Pentastomid infections in Nile crocodiles (*Crocodylus niloticus*) in the Kruger National Park, South Africa, with a description of the males of *Alofia simpsoni*

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ABSTRACT

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Two Nile crocodiles were obtained from two different localities in the Kruger National Park, one a healthy specimen, the other in a severely debilitated condition. Both were males over 3 m long and both harboured the three pentastome genera *Sebekia*, *Alofia* and *Leiperia*. The genus *Sebekia* was represented by three species, *Sebekia wedli* Giglioli, 1922, *Sebekia cesarisi* Giglioli, 1922 and *Sebekia okavangoensis* Riley & Huchzermeyer, 1995. Of the genus *Alofia* two species, *Alofia simpsoni* Riley, 1994 and *Alofia nilotici* Riley & Huchzermeyer, 1995 were found. The male of *A. simpsoni*, formerly unknown, is described and the description of the females emended. *Leiperia cincinnalis* Sambon, 1922 was the only *Leiperia* present. Whereas *Sebekia* and *Alofia* were recovered from the bronchioles and lung parenchyma, female *Leiperia* occurred in the trachea and bronchi, and infective larvae as well as immature males and females, were collected from the lungs, the heart and the aorta. Adult *Subtriquetra* (Family Subtriquetridae) were not present in the nasopharynx of either crocodile. The intensity of infection was low in the healthy crocodile and had no negative effect on the host. In contrast, the debilitated crocodile was heavily infected and its poor condition is ascribed to its high pentastome burden. Histopathology revealed lesions in the tracheal wall and the lungs accompanied by chronic granulomata with secondary fungal infection as well as severe chronic multifocal granulomatous pneumonia.

Keywords: *Alofia*, *Crocodylus niloticus*, histopathology, *Leiperia*, pentastomes, *Sebekia*

INTRODUCTION

Pentastomes are endoparasites that mature in the respiratory tract of their final hosts, more than 90% of which are reptilians, such as crocodiles, snakes and saurians (Baer 1952; Riley 1986). Of the existing eight families of pentastomes, two families, the Sebekidae and Subtriquetridae are known to infect crocodilians, using fish as intermediate hosts. The family Sebekidae comprises the genera *Sebekia* Sambon, 1922, *Alofia* Giglioli, 1922, *Selfia* Riley, 1994, *Leiperia* Sambon, 1922, *Agema* Riley, Hill & Huchzermeyer, 1997 and *Diesingia* Sambon, 1922. The first five genera, with the exception of a single species of *Sebekia*, which can reach maturity in freshwater chelonians (Dukes, Shealy & Rogers 1971), occur only in crocodilians while *Diesingia* has a chelonian definitive host (Overstreet, Self & Vliet 1985). The monogeneric family Subtriquetridae is exclusive to crocodilians (Riley, Spratt & Winch 1990).

The Nile crocodile, *Crocodylus niloticus*, is parasitised by three sebekiid genera, *Sebekia*, *Alofia* and *Leiperia* (Sambon 1922; Fain 1961). Most data were derived from studies conducted in Central Africa during the first part of this century and only recently have Riley & Huchzermeyer (1995) and Riley, Hill & Huchzermeyer (1997) studied new material.

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One of the *Alofia* species present in Africa, *Alofia simpsoni* Riley, 1994, has been described from only two females recovered from an unknown host in Ghana. In this article we add to the description of the females and describe the main characteristics of the males.

A fourth sebekiid genus present in Africa, *Agema*, has to date only been recorded from the slender-snouted crocodile, *Crocodylus cataphractus*, and the dwarf crocodile, *Osteolaemus tetraspis*, both of which occur in the equatorial rain forests of West and Central Africa (Riley *et al.* 1997). The only reports regarding pentastome infections in crocodilians in southern Africa are from a single Nile crocodile in Botswana (Riley & Huchzermeyer 1995) and two in the Kruger National Park, South Africa (Junker 1996; Junker, Boomker & Booyse 1998a, b).

