudal end, lateral view; B – spicules and gubernaculum; C – ♀ anterior part of body, lateral view; D – ♂ caudal end, ventral view. A – scale 500 µm; B – scale 100 µm; C, D – scale 250 µm

of an intestinal diverticulum, and, in the males, a pre-anal ventral sucker, two subequal spicules and a large gubernaculum (Fig. 3B, C, D).

A total of 34 specimens of *Spiroxys* sp. were found (14 males, 15 females, 5 larvae), mainly in *P. subrufa* and *P.*

castanoides. The species is characterised by two large pseudolabia with denticulated lobes, no marked division between the anterior and posterior part of the oesophagus, non-larvated eggs in the females, and subequal spicules and ten pairs of caudal papillae in the males.

Fig. 3. A – *Camallanus chelonius* ♀ anterior extremity, lateral view; B – *Falcaustra pelusios* ♀ anterior extremity, dorsal view; C – *Falcaustra pelusios* ♂ posterior end, lateral view; D – *Falcaustra pelusios*, spicules and gubernaculum. A – scale 250 µm; B – scale 100 µm; C – scale 1 mm; D – scale 500 µm

Discussion

Systematics and chorology of helminths

Atractis chabaudi is characterised by the morphology of the oesophagus; the length and morphology of the spicules (one of them long and lanceolate and the other short and

lagenoid); and the disposition of caudal papillae in the males (Petter, 1966; Bursey & Flanagan, 2002). With the same papillae pattern, our specimens match the original description of Petter (1966). This character separates *A. chabaudi* from the other Ethiopian species of the genus (Bursey & Flanagan, 2002). *A. chabaudi* has been found in the

tortoise *Pyxis arachnoides* (Testudinidae) from the south of Madagascar (Petter, 1966). Since we found it in freshwater turtles, we suggest that this species is a specialist (*sensu* Edwards & Bush, 1989) for the group Testudinata (tortoises, terrapins, turtles) suggesting a co-adaptation and co-evolution among species of two ancient parasite-host groups, as the Atractidae (see Baker, 1982; Adamson & Baccam, 1988) and the Testudinata (see Bellairs, 1969). The three species of turtles are new hosts for this nematode species.

With buccal valves each having 9 – 10 thick ridges (Baker, 1983), *Camallanus chelonius* is characterised by the morphology of its oral region. Our specimens had longitudinal ridges in the buccal valves undivided into dorsal and ventral groups, - a characteristic (division of the ridges) typical of the genus *Serpinema* (Yeh, 1960) – so they match the description by Baker (1983). Previously recorded from *Pelusios sinuatus*, this is the first record of this species in Madagascan turtles and corroborates the status of *C. chelonius* as an intermediate form in the evolution from *Camallanus* to *Serpinema* (see Petter, 1979; Baker, 1983). *Serpinema* first evolved in pleurodirans and later radiated with the major cryptodiran radiation around 120 – 90 mya (see Ferguson & Smales, 1998).

Falcaustra Lane, 1915 (= *Spirogonura* Leidy, 1856) contains many species with a worldwide distribution (Yamaguti, 1961; Manna & Mahapatra, 1989). The specimens found in *E. madagascariensis* have been identified as *F. pelusios* by the presence of two cheilostomal rings at the cephalic extremity, the short tail in the male, subequal and thick spicules, large gubernaculum, and more than 50 pairs of subventral pre-anal muscle cells posterior to the sucker, features that match the description by Baker (1983) of specimens from *Pelusios sinuatus*. Another species, *F. golvani* Chabaud and Brygoo, 1957, was described in a rhacophorid frog in Madagascar, but it lacks a cheilostomal ring (Chabaud & Brygoo, 1957). This is the first record of a *Falcaustra* species in *E. madagascariensis*, and the second in a pleurodiran turtle. Species of *Falcaustra* are much more common in cryptodirans (see Baker, 1983).

Most of the nematodes found in *P. castanoides* and *P. subrufa* had characteristics (see results) of the genus *Spiroxys* (see Yorke & Maplestone, 1969; Chabaud, 1974). Studies on their specific identity are in progress (Roca, in preparation). This is the first record of *Spiroxys* sp. in Madagascan freshwater turtles. Confirmation of *Spiroxys* spp. in turtles in the Ethiopian region was only known previously from two male specimens labelled *Spiroxys contorta* from *Pelusios subniger* (Pelomedusidae) in Nigeria, deposited in the British Museum (Natural History, reg. N° 1971.937) and confirmed as species of the genus (but not *S. contortus*) by Berry (1985).

This is also a new record of *Spiroxys* spp. in a pleurodiran turtle (previous records were in pleurodirans from Australia by Berry, 1985; Ferguson & Smales, 1998), since *Spiroxys* spp. occur widely in the Cryptodira group. In accordance with the hypothesis of Berry (1985) regarding the presence of *Spiroxys* spp. in Australian pleurodiran turtles,

we suggest that Ethiopian pleurodirans acquired *Spiroxys* from non-marine cryptodirans, perhaps from Palaearctic Bataguridae. This is supported by the presence of *Spiroxys contortus* and *Spiroxys* sp. in *Mauremys leprosa* (Bataguridae) from some areas of North Africa, Turkey, and the Iberian Peninsula (Schad *et al.*, 1960; Yamaguti, 1961; Roca *et al.*, 2005).

Helminth communities

Helminths occurred at moderate to low prevalence in the only representative in this sample, the host-species *E. madagascariensis*. This is in line with the typical pattern of helminth infection in many reptiles, i. e., few species occur frequently, few species occur with moderate prevalence, and many species are rare (Aho, 1990; Roca *et al.*, 2005). *A. chabaudi* can be considered as the core species (Table I). Its high prevalence might be related to the auto-infective cycle typical of the Atractidae, in which larvae hatch and develop to adulthood without passing to the external environment (Adamson & Baccam, 1988). With a low prevalence, *Spiroxys* sp. is a satellite species in the helminth community of *E. madagascariensis*.

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