In order to determine the pentastome fauna and their prevalence in crocodiles in the southern parts of Africa, a study on crocodile pentastomes was conducted in the Kruger National Park, South Africa during 1995 (Junker 1996). Some of the results of the unpublished thesis are presented in this paper.

MATERIALS AND METHODS

Hosts

Two Nile crocodiles were obtained from different localities in the Kruger National Park. Both specimens were male and measured 3,2 and 3,3 m in length, respectively. Crocodile A was caught in the Phabeni Dam (25°1'S, 31°15'E) in February 1995 with a baited cage-trap. It was immobilized with gallamine triethiodide (Flaxedil™) by means of an intra-muscular injection given with a pole dart. Subsequently the crocodile was transported to the laboratory at Skukuza where it was shot and examined immediately after death.

Crocodile B was in a severely debilitated condition and was shot at the Shimuwini Dam (23°42'S, 31°17'E) in June 1995. Its heart, lungs and trachea were placed in separate plastic bags filled with saline and kept cool. The organs were examined within 13 h of death. After removal of the trachea and the oesophagus, the nasopharynx, especially the area around the internal nostrils, was visually inspected for subtriquetrids.

Parasites

Pentastomes visible underneath the pleurae of the lungs were removed through an incision. Both lungs of each of the reptiles were opened along the bronchi and bronchioli with a pair of scissors and the parasites dissected out of the tissue. The hearts were opened with a pair of scissors, as well as the left and right aorta, and truncus pulmonalis.

All pentastome material was transferred into saline and used for experimental infections or fixed in 70% ethanol and mounted in Hoyer's medium for identification. Measurements were taken from whole mounted specimens according to the methods described by Riley *et al.* (1990).

The prevalence and intensity of pentastome infections were determined and the use of ecological terms is in accordance with the definitions given by Margolis, Esch, Holmes, Kuris & Schad (1982).

Pathology

Tissue samples of the trachea, lungs and heart were collected and fixed in 10% buffered formalin for histopathological examination. Tissue blocks were embedded in paraffin wax, sectioned at 5 mm and stained with eosin and haematoxylin. Fungi in the lung lesions were demonstrated by staining sections with Gomori's methenamine-silver nitrate (GMS) (Luna 1968) and the periodic acid-Schiff reaction (Pearse 1961).

RESULTS

Parasites

Both crocodiles harboured the three sebekiid genera *Leiperia*, *Sebekia* and *Alofia*. Female *Leiperia cincinnalis* occurred in the trachea and the bronchi, while *Sebekia* and *Alofia* were found in the bronchioles and the lung parenchyma. *Subtriquetra* was not found in the nasopharynx of either crocodile.

Fifteen adult pentastomes and 14 nymphs were obtained from Crocodile A. *Sebekia okavangoensis* was the dominant species with nine adult specimens being present. A single *S. okavangoensis* male was found in the aorta, the remainder being in the bronchioles. One *Sebekia wedli* male was collected from the lungs, as well as a single *Sebekia cesarisi* female and one male *Alofia nilotici*. All adult pentastomes were sexually mature specimens as indicated by the fully developed copulatory spicules of the males and the presence of eggs in the uteri of the females. Also present in the lungs were 11 infective sebekiid larvae other than *Leiperia*. Three infective larvae of *L. cincinnalis* were attached to the aorta, while three adult females were collected from the trachea.

Crocodile B was heavily parasitised and harboured six different pentastome species. A total of 177 adults and 62 infective larvae were recovered of which *S. wedli* from the lungs (n=75) accounted for nearly half of the adult collection. A single *S. cesarisi* female and seven males, one male and two female *S. okavangoensis* and six adult *Sebekia* spp. females that could not be identified to the species level were present in the lungs. Four infective larvae were recovered from the same site. The genus *Alofia* was

represented by 61 *A. simpsoni* together with one male and one female *A. nilotici*. A single infective *Alofia* sp. larva (ascribed to this genus because of the characteristically U-shaped oral cadre) occurred in the heart. The sex ratio was in favour of females, it being 91 % in *S. wedli* and 79 % in *A. simpsoni*.

Fifteen patent *L. cincinnalis* females were obtained from Crocodile B. Two were attached to the tracheal wall and three were recovered from the right bronchus. The remaining *Leiperia* females were lumped together in a mucous matrix in the left bronchus, severely obstructing the airflow. Males were not present. Infective *L. cincinnalis* larvae ($n = 57$) were collected from both lungs, the heart and from two big clusters in the pulmonary artery. The latter larvae were embedded in a mucous matrix that partially obstructed the lumen of the vessel. Also isolated from the clusters were seven specimens that carried simple hooks and retained the old cuticle of the infective larval stages. One immature male and an immature female were identified while the sex of the other five specimens remains undetermined.

Additions to the description of *Alofia simpsoni* Riley, 1994

Females ($n = 12$)

The body-shape is dominated by the bulbous caudal extremity. The body length is 29 ± 1,6 mm and the maximum width is 2,0 ± 0,2 mm. The oral cadre is 318,2 ± 27,3 µm long and 151,3 ± 16,4 µm wide, with an overall length of 366,7 ± 32,2 µm. Hooks are 124,6 ± 8,5 µm long and the fulcra measure 274,5 ± 30,9 µm. Annuli number 82 ± 2.

Males ($n = 13$)

Males of *A. simpsoni* are markedly smaller than the females and lack the bulbous tail. The body length averages 8,7 ± 0,9 mm and it is 1,1 ± 0,2 mm wide. The smooth hooks are long (104,4 ± 3,5 µm) and slender, bent almost through a right angle and are devoid of spines (Fig. 1A, B). The fulcra measure 230,1 ± 15,2 µm. The oral cadre is U-shaped, possesses a small peg-like extension into the pharynx, and is 207,2 ± 15,7 µm long and 104,1 ± 5,0 µm wide (Fig. 1A, C). It has an overall length of 267,6 ± 16,9 mm. The copulatory spicules are typically alofian in that the smooth-surfaced shorter extension of the base of the cowry-shell ends in a double hooked collar. The second projection is elongated and its surface marked by transverse grooves (Fig. 1A, D, E). The length of the cowry-shell, including the short extension, averages 300,4 ± 14,3 µm and the total length, including the longer extension, is 372,8 ± 30,5 µm. The opening in the cowry-shell is shaped like a long ellipse. The number of annuli varies from 79–83.

Pathology

Crocodile A was in good condition and the lungs and heart were not impaired in their functionality. The attachment sites of pentastomes in the lungs and trachea were characterized by an area of mild coagulative necrosis with eosinophilic and heterophilic infiltrates, with associated oedema and haemorrhage in the surrounding tissue. Migration tracts were seen as multifocal thin-walled cavities lined by scattered multinucleated giant cells and containing coagulated blood and haematoidin. A pentastome was present in the aorta lumen, attached to the endothelium. At the attachment site focal erosion of the endothelium, associated oedema, haemorrhage and infiltration of small numbers of macrophages and lymphocytes were seen.

Crocodile B was severely emaciated. It only weighed between 105 and 110 kg whereas the average normal weight of a crocodile of 3,3 m is around 155 kg (Loveridge & Blake 1972). Macroscopically part of the bronchi and pulmonary aorta were obstructed by females and infective larvae of *L. cincinnalis*, respectively. The outer surface of the trachea was covered by numerous brown nodules, which represented migration tracts and attachment sites of the pentastomes. Their histopathological appearance was as described for Crocodile A. The anterior part of a female embedded in the tracheal mucosa and attached to the wall of the trachea is illustrated in Fig. 2A. A chronic multifocal granulomatous pneumonia associated with many intralesional pentastome adults, larvae and eggs (Fig. 2B) was present. Several of the lesions in the trachea and lung were enlarged, containing abundant eosinophilic necrotic debris surrounded by multinucleated giant cells. Associated with some of these lesions were a myriad of fungal hyphae, 3–6 µm diameter, regularly septate with random branches at 90° angles. One such a fungal lesion is illustrated in Fig. 2C. Alternatively, these lesions contained many bacterial colonies within the necrotic centres. The hooks of the pentastomes attached to the pulmonary arterial wall elicited a moderate chronic multifocal granulomatous arteritis.

DISCUSSION

Parasites

The pentastomid fauna of *C. niloticus* is characterized by a high diversity. Three different genera of pentastomes were recovered from both the crocodiles examined and a total of six sebekiid species were present. Although adults of *Subtriquetra* were not recovered from either of the crocodiles, the presence of infective larvae of *Subtriquetra rileyi* in two cichlid fish species in the Kruger National Park indicates that Nile crocodiles probably also serve as hosts for this pentastome (Junker *et al.* 1998a). *A.*

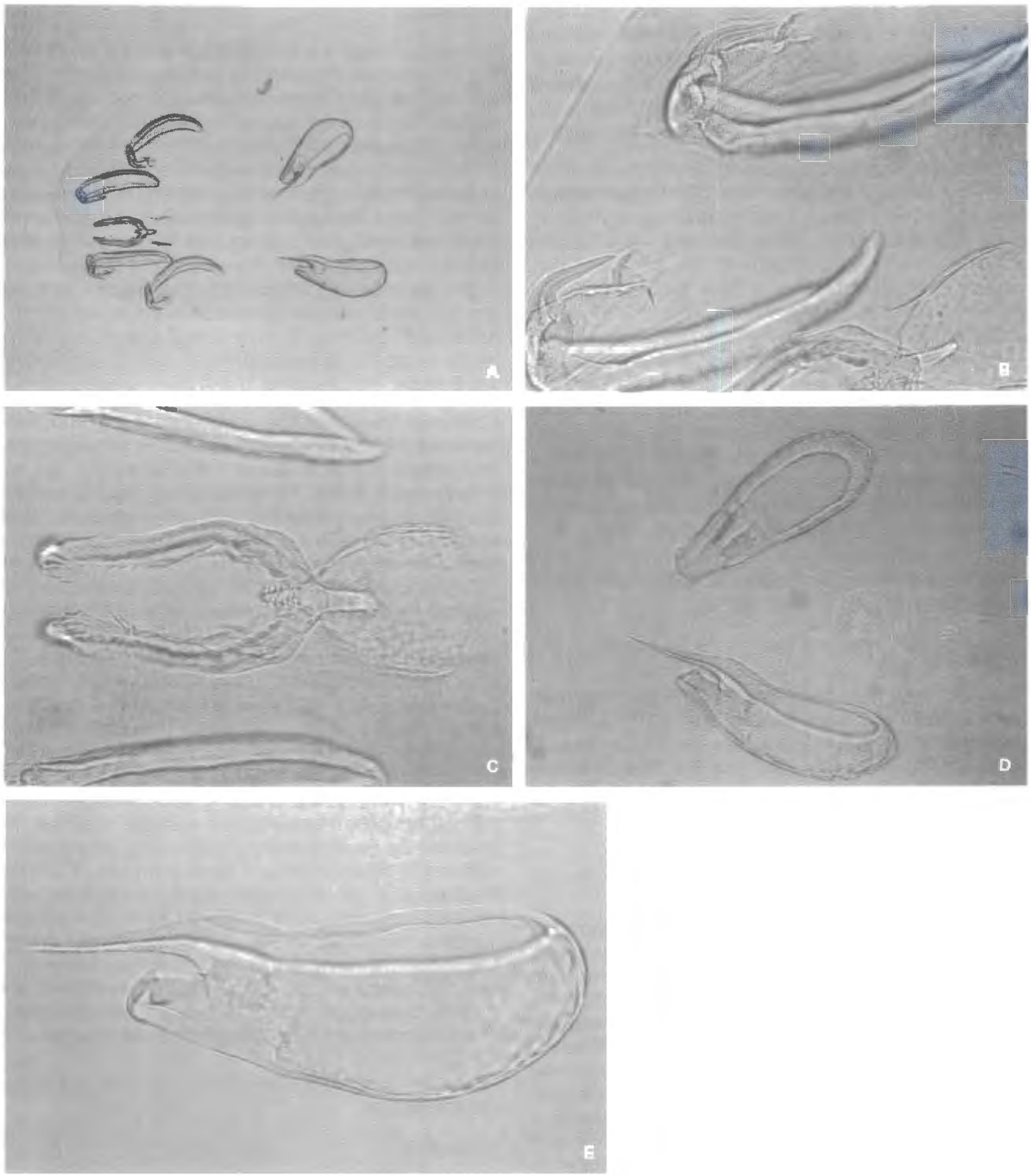


FIG. 1 *Alofia simpsoni*

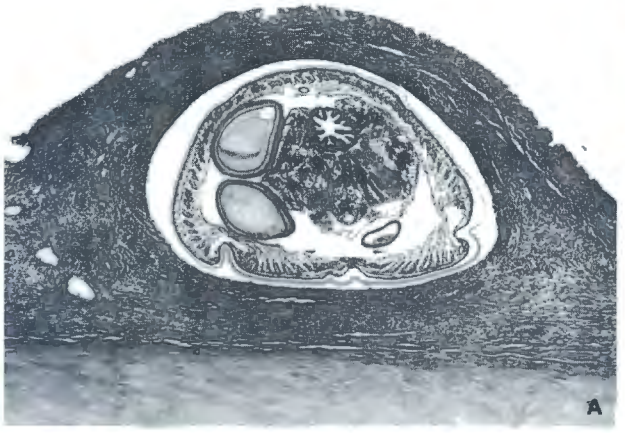
A Cephalothorax of a male, depicting the alignment of the hooks, the oral cadre and the copulatory spicules

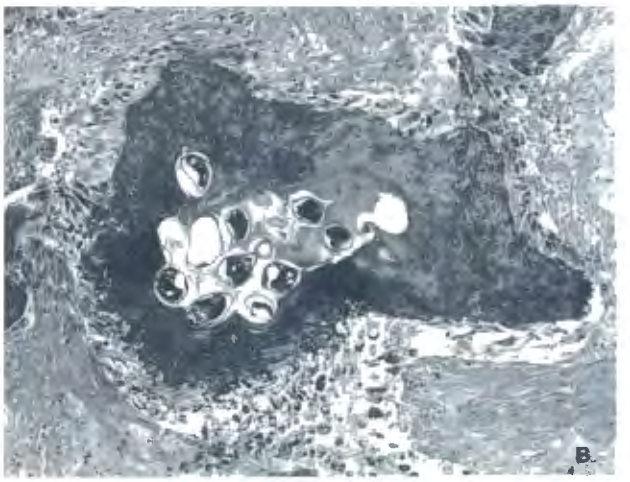
B Detail of the left posterior and anterior hook of a male. The hooks are smooth and bent at almost a right angle

C U-shaped oral cadre of a male showing the peg-like extension into the oesophagus

D Right and left copulatory spicule, in lateral and ventral view, respectively. Note the double hooked collar of the shorter extension of the cowry-shell

E Detail of right copulatory spicule seen in A. The long, spatulate extension of the cowry-shell is marked by chitinized ridges. In the right upper corner parts of the coiled cirrus are visible





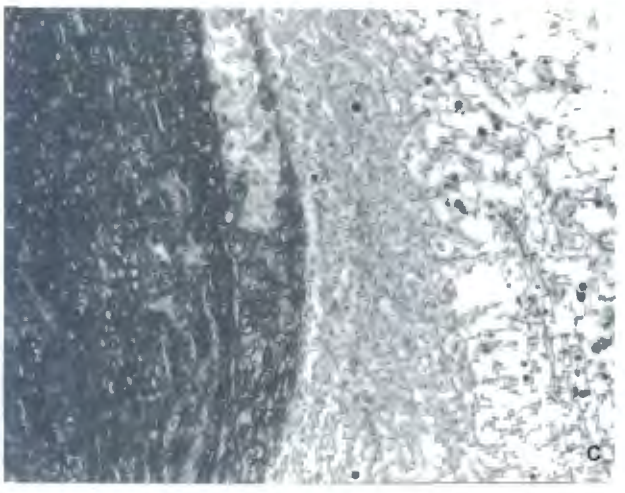


FIG. 2 A A transverse section of the anterior part of a female *Leiperia cincinnalis* embedded in the mucosa of the lumen of the trachea. HE, x 40

B A granulomatous lesion in the lung associated with pentastome eggs. HE, x 100

C A fungal granuloma (left) surrounded by fairly normal lung tissue (right). GMS, x 100

simpsoni has thus far only been recorded from an unknown host, probably a crocodilian (Riley 1994), and its presence in Nile crocodiles constitutes a new host record for the parasite.

As opposed to snakes in which multiple infections seldom occur (Fain 1961), multiple infections in crocodilians are common. Riley & Huchzermeyer (1995) found four different pentastome species in a single Nile crocodile from Botswana. Similarly, the Indo-pacific crocodile, *Crocodylus porosus*, is known to be the final host of seven species representing four genera (Riley 1994).

Most pentastomes were encountered at attachment sites considered typical for the genus and its developmental stages. Thus, infective larvae of *L. cincinnalis* were in the heart and aorta from where they invade the trachea and bronchi (Rodhain & Vuylsteke 1932; Heymons 1939).

The occurrence of a *S. okavangoensis* male in the aorta of Crocodile A and an infective larva of *A. simpsoni* in that of Crocodile B is unusual, since these sebekiids occur in the bronchioli and lung parenchyma (Fain 1961; Riley 1994). Adult pentastomes start migrating from the lung tissue following the death of the host (Overstreet *et al.* 1985) which is ascribed to declining oxygen levels (Riley & Huchzermeyer 1995). Crocodile A was necropsied immediately following its death but the muscle relaxant may have impaired its breathing. We therefore assume that the parasite's presence was due to post-mortal migration. Due to the long interval before the organs of Crocodile B were processed, we make the same assumption for *A. simpsoni*.

Some authors have observed that in spite of a balanced sex ratio in the infective larvae, the sex ratio in mature infections shifts in favour of the females (Leuckart 1860; Hett 1924; Riley 1972). Based on observations of the genera *Kiricephalus* and *Waddycephalus*, Riley & Self (1980; 1981) conclude that this is due to the comparatively shorter life span of pentastome males. Our findings, especially concerning *S. wedli* and *A. simpsoni*, support this.

Few data exist as regards the prevalence and intensity of pentastomid infections in the Nile crocodile.

The Phabeni Dam in the south-west and the Shimuwini Dam in the north-west of the Park are part of two unrelated river systems, the Sabie River/ Phabeni River in the south and the Letaba River in the north. The recovery of pentastomes in each of the Nile crocodiles indicates that the parasites are widespread in the Park and not limited to a single river system.

Apart from this study, only Riley & Huchzermeyer (1995) provide the intensity of pentastome infection of a single Nile crocodile in Botswana. This reptile harboured 94 adult pentastomids. Almost as little is known about the slender-snouted crocodile and the dwarf crocodile. Riley *et al.* (1997) collected eight pentastomids from a juvenile *C. cataphractus* from the Congo Republic. At the same occasion, pentastomes from 15 specimens of *O. tetraspis* were recovered. The prevalence of infection was 80 % with a mean intensity of 24 (Riley *et al.* 1997). More detailed information is available for North American alligators. Seven alligators from Georgia were infected with 30–40 pentastomids each (Deakins 1971), and 93 % of 30 alligators examined by Cherry & Ager (1982) had 10,6 (1–77) adults. The intensity of infection in the two crocodiles examined during this study differed considerably. While Crocodile A carried a light pentastome burden (15 adults), Crocodile B was heavily infected, and the recovery of 177 adults exceeds the intensities formerly recorded for crocodilians by far.

Alofia simpsoni Riley, 1994

The main characteristics of the females of *A. simpsoni* described in this paper fit in well with Riley's (1994) description. The overall length of the oral cadre was given as 355 µm, but according to Riley (1994) it was not possible to measure any other dimensions of the buccal complex due to the way in which the specimens were mounted.

There is a notable difference in our annulus counts when compared to that given by Riley (1994). We are not able to explain the discrepancy, but considering the number of specimens at our disposal we believe our counts to be representative.

The males of *A. simpsoni* are distinctly different from *A. nilotici*, in that the hooks of *A. simpsoni* are smooth whereas those of *A. nilotici* are equipped with a patch of minute spines. The copulatory spicules of *A. simpsoni* are markedly smaller than those of *A. nilotici* (372,8 µm long as opposed to 585 and 520 µm, respectively).

Pathology

Ladds & Sims (1990) necropsied 54 young crocodiles, *C. porosus* and *C. novaeguineae*, eight of which were infected with pentastomes. The same histopathological picture was evident in our crocodiles. In three of the cases, the infection with pentastomes was considered one of the main reasons for the poor condition of these animals (Ladds & Sims 1990). The presence of granulomata in the lungs and trachea of Crocodile B were often associated with bacterial colonies or fungal infiltration, which conforms to the findings of Deakins (1971). The damage caused to the lung epithelium by pentastomes often gives way to secondary infections (Deakins 1971). In alligators, *Sebekia* spp. facilitate infection with *Aeromonas* sp. (Shotts, Gaines, Martin & Prestwood 1972; Hazen, Aho, Murphy, Esch & Schmidt 1978).

The mild pentastome infection in Crocodile A had no apparent adverse effect, indicating that, under natural conditions, Nile crocodiles are able to tolerate pentastome infections. Boyce, Cardheilac, Lane, Buergelt & King (1984) came to the same conclusion when studying sebekiosis in alligators. The distinct clinical signs seen in Crocodile B, however, emphasize that given the right circumstances, pentastomids can have a serious impact on the host. We ascribe the poor condition of Crocodile B to the heavy infection, the pentastome activity causing extensive damage to the lungs and heart of the host. During post mortem examination no injuries accounting for Crocodile B's condition were found. A possible explanation for the large number of pentastomes may be found in the environmental circumstances at the Shimuwini Dam during the months prior to our studies: a large number of crocodiles congregated in front of the dam wall, feeding extensively on fish that got trapped against this structure.

Unfortunately, the prevalence and intensity of infection in fish at the Shimuwini Dam could not be established. However, infection rates in Mozambique bream, *Oreochromis mossambicus*, and red-breasted bream, *Tilapia rendalli*, from the Phabeni Dam were low (Junker *et al.* 1998a). This suggests that the high density of intermediate hosts and thus the high intake of fish by the final hosts, even though the infection rate in the fish might have been low, exposed the crocodiles to a concentration of infective pentastomid larvae that would otherwise not be encountered. These are important considerations as regard the conservation of the Nile crocodile. It illustrates that under certain conditions, pentastomes can pose a serious threat to their definitive hosts. Environmental destruction and decreasing water levels due to human activity imply that crocodiles are restricted to a decreasing number of suitable habitats. The resulting increase in population density may enhance the spreading of parasitic infections drastically.

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